I. BACTERIA

A. Prokaryotic organisms

1. Bacteria are unicellular organisms that lack a true nucleus and nuclear membrane. The lack of mitochondria, endoplasmic reticulum, and Golgi bodies also differentiates the prokaryotic bacteria from eukaryotes.

2. The bacterial genome is a single, closed, circular chromosome of double-stranded DNA called the nucleoid. Plasmids are small circular molecules of extrachromosomal circular DNA. Antibiotic resistance is often encoded by genes located on plasmids. Chromosomal or plasmid gene exchange via transformation, transduction, or conjugation are means of genetic recombination.

3. Reproduction of bacteria is achieved by binary fission, a means of asexual reproduction. Prokaryotes do not reproduce sexually.

4. Energy generation of bacteria is cytoplasmic membrane-associated via the electron transport chain.

5. Bacteria usually range in size from 0.2 to 2 um in diameter and 1 to 6 um in length. The four basic morphological types are cocci (spherical-shaped cells), bacilli (rod-shaped cells), spirilla (spiral-shaped cells), and vibrios (comma-shaped cells (Figure 7–1).

B. Growth and nutrition

1. Prokaryotic bacteria have three major nutritional needs for growth.
   a. A source of carbon is needed for the synthesis of cellular constituents.
   b. A source of nitrogen is necessary for the synthesis of protein.
   c. Energy (ATP) is needed for cellular functions.

2. The optimum pH for the growth of most bacteria is 7.0 to 7.5. Acidophiles grow at an acidic pH and alkaliphiles grow optimally at an alkaline pH.

3. A wide range of growth temperatures facilitate the growth of bacteria. The optimum growth temperature for most human bacterial pathogens is 35 °C to 37 °C, the temperature of the human body.

4. Bacteria may be classified based on the atmospheric requirement for the growth.
   a. Obligate aerobes require oxygen for growth.
   b. Aerotolerant aerobes can grow in the presence of oxygen, but grow best in an anaerobic environment.
   c. Facultative anaerobes grow in both aerobic and anaerobic environments.
   d. Obligate anaerobes cannot grow in the presence of oxygen.
   e. Capnophilic bacteria require concentrations of 5% to 10% CO₂ for optimal growth.
   f. Microaerophilic bacteria grow optimally in a reduced level of oxygen.
C. Metabolism

1. **Fermentation** is an anaerobic process by which bacteria catabolize carbohydrates to produce energy. The process is carried out by both obligate and facultative anaerobes, and the final electron acceptor is an organic compound.

2. **Respiration (oxidation)** is an efficient process by which obligate aerobes and facultative anaerobes generate energy. Molecular oxygen is the final electron acceptor. Respiration is a more efficient mechanism for energy generation than fermentation.

II. BACTERIAL CELL STRUCTURE

A. The **cell membrane** serves as an osmotic barrier and may be a site of antibiotic action. An intact membrane is essential for bacterial viability.

B. The **cell wall**

1. The most prominent layer of the **gram-positive cell wall** is the thick, rigid peptidoglycan layer; the site of action of the penicillins and cephalosporins. **Teichoic and lipoteichoic acids** are unique to the gram-positive cell wall (Figure 7–2).

2. More complex than the gram-positive cell wall, the **gram-negative cell wall** contains a thinner peptidoglycan layer, but also an outer lipopolysaccharide (LPS) layer. LPS is an **endotoxin** that is an important virulence factor. An endotoxin causes shock, sepsis, fever, disseminated intravascular coagulation (DIC), and leukopenia (Figure 7–3).

C. A **polysaccharide capsule** covers many bacteria and serves to prevent or inhibit phagocytosis. For many organisms (e.g., *Streptococcus pneumoniae*), the capsule is the chief determinant of virulence.
D. Pili, also called fimbriae, are short, hairlike structures that serve to attach bacteria to target cells. For many bacteria (e.g., *Neisseria gonorrhoeae*), interference with attachment prevents infection. The exchange of deoxyribonucleic acid (DNA) between bacteria during conjugation occurs through the pili.

E. Flagella determine motility and can be used in classification.

F. Endospores are a means of survival that make disease control very difficult. The two spore-forming genera of importance are *Bacillus* and *Clostridium*. 

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**Figure 7–3.** Gram-Negative Bacterial Cell Wall. Winn WC, Jr. *Color Atlas and Textbook of Diagnostic Microbiology.* 6th ed. Baltimore: Lippincott Williams & Wilkins; 2006.
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III. STAINS

A. Gram’s stain
1. Crystal violet is the primary stain. Iodine binds the crystal violet to the cell wall (mordant). Decolorizer washes out any unbound dye. Safranin O is a counterstain.
   a. Gram-positive cells retain the crystal violet and stain purple (see Web Color Images 7–1, 7–2, and 7–3).
   b. Gram-negative cells are decolorized, retain the safranin O counterstain, and stain red or pink (see Web Color Images 7–4 and 7–5).
2. Clinical use. Gram’s stain is especially useful for examining smears of clinical specimens. Initial treatment, and often a presumptive identification, can be made from Gram’s stain results. White and red blood cells (WBCs and RBCs), as well as cellular debris, stain pink. This can serve as an internal control.

B. Acridine orange stain is an orange fluorescent stain used to detect bacteria in body fluids in which numbers of bacteria may be few (e.g., blood and spinal fluid). The stain can also be used to detect bacteria in direct smears with excess cellular debris. It is very sensitive and can detect small numbers of bacteria that are living or dead. The procedure consists of flooding a methanol-fixed smear with acridine orange for 2 minutes, washing, and then observing with a fluorescence microscope (see Web Color Image 7–6).

C. Methylene blue stain is especially helpful for demonstrating metachromatic granules and characteristic morphology of *Corynebacterium diphtheriae* from Loeffler coagulated serum medium. The procedure consists of flooding a fixed smear with methylene blue, followed by washing (see Web Color Image 7–7).

D. Acid-fast stain is used to detect organisms that do not stain well with other conventional stains (e.g., *Mycobacterium* spp., *Nocardia, Actinomyces*). These organisms have a high lipid content in their cell walls. Once stained, they are very resistant to decolorization by acid alcohol.
   The most commonly used method is a carbol fuchsin stain, the modified Kinyoun stain. The primary stain is carbol fuchsin, which contains a surface-active detergent to facilitate penetration of the stain without heating. After washing, methylene blue is used as a counterstain. Acid-fast organisms appear red against a blue background. The Ziehl-Neelsen is a carbol fuchsin stain that utilizes heat to drive the stain into the mycobacterial cell wall (see Web Color Image 7–8).

E. Auramine-rhodamine stain is a fluorescent stain that detects mycolic acids and can be used for staining acid-fast organisms. The smear is stained with auramine-rhodamine, decolorized with acid alcohol, and then flooded with potassium permanganate. It is observed with a fluorescence microscope. The cells appear yellow against a dark background (see Web Color Image 7–9).

F. Calcofluor white stain binds specifically to chitin, which is found in fungal cell walls. It is used to detect yeast cells and hyphae in skin scrapings and other specimens. The fungal elements appear green or blue-white (see Web Color Image 7–10).

IV. NORMAL FLORA describes the microorganisms that are frequently found on or in the bodies of healthy persons.

A. General characteristics
1. Local conditions select for those organisms that are suited for growth in a particular area.
2. Resident flora colonize an area for months or years.
3. Transient flora are present at a site temporarily.
4. Organisms that live at the expense of the host are parasites.
5. Organisms that benefit the host are symbionts.
6. Commensals have a neutral effect on the host.
7. A carrier harbors the organism without manifesting symptoms, but is capable of transmitting infection (carrier state).
8. Opportunists are organisms that do not normally cause infection, but can do so if the condition of the host changes (e.g., immunosuppression).

B. Normal flora of the skin
1. The skin contains a wide variety of microorganisms that are not eliminated by washing or superficial antisepsis.
   a. Propionibacterium acnes colonizes the sebaceous glands.
   b. Micrococcus, Staphylococcus spp., and diphtheroids are common.
2. Intact skin is an effective barrier to microbial invasion.

C. Normal flora of the mouth. The mouth contains large numbers of bacteria, most commonly Streptococcus spp. (especially viridans species), coagulase-negative Staphylococcus, Peptostreptococcus, and other anaerobes.

D. Normal flora of the respiratory tract
1. The respiratory tract beyond the oropharynx is normally sterile.
2. The ciliary action of epithelial cells and mucus movement remove invading organisms.
3. The nose and nasopharynx contain Staphylococcus aureus, S. epidermidis, and Streptococcus spp. The following may be present transiently during community outbreaks of infection: Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis.
4. The normal flora in the oropharynx mirrors that of the mouth.

E. Normal flora of the gastrointestinal tract
1. Most microorganisms are destroyed in the stomach due to the acidic pH. The survivors multiply in the colon.
2. More than 90% of the microbial population is comprised of anaerobes.
3. Alteration of the normal flora by antibiotics may allow a superinfection by Clostridium difficile (necrotizing enterocolitis), Candida albicans, or S. aureus.
4. The most commonly found organisms in the gastrointestinal tract are: Bacteroides spp., Clostridium spp., Eubacterium spp., anaerobic streptococci, Enterococcus spp., and Enterobacteriaceae.

F. Normal flora of the genitourinary tract
1. The outermost segment of the urethra is colonized by skin organisms.
2. The vagina is colonized with Lactobacillus, anaerobic gram-negative rod-shaped bacteria, and gram-positive cocci.

V. PATHOGENESIS OF INFECTION

A. Host resistance
1. Innate immunity, or natural immunity, is a nonspecific mechanism of resistance to disease.
   a. Physical barriers such as the skin, mucous membranes, and cilia help prevent the invasion of pathogenic bacteria.
   b. The cleansing action of fluids in the eyes, respiratory, digestive, urinary, and genital tracts are also effective host resistance mechanisms.
   c. Antimicrobial substances such as secretory IgA, lysozyme, B-lysin, and interferon are produced in the human host.
   d. Indigenous microbial flora compete with invading bacteria for colonization sites, as well as produce inhibitory bacteriocins.
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e. Phagocytosis, inflammation, and complement activation are also important non-specific defense mechanisms of the innate immunity.

2. Acquired or specific immunity develops in response to a specific foreign antigen (e.g., invading bacterium). Acquired immunity may be active (a response to immunization or disease) or passive (through placent transfer or infusions of serum or plasma).
   a. The humoral response involves antibody production by B lymphocytes.
   b. Cell-mediated immunity involves cells, such as macrophages, cytokines, and T lymphocytes. The cellular response provides immunity against intracellular pathogens, including viruses, fungi, and mycobacteria.

B. Criteria for a successful pathogen

1. The degree of pathogenicity of a microorganism is directly related to its virulence. A virulent strain is characterized by its ability to evade or overcome host defenses and cause disease. Microbial virulence factors include adhesions, capsular polysaccharides, and production of extracellular toxins and enzymes that may cause damage to the host.

2. An appropriate portal of entry is necessary for successful transmission of disease. For example, respiratory pathogens enter the respiratory tract via inhalation of aerosolized respiratory secretions. Gastrointestinal pathogens enter the GI tract via ingestion of contaminated food and water.

3. A sufficient infective dose is needed for a microorganism to be able to cause disease in its host. Some organisms can establish an infection with a relatively low infective dose (e.g., Shigella), whereas others require a much higher infective dose to cause infection (e.g., Salmonella).

4. A successful pathogen must be able to overcome the host resistance mechanisms, including the immune responses, and cause disease in a susceptible host.

5. For continued transmission of disease, pathogenic microorganisms must be able to leave the host via an appropriate portal of exit. For most pathogens, it is the same as the portal of entry.

C. Transmission

1. Airborne transmission involves the respiratory spread of infectious disease via aerosolized respiratory secretions or contact with contaminated inanimate objects or fingers and hands.

2. Ingestion of contaminated food and water is the usual means for transmission of gastrointestinal infections. Infection can also occur via the fecal-oral route.

3. Sexual transmission is the route of infection for venereal diseases. Some diseases can be also transmitted via skin-to-skin contact or via direct transfer of saliva (e.g., kissing).

4. Animal and human bites can result in serious wound infections, usually caused by mouth flora. Rabies is an example of a viral infection transmitted from an animal bite. Opportunistic pathogens, including environmental bacteria, can enter the human via cuts and trauma wounds.

5. Arthropod vectors, such as ticks, fleas, and mites, are responsible for the transmission of diseases such as malaria, Lyme disease, plague, Rocky Mountain spotted fever, and hemorrhagic fevers. The infectious agent multiplies in the arthropod and is transmitted when the arthropod feeds off a human host.

6. Zoonoses are diseases of animals that are transmitted to humans. Transmission is usually via contact with infected animals, animal secretions, or animal products. Zoonoses can also be transmitted via animal bites or arthropod vectors.

VI. COLLECTION AND HANDLING OF CLINICAL SPECIMENS

A. Collection. A properly collected specimen is absolutely crucial to quality diagnostic information and patient care.

1. Safety
a. **Universal precautions** are followed throughout the collection and handling process. Persons collecting or handling specimens should wear gloves and a laboratory coat. Eye protection should also be worn if splashing is a potential hazard.

b. Accidents or injuries must be reported immediately.

2. **General guidelines**

a. The specimen should be from the infection site and not contaminated by the surrounding area (e.g., culture within a wound and not the surface or the surrounding skin).

b. Whenever possible, the specimen should be collected before antimicrobials are administered.

c. Appropriate collection devices and containers should be used and must be sterile. Aseptic technique is required.

d. The specimen container should be labeled with the patient’s identification, the date and time of collection, and the source of specimen.

3. **Collection from various body sites**

a. **Throat.** The tongue should be depressed before swabbing between the tonsillar pillars and behind the uvula. The cheek, tongue, and teeth should not be touched.

b. **Nasopharynx.** A flexible wire nasopharyngeal swab should be gently inserted through the nose into the posterior nasopharynx, rotated, and then removed.

c. **Sputum.** Whenever possible, the patient should gargle with water (not mouthwash) immediately before sampling. Early morning specimens are best. Expectorated specimens from a deep cough should be collected into a sterile specimen cup.

d. **Stool** should be collected in a clean, wide-mouthed container with a tight-fitting lid. If the specimen cannot be plated within 1 hour of collection, it should be mixed with a transport medium (e.g., buffered glycerol saline, Cary-Blair transport medium). The change in pH and temperature over time is detrimental to *Shigella* spp. Stool specimens should never be taken from the toilet and should not be contaminated with urine. Commercial systems with preservatives are available for collection of specimens for both bacterial culture and ova and parasite examination.

e. **Urine.** Midstream clean-catch is the most common collection method. Proper cleansing of the urethral area is important, especially in women. The first few milliliters, which flush out the urethra, are discarded. The specimen should be collected into a sterile specimen cup and transported immediately to the laboratory or refrigerated, because contaminants grow readily at room temperature. Cultures of catheterized urine specimens usually have less contaminating bacterial flora.

f. **Blood.** Two to three cultures should be collected at random times during a 24-hour period. Collecting more than three sets of cultures in a 24-hour period does not significantly increase the probability of detecting bacteremia. Skin is disinfected with 70% alcohol, followed by iodine. The disinfectant is allowed to dry. The puncture site should not be palpated after disinfection. Ideally, 20 to 30 mL of blood per culture is collected from an adult (1–5 mL from infants and small children). Iodine should be cleaned from the puncture site with alcohol following the venipuncture.

g. **Cerebrospinal fluid** should be collected aseptically by a physician. This specimen should be processed immediately and not exposed to heat or refrigeration.

h. **Abscess aspirates or exudates, as well as synovial, pericardial, and chest fluid** should be collected by a physician with a needle and syringe. The use of swabs may inhibit growth of anaerobes or increase the likelihood of contamination with indigenous bacteria flora from adjacent tissues (e.g., mucous membranes or superficial skin surfaces). Care should be taken not to inject an air bubble into the syringe.

i. **Genital tract**

1. **Men.** Exudates may be expressed from the urethral orifice or a small-diameter swab may be inserted 3 to 4 cm into the urethra. The specimen should be plated immediately on the appropriate media and not allowed to dry or be exposed to cold temperatures. A direct Gram’s stain smear should be prepared.

2. **Women.** Cervical specimens are obtained by a physician with the aid of a speculum. Lubricants, which may be lethal to *Neisseria gonorrhoeae*, should
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not be used on the speculum. The cervical mucus plug is removed, and a sterile swab is inserted into the cervix, rotated, and allowed to remain for a few seconds. The specimen should be plated immediately to the appropriate media, and swabs should not be refrigerated, as refrigeration may be lethal to genital pathogens. A smear for Gram’s stain should be prepared from the specimen.

B. Handling

1. Transport all specimens to the laboratory promptly. Anaerobic specimens must be transported in an anaerobic transport system. If transport cannot occur immediately, most specimens can be held at 2°C to 8°C. Exceptions to this include specimens that likely contain temperature-sensitive organisms (e.g., Neisseria), blood culture bottles, and cerebrospinal fluid (CSF). Generally, swabs are the least desirable collection and transport method. However, organisms can be successfully cultured if the swab is handled and transported properly (i.e., not allowed to dry out). Swabs are inappropriate for culturing anaerobes, although in some clinical settings, culturettes are often used. If swabs are used for the culture of anaerobes, it is essential that an anaerobic culturette be used. Use of aerobic culturettes for the culture of anaerobes is criteria for specimen rejection.

2. Processing

a. Media selection. Some general principles apply to the use of primary plating media. In most cases, the concern is that the primary media will grow and isolate all or a majority of the possible organisms from a clinical specimen. In those cases in which certain organisms are excluded, the decision of what media is used is based on time, cost, and probability of isolation information. In many cases, the choice of primary media is an individual laboratory choice. Selective and differential media may be used in addition to all-purpose and enriched agars. These specialized media are used for selective recovery and preliminary differentiation of specific bacteria. It is especially important that the microbiologist understands the range and purpose of each primary isolation medium, as well as the various reactions of the organisms, since individual organism groups may react differently on specific media.

(1) Most isolation protocols call for the use of blood agar (with 5% sheep RBC).
(2) Chocolate agar is used for fastidious isolates.
(3) Specialized media [e.g., mannitol salt agar, bismuth sulfite agar, Campylobacter agar, thiosulfate-citrate-bile salts-sucrose (TCBS) agar] are used when specific organisms are suspected.
(4) Substitutions may be made with acceptable results (e.g., MacConkey agar in place of Eosin Methylene Blue agar).
(5) Prereduced anaerobically sterilized (PRAS) culture media is recommended for the culture of anaerobes (see anaerobe section).

b. Incubation

(1) The normal incubation temperature for bacterial cultures is 35°C to 37°C. Culture plates may be incubated in ambient air, but incubation in an capnophilic atmosphere of 5% to 10% CO₂ is recommended to enhance the growth of fastidious bacterial isolates. Anaerobic cultures should be incubated anaerobically at 35°C to 37°C.
(2) Anaerobic bags, jars, or an anaerobic chamber are appropriate for incubation of anaerobic cultures.
(3) Recommended incubation of stool cultures for isolation of Campylobacter jejuni is in a microaerophilic, capnophilic atmosphere at 42°C to 45°C.

c. Specimen rejection criteria. Rejection criteria should be part of the written policy of every clinical laboratory. These criteria should be clearly listed and made available to anyone who might submit specimens for culture. Processing and culture of inappropriate specimens leads to increased costs and misinformation. In the event a specimen is rejected, the person submitting the request should be contacted and informed. In some cases, the difficulty of collection makes culturing necessary, although the results are not optimal. The following situations or
specimen types should be rejected; however, this is not intended to be a comprehensive list:
(1) Twenty-four-hour urine or sputum collections
(2) Specimens received in nonsterile or contaminated containers (including those in which the specimen has leaked out)
(3) Specimens contaminated with barium or other foreign substances
(4) Culturing of Foley catheter tips
(5) Saliva instead of sputum
(6) Unrefrigerated urine specimens 2 hours or more post-collection
(7) Anaerobic culturing of midstream urine, upper respiratory tract, superficial skin, or feces specimens (certain *Clostridium* species may be appropriately cultured from feces)

VII. MICROCOCCACEAE

A. General characteristics

1. Members of the family Micrococcaceae are gram-positive cocci (see Web Color Image 7–1), aerobic or facultative anaerobes, and catalase-positive (except *Stomatococcus*).
   a. The catalase test differentiates the Micrococcaceae from the gram-positive, catalase-negative Streptococcaceae.
   b. Hydrogen peroxide is converted to water and oxygen in the presence of the bacterial enzyme catalase. The observation of vigorous bubbling when the bacterium is mixed with a drop of 3% hydrogen peroxide is a positive test (see Web Color Image 7–11).

2. Most are members of the indigenous flora and are commonly isolated from a wide variety of diseases.

3. *Staphylococcus* (Table 7–1; Figure 7–4)
   a. The staphylococci are catalase-positive, nonmotile, facultative anaerobes that are normal inhabitants of the skin and mucous membranes. These organisms commonly cause human infections.
   b. Species are initially differentiated by the coagulase test (see Web Color Images 7–12 and 7–13). The most important coagulase-positive species is *S. aureus*. Some animal species produce coagulase, but are rarely isolated from human samples.
   c. Staphylococci that do not produce coagulase are called "coagulase-negative staphylococci." The most prominent species are *S. epidermidis* and *S. saprophyticus*.

4. *Micrococcus* (Table 7–1; Figure 7–4). Micrococci are opportunistic pathogens found only in immunocompromised persons. *Micrococcus* is of low pathogenic significance, but may be isolated as a contaminant or as part of the normal flora.

5. *Stomatococcus* (Table 7–1; Figure 7–4). This genus is part of the normal oral flora and is rarely isolated from infection. The colonies adhere strongly to the agar surface.

B. *Staphylococcus aureus*

1. Infections
   a. Skin and wound infections caused by *S. aureus* are suppurative and pyogenic. Some common skin infections are boils, carbuncles, furuncles, and folliculitis.

<table>
<thead>
<tr>
<th>Table 7–1</th>
<th>Differentiation of the Micrococcaceae</th>
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<tbody>
<tr>
<td>Characteristic</td>
<td>Staphylococcus</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
</tr>
<tr>
<td>Growth on 6.5% NaCl agar</td>
<td>+</td>
</tr>
<tr>
<td>Modified oxidase test</td>
<td>–</td>
</tr>
<tr>
<td>Resistance to bacitracin (0.04 μg) disk</td>
<td>+</td>
</tr>
<tr>
<td>Resistance to furazolidone (100 mcg) disk</td>
<td>–</td>
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</tbody>
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+= positive; –= negative
b. **Food poisoning.** Staphylococcal enterotoxins A and D are associated with food poisoning. The source of contamination is usually an infected food handler. Infection occurs when an individual ingests food contaminated with enterotoxin-producing strains. The heat-stable toxins are preformed in the food. Symptoms appear rapidly (2–6 hours after ingestion) and resolve within 8 to 10 hours. Symptoms may include nausea, vomiting, headache, abdominal pain, and severe cramping.

c. **Scalded skin syndrome (Ritter’s disease)** is an extensive exfoliative dermatitis that occurs primarily in newborns and is caused by staphylococcal exfoliative or epidermolytic toxin. It can also occur in adults, most frequently among those who have chronic renal failure or are immunocompromised. The mortality rate is low in children but high in adults.

d. **Toxic shock syndrome (TSS)** is a multisystem disease characterized by high fever, rash, hypotension, shock, desquamation of the hands and feet, and possible death. The etiologic agent is a strain of *S. aureus* that produces enterotoxin F [toxic-shock syndrome toxic-1 (TSST-1)]. There is an association between the use of tampons and TSS, although the disease may occur in both sexes if a toxin-producing strain of *S. aureus* has caused infection.

e. **Other staphylococcal infections**
   (1) Staphylococcal pneumonia secondary to influenza can occur. The mortality rate is high.
   (2) Osteomyelitis can occur secondary to bacteremia.

f. In addition to the toxins associated with specific infections (e.g., exfoliative toxin, enterotoxins A and D, TSST-1), other toxins and enzymes are also virulence factors of *S. aureus*. Coagulase is the major virulence marker of the species. Protein A is a cellular component in the cell wall that helps the bacterium avoid phagocytosis. Alpha and beta hemolysins cause the hemolysis of RBCs. In addition, hyaluronidase prevents the spread of infection and lipase facilitates colonization of the bacterium on the skin surface.

2. **Laboratory identification of *Staphylococcus aureus***

a. **Microscopic examination** of stained smears from clinical specimens can be especially helpful. Numerous gram-positive cocci with polymorphonuclear cells are usually seen.
b. *S. aureus* grows readily on common laboratory media. On sheep blood agar, colonies appear as round, smooth and white or pigmented (yellow-orange). They are usually β-hemolytic. (See Web Color Image 7–14.)

c. *S. aureus* is most often identified by the coagulase test. Isolates may show cell-bound (clumping factor) or free (extracellular) coagulase. Cell-bound coagulase is identified by mixing the suspected organism with a drop of rabbit plasma on a glass slide. (See Web Color Image 7–12.) If clumping occurs, the isolate demonstrates cell-bound coagulase and is identified as *S. aureus*. Isolates that do not clump are tested for free coagulase by the tube method (see Web Color Image 7–13), in which the organism is mixed with 0.5 mL of rabbit plasma, and following incubation at 37°C for 4 hours, is observed for clot formation.

d. Selective media that can be used to isolate *S. aureus* from heavily contaminated specimens or when it is the only isolate of concern are mannitol salt agar (MSA), phenylethyl alcohol (PEA) blood agar, and Columbia-naladixic acid Agar (CNA). (1) MSA provides mannitol as a fermentable carbohydrate source as well as 6.5% sodium chloride (NaCl). Generally, only *Staphylococcus* species grow on this medium because of the high salt content. *S. aureus* ferments the mannitol to produce acid, which turns the pink agar yellow. The colonies are identified by a yellow halo. This test is presumptive because some strains of *S. epidermidis* and few other species can also ferment mannitol. (2) PEA and CNA agars both inhibit the growth of gram-negative organisms, whereas the gram-positive bacteria, including *Staphylococcus* species, grow well. Five-percent sheep blood is incorporated in the medium for additional enrichment and detection of hemolytic reactions.

e. Rapid methods use plasma-coated latex particles. The plasma detects clumping factor and causes agglutination of the particles. Protein A in the cell wall of *S. aureus* (with IgG) may also be detected with rapid methods. Other species that produce clumping factor produce positive reactions, but are tube-coagulase-negative.

C. **Coagulase-negative staphylococci**

1. General characteristics. The coagulase-negative staphylococci are found as normal flora in humans and animals. The incidence of infection by these organisms has increased. They are often hospital-acquired (nosocomial). Predisposing factors include catheterization, prosthetic device implants, and immunosuppressive therapy. The most common species isolated from clinical infections are *S. epidermidis* and *S. saprophyticus*. *S. saprophyticus* has been associated with UTIs in young, sexually active women. Other species of coagulase-negative staphylococci are not isolated frequently. Three species that can cause a wide range of infections, but do so only occasionally, are *S. haemolyticus, S. lugdunensis,* and *S. schleiferi*. The latter two produce clumping factor and may yield a positive slide coagulase test.

2. Laboratory identification of coagulase-negative staphylococci

a. On sheep blood agar, colonies are usually round, smooth, and white without hemolysis. (See Web Color Image 7–15.)

b. The most common isolates are *S. epidermidis* and *S. saprophyticus*.

c. Urine isolates that are coagulase-negative are further tested to presumptively identify *S. saprophyticus*. This is done by testing for novobiocin susceptibility using a 5-mg novobiocin disk. *S. saprophyticus* is resistant to novobiocin, whereas other coagulase-negative staphylococci are susceptible.

d. Species identification of the coagulase-negative staphylococci requires differentiation using many biochemical tests. Various commercial identification systems exist and may be used if appropriate.

D. **Micrococcus species**

1. General characteristics. *Micrococcus* species are environmental organisms, as well as normal skin flora. They may also be normal flora in the respiratory tract or other sites
in the body, and are common contaminants. These coagulase-negative, gram-positive opportunists can easily be differentiated from the *Staphylococcus* species.

2. Laboratory identification of *Micrococcus* species
   a. Modified oxidase test. Modified oxidase reagent (6% tetramethylphenylene diamine hydrochloride in dimethyl sulfoxide) is added to a small amount of growth smeared onto a filter paper. Micrococci are **modified oxidase-positive** and turn dark blue within 2 minutes.
   b. Bacitracin susceptibility. An isolate is streaked onto a sheep blood Mueller-Hinton medium. A 0.04-U bacitracin disk is placed onto the streaked area, and the plate is incubated overnight and observed for a zone of inhibition. Micrococci are **susceptible to bacitracin**.
   c. Furazolidone susceptibility is tested exactly as for bacitracin susceptibility, except a disk containing 100-mcg furazolidone is used. Micrococci are **resistant to furazolidone**. (See Web Color Image 7–16.)

E. Antibiotic susceptibility
   1. Penicillin resistance is often so high, especially in *S. aureus* isolates (85% to 90%), that other antibiotics must often be used. There is variability in the susceptibility patterns.
   2. A common resistance mechanism of the staphylococci is a production of **β-lactamase**, an enzyme that inactivates the **β-lactam antibiotics**.
   3. Various **β-lactamase resistant penicillins** have been developed. Methicillin is the most frequently used. Oxacillin is used for in vitro susceptibility testing of methicillin resistance.
   4. Methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE) have increased in number. These may also be referred to as oxacillin-resistant *S. aureus* (ORSA) and oxacillin-resistant *S. epidermidis* (ORSE), respectively. Vancomycin has been used as an alternative treatment of methicillin-resistant strains. However, vancomycin resistance is increasing. MRSA are etiologic agents of serious nosocomial and community-associated infections. The increased virulence of this bacterium is a major concern of physicians, epidemiologists, and the health care community.

VIII. **STREPTOCOCCUS, ENTEROCOCCUS, AND RELATED GENERA**

A. General characteristics
   1. The organisms included in this group are catalase-negative, gram-positive cocci (old cells may stain gram-negative or gram-variable) that are arranged in pairs or chains (see Web Color Image 7–2) and are facultative anaerobes. Growth requirements may be complex, and the use of blood or enriched medium is necessary for isolation. Their role in human disease ranges from well-established and common, to rare but increasing.
   2. Hemolysis patterns on sheep blood agar (see Web Color Images 7–17, 7–18, and 7–19) are helpful in identification (Table 7–2).

B. Streptococcus
   1. *Streptococcus pneumoniae* (Table 7–3)
      a. *S. pneumoniae* is often part of the normal flora of the respiratory tract.

<table>
<thead>
<tr>
<th>Table 7–2</th>
<th>Streptococcus Hemolysis Patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Alpha (α)</td>
<td>Greenish discoloration in medium surrounding colony due to partial lysis of red blood cells</td>
</tr>
<tr>
<td>Alpha-prime (α′)</td>
<td>Small ring of no hemolysis around the colony, which is surrounded by a wider zone of complete hemolysis (also called “wide zone” hemolysis)</td>
</tr>
<tr>
<td>Beta (β)</td>
<td>Clearing of red blood cells surrounding the colony due to complete lysis</td>
</tr>
<tr>
<td>Nonhemolytic</td>
<td>No change</td>
</tr>
</tbody>
</table>
b. The key virulence factor is an antiphagocytic capsule. There are approximately 80 antigenic types.

c. *S. pneumoniae* is an important human pathogen, causing pneumonia, sinusitis, otitis media, bacteremia, and meningitis. It is frequently isolated as a pathogen and as a member of the normal respiratory flora. Direct smears often reveal leukocytes and numerous gram-positive cocci in pairs. The ends of the cells are slightly pointed, giving them an oval or lancet shape. (See Web Color Image 7–20.)

d. Complex media, such as brain-heart infusion agar, trypticase soy agar with 5% sheep blood, or chocolate agar are necessary for good growth. Isolates may require increased CO$_2$ for growth during primary isolation. Colonies are α-hemolytic. Young cultures produce a round, glistening, wet, mucoid, dome-shaped appearance.

e. Laboratory identification. *S. pneumoniae* is susceptible to optochin (ethylhydrocuprein hydrochloride). (See Web Color Image 7–21.) The bile solubility test is also used for identification. An α-hemolytic streptococcus that is optochin-susceptible or bile-soluble can be identified as *S. pneumoniae*. Other α-hemolytic streptococci are negative for both tests. Capsular subtypes of *S. pneumoniae* are detected using the Quellung test, a microscopic “precipitin test” in which the capsules surrounding the pneumococci appear to swell. (See Web Color Image 7–22.)

2. *Streptococcus pyogenes* (Table 7–3)

a. The cell wall contains the Lancefield group A carbohydrate. This organism is also referred to as group A streptococcus or β-hemolytic group A streptococcus.

b. Virulence factors

(1) The most well-defined virulence factor is M protein. There are more than 80 different serotypes. Resistance to infection is related to the presence of type-specific antibodies to the M protein. The M protein molecule causes the streptococcal cell to resist phagocytosis. It enables the bacterial cell to adhere to mucosal cells.

(2) Streptolysin O causes hemolysis of RBCs. Its role in virulence is unknown. Antibodies to streptolysin O indicate a recent infection (antistreptolysin O titer).

(3) Hyaluronidase (spreading factor) may favor the spread of the organism through the tissues.

(4) All strains form at least one deoxyribonuclease (DNAse). The most common is DNAse B. These enzymes are antigenic, and antibodies to DNAse can be detected following infection.
(5) Some strains of S. pyogenes cause a red spreading rash referred to as scarlet fever. This condition is caused by erythrogenic toxin.
(6) Protein F is a fibronectin-binding protein that facilitates adhesion to epithelial cells.
(7) Streptokinase causes the lysis of fibrin clots.

e. Infections

(1) Pharyngitis is one of the most common S. pyogenes infections. “Strep throat” is most frequently seen in children between the ages of 5 and 15 years. Diagnosis relies on a throat culture or a positive quick “strep” test, in which group A antigens are detected from a throat swab in a matter of minutes. A throat culture is recommended if the antigen-detecting test is negative.
(2) Skin infections include impetigo, necrotizing fasciitis, and pyoderma.
(3) Scarlet fever is a red rash that appears on the upper chest and spreads to the trunk and extremities following infection with S. pyogenes.
(4) Rheumatic fever and glomerulonephritis may result from infection at other sites in the body. Damage appears to result from cross-reactivity of the streptococcal antigens with host tissue antigens.
(5) Streptococcal TSS (toxic shock syndrome) is similar to that caused by Staphylococcus aureus.

d. Laboratory identification. Colonies of S. pyogenes on blood agar are small, transparent, and smooth, and they show β hemolysis. Gram’s stain reveals gram-positive cocci with some short chains. The bacterium is susceptible to bacitracin or Taxo A (see Web Color Image 7–23) and resistant to SXT. In addition, S. pyogenes hydrolyzes l-pyrrolidonyl-β-naphthylamide (PYR). A positive test is development of a red color after the addition of dimethylaminocinnamaldehyde reagent to an inoculated PYR disk (see Web Color Image 7–24).

3. Streptococcus agalactiae (Table 7–3)

a. The cell wall contains the Lancefield group B carbohydrate. This organism is also referred to as group B streptococcus. It may be found as normal flora in the genitourinary tract.

b. Virulence factors

(1) The capsule is the most important virulence factor.
(2) Other factors (e.g., DNAse, hyaluronidase) have not been shown to be factors in infection.

c. Infections

(1) Neonatal sepsis (usually manifest as pneumonia or meningitis) occurs soon after birth. The most important factor in infection is the presence of group B streptococcus in the vagina of the mother.
(2) Postpartum fever and sepsis may occur after birth and may manifest as endometritis or a wound infection.

d. Laboratory identification. Group B streptococci grow on blood agar as grayish white mucoid colonies surrounded by a small zone of β hemolysis. They are gram-positive cocci that form short chains in clinical specimens and long chains in culture. Group B streptococci are CAMP test-positive, demonstrating an arrowhead-shaped area of synergistic hemolysis when streaked perpendicular to a β-hemolytic S. aureus (see Web Color Image 7–25). Group B streptococci are also hippurate hydrolysis-positive, resistant to SXT, and PYR-negative.

4. Groups C and G

a. There are three hemolytic species in Lancefield group C that are occasionally isolated from clinical specimens: S. equi, S. zooepidemicus, and S. equisimilis. The major species found in group G is S. canis. It occasionally causes infection, and is part of the normal skin flora. Minute colony types of group G are part of the S. milleri group, with S. anginosus being the most prominent species.

b. These groups produce a variety of infections similar to those caused by groups A and B. Group C can cause pharyngitis.
c. Laboratory identification. Groups C and G can be identified by extensive biochemical tests. However, serologic tests to identify the group carbohydrate in the cell wall of the isolate (e.g., agglutination) are best.

5. Group D (Table 7–3)
   a. The group D streptococci include *S. bovis* and *S. equinus*. They may be found as normal intestinal flora.
   b. The group D streptococci may be etiologic agents of bacterial endocarditis, UTIs, and other infections, such as abscesses and wound infections. An association has been made between bacteremia due to *S. bovis* and the presence of gastrointestinal tumors. Isolation of *S. bovis* from a blood culture may be the first indication that the patient has an occult tumor.
   c. Laboratory Identification. Hemolysis is usually absent, or α-hemolysis is present. Key reactions of group D streptococci include a positive bile esculin test (formation of a black precipitate due to the hydrolysis of esculin) without growth in 6.5% NaCl broth. Group D can be separated from *Enterococcus* by the *l*-pyrrolidonyl-β-naphthylamide (PYR) test because it is negative and *Enterococcus* is positive. (The enterococci also grow in 6.5% NaCl broth.) Serotyping should be done to identify an isolate such as *S. bovis*, because it cannot be distinguished from some of the viridans group by biochemical tests alone.

6. *Enterococcus* (Table 7–3)
   a. This genus is found in the intestinal tract. The species found in this genus include *E. faecalis*, which is the most common isolate, *E. faecium*, *E. avium*, and *E. durans*. These enterococcal species share a number of characteristics with the group D streptococci, including the group D antigen. They show resistance to several of the commonly used antibiotics, so differentiation from Group D *Streptococcus* and susceptibility testing is important.
   b. The infections caused are similar to those caused by the group D streptococci. The most common is a urinary tract infection.
   c. Laboratory Identification. It is not difficult to differentiate between *Enterococcus* and group D—streptococci. In addition to being positive for bile esculin (black precipitate), *Enterococcus* species grow in 6.5% NaCl broth (see Web Color Image 7–26), are PYR-positive (see Web Color Image 7–24), and SXT-resistant.
   d. Enterococci may be screened for high-level aminoglycoside resistance because aminoglycosides are usually used in combination with ampicillin or penicillin for effective treatment of enterococcal infections. Resistant strains cannot be used for synergistic treatment. Gentamicin and streptomycin resistance can be detected with broth or agar dilution and disk diffusion tests.
   e. The emergence of vancomycin-resistant *Enterococcus* (VRE), encoded by the *vanA* gene, is a major concern of physicians, microbiologists, and hospital infection control personnel. *E. faecium* is the most common species, followed by *E. faecalis*. Most microbiology laboratories screen for VR colonization using vancomycin-containing agar. Susceptibility testing is performed only on clinically significant isolates.

7. Viridans streptococci (Table 7–3)
   a. The viridans group includes those α-hemolytic streptococci that lack Lancefield group antigens and do not meet the criteria for *S. pneumoniae*. They are part of the normal flora of the oropharynx and intestine.
   b. The most common infection caused by these organisms is subacute bacterial endocarditis.
   c. The viridans streptococci are fastidious and some strains require increased CO₂ for growth. Identification of the viridans streptococci to the species level is a difficult task. Part of the reason for this is that there is not widespread agreement on a classification scheme. Species of viridans streptococci include *S. mutans*, *S. salivarius*, *S. sanguis*, *S. mitis*, and *S. milleri* (not β hemolytic).

8. Nutritionally variant streptococci (NVS)
   a. The NVS subgroup of viridans streptococci are nutritionally deficient and have been isolated from patients who have endocarditis and otitis media. This
subgroup is also known as pyridoxal (vitamin B\textsubscript{6})-dependent, thiol-dependent, or symbiotic streptococci. Pyridoxal is not present in most liquid and solid bacteriologic media, so bacteriologic media must be supplemented with pyridoxal (vitamin B\textsubscript{6}) to support the growth of NVS. The NVS colonies are small, measuring 0.2 to 0.5 mm in diameter. When gram stained, the morphology can vary from classic gram-positive streptococci to gram-negative or gram-variable pleomorphic forms. As the optimal concentrations or required nutrients decrease, the cells become pleomorphic, even showing globular and filamentous forms.

b. NVS satellite around or grow adjacent to staphylococcal isolates. The staphylococci provide the growth requirements needed to facilitate the growth of the NVS.

c. A clue to the presence of NVS is a positive Gram’s stain, but negative cultures.

9. Treatment of streptococcal and enterococcal infections. Most species of \textit{Streptococcus} are susceptible to penicillin. \textit{S. agalactiae} is less susceptible than group A and may require a combination of ampicillin and an aminoglycoside. Group D is susceptible to penicillin, whereas \textit{Enterococcus} is usually resistant. \textit{Enterococcus} is often treated with a penicillin-aminoglycoside combination (synergy). Some isolates are resistant to this combination therapy. Although most pneumococcal isolates are susceptible to penicillin, some strains have shown resistance. Resistant streptococcal strains are often treated with erythromycin. Linezolid is often used for treatment of infections caused by vancomycin-resistant enterococci (VRE).

C. \textbf{Streptococcus-like organisms} (Table 7–3)

1. \textit{Aerococcus} is very similar to \textit{Enterococcus} on blood agar. The gram-positive coccus is susceptible to vancomycin and can be isolated from tissue samples of endocarditis and other varied infections.

2. \textit{Leuconostoc} is very similar to viridans streptococci on blood agar. It is found in the general environment. A Gram’s stain shows gram-positive coccobacilli in pairs and short chains. \textit{Leuconostoc} has been found in patients who have meningitis and endocarditis. It is intrinsically resistant to vancomycin.

3. \textit{Pediococcus} is also found in the general environment. A Gram’s stain shows gram-positive cocci in pairs, tetrads, and clusters. \textit{Pediococcus} is a rare isolate in patients who have septicemia. The bacterium is intrinsically resistant to vancomycin.

D. \textbf{Laboratory identification of Streptococci}

1. Hemolysis on blood agar is an important characteristic (Table 7–2). (See Web Color Images 7–17, 7–18, and 7–19.)

2. Bile solubility measures autolysis of bacteria under the influence of a bile salt (sodium deoxycholate). \textit{S. pneumoniae} is bile soluble.

3. Optochin (ethylhydrocuprein hydrochloride) susceptibility is determined by a zone of inhibition ($\geq$14 mm with a 5 mcg optochin disk) after growing the organism on blood agar with a filter paper disk containing optochin. Results correlate with bile solubility; that is, optochin-susceptible isolates are bile soluble. \textit{S. pneumoniae} is optochin susceptible. (See Web Color Image 7–21.)

4. Bacitracin (Taxo A) susceptibility is a characteristic of \textit{S. pyogenes}. The test is performed by placing a filter paper disk containing bacitracin on an inoculated blood agar plate, and measuring the zone of inhibition following incubation. (See Web Color Image 7–23.)

5. Group A and B streptococci are resistant to sulfamethoxazole-trimethoprim (SXT). This resistance can be measured with a filter paper disk or by incorporating SXT into blood agar. The latter technique allows for selective isolation. \textit{Enterococcus} species are also SXT-resistant.

6. \textit{Group B} streptococci hydrolyze hippurate. The glycine liberated can be detected by triketohydrindene hydrate (Ninhydrin), which imparts a purple color.

7. The Christie, Atkins, and Munch-Petersen (CAMP) test presumptively identifies \textit{group B} streptococci by measuring the enhanced hemolytic activity of staphylococcal \textit{β}-lysin by \textit{S. agalactiae}. \textit{Group B} streptococci, plated perpendicular to \textit{S. aureus}, demonstrate a characteristic \textbf{arrow-shaped hemolysis} pattern. (See Web Color Image 7–25.)
8. The ability of an organism to hydrolyze esculin is the basis of the esculin test. A positive result is a black precipitate in the agar surrounding the growth. Group D streptococci and Enterococcus are bile esculin positive. (See Web Color Image 7–26.)

9. Enterococcus is able to grow in nutrient broth containing 6.5% NaCl. (See Web Color Image 7–26.)

10. Hydrolysis of PYR can be detected by the development of a red color on the addition of cinnamaldehyde reagent. This test is specific for Enterococcus and S. pyogenes. (See Web Color Image 7–24.)

11. The LAP test (leucine aminopeptidase) is used to help differentiate Aerococcus and Leuconostoc from the other Streptococcus species. Both bacteria are LAP-negative, while other streptococci are LAP-positive. LAP hydrolyzes the substrate, leucine-β-naphthylamide, to β-naphthylamine. Development of a red color is detected upon addition of DMACA.

12. Serology testing for detection of the C carbohydrate of the cell wall is used for serogrouping of the β-hemolytic streptococci.

IX. AEROBIC GRAM-POSITIVE BACILLI

A. General characteristics
   1. The members of this group that are seen most frequently in the clinical laboratory are listed in Box 7–1.
   2. Except for Corynebacterium diphtheriae, these organisms are of low pathogenicity and usually require an immunocompromised host.
   3. With the exception of Bacillus, these organisms are all pleomorphic rods, and most grow well on standard media.

B. Listeria monocytogenes is widespread in the environment. It causes a wide variety of infections, especially in neonates, pregnant women, and immunosuppressed persons. Meningitis is a common outcome.
   1. Isolation is usually from blood, CSF, or swabs of lesions. L. monocytogenes grows well on blood agar and closely resembles group B streptococcus. Growth occurs at 4°C. This allows the use of the cold enrichment technique, which requires inoculation of the specimen into broth medium, followed by incubation at 4°C for several weeks. This technique has limited clinical importance.
   2. Gram stain shows a gram-positive rod or coccobacillus. (See Web Color Image 7–27.)
   3. The bacterium is catalase-positive, hydrolyzes hippurate and esculin, and is CAMP test-positive with block hemolysis. (See Web Color Image 7–28) A characteristic tumbling motility is demonstrated when the organism is grown in broth at room temperature and umbrella motility is observed in semisolid agar motility medium following room temperature incubation. (See Web Color Image 7–29) Identification of this organism is summarized in Box 7–2.

C. Erysipelothrix rhusiopathiae is an uncommon isolate. It is a pleomorphic gram-positive bacillus that often forms long filaments (see Web Color Image 7–30). The usual route of infection is through the skin. It is catalase negative, and forms hydrogen sulfide. The bacterium is nonmotile, but produces a characteristic “bottle brush” extension laterally from the streak line in soft gelatin agar (see Web Color Image 7–31).

Box 7–1 Most Commonly Isolated Aerobic Gram-Positive Bacilli

- Bacillus
- Corynebacterium
- Erysipelothrix
- Listeria
- Nocardia
Box 7–2  Characteristics of *Listeria monocytogenes*

- Gram-positive coccolbacillus
- Umbrella motility pattern (motility agar tube) at room temperature
- Hippurate hydrolysis positive
- CAMP test positive – block hemolysis pattern
- Esculin hydrolysis positive
- Growth at 4°C
- Catalase positive
- β hemolytic (very similar to group B streptococcus)

D. The most important species of *Corynebacterium* are *C. diphtheriae*, *C. jeikeium*, and *C. urealyticum*.

1. All species are pleomorphic, palisading, gram-positive bacilli and resemble *C. diphtheriae* on Gram’s stain. The morphology was, therefore, termed diphtheroid. The morphology may also be described as picket fence or Chinese letters (see Web Color Image 7–32).

2. *C. diphtheriae* is the cause of diphtheria. The disease has a presentation of local inflammation of the throat with a pseudomembrane caused by dead cells and exudate. The diphtheria toxin damages major organ systems and results in a high mortality rate when infected persons go untreated. Diphtheria occurs in nonimmunized populations. Treatment is with an antitoxin.
   a. Laboratory diagnosis consists of culture and testing for toxin production. Media that have been developed for the growth and identification of *C. diphtheriae* are summarized in Table 7–4. Suspicious colonies from cystine-tellurite or Tinsdale’s agar are gram stained. Catalase and urease tests are performed on gram-positive rods with diphtheroid morphology. (Loeffler’s agar can be used to enhance the pleomorphic microscopic morphology of the gram-positive rods.). Urease negative isolates are presumptively identified as *C. diphtheriae*.
   b. Toxin production may be determined by the Elek test, which detects toxin production by an isolate using an antitoxin-impregnated filter paper strip that is laid perpendicular to lines of bacterial growth. Precipitin lines are formed if the test stain of *C. diphtheriae* is a toxigenic strain.

3. *C. jeikeium* (group JK) is an extremely virulent organism. It may cause infections following implantation of prosthetic devices, and it is resistant to a wide range of antibiotics. This organism is suspected in those patients who are immunocompromised or have undergone invasive procedures or in whom an isolate with typical diphtheroid morphology is found.

4. *C. urealyticum* is a urinary pathogen that is slow growing (48 hours) and strongly urease positive. Urease production occurs within minutes following inoculation on a urea slant.

5. Differentiation between *Corynebacterium, Erysipelothrix*, and *Listeria* is outlined in Table 7–5.

E. The two species of *Bacillus* that are of medical importance are *B. anthracis* and *B. cereus*. These aerobic bacteria are catalase-positive, gram-positive spore formers that appear singly or in chains with a “boxcar” morphology on Gram’s stain (see Web Color Image 7–33).

1. *B. anthracis* is the cause of anthrax, a rare disease in the United States. It usually appears in the cutaneous form as a result of wounds contaminated with anthrax spores.

### Table 7–4 Media Used to Isolate *Corynebacterium diphtheriae*

<table>
<thead>
<tr>
<th>Media</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystine-tellurite</td>
<td>Colonies are black or gray</td>
</tr>
<tr>
<td>Tinsdale’s agar</td>
<td>Colonies are dark brown-to-black with brown-to-black halos</td>
</tr>
<tr>
<td>Loeffler agar</td>
<td>Supports growth and enhances pleomorphism</td>
</tr>
</tbody>
</table>
Table 7–5 Differentiation of Corynebacterium, Erysipelothrix, and Listeria

<table>
<thead>
<tr>
<th>Organism</th>
<th>Voges-Proskauer</th>
<th>Catalase</th>
<th>Growth at 35°C</th>
<th>Motility at Room Temperature</th>
<th>Esculin Hydrolysis</th>
<th>Hydrogen Sulfide H₂S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corynebacterium spp.</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Erysipelothrix rhusiopathiae</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

* + = positive; − = negative; V = variable

The lesion that is formed develops a characteristic center of necrosis, which has been termed a black eschar or malignant pustule. Handling of B. anthracis is extremely dangerous and should only be done within a biologic safety cabinet.

2. B. cereus is a common cause of food poisoning.

3. Laboratory identification is accomplished by growth on blood agar. Colonies of both species are large and flat. B. cereus is β-hemolytic. The differentiation between B. anthracis and B. cereus is outlined in Table 7–6.

F. Aerobic Actinomycetes include Nocardia, Actinomadura, and Streptomyces species. These microorganisms are similar morphologically to fungi. They are gram-positive bacilli, but do not produce spores.

1. Nocardia species are saprophytes and are found worldwide in soil and on plant material. They cause pulmonary and cutaneous infections in humans. Nocardia asteroides is the most commonly isolated member of Nocardia. This organism is usually found in immunocompromised patients as a chronic infection, particularly pulmonary. It is pleomorphic and partially acid-fast. (See Web Color Image 7–34). Growth is slow (up to 6 weeks). The colonial morphology is dry and heaped, similar to a fungus (see Web Color Images 7–35 and 7–36). It also has a soil or musty-basement odor. Exudates may demonstrate “sulfur granules,” which are masses of filamentous organisms with pus materials.

2. Actinomadura are etiologic agents of mycetomas. Their microscopic and macroscopic morphology is similar to that of the Nocardia species. However, the microorganisms are not acid-fast.

3. Streptomyces species are primarily saprophytes, found as soil organisms. They may cause opportunistic infections similar to those caused by the other aerobic actinomycetes.

G. Lactobacillus species is a pleomorphic, nonspore-forming, gram-positive bacillus. It is catalase-negative, and may grow better under anaerobic conditions. Lactobacilli are normal vaginal flora.

H. Arcanobacterium haemolyticum is a small, β-hemolytic, nonspore-forming, gram-positive bacillus. It resembles the β-hemolytic streptococci. The organism is the etiologic agent of pharyngitis and must be differentiated from Streptococcus pyogenes. It is catalase-negative and exhibits a reverse CAMP reaction.

X. NEISSERIA AND MORAXELLA CATARRHALIS

A. General characteristics

1. Members of this group are gram-negative cocci that are often seen in pairs. The adjacent sides are flattened, producing a kidney-bean shape (see Web Color Image 7–37).

Table 7–6 Differentiation of Bacillus anthracis and B. cereus

<table>
<thead>
<tr>
<th>Species</th>
<th>10 U Penicillin</th>
<th>β-Hemolysis</th>
<th>Gelatin Hydrolysis</th>
<th>&quot;String of Pearls&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. anthracis</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. cereus</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

S = susceptible, R = resistant, + = positive, − = negative.
2. They are all oxidase-positive and catalase-positive (see Web Color Images 7–38 and 7–41). Differentiation of species is often based on acid production from carbohydrate utilization.

3. Some of the pathogenic species may have fastidious growth requirements.

4. Proper specimen collection is essential for successful isolation. *N. gonorrhoeae* and *N. meningitidis* are especially sensitive to drying, cold, and chemicals (disinfectants and antiseptics). Dacron or rayon swabs are less inhibitory than calcium alginate and cotton and are preferred for collection. Specimens should be kept at room temperature and plating should occur as soon as possible following collection.

**B. Neisseria gonorrhoeae**

1. Infections

   a. Gonorrhea is a frequently seen venereal disease. The majority of infected men show symptoms such as burning and discharge from the urethra (gonococcal urethritis). Females may be asymptomatic, but infection in this gender may lead to pelvic inflammatory disease. Gonorrhea may cause sterility in males and females and disseminate to blood, skin, and joints.

   b. Ophthalmia neonatorum is primarily a gonococcal infection of the conjunctiva of newborns as a result of passage through an infected mother’s birth canal. It can be successfully treated at birth to prevent blindness.

   c. Penicillinase-producing *Neisseria gonorrhoeae* are a common problem. A β-lactamase test should be done on each isolate to detect resistance to the penicillin antibiotics.

2. Direct microscopic examination of gram stained urethral discharge from males is a valuable diagnostic procedure. The presence of gram-negative intracellular diplococci (see Web Color Image 7–37) from a symptomatic male with discharge has a 95% correlation rate with culture and is strong presumptive evidence of gonorrhea. Correlation is much lower in females because of the normal flora.

3. Culture of *N. gonorrhoeae* can be accomplished with various enriched and selective media.

   a. Specimens for culture should be collected from infected sources (cervix, urethra, rectum, or throat.) Swabs or exudates are acceptable specimens, and should be kept at room temperature prior to processing.

   b. Agar. *N. gonorrhoeae* grows on chocolate agar, but not on blood agar. In those cases in which normal flora may contaminate the medium, selective agars, inhibitory to organisms other than *Neisseria* species, may be used.

   (1) Selective media include Thayer-Martin and modified Thayer-Martin (MTM), Martin-Lewis, and New York City.

   (2) These media are often packaged in self-contained transport/incubation systems that are inoculated at the site of collection. Examples include Transgrow, Gono-Pak, and JEMBEC plates.

   c. Culture and identification. Plates are incubated at 35 °C in a 3% to 5% carbon dioxide, humidified atmosphere. Colonies of *N. gonorrhoeae* are flat, smooth, glistening, and gray to white. The oxidase-positive, catalase-positive, gram-negative diplococcus produces acid from glucose utilization. Identification/differentiation is outlined in Table 7–7. Various tests are available for direct detection of

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Oxidase</th>
<th>Catalase</th>
<th>Acid from Glucose</th>
<th>Acid from Maltose</th>
<th>DNAse</th>
<th>Growth on Nutrient Agar</th>
<th>Butyrate Esterase</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. gonorrhoeae</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Other <em>Neisseria</em> species</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

DNAse = deoxyribonuclease; V = variable; + = positive; − = negative.
N. gonorrhoeae from clinical specimens. These include detection of cellular antigens and those that detect gonococcal nucleic acid.

C. Neisseria meningitidis
1. Infections
   a. Meningococcal meningitis is transmitted by respiratory droplets. Prolonged close contact is necessary for infection. The onset is abrupt, with headache, stiff neck, and fever. Petechial skin lesions may be present.
   b. Meningococcemia, inflammation of both the brain substance and surrounding membranes, also involves the blood vessels and various major organs. Petechial skin lesions are common. This condition may progress to DIC.
2. Direct microscopic examination of CSF sediment may reveal intracellular and extracellular gram-negative diplococci (see Web Color Image 7–37).
3. Specimens for culture include CSF, blood, and joint fluid. Nasopharyngeal swabs are cultured to detect carriers. Specimens should be kept at room temperature and processed as soon as possible. Swabs should not be allowed to dry out. Growth is seen on blood and chocolate agar and is enhanced with increased carbon dioxide and humidity. Colonies of N. meningitidis are flat, smooth, glistening, and gray to white. Acid production from the utilization of glucose and maltose differentiates the bacterium from N. gonorrhoeae.
   Identification is outlined in Table 7–7.

D. Moraxella catarrhalis is part of the normal upper respiratory tract flora. It may cause otitis media, sinusitis, and respiratory infections. M. catarrhalis grows on blood, chocolate, and nutrient agars. Identification of the asaccharolytic gram-negative coccus is outlined in Table 7–7.

E. Other Neisseria species only rarely cause clinical infection. These species may need to be differentiated from the pathogenic species, because they are normal flora at the same sites at which the pathogenic species are located (Table 7–7).

XI. MISCELLANEOUS GRAM-NEGATIVE BACILLI

A. Haemophilus
1. Members of the genus Haemophilus are gram-negative, nonmotile bacilli and cocacobacilli, which are often pleomorphic. Members of Haemophilus require hemin (X factor) and/or nicotinamide adenine dinucleotide (NAD; V factor) for growth (except H. aphrophilus). These fastidious bacteria grow on chocolate agar, but not on blood agar, due to its absence of the NAD.
2. H. influenzae is the most important species, causing meningitis, otitis media, epiglottitis, pneumonia, and contagious conjunctivitis. Children, especially those who have not been immunized, are particularly at risk. H. influenzae is part of the normal upper respiratory tract flora. Capsular serotype b is the most common cause of disseminated infections, but widespread immunization with the Hib vaccine has resulted in a decreased incidence of these infections.
3. H. ducreyi is the cause of chancroid, a sexually transmitted disease. Organisms enter through breaks in the skin and multiply locally. Approximately 1 week later, a small papule appears that soon develops into a painful ulcer. A Gram’s stain of the lesion exudate may show small pleomorphic gram-negative bacilli in clusters (“school of fish” morphology).
4. H. aegyptius (Koch-Weeks bacillus) is associated with acute, contagious conjunctivitis, commonly referred to as “pink eye.”
5. H. parainfluenzae and H. aphrophilus are human normal oral flora and are seen primarily in endocarditis.
6. Differentiation of the Haemophilus species is summarized in Table 7–8.
   a. X and V factor requirements can be determined by placing X and V factor–impregnated filter paper strips onto a nutrient agar plate that has been inoculated
with the unknown species. X and V requirements are determined by growth patterns (e.g., growth around X indicates a requirement for that factor). As depicted in Web Color Image 7–39, H. influenzae requires both X and V factors for growth. H. parainfluenzae requires only V factor for growth (see Web Color Image 7–40).

b. Growth can also be seen surrounding colonies of S. aureus, S. pneumoniae, or Neisseria species. These organisms produce V factor as a metabolic byproduct and lyse the RBCs in the medium, releasing the X factor. This is called the satellite phenomenon (see Web Color Image 7–41).

c. The porphyrin test is a sensitive method to determine X factor requirements. Those species that do not require X factor yield a positive porphyrin test (e.g., H. influenzae is porphyrin negative).

d. Fermentation of carbohydrates (glucose, sucrose, lactose) can aid in identification of the species.

e. H. influenzae may be divided into biotypes. The site of infection by H. influenzae can be correlated with biotype. Biotypes are determined by an isolate’s activity with indole, urease, and ornithine decarboxylase. The disease association and biochemical characteristics of common biotypes are summarized in Table 7–9.

7. Antibiotic resistance. Resistance to the penicillins is common due to β-lactamases and other mechanisms.

B. Pasteurella multocida is the most commonly encountered species in the Pasteurella genus. It is found as normal flora in the respiratory tract of animals, especially dogs and cats. Isolation of P. multocida is always a strong possibility in an infected dog or cat bite or scratch. This species appears as a small gram-negative coccobacillus with bipolar staining. The key characteristics for differentiating P. multocida are listed in Box 7–3.

C. Bordetella species are very small gram-negative bacilli. There are three species that cause human infection: B. pertussis, B. parapertussis, and B. bronchiseptica. All cause respiratory tract infection, but B. pertussis causes the most serious infection, which is whooping cough.

1. Bordetella pertussis, the cause of pertussis or whooping cough, is found worldwide and is spread via droplets.

a. Disease progression. The disease begins with coldlike symptoms (i.e., runny nose, sneezing, malaise). This catarrhal stage is the most infectious. After 1 to 2 weeks,
Box 7–3 Key Characteristics of Pasteurella multocida

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-negative cocobacillus</td>
<td>with bipolar staining</td>
</tr>
<tr>
<td>Growth on blood agar</td>
<td>(may have a musty or mousy smell)</td>
</tr>
<tr>
<td>No growth on MacConkey agar</td>
<td></td>
</tr>
<tr>
<td>Catalase positive</td>
<td></td>
</tr>
<tr>
<td>Indole positive</td>
<td></td>
</tr>
<tr>
<td>Oxidase positive</td>
<td></td>
</tr>
<tr>
<td>Penicillin susceptible (2-U disk)</td>
<td></td>
</tr>
<tr>
<td>Glucose utilization</td>
<td></td>
</tr>
</tbody>
</table>

the paroxysmal stage begins, with violent coughs that often make it difficult for the infected person to take a breath. Convalescence can take weeks to months with secondary complications, such as pneumonia, seizures, and encephalopathy possible.

b. Specimen collection and transport. B. pertussis is very sensitive to drying and to trace toxic chemicals on swabs and in media. Specimen collection and transport must be done correctly for successful culture. Cotton swabs are toxic; calcium alginate or Dacron swabs should be used. The best specimen is a nasopharyngeal swab or aspirate. Immediate plating is preferred, because the organism does not readily survive transport. A swab should also be collected for a direct fluorescent antibody and Gram’s stain of smears.

1. Growth can be accomplished using Regan-Lowe or Bordet-Gengou agar (charcoal-horse blood agar). B. pertussis colonies, often described as “mercury drop” colonies, are small and pearl-like in appearance after 3 to 4 days. It does not grow on sheep blood agar and is urease-negative.

2. Identification is by microscopic and colonial morphology on selective media, biochemical reactions, and reactivity with specific antiserum, usually in a direct fluorescence test (DFA) (see Web Color Image 7–42).

2. B. parapertussis can be found in patients who have respiratory tract illness that resembles a mild form of pertussis. This species grows on sheep blood agar within 2 to 3 days and is urease positive within 24 hours. B. bronchiseptica is rarely found in humans but causes respiratory tract disease in animals (“kennel cough”). Growth is observed on sheep blood agar within 1 to 2 days and it is urease-positive within 4 hours (see Web Color Image 7–43).

D. Francisella tularensis is the causative agent of tularemia, a zoonotic disease. Transmission is via contact with infected animals (e.g., rabbits, deer), arthropod bites (e.g., ticks, fleas), or inhalation. Isolation generally requires extended incubation on media enriched with cystine or cysteine. The gram-negative rod/cocobacillus is very small and stains poorly on Gram’s stain. Definitive identification is made with specific antisera (direct fluorescence). This organism is very dangerous to work with in the laboratory. It should always be handled under a biologic safety hood with safety precautions strictly observed.

E. Members of the genus Brucella cause disease in animals. Human disease is normally a result of contact with the animals or their waste, meat, hides, or secretions. There are four species responsible for the majority of human disease: B. melitensis, B. abortus, B. suis, and B. canis.

1. The disease caused is brucellosis (also known as Bang’s disease or undulant fever). It is characterized by fever, chills, fatigue, weakness, and internal organ lesions. It can be chronic. Brucellosis is a CDC reportable infection.

2. Brucella species are most often isolated from blood or bone marrow. The organism is slow-growing, and blood cultures may need to be incubated for 4 to 6 weeks before they are considered negative. A Gram’s stain shows a faintly staining, small gram-negative cocobacillus. Serologic tests (agglutination of the isolate) are valuable in identification. The CO₂ requirement, along with urease, hydrogen sulfide, and
CHAPTER 7 Clinical Microbiology

Table 7–10 Differentiation of Brucella Species

<table>
<thead>
<tr>
<th>Species</th>
<th>CO₂ Required</th>
<th>Urease</th>
<th>H₂S</th>
<th>Time</th>
<th>Thionine</th>
<th>Fuchsin</th>
<th>Natural Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. abortus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>&lt;2 h</td>
<td>−</td>
<td>+</td>
<td>Cattle</td>
</tr>
<tr>
<td>B. melitensis</td>
<td>−</td>
<td>+ V</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Goats, sheep</td>
</tr>
<tr>
<td>B. suis</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>&lt;0.5 h</td>
<td>−</td>
<td>+</td>
<td>Swine</td>
</tr>
<tr>
<td>B. canis</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>&lt;0.5 h</td>
<td>−</td>
<td>−</td>
<td>Dogs</td>
</tr>
</tbody>
</table>

H₂S = hydrogen sulfide; + = positive; − = negative; V = variable

growth in the presence of thionin and basic fuchsin can also be used in differentiation of the species. Table 7–10 summarizes the differential characteristics of the Brucella species. Brucella organisms should be handled under biosafety level 3 conditions with safety precautions strictly observed.

F. *Antinobacillus actinomyctemcomitans* is a slow-growing, small, facultative gram-negative bacillus associated with endocarditis, bacteremia, and dental infections. It may be associated with *Actinomyces*. It is catalase positive and oxidase negative (Table 7–11).

G. *Kingella kingae* colonizes the upper respiratory tract and is primarily associated with infections of bones and joints, as well as endocarditis in children and young adults. It grows on sheep blood agar and is β-hemolytic. A Gram’s stain shows short, plump, gram-negative rods with square ends (Table 7–11). Other less pathogenic species in the genus are *K. denitrificans* and *K. oralis*.

H. *Capnocytophaga* is normal oral flora in humans. It may cause serious infections in immunosuppressed patients. It has been associated with dog bites and is usually isolated from blood or cerebrospinal fluid. It grows on sheep blood agar, but is capnophilic and must have CO₂ for growth. Colonies are beige or yellow and show a haze of growth at the periphery as a result of gliding motility (see Web Color Image 7–44). Gram’s stain shows fusiform, gram-negative bacillus (Table 7–11). (See Web Color Image 7–45.)

I. *Cardiobacterium hominis* is a pleomorphic, gram-negative rod that grows on blood, but not MacConkey agar. It is normal human oral flora and is found in patients who have endocarditis and bacteremia. The colonies are small, and growth is slow. Differentiation of the miscellaneous gram-negative bacilli and coccobacilli is summarized in Table 7–11.

Table 7–11 Differentiation of Gram-Negative Bacilli/Coccobacilli

<table>
<thead>
<tr>
<th>Organism</th>
<th>Oxidase</th>
<th>Catalase</th>
<th>Coccolid</th>
<th>Fusiform</th>
<th>Indole</th>
<th>Urease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobacillus actinomyctemcomitans</td>
<td>− V</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Capnocytophaga</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Cardiohemobacterium hominis</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Kingella kingae</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Brucella</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>2 h</td>
</tr>
<tr>
<td>Francisella tularensis</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>weak</td>
</tr>
</tbody>
</table>

+ = positive; − = negative; 2 h = 2 hours weak
XII. ENTEROBACTERIACEAE

A. General characteristics
1. The Enterobacteriaceae is the largest and most medically important family of gram-negative bacilli (see Web Color Image 7–4). Although there are numerous genera and species, more than 90% of the medically important isolates belong to just a few genera (Box 7–4).
2. The Enterobacteriaceae are found worldwide and are part of the normal flora of all animals. They are a common cause of nosocomial infections.
3. Commonly seen sites of infection with members of the Enterobacteriaceae are listed in Table 7–12.
4. All members ferment glucose, reduce nitrates to nitrites, and are oxidase-negative. The oxidase reaction is especially important in making a rapid distinction between the Enterobacteriaceae and the majority of the gram-negative nonfermenters. However, the oxidase-positive Plesiomonas shigelloides, formerly in the family Vibrionaceae, has been moved to the family Enterobacteriaceae. Phylogenetic studies have shown that Plesiomonas is closely related to members in this family, particularly the genus Proteus. (Identification of P. shigelloides will be discussed in Section XIII.)
5. Antigens of this group include the O or somatic antigen, found in the bacterial cell wall, the H or flagellar antigen, and the K or capsular antigen.

B. Various culture media and tests are used for the isolation, selection, differentiation, and identification of the Enterobacteriaceae.
1. Media used for isolation and selection of Salmonella and Shigella, along with expected reactions, are summarized in Table 7–13. (see Web Color Images 7–46, 7–47, and 7–48).
2. Media used for the isolation and detection of lactose fermenters are summarized in Table 7–14. (see Web Color Images 7–49, 7–50, and 7–51).

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of Enterobacteriaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Escherichia</td>
</tr>
<tr>
<td></td>
<td>Klebsiella</td>
</tr>
<tr>
<td></td>
<td>Enterobacter</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>Escherichia</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Salmonella</td>
</tr>
<tr>
<td></td>
<td>Shigella</td>
</tr>
<tr>
<td>Lower respiratory tract</td>
<td>Yersinia</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>Klebsiella</td>
</tr>
<tr>
<td></td>
<td>Enterobacter</td>
</tr>
<tr>
<td></td>
<td>Proteus</td>
</tr>
<tr>
<td></td>
<td>Escherichia</td>
</tr>
<tr>
<td></td>
<td>Klebsiella</td>
</tr>
</tbody>
</table>
Table 7–13 Media Used to Isolate and Select Salmonella and Shigella

<table>
<thead>
<tr>
<th>Media</th>
<th>Characteristic Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bismuth sulfite agar</td>
<td>Salmonella typhi are black colonies surrounded by a black zone of precipitate</td>
</tr>
<tr>
<td>Brilliant green agar</td>
<td>Proteus colonies are red to pink</td>
</tr>
<tr>
<td></td>
<td>Salmonella colonies are red to pink</td>
</tr>
<tr>
<td></td>
<td>Shigella is inhibited</td>
</tr>
<tr>
<td>Gram-negative broth</td>
<td>The growth of most gram-negative rods is inhibited, whereas Salmonella and Shigella</td>
</tr>
<tr>
<td></td>
<td>growth is enriched</td>
</tr>
<tr>
<td>Hektoen enteric agar</td>
<td><em>Escherichia coli</em> and lactose/sucrose fermenters are orange to salmon pink colonies</td>
</tr>
<tr>
<td></td>
<td>Proteus is usually inhibited</td>
</tr>
<tr>
<td></td>
<td>Salmonella colonies are blue to blue-green, with black centers if hydrogen sulfide is</td>
</tr>
<tr>
<td></td>
<td>produced</td>
</tr>
<tr>
<td></td>
<td>Shigella colonies are green</td>
</tr>
<tr>
<td>Salmonella-Shigella agar</td>
<td><em>E. coli</em> colonies are red</td>
</tr>
<tr>
<td></td>
<td>Proteus colonies are colorless, with black centers</td>
</tr>
<tr>
<td></td>
<td>Salmonella colonies are colorless, with or without black centers</td>
</tr>
<tr>
<td></td>
<td>Shigella colonies are colorless</td>
</tr>
<tr>
<td>Selenite broth</td>
<td>Salmonella stool specimens are enriched</td>
</tr>
<tr>
<td></td>
<td>Gram-positive organisms are inhibited</td>
</tr>
<tr>
<td>Xylose-lysine-deoxycholate agar</td>
<td><em>E. coli</em> colonies are yellow</td>
</tr>
<tr>
<td></td>
<td>Proteus colonies are clear or yellow</td>
</tr>
<tr>
<td></td>
<td>Salmonella colonies are red, with black centers</td>
</tr>
<tr>
<td></td>
<td>Shigella colonies are clear</td>
</tr>
</tbody>
</table>

3. Media and tests used for the identification of the Enterobacteriaceae include the following:

a. Triple sugar iron agar (TSI). This medium differentiates gram-negative bacilli by their ability to ferment glucose, lactose, and sucrose and to produce hydrogen sulfide (Web Color Image 7–52).

   1. Nonfermenters produce an alkaline slant and alkaline deep (no change in the red color of the medium).
   2. Nonlactose fermenters (but glucose fermenters) produce an alkaline slant (red) and acid (yellow) deep.
   3. Lactose fermenters and sucrose fermenters produce an acid (yellow) slant and acid (yellow) deep. A lactose-negative, sucrose-positive organism will produce an acid slant and acid deep.
   4. Hydrogen sulfide (H$_2$S) production is indicated by a black precipitate in the medium.
   5. Kliger’s iron agar (KIA) is a similar medium, but only incorporates the carbohydrates glucose and lactose. H$_2$S production can also be detected with this formulation (see Web Color Image 7–52).

b. Nitrate reduction. Nitrate test medium is inoculated and incubated overnight to determine the ability of microorganisms to reduce nitrates to nitrite. The presence of nitrates in the medium is detected by the addition of N,N-Dimethyl-$\alpha$-naphthylamine and sulfanilic acid. The formation of a red color after the addition of the two reagents indicates that nitrite is present (positive test). If a red color is

Table 7–14 Media Used for the Isolation and Detection of Lactose Fermenters

<table>
<thead>
<tr>
<th>Media</th>
<th>Characteristic Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosin-methylene blue agar</td>
<td><em>Escherichia coli</em> colonies are dark, with a green metallic sheen</td>
</tr>
<tr>
<td></td>
<td>Gram-positive organisms are inhibited</td>
</tr>
<tr>
<td></td>
<td>Lactose fermenters show pink to red colonies</td>
</tr>
<tr>
<td></td>
<td>Lactose nonfermenters have colorless colonies</td>
</tr>
<tr>
<td>MacConkey agar</td>
<td>Gram-positive organisms are inhibited</td>
</tr>
<tr>
<td></td>
<td>Lactose-fermenter colonies are pink to red</td>
</tr>
<tr>
<td></td>
<td>Lactose-nonfermenter colonies are colorless</td>
</tr>
</tbody>
</table>
not detected, all available nitrate may have been reduced to nitrite and then completely converted to nitrogen gas (N\(_2\)), nitric oxide (NO), or nitrous oxide (N\(_2\)O). No nitrite remains to react with the sulfanilic acid. This can be detected by adding a small pinch of zinc dust to the tube (metallic zinc reduces nitrate to nitrite). With the formation of nitrite upon addition of zinc, the reactions can take place, resulting in the red color. A red color at this point indicates that nitrate was still in the broth (negative test). Absence of red color after the addition of zinc indicates that no nitrate was left (positive test). All Enterobacteriaceae reduce nitrites to nitrates (see Web Color Image 7–53).

c. Oxidase. The oxidase test determines the presence of the cytochrome oxidase system that oxidizes reduced cytochrome with molecular oxygen. A positive test is the development of a purple when the bacterium is mixed with the reagent tetramethyl-p-phenylenediamine dihydrochloride. The Enterobacteriaceae are oxidase-negative, with the exception of Plesiomonas species (see Web Color Image 7–38).

d. Indole. The indole test detects an organism’s ability to produce the enzyme tryptophanase and deaminate tryptophan to indole, pyruvic acid, and ammonia. A positive reaction is detected with the addition of Ehrlich’s reagent, paradimethylaminobenzaldehyde, or Kovac’s reagent. A positive reaction is a pink color (see Web Color Images 7–54 and 7–55).

e. Methyl red-Voges-Proskauer (MR-VP) broth. The MR and VP tests are used to determine the method by which bacteria metabolize glucose: the mixed acid fermentation pathway or the butylene glycol pathway. The tests detect the end products of glucose fermentation, in accordance with the pathway an organism uses to metabolize glucose. Isolates are inoculated into MR-VP broth and allowed to grow for 48 hours. At that time, the broth is split into two fractions: one to measure methyl red and one for the Voges-Proskauer test (see Web Color Images 7–54 and 7–55).

(1) Those organisms that carry out mixed acid fermentation produce vast amounts of acid that will convert the methyl red indicator to a red color (pH <4.4).

(2) The Voges-Proskauer test measures the production of acetoin. The addition of 40% potassium hydroxide followed by α-naphthol results in a red complex (neutral pH), which indicates a positive test.

f. Citrate. The citrate test determines whether an organism can utilize sodium citrate as a sole source of carbon. Ammonium salts are the nitrogen source in the medium and utilization of these salts results in the release of ammonia, causing a pH change. The bromthymol blue indicator turns the medium from green to blue (a positive test). (See Web Color Images 7–54 and 7–55.)

g. Urea. Organisms that produce urease will hydrolyze urea to form ammonia. The ammonia reacts in solution to form ammonium carbonate, which increases the pH. This is detected by the phenol red in the medium and turns the medium a bright pink color (positive test). Christensen’s urea agar is generally a preferred test medium. (See Web Color Image 7–43.)

h. Gelatin hydrolysis. Bacteria that produce gelatinases that break down gelatin into amino acids. A slant of nutrient gelatin is inoculated and incubated at room temperature for up to several days. If incubation is at 35°C, the medium should be chilled in a refrigerator before reading the test results. Liquefaction of the gelatin is a positive test.

i. Hydrogen sulfide (H\(_2\)S). A bacterium utilizes the sodium thiosulfate sulfur source to form H\(_2\)S, a colorless gas. H\(_2\)S combines with the indicator, ferrous sulfate. Numerous media demonstrate the production of H\(_2\)S (e.g., sulfide-indole-motility agar, motility-indole-ornithine agar, Hektoen enteric agar, Salmomella-Shigella agar, Triple sugar iron agar, Kliger Iron agar, and lysine iron agar). (See Web Color Images 7–46, 7–47 and 7–56.)

j. Phenylalanine deaminase. The deamination of phenylalanine (removal of an amine group) results in the production of phenylpyruvic acid. Following overnight incubation and the addition of a 10% ferric chloride to an inoculated slant, a green
color indicates the presence of phenylpyruvic acid, a positive test. *Proteus, Morganella, and Providencia* species are phenylalanine deaminase-positive. (See Web Color Image 7–57.)

k. Decarboxylase and dihydrolase tests. The decarboxylation (removal of a carboxyl group, COOH) of *lysine, ornithine, and arginine* (dihydrolase reaction) may be detected by inoculating media with a specific amino acid and glucose, a carbohydrate source. Semisolid agar tubes are inoculated by stabbing. An acid pH and anaerobic environment are required for decarboxylation reactions to occur. A yellow color initially indicates glucose fermentation and results in an acid pH that is lowered enough to activate the decarboxylase enzymes. A positive test, caused by an alkaline pH shift, is a return to the original color of the uninoculated medium (purple). Text media include *Moeller decarboxylase base medium* (see Web Color Image 7–58), *motility-indole-ornithine* (MIO), or *lysine iron agar* (LIA).

l. β-galactosidase and the orthonitrophenyl-β-D-galactopyranoside (ONPG) test. Organisms that are late or slow lactose fermenters may appear as nonfermenters on primary media. The ONPG test determines if the organism is a slow or late lactose fermenter (e.g., lacks the permease that allows lactose to enter the cell, but has β-galactosidase, which splits lactose). Lactose nonfermenters lack the β-galactosidase. β-galactosidase acts on ONPG to form a yellow compound (positive test), which indicates that the organism is a lactose fermenter. (See Web Color Image 7–59.)

m. DNase. Bacterial DNases are endonucleases that cleave phosphodiester bonds in DNA, resulting in smaller subunits of the polynucleotide. A bacterial isolate is streaked onto a medium with 0.2% DNA and incubated for 24 hours at 35°C. Following incubation, 1N HCl is added to the surface of the medium. Unhydrolyzed DNA is insoluble in HCl and forms a precipitate. A positive reaction is the formation of a clear zone (halo) around the inoculum.

n. Motility. Motility can be demonstrated by microscopic examination of wet mounts of bacteria. In addition, semisolid medium, with agar concentrations of 0.4% or less, can be used to demonstrate motility. Following overnight incubation, movement away from the stab line or a hazy appearance throughout the medium is indicative of a positive reaction by a motile bacterium. (See Web Color Image 7–60.)

o. Carbohydrate fermentation. All Enterobacteriaceae ferment glucose, and lactose fermentation is used to differentiate groups of genera within the family. Other carbohydrates can be utilized by bacteria. Phenol red carbohydrates or CTA sugars with phenol red indicators are most commonly used to detect fermentation of specific carbohydrates. A yellow color indicate a fermentation and a positive reaction. (See Web Color Image 7–61.)

4. The identification of frequently encountered species in the family Enterobacteriaceae can be accomplished using the information presented in Table 7–15.

XIII. CAMPYLOBACTER, HELICOBACTER, AND VIBRIONACEAE

A. Campylobacter

1. The most clinically relevant species are *C. jejuni* subsp. *jejuni*, *C. coli*, and *C. fetus* subsp. *fetus*.

2. Diarrhea is the primary disease caused by *Campylobacter jejuni*. It is often transmitted by means of contaminated water and animals, especially poultry and carcasses. *Campylobacter fetus* is the causative agent of bacteremia.

3. The characteristic gram-stain reaction and microscopic morphology of *Campylobacter* is often described as faintly staining, "seagull-shaped" gram-negative rods (see Web Color Image 7–62). The organisms exhibit darting motility. Characteristics of *Campylobacter* species are summarized in Box 7–5.
### Table 7–15 Identification of the Enterobacteriaceae

<table>
<thead>
<tr>
<th>Species</th>
<th>Indole</th>
<th>Methyl Red</th>
<th>V-P</th>
<th>Citrate</th>
<th>H2S</th>
<th>Urea</th>
<th>FDA</th>
<th>LDC</th>
<th>ODC</th>
<th>Motility (22°C)</th>
<th>Galactin (22°C)</th>
<th>Lactose (25°C)</th>
<th>DNAase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>+</td>
<td></td>
<td>Y</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>±</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>±</td>
</tr>
<tr>
<td>Shigella* aerogroups A,B,C</td>
<td>Y</td>
<td>−</td>
<td></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>±</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>S. sonnet</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Salmonella, most serotypes</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>−</td>
</tr>
<tr>
<td>S. typhi</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>S. paratyphi A</td>
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<td>+</td>
<td>−</td>
<td>−</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<td>−</td>
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<td>−</td>
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<td>−</td>
</tr>
<tr>
<td>C. diversus</td>
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<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Edwardsiella tarda</td>
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<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>+</td>
<td>Y</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td>−</td>
<td>−</td>
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<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Hafnia alvei</td>
<td>−</td>
<td>Y</td>
<td>V</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>V</td>
<td>+</td>
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<td>Proteus</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>+</td>
<td>−</td>
<td>Y</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Providencia rettgeri</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>Y</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>T. pyov</td>
<td>−</td>
<td>Y</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

DNAse = deoxyribonuclease; H2S = hydrogen sulfide; LDC = lysine decarboxylase; ODC = ornithine decarboxylase; FDA = phenylalanine deaminase; V = 10% to 89% are positive; V-P = Voges-Proskauer test; ≥ = ≥90% are positive; ≤ = ≤10% are positive.

4. The isolation of *Campylobacter* is accomplished by inoculating the specimen (usually stool) to *Campylobacter blood agar*. This contains several antibiotics that suppress growth of normal fecal flora. The plate is incubated in a microaerophilic atmosphere.

**Incubation at 42°C** also inhibits growth of normal fecal flora. The specimen of choice for isolation of *C. jejus* is blood with incubation at 35°C to 37°C.

5. The laboratory identification of *Campylobacter* is summarized in Figure 7–5 and Table 7–16. The oxidase-positive, catalase-positive *Campylobacter* species can be

### Box 7–5 Characteristics of Campylobacter

- **Gram negative**
- Curved rods—“seagull-wing-shaped”
- Daring motility
- Microaerophilic
- Oxidase positive
- Catalase positive
differentiated based on growth at 42°C, hippurate hydrolysis, and susceptibility to nalidixic acid and cephalothin. *C. jejuni* grows at 42°C, is hippurate hydrolysis-positive, susceptible to nalidixic acid, and resistant to cephalothin. Reactions for *C. fetus* are the opposite.

**B. Helicobacter** is an organism very similar to *Campylobacter*.
1. Diseases caused by *Helicobacter* include gastritis and duodenal ulcers.
2. Specimens are usually gastric biopsy material. Isolates are strongly urease-positive (see Web Color Image 7–43).

**C. The Vibrionaceae** include *Vibrio* and *Aeromonas*. *Plesiomonas*, previously a genus in the family Vibrionaceae, has been moved to the family Enterobacteriaceae. (*Plesiomonas* will be discussed in this section.)
1. The most commonly isolated species of *Vibrio* include *V. cholerae* (O-1 and non-O-1), *V. parahaemolyticus*, *V. alginolyticus*, and *V. vulnificus*.
2. All of these species are found in water sources and are transmitted by contaminated food and water.
3. *Vibrio cholerae* is the cause of *cholera*, a disease in which vast quantities of fluid and electrolytes are lost from the intestinal tract. The liquid stools are often referred to as “rice-water” stools, because they are colorless and contain mucus flecks. Cholera-causing isolates have a somatic antigen referred to as O-1. Non-O-1 isolates do not cause cholera, but may cause other infections. *V. cholerae* can be differentiated from other species by a positive “string test” when mixed with sodium deoxycholate (see Web Color Image 7–63).
4. *Vibrio parahaemolyticus* and *Vibrio alginolyticus* usually cause gastroenteritis following ingestion of raw or improperly handled seafood and wound infections following exposure to sea water. Both organisms require salt for growth (halophilic).
5. *Vibrio vulnificus* is an extremely virulent organism that causes rapidly progressive wound infections after exposure to contaminated water and septicemia after eating raw oysters.
6. The laboratory isolation and identification of *Vibrio* is summarized in Tables 7–17 and 7–18. *Vibrio* species are usually described as “curved” gram-negative rods (see Web Color Image 7–64), but this morphology is often only seen in the initial Gram stain.
of the clinical specimen. Isolated colonies are straight to pleomorphic gram-negative rods. The organisms are usually isolated from stool specimens. *V. cholerae* can be enriched by using alkaline peptone water (pH 8.4). This suppresses the growth of other organisms. All *Vibrio* species grow well on routine media. The *Vibrio* species are “halophilic” or “salt-loving,” and with the exception of *V. cholerae* and *V. mimicus*, all species require salt for growth. The differential medium of choice is Thiosulfate citrate bile salts sucrose (TCBS) agar (see Web Color Images 7–65 and 7–66). All isolates are indole and oxidase-positive (see Web Color Image 7–38), and have a fermentative metabolism. The reactions of the various *Vibrio* species on TCBS are summarized in Table 7–17.

7. *Aeromonas* and *Plesiomonas* are found in fresh and salt waters. They may cause diarrheal disease as well as other miscellaneous infections. Normally, only patients who have underlying disease are treated. This type of diarrhea is usually self-limiting. The role of *Aeromonas* and *Plesiomonas* in diarrheal disease is not well established. These organisms grow well on blood and MacConkey agar, are oxidase positive, and ferment glucose. These bacteria are gram-negative rods. *Plesiomonas* may show long filamentous forms. Differentiation of *Aeromonas* and *Plesiomonas* is summarized in Table 7–19.

8. Differentiation of *Aeromonas*, *Plesiomonas*, and *Vibrio* is summarized in Table 7–20.

### XIV. GRAM-NEGATIVE NONFERMENTATIVE BACILLI

**A.** This large group of organisms uses biochemical pathways other than fermentation. They may be oxidizers (see Web Color Image 7–67) or they may be asaccharolytic (nonoxidizers). Gram-negative nonfermentative bacilli account for approximately 15% of gram-negative rod-shaped bacteria isolated in the clinical laboratory. They are found in the environment and cause disease in immunocompromised individuals. Virtually all of these organisms are opportunists. An isolate that grows on blood agar but not MacConkey agar should be suspected of being a nonfermenter. This is especially true if the isolate is also oxidase positive. Nonfermenters that do grow on MacConkey agar appear as lactose negative.

**B.** The most commonly isolated gram-negative, nonfermentative, rod-shaped bacteria are listed in Box 7–6.

**C.** The most frequently isolated gram-negative nonfermenter is *Pseudomonas aeruginosa*. This organism is commonly seen in patients who have serious burns and cystic fibrosis.

### Table 7–18 Identification of *Vibrio* Species

<table>
<thead>
<tr>
<th>Growth in NaCl</th>
<th>Acid from:</th>
<th>Susceptible to O/129</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Lactose</td>
<td>Sucrose</td>
</tr>
<tr>
<td><em>V. alginolyticus</em></td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td><em>V. cholerae</em></td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td><em>V. vulnificus</em></td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

V = variable; VP = Voges-Proskauer; O/129 = 2,4-diamino-6,7-diisopropylpteridine phosphate; + = positive; − = negative.
P. aeruginosa is also found to cause UTIs, endocarditis, and external otitis (swimmer’s ear). Infections may be difficult to control because of antibiotic resistance; this situation is usually only a problem in immunocompromised persons.

1. The growth requirements of P. aeruginosa are very simple. It has been found growing in distilled water. It also grows over a wide temperature range (4°C–42°C).

2. Identification of P. aeruginosa is not difficult and is summarized in Box 7–7. This organism grows well on most media and is easily recognized by its blue-green pigment (due to pyocyanin production) (see Web Color Image 7–68) and corn tortilla odor (some describe it as a grape-like odor). P. aeruginosa also produces the pyoverdin (see Web Color Image 7–69) that fluoresces under UV light and is oxidase-positive (see Web Color Image 7–38).

3. Other Pseudomonas species are of low virulence and rarely cause clinical disease. Many are environmental organisms. They can be opportunists, but are often considered as contaminants when isolated from clinical specimens.

b. P. stutzeri is a rare pathogen, but may cause infection in an immunocompromised host. The organism demonstrates characteristic yellow to brown wrinkled, leathery, adherent colonies.

D. Infections by Burkholderia (Pseudomonas) cepacia are primarily nosocomial infections related to contaminated disinfectants used for antisepsis. Community-acquired infections are rare except in intravenous drug abusers. The bacterium has been associated with pneumonia in patients with cystic fibrosis.

E. Pathogenic Burkholderia species are known to cause severe infections, but are seldom seen in the United States. These species are considered by government agencies to be potential agents of bioterrorism.

1. Burkholderia mallei causes glanders, which is a zoonosis primarily affecting livestock, and can produce local suppurative or acute pulmonary infections in humans.

2. Burkholderia (Pseudomonas) pseudomallei causes melioidosis, an aggressive granulomatous pulmonary disease. Endemic areas include Southeast Asia, Northern Australia, and Mexico.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Esculin Hydrolysis</th>
<th>LDC</th>
<th>ODC</th>
<th>Mannitol</th>
<th>Arabinose</th>
<th>Sucrose</th>
<th>Inositol</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. hydrophila</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. caviae</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>A. sobria</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>P. shigelloides</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

LDC = lysine decarboxylase; ODC = ornithine decarboxylase; + = positive; − = negative.

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<table>
<thead>
<tr>
<th>Organism</th>
<th>Esculin Hydrolysis</th>
<th>LDC</th>
<th>ODC</th>
<th>Mannitol</th>
<th>Arabinose</th>
<th>Sucrose</th>
<th>Inositol</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. hydrophila</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. caviae</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>A. sobria</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>P. shigelloides</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

LDC = lysine decarboxylase; ODC = ornithine decarboxylase; + = positive; − = negative.
Box 7–6  Most Commonly Isolated Gram-Negative, Nonfermentative Rods

- *Pseudomonas aeruginosa*
- Burkholderia (Pseudomonas) cepacia
- *Stenotrophomonas (Xanthomonas) maltophilia*
- *Acinetobacter*
- *Chryseobacterium (Flavobacterium) meningosepticum*
- *Eikenella corrodens*
- *Moraxella*
- Alcaligenes and Achromobacter

**F.** *Stenotrophomonas* (*Xanthomonas*) *maltophilia* is found in the environment and causes a wide range of nosocomial infections.

**G.** *Acinetobacter* species are opportunists found in soil and water. They cause pneumonia and UTIs. On Gram’s stain, this genus is characteristically a fat coccobacillus. The two species most commonly seen in clinical specimens are *A. baumannii* (previously *A. calcoaceticus* var. *anitratus*) and *A. lwoffii* (previously *A. calcoaceticus* var. *lwoffii*).

**H.** *Chryseobacterium* (*Flavobacterium*) *meningosepticum* is a cause of neonatal meningitis or septicemia, especially in premature infants. The bacterium can cause pneumonia, endocarditis, bacteremia, and meningitis in adults.

**I.** *Eikenella corrodens* is found as normal mouth and nasopharyngeal flora. Trauma to the face or mouth, including dental work, may predispose an individual to infection. Human bite wounds are another source of infection. This isolate is often found as part of a mixed infection. The name is derived from the corroding or pitting of the agar by the colonies.

**J.** *Moraxella* species are normal flora of mucous membranes. They infrequently cause infection. *Conjunctivitis* is caused by *M. lacunata*. *M. catarrhalis* is normal oral flora of the respiratory tract and may cause *otitis media*, *sinusitis*, and respiratory infections (see Section X).

**K.** Alcaligenes and Achromobacter are divided into asaccharolytic and saccharolytic species. These opportunists are found in water and are resistant to disinfectants. The asaccharolytic *Alcaligenes faecalis* is most often seen in clinical specimens and has been isolated from urine, sputum, wound, and blood. *Achromobacter xylosoxidans* subsp. *xylosoxidans*, an oxidizer, has been associated with otitis media, meningitis, pneumonia, surgical wound infections, UTIs, peritonitis, and bacteremia.

**L.** The antibiotic susceptibility patterns of gram-negative nonfermentative bacilli are similar to those of the Enterobacteriaceae. Some nonfermenters are resistant to most of the antibiotics used.

Box 7–7  Identification of *Pseudomonas aeruginosa*

- Gram-negative rod
- Grows on most media
- Colonies have a feathered, ground (frosted) glass appearance
- β-hemolytic
- Corn tortilla odor (some prefer to describe it as a grapelike odor)
- Blue-green pigment (pyocyanin)
- Fluorescence upon exposure to ultraviolet light (pyoverdin)
- Grows between 4 C and 42 C
- Oxidase positive
- Oxidizers
Table 7–21 Identification of Nonfermentative, Gram-Negative Bacilli and Cocccobacilli

<table>
<thead>
<tr>
<th>Species</th>
<th>Oxidase</th>
<th>Catalase</th>
<th>Indole</th>
<th>Growth on MacConkey Agar</th>
<th>Motility</th>
<th>O/F Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Acinetobacter lwoffi</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Burkholderia cepacia</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavobacterium meningosepticum</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Eikenella corrodens</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Moraxella species</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Alcaligenes faecalis</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Achromobacter xylosoxidans subsp. xylosoxidans</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

O/F = oxidation/fermentation; + = positive; − = negative.

M. The laboratory identification of the most common bacteria in this group is summarized in Table 7–21.

XV. MISCELLANEOUS GENERA

A. *Legionella* species are found worldwide in the environment. Most infections result from contaminated water sources. *Legionella pneumophila* is the most common isolate.

1. Pneumonia (Legionnaires’ disease) is the most common infection caused by *Legionella*. Pontiac fever is a nonpneumonic form of *Legionella* infection with flu-like symptoms. Its incidence in the general population is unknown.

2. Specimens for culture are generally from pulmonary sources.

3. Various staining methods can be used to visualize *Legionella*. The organisms are small gram-negative rods and stain weakly on Gram’s stain. Giemsa stain may be used. A direct fluorescent antibody test is available and is very useful for detection from direct specimens.

4. Culture can be accomplished using special media, because *Legionella* does not grow on blood agar. *Cysteine* is required for growth. The recommended medium for isolation is buffered charcoal yeast extract agar (BCYE). After several days of incubation, the colonies are gray-white to blue-green. Definitive identification can be made with fluorescent antibody or DNA probes.

B. *Chromobacterium* is characterized by a violet pigment. It is an opportunist that causes wound infections and bacteremia with a skin lesion as the typical portal of entry. *Chromobacterium* is found in soil and water, most commonly in tropical and subtropical climates. However, it has been found in the southeastern United States.

C. *Gardnerella vaginalis* is often associated with bacterial vaginosis. The organism is not thought to be the etiologic agent, but confections with the anaerobe *Mobiluncus* and other anaerobic bacteria are common. *Human blood Tween agar* is used for isolation with incubation at 48 hours in 5% to 10% carbon dioxide. Colonies are β hemolytic. *Gram’s stain shows small gram-variable or gram-negative cocccobacilli*. A presumptive identification can be made when *clue cells*, squamous epithelial cells covered with tiny bacilli, are observed in wet mounts or direct smears of vaginal discharge.

D. *Streptobacillus moniliformis* is the agent of rat-bite fever (streptobacillosis), an infection characterized by fever, flu-like symptoms, a macropapular rash, and lymph node
involvement. Gram’s stain shows pleomorphic, long filamentous forms. In blood culture media, the organism grows as “fluff balls” or “bread crumbs.” Serum antibodies can be detected by agglutination tests.

E. *Spirillum minus* (minor) is also the agent of rat-bite fever (spirillosis). Symptoms of infection are the same as those for streptobacillosis. Serum antibodies can also be detected by agglutination tests. Penicillin G is the treatment of choice for both infections.

F. *Bartonella bacilliformis* is the etiologic agent of bartonellosis, also known as verruca peruana. Geographically, the disease is restricted to the high-altitude valleys of Peru, Ecuador, and southwest Columbia. The febrile systemic infection is transmitted to humans by the bite of infected sandflies. The organism is an aerobic, pleomorphic, poorly staining, gram-negative bacterium.

XVI. **MYCOPLASMA, UREAPLASMA, AND THE CHLAMYDIACEAE**

A. The organisms in the family Mycoplasmataceae are small, free-living organisms that lack a cell wall. The three most common isolates are *Mycoplasma pneumoniae*, *Mycoplasma hominis*, and *Ureaplasma urealyticum*.

1. *M. pneumoniae* is the causative agent of atypical or walking pneumonia. This organism is not normal flora, and isolation is always significant.

2. *U. urealyticum* and *M. hominis* colonize the lower genitourinary tracts of up to 60% to 70% of the normal population. They cause opportunistic infections such as nongonococcal urethritis in males and postpartum infections in females.

3. The organisms can be cultured on special enriched media. *Mycoplasma* colonies are very small, requiring a dissecting microscope for observation. They demonstrate a characteristic “fried egg” appearance (raised center with flat edges). Colonies of *Ureaplasma* are much smaller.

4. Dienes (see Web Color Image 7–70) or methylene blue stains can be used to visualize the colonies on agar media. Immunofluorescent stains and serology testing are used for laboratory diagnosis. Differentiation of colonization and infection may be difficult in test results positive for *U. urealyticum* or *M. hominis*, because both can be normal flora.

B. Members of the family Chlamydiaceae are obligate intracellular parasites. The three most common species are *Chlamydia trachomatis*, *Chlamydophila pneumoniae* (previously *Chlamydia pneumoniae*), and *Chlamydophila psittaci* (previously *Chlamydia psittaci*). There are two forms in the growth cycle of the organism: the **elementary body** is the infectious form, and the noninfectious **reticulate body** is the intracellular reproductive form.

1. *C. trachomatis* is the causative agent of trachoma, lymphogranuloma venereum, and various other sexually transmitted diseases. Trachoma is a leading cause of blindness worldwide. *C. trachomatis* is the most common sexually transmitted bacterial pathogen in the United States.

2. *C. pneumoniae* is an important cause of pneumonia and pharyngitis.

3. *C. psittaci* is the cause of psittacosis (ornithosis or parrot fever), a respiratory tract disease seen in patients exposed to birds.

4. Laboratory diagnosis can be accomplished several ways. Shell vial tissue cultures are used for detection of *Chlamydia*. Cell lines, usually McCoy cells, can be inoculated with specimens suspected of harboring *Chlamydia*. After 72 hours, staining with iodine shows darkly stained inclusion bodies within the cells. Fluorescein-labeled monoclonal antibodies can also be used to detect the chlamydial inclusions in Shell vial cultures. Other methods of laboratory diagnosis available include immunofluorescence, enzyme immunoassay, nucleic acid probes, and polymerase chain reaction.
 SPIROCHETES

A. Three genera in the order Spirochaetales cause human disease: *Treponema*, *Leptospira*, and *Borrelia*. These bacteria are characterized by their helical shape with spiral coils (see Web Color Image 7–71). The pathogenic spirochetes and associated infections are listed in Table 7–22.

B. *Treponema pallidum ssp. pallidum* is the etiologic agent of syphilis. The infection progresses through three phases: primary, secondary, and tertiary. Skin lesions called chancres are characteristic of primary and secondary syphilis. Chancres are extremely infectious and fluid from these lesions can be used to perform darkfield microscopy. The observation of motile, spiral-shaped treponemes from the chancre in the primary and secondary stages of syphilis is diagnostic. Diagnosis is usually done by serologic testing, which requires either a nonspecific or specific treponemal test.

1. The nonspecific tests include the rapid plasma reagin (RPR) and venereal disease research laboratory tests (VDRL).
2. Treponemal tests detect antibodies specific for treponemal antigens. These tests include the fluorescent treponemal antibody absorption test (FTA-ABS) and the TP-PA test, a modification of the MHA-TP test (microhemagglutination test for *T. pallidum*).

C. *Treponema* species are the etiologic agents of nonveneral treponemal diseases. These diseases are endemic to developing countries, humid tropical areas, or arid dry areas. They are not found in the United States. Transmission is via direct contact, person-to-person, or sharing contaminated eating utensils. Skin lesions, gummas, and dissemination are characteristic clinical manifestations.

1. *Treponema pallidum ssp. pertenue* is the etiologic agent of Yaws.
2. *Treponema pallidum ssp. endemicum* is the etiologic agent of Bejel or endemic syphilis.
3. *Treponema carateum* is the etiologic agent of Pinta.

D. *Borrelia* is the cause of relapsing fever (*B. recurrentis*) and Lyme disease (*B. burgdorferi*), both of which are spread by ticks. Relapsing fever is also associated with house-borne transmission. Erythema chronicum migrans (EMC) is associated with lyme disease, and is characterized by classic skin lesions at the site of the tick bite. Relapsing febrile episodes are caused by antigenic variation in the spirochete and are characteristic of infections caused by *B. recurrentis*. Laboratory diagnosis of relapsing fever is achieved.
by observation of spirochetes in blood smears stained with the Giemsa or Wright stain. Diagnosis of Lyme disease is determined by serologic means. Antibody in the patient’s serum may be detected after the third week of the illness. Sensitivity of the various methods varies widely from poor to good.

E. *Leptospira interrogans* is the cause of leptospirosis or Weil’s disease. Animals reservoirs are dogs, rats, and rodents. The organism is transmitted via exposure to the urine of carriers or urine-contaminated soil or water. The organism can be cultured using Fletcher medium, a semi-solid, tubed medium. Growth occurs in a ring just beneath the medium surface. Stuart liquid media can also be used for culture. Diagnosis may be made from microscopy (dark-field, phase-contrast, or immunofluorescent microscopy) or culture. Serological diagnosis, most commonly used, is via slide agglutination or IgM dot-ELISA.

XVIII. MYCOBACTERIA

A. The mycobacteria are aerobic bacilli. The cell wall is rich in lipids, which makes them resistant to Gram’s stain. After staining, the bacilli are difficult to decolorize with acid solutions (e.g., they are acid-fast). Most members of this group grow slowly with cellular division every 12 to 24 hours. Identification of a Mycobacterium species isolate is determined by the characteristics outlined in Box 7–8.

B. The most common specimen collected and processed is respiratory secretions, although mycobacteria can be recovered from virtually any body site. With sputum and other specimens collected from nonsterile sites, digestion and decontamination must be done before inoculation of growth media. See Box 7–9 for digestion and decontamination agents.

C. Following digestion and decontamination, the suspension is diluted with buffer and centrifuged to concentrate any organisms present. The sediment is inoculated onto mycobacterial growth media and used to make a smear for acid-fast staining.

1. Mycobacterial growth media
   a. The most commonly used egg-based medium is Lowenstein-Jensen.
   b. Agar-based media are variations of Middlebrook 7H10 medium. All contain some inhibitory agents to suppress the growth of contaminating bacteria.

2. Acid-fast stains
   a. Carbol-fuchsin stains include the Ziehl-Neelsen and Kinyoun, which use carbol-fuchsin as the primary stain, acid alcohol for the decolorizing agent, and a methylene blue counterstain. The Ziehl-Neelsen stain utilizes heat to drive the stain into the mycobacterial cell wall. The Kinyoun stain is a “cold” variation of

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Box 7-8 Characteristics Used to Identify Mycobacterium Isolates

<table>
<thead>
<tr>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid fast</td>
</tr>
<tr>
<td>Colony morphology</td>
</tr>
<tr>
<td>Colony pigmentation and photoreactivity</td>
</tr>
<tr>
<td>Growth rate</td>
</tr>
<tr>
<td>Growth temperature</td>
</tr>
<tr>
<td>Biochemical tests</td>
</tr>
</tbody>
</table>

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Box 7-9 Decontamination and Digestion Agents

<table>
<thead>
<tr>
<th>Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>N-acetyl-L-cysteine</td>
</tr>
<tr>
<td>Benzalkonium chloride</td>
</tr>
<tr>
<td>Oxalic acid</td>
</tr>
</tbody>
</table>
Table 7–23 Characteristics of Mycobacterial Photoreactivity Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Pigment Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photochromogens</td>
<td>Produce pigment on exposure to light</td>
</tr>
<tr>
<td>Scottochromogens</td>
<td>Produce pigment in the light or dark</td>
</tr>
<tr>
<td>Nonchromogens</td>
<td>No pigment produced in the light or dark</td>
</tr>
</tbody>
</table>

the stain. Stained smears are viewed with a light microscope. Acid-fast organisms stain pink or red against a blue background. Nonacid-fast organisms stain blue (see Web Color Image 7–8).

b. The fluorochrome stains—auramine and auramine-rhodamine—are very sensitive and require a fluorescence microscope. Acid-fast organisms fluoresce a yellow-orange against a dark background (see Web Color Image 7–9).

D. Pigment groups are helpful in a presumptive identification of the mycobacteria. The Runyon classification system was designed to classify the species within the genus. Three groups are based on photosensitivity: photochromogens (Group I), scotochromogens (Group II), and nonphotochromogens (Group III). The “rapid growers” are Group IV. The species within the MTB group, although nonchromogens, are categorized separately. The Mycobacterium species are still categorized according to photosensitivity, but the designation as “Runyon groups” is not often noted.

1. To determine pigment groups, or photoreactivity, a specimen is inoculated to two tubes or plates of mycobacterial media. One tube is incubated in the light, and the other is incubated in the dark. Following growth, pigment production of each is noted. The medium initially incubated in the dark is incubated in bright light for several hours, and pigment production, if any, is noted.

2. The nonphotochromogenic (nonchromogenic) mycobacteria do not produce pigment in either light or dark. Photochromogens produce pigment in light, whereas scotochromogens produce pigment in the light or dark. Pigment color may range from light yellow to a dark orange (see Web Color Images 7–72 and 7–73). The mycobacterial photoreactivity groups are summarized in Table 7–23.

E. The growth rate is determined as rapid or slow. Rapid growers produce colonies in fewer than 7 days. Slow growers require longer than 7 days for production of colonies. Table 7–24 lists some of the medically significant mycobacterial species arranged according to pigment groups and growth rate.

F. The optimal growth temperature for most Mycobacterium species is 35°C to 37°C. M. marinum grows best at 30°C, whereas M. xenopi grows best at 42°C.

Table 7–24 Medically Important Mycobacteria According to Pigment Group and Growth Rate

<table>
<thead>
<tr>
<th>Group</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photochromogen</td>
<td>Mycobacterium kansasii</td>
</tr>
<tr>
<td></td>
<td>M. marinum</td>
</tr>
<tr>
<td></td>
<td>M. simiae</td>
</tr>
<tr>
<td>Scottochromogen</td>
<td>M. gordonae</td>
</tr>
<tr>
<td></td>
<td>M. scrofulaceum</td>
</tr>
<tr>
<td></td>
<td>M. szulgai</td>
</tr>
<tr>
<td></td>
<td>M. xenopi (N/S)</td>
</tr>
<tr>
<td></td>
<td>M. faurceae</td>
</tr>
<tr>
<td>Nonchromogen</td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td></td>
<td>M. bovis</td>
</tr>
<tr>
<td></td>
<td>M. avium—M. intracellulare*</td>
</tr>
<tr>
<td>Rapid growers</td>
<td>M. chelonae</td>
</tr>
<tr>
<td></td>
<td>M. fortuitum</td>
</tr>
</tbody>
</table>

* N = nonchromogen, F = scotochromogen.
* Some isolates (15%) are pigmented.
Table 7–25 Identification of Mycobacteria

<table>
<thead>
<tr>
<th>Organism</th>
<th>Photochromogenic</th>
<th>Niacin</th>
<th>Susceptible to TCH</th>
<th>Nitrate Reduction</th>
<th>SQ Catalase</th>
<th>Tween Hydrolysis</th>
<th>Growth in 5% NaCl</th>
<th>Iron Uptake</th>
<th>Arylsulfatase</th>
<th>Urease</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. marinum</td>
<td>Photochromogenic</td>
<td>V</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>M. kansasii</td>
<td>Photochromogenic</td>
<td>V</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>M. scrofulaceum</td>
<td>Nonchromogenic</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>M. szulgai</td>
<td>Nonchromogenic</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>M. xenopi</td>
<td>Scotochromogenic</td>
<td>V</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>M. flavescens</td>
<td>Scotochromogenic</td>
<td>V</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>M. fortuitum</td>
<td>Rapid grower</td>
<td>V</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>M. chelonei</td>
<td>Rapid grower</td>
<td>V</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

SQ = semi-quantitative; TCH = thiophen-2-carboxylic acid hydrazide; V = variable; + = positive; − = negative.

There are several biochemical tests that are valuable in identification of the mycobacteria (Table 7–25). Most of these tests are performed only in those laboratories that do complete identification. Some tests, such as niacin and nitrate tests, are easy to perform and can give presumptive identification of *M. tuberculosis*.

1. **Niacin accumulation** is detected by measuring nicotinic acid, which reacts with cyanogen bromide in the presence of aniline to form a yellow compound.
2. **Nitrate reduction** is performed as with the method used for Enterobacteriaceae. A positive test is a red pigment.
3. All mycobacteria are catalase-positive (see Web Color Image 7–11). The quantity of catalase and production of heat-stable catalase are species-specific. Semi-quantitative catalase production (see Web Color Image 7–74) is determined by measuring the height of the column of bubbles when hydrogen peroxide and Tween 80 are added to a deep with mycobacterial growth. Catalase heat stability is determined by heating the specimen to 68°C for 20 minutes prior to the addition of hydrogen peroxide. Production of bubbles is a positive reaction.
4. ** Tween hydrolysis** measures the presence of a lipase. Hydrolysis causes a pink color change.
5. NaCl tolerance is determined by inoculating an egg-based media with 5% NaCl and observing growth or no growth following incubation.
6. Iron uptake is determined by adding ferric ammonium citrate to the mycobacterial colonies. Dusty-brown colonies are a positive reaction for iron uptake and the formation of iron oxide.
7. **Arylsulfatase** activity is detected by adding phenolphthalein to the colony/substrate mixture and observing the formation of a pink color.
8. The urease test detects an organism’s ability to produce urease and hydrolyze urea to form ammonia, which produces an alkaline reaction. The increase in pH is detected by a change of color to pink or red.
9. **Susceptibility to thiophen-2-carboxylic acid hydrazide (TCH)** is determined by observing growth or no growth of mycobacteria following incubation with this compound.
10. Nucleic acid amplification is available for identification of some species of mycobacteria, including MTB complex and MAC complex. Specificity is very good, but use with clinical specimens may give false-negative results. Identification with this technique is usually performed in reference laboratories.
11. Gas-liquid chromatography and high-performance liquid chromatography are methods to analyze mycobacterial lipids.

**H. Mycobacterium tuberculosis** is a member of the MTB complex. MTB causes tuberculosis (TB), a pulmonary infection transmitted by the inhalation of droplet nuclei. *M. bovis* causes tuberculosis in humans, cattle, and other animals.
Table 7–26 Summary of Clinical Infections Caused by Mycobacteria

<table>
<thead>
<tr>
<th>Pigment Group</th>
<th>Organism</th>
<th>Clinical Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photochromogen</td>
<td>M. marinum</td>
<td>Cutaneous infections from water exposure</td>
</tr>
<tr>
<td></td>
<td>M. kansasi</td>
<td>Pulmonary disease</td>
</tr>
<tr>
<td></td>
<td>M. simiae</td>
<td>Pulmonary disease (rare)</td>
</tr>
<tr>
<td>Nonchromogen</td>
<td>M. tuberculosis</td>
<td>Pulmonary disease; other sites</td>
</tr>
<tr>
<td></td>
<td>M. bovis</td>
<td>Pulmonary disease (rare in the United States)</td>
</tr>
<tr>
<td></td>
<td>M. avium-M. intracellulare</td>
<td>Pulmonary disease (especially AIDS patients)</td>
</tr>
<tr>
<td>Scochochromogen</td>
<td>M. gordonae</td>
<td>Common contaminant (“tap-water bacillus”)</td>
</tr>
<tr>
<td></td>
<td>M. scrofulaceum</td>
<td>Cervical lymphadenitis in children</td>
</tr>
<tr>
<td></td>
<td>M. szulgai</td>
<td>Pulmonary disease</td>
</tr>
<tr>
<td></td>
<td>M. xenopi</td>
<td>Pulmonary disease in immunosuppressed patients</td>
</tr>
<tr>
<td>Rapid grower</td>
<td>M. fortuitum</td>
<td>Pulmonary disease; other sites in immunosuppressed patients</td>
</tr>
<tr>
<td></td>
<td>M. chelonei</td>
<td>Pulmonary disease; other sites in immunosuppressed patients</td>
</tr>
</tbody>
</table>

AIDS = acquired immunodeficiency syndrome.

1. Primary tuberculosis is the initial infection. The mycobacterium is eradicated by the host cellular immune response or walled off in a granuloma in the lung. Reactivation of latent infections can occur in immunocompromised individuals and cause secondary tuberculosis. Military TB is a disseminated infection with multiple organ involvement.

2. The tuberculin skin test is used to detect MTB-infected individuals. A purified protein derivative (PPD) is the MTB antigen. A hypersensitivity reaction at the injection site within 72 hours is a positive test. A positive skin test does not distinguish patients with active disease from those with latent infections.

3. Identifying characteristics of M. tuberculosis: slow growth, nonchromogenic, “serpentine cording,” niacin accumulation-positive, nitrate-positive, and susceptible to NAP. Web Color Image 7–75 depicts a Kinyoun acid-fast stain with the characteristic cell aggregates of M. tuberculosis, due to the cording factor.

I. Mycobacterium avium complex (MAC) consists of the environmental organisms M. avium and M. intracellulare. It may colonize healthy individuals or cause opportunistic infections in immunocompromised individuals.

1. It is most commonly associated with disseminated disease in AIDS patients.

2. Identifying characteristics of MAC: slow growth, semiquantitative catalase >45 mm, and most are tellurite-positive. Otherwise, it is relatively inactive biochemically.

J. MOTT. Mycobacterium other than tuberculosis include several species that are environmental organisms that may colonize individuals or cause a variety of opportunistic infections. They are classified according to growth rate and photosensitivity. The mycobacteria are also known as “atypical mycobacteria” or NTM, mycobacteria other than the tuberculous mycobacteria.

K. Mycobacterium leprae is the etiologic agent of leprosy or Hansen’s disease. The mycobacterium cannot be cultured in vitro, but can be grown in mouse footpads or the armadillos.

L. Identifying characteristics of select Mycobacterium species are summarized in Table 7–25. Infections caused by mycobacteria are summarized in Table 7–26.

XIX. ANAEROBES

A. Atmospheric requirements. Anaerobes constitute the majority of bacteria found in and on the human host. Obligate anaerobes grow in the absence of oxygen and vary in their
tolerance to oxygen. Oxygen and its derivatives (e.g., hydrogen peroxide) are toxic. Most require a low redox potential, because an environment with a high redox potential is oxidized and harmful to anaerobic bacteria. These bacteria can be divided into moderate and strict anaerobes. The majority of medically significant anaerobes are moderate anaerobes.

1. **Moderate obligate anaerobes** can tolerate an atmosphere containing low levels of oxygen (2%–8%).

2. **Strict obligate anaerobes** are killed by exposure to oxygen. They cannot tolerate more than 0.5% oxygen.

3. **Aerotolerant anaerobes** grow very poorly in ambient air (21% oxygen), but grow well under anaerobic conditions.

4. **Facultative anaerobes** can grow aerobically and anaerobically.

B. **Direct examination** of a specimen using Gram’s stain is very helpful. Typical anaerobe morphology (pleomorphic rods; gram-positive, boxcar-shaped cells; thin rods with pointed ends) may be evident. Also, a positive Gram’s stain with negative aerobic culture results may indicate the need for anaerobic culture. If aerobic and anaerobic cultures have been performed, negative results may indicate a problem with collection, transport, or culture conditions. The Gram’s stain can also determine the culture conditions and media to be used.

A list of the commonly isolated anaerobes with their gram reactions are listed in Table 7–27. Table 7–28 summarizes the microscopic morphology of the more common anaerobes.

C. The media that are used to grow and identify anaerobes are as follows. Anaerobic bacteria grow best on pre-reduced, anaerobically sterilized media (PRAS) that are sterilized and stored under anaerobic conditions. Culture media may be enriched with hemin and vitamin K.

1. **Anaerobic sheep blood agar** is a general growth medium for all anaerobes. It is supplemented with vitamin K and hemin, and the type of agar base may vary (e.g., Columbia, Schaedler, brain-heart infusion). CDC blood agar and Brucella blood agar (BRU-BA) are most commonly used as all-purpose, direct plating media.
2. Bacteroides bile esculin (BBE) agar is used for the selection and presumptive identification of Bacteroides fragilis group. The high concentration of bile is inhibitory to other organisms. Colonies of the B. fragilis group hydrolyze esculin and appear black, surrounded by a black halo (see Web Color Image 7–76).

3. Phenylethyl alcohol blood agar (anaPEA) inhibits the gram-negative facultative bacilli, whereas most anaerobes grow well on the medium.

4. Kanamycin-vancomycin laked blood agar (KVLB) selects for Prevotella and Bacteroides. Other gram-positive and gram-negative rods are inhibited by the kanamycin and vancomycin. Laked blood enhances production of a brown-black pigment by certain Prevotella species.

5. Egg-yolk agar is used to determine if an isolate produces lecithinase or lipase. The lecithin in egg yolk is split by lecithinase, which results in an opaque halo around the colony (see Web Color Image 7–77). Lipase degrades triglycerides into glycerol and free fatty acids, and is detected by observing an oily or “mother-of-pearl” sheen on the colony surface (see Web Color Image 7–78). Proteolysis results in a small clear halo around the colonial growth.

6. Cycloserine-cefoxitin-fructose agar (CCFA) is a selective and differential medium for the isolation of Clostridium difficile. The organism produces characteristic yellow, ground-glass colonies on CCFA (see Web Color Image 7–79).

D. Tests used for the identification of anaerobes are as follows.

1. The catalase test for anaerobes uses 15% hydrogen peroxide instead of 3%.
2. A spot indole test is easily and quickly performed. This test uses p-dimethyl-aminocinnamaldehyde as the developing reagent. A blue color is produced when the test is positive (see Web Color Image 7–80).
3. Special potency antibiotic disks can be very helpful in the identification of anaerobic bacteria. The susceptibility of anaerobes to colistin (10 mcg), vancomycin (5 mcg), and kanamycin (1 mg) varies. Most gram-negative anaerobes are resistant to vancomycin, while most gram-positive anaerobes are susceptible to vancomycin and resistant to colistin. Gram-negative anaerobes vary in their susceptibility to colistin.
4. Growth in 20% bile separates the bile-resistant B. fragilis group from Prevotella. Bile disks, broth, or bile-containing agar can be used to perform the test. Esculin hydrolysis (see Web Color Image 7–76) is often detected with the same agar, and the reaction may also be helpful in the identification of anaerobic gram-negative rods.
5. Susceptibility to a 1-mg sodium polyanethol sulfonate (SPS) disk is characteristic of Peptostreptococcus anaerobius.
6. The nitrate test is performed with a disk placed on an inoculated anaBAP with the test organism. Following incubation, sulfanilic acid, dimethyl-α-naphthylamine, and zinc (if necessary) are added to the disk. The test is interpreted in the same manner as the tube test.
7. A reverse CAMP test identifies Clostridium perfringens. In this test, the Clostridium isolate is streaked perpendicular to a known group B Streptococcus agalactiae. A positive test demonstrates characteristic arrowhead hemolysis.
8. The Nagler Test is a laboratory test for the detection of lecithinase. This test uses C. perfringens type A antitoxin to neutralize lecithinase. The antitoxin inhibits the lecithinase reaction on egg-yolk agar. The test has been used for the presumptive identification of C. perfringens. However, it is not used as frequently, because other Clostridium species are now known to be Nagler test-positive.
9. Lecithinase, lipase, and proteolysis are all detected on egg-yolk agar (Color Images 7–77 and 7–78). Characteristic reactions are described above in the anaerobic media section.

E. Alternate methods may be used for the identification of anaerobic bacteria.

1. Gas-liquid chromatography (GLC) is used to detect metabolic end products (e.g., propionic acid, lactic acid) or cellular fatty acids (membrane components). Certain anaerobic bacteria have a characteristic GLC pattern.
Table 7–29 Identification of Clinically Encountered Anaerobic Gram-Negative Bacilli

<table>
<thead>
<tr>
<th>Species</th>
<th>Vancomycin</th>
<th>Kanamycin</th>
<th>Colistin</th>
<th>Indole</th>
<th>Lipase</th>
<th>Esculin Hydrolysis</th>
<th>Growth in 20% Bile</th>
<th>Catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides fragilis</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. thetaiotaomicron</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. vulgatus</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>B. oralis</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Prevotella intermedia</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Porphyromonas saccharolytica</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>V</td>
</tr>
<tr>
<td>Fusobacterium nucleatum</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>F. necrophorum</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>F. mortiferum</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

R = resistant; S = susceptible; + = positive; − = negative.

2. Conventional biochemical tubes ("roll-tubes") are the "gold standard" and are performed in reference laboratories. PRAS media supplemented with substrates is used for biochemical testing.

3. Commercial identification systems are miniaturized biochemical test panels or rapid enzymatic systems that detect preformed enzymes.

F. The characteristics of the more commonly isolated anaerobes, including the types of infections caused by each, are as follows.

1. The gram-negative bacilli cause a wide variety of infections. These organisms are part of the normal flora in the gastrointestinal tract, the female genital tract, and the oropharynx. Key characteristics of the most common species are summarized in Table 7–29.
   a. Members of the Bacteroides fragilis group are bile-resistant, and therefore grow in broth with 20% bile. Growth occurs on BBE agar with characteristic black colonies, because the organisms also hydrolyze esculin (see Web Color Image 7–76) with few exceptions. This group of anaerobic gram-negative rods is resistant to vancomycin, kanamycin, and colistin.
   (1) B. fragilis is the most common anaerobic isolate. In addition to the above characteristics, it is indole-negative and catalase-positive.
   (2) B. vulgatus, a member of the B. fragilis group, is indole-negative, catalase-positive, and esculin-negative.
   (3) Presumptive identification of B. thetaiotaomicron is based on positive indole and catalase reactions.

b. The Bacteroides uniformis group and Bilophila wadsworthia are differentiated from the B. fragilis group in that they are nitrate-positive, vancomycin-resistant, kanamycin-susceptible, and colistin-susceptible.

c. Prevotella and Porphyromonas are bile sensitive. Most of the clinically important species are pigmented dark brown or black (see Web Color Image 7–81). Laked blood agar enhances pigment production by these anaerobes. Both demonstrate a brick-red fluorescence when exposed to ultraviolet light.

d. Porphyromonas species are susceptible to vancomycin, whereas other gram-negative anaerobic bacilli are vancomycin-resistant. Porphyromonas species will not grow on kanamycin-vancomycin laked blood agar (KVLB). They require hemin and vitamin K for growth.

e. Fusobacterium nucleatum and F. necrophorum are normal flora in the respiratory and gastrointestinal tracts. F. nucleatum is a long, slender, gram-negative rod with tapered ends (see Web Color Image 7–82) and is indole-positive and lipase-negative. F. necrophorum is a pleomorphic gram-negative rod that is indole-positive and lipase-positive. Both demonstrate chartreuse fluorescence when exposed to ultraviolet light.
CHAPTER 7 Clinical Microbiology

Table 7–30 Identification of Clostridium Species

<table>
<thead>
<tr>
<th>Organism</th>
<th>Lecithinase</th>
<th>Lipase</th>
<th>Esculin Hydrolysis</th>
<th>Double Zone Hemolysis</th>
<th>Reverse Camp</th>
<th>Motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. perfringens</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>C. novyi</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>C. septicum</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>C. sporogenes</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

CAMP = Christie, Atkins, and Munch-Petersen; + = positive; − = negative.

2. The gram-positive bacilli are found as normal flora and are also widely distributed in the environment (e.g., Clostridium species).

a. Clostridium species (Table 7–30) cause a wide variety of infections. These gram-positive bacilli produce endospores that survive in adverse environmental conditions and germinate when conditions are favorable for bacterial growth.

(1) C. botulinum is the etiologic agent of botulism. The botulin neurotoxin is the most potent toxin known.

(2) C. tetani causes tetanus (lockjaw). It is characterized by swarming colonies and rods with round, terminal spores and a “drumstick” or “tennis racket” appearance (see Web Color Image 7–83). The clinical laboratory has little role in the diagnosis of either C. botulinum or C. tetani. The diagnosis is based on the clinical symptoms.

(3) C. difficile is associated with antibiotic-associated diarrhea and pseudomembranous enterocolitis.

(a) Treatment with broad-spectrum antibiotics may suppress the normal intestinal flora.

(b) C. difficile, which produces a toxin, is able to proliferate, resulting in diarrhea or colitis.

(c) Tests to detect C. difficile toxin are available (e.g., tissue culture, latex agglutination, enzyme-immunoassay), and may be performed on bacterial isolates or stool specimens. Toxin tests should be performed, because not all strains are toxigenic.

(d) C. difficile has a characteristic “horse-stable” odor, produces yellow, ground-glass colonies on CCFA agar (see Web Color Image 7–79), and demonstrates a chartreuse fluorescence upon exposure to ultraviolet light.

(4) Gas gangrene (myonecrosis) can be caused by C. perfringens, C. septicum, C. sporogenes, and C. novyi. Clostridial endospores are introduced into tissue by trauma or surgery. The spores germinate in vivo, and the organisms produce gas and cause extensive muscle and tissue necrosis.

(a) C. perfringens demonstrates a characteristic double-zone hemolysis (see Web Color Image 7–84) on anaerobic blood agar, is Nagler test-positive, lecithinase-positive, and reverse Camp test-positive. Presumptive identification of C. perfringens is summarized in Table 7–31.

(b) C. septicum is nonhemolytic and its colonies swim and have a characteristic “medusa-head” appearance. It is lecithinase-negative and hydrolyzes esculin.

b. The gram-positive, nonspore-forming bacilli are normal flora in various body sites. Their significance of infections is secondary when compared with infections caused by the Clostridium.

Table 7–31 Presumptive Identification of Anaerobic Gram-Positive Bacilli

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Spores</th>
<th>Kanamycin</th>
<th>Vancomycin</th>
<th>Collistin</th>
<th>Indole</th>
<th>Catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium perfringens</td>
<td>+</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>−</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Eubacterium lentum</td>
<td>−</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

R = resistant; S = susceptible; + = positive; − = negative.
1. Actinomyces israelii is the most frequently isolated member of the anaerobic genus Actinomyces. Infections often involve periodontal disease. Sulfur granules may be present in exudate. The gram-positive rods have a pleomorphic, beaded and branching appearance (see Web Color Image 7–85), and older colonies have a characteristic “molar-tooth” morphology (see Web Color Image 7–86).

2. Bifidobacterium species are part of the normal intestinal flora. The rods are pleomorphic and may have two forks. Identification is difficult unless laboratory personnel are experienced with anaerobes.

3. Eubacterium species are normal intestinal and oral flora and also are difficult to identify. E. lentum is a pleomorphic gram-positive rod and is nitrate-positive and catalase-negative. Characteristics used for presumptive identification are summarized in Table 7–31.

4. Propionibacterium are common on skin. P. acnes is the most common isolate. This organism is seen as a contaminant in much the same fashion as coagulase-negative staphylococci. The propionibacteria are often referred to as “anaerobic diphtheroids.” A pleomorphic, gram-positive rod that is catalase and indole-positive is presumptively identified as P. acnes (Table 7–31).

5. Lactobacillus species may be facultative or obligate anaerobes. They are normal flora of the mouth, intestinal tract, and vagina. The lactobacilli are long chains of gram-positive rods and are catalase-negative.

6. Mobiluncus species are curved gram-positive rods that stain gram-variable. They are associated with bacterial vaginosis, a poly-microbial infection. Microscopic observation of “clue cells” in vaginal exudates may be indicative of an infection. Gardnerella vaginalis is often associated with Mobiluncus and bacterial vaginosis.

3. Anaerobic cocci are normal flora in the mouth, gastrointestinal tract, female genital tract, and on the skin. Presumptive identification of the anaerobic cocci is simple.

a. Veillonella species are anaerobic gram-negative cocci (see Web Color Image 7–5). They are nitrate-positive, vancomycin-resistant, and may fluoresce red upon exposure to ultraviolet light.

b. Peptostreptococcus species are anaerobic gram-positive cocci. P. anaerobius is susceptible to sodium polyanethol sulfonate (SPS). P. asaccharolyticus is SPS-resistant, indole-positive, and nitrate-negative. P. indolicus is SPS-resistant, indole-positive, and nitrate-positive.

c. Peptococcus niger is the only species in the genus Peptococcus and is rarely isolated. It is also a gram-positive coccus, demonstrates black pigmented colonies, and is catalase-positive.

XX. ZOONOTIC AND RICKETTSIAL INFECTIONS

A. Zoonotic infections are diseases of animals that infect humans who have contact with infected animals. Animals are the natural hosts, and transmission to humans may be via inhalation, contact with animal secretions, carcasses or products, animal bites and scratches, or by arthropod vectors. Refer to Table 7–32 for a list of bacterial agents and associated with zoonotic infections. The natural host, vector, mode of transmission and associated infections are indicated for each bacterium.

B. Members of the Family Rickettsiaceae are obligate intracellular pathogens and causative agents of arthropod-borne human infections (Table 7–33). Animals or rodents are the Rickettsia rickettsii, R. typhi, R. prowazekii, and R. akari.

1. Rocky mountain spotted fever is transmitted by the tick and is caused by R. rickettsii. Humans are accidental hosts.

2. Endemic typhus, also called murine typhus, is transmitted by rat fleas and caused by R. typhi. Rats are reservoirs, whereas humans and other animals are accidental hosts.
Table 7–32 Zoonotic Infections and Causative Agents

<table>
<thead>
<tr>
<th>Zoonotic Infection</th>
<th>Causative Agent</th>
<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plague</td>
<td>Yersinia pestis</td>
<td>Fleas</td>
</tr>
<tr>
<td>Lyme borreliosis</td>
<td>Borrelia burgdorferi</td>
<td>Ticks</td>
</tr>
<tr>
<td>Pasteurellosis</td>
<td>Pasteurella multocida</td>
<td>Cats and dogs—bites or scratches</td>
</tr>
<tr>
<td>Erysipeloid</td>
<td>Erysipelothrix rhusiopathiae</td>
<td>Contact with infected animals or animal products</td>
</tr>
<tr>
<td>Capnocytophaga canimorsus infection</td>
<td>Capnocytophaga canimorsus</td>
<td>Cat or dog bites</td>
</tr>
<tr>
<td>Cat scratch disease</td>
<td>Bartonella</td>
<td>Cat scratch</td>
</tr>
<tr>
<td>Bacillary rat-bite fever</td>
<td>Streptobacillus moniliformis</td>
<td>Rat bite; contaminated milk</td>
</tr>
<tr>
<td>Spiroillary rat-bite fever</td>
<td>Spirillum minus</td>
<td>Rat bite; contaminated milk</td>
</tr>
<tr>
<td>Anthrax</td>
<td>Bacillus anthracis</td>
<td>Inhalation; direct or indirect contact with animals or animal products</td>
</tr>
<tr>
<td>Tularemia</td>
<td>Francisella tularensis</td>
<td>Ticks, mosquitos, rodents, and rabbits</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>Brucella species</td>
<td>Contact with infected animals and animal products; contaminated milk</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>Leptospira interrogans</td>
<td>Contact with urine of animal reservoirs—rodents and domestic animals; contaminated soil or water</td>
</tr>
</tbody>
</table>

3. Epidemic typhus is caused by *R. prowazekii*. The infection is transmitted by lice and fleas. Flying squirrels and humans are the reservoirs.

4. Rickettsial pox, a spotted fever, is transmitted by mites and caused by *R. akari*. The reservoir is the common house mouse.

C. Other arthropod-borne infections include *ehrlichiosis*, *scrub typhus*, and *Q fever*. 
1. *Ehrlichiosis* is transmitted ticks and natural hosts are dogs, deer, and other mammals. *Ehrlichia* species are major veterinary pathogens.
2. *Scrub typhus* is transmitted by chiggers and rats are the reservoirs. The etiologic agent is *Orientia tsutsugamushi*, a major veterinary pathogen.
3. *Q fever* is transmitted by ticks or inhalation of infected dust. It is caused by *Coxiella burnetii*.
4. Arthropod-borne infections caused by *Ricketttsi*, *Ehrlichia*, *Orientia*, and *Coxiella* species are summarized in Table 7–33.

XXI. AGENTS OF BIOTERRORISM

A. Many highly infectious bacteria and viruses have been classified as potential agents of bioterrorism. A massive outbreak of infection in a population within a geographic area could result in high rates of morbidity and mortality, as well as contamination of food supplies, destruction of vegetation, and infections in livestock. Inhalation of aerosols or ingestion would be the most likely means of transmission.

1. Bioterroristic agents are classified in Categories A, B, or C according to their pathogenicity. *Category A* agents are the most infectious because of the potential

<table>
<thead>
<tr>
<th>Table 7–33 Ricketttsi, Ehrlichia, Orienta, and Coxiiela</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthropod-borne Infection</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Rocky Mountain Spotted Fever</td>
</tr>
<tr>
<td>Endemic louse-borne typhus</td>
</tr>
<tr>
<td>Epidemic louse-borne typhus</td>
</tr>
<tr>
<td>Rickettsial pox</td>
</tr>
<tr>
<td>Ehrlichiosis</td>
</tr>
<tr>
<td>Scrub typhus</td>
</tr>
<tr>
<td>Q fever</td>
</tr>
</tbody>
</table>
Table 7–34 Category A Agents of Bioterrorism

<table>
<thead>
<tr>
<th>Bioterroristic Agent</th>
<th>Infection</th>
<th>Characteristic Clinical Manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus anthracis</td>
<td>Anthrax – inhalational, gastrointestinal, and cutaneous</td>
<td>Black eschar</td>
</tr>
<tr>
<td>Francisella tularensis</td>
<td>Tularemia</td>
<td>Acute granulomatous disease; primary pleuropulmonary disease</td>
</tr>
<tr>
<td>Yersinia pestis</td>
<td>Plague – bubonic, pneumonic, and septicemic</td>
<td>Multi-system disease</td>
</tr>
<tr>
<td>Botulinum toxin</td>
<td>Botulism – wound, gastrointestinal, and infant botulism</td>
<td>Paralytic illness</td>
</tr>
<tr>
<td>Variola major virus</td>
<td>Smallpox</td>
<td>Focal skin lesions progress to hemorrhagic smallpox</td>
</tr>
<tr>
<td>Hemorrhagic fever viruses</td>
<td>Hemorrhagic fevers</td>
<td>Severe multi-system syndrome with vascular hemorrhage</td>
</tr>
</tbody>
</table>

threat to humans and the ease with which these agents can be transmitted. These agents are listed in Table 7–34 with their respective clinical infections.

2. The Center for Disease Control (CDC) has a Laboratory Network Response (LNR) to decentralize testing capabilities and link state and local laboratories with advanced-capacity labs. LRN reference labs perform all confirmatory testing on biothreat agents.

3. Biosafety level laboratories are categorized in accordance with their capabilities to safely work with bioterrorist agents and minimize exposure to workers and the environment. Facilities are classified according to the safety equipment in the laboratory, as well as the training, procedures, and capability to perform adequate testing on the infectious agents. Laboratories are classified as BSL-1, BSL-2, BSL-3, and BSL-4. The most infectious agents are handled only in BSL-4 Laboratories, such as the CDC.

XXII. ANTIMICROBIAL SUSCEPTIBILITY TESTING

A. Background. The changing pattern of antimicrobial resistance of clinical isolates makes susceptibility testing of each isolate increasingly important. The in vitro results do not always give the complete picture. The antibiotic that should be used depends on other variables, such as host conditions, site of infection (e.g., CSF versus urine), route of administration, cost, and side effects.

B. Standardization. An important part of any susceptibility technique is use of a standard inoculum of bacteria. The most common procedure is to compare the turbidity of the inoculum with a McFarland turbidity standard. The 0.5 McFarland standard is usually used, and this is equivalent to $1.5 \times 10^8$ cfu/mL. The turbidity of a chemical precipitate in the standard correlates with the number of colony forming units of the bacterium per milliliter of inoculum. Regardless of the test used, each susceptibility method is highly standardized. The most commonly used tests are the Kirby-Bauer disk diffusion test and the minimum inhibitory concentration (MIC). Table 7–35 summarizes the antimicrobial susceptibility test procedures. Box 7–10 defines the areas of standardization that apply to both the Kirby-Bauer and MIC tests.

C. Interpretation of results. Results may be interpreted according to the following four categories:

1. Susceptible. The organism should respond to the usual doses of the drug.

2. Moderately susceptible. The isolate may be inhibited by concentrations of a drug that are achieved when the maximum parenteral doses are given.

3. Intermediate. The results are equivocal or indeterminate.

4. Resistant. The bacterium is not inhibited by achievable concentrations of drug.
Table 7–35 Summary of Antimicrobial Tests

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<th>Procedure</th>
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<td>Minimum inhibitory concentration</td>
<td>Lowest concentration of antibiotic that inhibits visible growth</td>
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<tr>
<td>Disk diffusion (Kirby-Bauer)</td>
<td>Measure diameter of growth inhibition around filter paper disk containing antibiotics</td>
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<td>E-test</td>
<td>Elliptical zone of inhibition intersects with strip containing antibiotics in a concentration gradient to determine MIC</td>
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<td>β-lactamase test</td>
<td>Detection of bacterial β-lactamase enzyme; positive test indicates resistance to β-lactam antibiotics</td>
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<td>Antibiotic level</td>
<td>Concentration of antibiotic in serum</td>
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D. The MIC is the lowest concentration of an antimicrobial agent that inhibits growth of the organism, as detected by lack of turbidity.

1. The antibiotic is added to broth in serial twofold dilutions (e.g., 0.5 mcg, 1.0 mcg, 2.0 mcg). A standardized inoculum of the test bacterium is added to each concentration of the antimicrobial agent. The MIC is the concentration of the first well that shows no growth or turbidity. Most routine MIC tests use commercially prepared broth microdilution trays with preselected antibiotics. MIC tube macrodilutions and microdilution trays are depicted in Web Color Images 7–87 and 7–88, respectively.

2. A sterility well monitors contamination. This well should have no growth. A growth well monitors organism growth and should have growth. If there is bacterial growth in the sterility well or the absence of growth in the growth well, the test is invalid and should not be read, but repeated.

3. Quality control testing with known bacterial strains (e.g., American Type Culture Collection (ATCC) strains) is performed weekly to ensure the validity of test results.

E. The Kirby-Bauer or disk diffusion (see Web Color Image 7–89) method measures the diameter of inhibition around an antibiotic-impregnated filter paper disk. A standardized bacterial suspension is plated to Mueller Hinton agar so as to achieve a confluent “lawn of growth.” Antimicrobial disks are dropped on the agar surface. As soon as the disk comes in contact with the agar surface, water is absorbed into the filter paper, and the antibiotic diffuses into the surrounding medium. The concentration of the antibiotic decreases with increased distance from the disk. With overnight incubation at 37 °C, a zone of inhibition surrounds the antibiotic disk in accordance with the bacterium’s susceptibility or resistance to the antimicrobial agent. If the test is properly performed, the edges of the zone of inhibition are clear and easy to measure. There are times when the zone is not obvious.
1. Swarming by *Proteus* may result in a thin film of growth beyond the outer margin. The zone of swarming should be ignored, and the outer margin should be measured.

2. Occasionally, colonies grow within the zone of inhibition. This represents either resistant mutants or a mixed culture. If the inoculum is a pure culture, the colonies represent a mutant, and the isolate is considered resistant.

3. The disks must be placed so that the zones of inhibition do not overlap. Overlapping zones may interfere with accurate interpretation of the results.

4. *Quality control* testing is performed weekly using stock cultures with known susceptibility results (e.g., ATCC strains).

F. The minimum bactericidal concentration (MBC) measures the lowest concentration of antibiotic that kills 99.9% of a bacterial isolate. It is used to demonstrate tolerance to an antibiotic. Tolerance is determined when the MBC endpoints are five or more twofold dilutions greater than the MIC.

G. Synergy describes the enhanced antibacterial activity achieved by using a combination of two drugs rather than either drug separately. This is helpful information in treating cases with combination drug therapy (e.g., *Enterococcus*). A checkerboard MIC plate is used. Antibiotics are tested separately and together at various concentrations with the test bacterium to determine what concentration of each drug results in synergy.

H. The serum bactericidal titer tests the bacterial isolate from the patient with the patient’s own serum (containing the antibiotic). The lowest dilution of patient serum that kills a standard inoculum of the bacterium is called the serum bactericidal level. The Schlichter test is the most commonly used test protocol; however, it is not routinely performed.

I. E-test is similar to the disk diffusion test, but produces MIC results. Agar plates are inoculated in the same manner as those for disk diffusion tests. A plastic strip containing a gradient of the antimicrobial agent is placed on the plate. Following incubation, plates are examined for an elliptical zone of inhibition to determine the MIC value (see Web Color Image 7–90).

J. β-lactamase tests indicate that bacterial isolates produce β-lactamase, but cannot be used to predict the organism’s susceptibility or resistance to a particular drug. In general, β-lactamase-positive bacteria are usually resistant to the β-lactam antibiotics. Negative β-lactamase tests do not mean that an organism is susceptible to the β-lactam antibiotics. Direct β-lactamase tests are not appropriate for the Enterobacteriaceae and *Pseudomonas aeruginosa*.

K. Antibiotic levels in serum (e.g., gentamicin, vancomycin) are measured using immunoassays or other chemical methods to ensure that therapeutic levels of the antimicrobial agent are achieved in the serum. In addition, such monitoring ensures that toxic levels of the antibiotic are not present in the serum.

L. Antimicrobial agents include antiseptics, antibiotics, sterilants, and disinfectants. All have the capacity to kill or suppress the growth of microorganisms. Antibiotics are used to treat infections. These agents may be natural, semi-synthetic, or synthetic in accordance with how the agents are produced. Antibiotics may be grouped in accordance with the target site or mechanism of action. Antibiotic target sites include the following:

1. Interruption of structural integrity: interference with cell wall synthesis or cell membrane composition (e.g., penicillins, cephalosporins, carbapenems, vancomycin, and polymyxins)

2. Inhibition of protein synthesis (e.g., aminoglycosides, tetracyclines, macrolides, chloramphenicol)

3. Interference with nucleic acid metabolism (e.g., rifampin, metronidazole, nalidixic acid, fluorinated quinolones)
4. Inhibition of essential metabolites (e.g., sulfonamides, trimethoprim, septra/bactrim).

M. Some nonfastidious bacteria may require special procedures to detect clinically significant antibiotic resistance.

1. *Staphylococcus aureus*
   
a. Methicillin-resistant (oxacillin-resistant) *S. aureus* (MRSA or ORSA) are resistant to the penicillinase-resistant penicillins, the drug class of choice for treating staphylococcal infections. The resistance is due to the presence of a penicillin-binding protein (PBP2a or PBP’2) that is encoded by the *meA* gene. Oxacillin screening with Mueller Hinton agar supplemented with 4% NaCl and 6 mcg/mL oxacillin is used to detect MRSA. MRSA grows on the medium, whereas methicillin-susceptible strains are inhibited.

b. Although uncommon, vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) have apparently acquired a plasmid containing the vanA vancomycin-resistant gene from vancomycin-resistant enterococci. The CLSI recommends use of broth microdilution (MIC) tests or vancomycin screening agar (BHI with 6 mcg/mL vancomycin) for detection of these bacteria.

c. If a staphylococcal isolate is erythromycin-resistant but clindamycin-susceptible, the D-zone test must be performed for detection of inducible clindamycin resistance. The test is a disk diffusion test in which erythromycin and clindamycin disks are placed adjacent to each other on an inoculated agar plate. Following overnight incubation, a flattened clindamycin zone between the two disks indicates inducible clindamycin resistance due to the *erm* gene. The isolate must be reported as clindamycin-resistant. No flattening indicated the isolate is erythromycin-resistant only (due to *msrA* gene), and susceptible to clindamycin.

2. *Enterococcus* species
   
a. Vancomycin-resistant enterococci (VRE) contain the vanA vancomycin-resistant gene that encodes for resistance. These are detected by use of the vancomycin agar screen agar plate (BHI with 6 mcg/mL vancomycin).

b. High-level aminoglycoside resistance in enterococci is detected by use of broth, agar, or disk diffusion methods, testing for gentamicin and streptomycin resistance.

3. Resistance to later-generation penicillins, cephalosporins and aztreonam is due to extended spectrum β-lactamases (ESBLs) in *Klebsiella* species and *E. coli*. ESBL production is detected by disk diffusion screening or MIC breakpoints with the used of “indicator drugs,” followed by confirmatory testing. ESBL-producing strains should be reported as clinically resistant to all cephalosporins, penicillins, and aztreonam, regardless of in vitro susceptibility test results.

XXIII. DISINFECTION AND STERILIZATION

A. Disinfection is the elimination of a defined scope of microorganisms, including some spores.

1. Disinfectants are chemical agents applied to inanimate objects.

2. Antiseptics are applied to the skin to eliminate or reduce the numbers of bacteria present. These agents do not kill spores.

3. Pasteurization & boiling achieve disinfection but not sterilization, as endospores are not destroyed.

B. Sterilization refers to the destruction of all life forms, including bacterial spores. There are chemical and physical means of sterilization.

1. Physical means of sterilization include moist heat (autoclaves—heat under steam pressure) dry heat, filtration, and radiation.

2. Chemical agents used for sterilization are known as chemosterilizers. These agents damage the bacterial cytoplasmic membranes, destroy cellular proteins, or damage bacterial RNA and DNA.
MOLECULAR TESTING

A. The increased use of molecular diagnostic techniques in the clinical laboratory is, in part, due to the increased sensitivity and specificity of these assays over routine culture techniques. The techniques allow for rapid detection of microorganisms and rapid answers for treatment options. Antibacterial resistance can be determined by the detection of resistance genes (e.g., the vanA gene in vancomycin-resistant enterococci). Three basic diagnostic techniques are as follows:

1. Nucleic acid hybridization techniques: detection of nucleic acid targets with labeled probes
2. Amplication techniques: exponential increase of the target nucleic acid or the signal that binds to the target nucleic (e.g., PCR).
3. Strain typing techniques: used in epidemiologic studies to determine strain similarities in outbreaks

B. The simultaneous detection of Chlamydia trachomatis and Neisseria gonorrhoeae from the same specimen is one example of molecular testing in use in the clinical microbiology laboratory. Refer to the chapter on molecular testing for a summary of diagnostic molecular procedures in the clinical laboratory.

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Go to thePoint.lww.com/Hubbard2e for the following:
- Web Color Images 7–1 through 7–90
- Study Questions and Answers