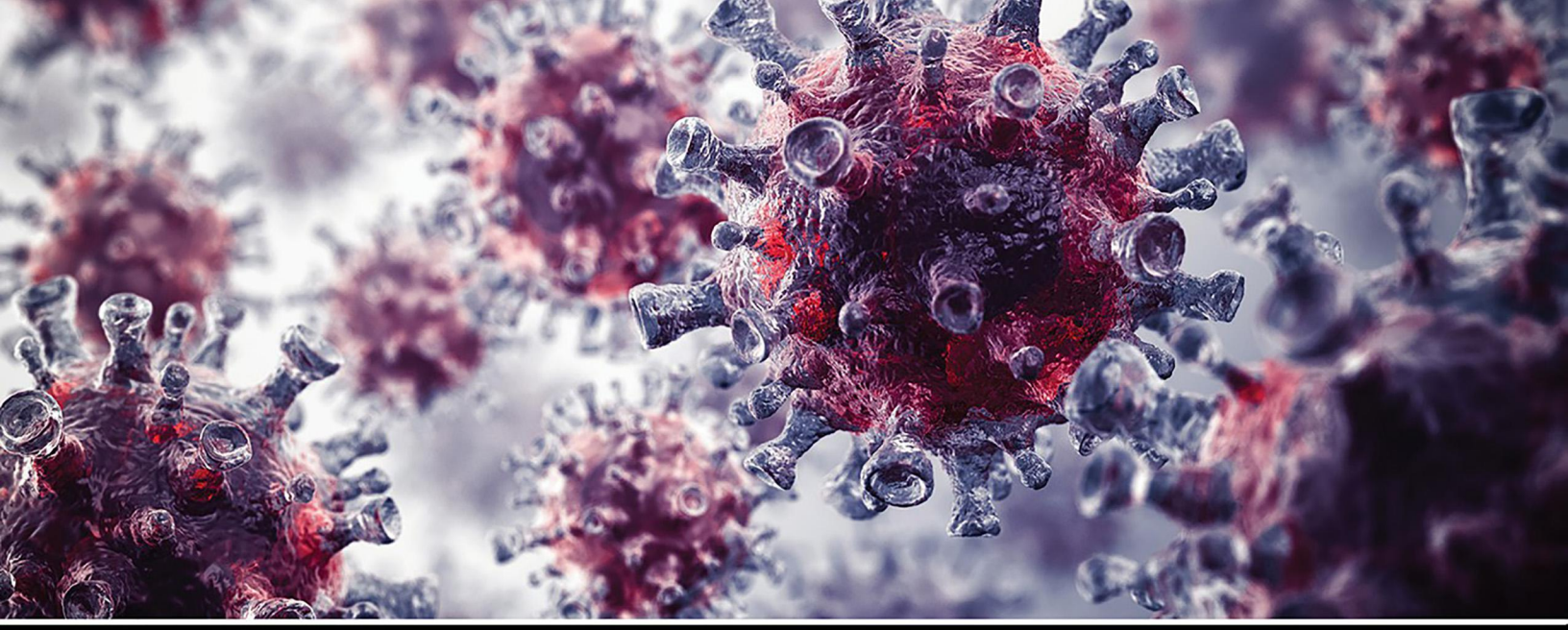

REVIEW OF Medical Microbiology and Immunology

A Guide to Clinical Infectious Diseases

Sixteenth Edition

WARREN LEVINSON
PETER CHIN-HONG
ELIZABETH A. JOYCE
JESSE NUSSBAUM
BRIAN SCHWARTZ

Mc Graw Hill LANGE®

a LANGE medical book

Review of Medical Microbiology & Immunology

A Guide to Clinical Infectious Diseases

Sixteenth Edition

Senior Author
Warren Levinson, MD, PhD

Authors
Peter Chin-Hong, MD
Elizabeth A. Joyce, PhD
Jesse Nussbaum, MD
Brian Schwartz, MD

New York Chicago San Francisco Athens London Madrid Mexico City
Milan New Delhi Singapore Sydney Toronto

ISBN: 978-1-26-011672-4
MHID: 1-26-011672-7

The material in this eBook also appears in the print version of this title: ISBN: 978-1-26-011671-7,
MHID: 1-26-011671-9.

eBook conversion by codeMantra
Version 1.0

Notice

Medicine is an ever-changing science. As new research and clinical experience broaden our knowledge, changes in treatment and drug therapy are required. The authors and the publisher of this work have checked with sources believed to be reliable in their efforts to provide information that is complete and generally in accord with the standards accepted at the time of publication. However, in view of the possibility of human error or changes in medical sciences, neither the authors nor the publisher nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they disclaim all responsibility for any errors or omissions or for the results obtained from use of the information contained in this work. Readers are encouraged to confirm the information contained herein with other sources. For example and in particular, readers are advised to check the product information sheet included in the package of each drug they plan to administer to be certain that the information contained in this work is accurate and that changes have not been made in the recommended dose or in the contraindications for administration. This recommendation is of particular importance in connection with new or infrequently used drugs.

Contents

Authors

Senior Author

Warren Levinson, MD, PhD
Professor of Microbiology
Department of Microbiology & Immunology
University of California, San Francisco
San Francisco, California

Authors

Peter Chin-Hong, MD
Professor of Medicine
Department of Medicine
Division of Infectious Diseases
University of California, San Francisco
San Francisco, California

Elizabeth A. Joyce, PhD
Associate Professor of Microbiology
Department of Microbiology & Immunology
University of California, San Francisco
San Francisco, California

Jesse Nussbaum, MD
Assistant Adjunct Professor of Medicine
Department of Medicine
Division of Infectious Diseases
University of California, San Francisco
San Francisco, California

Brian Schwartz, MD
Professor of Medicine
Department of Medicine
Division of Infectious Diseases
University of California, San Francisco
San Francisco, California

This page intentionally left blank

Preface

This book is a concise review of the medically important aspects of microbiology and immunology. It covers both the basic and clinical aspects of bacteriology, virology, mycology, parasitology, and immunology. It also discusses important infectious diseases, using an organ system approach.

Its two major aims are (1) to assist those who are preparing for the USMLE (National Boards) and (2) to provide students who are currently taking medical microbiology courses with a brief and up-to-date source of information. The goal is to provide the reader with an accurate source of clinically relevant information at a level appropriate for those beginning their medical education.

This new edition presents current, medically important information in the rapidly changing fields of microbiology and immunology. It contains many color micrographs of stained microorganisms as well as images of important laboratory tests. It also includes many images of clinical lesions and highlights current information on antimicrobial drugs and vaccines.

These aims are achieved by using several different formats, which should make the book useful to students with varying study objectives and learning styles:

1. A narrative text for complete information.
2. A separate section containing summaries of important microorganisms for rapid review of the high-yield essentials.
3. Sample questions in the USMLE (National Board) style, with answers provided after each group of questions.
4. A USMLE (National Board) practice examination consisting of 80 microbiology and immunology questions. These questions are written in a clinical case format and simulate the computer-based examination. Answers are provided at the end of each block of 40 questions.
5. Self-assessment questions at the end of the chapters so you can evaluate whether the important information has been mastered. Answers are provided.
6. Clinical case vignettes to provide both clinical information and practice for the USMLE.
7. A section titled "Pearls for the USMLE" describing important epidemiologic information helpful in answering questions on the USMLE.
8. Many images of clinically important lesions seen in patients with infectious diseases described in this book are available on the McGraw-Hill Online Learning Center's Web site (www.langetextbooks.com).

The following features are included to promote a successful learning experience for students using this book:

1. The information is presented succinctly, with stress on making it clear, interesting, and up to date.
2. There is strong emphasis in the text on the clinical application of microbiology and immunology to infectious diseases.
3. In the clinical bacteriology and virology sections, the organisms are separated into major and minor pathogens. This allows the student to focus on the most important clinically relevant microorganisms.
4. Key information is summarized in useful review tables. Important concepts are illustrated by figures using color.
5. Important facts called "Pearls" are listed at the end of each basic science chapter.
6. Self-assessment questions with answers are included at the end of the chapters.
7. The 654 USMLE (National Board) practice questions cover the important aspects of each of the subdisciplines on the USMLE: Bacteriology, Virology, Mycology, Parasitology, and Immunology. A separate section containing *extended* matching questions is included. In view of the emphasis placed on clinical relevance in the USMLE, another section provides questions set in a clinical case context.
8. Brief summaries of medically important microorganisms are presented together in a separate section to facilitate rapid access to the information and to encourage comparison of one organism with another.
9. Fifty clinical cases are presented as unknowns for the reader to analyze in a brief, problem-solving format. These cases illustrate the importance of basic science information in clinical diagnosis.
10. Color images depicting clinically important findings, such as infectious disease lesions, Gram stains of bacteria, electron micrographs of viruses, and microscopic images of fungi, protozoa, and worms, are included in the text.
11. There are 11 chapters on infectious diseases from an organ system perspective. They are written concisely and are appropriate for a medical student's introduction to this subject. These chapters include Bone & Joint Infections, Cardiac Infections, Central Nervous System Infections, Gastrointestinal Tract Infections, Pelvic Infections, Upper Respiratory Tract Infections, Lower Respiratory Tract

Infections, Infections of the Skin & Skin Structures, Urinary Tract Infections, Sepsis & Septic Shock, and Eye Infections.

After teaching both medical microbiology and clinical infectious disease for many years, I believe that students appreciate a book that presents the essential information in a readable, interesting, and varied format. I hope you find that this book meets those criteria.

Warren Levinson, MD, PhD
San Francisco, California
January 2020

Acknowledgments

In this 16th edition, the senior author, Warren Levinson, would like to express great appreciation for the ongoing valuable, informative writings of the four coauthors: Elizabeth A. Joyce, Jesse Nussbaum, Brian S. Schwartz, and Peter Chin-Hong.

Elizabeth A. Joyce, PhD, is an Associate Professor of Microbiology and Immunology in the School of Medicine, University of California, San Francisco. Elizabeth teaches in and directs courses in the microbiology curriculum in the Schools of Medicine, Dentistry, and Pharmacy.

Jesse Nussbaum, MD, is an Assistant Adjunct Professor of Medicine in the Division of Infectious Diseases in the School of Medicine, University of California, San Francisco (UCSF). Jesse is an infectious disease specialist who cares for patients at UCSF HIV/AIDS clinics. In his research, he studies how the innate immune system interacts with T lymphocytes.

Brian S. Schwartz, MD, is an Associate Professor of Clinical Medicine in the School of Medicine, University of California, San Francisco, specializing in infectious diseases. His clinical and research interests include the diagnosis, treatment, and prevention of infections in immunocompromised patients. He and Dr. Peter Chin-Hong share the leadership of the Microbiology course for medical students.

Peter Chin-Hong, MD, is a Professor of Clinical Medicine in the School of Medicine, University of California, San Francisco, specializing in infectious diseases. He directs the immunocompromised host infectious diseases program at UCSF. His research focuses on human papillomavirus, Chagas disease, and other donor-derived infections in transplant recipients. He and Dr. Brian S. Schwartz share the leadership of the Microbiology course for medical students.

The senior author thanks his cousin, Ralph Levinson, MD, for his expert review of Chapter 80 on Eye Infections. Ralph Levinson is a Health Sciences Professor of Ophthalmology in the Jules Stein Eye Institute at the University of California, Los Angeles.

The senior author is indebted to the editor of the first five editions, Yvonne Strong; to the editor of the sixth edition, Cara Lyn Coffey; to the editor of the seventh and ninth editions, Jennifer Bernstein; to the editor of the eighth edition, Linda Conheady; to the editor of the tenth and eleventh editions, Sunita Dogra; to the editor of the twelfth edition, Rebecca Kerins; to the editor of the thirteenth and fifteenth editions, Caroline Define; to the editor of the fourteenth edition, Nupur Mehra; and to the editor of the sixteenth edition, Dr Anchal Kaushik; all of whom ensured that the highest standards of grammar, spelling, and style were met.

The invaluable assistance of the senior author's wife, Barbara, in making this book a reality is also gratefully acknowledged.

The senior author dedicates this book to his father and mother, who instilled a love of scholarship, the joy of teaching, and the value of being organized.

This page intentionally left blank

How to Use This Book

1. **CHAPTER CONTENTS:** The main headings in each chapter are listed so the reader can determine, at a glance, the topics discussed in the chapter.
2. **TEXT:** A concise, complete description of medically important information for the professional student. Includes basic and clinical bacteriology (pages 1-218), basic and clinical virology (pages 219-394), mycology (fungi) (pages 395-422), parasitology (pages 423-488), immunology (pages 489-598), and ectoparasites (pages 599-606).

   The text also includes 11 chapters on infectious diseases. These chapters include Bone & Joint Infections (pages 607-611), Cardiac Infections (pages 612-617), Central Nervous System Infections (pages 618-626), Gastrointestinal Tract Infections (pages 627-634), Pelvic Infections (pages 635-642), Upper Respiratory Tract Infections (pages 643-648), Lower Respiratory Tract Infections (pages 649-654), Infections of the Skin & Skin Structures (pages 655-661), Urinary Tract Infections (pages 662-665), Sepsis & Septic Shock (pages 666-669), and Eye Infections (pages 670-676).
3. **SUMMARIES OF ORGANISMS:** A quick review for examinations describing the important characteristics of the organisms (pages 677-716).
4. **SELF-ASSESSMENT QUESTIONS:** USMLE-style questions with answers are included at the end of the chapters.
5. **PEARLS FOR THE USMLE:** Eleven tables containing important clinical and epidemiologic information that will be useful for answering questions on the USMLE (pages 727-734).
6. **USMLE-TYPE QUESTIONS:** 654 practice questions that can be used to review for the USMLE and class examinations (pages 735-776).
7. **USMLE PRACTICE EXAM:** Two 40-question practice examinations in USMLE format (pages 777-786).
8. **PEARLS:** Summary points at the end of each basic science chapter.
9. **CLINICAL CASES:** 50 cases describing important infectious diseases with emphasis on diagnostic information (pages 717-726).
10. **CLINICAL IMAGES:** More than 100 images of clinically important lesions illustrate the text. Additional clinical lesions can be seen on the McGraw-Hill Online Learning Center's Web site (www.langetextbooks.com/levinson/gallery/).

This page intentionally left blank

PART I BASIC BACTERIOLOGY

CHAPTER 1

Bacteria Compared With Other Microorganisms

CHAPTER CONTENTS

MICROBES THAT CAUSE INFECTIOUS DISEASES

The agents of human infectious diseases belong to five major groups of organisms: bacteria, fungi, protozoa, helminths, and viruses. Bacteria belong to the Bacteria domain, whereas fungi (yeasts and molds), protozoa, and helminths (worms) are classified in the Eukarya domain (Table 1–1). Protists and fungi are distinguished from animals and plants by being either unicellular or relatively simple multicellular organisms. In contrast, helminths are complex multicellular organisms. Viruses are quite distinct from other organisms. They are noncellular; that is, they do not have a nucleus and cytoplasm, cannot make their own energy, and are unable to synthesize proteins. They are completely reliant upon host cells for replication and are thus considered obligate intracellular pathogens.

IMPORTANT FEATURES OF MICROBES

Many of the essential characteristics of these organisms are described in Table 1–2. One salient feature is that bacteria, fungi, protozoa, and helminths are cellular, whereas viruses are not. This distinction is based primarily on three criteria:

(1) **Structure.** Cells have a nucleus or nucleoid (see below), which contains DNA; this is surrounded by cytoplasm, where proteins are synthesized and energy is generated. Viruses have an inner core of genetic material (either DNA or RNA) but no cytoplasm, and so they depend on host cells to provide the machinery for protein synthesis and energy generation.

(2) **Method of replication.** Cells replicate either by binary fission or by mitosis, during which one parent cell divides to make two progeny cells while retaining its cellular structure. Prokaryotic cells (e.g., bacteria) replicate by binary fission, whereas eukaryotic cells replicate by mitosis. In contrast, viruses disassemble, produce many copies of their nucleic acid and protein, and then reassemble into multiple progeny viruses. Furthermore, viruses must replicate within host cells because, as mentioned previously, they lack protein-synthesizing and energy-generating systems. With the exception of rickettsiae and chlamydiae, which also require living host cells for growth, bacteria can replicate extracellularly.

(3) **Nature of the nucleic acid.** Cells contain both DNA and RNA, whereas viruses contain either DNA or RNA, but not both.

TABLE 1–1 Biologic Relationships of Pathogenic Microorganisms

Domain (Superkingdom)	Kingdom	Pathogenic Microorganisms	Type of Cells
Eukarya	Animal	Helminths (Worms)	Eukaryotic
Eukarya	Protist	Protozoa	Eukaryotic
Eukarya	Fungi	Fungi (Yeasts and Molds)	Eukaryotic
Bacteria	Prokaryote	Bacteria Viruses	Prokaryotic Noncellular

EUKARYOTES & PROKARYOTES

Cells have evolved into two fundamentally different types, **eukaryotic** and **prokaryotic**, which can be distinguished based on their structure and the complexity of their organization. Fungi, protozoa, and helminths are eukaryotic, whereas bacteria are prokaryotic.

(1) The eukaryotic cell has a true **nucleus** with multiple chromosomes surrounded by a nuclear membrane and uses a mitotic apparatus to ensure equal allocation of the chromosomes to progeny cells.

(2) The **nucleoid** of a prokaryotic cell typically consists of a single circular molecule of loosely organized DNA and lacks a nuclear membrane and mitotic apparatus (Table 1–3).

In addition to the different types of nuclei, the two classes of cells are distinguished by several other characteristics:

(1) Eukaryotic cells contain **organelles**, such as mitochondria and lysosomes, and larger (80S) ribosomes, whereas prokaryotes contain no organelles and smaller (70S) ribosomes.

TABLE 1–3 Characteristics of Prokaryotic and Eukaryotic Cells

Characteristic	Prokaryotic Bacterial Cells	Eukaryotic Human Cells
DNA within a nuclear membrane	No	Yes
Mitotic division	No	Yes
Chromosome number	Usually 1	More than 1
Membrane-bound organelles, such as mitochondria and lysosomes	No	Yes
Size of ribosome	70S	80S
Cell wall containing peptidoglycan	Yes	No

(2) Most prokaryotes have a rigid external cell wall that contains **peptidoglycan**, a polymer of amino acids and sugars, as its unique structural component. Eukaryotes, on the other hand, do not contain peptidoglycan. Either they are bound by a flexible cell membrane, or, in the case of fungi, they have a rigid cell wall with chitin, a homopolymer of *N*-acetylglucosamine, typically forming the framework.

(3) The eukaryotic cell membrane contains **sterols**, whereas no prokaryote, except the wall-less *Mycoplasma*, has sterols in its membranes.

Motility is another characteristic by which these organisms can be distinguished. Most protozoa and some bacteria are motile, whereas fungi and viruses are nonmotile. The protozoa are a heterogeneous group that possesses three different organs of locomotion: flagella, cilia, and pseudopods. The motile bacteria move only by means of flagella.

TABLE 1–2 Comparison of Medically Important Organisms

Characteristic	Viruses	Bacteria	Fungi	Parasites
Cell wall	No	Yes	Yes	+/–[1]
Approximate diameter (μm)[2]	0.02–0.2	1–5	3–10 (yeasts)	15–25 (trophozoites)
Nucleic acid	Either DNA or RNA	Both DNA and RNA	Both DNA and RNA	Both DNA and RNA
Type of nucleus	None	No distinct nuclear compartment	Membrane-bound nucleus	Membrane-bound nucleus
Ribosomes	Absent	70S	80S	80S
Mitochondria	Absent	Absent	Present	Present
Nature of outer surface	Protein capsid and lipoprotein envelope	Rigid wall containing peptidoglycan	Rigid wall containing chitin	Flexible membrane
Motility	None	Some	None	Most
Method of replication	Not binary fission	Binary fission	Budding or mitosis[3]	Mitosis[4]

[1]The cyst forms of parasites have cell walls.

[2]For comparison, a human red blood cell has a diameter of 7 μm.

[3]Yeasts divide by budding, whereas molds divide by mitosis.

[4]Helminth cells divide by mitosis, but the organism reproduces itself by complex, sexual life cycles.

TERMINOLOGY

Bacteria, fungi, protozoa, and helminths are named according to the binomial Linnaean system that uses genus and species. For example, regarding the name of the well-known bacteria *Escherichia coli*, *Escherichia* is the genus and *coli* is the species name. Similarly, the name of the yeast *Candida albicans* consists of *Candida* as the genus and *albicans* as the species. Viruses typically have a single name, such as poliovirus, measles virus, or rabies virus. Some viruses have names with two words, such as herpes simplex virus, but those do not represent genus and species.

PEARLS

- The agents of human infectious diseases are **bacteria, fungi (yeasts and molds), protozoa, helminths (worms),** and **viruses.**
- Bacterial cells have a **prokaryotic** nucleus, whereas human, fungal, protozoan, and helminth cells have a **eukaryotic** nucleus. Viruses are not cells and do not have a nucleus.
- All cells contain both DNA and RNA, whereas viruses contain either DNA or RNA, but not both.
- Bacterial and fungal cells are surrounded by a rigid cell wall, whereas human, protozoan, and helminth cells have a flexible cell membrane.
- Most bacteria have cell walls that contain **peptidoglycan**, whereas the fungal cell wall contains chitin.

SELF-ASSESSMENT QUESTIONS

1. You're watching a television program that is discussing viruses called bacteriophages that can kill bacteria. Your roommate says, "Wow, maybe viruses can be used to kill the bacteria that infect people! You're taking the Microbiology course now; what's the difference between viruses and bacteria?" Which one of the following would be the most accurate statement to make?

(A) Viruses do not have mitochondria, whereas bacteria do.
(B) Viruses do not have a nucleolus, whereas bacteria do.
(C) Viruses do not have ribosomes, whereas bacteria do.
(D) Viruses replicate by binary fission, whereas bacteria replicate by mitosis.
(E) Viruses are prokaryotic, whereas bacteria are eukaryotic.

2. Bacteria, fungi (yeasts and molds), viruses, and protozoa are important causes of human disease. Which one of the following microbes contains either DNA or RNA but not both?

(A) Bacteria
(B) Molds
(C) Protozoa
(D) Viruses
(E) Yeasts

3. Which one of the following contains DNA that is not surrounded by a nuclear membrane?

(A) Bacteria
(B) Molds
(C) Protozoa
(D) Yeasts

ANSWERS

(1) **(C)**
(2) **(D)**
(3) **(A)**

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 735. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 777.

CHAPTER 2

Structure of Bacterial Cells

CHAPTER CONTENTS

SHAPE & SIZE OF BACTERIA

Bacteria are classified by shape into three basic groups: **cocci, bacilli,** and **spirochetes** (Figure 2–1). The cocci are round, the bacilli are rods, and the spirochetes are spiral-shaped. Some bacteria are variable in shape and are said to be **pleomorphic** (heterogeneous shape). The shape of a bacterium is determined by its rigid cell wall. The microscopic appearance of a bacterium is one of the most important criteria used in its identification.

In addition to their characteristic shapes, the arrangement of bacteria is important. For example, certain cocci occur in pairs (**diplococci**), some in chains (**streptococci**), and others in grapelike clusters (**staphylococci**). These arrangements are determined by the orientation and degree of attachment of the bacteria at the time of cell division. The arrangement of rods and spirochetes is medically less important and is not described in this introductory chapter.

Bacteria range in size from about 0.2 to 5 μm (Figure 2–2). The smallest bacteria (*Mycoplasma*) are about the same size as the largest viruses (poxviruses) and are the smallest organisms capable of existing outside a host. The longest bacteria are the size of some yeasts and human red blood cells (7 μm).

STRUCTURE OF BACTERIA

The structure of a typical bacterium is illustrated in Figure 2–3, and the important features of each component are presented in Table 2–1.

Cell Wall

The cell wall is the outermost component common to all bacteria (except *Mycoplasma* species, which are bounded by a cell membrane, not a cell wall). Some bacteria have surface features external to the cell wall, such as capsule, flagella, and pili, which are less common components and are discussed next.

The cell wall is located external to the cytoplasmic membrane and is composed of **peptidoglycan** (see page 6). The peptidoglycan provides structural support and maintains the characteristic shape of the cell.

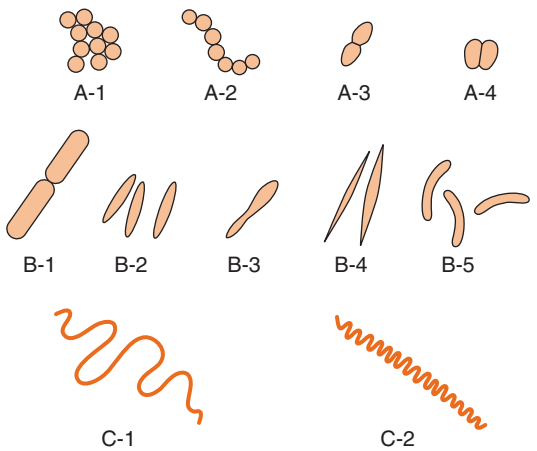

FIGURE 2–1 Bacterial morphology. **A:** Cocci in clusters (e.g., *Staphylococcus;* A-1); in chains (e.g., *Streptococcus;* A-2); in pairs with pointed ends (e.g., *Streptococcus pneumoniae;* A-3); in pairs with kidney bean shape (e.g., *Neisseria;* A-4). **B:** Rods (bacilli): with square ends (e.g., *Bacillus;* B-1); with rounded ends (e.g., *Salmonella;* B-2); club-shaped (e.g., *Corynebacterium;* B-3); fusiform (e.g., *Fusobacterium;* B-4); comma-shaped (e.g., *Vibrio;* B-5). **C:** Spirochetes: relaxed coil (e.g., *Borrelia;* C-1); tightly coiled (e.g., *Treponema;* C-2). (Reproduced with permission from Joklik WK, Willett HP, Amos DB: *Zinsser Microbiology,* 20th ed. New York, NY: McGraw-Hill Education; 1992.)

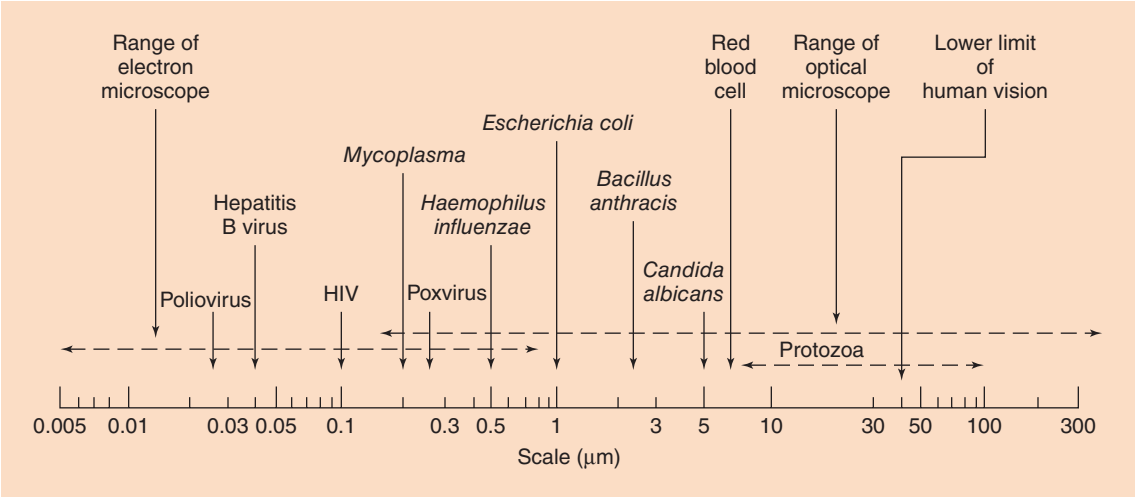

FIGURE 2–2 Sizes of representative bacteria, viruses, yeasts, protozoa, and human red cells. The bacteria range in size from *Mycoplasma,* the smallest, to *Bacillus anthracis,* one of the largest. The viruses range from poliovirus, one of the smallest, to poxviruses. Yeasts, such as *Candida albicans,* are generally larger than bacteria. Protozoa have many different forms and a broad size range. HIV, human immunodeficiency virus.
(Reproduced with permission from Joklik WK, Willett HP, Amos DB: *Zinsser Microbiology,* 20th ed. New York, NY: McGraw-Hill Education; 1992.)

Cell Walls of Gram-Positive and Gram-Negative Bacteria

The structure, chemical composition, and thickness of the cell wall differ in gram-positive and gram-negative bacteria (Table 2–2, Figure 2–4A, and "Gram Stain" box).

(1) The peptidoglycan layer is much thicker in gram-positive than in gram-negative bacteria. Many gram-positive bacteria also have fibers of teichoic acid that protrude outside the peptidoglycan, whereas gram-negative bacteria do not have teichoic acids.

(2) In contrast, the gram-negative bacteria have a complex outer layer consisting of lipopolysaccharide (LPS), lipoprotein, and phospholipid. Together with the cell wall, this gram-negative architecture is referred to as the "envelope." Lying between the outer-membrane layer and the cytoplasmic membrane in gram-negative bacteria is the **periplasmic space**, which is the site, in some species, of enzymes called β-lactamases that degrade penicillins and other β-lactam drugs.

The cell wall in gram-positive organisms or cell envelope in gram-negative organisms has several other important properties:

(1) In gram-negative bacteria, the envelope contains **endotoxin**, an LPS (see pages 8 and 43).

(2) Both gram-positive and gram-negative bacteria contain polysaccharides and proteins on their surface that are antigens useful in laboratory identification.

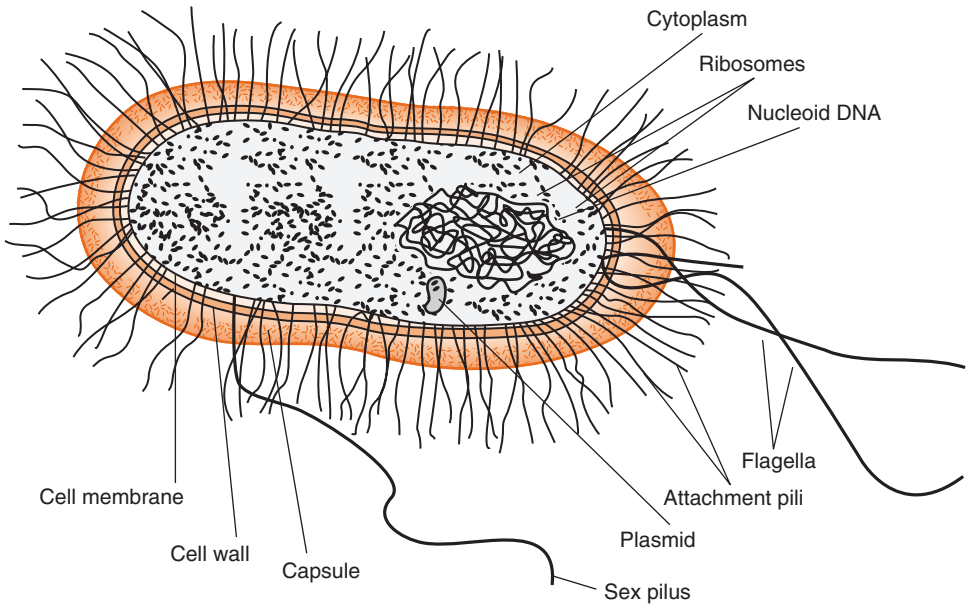

FIGURE 2–3 Bacterial structure. (Reproduced with permission from Ryan K: *Sherris Medical Microbiology*, 4th ed. New York, NY: McGraw-Hill Education; 2004.)

TABLE 2–1 Bacterial Structures

Structure	Chemical Composition	Function
Essential components		
Cell wall		
Peptidoglycan	Glycan (sugar) backbone with peptide side chains that are cross-linked	Gives rigid support, protects against osmotic pressure, is the site of action of penicillins and cephalosporins, and is degraded by lysozyme
Outer membrane of gram-negative bacteria	1. Lipid A	Toxic component of endotoxin
	2. Polysaccharide	Major surface antigen used frequently in laboratory diagnosis
Surface fibers of gram-positive bacteria	Teichoic acid	Major surface antigen but rarely used in laboratory diagnosis
Plasma membrane	Lipoprotein bilayer without sterols	Site of oxidative and transport enzymes
Ribosome	RNA and protein in 50S and 30S subunits	Protein synthesis; site of action of aminoglycosides, erythromycin, tetracyclines, and chloramphenicol
Nucleoid	DNA	Genetic material
Mesosome	Invagination of plasma membrane	Participates in cell division and secretion
Periplasm	Space between plasma membrane and outer membrane	Contains many hydrolytic enzymes, including β-lactamases
Nonessential components		
Capsule	Polysaccharide[1]	Protects against phagocytosis
Pilus or fimbria	Glycoprotein	Two types: (1) mediates attachment to cell surfaces; (2) sex pilus mediates attachment of two bacteria during conjugation
Flagellum	Protein	Motility
Spore	Keratin-like coat, dipicolinic acid	Provides resistance to dehydration, heat, and chemicals
Plasmid	DNA	Contains a variety of genes for antibiotic resistance and toxins
Granule	Glycogen, lipids, polyphosphates	Site of nutrients in cytoplasm
Glycocalyx	Polysaccharide	Mediates adherence to surfaces

[1]Except in *Bacillus anthracis,* in which it is a polypeptide of D-glutamic acid.

(3) **Porin** proteins play a role in facilitating the passage of small, hydrophilic molecules into the cell. Several types of porin proteins can be found in the outer membrane of gram-negative bacteria, allowing the entry of essential substances such as sugars, amino acids, vitamins, and metals as well as many antimicrobial drugs such as penicillins. Porins have also been identified in gram-positive bacteria, where they are anchored to the cell wall.

Cell Walls of Acid-Fast Bacteria

Mycobacteria (e.g., *Mycobacterium tuberculosis*) have an unusual cell wall, resulting in their inability to be Gram-stained (Figure 2–4B). These bacteria are said to be **acid-fast** because they resist decolorization with acid–alcohol after being stained with carbolfuchsin. This property is related to the high concentration of lipids, called **mycolic acids**, in the cell wall of mycobacteria.

Note that *Nocardia asteroides*, a bacterium that can cause lung and brain infections in immunocompromised individuals, is **weakly acid-fast**. The meaning of the term "weakly" is that if the acid-fast staining process uses a weaker solution of hydrochloric acid to decolorize than that used in the stain for mycobacteria, then *N. asteroides* will *not* decolorize. However, if the regular-strength hydrochloric acid is used, *N. asteroides* will decolorize.

In view of their importance, three components of the cell wall (i.e., peptidoglycan, LPS, and teichoic acid) are discussed in detail here.

TABLE 2–2 Comparison of Cell Walls of Gram-Positive and Gram-Negative Bacteria

Component	Gram-Positive Cells	Gram-Negative Cells
Peptidoglycan	Thicker; multilayer	Thinner; few layers
Teichoic acids	Yes	No
Lipopolysaccharide (endotoxin)	No	Yes

Peptidoglycan

Peptidoglycan is a complex, interwoven network that surrounds the entire cell and is composed of a single covalently linked macromolecule. It is found *only* in bacterial cell walls. It provides rigid support for the cell, is important in maintaining the characteristic shape of the cell, and allows the cell to withstand low osmotic pressure. A representative segment of the peptidoglycan layer is shown in Figure 2–5. The term **peptidoglycan** is derived from the peptides and the sugars (glycan) that make up the molecule. Synonyms for peptidoglycan are **murein** and **mucopeptide**.

Figure 2–5 illustrates the carbohydrate backbone, which is composed of alternating *N*-acetylmuramic acid and *N*-acetylglucosamine molecules. Attached to each of the muramic acid molecules is a tetrapeptide consisting of both D- and L-amino acids, the precise composition of which differs from one bacterium to another. Two of these amino acids are worthy of special mention: diaminopimelic acid, which is unique to bacterial cell

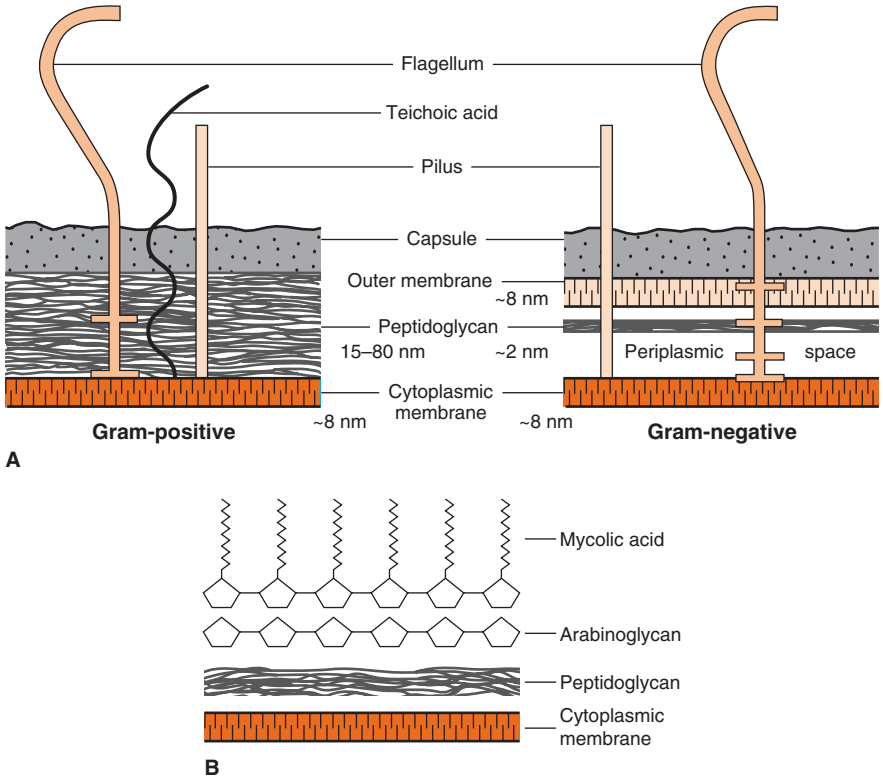

FIGURE 2–4 Bacterial cell wall structure. **A:** Cell walls of gram-positive and gram-negative bacteria. Note that the peptidoglycan in gram-positive bacteria is much thicker than in gram-negative bacteria. Note also that only gram-negative bacteria have an outer membrane containing endotoxin (lipopolysaccharide [LPS]) and thus have a periplasmic space where β-lactamases are found. Several important gram-positive bacteria, such as staphylococci and streptococci, have teichoic acids. (Reproduced with permission from Ingraham JL, Maaløe O, Neidhardt FC. *Growth of the Bacterial Cell.* Sunderland, MA: Sinauer Associates; 1983.) **B:** Cell wall of *Mycobacterium tuberculosis:* Note the layers of mycolic acid and arabinoglycan that are present in members of the genus *Mycobacterium* but not in most other genera of bacteria.

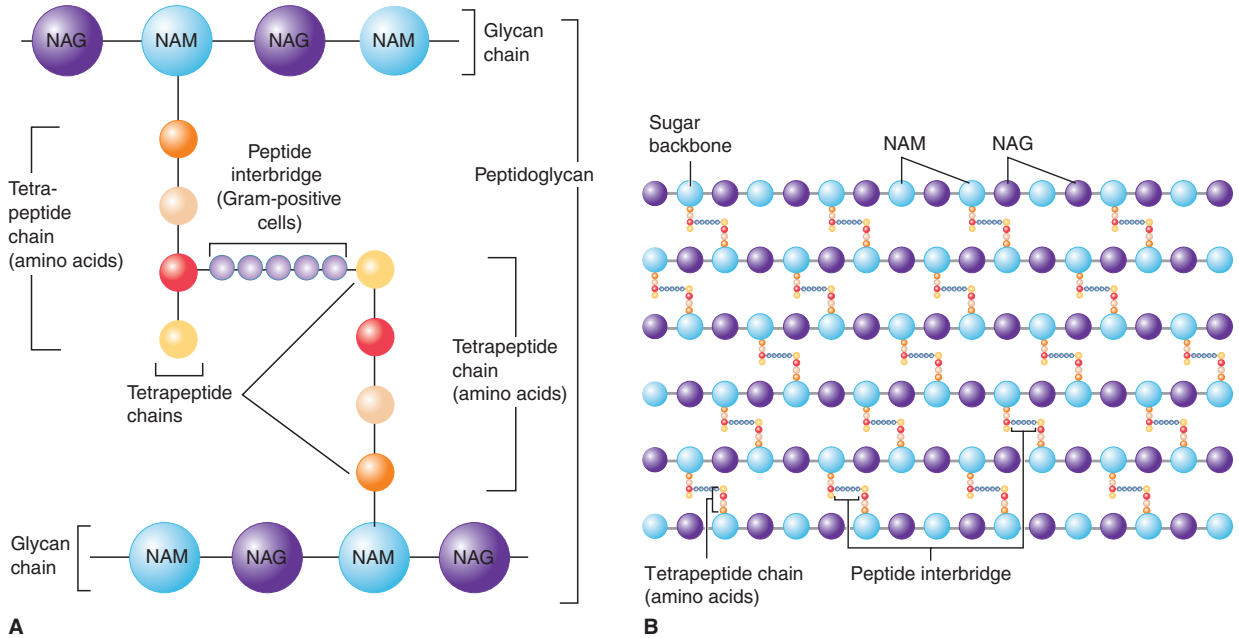

FIGURE 2–5 Peptidoglycan structure. **A:** Peptidoglycan is composed of a glycan chain (NAM and NAG), a tetrapeptide chain, and a cross-link (peptide interbridge). **B:** In the cell wall, the peptidoglycan forms a multilayered, three-dimensional structure. NAG, *N*-acetylglucosamine; NAM, *N*-acetylmuramic acid. (Reproduced with permission from Nester EW, Anderson D, Roberts CE, et al. *Microbiology: A Human Perspective,* 6th ed. New York, NY: McGraw-Hill Education; 2009.)

GRAM STAIN

This staining procedure, developed in 1884 by the Danish physician Christian Gram, is the most important staining procedure in microbiology. It separates most bacteria into two groups: the gram-positive bacteria, which stain purple, and the gram-negative bacteria, which stain red. The Gram stain involves the following four-step procedure:

(1) The crystal violet dye stains all cells purple.

(2) The iodine solution (a mordant) is added to form a crystal violet–iodine complex; all cells continue to appear purple.

(3) The organic solvent, such as acetone or ethanol, extracts the purple dye complex from the lipid-rich, thin-walled, gram-negative bacteria to a greater degree than from the lipid-poor, thick-walled, gram-positive bacteria. The gram-negative organisms appear colorless; the gram-positive bacteria remain purple.

(4) The red dye safranin stains the decolorized gram-negative cells red/pink; the gram-positive bacteria remain purple.

The Gram stain is useful in two ways:

(1) In the identification of many bacteria.

(2) In influencing the choice of antibiotic because, in general, gram-positive bacteria are more susceptible to penicillin G than are gram-negative bacteria.

However, not all bacteria can be seen in the Gram stain. Table 2–3 lists the medically important bacteria that cannot be seen and describes the reason why. The alternative microscopic approach to the Gram stain is also described.

Note that it takes approximately 100,000 bacteria/mL to see 1 bacterium per microscopic field using the oil immersion (100×) lens. So the sensitivity of the Gram stain procedure is low. This explains why a patient's blood is rarely stained immediately but rather is incubated in blood cultures overnight to allow the bacteria to multiply. One important exception to this is meningococcemia in which very high concentrations of *Neisseria meningitidis* can occur in the blood.

walls, and D-alanine, which is involved in the cross-links between the tetrapeptides and in the action of penicillin. Note that this tetrapeptide contains the rare D-isomers of amino acids; most proteins contain the L-isomer. The other important component in this network is the peptide cross-link between the two tetrapeptides. The cross-links vary among species; in *Staphylococcus aureus,* for example, five glycines link the terminal D-alanine to the penultimate L-lysine.

Because peptidoglycan is present in bacteria but not in human cells, it is a good target for antibacterial drugs. Several of these drugs, such as penicillins, cephalosporins, and vancomycin, inhibit the synthesis of peptidoglycan by inhibiting the transpeptidase that makes the cross-links between the two adjacent tetrapeptides (see Chapter 10).

Lysozyme, an enzyme present in human tears, mucus, and saliva, can cleave the peptidoglycan backbone by breaking its glycosyl bonds, thereby contributing to the natural resistance of the host to microbial infection. Lysozyme-treated bacteria may swell and rupture as a result of the entry of water into the cells, which have a high internal osmotic pressure. However, if the lysozyme-treated cells are in a solution with the same osmotic pressure as that of the bacterial interior, they will survive as spherical forms, called **protoplasts**, surrounded only by a cytoplasmic membrane.

Lipopolysaccharide

The LPS of the outer membrane of the cell wall of gram-negative bacteria is **endotoxin**. It is responsible for many of the features of disease, such as fever and shock (especially hypotension), caused

TABLE 2–3 Medically Important Bacteria That Cannot Be Seen in the Gram Stain

Name	Reason	Alternative Microscopic Approach
Mycobacteria, including *M. tuberculosis*	Too much lipid in cell wall so dye cannot penetrate	Acid-fast stain
Treponema pallidum	Too thin to see	Dark-field microscopy or fluorescent antibody
Mycoplasma pneumoniae	No cell wall; very small	None
Legionella pneumophila	Poor uptake of red counterstain	Prolong time of counterstain
Chlamydiae, including *C. trachomatis*	Intracellular; very small	Inclusion bodies in cytoplasm
Rickettsiae	Intracellular; very small	Giemsa or other tissue stains

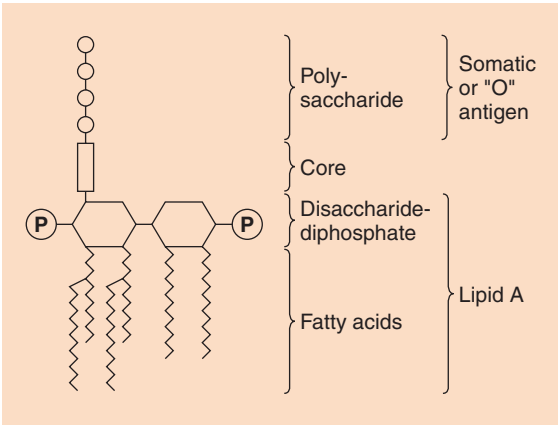

FIGURE 2–6 Endotoxin (lipopolysaccharide [LPS]) structure. The O-antigen polysaccharide is exposed on the exterior of the cell, whereas the lipid A faces the interior. (Reproduced with permission from Brooks GF, Jawetz E: *Medical Microbiology,* 19th ed. New York, NY: McGraw-Hill Education; 1991.)

by these organisms (see page 43). It is called endotoxin because it is an integral part of the cell envelope, in contrast to exotoxins, which are actively secreted from the bacteria. The constellation of symptoms caused by the endotoxin of one gram-negative bacterium is similar to another, but the severity of the symptoms can differ greatly. In contrast, the symptoms caused by exotoxins of different bacteria are usually quite different.

The LPS is composed of three distinct units (Figure 2–6):

(1) A phospholipid called lipid A, which is responsible for the toxic effects.

(2) A core polysaccharide of five sugars linked through ketodeoxyoctulonate (KDO) to lipid A.

(3) An outer polysaccharide consisting of up to 25 repeating units of three to five sugars. This outer polymer is the important somatic, or O, antigen of several gram-negative bacteria that is used to identify certain organisms in the clinical laboratory. Some bacteria, notably members of the genus *Neisseria*, have an outer lipooligosaccharide (LOS) containing very few repeating units of sugars.

Teichoic Acid

Teichoic acids are **fibers located in the outer layer of the gram-positive cell wall** and extend from it. They are composed of polymers of either glycerol phosphate or ribitol phosphate. Some polymers of glycerol teichoic acid penetrate the peptidoglycan layer and are covalently linked to the lipid in the cytoplasmic membrane, in which case they are called **lipoteichoic acid**; others anchor to the muramic acid of the peptidoglycan.

The medical importance of teichoic acids lies in their ability to **induce inflammation and septic shock when caused by certain gram-positive bacteria**; that is, they activate the same pathways as does endotoxin (LPS) in gram-negative bacteria. Teichoic acids also mediate the attachment of staphylococci to mucosal cells. Gram-negative bacteria do not have teichoic acids.

Cytoplasmic Membrane

Just inside the peptidoglycan layer of the cell wall lies the cytoplasmic membrane, which is composed of a phospholipid bilayer similar in microscopic appearance to that in eukaryotic cells. They are chemically similar, but eukaryotic membranes contain sterols, whereas prokaryotes generally do not. The only prokaryotes that have sterols in their membranes are members of the genus *Mycoplasma.* The membrane has four important functions: (1) active transport of molecules into the cell, (2) energy generation by oxidative phosphorylation, (3) synthesis of precursors of the cell wall, and (4) secretion of enzymes and toxins.

Cytoplasm

The cytoplasm has two distinct areas when seen in the electron microscope:

(1) An amorphous matrix that contains ribosomes, nutrient granules, metabolites, and plasmids.

(2) An inner, nucleoid region composed of DNA.

Ribosomes

Bacterial ribosomes are the site of protein synthesis as in eukaryotic cells, but they differ from eukaryotic ribosomes in size and chemical composition. Bacterial ribosomes are 70S in size, with 50S and 30S subunits, whereas eukaryotic ribosomes are 80S in size, with 60S and 40S subunits. The differences in both the ribosomal RNAs and proteins constitute the basis of the selective action of several antibiotics that inhibit bacterial, but not human, protein synthesis (see Chapter 10).

Granules

The cytoplasm contains several different types of granules that serve as storage areas for nutrients and stain characteristically with certain dyes. For example, volutin is a reserve of high energy stored in the form of polymerized metaphosphate. It appears as a "metachromatic" granule since it stains red with methylene blue dye instead of blue as one would expect. Metachromatic granules are a characteristic feature of *Corynebacterium diphtheriae,* the cause of diphtheria.

Nucleoid

The nucleoid is the area of the cytoplasm in which DNA is located. The DNA of most prokaryotes is a single, circular molecule; however, there are important exceptions. For instance, the genome of *Vibrio cholerae,* the causative agent of cholera, is composed of two circular chromosomes. *Borrelia burgdorferi*, the spirochete that causes Lyme disease, is composed of a linear chromosome and multiple circular and linear plasmids (see below). The size of bacterial genomes varies widely, with the smallest genome containing just over 130 genes and the largest containing approximately 11,600 genes. By contrast, human DNA has approximately 25,000 genes.

Because the bacterial nucleoid contains no nuclear membrane, no nucleolus, no mitotic spindle, and no histones, there

is little resemblance to the eukaryotic nucleus. One major difference between bacterial DNA and eukaryotic DNA is that bacterial DNA has no introns, whereas eukaryotic DNA does.

Plasmids

Plasmids are extrachromosomal, double-stranded, circular DNA molecules that are capable of replicating independently of the bacterial chromosome. Although plasmids are usually extrachromosomal, they can be integrated into the bacterial chromosome. Plasmids occur in both gram-positive and gram-negative bacteria, and several different types of plasmids can exist in one cell:

(1) **Transmissible** plasmids can be transferred from cell to cell by conjugation (see Chapter 4 for a discussion of conjugation). They are large (molecular weight [MW] 40–100 million), since they contain about a dozen genes responsible for synthesis of the sex pilus and for the enzymes required for transfer. They are usually present in a few (1–3) copies per cell.

(2) **Nontransmissible** plasmids are small (MW 3–20 million) since they do not contain the transfer genes; they are frequently present in many (10–60) copies per cell.

Plasmids carry the genes for the following functions and structures of medical importance:

(1) Antibiotic resistance, which is mediated by a variety of enzymes, such as the β-lactamase of *S. aureus, Escherichia coli,* and *Klebsiella pneumoniae.*

(2) Exotoxins, such as the enterotoxins of *E. coli,* anthrax toxin of *Bacillus anthracis,* exfoliative toxin of *S. aureus*, and tetanus toxin of *Clostridium tetani.*

(3) Pili (fimbriae), which mediate the adherence of bacteria to epithelial cells.

(4) Resistance to heavy metals, such as mercury, the active component of some antiseptics (e.g., Merthiolate and Mercurochrome), and silver, which is mediated by a reductase enzyme.

(5) Resistance to ultraviolet light, which is mediated by DNA repair enzymes.

(6) Bacteriocins, which are toxic proteins produced by certain bacteria that are lethal for other bacteria. Two common mechanisms of action of bacteriocins are (i) degradation of bacterial cell membranes by producing pores in the membrane and (ii) degradation of bacterial DNA by deoxyribonuclease (DNase). Examples of bacteriocins produced by medically important bacteria are colicins made by *E. coli* and pyocins made by *Pseudomonas aeruginosa*. Bacteria that produce bacteriocins have a selective advantage in the competition for food sources over those that do not. However, the medical importance of bacteriocins is that they may be useful in treating infections caused by antibiotic-resistant bacteria.

Transposons

Transposons are pieces of DNA that move readily from one site to another either within or between the DNAs of bacteria, plasmids, and bacteriophages. Because of their unusual ability to move, they are nicknamed "jumping genes." Some transposons move by replicating their DNA and inserting the new copy into another site (replicative transposition), whereas others are excised from the site without replicating and then inserted into the new site (direct transposition). Transposons can code for drug-resistant enzymes, toxins, or a variety of metabolic enzymes and can either cause mutations in the gene into which they insert or alter the expression of nearby genes.

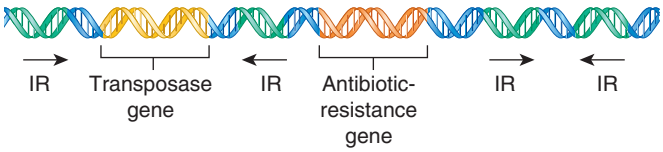

FIGURE 2–7 Transposon genes. This transposon is carrying a drug-resistance gene. IR, inverted repeat. (Reproduced with permission from Willey JM, Sherwood L, Woolverton: *Prescott's Principles of Microbiology.* New York, NY: McGraw-Hill Education; 2009.)

Transposons typically have four identifiable domains. On each end is a short DNA **sequence of inverted repeats**, which are involved in the integration of the transposon into the recipient DNA. The second domain is the gene for the transposase, which is the enzyme that mediates the excision and integration processes. The third region is the gene for the repressor that regulates the synthesis of both the transposase and the protein encoded by the fourth domain, which, in many cases, is an enzyme mediating antibiotic resistance (Figure 2–7). Note that for simplicity, the repressor gene is not shown in Figure 2–7.

Antibiotic resistance genes are transferred from one bacterium to another primarily by **conjugation** (see Chapter 4). This transfer is mediated primarily by plasmids, but some transposons, called **conjugative transposons**, are capable of transferring antibiotic resistance as well.

In contrast to plasmids or bacterial viruses, transposons are not capable of independent replication; they replicate as part of the DNA in which they are integrated. More than one transposon can be located in the DNA; for example, a plasmid can contain several transposons carrying drug-resistant genes. **Insertion sequences** are a type of transposon that have fewer bases (800–1500 base pairs), since they do not code for their own integration enzymes. They can cause mutations at their site of integration and can be found in multiple copies at the ends of larger transposon units.

Structures Outside the Cell Wall

Capsule

The capsule is a gelatinous layer covering the entire bacterium. It is typically composed of polysaccharide. The sugar components of the polysaccharide vary from one species of bacteria to another and frequently determine the serologic type (serotype) within a species. For example, there are 91 different serotypes of *Streptococcus pneumoniae,* which are distinguished by the antigenic differences of the sugars in the polysaccharide capsule.

The capsule is important for four reasons:

(1) It is a determinant of virulence of many bacteria since it limits the ability of phagocytes to engulf the bacteria. Negative charges on the capsular polysaccharide repel the negatively

charged cell membrane of the neutrophil and prevent it from ingesting the bacteria. Variants of encapsulated bacteria that have lost the ability to produce a capsule are usually nonpathogenic.

(2) Specific identification of an organism can be made by using antiserum against the capsular polysaccharide. In the presence of the homologous antibody, the capsule will swell greatly. This swelling phenomenon, which is used in the clinical laboratory to identify certain organisms, is called the **Quellung reaction**.

(3) Capsular polysaccharides are used as the antigens in certain vaccines because they are capable of eliciting protective antibodies. For example, the capsular polysaccharide of *S. pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae* is the immunogen in the current vaccine against these bacteria.

(4) The capsule may play a role in the adherence of bacteria to human tissues, which is an important initial step in causing infection.

Flagella

Flagella are long, whip-like appendages that move the bacteria toward nutrients and other attractants, a process called **chemotaxis**. The long filament, which acts as a propeller, is composed of many subunits of a single protein, flagellin, arranged in several intertwined chains. The energy for movement, the **proton motive force**, is provided by adenosine triphosphate (ATP), derived from the passage of ions across the membrane.

Flagellated bacteria have a characteristic number and location of flagella: some bacteria have one, and others have many; in some, the flagella are located at one end, and in others, they are all over the outer surface. Only certain bacteria have flagella. Many rods do, but most cocci do not and are therefore nonmotile. Spirochetes move by using a flagellum-like structure called the **axial filament**, which wraps around the spiral-shaped cell to produce an undulating motion.

Flagella are medically important for two reasons:

(1) Some species of motile bacteria (e.g., *E. coli* and *Proteus* species) are common causes of urinary tract infections. Flagella may play a role in pathogenesis by propelling the bacteria up the urethra into the bladder.

(2) Some species of bacteria (e.g., *Salmonella* species) are identified in the clinical laboratory by the use of specific antibodies against flagellar proteins.

Pili (Fimbriae)

Pili are hairlike filaments that extend from the cell surface. They are shorter and straighter than flagella and are composed of subunits of pilin, a protein arranged in helical strands. They are found mainly on gram-negative organisms.

Pili have two important roles:

(1) They mediate the **attachment** of bacteria to specific receptors on the human cell surface, which is a necessary step in the initiation of infection for some organisms. Mutants of *Neisseria gonorrhoeae* that do not form pili are nonpathogens.

(2) A specialized kind of pilus, the sex pilus, forms the attachment between the donor and the recipient bacteria during conjugation (see Chapter 4).

Glycocalyx (Slime Layer)

The glycocalyx is a polysaccharide coating that is secreted by many bacteria. It covers surfaces like a film and allows the bacteria to **adhere firmly** to various structures (e.g., skin, heart valves, prosthetic joints, and catheters). The glycocalyx is an important component of biofilms (see page 36). The medical importance of the glycocalyx is illustrated by the finding that it is the glycocalyx-producing strains of *P. aeruginosa* that cause respiratory tract infections in cystic fibrosis patients, and it is the glycocalyx-producing strains of *Staphylococcus epidermidis* and viridans streptococci that cause endocarditis. The glycocalyx also mediates adherence of certain bacteria to the surface of teeth. This plays an important role in the formation of plaque.

Bacterial Spores

These highly resistant structures are formed in response to adverse conditions by two genera of medically important gram-positive rods: the genus *Bacillus,* which includes the agent of anthrax, and the genus *Clostridium,* which includes the agents of tetanus and botulism. Spore formation (sporulation) occurs when nutrients, such as sources of carbon and nitrogen, are depleted (Figure 2–8). The spore forms inside the cell and contains bacterial DNA, a small amount of cytoplasm, cell membrane, peptidoglycan, very little water, and most importantly, a thick, keratin-like coat that is responsible for the remarkable resistance of the spore to heat, dehydration, radiation, and chemicals. This resistance may be mediated by **dipicolinic acid**, a calcium ion chelator found only in spores.

Once formed, the spore has no metabolic activity and can remain dormant for many years. Upon exposure to water and the appropriate nutrients, specific enzymes degrade the coat, water and nutrients enter, and germination into a potentially pathogenic bacterial cell occurs. Note that this differentiation

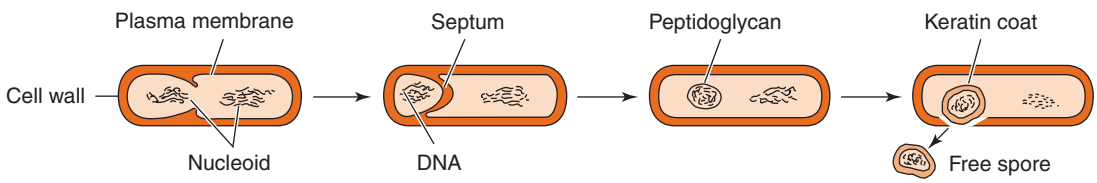

FIGURE 2–8 Bacterial spores. The spore contains the entire DNA genome of the bacterium surrounded by a thick, resistant coat.

TABLE 2–4 Important Features of Spores and Their Medical Implications

Important Features of Spores	Medical Implications
Highly resistant to heating; spores are not killed by boiling (100°C), but are killed at 121°C.	Medical supplies must be heated to 121°C for at least 15 minutes to be sterilized.
Highly resistant to many chemicals, including most disinfectants, due to the thick, keratin-like coat of the spore.	Only solutions designated as sporicidal will kill spores.
They can survive for many years, especially in the soil.	Wounds contaminated with soil can be infected with spores and cause diseases such as tetanus (*C. tetani*) and gas gangrene (*C. perfringens*).
They exhibit no measurable metabolic activity.	Antibiotics are ineffective against spores because antibiotics act by inhibiting certain metabolic pathways of bacteria. Also, spore coat is impermeable to antibiotics.
Spores form when nutrients are insufficient but then germinate to form bacteria when nutrients become available.	Spores are not often found at the site of infections because nutrients are not limiting. Bacteria rather than spores are usually seen in Gram-stained smears.
Spores are produced by members of only two genera of bacteria of medical importance, *Bacillus* and *Clostridium*, both of which are gram-positive rods.	Infections transmitted by spores are caused by species of either *Bacillus or Clostridium*.

process is *not* a means of reproduction since one cell produces one spore that germinates into one cell.

The medical importance of spores lies in their **extraordinary resistance to heat** and chemicals. As a result of their resistance to heat, sterilization cannot be achieved by boiling. Steam heating under pressure (autoclaving) at 121°C, for at least 15 minutes, is required to ensure the sterility of products for medical use. Spores are often not seen in clinical specimens recovered from patients infected by spore-forming organisms because the supply of nutrients is adequate.

Table 2–4 describes the medically important features of bacterial spores.

PEARLS

Shape & Size

- Bacteria have three shapes: **cocci** (spheres), **bacilli** (rods), and **spirochetes** (spirals).
- Cocci are arranged in three patterns: pairs (diplococci), chains (streptococci), and clusters (staphylococci).
- The size of most bacteria ranges from 1 to 3 µm. *Mycoplasma,* the smallest bacteria (and therefore the **smallest cells**), are 0.2 µm. Some bacteria, such as *Borrelia,* are as long as 10 µm; that is, they are longer than a human red blood cell, which is 7 µm in diameter.

Bacterial Cell Wall

- All bacteria have a cell wall composed of **peptidoglycan** except *Mycoplasma,* which are surrounded *only* by a cell membrane.
- Gram-negative bacteria have a **thin** peptidoglycan covered by an outer lipid-containing membrane, whereas gram-positive bacteria have a **thick** peptidoglycan and no outer membrane. These differences explain why gram-negative bacteria lose the stain when exposed to a lipid solvent in the Gram stain process, whereas gram-positive bacteria retain the stain and remain purple.
- The outer membrane of gram-negative bacteria contains **endotoxin** (**lipopolysaccharide, LPS**), the main inducer of septic shock. Endotoxin consists of **lipid A**, which causes the fever and hypotension seen in septic shock, and a polysaccharide called **O antigen**, which is useful in laboratory identification.
- Between the inner cell membrane and the outer membrane of gram-negative bacteria lies the **periplasmic space**, which is the location of **β-lactamases**—the enzymes that degrade β-lactam antibiotics, such as penicillins and cephalosporins.
- Peptidoglycan is found *only* in bacterial cells. It is a network that covers the entire bacterium and gives the organism its shape. It is composed of a sugar backbone (**glycan**) and peptide side chains (**peptido**). The side chains are cross-linked by **transpeptidase**—the enzyme that is inhibited by penicillins and cephalosporins.
- The cell wall of mycobacteria (e.g., *M. tuberculosis*) has **more lipid** than either the gram-positive or gram-negative bacteria. As a result, the dyes used in the Gram stain do not penetrate into (do not stain) mycobacteria. The **acid-fast stain** does stain mycobacteria, and these bacteria are often called acid-fast bacilli (acid-fast rods).

- **Lysozymes** kill bacteria by cleaving the glycan backbone of peptidoglycan.
- The cytoplasmic membrane of bacteria consists of a phospholipid bilayer (without sterols) located just inside the peptidoglycan. It regulates active transport of nutrients into the cell and the secretion of toxins out of the cell.

Gram Stain

- **Gram stain** is the most important staining procedure. Gram-positive bacteria stain *purple,* whereas gram-negative bacteria stain *pink*. This difference is due to the ability of gram-positive bacteria to *retain the crystal violet–iodine complex in the presence of a lipid solvent,* usually acetone–alcohol. Gram-negative bacteria, because they have an outer lipid-containing membrane and thin peptidoglycan, lose the purple dye when treated with acetone–alcohol. They become colorless and then stain pink when exposed to a red dye such as safranin.
- Not all bacteria can be visualized using Gram stain. Some important human pathogens, such as the bacteria that cause tuberculosis and syphilis, cannot be seen using this stain.

Bacterial DNA

- The bacterial genome typically consists of a **single chromosome of circular DNA** located in the nucleoid.
- **Plasmids** are extrachromosomal pieces of circular DNA that encode both exotoxins and many enzymes that cause antibiotic resistance.
- **Transposons** are small pieces of DNA that move frequently between chromosomal DNA and plasmid DNA. They carry antibiotic-resistant genes.

Structures External to the Cell Wall

- **Capsules** are antiphagocytic; that is, they limit the ability of neutrophils to engulf the bacteria. Almost all capsules are composed of *polysaccharide;* the polypeptide capsule of anthrax bacillus is the only exception. Capsules are also the antigens in several vaccines, such as the pneumococcal vaccine. Antibodies against the capsule neutralize the antiphagocytic effect and allow the bacteria to be engulfed by neutrophils. **Opsonization** is the process by which antibodies enhance the phagocytosis of bacteria.
- **Pili** are filaments of protein that extend from the bacterial surface and mediate **attachment** of bacteria to the surface of human cells. A different kind of pilus, the sex pilus, functions in conjugation (see Chapter 4).
- The **glycocalyx** is a polysaccharide "slime layer" secreted by certain bacteria. It **attaches bacteria firmly** to the surface of human cells and to the surface of catheters, prosthetic heart valves, and prosthetic hip joints.

Bacterial Spores

- **Spores** are medically important because they are **highly heat resistant** and are not killed by many disinfectants. Boiling will *not* kill spores. They are formed by certain gram-positive rods, especially *Bacillus* and *Clostridium* species.
- Spores have a thick, keratin-like coat that allows them to survive for many years, especially in the soil. Spores are formed when nutrients are in short supply, but when nutrients are restored, spores germinate to form bacteria that can cause disease. Spores are *metabolically inactive* but contain DNA, ribosomes, and other essential components.

SELF-ASSESSMENT QUESTIONS

1. The initial step in the process of many bacterial infections is adherence of the organism to mucous membranes. The bacterial component that mediates adherence is the:
(A) lipid A
(B) nucleoid
(C) peptidoglycan
(D) pilus
(E) plasmid

2. In the Gram stain procedure, bacteria are exposed to 95% alcohol or to an acetone/alcohol mixture. The purpose of this step is:
(A) to adhere the cells to the slide
(B) to retain the purple dye within all the bacteria
(C) to disrupt the outer cell membrane so the purple dye can leave the bacteria
(D) to facilitate the entry of the purple dye into the gram-negative cells
(E) to form a complex with the iodine solution

3. In the process of studying how bacteria cause disease, it was found that a rare mutant of a pathogenic strain failed to form a capsule. Which one of the following statements is the most accurate in regard to this unencapsulated mutant strain?
(A) It was nonpathogenic primarily because it was easily phagocytized.
(B) It was nonpathogenic primarily because it could not invade tissue.
(C) It was nonpathogenic primarily because it could only grow anaerobically.
(D) It was highly pathogenic because it could secrete larger amounts of exotoxin.
(E) It was highly pathogenic because it could secrete larger amounts of endotoxin.

4. *Mycobacterium tuberculosis* stains well with the acid-fast stain, but not with the Gram stain. Which one of the following is the most likely reason for this observation?
(A) It has a large number of pili that absorb the purple dye.
(B) It has a large amount of lipid that prevents entry of the purple dye.
(C) It has a very thin cell wall that does not retain the purple dye.
(D) It is too thin to be seen in the Gram stain.
(E) It has histones that are highly negatively charged.

5. Of the following bacterial components, which one exhibits the most antigenic variation?
- **(A)** Capsule
- **(B)** Lipid A of endotoxin
- **(C)** Peptidoglycan
- **(D)** Ribosome
- **(E)** Spore

6. β-Lactamases are an important cause of antibiotic resistance. Which one of the following is the most common site where β-lactamases are located?
- **(A)** Attached to DNA in the nucleoid
- **(B)** Attached to pili on the bacterial surface
- **(C)** Free in the cytoplasm
- **(D)** Within the capsule
- **(E)** Within the periplasmic space

7. Which one of the following is the most accurate description of the structural differences between gram-positive bacteria and gram-negative bacteria?
- **(A)** Gram-positive bacteria have a thick peptidoglycan layer, whereas gram-negative bacteria have a thin layer.
- **(B)** Gram-positive bacteria have an outer lipid-rich membrane, whereas gram-negative bacteria do not.
- **(C)** Gram-positive bacteria form a sex pilus that mediates conjugation, whereas gram-negative bacteria do not.
- **(D)** Gram-positive bacteria have plasmids, whereas gram-negative bacteria do not.
- **(E)** Gram-positive bacteria have capsules, whereas gram-negative bacteria do not.

8. Bacteria that cause nosocomial (hospital-acquired) infections often produce extracellular substances that allow them to stick firmly to medical devices, such as intravenous catheters. Which one of the following is the name of this extracellular substance?
- **(A)** Axial filament
- **(B)** Endotoxin
- **(C)** Flagella
- **(D)** Glycocalyx
- **(E)** Porin

9. Lysozyme in tears is an effective mechanism for preventing bacterial conjunctivitis. Which one of the following bacterial structures does lysozyme degrade?
- **(A)** Endotoxin
- **(B)** Nucleoid DNA
- **(C)** Peptidoglycan
- **(D)** Pilus
- **(E)** Plasmid DNA

10. Several bacteria that form spores are important human pathogens. Which one of the following is the most accurate statement about bacterial spores?
- **(A)** They are killed by boiling for 15 minutes.
- **(B)** They are produced primarily by gram-negative cocci.
- **(C)** They are formed primarily when the bacterium is exposed to antibiotics.
- **(D)** They are produced by anaerobes only in the presence of oxygen.
- **(E)** They are metabolically inactive, yet can survive for years in that inactive state.

ANSWERS

(1) **(D)**
(2) **(C)**
(3) **(A)**
(4) **(B)**
(5) **(A)**
(6) **(E)**
(7) **(A)**
(8) **(D)**
(9) **(C)**
(10) **(E)**

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 735. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 777.

CHAPTER

3

Growth

CHAPTER CONTENTS

GROWTH CYCLE

Bacteria reproduce by **binary fission**, a process by which one parent cell divides to form two progeny cells. Because one cell gives rise to two progeny cells, bacteria are said to undergo exponential growth (logarithmic growth). The concept of exponential growth can be illustrated by the following relationship:

Number of cells	1	2	4	8	16
Exponential	2^0	2^1	2^2	2^3	2^4

Thus, 1 bacterium will produce 16 bacteria after 4 generations.

The doubling (generation) time of bacteria ranges from as little as 20 minutes for *Escherichia coli* to as long as 18 hours for *Mycobacterium tuberculosis*. The exponential growth and the short doubling time of some organisms result in rapid production of very large numbers of bacteria. For example, 1 *E. coli* organism will produce over 1000 progeny in about 3 hours and over 1 million in about 7 hours. The doubling time varies not only with the species, but also with the amount of nutrients, the temperature, the pH, and other environmental factors.

The growth cycle of bacteria has four major phases. If a small number of bacteria are inoculated into a liquid nutrient medium and the bacteria are counted at frequent intervals, the typical phases of a standard growth curve can be demonstrated (Figure 3–1).

(1) The first is the **lag** phase, during which vigorous metabolic activity occurs but cells do not divide. This can last for a few minutes up to many hours.

(2) The **log** (logarithmic) phase is when rapid cell division occurs. Many antibiotics, such as penicillin, are most efficacious during this phase because they act by disrupting biosynthetic processes carried out by the bacterial cell during active growth

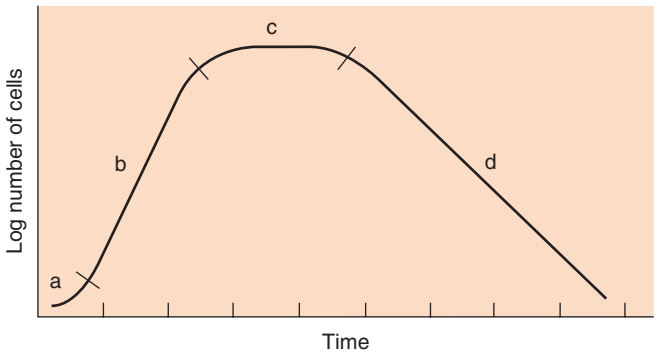

FIGURE 3–1 Growth curve of bacteria: a, lag phase; b, log phase; c, stationary phase; d, death phase. (Reproduced with permission from Joklik WK, Willett HP, Amos DB: *Zinsser Microbiology,* 20th ed. New York, NY: McGraw-Hill Education; 1992.)

(i.e., when they are dividing). The log phase is also known as the **exponential** phase.

(3) The **stationary** phase occurs when nutrient depletion or toxic products cause growth to slow until the number of new cells produced balances the number of cells that die, resulting in a steady state. Cells grown in a special apparatus called a "chemostat," into which fresh nutrients are added and from which waste products are removed continuously, can remain in the log phase and do not enter the stationary phase.

(4) The final phase is the **death** phase, which is marked by a decline in the number of viable bacteria.

OBLIGATE INTRACELLULAR GROWTH

Most bacterial pathogens of humans are capable of growing on artificial media in the clinical laboratory. The term *artificial* means that the medium is composed of purified chemicals such as sugars, amino acids, and salts, such as sodium chloride. Often

blood is added in the form of sheep's blood, but that is for nutritional purposes, not because the bacteria need to grow within the red blood cells.

However, certain bacterial pathogens of humans, notably *Chlamydia* and *Rickettsia* (see Chapters 25 and 26, respectively) and *Ehrlichia* and *Anaplasma* (see Chapter 26), can *only* grow within living cells and are referred to as **obligate intracellular pathogens**. The main reason for this is that they lack the ability to produce sufficient adenosine triphosphate (ATP) and must use ATP produced by the host cells.

AEROBIC & ANAEROBIC GROWTH

For most organisms, an adequate supply of oxygen enhances metabolism and growth. The oxygen acts as the hydrogen acceptor in the final steps of energy production catalyzed by the flavoproteins and cytochromes. Because the use of oxygen generates two toxic molecules, hydrogen peroxide (H_2O_2) and the free radical superoxide (O_2^-), bacteria require two enzymes to detoxify these molecules when oxygen is used. The first is **superoxide dismutase**, which catalyzes the following reaction:

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

The second is **catalase**, which catalyzes the following reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The response to oxygen is an important criterion for classifying bacteria and has a great practical significance because specimens from patients must be incubated in a proper atmosphere for the bacteria to grow.

(1) Some bacteria, such as *M. tuberculosis*, are **obligate aerobes**; that is, they require oxygen to grow because their ATP-generating system is dependent on oxygen as the hydrogen acceptor.

(2) Other bacteria, such as *E. coli*, are **facultative anaerobes**; they use oxygen, if it is present, to generate energy by respiration, but they can use the fermentation pathway to synthesize ATP in the absence of sufficient oxygen.

(3) The third group of bacteria consists of the **obligate anaerobes**, such as *Clostridium tetani*, which cannot grow in the presence of oxygen because they lack either superoxide dismutase or catalase, or both. Obligate anaerobes vary in their response to oxygen exposure; some can survive but are not able to grow, whereas others are killed rapidly.

FERMENTATION OF SUGARS

In the clinical laboratory, identification of several important human pathogens is based on the fermentation of certain sugars. For example, *Neisseria gonorrhoeae* and *Neisseria meningitidis* can be distinguished from each other on the basis of fermentation of either glucose or maltose (see page 130), and *E. coli* can be differentiated from *Salmonella* and *Shigella* on the basis of fermentation of lactose (see page 149).

The term **fermentation** refers to the breakdown of a sugar (such as glucose or maltose or galactose) to pyruvic acid and then, usually, to lactic acid. Note that lactose is a disaccharide composed of glucose and galactose and therefore must be cleaved by β-galactosidase in *E. coli* before fermentation can occur. Fermentation is also called the glycolytic (glyco = sugar, lytic = breakdown) cycle, and this is the process by which facultative bacteria generate ATP in the absence of oxygen.

If oxygen is present, the pyruvate produced by fermentation enters the Krebs cycle (oxidation cycle, tricarboxylic acid cycle) and is metabolized to two final products, CO_2 and H_2O. The Krebs cycle generates much more ATP than the glycolytic cycle; therefore, facultative bacteria grow faster in the presence of oxygen. Facultative and anaerobic bacteria ferment, but aerobes, which can grow only in the presence of oxygen, do not. Aerobes, such as *Pseudomonas aeruginosa*, produce metabolites that enter the Krebs cycle by processes other than fermentation, such as the deamination of amino acids.

During fermentation, acidic end products (pyruvate and lactate) are generated, which can be detected by an indicator that changes color upon changes in pH. For example, if a sugar is fermented in the presence of phenol red (an indicator), the pH becomes acidic and the medium turns yellow. If, however, the sugar is not fermented, no acid is produced and the phenol red remains red.

IRON METABOLISM

Iron, in the form of ferric ion, is required for the growth of bacteria because it is an essential component of cytochromes and other enzymes. The amount of iron available for pathogenic bacteria in the human body is very low because the iron is sequestered in iron-binding proteins such as transferrin. To obtain iron for their growth, bacteria produce iron-binding compounds called **siderophores**. Siderophores, such as enterobactin produced by *E. coli*, are secreted by the bacteria, capture iron by chelating it, then attach to specific receptors on the bacterial surface, and are actively transported into the cell where the iron becomes available for use. The fact that bacteria have such a complex and specific mechanism for obtaining iron testifies to its importance in the growth and metabolism of bacteria.

PEARLS

- Bacteria reproduce by **binary fission**, whereas eukaryotic cells reproduce by mitosis.
- The bacterial growth cycle consists of four phases: the **lag** phase, during which nutrients are incorporated; the **log** phase, during which rapid cell division occurs; the **stationary** phase, during which as many cells are dying as are being formed; and the **death** phase, during which most of the cells are dying because nutrients have been exhausted.

- Some bacteria can grow in the presence of oxygen (**aerobes** and **facultatives**), but others die in the presence of oxygen (**anaerobes**). The use of oxygen by bacteria generates toxic products such as **superoxide** and **hydrogen peroxide**. Aerobes and facultatives have enzymes, such as **superoxide dismutase** and **catalase**, that detoxify these products, but anaerobes do not and are killed in the presence of oxygen.
- The fermentation of certain sugars is the basis of the laboratory identification of some important pathogens. Fermentation of sugars, such as glucose, results in the production of ATP and acidic products (pyruvic acid or lactic acid). These acids lower the pH, and this can be detected by the change in color of indicator dyes.

SELF-ASSESSMENT QUESTIONS

1. Figure 3–1 depicts a bacterial growth curve divided into phases a, b, c, and d. In which one of the phases are antibiotics such as penicillin most likely to kill bacteria?

(A) Phase a
(B) Phase b
(C) Phase c
(D) Phase d

2. Some bacteria are obligate anaerobes. Which of the following statements best explains this phenomenon?

(A) They can produce energy both by fermentation (i.e., glycolysis) and by respiration using the Krebs cycle and cytochromes.
(B) They cannot produce their own ATP.
(C) They do not form spores.
(D) They lack superoxide dismutase and catalase.
(E) They do not have a capsule.

ANSWERS

(1) **(B)**
(2) **(D)**

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 735. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 777.

CHAPTER

4

Genetics

CHAPTER CONTENTS

INTRODUCTION

There are several unique aspects of microbial genetics that largely account for the great genotypic and phenotypic diversity, the ability to cause disease, and the propensity to develop resistance to virtually any antibiotic observed in bacteria. Bacteria have a simple genetic organization relative to eukaryotic organisms. They are haploid, usually possessing a single chromosome and therefore a single copy of each gene. This is in contrast to eukaryotic cells (such as human cells), which are **diploid**, meaning they have a pair of each chromosome and therefore have two copies of each gene. In diploid cells, one copy of a gene (allele) may be expressed as a protein (i.e., be dominant), whereas another allele may not be expressed (i.e., be recessive). In haploid cells, any gene that has acquired a mutation will result in a cell synthesizing either a mutant protein or no protein at all depending on the type of mutation.

MUTATIONS

A mutation is a change in the base sequence of DNA that can result in the insertion of a different amino acid or stop codon into a protein and the appearance of an altered phenotype. Mutations result from three types of molecular changes:

(1) The first type is the **base substitution**. This occurs when one base is inserted in place of another. It takes place at the time of DNA replication, either because the DNA polymerase makes an error or because a mutagen alters the hydrogen bonding of the base being used as a template in such a manner that the wrong base is inserted. When the base substitution results in a codon that simply causes a different amino acid to be inserted, the mutation is called a **missense mutation**; when the base substitution generates a termination codon that stops protein synthesis prematurely, the mutation is called a **nonsense mutation**. Nonsense mutations almost always destroy protein function.

(2) The second type of mutation is the **frameshift mutation**. This occurs when one or more base pairs are added or deleted, which shifts the reading frame on the ribosome and results in incorporation of the wrong amino acids "downstream" from the mutation and in the production of an inactive protein.

(3) The third type of mutation occurs when **transposons** or **insertion sequences** are integrated into the DNA. These newly inserted pieces of DNA can cause profound changes in the genes into which they insert and in adjacent genes.

Mutations can be caused by chemicals, radiation, or viruses. Chemicals act in several different ways.

(1) Some, such as nitrous acid and alkylating agents, alter the existing base so that it forms a hydrogen bond preferentially with the wrong base (e.g., adenine would no longer pair with thymine but with cytosine).

(2) Some chemicals, such as 5-bromouracil, are base analogues, since they resemble normal bases. Because the bromine atom has an atomic radius similar to that of a methyl group, 5-bromouracil can be inserted in place of thymine (5-methyluracil). However, 5-bromouracil has less hydrogen-bonding fidelity than does thymine, and so it binds to guanine with greater frequency. This results in a transition from an A-T base pair to a G-C base pair, thereby producing a mutation. The antiviral drug iododeoxyuridine acts as a base analogue of thymidine.

(3) Some chemicals, such as benzpyrene, which is found in tobacco smoke, bind to the existing DNA bases and cause

frameshift mutations. These chemicals, which are frequently carcinogens as well as mutagens, intercalate between the adjacent bases, thereby distorting and offsetting the DNA sequence.

X-rays and ultraviolet light can also cause mutations.

(1) X-rays have high energy and can damage DNA in three ways: (a) by breaking the covalent bonds that hold the ribose phosphate chain together, (b) by producing free radicals that can attack the bases, and (c) by altering the electrons in the bases and thus changing their hydrogen bonding.

(2) Ultraviolet radiation, which has lower energy than X-rays, causes the cross-linking of the adjacent pyrimidine bases to form dimers. This cross-linking (e.g., of adjacent thymines to form a thymine dimer) results in inability of the DNA to replicate properly.

Certain viruses, such as the bacterial virus Mu (mutator bacteriophage), cause a high frequency of mutations when their DNA is inserted into the bacterial chromosome. Since the viral DNA can insert into many different sites, mutations in various genes can occur. These mutations are either frameshift mutations or deletions.

Conditional lethal mutations are of medical interest because they may be useful in vaccines (e.g., influenza vaccine). The word *conditional* indicates that the mutation is expressed only under certain conditions. The most important conditional lethal mutations are the temperature-sensitive ones. Temperature-sensitive organisms can replicate at a relatively low, permissive temperature (e.g., 32°C) but cannot grow at a higher, restrictive temperature (e.g., 37°C). This behavior is due to a mutation that causes an amino acid change in an essential protein, allowing it to function normally at 32°C but not at 37°C because of an altered conformation at the higher temperature. An example of a conditional lethal mutant of medical importance is a strain of influenza virus currently used in an experimental vaccine. This vaccine contains a virus that cannot grow at 37°C and hence cannot infect the lungs and cause pneumonia, but it can grow at 32°C in the nose, where it can replicate and induce immunity.

TRANSFER OF DNA WITHIN BACTERIAL CELLS

Transposons transfer DNA from one site on the bacterial chromosome to another site or to a plasmid. They do so by synthesizing a copy of their DNA and inserting the copy at another site in the bacterial chromosome or the plasmid. The structure and function of transposons are described in Chapter 2, and their role in antimicrobial drug resistance is described in Chapter 11. The transfer of a transposon to a plasmid and the subsequent transfer of the plasmid to another bacterium by conjugation (see below) contribute significantly to the spread of antibiotic resistance.

Transfer of DNA within bacteria also occurs by **programmed rearrangements** (Figure 4–1). These gene rearrangements account for many of the antigenic changes seen in *Neisseria gonorrhoeae* and *Borrelia recurrentis*, the cause of relapsing fever. (They also occur in trypanosomes, which are discussed in Chapter 52.) A programmed rearrangement consists of the movement of a gene from a silent storage site where the gene

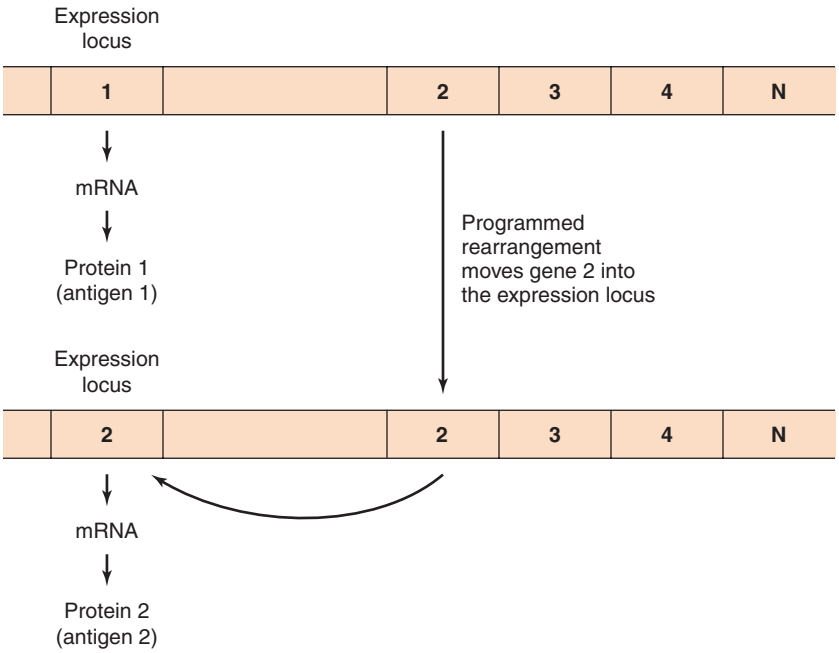

FIGURE 4–1 Programmed rearrangements. In the top part of the figure, the gene for protein 1 is in the expression locus, and the mRNA for protein 1 is synthesized. At a later time, a copy of gene 2 is made and inserted into the expression locus. By moving only the copy of the gene, the cell always keeps the original DNA for use in the future. When the DNA of gene 2 is inserted, the DNA of gene 1 is excised and degraded.

is not expressed to an active site where transcription and translation occur. There are many silent genes that encode variants of the antigens, and the insertion of a new gene into the active site in a sequential, repeated programmed manner is the source of the consistent antigenic variation.

These movements are not induced by an immune response but have the effect of allowing the organism to evade it. They occur even when the organism is grown in culture in the laboratory, in the absence of the host immune response.

TRANSFER OF DNA BETWEEN BACTERIAL CELLS

The transfer of genetic information from one cell to another can occur by three methods: conjugation, transduction, and transformation (Table 4–1). From a medical viewpoint, the two most important consequences of DNA transfer are (1) **that antibiotic resistance genes are spread from one bacterium to another primarily by conjugation** and (2) **that several important exotoxins are encoded by bacteriophage genes and are transferred by transduction**.

1. Conjugation

Conjugation is the mating of two bacterial cells, during which DNA is transferred from the donor to the recipient cell (Figure 4–2). The mating process is controlled by an **F (fertility) plasmid** (F factor), which carries the genes for the proteins required for conjugation. One of the most important proteins is pilin, which forms the **sex pilus** (conjugation tube). Mating begins when the pilus of the donor bacterium carrying the F factor (F^+) attaches to a receptor on the surface of a recipient bacterium, which does not contain an F factor (F^-), resulting in a direct connection between the cytoplasm of the donor and recipient cells. After an enzymatic cleavage of the F factor DNA, one strand is transferred across the conjugal bridge (mating bridge) into the recipient cell. The process is completed by synthesis of the complementary strand to form a double-stranded F factor plasmid in both the donor and recipient cells. The recipient is now an F^+ cell that is capable of transmitting the plasmid further. Note that in this instance only the F factor, and not the bacterial chromosome, has been transferred.

Some F^+ cells have their F plasmid integrated into the bacterial DNA and thereby acquire the capability of transferring the chromosome into another cell. These cells are called **Hfr (high-frequency recombination)** cells (Figure 4–3). During this transfer, the single strand of DNA that enters the recipient F^- cell contains a piece of the F factor at the leading end followed by the bacterial chromosome and then by the remainder of the F factor. The time required for complete transfer of the bacterial DNA is approximately 100 minutes. Most matings result in the transfer of only a portion of the donor chromosome because the attachment between the two cells can break. The donor cell genes that are transferred vary since the F plasmid can integrate at several different sites in the bacterial DNA. The bacterial genes adjacent to the leading piece of the F factor are the first and therefore the most frequently transferred. The newly acquired DNA can recombine into the recipient's DNA and become a stable component of its genetic material.

Resistance plasmids (R plasmids) can also be transferred by conjugation. R plasmids can carry one or more genes for

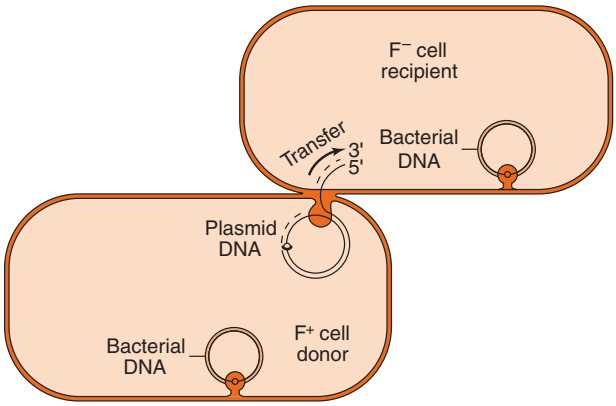

FIGURE 4–2 Conjugation. An F plasmid is being transferred from an F^+ donor bacterium to an F^- recipient. The transfer is at the contact site made by the sex pilus. The new plasmid in the recipient bacterium is composed of one parental strand (solid line) and one newly synthesized strand (dashed line). The previously existing plasmid in the donor bacterium now consists of one parental strand (solid line) and one newly synthesized strand (dashed line). Both plasmids are drawn with only a short region of newly synthesized DNA (dashed lines), but at the end of DNA synthesis, both the donor and the recipient contain a complete copy of the plasmid DNA.

TABLE 4–1 Comparison of Conjugation, Transduction, and Transformation

Transfer Procedure	Process	Type of Cells Involved	Nature of DNA Transferred
Conjugation	DNA transferred from one bacterium to another	Prokaryotic	Chromosomal or plasmid
Transduction	DNA transferred by a virus from one cell to another	Prokaryotic	Any gene in generalized transduction; only certain genes in specialized transduction
Transformation	Naked DNA in the immediate environment taken up by a cell	Prokaryotic	Any DNA

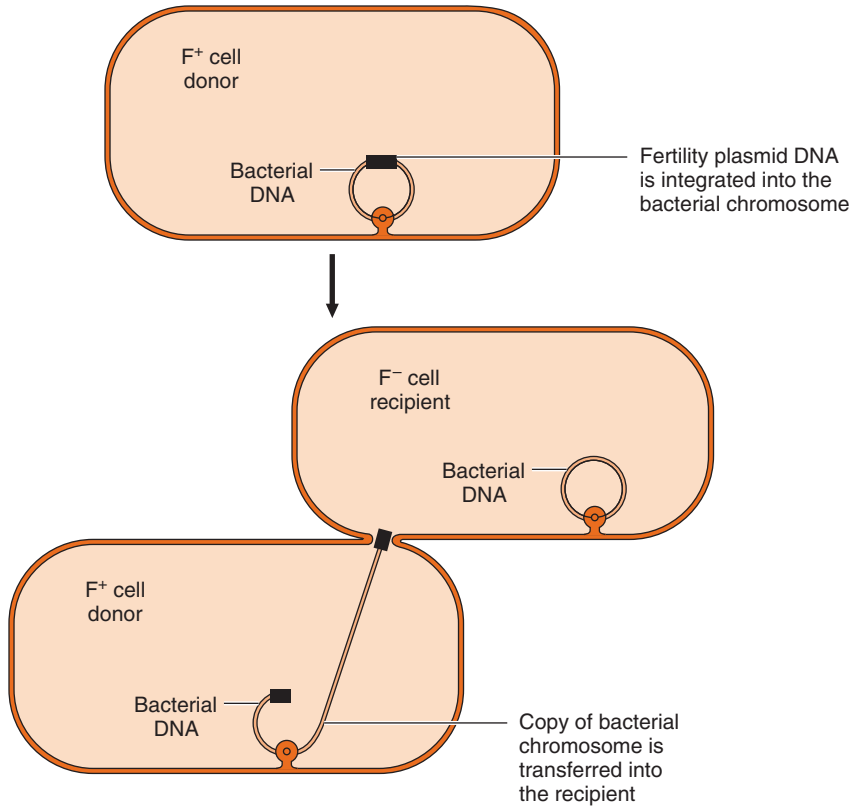

FIGURE 4–3 High-frequency recombination. **Top:** A fertility (F) plasmid has integrated into the bacterial chromosome. **Bottom:** The F plasmid mediates the transfer of the bacterial chromosome of the donor into the recipient bacteria.

a variety of enzymes that can degrade antibiotics and modify membrane transport systems. For example, R plasmids encode the β-lactamases of *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. In addition, they encode the proteins of the transport system that actively export sulfonamides out of the bacterial cell. Note that R plasmids can be transferred not only to cells of the same species, but also to other species and genera. (See Chapter 11 for more information about R plasmids.)

2. Transduction

Transduction is the transfer of cell DNA by means of a bacterial virus (**bacteriophage, phage**) (Figure 4–4). During the growth of the virus within the cell, a piece of bacterial DNA is incorporated into the virus particle and is carried into the recipient cell at the time of infection. Within the recipient cell, the phage DNA can integrate into the cell DNA and the cell can acquire a new trait—a process called **lysogenic conversion** (see the

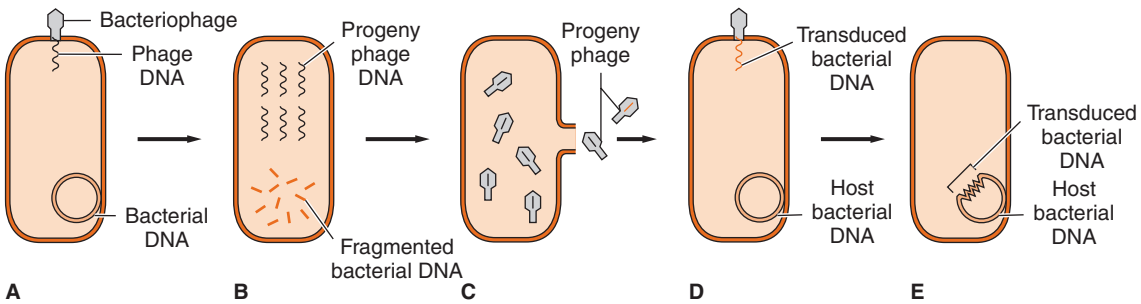

FIGURE 4–4 **Transduction. A:** A bacteriophage infects a bacterium, and phage DNA enters the cell. **B:** The phage DNA replicates, and the bacterial DNA fragments. **C:** The progeny phages assemble and are released; most contain phage DNA, and a few contain bacterial DNA. **D:** Another bacterium is infected by a phage-containing bacterial DNA. **E:** The transduced bacterial DNA integrates into host DNA, and the host acquires a new trait. This host bacterium survives because no viral DNA is transduced; therefore, no viral replication can occur. (Another type of transduction mechanism is depicted in Figure 29–10.)

end of Chapter 29). This process can change a nonpathogenic organism into a pathogenic one. Diphtheria toxin, botulinum toxin, cholera toxin, shiga toxin of *E. coli* and erythrogenic toxin (*Streptococcus pyogenes*) are encoded by bacteriophages and can be transferred by transduction.

There are two types of transduction: generalized and specialized. The **generalized** type occurs when the virus carries a segment from any part of the bacterial chromosome. This occurs because the cell DNA is fragmented after phage infection and pieces of cell DNA the same size as the viral DNA are incorporated into the virus particle at a frequency of about 1 in every 1000 virus particles. The **specialized** type occurs when the bacterial virus DNA that has integrated into the cell DNA is excised and carries with it an adjacent part of the cell DNA. Since most lysogenic (temperate) phages integrate at specific sites in the bacterial DNA, the adjacent cellular genes that are transduced are usually specific to that virus.

3. Transformation

Transformation is the transfer of DNA itself from one cell to another. This occurs by either of the two following methods. First, in nature, dying bacteria may release their DNA, which may be taken up by recipient cells. Certain bacteria, such as *Neisseria*, *Haemophilus*, and *Streptococci*, synthesize receptors on the cell surface that play a role in the uptake of DNA from the environment.

Second, in the laboratory, an investigator may extract DNA from one type of bacteria and introduce it into genetically different bacteria. The experimental use of transformation has revealed important information about DNA. In 1944, it was shown that DNA extracted from encapsulated smooth pneumococci could transform nonencapsulated rough pneumococci into encapsulated smooth organisms. This demonstration that the transforming principle was DNA marked the first evidence that DNA was the genetic material.

RECOMBINATION

Once the DNA is transferred from the donor to the recipient cell by one of the three processes just described, it can integrate into the host cell chromosome by recombination. There are two types of recombination:

(1) **Homologous recombination**, in which two pieces of DNA that have extensive homologous regions pair up and exchange pieces by the processes of breakage and reunion.

(2) **Nonhomologous recombination**, in which little, if any, homology is necessary.

Different genetic loci govern these two types, and so it is presumed that different enzymes are involved. Although it is known that a variety of endonucleases and ligases are involved, the precise sequence of events is unknown.

PEARLS

- Bacteria have only one copy of their genome DNA (i.e., they are **haploid**). In contrast, eukaryotic cells have two copies of their genome DNA (i.e., they are **diploid**). Bacterial DNA is typically circular; human nuclear DNA is linear.
- The transfer of DNA within bacterial cells occurs by two processes: movement of transposons and programmed rearrangements. **Transposons** are small pieces of DNA that move readily from one site on the bacterial chromosome to another or from the bacterial chromosome to a plasmid. Medically, transposons are important because they commonly **carry antibiotic resistance genes**. The transfer of transposons on plasmids to other bacteria by conjugation contributes significantly to antibiotic resistance.
- **Programmed rearrangements** are the movement of genes from inactive (storage) sites into active sites, where they are expressed as new proteins. Medically, this is important because bacteria can acquire new proteins (antigens) on their surface and evade the immune system. Two important organisms in which this occurs are *Neisseria gonorrhoeae*, the cause of gonorrhea, and *Trypanosoma brucei*, a protozoan that causes African sleeping sickness.
- The transfer of DNA between bacterial cells occurs mainly by two processes: conjugation and transduction. **Conjugation** is the process by which DNA, either plasmid or chromosomal, is transferred directly from one bacterium to another. For conjugation to occur, the donor bacterium must have a "fertility" plasmid (F plasmid) that encodes the proteins that mediate this process, the most important of which are the proteins that form the **sex pilus**. The DNA transferred by conjugation to the recipient bacterium is a new copy that allows the donor to keep a copy of the DNA. Plasmids carrying antibiotic resistance genes are commonly transferred by conjugation.
- **Transduction** is the process by which DNA, either plasmid or chromosomal, is transferred from one bacterium to another by a **virus**. The transferred DNA integrates into the chromosomal DNA of the recipient, and new proteins, such as exotoxins, are made—a process called **lysogenic conversion**.
- **Transformation** is the process by which DNA itself, either DNA released from dying cells or DNA purified in the laboratory, enters a recipient bacterium.

SELF-ASSESSMENT QUESTIONS

1. The emergence of antibiotic-resistant bacteria, especially in enteric gram-negative rods, is a medically important phenomenon. This most commonly occurs by a process that involves a sex pilus and the subsequent transfer of plasmids carrying one or more transposons. Which one of the following is the name that best describes this process?

(A) Conjugation
(B) Transduction
(C) Transformation
(D) Translocation
(E) Transposition

2. Several important pathogenic bacteria have the ability to translocate pieces of their DNA in a process called *programmed rearrangements*. Which one of the following is the most important known consequence of this ability?

(A) The number of plasmids increases significantly, which greatly enhances antibiotic resistance.
(B) The amount of endotoxin increases significantly, which greatly enhances the ability to cause septic shock.
(C) The surface antigens of the bacteria vary significantly, which greatly enhances the ability to avoid opsonization by antibody.
(D) The ability of the bacterium to be lysogenized is significantly increased, which greatly enhances the ability to produce increased amounts of exotoxins.
(E) The ability of the bacterium to survive intracellularly is greatly increased.

3. Which statement is the most accurate regarding transposons?

(A) They encode enzymes that degrade the ends of the bacterial chromosome.
(B) They are short sequences of DNA that often encode enzymes that mediate antibiotic resistance.
(C) They are short sequences of RNA that silence specific regulatory genes.
(D) They are a family of transfer RNAs that enhance mutations at "hot spots" in the bacterial genome.

4. *Corynebacterium diphtheriae* causes the disease diphtheria by producing diphtheria toxin. The gene encoding the toxin is integrated into bacterial genome during lysogenic conversion. The toxin gene was acquired by which process?

(A) Conjugation
(B) Transduction
(C) Transformation
(D) Translocation
(E) Transposition

ANSWERS

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

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 735. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 777.

CHAPTER 5

Classification of Medically Important Bacteria

CHAPTER CONTENTS

PRINCIPLES OF CLASSIFICATION

The current classification of bacteria is based primarily on morphologic and biochemical characteristics. A scheme that divides the medically important organisms by genus is shown in Table 5–1. For pedagogic purposes, this classification scheme deviates from those derived from strict taxonomic principles in two ways:

(1) Only organisms that are described in this book in the section on medically important bacteria are included.

(2) Because there are so many gram-negative rods, they are divided into three categories: respiratory organisms, zoonotic organisms, and enteric and related organisms.

The initial criterion used in the classification is the nature of the cell wall (i.e., is it rigid, flexible, or absent?). Bacteria with rigid, thick walls can be subdivided into free-living bacteria, which are capable of growing on laboratory medium in the absence of human or other animal cells, and non–free-living bacteria, which are obligate intracellular parasites and therefore can grow only within human or other animal cells. The free-living organisms are further subdivided according to shape and staining reaction into a variety of gram-positive and gram-negative cocci and rods with different oxygen requirements and spore-forming abilities. Bacteria with flexible, thin walls (the spirochetes) and those without cell walls (the mycoplasmas) form separate units.

Using these criteria, along with various biochemical reactions, many bacteria can be readily classified into separate genera and species. However, there have been several examples of these criteria placing bacteria into the same genus when DNA sequencing of their genome reveals they are significantly different and should be classified in a new or different genus. For example, an organism formerly known as *Pseudomonas cepacia* has been reclassified as *Burkholderia cepacia* because the base sequence of its DNA was found to be significantly different from the DNA of the members of the genus *Pseudomonas*.

PEARLS

- The classification of bacteria is based on various criteria, such as the nature of the cell wall, staining characteristics, ability to grow in the presence or absence of oxygen, and ability to form spores.
- The criterion currently used is the base sequence of the genome DNA. Several bacteria have been reclassified based on this information.

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 735. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 777.

TABLE 5–1 Classification of Medically Important Bacteria

Characteristics	Genus	Representative Diseases
I. Rigid, thick-walled cells		
A. Free-living (extracellular bacteria)		
1. Gram-positive		
a. Cocci	*Streptococcus*	Pneumonia, pharyngitis, cellulitis
	Staphylococcus	Abscess of skin and other organs
b. Spore-forming rods		
(1) Aerobic	*Bacillus*	Anthrax
(2) Anaerobic	*Clostridium*	Tetanus, gas gangrene, botulism
c. Non–spore-forming rods		
(1) Nonfilamentous	*Corynebacterium*	Diphtheria
	Listeria	Meningitis
(2) Filamentous	*Actinomyces*	Actinomycosis
	Nocardia	Nocardiosis
2. Gram-negative		
a. Cocci	*Neisseria*	Gonorrhea, meningitis
b. Rods		
(1) Facultative		
(a) Straight		
(i) Respiratory organisms	*Haemophilus*	Meningitis
	Bordetella	Whooping cough
	Legionella	Pneumonia
(ii) Zoonotic organisms	*Brucella*	Brucellosis
	Francisella	Tularemia
	Pasteurella	Cellulitis
	Yersinia	Plague
(iii) Enteric and related organisms	*Escherichia*	Urinary tract infection, diarrhea
	Enterobacter	Urinary tract infection
	Serratia	Pneumonia
	Klebsiella	Pneumonia, urinary tract infection
	Salmonella	Enterocolitis, typhoid fever
	Shigella	Enterocolitis
	Proteus	Urinary tract infection
(b) Curved	*Campylobacter*	Enterocolitis
	Helicobacter	Gastritis, peptic ulcer
	Vibrio	Cholera
(2) Aerobic	*Pseudomonas*	Pneumonia, urinary tract infection
(3) Anaerobic	*Bacteroides*	Peritonitis
3. Acid-fast	*Mycobacterium*	Tuberculosis, leprosy
B. Non–free-living (obligate intracellular parasites)	*Rickettsia*	Rocky Mountain spotted fever, typhus, Q fever
	Chlamydia	Urethritis, trachoma, psittacosis
II. Flexible, thin-walled cells (spirochetes)	*Treponema*	Syphilis
	Borrelia	Lyme disease
	Leptospira	Leptospirosis
III. Wall-less cells	*Mycoplasma*	Pneumonia

CHAPTER 6

The Human Microbiome

CHAPTER CONTENTS

THE HUMAN MICROBIOME

The **human microbiome** is the term used to describe the distinct microbial communities that inhabit different host environments on the body's skin and mucosal surfaces. Historically, microbiologists referred to microbial populations routinely found on and in the body as **normal flora**. The term *microbiome* also encompasses all of the genetic material associated with these normal constituents. As you will read below, the *genetic* capabilities of any given normal flora organism can have profound and important impacts on the interactions that the microbe has with the host. The establishment of the human microbiome is initiated immediately after birth and is a necessary and normal part of human development.

Until relatively recently, our understanding of the organisms that compose the human microbiome relied on cultivation to isolate organisms in pure culture. This approach is limited in its usefulness for several reasons. First, the vast majority of microbes associated with humans cannot be cultivated ex vivo. Second, the ability to culture a microbe does not yield any information on the relative abundance of that organism in the niche under investigation. Finally, growing an organism out of its environment in pure culture gives little, if any, information on the complexity and interdependence of the microbial communities in that niche.

The development of sophisticated molecular techniques over the past decade (see Chapter 9 for more detail) has revealed enormous numbers of bacteria, yeasts, and protozoa that are associated with the human microbiome, many of which were previously unknown. Current estimates suggest that there is an approximately equal number of prokaryotic cells on and in the human body as there are human cells, most of which are gut-associated. This remarkable statistic is even more notable considering that the average adult gut is home to ~1000 bacterial species, each of which contains ~2000 genes, cataloging a total of 2,000,000 gut-associated microbial genes. This is 100 times more than the ~20,000 genes encoded in the entire human genome.

Variation in the abundance and complexity of the microbiome constituents is observed within an individual over time and certainly between individuals. However, longitudinal characterization of the human gut microbiome has shown that within the first few years of life, our microbial communities mature and become relatively stable and unique to each individual unless perturbed, such as by antibiotic treatment.

Once established, members of the microbiome are considered **permanent residents** of the associated body sites, such as the skin, oropharynx, colon, and vagina (Tables 6–1 and 6–2). These microbes are often referred to as **commensals**, which are organisms that derive benefit from another host but do not damage that host.

Members of the microbiota vary in both abundance and type from one body site to another. Internal organs usually are sterile, although the central nervous system, blood, lower bronchi and alveoli, liver, spleen, kidneys, and bladder experience occasional transient microbial intrusions, often introduced after modest trauma (after flossing of teeth) or abrasions on the skin.

We make a distinction between established members of the microbiome and something called the **carrier state**. The term *carrier* implies that an individual has become **colonized** with a potential pathogen and therefore can be a source of infection of others. It is most frequently used in reference to a person with an asymptomatic infection or to someone who has recovered from a disease but continues to carry the organism and can serve as a reservoir of infection for others.

We have known for some time that individual members of the normal flora can cause disease when they gain access to other body sites. Examples of this include *Escherichia coli*

TABLE 6–1 Summary of the Members of Normal Flora and Their Anatomic Locations

Members of the Normal Flora[1]	Anatomic Location
Bacteroides species	Colon, throat, vagina
Candida albicans	Mouth, colon, vagina
Clostridium species	Colon
Corynebacterium species (diphtheroids)	Nasopharynx, skin, vagina
Enterococcus faecalis	Colon
Escherichia coli and other coliforms	Colon, vagina, outer urethra
Gardnerella vaginalis	Vagina
Haemophilus species	Nasopharynx
Lactobacillus species	Mouth, colon, vagina
Neisseria species	Mouth, nasopharynx
Propionibacterium acnes	Skin
Pseudomonas aeruginosa	Colon, skin
Staphylococcus aureus	Nose, skin
Staphylococcus epidermidis	Skin, nose, mouth, vagina, urethra
Viridans streptococci	Mouth, nasopharynx

[1]In alphabetical order.

and *Bacteroides fragilis*, both are normal flora organisms of the intestinal tract, which cause urinary tract infections and peritonitis, respectively. However, there is mounting evidence that the dynamic nature of the microbiome composition plays important roles both in the maintenance of health and in the etiology of disease. Dysbioses of the microbiome, which refer to any change in the composition of resident commensal communities relative to the community found in healthy individuals, are associated with an expanding list of chronic diseases including obesity, inflammatory bowel diseases (IBDs), diabetes, cardiovascular disease, colon cancer, rheumatoid arthritis, major depression, Parkinson's disease, and autism spectrum disorder.

There are three major ways in which the microbiome is thought to contribute to health and disease:

(1) **A healthy microbiome provides indispensable instruction to the developing immune system**. It is now well established that the microbiome is important for the development of intestinal immune responses. A large body of research in germ-free animal models reveals that the gut microbiota plays a critical immunomodulatory role in the development of gut-associated lymphoid tissues (GALT). Germ-free animals show low serum levels of antibodies and do not produce CD8 intraepithelial lymphocytes. In addition, variation in microbiome composition influences the proportion of Th1, Th2, and Th17 T cells (see Chapter 60). These observations all suggest that an intact healthy microbiota impacts the development of adaptive immune responses.

(2) **The human microbiome can confer susceptibility or resistance to pathogen colonization depending on its composition and harbors a diverse reservoir of antibiotic resistance genes**. The healthy human gut microbiome is populated most prominently by Bacteroidetes and Firmicutes, followed by Proteobacteria and Actinobacteria (see below). These predominantly nonpathogenic resident bacteria occupy attachment sites

TABLE 6–2 Medically Important Members of the Normal Flora

Location	Important Organisms[1]	Less Important Organisms[2]
Skin	*Staphylococcus epidermidis*	*Staphylococcus aureus, Corynebacterium* (diphtheroids), various streptococci, *Pseudomonas aeruginosa,* anaerobes (e.g., *Propionibacterium*), yeasts (e.g., *Candida albicans*)
Nose	*S. aureus*[3]	*S. epidermidis, Corynebacterium* (diphtheroids), various streptococci
Mouth	Viridans streptococci	Various streptococci, *Eikenella corrodens*
Dental plaque	*Streptococcus mutans*	*Prevotella intermedia, Porphyromonas gingivalis*
Gingival crevices	Various anaerobes (e.g., *Bacteroides, Fusobacterium,* streptococci, *Actinomyces*)	
Throat	Viridans streptococci	Various streptococci (including *Streptococcus pyogenes* and *Streptococcus pneumoniae*), *Neisseria* species, *Haemophilus influenzae, S. epidermidis*
Colon	*Bacteroides fragilis, Escherichia coli*	*Bifidobacterium, Eubacterium, Fusobacterium, Lactobacillus,* various aerobic gram-negative rods, *Enterococcus faecalis* and other streptococci, *Clostridium*
Vagina	*Lactobacillus, E. coli,*[3] group B streptococci[3]	Various streptococci, various gram-negative rods. *B. fragilis, Corynebacterium* (diphtheroids), *C. albicans*
Urethra		*S. epidermidis, Corynebacterium* (diphtheroids), various streptococci, various gram-negative rods (e.g., *E. coli*)[3]

[1]Organisms that are medically significant or present in large numbers.

[2]Organisms that are less medically significant or present in smaller numbers.

[3]These organisms are not part of the normal flora in this location but are important colonizers.

on the mucosa that can interfere with colonization by pathogenic bacteria. The ability of members of the normal flora to limit the growth of pathogens is called **colonization resistance**. If the composition of normal flora is altered (e.g., by diet) or suppressed by antibiotics, pathogens may grow and cause disease. For example, certain diets have been shown to affect colonization by enterohemorrhagic *E. coli* serotype O157:H7 and the severity and length of the resulting disease. Antibiotic use can reduce the normal colonic flora, allowing the growth of *Clostridium difficile*, which can lead to pseudomembranous colitis.

There is a substantial repertoire of resistance genes in the gut microbiome that is much more diverse and extensive than previously thought. Since antibiotic resistance determinants are readily exchanged between bacteria through horizontal gene transfer, these genes can serve as a reservoir of resistance that is accessible to pathogens.

(3) **The human microbiota contributes to nutrition and human health**. Gut bacteria aid digestion by breaking down otherwise indigestible plant fibers into short-chain fatty acids that intestinal cells can access. They also synthesize a variety of micronutrients including several of the B vitamins and vitamin K and have a major impact on the absorption of key minerals, such as iron.

MICROBIOME OF THE INTESTINAL TRACT

In normal fasting people, the stomach contains few organisms, primarily because of its low pH. The small intestine usually contains small numbers of streptococci, lactobacilli, and yeasts, particularly *Candida albicans*. Larger numbers of these organisms are found in the terminal ileum.

The largest and most complex microbial population in humans resides in the colon. Roughly 20% of feces consists of primarily anaerobic bacteria at approximately 10^{11} organisms/g. Within the colon, the two largest phyla of bacteria are the Firmicutes (64%) and the Bacteroidetes (23%). The Firmicutes are gram-positive rods and members of the genera *Clostridium* and *Faecalibacterium* are prominent organisms. The Bacteroidetes are gram-negative rods and the genera *Bacteroides* and *Prevotella* are important members. Species of Proteobacteria (gram-negative rods such as *Escherichia* and *Salmonella*) and Actinobacteria (gram-positive rods such as *Actinomyces*) make up the bulk of the remainder. The major bacteria found in the colon are listed in Table 6–3.

There is mounting evidence that the microbiome composition plays important roles in several disease states, such as weight control (obesity), and several inflammatory diseases, such as the two main IBDs—Crohn disease and ulcerative colitis. The effect on obesity is revealed by studies involving the transfer of fecal bacteria between strains of inbred mice. For example, fecal bacteria from obese mice transplanted into germ-free strains of nonobese mice resulted in the nonobese mice becoming obese. It appears that the fecal bacteria metabolize more of the input food, making more calories available to the mice. In other experiments, fecal transplants from identical (monozygotic) human twins, one obese and the other not obese, were transplanted into germ-free mice. The mice that received the fecal transplant from the obese twin gained significantly more weight than the mice that received the fecal transplant from the nonobese twin.

IBD is characterized by dysbiosis of the microbiome. Several studies have suggested that the microbiomes of patients with IBD have significantly lower abundances of putative beneficial microorganisms, particularly from the phyla Bacteroidetes and Firmicutes, and more from the phyla Actinobacteria and Proteobacteria, than healthy subjects. In addition, the composition of the microbiomes from patients with IBD fluctuated considerably more than those of healthy individuals.

TABLE 6–3 Major Bacteria Found in the Colon

Bacterium[1]	Number/g of Feces	Important Pathogen
Bacteroides, especially *B. fragilis*	10^{10}–10^{11}	Yes
Bifidobacterium	10^{10}	No
Eubacterium	10^{10}	No
Coliforms	10^{7}–10^{8}	Yes
Enterococcus, especially *E. faecalis*	10^{7}–10^{8}	Yes
Lactobacillus	10^{7}	No
Clostridium, especially *C. perfringens*	10^{6}	Yes

[1] *Bacteroides, Bifidobacterium*, and *Eubacterium* (which make up more than 90% of the fecal flora) are anaerobes. Coliforms (*Escherichia coli, Enterobacter* species, and other gram-negative organisms) are the predominant facultative anaerobes.

MICROBIOME OF THE SKIN

The gut is not the only place where microbial communities are established. The skin has a microbiome that is less complex than that of the gut. The predominant organism on the skin is *Staphylococcus epidermidis*, which in this location is a nonpathogen but can cause disease when it reaches certain sites, such as artificial heart valves and prosthetic joints. It is found on the skin much more frequently than its pathogenic relative *Staphylococcus aureus* (see Table 6–2). There are about 10^{3} to 10^{4} organisms/cm^{2} of skin. Most of them are located superficially in the stratum corneum, but some are found in the hair follicles and act as a reservoir to replenish the superficial flora after hand washing. Anaerobic organisms, such as *Propionibacterium* and *Peptococcus*, are situated in the deeper follicles in the dermis, where oxygen tension is low. *Propionibacterium acnes* is a common skin anaerobe that is implicated in the pathogenesis of acne.

The yeast *C. albicans* is also a member of the normal flora of the skin. It can enter a person's bloodstream when needles pierce the skin (e.g., in patients with intravenous catheters or in those who use intravenous drugs). It is an important cause

of systemic infections in patients with reduced cell-mediated immunity.

MICROBIOME OF THE RESPIRATORY TRACT

A wide spectrum of organisms colonize the nose, throat, and mouth, but the lower bronchi and alveoli typically contain few, if any, organisms. The nose is colonized by a variety of streptococcal and staphylococcal species, the most significant of which is the pathogen *S. aureus.* Occasional outbreaks of disease due to this organism, particularly in newborns, can be traced to nasal, skin, or perianal carriage by health care personnel.

The throat contains a mixture of viridans streptococci, *Neisseria* species, and *S. epidermidis* (see Table 6–2). These nonpathogens occupy attachment sites on the pharyngeal mucosa and inhibit the growth of the pathogens *Streptococcus pyogenes, Neisseria meningitidis,* and *S. aureus*, respectively.

In the mouth, viridans streptococci make up about half of the bacteria and are found on a variety of oral surfaces, including the teeth. Plaque that builds up on the enamel surface of teeth is composed of salivary proteins that deposit on the enamel as well as gelatinous, high-molecular-weight glucans secreted by colonizing streptococcal bacteria, which form a structure for an ordered succession of different organisms to colonize. *Streptococcus mutans*, a member of the viridans group, is of special interest since it is found in large numbers (10^{10}/g) in the dental plaque of patients with dental caries. The *S. mutans* established in the plaque produces a large amount of acid, which demineralizes the enamel and initiates caries. Other viridans streptococci found in the oral cavity, such as *Streptococcus sanguinis*, are also among the leading causes of subacute bacterial (infective) endocarditis. These organisms can enter the bloodstream and attach to damaged heart valves.

Anaerobic bacteria, such as species of *Bacteroides, Prevotella, Fusobacterium, Clostridium,* and *Peptostreptococcus*, are found in the gingival crevices, where the oxygen concentration is very low. If aspirated, these organisms can cause lung abscesses, especially in debilitated patients with poor dental hygiene. In addition, the gingival crevices are the natural habitat of *Actinomyces israelii*—an anaerobic actinomycete that can cause abscesses of the jaw, lungs, or abdomen.

MICROBIOME OF THE GENITOURINARY TRACT

The vaginal flora of adult women consists primarily of *Lactobacillus* species (see Table 6–2). Lactobacilli are responsible for producing the acid that keeps the pH of the adult woman's vagina low. Before puberty and after menopause, when estrogen levels are low, lactobacilli are rare and the vaginal pH is high. Lactobacilli appear to prevent the growth of potential pathogens, since their suppression by antibiotics can lead to overgrowth by *C. albicans.* Overgrowth of this yeast can result in *Candida* vaginitis.

The vagina is located close to the anus and can be colonized by members of the fecal flora. For example, women who are prone to recurrent urinary tract infections harbor organisms such as *E. coli* and *Enterobacter* in the opening of the vaginal cavity. About 15% to 20% of women of childbearing age carry group B streptococci in the vagina. This organism is an important cause of sepsis and meningitis in the newborn and is acquired during passage through the birth canal. The vagina is colonized by *S. aureus* in approximately 5% of women, which predisposes them to toxic shock syndrome.

Urine in the bladder is sterile in the healthy person, but during passage through the outermost portions of the urethra, it often becomes contaminated with *S. epidermidis*, coliforms, diphtheroids, and nonhemolytic streptococci. The area around the urethra of women and uncircumcised men contains secretions that carry *Mycobacterium smegmatis*, an acid-fast organism. The skin surrounding the genitourinary tract is the site of *Staphylococcus saprophyticus*, a cause of urinary tract infections in women.

PEARLS

- **Normal flora** are those microorganisms that are the **permanent residents** of the body in all humans. The **microbiome** refers to normal flora organisms and additionally includes the genetic composition and capabilities of these organisms. Some people can be transiently **colonized**, either for short or long periods, with certain organisms, but those are not considered members of the normal flora. **Carriers** (also called chronic carriers) are individuals in whom pathogenic organisms are present in significant numbers and therefore are a source of infection for others.
- Normal flora organisms inhabit the body surfaces exposed to the environment, such as the **skin, oropharynx, intestinal tract,** and **vagina**. Members of the normal flora differ in number and kind at various anatomic sites.
- Members of the normal flora are **low-virulence** organisms. In their usual anatomic site, they are nonpathogenic. However, if they leave their usual anatomic site, especially in an immunocompromised individual, they can cause disease. Normal flora organisms can also horizontally acquire genes from other members of the microflora, which can impact their virulence.
- **Colonization resistance** occurs when members of the normal flora occupy receptor sites on the skin and mucosal surfaces, thereby preventing pathogens from binding to those receptors.

Important Members of the Normal Flora

- **Skin.** The predominant member of the normal flora of the skin is *Staphylococcus epidermidis*. It is an important cause of infections of prosthetic heart valves and prosthetic joints. *Candida albicans*, **a yeast** also found on the skin, can enter the bloodstream and cause disseminated infections, such as endocarditis in intravenous drug users. *Staphylococcus aureus* is also present on the skin, but its **main site is in the nose**. It causes abscesses in the skin and in many other organs.
- **Oropharynx.** The main members of the normal flora of the mouth and throat are the **viridans streptococci**, such as *Streptococcus sanguinis* and *S. mutans*. Viridans streptococci are the most common cause of subacute endocarditis.
- **Gastrointestinal tract.** The stomach contains very few organisms because of the low pH. The colon contains the **largest number of normal flora** and the most diverse species, including both anaerobic and facultative bacteria. There are both gram-positive and gram-negative rods and cocci. The members of the colonic normal flora are an important cause of disease outside of the colon. The two most important members of the colonic flora that cause disease are the anaerobe *Bacteroides fragilis* and the facultative *Escherichia coli*. *Enterococcus faecalis*, a facultative bacterium, is also an important pathogen.
- **Vagina. Lactobacilli** are the predominant normal flora organisms in the vagina. They keep the pH of the vagina low, which inhibits the growth of organisms such as *C. albicans*, an important cause of vaginitis.
- **Urethra.** The outer third of the urethra contains a mixture of bacteria, primarily *S. epidermidis*. The female urethra can become colonized with fecal flora such as *E. coli*, which predisposes to urinary tract infections.

SELF-ASSESSMENT QUESTIONS

1. The colon is the site of the largest number of normal flora bacteria. Which of the following bacteria is found in the greatest number in the colon?

(A) *Bacteroides fragilis*
(B) *Clostridium perfringens*
(C) *Enterococcus faecalis*
(D) *Escherichia coli*
(E) *Lactobacillus* species

2. A 76-year-old woman with a prosthetic (artificial) hip comes to you complaining of fever and pain in that joint. You are concerned about an infection by *Staphylococcus epidermidis*. Using your knowledge of normal flora, what is the most likely source of this organism?

(A) Dental plaque
(B) Mouth
(C) Skin
(D) Stomach
(E) Vagina

3. Your patient is a 30-year-old woman with a previous history of rheumatic fever who has had fever for the past 2 weeks. On examination, you find a new heart murmur. You suspect endocarditis and do a blood culture, which grows a viridans group *Streptococcus* later identified as *S. sanguinis*. Using your knowledge of normal flora, what is the most likely source of this organism?

(A) Duodenum
(B) Skin
(C) Throat
(D) Urethra
(E) Vagina

4. An outbreak of postsurgical wound infections caused by *S. aureus* has occurred in the hospital. The infection control team was asked to determine whether the organism could be carried by one of the operating room personnel. Using your knowledge of normal flora, which of the following body sites is the most likely location for this organism?

(A) Colon
(B) Gingival crevice
(C) Mouth
(D) Nose
(E) Throat

ANSWERS

(1) **(A)**
(2) **(C)**
(3) **(C)**
(4) **(D)**

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 735. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 777.

CHAPTER

7

Pathogenesis

CHAPTER CONTENTS

PRINCIPLES OF PATHOGENESIS

A microorganism is a **pathogen** if it is capable of causing disease; however, some organisms are highly pathogenic (i.e., they often cause disease), whereas others cause disease rarely. **Opportunistic** pathogens are those that rarely, if ever, cause disease in immunocompetent people but can cause serious infection in patients with reduced host defenses (immunocompromised) and, as discussed in Chapter 6, are frequent members of the body's normal flora.

Virulence is a quantitative measure of pathogenicity and is measured by the number of organisms required to cause disease. The 50% lethal dose (LD_{50}) is the number of organisms needed to kill half of the hosts that are exposed to the pathogen, while the 50% infectious dose (ID_{50}) is the number needed to cause infection in half of the exposed hosts. Organisms with a *lower* LD_{50} (or ID_{50}) are said to be *more* virulent than those with a higher LD_{50} (or ID_{50}) because fewer organisms are needed to cause death or disease.

The **infectious dose** of an organism required to cause disease varies greatly among the pathogenic bacteria. For example, *Shigella* and *Salmonella* both cause diarrhea by infecting the gastrointestinal tract, but the infectious dose of *Shigella* is less than 100 organisms, whereas the infectious dose of *Salmonella* is on the order of 100,000 organisms. The infectious dose of bacteria depends primarily on their **virulence factors** (e.g., whether their pili allow them to adhere well to mucous membranes, whether they produce exotoxins or endotoxins, whether they possess a capsule to protect them from phagocytosis, and whether they can survive various nonspecific host defenses such as acid in the stomach).

There are two uses of the word **parasite**. Within the context of this chapter, the term refers to the parasitic relationship of the bacteria to the host cells (i.e., the presence of the bacteria is **detrimental** to the host cells). Bacteria that are human pathogens can be thought of, therefore, as parasites. Some bacterial pathogens are **obligate intracellular parasites** (e.g., *Chlamydia* and *Rickettsia*), because they can grow only within host cells. Many bacteria are facultative parasites because they can grow within cells, outside cells, or on bacteriologic media. The other use of the term *parasite* refers to the protozoa and the helminths, which are discussed in Part VI of this book.

WHY DO PEOPLE GET INFECTIOUS DISEASES?

People get infectious diseases when microorganisms overpower our host defenses (i.e., when the balance between the organism and the host shifts in favor of the organism). The organism or its products are then present in sufficient amount to induce various symptoms, such as fever and inflammation, which we interpret as those of an infectious disease.

From the organism's perspective, the two critical determinants in overpowering the host are the **number of organisms** to which a person is exposed and the **virulence** of these

organisms. Clearly, the greater the number of organisms, the greater is the likelihood of infection. It is important to realize, however, that a small number of highly virulent organisms can cause disease just as a large number of less virulent organisms can. The virulence of an organism is determined by its ability to produce various **virulence factors**, several of which were described previously.

The production of specific virulence factors also determines what disease the bacteria cause. For example, a strain of *Escherichia coli* that produces one type of exotoxin causes watery (nonbloody) diarrhea, whereas a different strain of *E. coli* that produces another type of exotoxin causes bloody diarrhea. This chapter describes several important examples of specific diseases related to the production of various virulence factors.

From the host's perspective, the two main arms of our host defenses are innate immunity and acquired immunity, the latter of which includes both antibody-mediated and cell-mediated immunity. A reduction in the functioning of any component of our host defenses shifts the balance in favor of the organism and increases the chance that an infectious disease will occur. Some important causes of a reduction in our host defenses include genetic immunodeficiencies such as agammaglobulinemia and acquired immunodeficiencies such as acquired immunodeficiency syndrome (AIDS), drug-induced immunosuppression in patients with organ transplants, and cancer patients who are receiving chemotherapy. Patients with diabetes and autoimmune diseases also may have reduced host defenses. An overview of our host defenses is presented in Chapters 8 and 57.

In many instances, a person acquires an organism, but no infectious disease occurs because the host defenses were successful. Such **asymptomatic infections** are common and are typically recognized by detecting antibody against the organism in the patient's serum.

TYPES OF BACTERIAL INFECTIONS

The term **infection** has more than one meaning. One meaning is that an organism has infected the person (i.e., it has entered the body of that person). For example, a person can be infected with an organism of low pathogenicity and not develop symptoms of disease. Another meaning of the term *infection* is to describe an infectious disease, such as when a person says, "I have an infection." In this instance, infection and disease are being used interchangeably, but it is important to realize that according to the first definition, the word infection does not have to be equated with disease. Usually, the meaning will be apparent from the context.

Bacteria cause disease by two major mechanisms: (1) **toxin production** and (2) **invasion** and **inflammation**. Toxins fall into two general categories: **exotoxins** and **endotoxins**. Exotoxins are proteins secreted by the cell, whereas endotoxins are lipopolysaccharides (LPS) that form an integral part of the cell wall of gram-negative bacteria. Endotoxins are not actively released from the cell and cause fever, shock, and other generalized symptoms. Both exotoxins and endotoxins by themselves can cause symptoms; the presence of the bacteria in the host is not required. Invasive bacteria, on the other hand, grow to large numbers locally and induce an inflammatory response consisting of erythema, edema, warmth, and pain. Invasion and inflammation are discussed later in the section entitled "Determinants of Bacterial Pathogenesis."

Many, but not all, infections are **communicable** or **contagious** (i.e., they are spread from host to host). For example, tuberculosis is communicable (i.e., it is spread from person to person via airborne droplets produced by coughing), but botulism is not, because the exotoxin produced by the organism in the contaminated food affects only those eating that food.

An infection is **epidemic** if it occurs much more frequently than usual; it is **pandemic** if it has a worldwide distribution. An **endemic** infection is constantly present at a low level in a specific population. In addition to infections that result in overt symptoms, many are **inapparent** or **subclinical** and can be detected only by demonstrating a rise in antibody titer or by isolating the organism. Some infections result in a **latent** state, after which reactivation of the growth of the organism and recurrence of symptoms may occur. Certain other infections lead to a **chronic carrier** state, in which the organisms continuously grow with or without producing symptoms in the host. Chronic carriers (e.g., "Typhoid Mary") are an important source of infection of others and hence are a public health hazard.

The determination of whether an organism recovered from a patient is actually the cause of the disease involves an awareness of two phenomena: normal flora and colonization. Members of the **normal flora** are permanent residents of the body and vary in type according to anatomic site (see Chapter 6). When an organism is obtained from a patient's specimen, the question of whether it is a member of the normal flora is important in interpreting the finding. **Colonization** refers to the presence of a new organism that is neither a member of the normal flora nor the cause of symptoms. It can be a difficult clinical dilemma to distinguish between a pathogen and a colonizer, especially in specimens obtained from the respiratory tract, such as throat cultures and sputum cultures.

STAGES OF BACTERIAL PATHOGENESIS

Most bacterial infections are acquired from an external source. However, some bacterial infections are caused by members of the normal flora and, as such, are not transmitted directly prior to the onset of infection.

A generalized sequence of the stages of infection is as follows:

(1) Transmission from an external source into the portal of entry.

(2) Evasion of primary host defenses such as skin or stomach acid.

(3) Adherence to mucous membranes, usually by bacterial pili.

(4) Colonization by growth of the bacteria at the site of adherence.

(5) Disease symptoms caused by toxin production or invasion accompanied by inflammation.

(6) Host responses, both nonspecific and specific (immunity), during steps 3, 4, and 5.

(7) Progression or resolution of the disease.

DETERMINANTS OF BACTERIAL PATHOGENESIS

1. Transmission

An understanding of the mode of transmission of bacteria and other infectious agents is extremely important from a public health perspective, because interrupting the **chain of transmission** is an excellent way to prevent infectious diseases. The mode of transmission of many infectious diseases is "human-to-human," but infectious diseases are also transmitted from nonhuman sources such as soil, water, and animals. **Fomites** are inanimate objects, such as towels, that serve as a source of microorganisms that can cause infectious diseases. Table 7–1 describes some important examples of these modes of transmission.

Although some infections are caused by members of the normal flora, most are acquired by transmission from external sources. Pathogens exit the infected patient most frequently from the respiratory and gastrointestinal tracts; hence, transmission to the new host usually occurs via airborne respiratory droplets or fecal contamination of food and water. Organisms can also be transmitted by sexual contact, urine, skin contact, blood transfusions, contaminated needles, or biting insects. The transfer of blood, either by transfusion or by sharing needles during intravenous drug use, can transmit various bacterial and viral pathogens. The screening of donated blood for *Treponema pallidum*, human immunodeficiency virus (HIV), human T-cell lymphotropic virus, hepatitis B virus, hepatitis C virus, and West Nile virus has greatly reduced the risk of infection by these organisms.

The major bacterial diseases **transmitted by ticks** in the United States are Lyme disease, Rocky Mountain spotted fever, ehrlichiosis, relapsing fever, and tularemia. Of these five diseases, Lyme disease is by far the most common. Ticks of the genus *Ixodes* (deer tick) transmit three infectious diseases: Lyme disease, ehrlichiosis, and babesiosis, a protozoan disease. *Dermacentor* ticks (dog tick) transmit several diseases: Rocky Mountain spotted fever, tularemia, ehrlichiosis, anaplasmosis, and tick paralysis.

Bacteria, viruses, and other microbes can also be transmitted from mother to offspring, a process called **vertical transmission**. The three modes by which organisms are transmitted vertically are across the placenta, within the birth canal during birth, and via breast milk. Table 7–2 describes some medically important organisms that are transmitted vertically. (**Horizontal transmission**, by contrast, is person-to-person transmission that is not from mother to offspring.)

There are four important portals of entry: respiratory tract, gastrointestinal tract, genital tract, and skin (Table 7–3). Important microorganisms and diseases transmitted by water are described in Table 7–4.

The important bacterial diseases transmitted by foods are listed in Table 7–5, and those transmitted by insects are listed in Table 7–6. The specific mode of transmission of each organism is described in the subsequent section devoted to that organism.

Animals are also an important source of organisms that infect humans. They can be either the source (**reservoir**) or the mode of transmission (**vector**) of certain organisms. Diseases for which animals are the reservoirs are called **zoonoses**. The important zoonotic diseases caused by bacteria are listed in Table 7–7.

2. Adherence to Cell Surfaces

Certain bacteria have specialized structures (e.g., **pili**) or produce substances (e.g., **capsules** or **glycocalyces**) that allow them to adhere to the surface of human cells, thereby enhancing their ability to cause disease. These adherence mechanisms are essential for organisms that attach to mucous membranes; mutants

TABLE 7–1 Important Modes of Transmission

Mode of Transmission	Clinical Example	Comment
I. Human to human		
A. Direct contact	Gonorrhea	Intimate contact (e.g., sexual or passage through birth canal)
B. No direct contact	Dysentery	Fecal–oral (e.g., excreted in human feces, then ingested in food or water)
C. Transplacental	Congenital syphilis	Bacteria cross the placenta and infect the fetus
D. Bloodborne	Hepatitis B	Transfused blood or intravenous drug use can transmit bacteria and viruses; screening of blood for transfusions has greatly reduced this risk
II. Nonhuman to human		
A. Soil source	Tetanus	Spores in soil enter wound in skin
B. Water source	Legionnaire's disease	Bacteria in water aerosol are inhaled into lungs
C. Animal source		
1. Directly	Cat-scratch fever	Bacteria enter in cat scratch
2. Via insect vector	Lyme disease	Bacteria enter in tick bite
3. Via animal excreta	Hemolytic-uremic syndrome caused by *E. coli* O157	Bacteria in cattle feces are ingested in undercooked hamburger
D. Fomite source	Staphylococcal skin infection	Bacteria on an object (e.g., a towel) are transferred onto the skin

TABLE 7–2 Vertical Transmission of Some Important Pathogens

Mode of Transmission	Pathogen	Type of Organism[1]	Disease in Fetus or Neonate
Transplacental	*Treponema pallidum*	B	Congenital syphilis
	Listeria monocytogenes[2]	B	Neonatal sepsis and meningitis
	Cytomegalovirus	V	Congenital abnormalities
	Parvovirus B19	V	Hydrops fetalis
	Toxoplasma gondii	P	Toxoplasmosis
Within birth canal/at the time of birth	*Streptococcus agalactiae* (group B *Streptococcus*)	B	Neonatal sepsis and meningitis
	Escherichia coli	B	Neonatal sepsis and meningitis
	Chlamydia trachomatis	B	Conjunctivitis or pneumonia
	Neisseria gonorrhoeae	B	Conjunctivitis
	Herpes simplex type 2	V	Skin, CNS, or disseminated infection (sepsis)
	Hepatitis B virus	V	Hepatitis B
	Human immunodeficiency virus[3]	V	Asymptomatic infection
	Candida albicans	F	Thrush
Breast milk	*Staphylococcus aureus*	B	Oral or skin infections
	Cytomegalovirus	V	Asymptomatic infection
	Human T-cell leukemia virus	V	Asymptomatic infection

CNS = central nervous system.

[1]B, bacterium; V, virus; F, fungus; P, protozoa.

[2]*L. monocytogenes* can also be transmitted at the time of birth.

[3]HIV is transmitted primarily at the time of birth but is also transmitted across the placenta and in breast milk.

TABLE 7–3 Portals of Entry of Some Common Pathogens

Portal of Entry	Pathogen	Type of Organism[1]	Disease
Respiratory tract	*Streptococcus pneumoniae*	B	Pneumonia
	Neisseria meningitides	B	Meningitis
	Haemophilus influenzae	B	Meningitis
	Mycobacterium tuberculosis	B	Tuberculosis
	Influenza virus	V	Influenza
	Rhinovirus	V	Common cold
	Epstein-Barr virus	V	Infectious mononucleosis
	Coccidioides immitis	F	Coccidioidomycosis
	Histoplasma capsulatum	F	Histoplasmosis
Gastrointestinal tract	*Shigella dysenteriae*	B	Dysentery
	Salmonella typhi	B	Typhoid fever
	Vibrio cholerae	B	Cholera
	Norovirus	V	Gastroenteritis
	Rotavirus	V	Gastroenteritis
	Hepatitis A virus	V	Hepatitis A
	Poliovirus	V	Poliomyelitis
	Trichinella spiralis	H	Trichinosis
Skin	*Staphylococcus aureus*	B	Impetigo, boils, cellulitis, folliculitis
	Clostridium tetani	B	Tetanus
	Rickettsia rickettsii	B	Rocky Mountain spotted fever
	Rabies virus	V	Rabies
	Trichophyton rubrum	F	Tinea pedis (athlete's foot)
	Plasmodium vivax	P	Malaria
Genital tract	*Neisseria gonorrhoeae*	B	Gonorrhea
	Treponema pallidum	B	Syphilis
	Chlamydia trachomatis	B	Urethritis
	Human papillomavirus	V	Genital warts
	Herpes simplex virus 2	V	Genital herpes
	Candida albicans	F	Vaginitis

[1]B, bacterium; V, virus; F, fungus; P, protozoa; H, helminth.

TABLE 7–4 Transmission of Important Waterborne Diseases

Portal of Entry	Pathogen	Type of Organism[1]	Disease
Gastrointestinal tract			
1. Ingestion of drinking water	*Salmonella* species	B	Diarrhea
	Shigella species	B	Diarrhea
	Campylobacter jejuni	B	Diarrhea
	Norovirus	V	Diarrhea
	Giardia lamblia	P	Diarrhea
	Cryptosporidium parvum	P	Diarrhea
2. Ingestion of water while swimming[2]	*Leptospira interrogans*	B	Leptospirosis
Respiratory tract			
Inhalation of water aerosol	*Legionella pneumophila*	B	Pneumonia (Legionnaire's disease)
Skin			
Penetration through skin	*Pseudomonas aeruginosa*	B	Hot-tub folliculitis
	Schistosoma mansoni	H	Schistosomiasis
Nose			
Penetration through cribriform plate into meninges and brain	*Naegleria fowleri*	P	Meningoencephalitis

[1]B, bacterium; V, virus; P, protozoa; H, helminth.

[2]All of the organisms that cause diarrhea by ingestion of drinking water also cause diarrhea by ingestion of water while swimming.

TABLE 7–5 Bacterial Diseases Transmitted by Foods

Bacterium	Typical Food	Main Reservoir	Disease
I. Diarrheal diseases			
Gram-positive cocci			
Staphylococcus aureus	Custard-filled pastries; potato, egg, or tuna fish salad	Humans	Food poisoning, especially vomiting
Gram-positive rods			
Bacillus cereus	Reheated rice	Soil	Diarrhea
Clostridium perfringens	Cooked meat, stew, and gravy	Soil, animals, or humans	Diarrhea
Listeria monocytogenes	Unpasteurized milk products	Soil, animals, or plants	Diarrhea, neonatal sepsis
Gram-negative rods			
Escherichia coli	Various foods and water	Humans	Diarrhea
E. coli O157:*H7* strain	Undercooked meat	Cattle	Hemorrhagic colitis, hemolytic-uremic syndrome (HUS)
Salmonella enteritidis	Poultry, meats, and eggs	Domestic animals, especially poultry	Diarrhea
Salmonella typhi	Various foods	Humans	Typhoid fever
Shigella species	Various foods and water	Humans	Diarrhea (dysentery)
Vibrio cholerae	Various foods (e.g., seafood) and water	Humans	Diarrhea
Vibrio parahaemolyticus	Seafood	Warm salt water	Diarrhea
Campylobacter jejuni	Various foods	Domestic animals	Diarrhea
Yersinia enterocolitica	Various foods	Domestic animals	Diarrhea
II. Nondiarrheal diseases			
Gram-positive rods			
Clostridium botulinum	Improperly canned vegetables and smoked fish	Soil	Botulism
Listeria monocytogenes	Unpasteurized milk products	Cows	Sepsis in neonate or mother
Gram-negative rods			
Vibrio vulnificus	Seafood	Warm salt water	Sepsis
Brucella species	Meat and milk	Domestic animals	Brucellosis
Francisella tularensis	Meat	Rabbits	Tularemia
Mycobacteria			
Mycobacterium bovis	Milk	Cows	Intestinal tuberculosis

TABLE 7–6 Bacterial Diseases Transmitted by Insects

Bacterium	Insect	Reservoir	Disease
Gram-negative rods			
Yersinia pestis	Rat fleas	Rodents (e.g., rats and prairie dogs)	Plague
Francisella tularensis	Ticks (*Dermacentor*)	Many animals (e.g., rabbits)	Tularemia
Spirochetes			
Borrelia burgdorferi	Ticks (*Ixodes*)	Mice	Lyme disease
Borrelia recurrentis	Lice	Humans	Relapsing fever
Rickettsiae			
Rickettsia rickettsii	Ticks (*Dermacentor*)	Dogs, rodents, and ticks (*Dermacentor*)	Rocky Mountain spotted fever
Rickettsia prowazekii	Lice	Humans	Epidemic typhus
Ehrlichia chaffeensis	Ticks (*Dermacentor, Ixodes*)	Dogs	Ehrlichiosis
Anaplasma phagocytophilum	Ticks (*Ixodes*)	Dogs, rodents	Anaplasmosis

that lack these mechanisms are often nonpathogenic. For example, the **pili** of *Neisseria gonorrhoeae* and *E. coli* mediate the attachment of the organisms to the urinary tract epithelium, and the **glycocalyx** of *Staphylococcus epidermidis* and certain viridans streptococci allows the organisms to adhere strongly to the endothelium of heart valves. The various molecules that mediate adherence to cell surfaces are called **adhesins**.

After the bacteria attach, they often form a protective matrix called a **biofilm** consisting of various polysaccharides and proteins. Biofilms form especially on foreign bodies such as prosthetic joints, prosthetic heart valves, and intravenous catheters, but they also form on native structures such as heart valves. Biofilms protect bacteria from both antibiotics and host immune defenses such as antibodies and neutrophils. They also retard

TABLE 7–7 Zoonotic Diseases Caused by Bacteria

Bacterium	Main Reservoir	Mode of Transmission	Disease
Gram-positive rods			
Bacillus anthracis	Domestic animals	Direct contact	Anthrax
Listeria monocytogenes	Domestic animals	Ingestion of unpasteurized milk products	Sepsis in neonate or mother
Erysipelothrix rhusiopathiae	Fish	Direct contact	Erysipeloid
Gram-negative rods			
Bartonella henselae	Cats	Skin scratch	Cat-scratch disease
Brucella species	Domestic animals	Ingestion of unpasteurized milk products; contact with animal tissues	Brucellosis
Campylobacter jejuni	Domestic animals	Ingestion of contaminated meat	Diarrhea
Escherichia coli O157:H7	Cattle	Fecal–oral	Hemorrhagic colitis
Francisella tularensis	Many animals, especially rabbits	Tick bite and direct contact	Tularemia
Pasteurella multocida	Cats	Cat bite	Cellulitis
Salmonella enteritidis	Poultry, eggs, and cattle	Fecal–oral	Diarrhea
Yersinia enterocolitica	Domestic animals	Fecal–oral	Diarrhea
Yersinia pestis	Rodents, especially rats and prairie dogs	Rat flea bite	Sepsis
Mycobacteria			
Mycobacterium bovis	Cows	Ingestion of unpasteurized milk products	Intestinal tuberculosis
Spirochetes			
Borrelia burgdorferi	Mice	Tick bite (*Ixodes*)	Lyme disease
Leptospira interrogans	Rats and dogs	Urine	Leptospirosis
Chlamydiae			
Chlamydia psittaci	Psittacine birds, especially parrots and parakeets	Inhalation of aerosols	Psittacosis
Rickettsiae			
Rickettsia rickettsii	Rats and dogs	Tick bite (*Dermacentor*)	Rocky Mountain spotted fever
Coxiella burnetii	Sheep	Inhalation of aerosols of amniotic fluid	Q fever
Ehrlichia chaffeensis	Dogs	Tick bite (*Dermacentor*)	Ehrlichiosis
Anaplasma phagocytophilum	Dogs, rodents	Tick bite (*Ixodes*)	Anaplasmosis

wound healing, resulting in chronic wound infections, especially in diabetics. Biofilms play an important role in the persistence of *Pseudomonas* in the lungs of cystic fibrosis patients and in the formation of dental plaque, the precursor of dental caries.

The production of biofilms by bacteria such as *Pseudomonas* is controlled by the process of **quorum sensing**, which allows bacteria to coordinate the synthesis of particular proteins according to the density of the bacterial population. When the concentration of bacteria is low, these proteins are not expressed; but once the population reaches a critical high cell density, the individual members sense this and begin to synthesize these proteins, resulting in phenotypic changes that benefit the population as a whole. Examples of behaviors that are controlled by quorum sensing include biofilm formation, expression of virulence, and antibiotic resistance, all of which can contribute to pathogenesis.

Foreign bodies, such as artificial heart valves and artificial joints, predispose to infections. Bacteria can adhere to these surfaces, but phagocytes adhere poorly owing to the absence of selectins and other binding proteins on the artificial surface (see Chapter 8).

3. Invasion, Inflammation, & Intracellular Survival

One of the two main mechanisms by which bacteria cause disease is **invasion** of tissue followed by **inflammation**. (The inflammatory response is described in Chapter 8.) The other main mechanism, **toxin production**, and a third mechanism, **immunopathogenesis**, are described later in this chapter.

Several enzymes secreted by invasive bacteria play a role in pathogenesis. Among the most prominent are the following:

(1) **Collagenase** and **hyaluronidase**, which degrade collagen and hyaluronic acid, respectively, thereby allowing the bacteria to spread through subcutaneous tissue; they are especially important in cellulitis caused by *Streptococcus pyogenes.*

(2) **Coagulase**, which is produced by *Staphylococcus aureus* and accelerates the formation of a fibrin clot from its precursor, fibrinogen (this clot may protect the bacteria from phagocytosis by walling off the infected area and by coating the organisms with a layer of fibrin). Coagulase is also produced by *Yersinia pestis*, the cause of bubonic plague. See Chapter 20 for the role of coagulase in the pathogenesis of plague.

(3) **Immunoglobulin proteases**. There are several examples of organisms that produce enzymes that degrade immunoglobulin (Ig) A and IgG. *N. gonorrhoeae, Haemophilus influenzae,* and *Streptococcus pneumoniae* produce IgA proteases, which inactivate this immunoglobulin at the mucosal surface. This leads to better adherence of these organisms to mucous membranes. *S. pyogenes* produces an enzyme that specifically cleaves IgG heavy chains, which reduces opsonization and complement activation, enhancing the virulence of this organism.

In addition to these enzymes, several virulence factors contribute to invasiveness by limiting the ability of the host defense mechanisms, especially phagocytosis, to operate effectively.

(1) The most important of these antiphagocytic factors is the **capsule** external to the cell wall of several important pathogens such as *S. pneumoniae* and *Neisseria meningitidis.* The polysaccharide capsule prevents the phagocyte from adhering to the bacteria; anticapsular antibodies allow more effective phagocytosis to occur (a process called **opsonization**) (see Chapter 8). The vaccines against *S. pneumoniae, H. influenzae,* and *N. meningitidis* contain capsular polysaccharides that induce protective anticapsular antibodies.

(2) A second group of antiphagocytic factors are the cell wall proteins of the gram-positive cocci, such as the M protein of the group A streptococci (*S. pyogenes*) and protein A of *S. aureus.* The M protein is antiphagocytic, and protein A binds to the Fc portion of IgG and prevents the activation of complement. These virulence factors are summarized in Table 7–8.

TABLE 7–8 Surface Virulence Factors Important for Bacterial Pathogenesis

Organism	Virulence Factor	Used in Vaccine	Comments
Gram-positive cocci			
Streptococcus pneumoniae	Polysaccharide capsule	Yes	Determines serotype
Streptococcus pyogenes	M protein	No	Determines serotype[1]
Staphylococcus aureus	Protein A	No	Binds to Fc region of IgG, which prevents activation of complement
Gram-negative cocci			
Neisseria meningitides	Polysaccharide capsule	Yes	Determines serotype
Gram-positive rods			
Bacillus anthracis	Polypeptide capsule	No	
Gram-negative rods			
Haemophilus influenzae	Polysaccharide capsule	Yes	Determines serotype
Klebsiella pneumoniae	Polysaccharide capsule	No	
Escherichia coli	Protein pili	No	Causes adherence
Salmonella typhi	Polysaccharide capsule	Yes	Not important for other salmonellae
Yersinia pestis	V and W proteins	No	

[1]Do not confuse the serotype with the grouping of streptococci, which is determined by the polysaccharide in the cell wall.

(3) Leukocidins are pore-forming toxins that degrade the cell membrane of neutrophils and macrophages. The Panton-Valentine leukocidin produced by *S. aureus* is a good example.

Bacteria can cause two types of inflammation: **pyogenic** and **granulomatous**. In pyogenic (pus-producing) inflammation, neutrophils are the predominant cells. Some of the most important pyogenic bacteria are the gram-positive and gram-negative cocci listed in Table 7–8. In granulomatous inflammation, macrophages and helper T cells predominate. The most important organism in this category is *Mycobacterium tuberculosis.* No bacterial enzymes or toxins that induce granulomas have been identified. Rather, it appears that bacterial antigens stimulate the cell-mediated immune system, resulting in sensitized T-lymphocyte and macrophage activity. Phagocytosis by macrophages kills most of the bacteria, but some survive and grow within the macrophages in the granuloma.

Intracellular survival is an important attribute of certain bacteria that enhances their ability to cause disease. These bacteria are called "intracellular" pathogens and commonly cause granulomatous lesions. The best-known of these bacteria belong to the genera *Mycobacterium, Legionella, Brucella,* and *Listeria.* The best-known fungus is *Histoplasma.* These organisms can be cultured on microbiologic media in the laboratory and therefore are *not* obligate intracellular parasites, which distinguishes them from *Chlamydia* and *Rickettsia.* The intracellular location provides a protective niche from antibody and neutrophils that function extracellularly.

Intracellular bacteria use several different mechanisms to allow them to survive and grow inside cells. These include (1) inhibition of the fusion of the phagosome with the lysosome, which allows the organisms to avoid the degradative enzymes in the lysosome; (2) inhibition of acidification of the phagosome, which reduces the activity of the lysosomal degradative enzymes; and (3) escape from the phagosome into the cytoplasm, where there are no degradative enzymes. Members of the genera *Mycobacterium* and *Legionella* are known to use the first and second mechanisms, whereas *Listeria* species use the third.

The invasion of cells by bacteria is dependent on the interaction of specific bacterial surface proteins called **invasins** and specific cellular receptors belonging to the integrin family of transmembrane adhesion proteins. The movement of bacteria into the cell is a function of actin microfilaments. Once inside the cell, these bacteria typically reside within cell vacuoles such as phagosomes. Some remain there, others migrate into the cytoplasm, and some move from the cytoplasm into adjacent cells. Infection of the surrounding cells in this manner allows the bacteria to evade host defenses. For example, *Listeria monocytogenes* aggregates actin filaments on its surface and is propelled in a "sling-shot" fashion, called **actin rockets**, from one host cell to another.

The "Yops" (*Yersinia* outer-membrane proteins) produced by several *Yersinia* species are important examples of bacterial virulence factors that act primarily after invasion of human cells by the organism. The most important effects of the Yops are to inhibit phagocytosis by neutrophils and macrophages and to inhibit cytokine production (e.g., tumor necrosis factor [TNF] production) by macrophages. For example, one of the Yops of *Y. pestis* (Yop J) is a protease that cleaves signal transduction proteins required for the induction of TNF synthesis. This inhibits the activation of our host defenses and contributes to the ability of the organism to cause bubonic plague.

The genes that encode many virulence factors in bacteria are clustered in **pathogenicity islands** located on the bacterial chromosome or plasmids. For example, in many bacteria, the genes encoding adhesins, invasins, and exotoxins are adjacent to each other on these islands. Nonpathogenic variants of these bacteria do not have these pathogenicity islands. It appears that these large regions of the bacterial genome were transferred as a block via conjugation or transduction. Pathogenicity islands are found in many gram-negative rods, such as *E. coli, Salmonella, Shigella, Pseudomonas,* and *Vibrio cholerae*, and in gram-positive cocci, such as *S. pneumoniae*.

After bacteria have colonized and multiplied at the portal of entry, they may invade the bloodstream and spread to other parts of the body. Receptors for the bacteria on the surface of cells determine, in large part, the organs affected. For example, certain bacteria or viruses infect the brain because receptors for these microbes are located on the surface of brain neurons. The *blood–brain barrier,* which limits the ability of certain drugs to penetrate the brain, is not thought to be a determinant of microbial infection of the brain. The concept of a blood–brain barrier primarily refers to the inability of hydrophilic (charged, ionized) drugs to enter the lipid-rich brain parenchyma, whereas lipophilic (lipid-soluble) drugs enter well.

Two important diseases, diphtheria and pseudomembranous colitis, are characterized by inflammatory lesions called **pseudomembranes**. Pseudomembranes are thick, adherent, grayish or yellowish exudates on the mucosal surfaces of the throat in diphtheria and on the colon in pseudomembranous colitis. The term *pseudo* refers to the abnormal nature of these membranes in contrast to the normal anatomic membranes of the body, such as the tympanic membrane and the placental membranes.

4. Toxin Production

The second major mechanism by which bacteria cause disease is the production of toxins. A comparison of the main features of **exotoxins** and **endotoxins** is shown in Table 7–9.

Exotoxins

Exotoxins are produced by several gram-positive and gram-negative bacteria, in contrast to endotoxins, which are present only in gram-negative bacteria. The essential characteristic of exotoxins is that they are **secreted** by the bacteria, whereas endotoxin is a component of the cell wall. Exotoxins are polypeptides whose genes are frequently located on plasmids or lysogenic bacterial viruses (bacteriophages). Some important exotoxins encoded by bacteriophage DNA are diphtheria toxin, cholera toxin, and botulinum toxin.

TABLE 7–9 Main Features of Exotoxins and Endotoxins

	Comparison of Properties	
Property	**Exotoxin**	**Endotoxin**
Source	Certain species of gram-positive and gram-negative bacteria	Cell wall of gram-negative bacteria
Secreted from cell	Yes	No
Chemistry	Polypeptide	Lipopolysaccharide
Location of genes	Plasmid or bacteriophage	Bacterial chromosome
Toxicity	High (fatal dose on the order of 1 μg)	Low (fatal dose on the order of hundreds of micrograms)
Clinical effects	Various effects (see text)	Fever, shock
Mode of action	Various modes (see text)	Includes TNF and interleukin-1
Antigenicity	Induces high-titer antibodies called antitoxins	Poorly antigenic
Vaccines	Toxoids used as vaccines	No toxoids formed and no vaccine available
Heat stability	Destroyed rapidly at 60°C (except staphylococcal enterotoxin)	Stable at 100°C for 1 hour
Typical diseases	Tetanus, botulism, diphtheria	Meningococcemia, sepsis by gram-negative rods

TNF = tumor necrosis factor.

Exotoxins are among the **most toxic** substances known. For example, the fatal dose of tetanus toxin for a human is estimated to be less than 1 μg. Because some purified exotoxins can reproduce all aspects of the disease, we can conclude that certain bacteria play no other role in pathogenesis than to synthesize the exotoxin. Exotoxin polypeptides are good antigens and induce the synthesis of protective antibodies called antitoxins, some of which are useful in the prevention or treatment of diseases such as botulism and tetanus. When treated with formaldehyde (or acid or heat), the exotoxin polypeptides are converted into **toxoids**, which are used in protective vaccines because they retain their antigenicity but have lost their toxicity.

Many exotoxins have an **A–B subunit** structure; the A (or active) subunit possesses the toxic activity, and the B (or binding) subunit is responsible for binding the exotoxin to specific receptors on the membrane of the human cell. The binding of the B subunit determines the specific site of the action of the exotoxin. For example, botulinum toxin acts at the neuromuscular junction because the B subunit binds to specific receptors on the surface of the motor neuron at the junction. Important exotoxins that have an A–B subunit structure include diphtheria toxin, tetanus toxin, botulinum toxin, cholera toxin, and the enterotoxin of *E. coli* (Figure 7–1).

The A subunit of several important exotoxins acts by catalyzing the addition of adenosine diphosphate ribose (ADP-ribose) to the target protein in the human cell (**ADP-ribosylation**). The modification of target proteins with ADP-ribose often inactivates it but can also hyperactivate it, either of which can cause the symptoms of disease. For example, diphtheria toxin and *Pseudomonas* exotoxin A ADP-ribosylate elongation factor-2 (EF-2), an essential factor required for eukaryotic protein synthesis. This modification inactivates EF-2, freezing the translocation complex, and results in the inhibition of protein synthesis.

On the other hand, cholera toxin and *E. coli* toxin ADP-ribosylate G_s protein, thereby activating it. This causes an increase in adenylate cyclase activity, a consequent increase in the amount of cyclic adenosine monophosphate (AMP), and the production of watery diarrhea. Pertussis toxin is an interesting variation on the theme. It ADP-ribosylates G_i protein and inactivates it. Inactivation of the inhibitory G proteins turns on adenylate cyclase, causing an increase in the amount of cyclic AMP, which plays a role in causing the symptoms of whooping cough.

Exotoxins are released from bacteria by specialized structures called **secretion systems**. Some secretion systems transport the exotoxins into the extracellular space, but others transport the exotoxins directly into the mammalian cell. Those that transport the exotoxins directly into the mammalian cell are especially effective because the exotoxin is not exposed to antibodies in the extracellular space.

Several classes of bacterial secretion systems (six and counting) have been identified, but the **type III secretion system** (also called an injectosome) is particularly important in virulence. This secretion system is mediated by a needlelike projection (sometimes called a "molecular syringe") and by

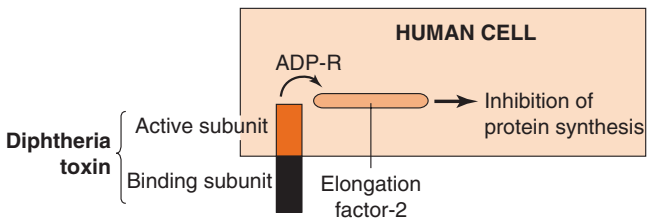

FIGURE 7–1 Mode of action of diphtheria toxin. The toxin binds to the cell surface via its binding subunit, and the active subunit enters the cell. The active subunit is an enzyme that catalyzes the addition of ADP-ribose (ADP-R) to elongation factor-2 (EF-2). This inactivates EF-2, and protein synthesis is inhibited.

TABLE 7–10 Important Bacterial Exotoxins

Bacterium	Disease	Mode of Action	Toxoid Vaccine
Gram-positive rods			
Corynebacterium diphtheriae	Diphtheria	Inactivates EF-2 by ADP-ribosylation	Yes
Clostridium tetani	Tetanus	Blocks release of the inhibitory neurotransmitter glycine by proteolytic cleavage of releasing proteins	Yes
Clostridium botulinum	Botulism	Blocks release of acetylcholine by proteolytic cleavage of releasing proteins	Yes[1]
Clostridium difficile	Pseudomembranous colitis	Exotoxins A and B inactivate GTPases by glucosylation	No
Clostridium perfringens	Gas gangrene	Alpha toxin is a lecithinase; enterotoxin is a superantigen	No
Bacillus anthracis	Anthrax	Edema factor is an adenylate cyclase; lethal factor is a protease that cleaves MAP kinase, which is required for cell division	No
Gram-positive cocci			
Staphylococcus aureus	1. Toxic shock syndrome	Is a superantigen; binds to class II MHC protein and T-cell receptor; induces IL-1 and IL-2	No
	2. Food poisoning	Is a superantigen acting locally in the gastrointestinal tract	No
	3. Scalded skin syndrome	Is a protease that cleaves desmoglein in desmosomes	No
Streptococcus pyogenes	Scarlet fever	Is a superantigen; action similar to toxic shock syndrome toxin of *S. aureus*	No
Gram-negative rods			
Escherichia coli	1. Watery diarrhea	Labile toxin stimulates adenylate cyclase by ADP-ribosylation; stable toxin stimulates guanylate cyclase	No
	2. Bloody diarrhea	Shiga toxin inhibits protein synthesis in enterocytes by removing adenine from 28S ribosomal RNA	No
Shigella dysenteriae	Bloody diarrhea	Shiga toxin inhibits protein synthesis in enterocytes by removing adenine from 28S ribosomal RNA	No
Vibrio cholerae	Cholera	Stimulates adenylate cyclase by ADP-ribosylation	No
Bordetella pertussis	Whooping cough	Stimulates adenylate cyclase by ADP-ribosylation; inhibits chemokine receptor	Yes[2]

[1]For high-risk individuals only.

[2]The acellular vaccine contains pertussis toxoid and four other proteins.

transport pumps in the bacterial cell membrane. The importance of the type III secretion system is illustrated by the finding that the strains of *Pseudomonas aeruginosa* that have this secretion system are significantly more virulent than those that do not. Other medically important gram-negative rods that utilize injectosomes include *Shigella* species, *Salmonella* species, *E. coli*, and *Y. pestis*.

The mechanisms of action of the important exotoxins produced by toxigenic bacteria are described in the following discussion and summarized in Tables 7–10, 7–11, and 7–12.

TABLE 7–11 Important Mechanisms of Action of Bacterial Exotoxins

Mechanism of Action	Exotoxin
ADP-ribosylation	Diphtheria toxin, cholera toxin, *Escherichia coli* heat-labile toxin, and pertussis toxin
Superantigen	Toxic shock syndrome toxin, staphylococcal enterotoxin, and erythrogenic toxin
Protease	Tetanus toxin, botulinum toxin, lethal factor of anthrax toxin, and scalded skin toxin
Lecithinase	*Clostridium perfringens* alpha toxin

The main location of symptoms of disease caused by bacterial exotoxins is described in Table 7–13.

Gram-Positive Bacteria

The exotoxins produced by gram-positive bacteria have several different mechanisms of action and produce different clinical effects. Some important exotoxins include diphtheria toxin, which inhibits protein synthesis by inactivating EF-2; tetanus toxin and botulinum toxin, which are neurotoxins that prevent

TABLE 7–12 Exotoxins That Increase Intracellular Cyclic AMP

Bacterium	Exotoxin	Mode of Action
Vibrio cholerae	Cholera toxin	ADP-ribosylates G_s factor, which activates it, thereby stimulating adenylate cyclase
Escherichia coli	Labile toxin	Same as cholera toxin
Bordetella pertussis	Pertussis toxin	ADP-ribosylates G_i factor, which inactivates it, thereby stimulating adenylate cyclase
Bacillus anthracis	Edema factor of anthrax toxin	Is an adenylate cyclase

TABLE 7–13 Main Location of Symptoms of Disease Caused by Bacterial Exotoxins

Main Location of Symptoms	Organism	Mode of Action Exotoxin
Gastrointestinal tract		
1. Gram-positive cocci	*Staphylococcus aureus*	Enterotoxin is a superantigen
2. Gram-positive rods	*Clostridium difficile*	Inactivates GTPases in enterocytes
	Clostridium perfringens	Superantigen
	Bacillus cereus	Superantigen
3. Gram-negative rods	*Vibrio cholera*	Stimulates adenylate cyclase
	Toxigenic *Escherichia coli*	Stimulates adenylate cyclase
	E. coli O157	Inactivates protein synthesis
Nervous system		
1. Gram-positive rods	*Clostridium tetani*	Inhibits glycine release
	Clostridium botulinum	Inhibits acetylcholine release
Respiratory tract		
1. Gram-positive rods	*Corynebacterium diphtheriae*	Inactivates protein synthesis
2. Gram-negative rods	*Bordetella pertussis*	Stimulates adenylate cyclase; inhibits chemokine receptor
Skin, soft tissue, or muscle		
1. Gram-positive cocci	*S. aureus* (scalded skin syndrome)	Protease cleaves desmosome in skin
	S. aureus (MRSA strains)	PV leukocidin is a pore-forming toxin that disrupts cell membrane
	Streptococcus pyogenes (scarlet fever)	Erythrogenic toxin is a superantigen
2. Gram-positive rods	*C. perfringens*	Lecithinase cleaves cell membranes
	Bacillus anthracis	Edema factor is an adenylate cyclase; lethal factor is a protease
Systemic		
1. Gram-positive cocci	*S. aureus*	Toxic shock syndrome toxin is a superantigen

MRSA = methicillin-resistant *Staphylococcus aureus*; PV = Panton-Valentine.

the release of neurotransmitters; and toxic shock syndrome toxin (TSST), which acts as a superantigen causing the release of large amounts of cytokines from helper T cells and macrophages. The mechanisms of action and the clinical effects of exotoxins produced by gram-positive bacteria are described next.

(1) Diphtheria toxin, produced by *Corynebacterium diphtheriae*, inhibits protein synthesis by ADP-ribosylation of EF-2 (see Figure 7–1).[1]

The resulting death of the affected cells leads to two prominent symptoms of diphtheria: pseudomembrane formation in the throat and myocarditis.

The exotoxin activity depends on two functions mediated by different domains of the molecule. The toxin is synthesized as a single polypeptide that is nontoxic because the active site of the enzyme is masked. This molecule is cleaved and modified to yield two active polypeptides. Fragment A, derived from the amino-terminal end of the exotoxin, yields an enzyme that catalyzes the transfer of ADP-ribose from nicotinamide adenine dinucleotide (NAD) to EF-2, inhibiting protein synthesis. Fragment B, derived from the carboxy-terminal end, binds to receptors on the outer membrane of eukaryotic cells and mediates transport of fragment A into the cells.

As the bacteria synthesize and secrete the full-length exotoxin, the carboxy-terminal end binds to host cell membrane receptors. The toxin is transported across the cell membrane, triggering cleavage and modification that result in active fragment A, which then targets and inactivates EF-2. The specificity for this protein is due to a unique amino acid, a modified histidine called diphthamide, that is present only on EF-2. Since all eukaryotic cells carry out protein synthesis, there is no tissue or organ specificity. Prokaryotic and mitochondrial protein synthesis are not affected because a different, nonsusceptible elongation factor is involved. The enzyme activity is remarkably potent; a single molecule of fragment A will kill a cell within a few hours. Other organisms whose exotoxins act by ADP-ribosylation are *E. coli, V. cholerae,* and *Bordetella pertussis*.

The *tox* gene, which codes for this exotoxin, is carried by a lysogenic bacteriophage called beta phage. As a result, only *C. diphtheriae* strains lysogenized by this phage cause diphtheria. (Nonlysogenized *C. diphtheriae* can be found in the throat of some healthy people.) This is an important example of *lysogenic conversion*, the process by which bacteria acquire new traits when lysogenized by a bacteriophage (see Chapter 4). Regulation of exotoxin synthesis is controlled by the interaction of iron in the medium with a *tox* gene repressor synthesized by the bacterium. As the concentration of iron increases, the iron-repressor complex inhibits the transcription of the *tox* gene.

(2) Tetanus toxin, produced by *Clostridium tetani*, is a **neurotoxin** that prevents release of an inhibitory neurotransmitter involved in muscle relaxation. When the inhibitory neurons are nonfunctional, the excitatory neurons are unopposed, leading to muscle spasms and a spastic paralysis. Tetanus toxin (tetanospasmin) is composed of two polypeptide subunits encoded by plasmid DNA. The heavy chain of the polypeptide binds to gangliosides in the membrane of the

[1] *Pseudomonas aeruginosa* exotoxin A has the same mode of action.

neuron; the light chain is a protease that degrades the protein(s) responsible for the release of the inhibitory neurotransmitters (γ-aminobutyric acid [GABA] and glycine). The toxin released at the site of the peripheral wound may travel either by retrograde axonal transport or in the bloodstream to the anterior horn and interstitial neurons of the spinal cord. Inhibiting the release of the GABA and glycine leads to convulsive contractions of the voluntary muscles, best exemplified by spasm of the jaw and neck muscles ("lockjaw").

(3) Botulinum toxin, produced by *Clostridium botulinum*, is a **neurotoxin** that blocks the release of a different neurotransmitter, acetylcholine, at the synapse of the neuromuscular junction, producing a flaccid paralysis. Approximately 1 μg is lethal for humans; it is one of the most toxic compounds known. The toxin is composed of two polypeptide subunits held together by disulfide bonds. One of the subunits binds to a receptor on the neuron; the other subunit is a protease that degrades the protein(s) responsible for the release of acetylcholine. There are six serotypes of botulinum toxin (A–F), with toxins A, B, E, and F being the most important for human disease. Some serotypes are encoded on a plasmid, some on a temperate bacteriophage, and some on the bacterial chromosome.

(4) Two exotoxins are produced by *Clostridium difficile*, both of which are involved in the pathogenesis of pseudomembranous colitis. Exotoxin A is an enterotoxin that causes watery diarrhea. Exotoxin B is a **cytotoxin** that damages the colonic mucosa and causes pseudomembranes to form. Exotoxins A and B are glucosyltransferases that modify target signal transduction proteins (Rho GTPases), which interferes with their signal transduction function. Glucosylation by exotoxin B causes disaggregation of actin filaments in the cytoskeleton, leading to apoptosis and cell death.

(5) Multiple toxins are produced by *Clostridium perfringens* and other species of clostridia that cause gas gangrene. A total of seven lethal factors and five enzymes have been characterized, but no species of *Clostridium* makes all 12 products. The best characterized is the **alpha toxin**, which is a **lecithinase** that hydrolyzes lecithin in the cell membrane, resulting in destruction of the membrane and widespread cell death. The other four enzymes are collagenase, protease, hyaluronidase, and deoxyribonuclease (DNase). The seven lethal toxins are a heterogeneous group with hemolytic and necrotizing activity. Certain strains of *C. perfringens* produce an enterotoxin that causes watery diarrhea. This enterotoxin acts as a superantigen similar to the enterotoxin of *S. aureus* (described below).

(6) Three exotoxins are produced by *Bacillus anthracis*, the agent of anthrax: edema factor, lethal factor, and protective antigen. The three exotoxins associate with each other, but each component has a distinct function. **Edema factor** is an adenylate cyclase that raises the cyclic AMP concentration within the cell, resulting in loss of chloride ions and water and consequent edema formation in the tissue (see Table 7–12). **Lethal factor** is a protease that cleaves a phosphokinase required for the signal transduction pathway that controls cell growth. Loss of the phosphokinase results in the failure of cell growth and consequent cell death. **Protective antigen** binds to a cell surface receptor and forms pores in the human cell membrane that allow edema factor and lethal factor to enter the cell. The name *protective antigen* is based on the finding that antibody against this protein protects against disease. The antibody blocks the binding of protective antigen, thereby preventing edema factor and lethal factor from entering the cell.

(7) TSST is a **superantigen** produced primarily by certain strains of *S. aureus* but also by certain strains of *S. pyogenes*. TSST binds directly to class II major histocompatibility (MHC) proteins on the surface of antigen-presenting cells (macrophages) without intracellular processing. This complex interacts with the T-cell receptor of many helper T cells, resulting in activation of these T cells (see the discussion of superantigens in Chapter 58). This causes the release of large amounts of interleukins, especially interleukin-1, interleukin-2, and TNF. These cytokines produce many of the signs and symptoms of toxic shock.

(8) Staphylococcal enterotoxin is also a superantigen, but because it is ingested, it acts locally on the lymphoid cells lining the small intestine. The enterotoxin is produced by *S. aureus* in the contaminated food and causes food poisoning, usually within 1–6 hours after ingestion. The main symptoms are vomiting and watery diarrhea. The prominent vomiting seen in food poisoning is caused by cytokines released from the lymphoid cells stimulating the enteric nervous system, which activates the vomiting center in the brain.

(9) Exfoliatin is a protease produced by *S. aureus* that causes scalded skin syndrome. Exfoliatin cleaves desmoglein, a protein in the desmosomes of the skin, resulting in the detachment of the superficial layers of the skin. Exfoliatin is also called epidermolytic toxin.

(10) Panton-Valentine (PV) leukocidin is a pore-forming exotoxin produced by methicillin-resistant strains of *S. aureus* (MRSA). It destroys white blood cells, skin, and subcutaneous tissue. The two subunits of the toxin assemble in the cell membrane to form a pore through which cell contents exit into the extracellular space.

(11) Erythrogenic toxin, produced by *S. pyogenes*, causes the rash characteristic of scarlet fever. Its mechanism of action is similar to that of TSST (i.e., it acts as a superantigen). The DNA that codes for the toxin resides on a lysogenic bacteriophage. Nonlysogenic bacteria do not cause scarlet fever, although they can cause pharyngitis.

(12) Exotoxin B is a protease produced by strains of *S. pyogenes* that cause necrotizing fasciitis. These strains are called "flesh-eating" streptococci.

Gram-Negative Bacteria

The exotoxins produced by gram-negative bacteria also have several different mechanisms of action and produce different clinical effects. Two very important exotoxins are the enterotoxins of *E. coli* and *V. cholerae* (cholera toxin), which induce an increase in the amount of cyclic AMP within the enterocyte,