TEXTBOOK OF DIAGNOSTIC MICROBIOLOGY



FOURTH EDITION

Connie R. Mahon
Donald C. Lehman
George Manuselis

SAUNDERS
ELSEVIER

evolve
learning system

SAUNDERS
ELSEVIER

3251 Riverport Lane
Maryland Heights, Missouri 63043

TEXTBOOK OF DIAGNOSTIC MICROBIOLOGY ISBN: 978-1-4160-6165-6
FOURTH EDITION
Copyright © 2023 by W.B. Saunders Company

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without written permission of the publisher.

Notice

Medicine is an ever-changing field. Standard safety precautions must be followed, but as new research and clinical experience broaden our knowledge, changes in treatment and drug therapy may become necessary or appropriate. Readers are advised to check the most current product information provided by the manufacturer of each drug to be administered to verify the recommended dose, the method and duration of administration, and contraindications. It is the responsibility of the treating physician, relying on experience and knowledge of the patient, to determine dosages and the best treatment for each individual patient. Neither the Publisher nor the editor assume any liability for any injury and/or damage to persons or property arising from this publication.

Permission to photocopy or reproduce solely for internal or personal use is permitted for libraries or other users registered with the Copyright Clearance Center, provided that the base fee of $4.00 per chapter plus $.10 per page is paid directly to the Copyright Clearance Center, 222 Rosewood Drive, Danvers, MA, 01923. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promotional purposes, for creating new collected works, or for resale.

Previous editions copyrighted 2015, 2007, 2000, 1995

Library of Congress Cataloging-in-Publication Data

Textbook of diagnostic microbiology / [edited by] Connie R. Mahon, Donald C. Lehman, George Manuselis.—4th ed.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-1-4160-6165-6 (pbk. : alk. paper) 1. Diagnostic microbiology. I. Mahon, Connie R. II. Lehman, Donald C. III. Manuselis, George.
[DNLM: 1. Microbiological Techniques. 2. Communicable Diseases—diagnosis. 3. Laboratory Techniques and Procedures. QW 25 T355 2011]
QR67.T49 2011
616.9′041—dc22

2009036108

Publishing Director: Andrew Allen
Managing Editor: Ellen Wurm-Cutter
Publishing Services Manager: Patricia Tannian
Senior Project Manager: Sharon Corell
Designer: Teresa McBryan

Printed in USA

Last digit is the print number: 9 8 7 6 5 4 3 2 1

Working together to grow
libraries in developing countries
www.elsevier.com | www.bookaid.org | www.sabre.org
ELSEVIER BOOK AID International Sabre Foundation

Acknowledgments

We are grateful to all contributing authors, students, and instructors, and to many other individuals who have made significant suggestions and invaluable comments on ways to improve this edition.

Connie R. Mahon
Donald C. Lehman
George Manuselis

Contents

PART I
INTRODUCTION TO CLINICAL MICROBIOLOGY

1 Bacterial Cell Structure, Physiology, Metabolism, and Genetics 2
2 Host-Parasite Interaction 23
3 Laboratory Role in Infection Control 48
4 Control of Microorganisms 61
5 Performance Improvement in the Microbiology Laboratory 93
6 Specimen Collection and Processing 111
7 Microscopic Examination of Infected Materials 126
8 Use of Colonial Morphology for the Presumptive Identification of Microorganisms 170
9 Biochemical Identification of Gram-Negative Bacteria 182
10 Immunodiagnosis of Infectious Diseases 200
11 Applications of Molecular Diagnostics 232
12 Antibiotic Mechanisms of Action and Resistance 261
13 Antimicrobial Susceptibility Testing 276

PART II
LABORATORY IDENTIFICATION OF SIGNIFICANT ISOLATES

14 Staphylococci 316
15 *Streptococcus, Enterococcus,* and Other Catalase-Negative Gram-Positive Cocci 330
16 Aerobic Gram-Positive Bacilli 352
17 *Neisseria* Species and *Moraxella catarrhalis* 376
18 *Haemophilus* and Other Fastidious Gram-Negative Bacilli 395
19 Enterobacteriaceae 427
20 *Vibrio, Aeromonas, Plesiomonas* and *Campylobacter* Species 462
21 Nonfermenting and Miscellaneous Gram-Negative Bacilli 482
22 Anaerobes of Clinical Importance 502
23 The Spirochetes 539
24 *Chlamydia* and *Rickettsia* 548
25 *Mycoplasma* and *Ureaplasma* 564
26 *Mycobacterium tuberculosis* and Other Nontuberculosis Mycobacteria 575
27 Medically Significant Fungi 603
28 Diagnostic Parasitology 639
29 Clinical Virology 703
30 Agents of Bioterror 741
31 Biofilms: Architects of Disease 758

PART III
LABORATORY DIAGNOSIS OF INFECTIOUS DISEASES: AN ORGAN SYSTEM APPROACH TO DIAGNOSTIC MICROBIOLOGY

32 Upper and Lower Respiratory Tract Infections 770
33 Skin and Soft Tissue Infections 805
34 Gastrointestinal Infections and Food Poisoning 834
35 Infections of the Central Nervous System 853
36 Bacteremia and Sepsis 867
37 Urinary Tract Infections 883
38 Genital Infections and Sexually Transmitted Diseases 901
39 Infections in Special Populations 927
40 Zoonotic Diseases 935
41 Ocular Infections 948

PART I

Introduction to Clinical Microbiology

CHAPTER 1

Bacterial Cell Structure, Physiology, Metabolism, and Genetics

*George Manuselis, Connie R. Mahon**

- SIGNIFICANCE
- OVERVIEW OF THE MICROBIAL WORLD
  - Bacteria
  - Parasites
  - Fungi
  - Viruses
- CLASSIFICATION/TAXONOMY
  - Nomenclature
  - Classification by Phenotypic and Genotypic Characteristics
  - Classification by Cellular Type: Prokaryotes, Eukaryotes, and Archaeobacteria
- COMPARISON OF PROKARYOTIC AND EUKARYOTIC CELL STRUCTURE
  - Prokaryotic Cell Structure
  - Eukaryotic Cell Structure
- BACTERIAL MORPHOLOGY
  - Microscopic Shapes
  - Common Stains Used for Microscopic Visualization
- MICROBIAL GROWTH AND NUTRITION
  - Nutritional Requirements for Growth
  - Environmental Factors Influencing Growth
  - Bacterial Growth
- BACTERIAL BIOCHEMISTRY AND METABOLISM
  - Metabolism
  - Fermentation and Respiration
  - Biochemical Pathways from Glucose to Pyruvic Acid
  - Anaerobic Utilization of Pyruvic Acid (Fermentation)
  - Aerobic Utilization of Pyruvate (Oxidation)
  - Carbohydrate Utilization and Lactose Fermentation
- BACTERIAL GENETICS
  - Anatomy of a DNA and RNA Molecule
  - Terminology
  - Genetic Elements and Alterations
  - Mechanisms of Gene Transfer

OBJECTIVES

After reading and studying this chapter, you should be able to:

1. Describe microbial classification (taxonomy) and accurately apply the rules of scientific nomenclature for bacterial names.
2. List and define five methods used by epidemiologists to subdivide bacterial species.
3. Differentiate between prokaryotic (bacterial and archaeobacteria) and eukaryotic cell types.
4. Compare and contrast prokaryotic and eukaryotic cytoplasmic and cell envelope structures and functions.
5. Describe the cell walls of gram-positive and gram-negative bacteria. Explain the Gram stain reaction of each cell wall type. Describe two other bacterial cell wall types, and give microbial examples of each.
6. Explain the use of the following stains in the diagnostic microbiology laboratory: Gram stain, acid-fast stains (Ziehl-Neelsen, Kinyoun, auramine-rhodamine), acridine orange, methylene blue, calcofluor white, lactophenol cotton blue, and India ink).
7. List the nutritional and environmental requirements for bacterial growth and define the categories of media used for culturing bacteria in the laboratory.
8. Define the atmospheric requirements of obligate aerobes, microaerophiles, facultative anaerobes, obligate anaerobes, and capnophilic bacteria.
9. Describe the stages in the growth of bacterial cells.
10. Describe the importance of microbial metabolism in clinical microbiology.
11. Differentiate between fermentation and oxidation (respiration).

*This chapter was prepared by C.R. Mahon in her private capacity. No official support or endorsement by the FDA is intended or implied.

12. Name and compare three biochemical pathways that bacteria use to convert glucose to pyruvate.
13. Identify and compare the two types of fermentation that explain positive results with the methyl red or Voges-Proskauer test.
14. Define the following genetic terms: *genotype, phenotype, constitutive, inducible, replication, transcription, translation, genome, chromosome, plasmids, IS element, transposon, point mutations, frame-shift mutations,* and *recombination.*
15. Discuss the development and transfer of antibiotic resistance in bacteria.
16. Differentiate among the mechanisms of transformation, transduction, and conjugation in the transfer of genetic material from one bacterium to another.
17. Define the terms *bacteriophage, lytic phage, lysogeny,* and *temperate phage.*
18. Define *restriction endonuclease enzyme* and explain the use of such enzymes in the clinical microbiology laboratory.

Case in Point

A 4-year-old girl had the presenting symptoms of redness, burning, and light sensitivity in both eyes. She also complained of her eyelids sticking together because of exudative discharge. A Gram stain of the conjunctival exudates (product of acute inflammation with white blood cells and fluid) showed gram-positive intracellular and extracellular, faint-staining, coccobacillary organisms. The organisms appeared to have small, clear, nonstaining "halos" surrounding each one. This clear area was noted to be between the stained organism and the amorphous (no definite form; shapeless) background material. The Gram stain of the stock staphylococci (gram-positive) *Escherichia coli* (gram negative) showed gram-positive reactions for both organisms on review of the stained quality control organisms.

Issues to Consider

After reading the case study, consider:

- The role of microscopic morphology in identification
- Significance of the clear halo surrounding the organism
- Importance of quality control procedures in the identification process
- Unique characteristics; for example, metabolic and physiologic pathways, and cell structure, which produce infection and disease in hosts

Key Terms

Acid-fast
Aerotolerant anaerobes
Anticodon
Archaea
Archaeobacteria
Autotrophs
Bacteria
Bacteriophage
Capnophilic
Capsule
Codon
Competent
Conjugation
Differential media
Dimorphic
Eukarya
Eukaryotes
Facultative anaerobes
Family
Fermentation
Fimbriae
Flagella
Fusiform
Genotype
Genus
Gram-negative
Gram-positive
Heterotrophs
Hyphae
Lysogeny
Mesophiles
Microaerophilic
Minimal medium
Mycelia
Nutrient media
Obligate aerobes
Obligate anaerobes
Pathogenic bacteria
Phenotype
Phyla
Pili
Plasmids
Pleomorphic
Prokaryotes
Protein expression
Psychrophiles
Respiration
Restriction enzymes
Selective media
Species
Spores
Strains
Taxa
Taxonomy
Temperate
Thermophiles
Transduction
Transformation
Transport medium

In this chapter the basic concepts of prokaryotic and eukaryotic cells, viral and bacterial cell structure physiology, metabolism, and genetics are reviewed. Common stains used to visualize microorganisms microscopically also are presented. The reader is made aware of the practical importance of each topic to diagnostic microbiologists in their efforts to culture, identify, and characterize the microbes that cause disease in humans. As presented in the opening Case in Point, proper characterization of the bacterial cells in human samples is critical in the correct identification of the infecting organism.

SIGNIFICANCE

Microbial inhabitants have evolved to survive in a variety of ecologic niches (way in which an organism uses its resources) and habitats (organism's location and where its resources may be found). Some grow rapidly, some slowly. Some can replicate with a minimal number of nutrients present, whereas others require enriched nutrients to survive. Variation exists in atmospheric growth conditions, temperature requirements,

and cell structure. This diversity is also found in the microorganisms that inhabit the human body as normal biota (normal flora), as opportunistic pathogens, or as true pathogens. Each microbe has its own unique physiology and metabolic pathways that allow it to survive in its particular habitat. One of the main roles of a diagnostic or clinical microbiologist is to isolate, identify, and analyze the bacteria that cause disease in humans. Knowledge of microbial structure and physiology is extremely important to clinical microbiologists in three areas:

- Culture of organisms from patient specimens
- Classification and identification of organisms after they have been isolated
- Prediction and interpretation of antimicrobial susceptibility patterns

Understanding the growth requirements of a particular bacterium enables the microbiologist to select the correct medium for primary culture and optimize the chance of isolating the pathogen. Determination of staining characteristics, based on differences in cell wall structure, is the first step in bacterial classification. Metabolic biochemical differences between organisms form the basis for most bacterial identification systems in use today. The cell structure and biochemical pathways of an organism determine its susceptibility to various antibiotics.

The ability of microorganisms to change rapidly, acquire new genes, and undergo mutations presents continual challenges to diagnostic microbiologists as they isolate and characterize the microorganisms associated with humans.

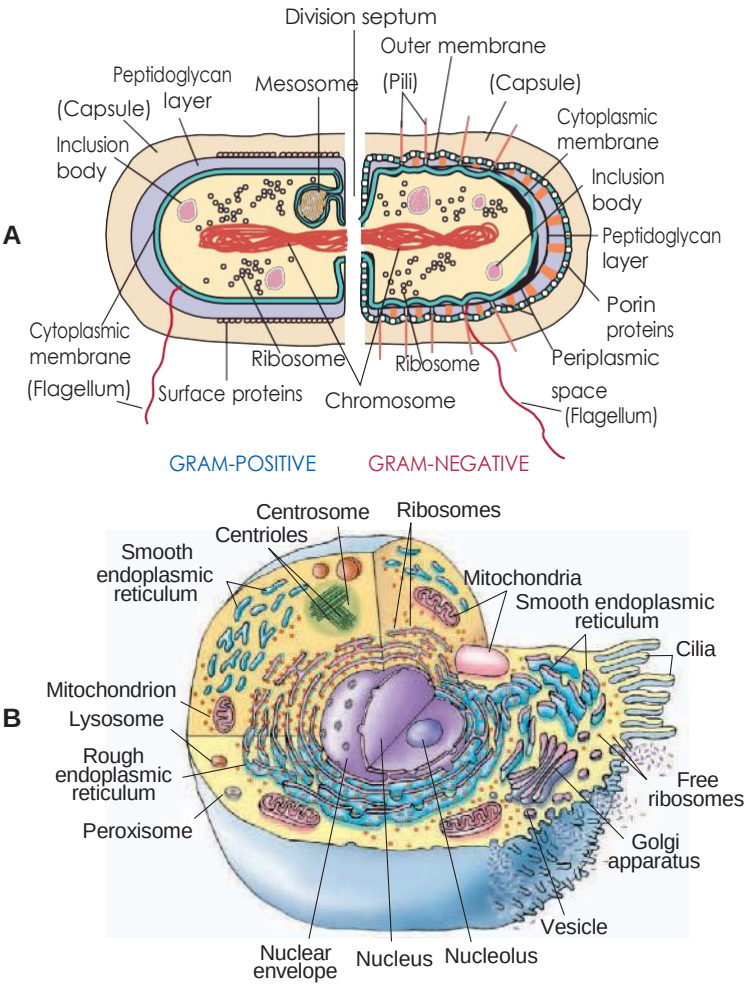

FIGURE 1-1 Comparison of prokaryotic and eukaryotic cell organization and structures. **A,** Prokaryotic gram-positive and gram-negative bacteria. (From Murray PR, Rosenthal KS, Pfaller MA: *Medical microbiology*, ed 6, Philadelphia, 2009 Mosby.) **B,** Structure of the generalized eukaryotic cell. (From Thibodeau GA, Patton KT: *Anatomy and physiology*, ed 6, St Louis, 2007, Mosby.)

OVERVIEW OF THE MICROBIAL WORLD

The study of microorganisms by the Dutch biologist/lens maker Anton van Leeuwenhoek has evolved immensely from its early historical beginnings. Because of Leeuwenhoek's discovery of what he affectionately called *beasties* in a water droplet in his homemade microscope, the scientific community acknowledged him as the "father of protozoology and bacteriology."

Today we know that there are enormous numbers of microbes in, on, and around us in our environment. Many of these microbes do not cause disease. The focus of this chapter and this textbook is on microbes that are associated with disease.

Bacteria

Bacteria are unicellular organisms that lack a nuclear membrane and true nucleus. They are classified as **prokaryotes** (Gr: before kernel [nucleus]), having no mitochondria, endoplasmic reticulum (ER), or Golgi bodies. The absence of the preceding bacterial cell structures differentiates them from **eukaryotes.** Table 1-1 compares prokaryotic and eukaryotic cell organization; Figure 1-1 shows both types of cells.

Parasites

Eukaryotic parasites exist as unicellular organisms of microscopic size, whereas others are multicellular organisms. Protozoa are unicellular organisms within the kingdom Protista, which obtain their nutrition through ingestion. Some are capable of locomotion (motile), whereas others are nonmotile. They are categorized by their locomotive structures: flagella (Latin: whiplike), pseudopodia (Gr: false feet), or cilia (Latin: eyelash). Many multicellular parasites (e.g., tapeworms) may be as long as 7 to 10 meters (see Chapter 28).

Fungi

Fungi are heterotrophic eukaryotes that obtain nutrients through absorption. Yeasts are a group of unicellular fungi that reproduce asexually. "True" yeasts do not form **hyphae** or **mycelia.** Most fungi are multicellular and many can reproduce sexually and asexually. The bodies of multicellular fungi are composed of filaments called *hyphae*, which interweave to form mats called *mycelia.* Moulds are filamentous forms that can reproduce asexually and sexually. Certain fungi can assume both morphologies (yeast and hyphae/mycelial forms), growing as yeast at incubator or human temperature, and the filamentous form at room temperature. These fungi are called **dimorphic.** Many of the dimorphic fungi produce systemic diseases in human hosts (see Chapter 27).

TABLE 1-1 Comparison of Prokaryotic and Eukaryotic Cell Organization

Characteristic	Prokaryote	Eukaryote
Typical size	0.4-2 μm in diameter 0.5-5 μm in length	10-100 μm in diameter >10 μm in length
Nucleus	No nuclear membrane; nucleoid region of the cytosol	Classic membrane-bound nucleus
Genome		
Location	In the nucleoid, at the mesosome	In the nucleus
Chromosomal DNA	Circular; complexed with RNA	Linear; complexed with basic histones and other proteins
Genome: extrachromosomal circular DNA	Plasmids, small circular molecule of DNA containing accessory information; most commonly found in gram-negative bacteria; each carries genes for its own replication; can confer resistance to antibiotics	In mitochondria and chloroplasts
Reproduction	Asexual (binary fission)	Sexual and asexual
Membrane-bound organelles	Absent	All
Golgi bodies	Absent in all	Present in some
Lysosomes	Absent in all	Present in some; contain hydrolytic enzymes
Endoplasmic reticulum	Absent in all	Present in all; lipid synthesis, transport
Mitochondria	Absent in all	Present in most
Nucleus	Absent in all	Present in all
Chloroplasts for photosynthesis	Absent in all	Present in algae and plants
Ribosomes: site of protein synthesis (nonmembranous)	Present in all	Present in all
Size	70S in size, consisting of 50S and 30S subunits	80S in size, consisting of 60S and 40S subunits
Electron transport for energy	In the cell membrane if present; no mitochondria present	In the inner membrane of mitochondria and chloroplasts
Sterols in cytoplasmic membrane	Absent, except in *Mycoplasma* spp.	Present
Plasma membrane	Lacks carbohydrates	Also contains glycolipids and glycoproteins
Cell wall, if present	Peptidoglycan in most bacteria	Cellulose, phenolic polymers, lignin (plants), chitin (fungi), other glycans (algae)
Glycocalyx	Present in most as an organized capsule or unorganized slime layer	Present; some animal cells
Cilia	Absent	Present; see description of flagella
Flagella, if present	Simple flagella; composed of polymers of flagellin; movement by rotary action at the base; spirochetes have MTs	Complex cilia of flagella composed of MTs, polymers of tubulin with dynein connecting the MTs; movement by sliding filaments by one another
Pili and fimbriae	Present	Absent

MT, Microtubule.

Viruses

Viruses are the smallest infectious particles (virions); they cannot be seen under an ordinary light microscope. They are neither prokaryotic nor eukaryotic. Many times we can see their effects on cell lines, such as inclusions, rounding up of cells, and syncytium (cell fusion of host cells into multinucleated infected forms), where these characteristics become diagnostic for many viral diseases. They are distinguished from living cells by the following characteristics:

- Viruses consist of DNA or RNA, but not both. Their genome may be double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), double-stranded RNA (dsRNA), or single-stranded RNA (ssRNA).
- They are acellular (not composed of cells), lack cytoplasmic membranes, and are surrounded by a protein coat.
- They are obligate intracellular parasites that require host cells for replication (increase in number does not involve mitosis, meiosis, or binary fission) and metabolism. Because they lack enzymes, ribosomes, and other metabolites, they "take over" host cell function to produce virus. Growth (increase in size) does not occur in viruses.
- Viruses are mostly host and/or host cell specific. For example, human immunodeficiency virus (HIV) infects T helper lymphocytes, not muscle cells, in humans; others such as the rabies virus can infect dogs, skunks, bats, and humans. A virus that infects and possibly destroys bacterial cells is known as a bacteriophage (Gr *phage*: to eat).

Viruses are becoming better known by their DNA or RNA makeup, host disease signs and symptoms, chemical makeup, geographic distribution, resistance to lipid solvents and detergents, resistance to changes in pH and temperature, and antigenicity (serologic methods). Currently, the organization and type (either DNA or RNA), of the virus's genome, how the

virus replicates, and the virus's virion (a virus outside of a cell) structure are used to differentiate virus order, family, and genera. More than 2000 descriptions of viruses can be found in The Universal Virus Database of the International Committee on Taxonomy of Viruses (www.ncbi.nlm.nih.gov/ICTVdb/). See Chapter 29 for a further discussion of viruses.

CLASSIFICATION/TAXONOMY

Taxonomy (Gr *taxes*: arrangement; Gr *nomos*: law) is the orderly classification and grouping of organisms into **taxa** (categories). Taxonomy involves three structured, interrelated categories: classification/taxonomy, nomenclature, and identification. It is based on similarities and differences in **genotype** (genetic makeup of an organism, or combinations of forms of one or a few genes under scrutiny in an organism's genome) and **phenotype** (readily observable physical and functional features of an organism expressed by its genotype). Examples of genotypic characteristics include base sequencing of DNA or RNA and DNA base composition ratio to measure the degree of relatedness of two organisms (see later in this chapter and Chapter 11). Examples of phenotypic characteristics include macroscopic (colony morphology on media) and microscopic (size, shape, arrangement into groups or chains of organisms) morphology, staining characteristics (gram-positive or gram-negative), nutritional requirements, physiologic and biochemical characteristics, and susceptibility or resistance to antibiotics or chemicals. See Chapters 5, 8, and 9 for more detailed information.

Taxa (plural of *taxon*), for example the levels of classification, are the categories or subsets in taxonomy. The formal levels of bacterial classification in successively smaller taxa or subsets are as follows: domain, kingdom, division (or phylum in kingdom Animalia), class, order, family, tribe, genus, species, and subspecies. Below the subspecies level, designations, such as serotype or biotype, may be given to organisms that share specific minor characteristics. Protists (protozoans) of clinical importance are named like animals; instead of divisions, one uses **phyla** (plural of *phylum*), but the names of the others remain the same. Bacteria are placed in domains Bacteria and Archaea, separate from the animals; plants and protists are placed in domain Eukarya. The domains Bacteria and Archaea include unicellular prokaryotic organisms.

Diagnostic microbiologists traditionally emphasize placement and naming of bacterial species into three (occasionally four or five) categories: the **family** (similar to a human "clan"), a genus (equivalent to a human last name), and a species (equivalent to a human first name). The plural of **genus** is *genera*, and there are many genera in the family Enterobacteriaceae. The proper word for the name of a **species** is an *epithet*. Although order and tribe may be useful for the classification of plants and animals, these taxa are not always used for the classification of bacteria. For example, *Staphylococcus* (genus) *aureus* (species epithet) belongs to the family Micrococcaceae. In addition, there are usually different **strains** within a given species of the same genus. For example, there are many different strains of *S. aureus*. If the *S. aureus* isolated from one patient is resistant to penicillin and another *S. aureus* from another patient is susceptible to penicillin, then the two isolates are considered to be different strains of the same species. For an additional example, see *Corynebacterium diphtheriae* in the Transduction section later in this chapter.

Nomenclature

Nomenclature provides naming assignments for each organism in this textbook. The following standard rules for denoting bacterial names are used. The family name is capitalized and has an *-aceae* ending (e.g., Micrococcaceae). The genus name is capitalized and followed by the species epithet, which begins with a lowercase letter; both the genus and species should be italicized in print but underlined when written in script (e.g., *Staphylococcus aureus* or Staphylococcus aureus). Often the genus name is abbreviated by using the first letter (capitalized) of the genus followed by a period and the species epithet (e.g., *S. aureus*). To eliminate confusion, the first two letters or the first syllable are used when two or more genera names begin with the same first letter (e.g., *Staph.* and *Strept.* for when *Staphylococcus* and *Streptococcus* are discussed). The reason for combining the two allows the species epithet to be used for a different species in another genus. For example, *Escherichia coli* (*E. coli* or *Esch. coli*) is a bacterium, but *Entamoeba coli* (*Ent. coli*) is an intestinal parasite. The genus name followed by the word *species* (e.g., *Staphylococcus* species) may be used to refer to the genus as a whole. Species are abbreviated "sp." (singular) or "spp." (plural) when the species is not specified. When bacteria are referred to as a group, their names are neither capitalized nor underlined (e.g., staphylococci).

Classification by Phenotypic and Genotypic Characteristics

The traditional method of placing an organism into a particular genus and species is based on the similarity of all members in a number of phenotypic characteristics. In the diagnostic microbiology laboratory, this is accomplished by testing each bacterial culture for a variety of metabolic characteristics and comparing the results with those listed in established charts. In many rapid identification systems, a numeric taxonomy is used, in which phenotypic characteristics are assigned a numeric value and the derived number indicates the genus and species of the bacterium.

Epidemiologists constantly seek means of further subdividing bacterial species to follow the spread of bacterial infections. Species may be subdivided into subspecies, based on phenotypic differences (abbreviated "subsp."); serovarieties, based on serologic differences (abbreviated "serovar"); or biovarieties, based on biochemical test result differences (abbreviated "biovar"). Phage typing (based on susceptibility to specific bacterial phages) has also been used for this purpose. Current technology has allowed the analysis of genetic relatedness (DNA and RNA structure and homology) for taxonomic purposes. The analysis of ribosomal RNA

(rRNA) has proved particularly useful for this purpose. The information obtained from these studies has resulted in the reclassification of some bacteria.

Classification by Cellular Type: Prokaryotes, Eukaryotes, and Archaeobacteria

Another method of classifying organisms is by cell organization. It is now recognized that organisms fall into three distinct groups based on type of cell organization and function: the prokaryotes, the eukaryotes, and the **archaeobacteria.** However, recently taxonomists have placed all organisms into three domains that have replaced some kingdoms: **Bacteria, Archaea,** and **Eukarya.** These three domains are the largest and most inclusive taxa. Each of these domains is divided into kingdoms based on the similarities of RNA, DNA, and protein sequences. Prokaryotes ("before nucleus") includes the domains Archaea and Bacteria (Eubacteria), whereas fungi, algae, protozoa, animals, and plants are eukaryotic in nature.

The domain Archaea (archaeobacteria) cell type appears to be more closely related to eukaryotic cells than to prokaryotic cells and is found in microorganisms that grow under extreme environmental conditions. Archaeal cell walls lack peptidoglycan, a major reason they are placed in a domain separate from bacteria. These microbes share some common characteristics with bacteria; they too can stain gram-positive and gram-negative. Gram-positive archaea have a thick wall and stain purple. Gram-negative archaeal cells, unlike the typical gram-negative bacterial lipid membrane, have a layer of protein covering the cell wall and stain pink. See the Gram stain discussion later in this chapter.

The structure of the cell envelope and enzymes of *archaea* (Gr: ancient, origin from the earliest cells) allows them to survive under stressful or extreme (*extremophiles*; lovers of the extreme) conditions. Examples include halophiles (salt-loving cells) in Utah's Great Salt Lake or thermophiles (heat-loving cells) in hot springs and deep ocean vents, and the anaerobic methanogens that give off swamp gas and inhabit the intestinal tracts of animals. Because *archaea* are not encountered in clinical microbiology, they are not discussed further in this chapter.

In general, the interior organization of eukaryotic cells is more complex than that of prokaryotic cells (see Figure 1-1). The eukaryotic cell is usually larger and contains membrane-encased organelles ("little organs") or compartments that serve specific functions; the prokaryotic cell is noncompartmentalized. Differences also exist in the processes of DNA synthesis, protein synthesis, and cell envelope synthesis and structure. Table 1-1 compares the major characteristics of eukaryotic and prokaryotic cells.

Pathogenic (disease-causing) **bacteria** are prokaryotic cells that infect eukaryotic hosts. Targeting antibiotic action against unique prokaryotic structures and functions inhibits bacterial growth without harming eukaryotic host cells. This is one reason that pharmaceutical companies have been so successful in developing effective antibiotics against bacterial pathogens but have been less successful in finding drugs effective against parasites, medically important fungi, and viruses, which are eukaryotic like their human hosts.

COMPARISON OF PROKARYOTIC AND EUKARYOTIC CELL STRUCTURE

Prokaryotic Cell Structure

The bacterial cell is smaller and less compartmentalized than the eukaryotic cell. A variety of structures are, however, unique to prokaryotic cells (see Figure 1-1).

Cytoplasmic Structures

Bacteria do not contain a membrane-bound nucleus. Their genome consists of a single circular chromosome. This appears as a diffuse nucleoid or chromatin body (nuclear body), which is attached to a mesosome, a saclike structure in the cell membrane.

Bacterial ribosomes, consisting of RNA and protein, are found free in the cytoplasm and attached to the cytoplasmic membrane. They are the site of protein biosynthesis. They are 70S in size and dissociate into two subunits, 50S and 30S in size (see Table 1-1). The *S* stands for *Svedberg* units that refer to sedimentation rates (unit of time) during high-speed centrifugation. It is named for Theodor Svedberg, Nobel prize winner, and inventor of the ultracentrifuge. Larger particles have higher S values. It is important to remember that the S value is *not additive.* When the two subunits 50S and 30S above bind together, there is a loss of surface area, and therefore the two subunits will add up to only 70S in size. The same occurs in the eukaryotic cell where the two subunits, 60S and 40S, add up to 80S.

Stained bacteria sometimes reveal the presence of granules in the cytoplasm (cytoplasmic granules). These granules are storage deposits and may consist of polysaccharides such as glycogen, lipids such as poly-β-hydroxybutyrate, or polyphosphates.

Certain genera, such as *Bacillus* and *Clostridium*, produce endospores in response to harsh environmental conditions. Endospores are small, dormant (inactive), asexual spores that develop inside the bacterial cell (active vegetative cell) as a means of survival, although they do become vegetative when the harsh conditions are removed. Their thick protein coat makes them highly resistant to chemical agents, temperature change, starvation, dehydration, ultraviolet and gamma radiation, and desiccation. It is important to remember that endospores are not a means of reproduction. Each vegetative cell (active, capable of growing and dividing), under harsh conditions, produces internally one endospore (inactive), which germinates under favorable environmental conditions into one vegetative cell (active). Endospores should not confused with the reproductive spores of fungi (see Chapter 27).

Spores appear as highly refractile bodies in the cell. Spores are visualized microscopically as unstained areas in a cell with the use of traditional bacterial stains (Gram) or by using specific spore stains. *Schaeffer-Fulton* is the most commonly used endospore stain. The size, shape, and interior location of the spore, for example at one end (terminal), subterminal,

or central, can be used as identifying characteristics. For example, the terminal spore of *Clostridium tetani*, the etiologic (causative) agent of tetanus, gives the organism a characteristic "racquet (tennis) or lollipop" shaped appearance.

Cell Envelope Structures

The cell envelope consists of the membrane and structures surrounding the cytoplasm. In bacteria, these are the cell membrane and the cell wall. Some species also produce capsules and slime layers.

Plasma Membrane (Cell Membrane). The plasma membrane is a phospholipid bilayer with embedded proteins that envelop the cytoplasm. The prokaryotic plasma membrane is made of phospholipids and proteins but does not contain sterols, unlike eukaryotic plasma membranes (except for *Mycoplasma*). The plasma membrane acts as an osmotic barrier (prokaryotes have a high osmotic pressure inside the cell) and is the location of the electron transport chain, where energy is generated. The general functions of the prokaryotic plasma membrane are identical to those in eukaryotes (see Figure 1-2).

Cell Wall. The cell wall of prokaryotes is a rigid structure that maintains the shape of the cell and prevents bursting of the cell from the high osmotic pressure inside it. There are several different types of cell wall structures in bacteria, which have traditionally been categorized according to their staining characteristics. The two major types of cell walls are the **gram-positive** and the **gram-negative** types. In addition, mycobacteria have a modified gram-positive cell wall called an **acid-fast** cell wall, although they do stain gram-positive, and mycoplasmas have no cell wall.

Gram-Positive Cell Wall. The gram-positive cell wall is composed of a very thick protective peptidoglycan (murein) layer. Because the peptidoglycan layer is the principal component of the gram-positive cell wall, many antibiotics effective against gram-positive organisms (e.g., penicillin) act by preventing synthesis of peptidoglycan. Gram-negative bacteria, which have a thinner layer of peptidoglycan and a different cell wall structure, are less affected by these antibiotics.

The peptidoglycan or murein layer consists of glycan (polysaccharide) chains of alternating *N*-acetyl-d-glucosamine (NAG) and *N*-acetyl-d-muramic acid (NAM) (Figure 1-3). Short peptides, each consisting of four amino acid residues, are attached to a carboxyl group on each NAM residue. The chains are then cross-linked to form a thick network via a peptide bridge (varying in number of peptides) connected to the tetrapeptides on the NAM.

Other components of the gram-positive cell wall that penetrate to the exterior of the cell are teichoic acid (anchored to the peptidoglycan) and lipoteichoic acid (anchored to the plasma membrane). These two components are unique to the gram-positive cell wall. Other antigenic polysaccharides may be present on the surface of the peptidoglycan layer.

Gram-Negative Cell Wall. The cell wall of gram-negative microorganisms is composed of two layers. The inner peptidoglycan layer is much thinner than in gram-positive cell walls. Outside the peptidoglycan layer is an additional outer membrane unique to the gram-negative cell wall. The outer membrane contains proteins, phospholipids, and lipopolysaccharide (LPS). LPS contains three regions: an antigenic O–specific polysaccharide, a core polysaccharide, and an inner lipid A (also called *endotoxin*). The lipid A moiety is responsible for producing fever and shock conditions in patients infected with gram-negative bacteria. The outer membrane functions in the following ways:

- It acts as a barrier to hydrophobic compounds and harmful substances.
- It acts as a sieve, allowing water-soluble molecules to enter through protein-lined channels called *porins*.
- It provides attachment sites that enhance attachment to host cells.

Between the outer membrane and the inner membrane, and encompassing the thin peptidoglycan layer, is an area referred to as the *periplasmic space.* Within the periplasmic

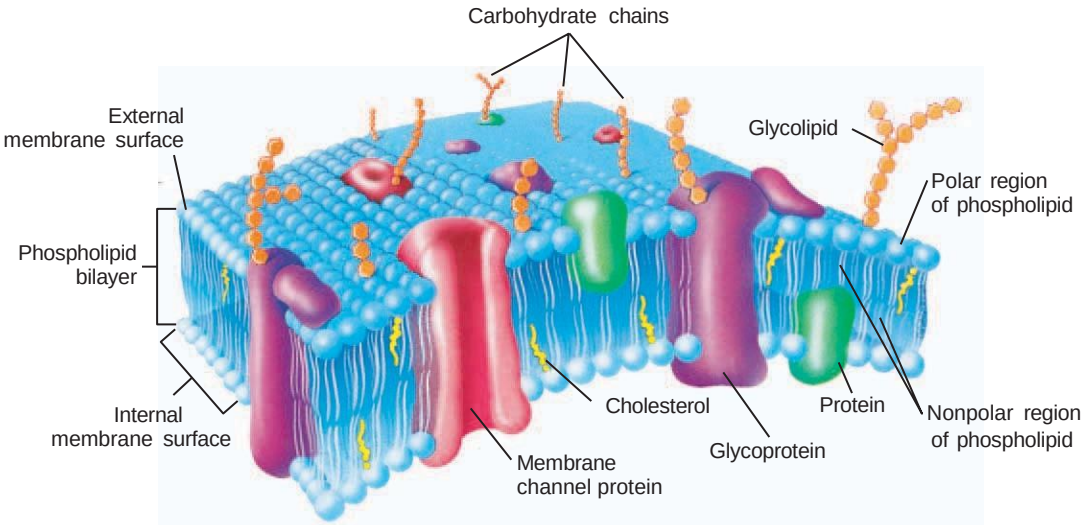

FIGURE 1-2 Structure of the plasma membrane. (From Thibodeau GA, Patton KT: *Anatomy & physiology*, ed 6, St Louis, 2007, Mosby.)

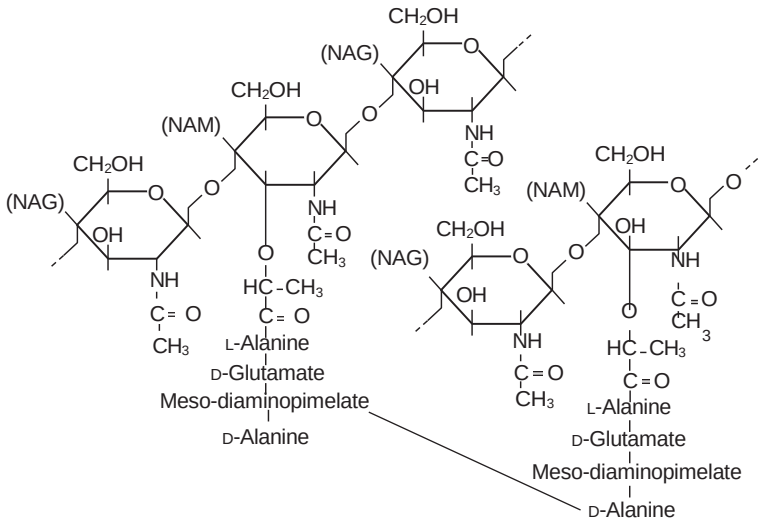

FIGURE 1-3 A diagram that demonstrates the structure of the peptidoglycan layer in the cell wall of *Escherichia coli*. The amino acids in the cross-linking tetrapeptides may vary among species. NAG, *N*-acetyl-d-glucosamine; NAM, *N*-acetyl-d-muramic acid. (From Neidhardt FC, Ingraham M, Schaechter M: *Physiology of bacterial cell: a molecular approach*, Sunderland, Mass, 1990, Sinauer Associates.)

space is a gel-like matrix containing nutrient-binding proteins and degradative and detoxifying enzymes. The periplasmic space is absent in gram-positive bacteria.

Acid-Fast Cell Wall. Certain genera (*Mycobacterium* and *Nocardia*) have a gram-positive cell wall structure but, in addition, contain a waxy layer of glycolipids and fatty acids (mycolic acid) bound to the exterior of the cell wall. More than 60% of the cell wall is lipid, and the major lipid component is mycolic acid. This is a strong "hydrophobic" molecule that forms a lipid shell around the organism and affects its permeability. This makes *Mycobacterium* spp. difficult to stain with the Gram stain. Because of their gram-positive nature, they stain a faint blue (gram-positive) color. The mycobacteria and nocardiae are best stained with an acid-fast stain, in which the bacteria are stained with carbolfuchsin, followed by acid-alcohol as a decolorizer. Other bacteria are decolorized by acid-alcohol, whereas mycobacteria and nocardiae retain the stain. They have therefore been designated *acid-fast bacteria*.

Absence of Cell Wall. Prokaryotes that belong to the *Mycoplasma* and *Ureaplasma* genera are unique in that they lack a cell wall and contain sterols in their cell membranes. Because they lack the rigidity of the cell wall, they are seen in a variety of shapes microscopically. Gram-positive and gram-negative cells can lose their cell walls and grow as L-forms in media supplemented with serum or sugar to prevent osmotic rupture of the cell membrane.

Surface Polymers

A variety of pathogenic bacteria produce a discrete organized covering termed a **capsule.** Capsules are usually made of polysaccharide polymers, although they may also be made of polypeptides. Capsules act as virulence factors in helping the pathogen evade phagocytosis. During identification of certain bacteria by serologic typing, capsules sometimes must be removed in order to detect the somatic (cell wall) antigens present underneath them. Capsule removal is accomplished by boiling a suspension of the microorganism. *Salmonella typhi* must have its capsular (Vi) antigen removed in order for the technologist to observe agglutination with *Salmonella* somatic (O) antisera. The capsule does not ordinarily stain with use of common laboratory stains, such as Gram or India ink. Instead, it appears as a clear area ("halolike") between or surrounding the stained organism and the stained amorphous background material in a direct smear from a clinical specimen.

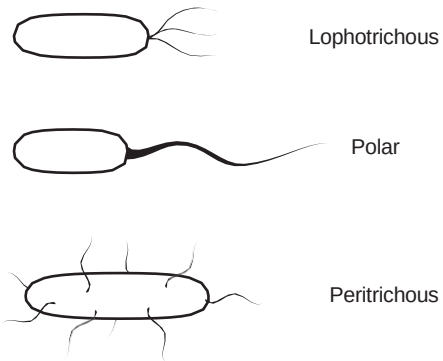

FIGURE 1-4 Diagram of three flagellar arrangements that occur in bacteria. Other variations do occur.

Slime layers are similar to capsules but are more diffuse layers surrounding the cell. They also are made of polysaccharides and serve either to inhibit phagocytosis or, in some cases, to aid in adherence to host tissue or synthetic implants.

Cell Appendages. The flagellum is the organ of locomotion. **Flagella** are exterior protein filaments that rotate and cause bacteria to be motile. Bacterial species vary in their possession of flagella from none (nonmotile) to many (Figure 1-4). Flagella that extend from one end of the bacterium are

polar. Polar flagella may occur singly at one or both ends or multiply in tufts at one end termed *lophotrichous.* Flagella that occur on all sides of the bacterium are peritrichous. The number and arrangement of flagella are sometimes used for identification purposes. Flagella can be visualized microscopically with special flagellum stains.

Pili (plural of *pilus*), also known as *conjugation pili*, are nonmotile, long, hollow protein tubes that connect two bacterial cells and mediate DNA exchange. **Fimbriae** (plural of *fimbria*) are nonflagellar, sticky, proteinaceous, hairlike appendages that adhere some bacterial cells to one another and to environmental surfaces.

Eukaryotic Cell Structure

The following structures are associated with eukaryotic cells (see Table 1-1 and Figure 1-1). In the diagnostic microbiology laboratory, the eukaryotic cell type occurs in medically important fungi and in parasites.

Cytoplasmic Structures

The nucleus of the eukaryotic cell contains the DNA of the cell in the form of discrete chromosomes (structures in the nucleus that carry genetic information; the genes). They are covered with basic proteins called *histones*. The number of chromosomes in the nucleus varies according to the particular organism.

A rounded, refractile body called a *nucleolus* is also located within the nucleus. The nucleolus is the site of ribosomal RNA synthesis. The nucleus is bounded by a bilayered lipoprotein nuclear membrane.

The ER is a system of membranes that occur throughout the cytoplasm. It is found in two forms. The "rough" ER is covered with ribosomes, the site of protein synthesis. It is the ribosomes that give the ER the rough appearance. The smooth ER does not have ribosomes on the outer surface of its membrane, hence the smooth appearance. Smooth ER does not synthesize proteins, but it does synthesize phospholipids (like rough ER). The major function of the Golgi apparatus or complex is to modify and package proteins sent to it by the rough ER depending on the protein's final destination.

Eukaryotic ribosomes, where protein synthesis occurs, are 80S in size and dissociate into two subunits: 60S and 40S. They are attached to the rough ER. Eukaryotic cells contain several membrane-enclosed organelles. Mitochondria are the main sites of energy production. They contain their own DNA and the electron transport system that produces energy for cell functions. Lysosomes contain hydrolytic enzymes for degradation of macromolecules and microorganisms within the cell. Peroxisomes contain protective enzymes that break down hydrogen peroxide and other peroxides generated within the cell. Chloroplasts, found in plant cells, are the sites of photosynthesis. Chloroplasts are the sites of energy production. Photosynthesis produces glucose from carbon dioxide and water. Fungi are not plants and therefore have no chloroplasts.

Cell Envelope Structures

Plasma Membrane. The plasma membrane (PM) (see Figure 1-2) is a phospholipid bilayer with embedded proteins that envelops the cytoplasm and regulates transport of macromolecules into and out of the cell. A substantial amount of cholesterol is found. Cholesterol has a stabilizing effect and helps keep the membrane fluid. The polar heads of the phospholipids are hydrophilic (water loving) and lie on both the intracellular and extracellular fluids; their nonpolar tails are hydrophobic (water hating) and avoid water by lining up in the center of the PM "tail to tail." It is this type of hydrophobic makeup of the interior of the PM that makes it potentially impermeable to water-soluble molecules. Proteins perform several important functions of the membrane. They may act as enzymes, hormone receptors, pore channels, and carriers. The presence of sterols is also a trait of eukaryotic cell membranes.

Cell Wall. The function of a cell wall is to provide rigidity and strength to the exterior of the cell. Most eukaryotic cells do not have cell walls. Fungi, however, have cell walls principally made of polysaccharides such as chitin, mannan, and glucan. Chitin is a distinct component of fungal cell walls.

Motility Organelles. Cilia are short projections (3 to 10 μm), usually numerous, that extend from the cell surface and are used for locomotion. They are found in certain protozoa and in ciliated epithelial cells of the respiratory tract. Flagella are longer projections (greater than 150 μm) used for locomotion by cells such as spermatozoa. The basal body, or kinetosome, is a small structure located at the base of cilia or flagella, where microtubule proteins involved in movement originate.

BACTERIAL MORPHOLOGY

Microscopic Shapes

Bacteria vary in size from 0.4 to 2 μm. They occur in three basic shapes (Figure 1-5):

- Cocci (spherical)
- Bacilli (rod-shaped)
- Spirochetes (spiral)

Individual bacteria may form characteristic groupings. Cocci (plural of *coccus*) may occur singly, in pairs (diplococci), in chains (streptococci), or in clusters (staphylococci). Bacilli (plural of *bacillus*) may vary greatly in size and length from very short coccobacilli to long filamentous rods. The ends may be square or rounded. Bacilli with tapered, pointed ends are termed **fusiform.** Some bacilli are curved. When a species varies in size and shape within a pure culture, the bacterium is **pleomorphic.** Bacilli may occur as single rods or in chains or may align themselves side by side (palisading). Spirochetes vary in length and in the number of helical turns (not all helical bacteria are called *spirochetes*).

Common Stains Used for Microscopic Visualization

Stains that impart color or fluorescence are needed to visualize bacteria under the microscope. The microscopic staining characteristics, shapes, and groupings are used in the classification of microorganisms (Figure 1-6).

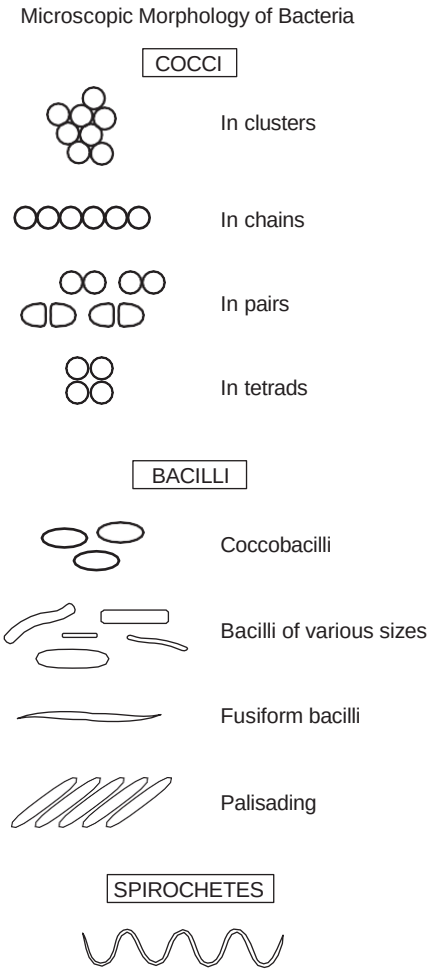

FIGURE 1-5 Diagram of the microscopic shapes and arrangements of bacteria.

Gram Stain. The Gram stain is the most commonly used stain in the clinical microbiology laboratory. It places bacteria into one of the two main groups: gram-positive (blue to purple) or gram-negative (pink) (see Figure 1-6, *A* and *B*). Some organisms are gram variable or do not stain at all. As mentioned previously, the cell wall structure determines the Gram-staining characteristics of a species. The Gram stain consists of gentle heat fixing (methyl alcohol may also be used to fix) of the smear and the addition of four sequential components: crystal violet (the primary stain, 1 minute), iodine (the mordant or fixative, 1 minute), alcohol or an alcohol acetone solution (the decolorizer, quick on and rinse), and safranin (the counterstain, 30 seconds). The time frames listed are not exact and vary with the organism; rinsing with water between each step is important. The bacteria are initially stained purple by the crystal violet, which is bound to the cell wall with the aid of iodine. When decolorizer is applied to bacteria with a gram-negative type of cell wall structure, the crystal violet washes out of the cells, which then take up the pink counterstain, safranin. Gram-negative bacteria therefore appear pink under the light microscope. Bacteria with a gram-positive cell wall retain the primary crystal violet stain during the decolorizing treatment and therefore remain purple. Cells in a direct smear from a patient specimen, such as epithelial cells, white blood cells, red blood cells, and amorphous background material, should appear pink (gram-negative) if the Gram stain was performed correctly. As presented in the case in point at the beginning of the chapter, review of quality control slides is important in the detection of errors in the performance of the Gram stain procedure and in interpretation.

Acid-Fast Stains. Acid-fast stains are used to stain bacteria that have a high lipid and wax content in their cell walls and do not stain well with traditional bacterial stains. Carbolfuchsin (a red dye) is used as the primary stain (see Figure 1-6, *C*). The cell wall is treated to allow penetration of the dye either by heat (Ziehl-Neelsen method) or by a detergent (Kinyoun method). Acidified alcohol is used as a decolorizer, and methylene blue is the counterstain. Acid-fast bacteria retain the primary stain and are red. Bacteria that are not acid-fast are blue.

Two other gram-positive genera, *Nocardia* and *Rhodococcus*, may stain acid-fast by a modified method. Acid-fast staining is used to identify a yeast, *Saccharomyces*, and coccidian parasites, such as *Isospora belli*, *Cryptosporidium*, and other coccidia-like bodies. A fluorochrome (i.e., fluorescent) stain, auramine-rhodamine, has also been used to screen for acid-fast bacteria (see Figure 1-6, *D*). This stain is selective for the cell wall of acid-fast bacteria. Acid-fast bacteria appear yellow or orange under a fluorescent microscope, making them easier to find.

Acridine Orange. Acridine orange is a fluorochrome dye that stains both gram-positive and gram-negative bacteria, living or dead. It binds to the nucleic acid of the cell and fluoresces as a bright orange when a fluorescent microscope is used. Acridine orange is used to locate bacteria in blood cultures and other specimens where discerning bacteria might otherwise be difficult (see Figure 1-6, *E*).

Calcofluor White. Calcofluor white is a fluorochrome that binds to chitin in fungal cell walls. It fluoresces as a bright apple-green or blue-white, allowing visualization of fungal structures with a fluorescent microscope. This was the original "blueing" used in high-volume laundries to whiten yellow-appearing white cotton and other fabrics.

Methylene Blue. Methylene blue has been traditionally used to stain *Corynebacterium diphtheriae* for observation of metachromatic granules (see Figure 1-6, *F*). It is also used as a counterstain in the acid-fast staining procedures.

Lactophenol Cotton Blue. Lactophenol cotton blue is used to stain the cell walls of medically important fungi grown in slide culture (see Figure 1-6, *G*).

India Ink. India ink is a negative stain used to visualize capsules surrounding certain yeasts, such as *Cryptococcus* spp. (see Figure 1-6, *H*). The fine ink particles are excluded from the capsule, leaving a dark background and a clear capsule surrounding the yeast.

Endospore Stain. To a heat-fixed smear, the primary stain, malachite green, is applied (flooded) and heated to steaming for about 5 minutes. Then the preparation is washed for about 30 seconds to remove the primary stain. Next, the counterstain safranin is applied to the smear. The endospores appear green, within pink- or red-appearing bacterial cells.

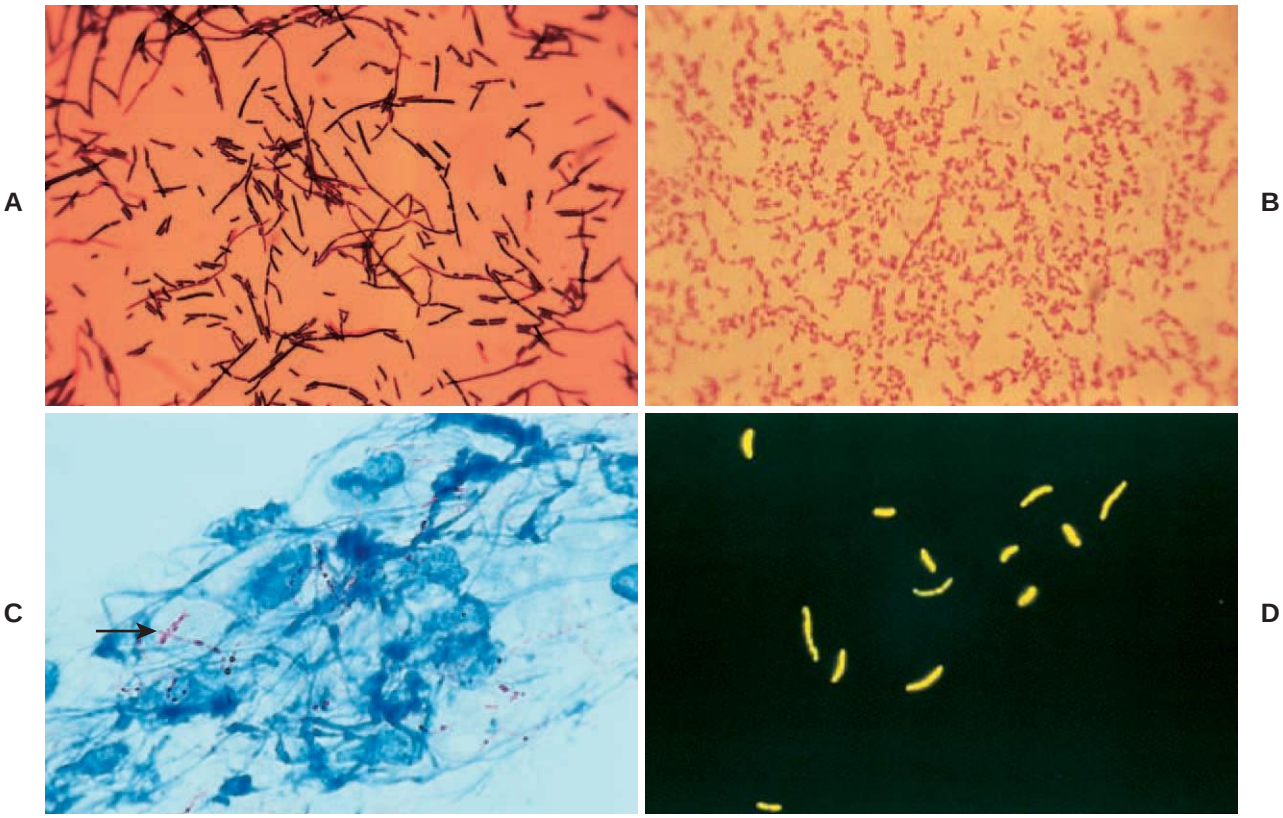

FIGURE 1-6 **A,** Gram stain of *Lactobacillus* species illustrating gram-positive bacilli, singly and in chains. A few gram-negative–staining bacilli are also present. (Courtesy Dr. Andrew G. Smith, Baltimore.) **B,** Gram stain of *Escherichia coli* illustrating short gram-negative bacilli. (Courtesy Dr. Andrew G. Smith, Baltimore.) **C,** Acid-fast stain, carbolfuchsin based. A sputum smear demonstrating the presence of acid-fast *Mycobacterium* species stained by the Kinyoun or Ziehl-Neelsen carbolfuchsin method. **D,** Acid-fast stain, fluorochrome based. *Mycobacterium* species stained with the acid-fast fluorescent auramine-rhodamine stain. This stain is useful for screening for the presence of acid-fast bacteria in clinical specimens. (Courtesy Clinical Microbiology Audiovisual Study Units, Health and Education Resources, Inc., Bethesda, Md.)

MICROBIAL GROWTH AND NUTRITION

All bacteria have three major nutritional needs for growth:

- A source of carbon (for making cellular constituents). Carbon represents 50% of the dry weight of a bacterium.
- A source of nitrogen (for making proteins). Nitrogen makes up 14% of the dry weight.
- A source of energy (ATP, for performing cellular functions).

Smaller amounts of molecules, such as phosphate for nucleic acids and phospholipids of cell membranes, and sulfur for protein synthesis make up an additional 4% of the weight. A variety of metals and ions for enzymatic activity must also be present. Important mineral ions, such as Na^+, K^+, Cl^-, and Ca^{2+}, are also required. Although the basic building blocks required for growth are the same for all cells, bacteria vary widely in their ability to use different sources of these molecules.

Nutritional Requirements for Growth

Bacteria are classified into two basic groups according to how they meet their nutritional needs. Members of the first group, the **autotrophs** (lithotrophs), are able to grow simply, using carbon dioxide as the sole source of carbon, with only water and inorganic salts required in addition. Autotrophs obtain energy either photosynthetically (phototrophs) or by oxidation of inorganic compounds (chemolithotrophs). Autotrophs occur in environmental milieus.

The second group of bacteria, the **heterotrophs,** require more complex substances for growth. They require an organic source of carbon, such as glucose, and obtain energy by oxidizing or fermenting organic substances. Often, the same substance (e.g., glucose) is used as both the carbon source and the energy source.

All bacteria that inhabit the human body fall into the heterotrophic group. Within this group, however, nutritional needs vary greatly. Bacteria such as *E. coli* and *Pseudomonas aeruginosa* can use a wide variety or organic compounds as carbon sources and therefore grow on most simple laboratory media. Other pathogenic bacteria, such as *Haemophilus influenzae* and the anaerobes, are fastidious, requiring additional metabolites such as vitamins, purines, pyrimidines, and hemoglobin supplied in the growth medium. Some pathogenic bacteria, such as *Chlamydia* spp., cannot be cultured on laboratory

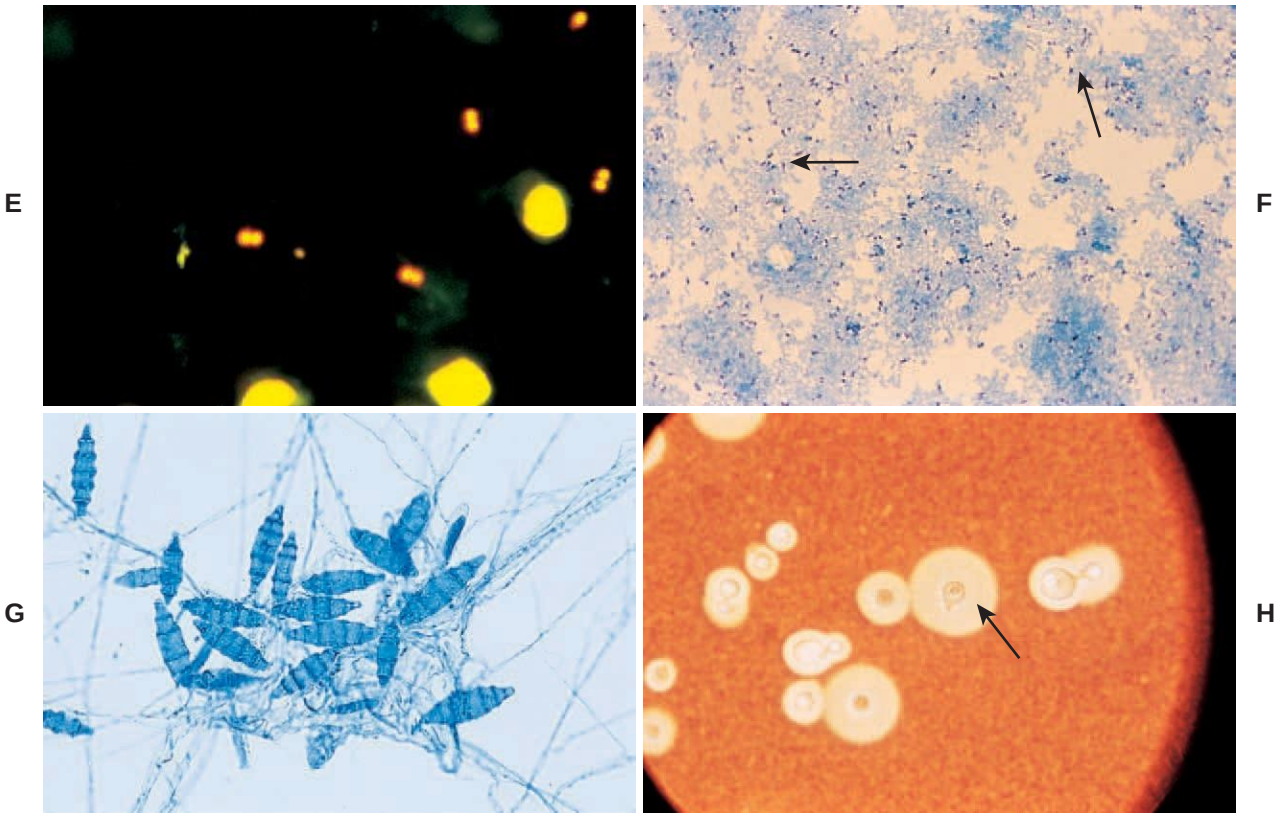

FIGURE 1-6, cont'd **E,** Acridine orange stain. A fluorescent stain demonstrating the presence of staphylococci in a blood culture broth. This stain is useful for detecting bacteria in situations where debris may mask the bacteria. (Courtesy Dr. John E. Peters Baltimore.) **F,** Methylene blue stain. A methylene blue stain demonstrating the typical morphology of *Corynebacterium diphtheriae (arrows)*. **G,** Lactophenol cotton blue stain. Lactophenol cotton blue-stained slide of macroconidia and hyphae of the fungal dermatophyte *Microsporum gypseum*. **H,** India ink. An India ink wet mount of *Cryptococcus neoformans* demonstrating the presence of a capsule *(arrow)*. (Courtesy Dr. Andrew G. Smith, Baltimore.)

media at all and must be grown in tissue culture or detected by other means.

Types of Growth Media

A laboratory growth medium whose contents are simple and completely defined is termed **minimal medium.** This type of medium is not usually used in the diagnostic microbiology laboratory. Media that are more complex and made of extracts of meat or soybeans are termed **nutrient media** (e.g., nutrient broth, trypticase soy broth). A growth medium that contains added growth factors, such as blood, vitamins, and yeast extract, is referred to as *enriched* (e.g., blood agar, chocolate agar). Media containing additives that inhibit the growth of some bacteria but allow others to grow are called **selective media** (e.g., MacConkey agar [MAC] selective for gram-negatives while inhibiting gram positives and colistin-nalidixic acid [CNA] selective for gram-positives while inhibiting gram negatives). Ingredients in media that allow visualization of metabolic differences between groups or species of bacteria are called **differential media.** Therefore MacConkey agar can also be a differential medium because it distinguishes between lactose fermenters (pink) and nonlactose fermenters (clear). A blood agar plate can also be, in a nonstrict sense, differential, because it distinguishes between hemolytic and nonhemolytic organisms. When a delay between collection of the specimen and culturing the specimen is necessary, a **transport medium** is used. A transport medium is a holding medium designed to preserve the viability of microorganisms in the specimen but not allow multiplication. Stuart broth and Amies and Cary-Blair transport media are common examples.

Environmental Factors Influencing Growth

Three environmental factors influence the growth rate of bacteria and must be considered when bacteria are cultured in the laboratory:

- pH
- Temperature
- Gaseous composition of the atmosphere

Most pathogenic bacteria grow best at a neutral pH. Diagnostic laboratory media for bacteria are usually adjusted to a final pH between 7.0 and 7.5. Temperature influences the rate of growth of a bacterial culture. Microorganisms

have been categorized according to their optimal temperature for growth. Bacteria that grow best at cold temperatures are called **psychrophiles** (optimal growth at 10° to 20° C). Bacteria that grow optimally at moderate temperatures are called **mesophiles** (optimal growth at 20° to 40° C). Bacteria that grow best at high temperatures are called **thermophiles** (optimal growth at 50° to 60° C). Psychrophiles and thermophiles are found environmentally in places such as the arctic seas and hot springs, respectively. Most bacteria that have adapted to humans are mesophiles that grow best near human body temperature (37° C). Diagnostic laboratories routinely incubate cultures for bacterial growth at 35° C. Some pathogenic species, however, prefer a lower temperature for growth; when these organisms are suspected, the specimen plate is incubated at a lower temperature. Fungal cultures are incubated at 30° C. The ability to grow at room temperature (25° C) or at an elevated temperature (42° C) is used as diagnostic characteristics for some bacteria.

Bacteria that grow on humans vary in their atmospheric requirements for growth. **Obligate aerobes** require oxygen for growth. **Aerotolerant anaerobes,** previously referred to as *facultative aerobes*, can survive in the presence of oxygen but do not use oxygen in metabolism (e.g., certain *Clostridium* spp.). **Obligate anaerobes** cannot grow in the presence of oxygen. **Facultative anaerobes** can grow either with or without oxygen. **Capnophilic** organisms grow best when the atmosphere is enriched with extra carbon dioxide (5% to 10%).

Air contains approximately 21% oxygen and 1% carbon dioxide. When the carbon dioxide content of an aerobic incubator is increased to 10%, the oxygen content of the incubator is lowered to approximately 18%. Obligate aerobes must have oxygen to grow; incubation in air or an aerobic incubator with 10% carbon dioxide present satisfies their oxygen requirement. **Microaerophilic** bacteria require a reduced level of oxygen to grow. An example of a pathogenic microaerophile is *Campylobacter* spp., which requires 5% to 6% oxygen. This type of atmosphere can be generated in culture jars or pouches using a commercially available microaerophilic atmosphere-generating system. Obligate anaerobes must be grown in an atmosphere either devoid of oxygen or with a significantly reduced oxygen content. Facultative anaerobes (aerobes that can grow anaerobically) are routinely cultured in an aerobic atmosphere because aerobic culture is easier and less expensive than anaerobic culture; an example is *E. coli*. Capnophilic bacteria require extra carbon dioxide (5% to 10%) for growth; an example is *H. influenzae*. Because many bacteria grow better in the presence of increased carbon dioxide, diagnostic microbiology laboratories often maintain their aerobic incubators at a 5% to 10% carbon dioxide level.

Bacterial Growth

Generation Time

Bacteria replicate by binary fission, with one cell dividing into two cells. The time required for one cell to divide into two cells is called the *generation time* or *doubling time*. The generation time of a bacterium in culture can be as little as 20 minutes for a fast-growing bacterium such as *E. coli* or as long as 24 hours for a slow-growing bacterium such as *Mycobacterium tuberculosis*.

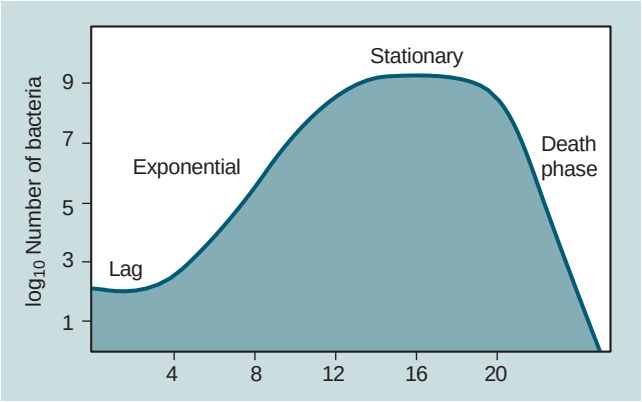

FIGURE 1-7 Typical growth curve of a bacterial culture.

Growth Curve

If bacteria are in a balanced growth state, with enough nutrients and no toxic products present, the increase in bacterial numbers is proportional to the increase in other bacterial properties, such as mass, protein content, and nucleic acid content. Thus measurement of any of these properties can be used as an indication of bacterial growth. When the growth of a bacterial culture is plotted during balanced growth, the resulting curve shows four phases of growth: (1) a lag phase, during which bacteria are preparing to divide, (2) a log phase, during which bacteria numbers increase logarithmically, (3) a stationary phase, in which nutrients are becoming limited and the numbers of bacteria remain constant (although viability may decrease), and (4) a death phase, when the number of nonviable bacterial cells exceeds the number of viable cells. An example of such a growth curve is shown in Figure 1-7.

Determination of Cell Numbers

In the diagnostic laboratory the number of bacterial cells present is determined in one of three ways:

- *Direct counting under the microscope:* This method can be used to estimate the number of bacteria present in a specimen. It does not distinguish between live and dead cells.
- *Direct plate count:* By growing dilutions of broth cultures on agar plates, one can determine the number of colony-forming units per milliliter (CFU/mL). This provides a count of viable cells only. This method is used in determining the bacterial cell count in urine cultures.
- *Density measurement:* The density (referred to as *cloudiness* or *turbidity*) of a bacterial broth culture in log phase can be correlated to CFU/mL of the culture. This method is used to prepare a standard inoculum for antimicrobial susceptibility testing.

BACTERIAL BIOCHEMISTRY AND METABOLISM

Metabolism

Microbial metabolism consists of the biochemical reactions bacteria use to break down organic compounds as well as those they use to synthesize new bacterial parts from the resulting carbon skeletons. Energy for the new constructions is generated during the metabolic breakdown of the substrate.

The occurrence of all biochemical reactions in the cell depends on the presence and activity of specific enzymes. Thus metabolism can be regulated in the cell either by regulating the production of an enzyme itself (a genetic type of regulation, in which production of the enzyme can be induced or suppressed by molecules present in the cell) or by regulating the activity of the enzyme (via feedback inhibition, in which the products of the enzymatic reaction or a succeeding enzymatic reaction inhibit the activity of the enzyme).

Bacteria vary widely in their ability to use various compounds as substrates and in the end products generated. A variety of biochemical pathways exist for substrate breakdown in the microbial world, and the particular pathway used determines the end product and final pH of the medium (Figure 1-8). Microbiologists use these metabolic differences as phenotypic markers in the identification of bacteria. Diagnostic schemes analyze each unknown microorganism for (1) utilization of a variety of substrates as a carbon source, (2) production of specific end products from various substrates, and (3) production of an acid or alkaline pH in the test medium. Thus knowledge of the biochemistry and metabolism of bacteria is important in the clinical laboratory.

Fermentation and Respiration

Bacteria use biochemical pathways to catabolize (break down) carbohydrates and produce energy by two mechanisms—**fermentation** and **respiration** (commonly referred to as *oxidation*). Fermentation is an anaerobic process carried out by both obligate and facultative anaerobes. In fermentation, the electron acceptor is an organic compound. Fermentation is less efficient in energy generation than respiration (oxidation) because the beginning substrate is not completely reduced; therefore all the energy in the substrate is not released. When fermentation occurs, a mixture of end products (e.g., lactate, butyrate, ethanol, and acetoin) accumulates in the medium. Analysis of these end products is particularly useful for the identification of anaerobic bacteria. End-product determination is also used in the Voges-Proskauer (VP) and methyl red tests, two important diagnostic tests used in the identification of the Enterobacteriaceae. (The term *fermentation* is often used loosely in the diagnostic microbiology laboratory to indicate any type of utilization—fermentative or oxidative—of a carbohydrate—sugar—with the resulting production of an acid pH.)

Respiration (not an act of breathing) is an efficient energy-generating process in which molecular oxygen is the final electron acceptor. Obligate aerobes and facultative anaerobes carry out aerobic respiration, in which oxygen is the final electron acceptor. Certain anaerobes can carry out anaerobic respiration, in which inorganic forms of oxygen, such as nitrate and sulfate, act as the final electron acceptors.

Biochemical Pathways from Glucose to Pyruvic Acid

The starting carbohydrate for bacterial fermentations or oxidations is glucose. When bacteria use other sugars as a carbon source, they first convert the sugar to glucose, which is then processed by one of three pathways. These pathways are designed to generate pyruvic acid, a key three-carbon intermediate. The three major biochemical pathways bacteria use to break down glucose to pyruvic acid are (1) the Embden-Meyerhof-Parnas (EMP) glycolytic pathway (Figure 1-9), (2) the pentose phosphate pathway (Figure 1-10), and (3) the Entner-Doudoroff pathway (see Figure 1-10). Pyruvate can then be further processed either fermentatively or oxidatively.

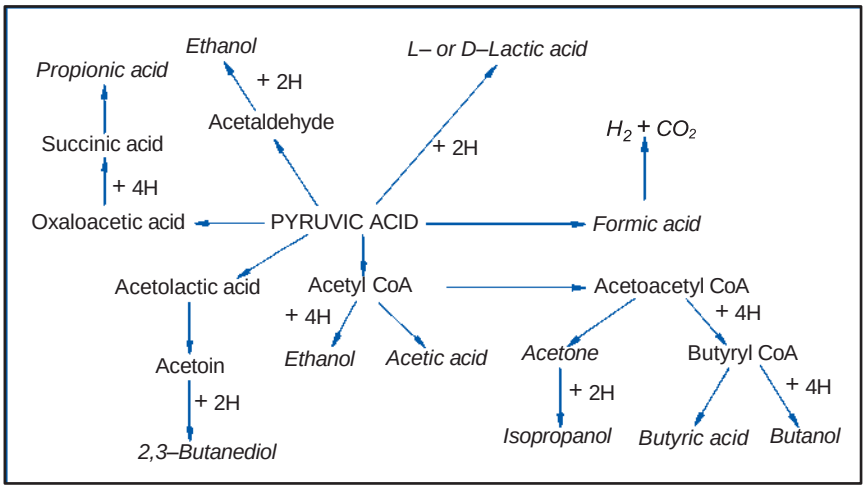

FIGURE 1-8 The fate of pyruvate in major fermentation pathways by microorganisms. (From Joklik WK et al: *Zinsser microbiology*, ed 20, Norwalk, Conn, 1992, Appleton & Lange.)

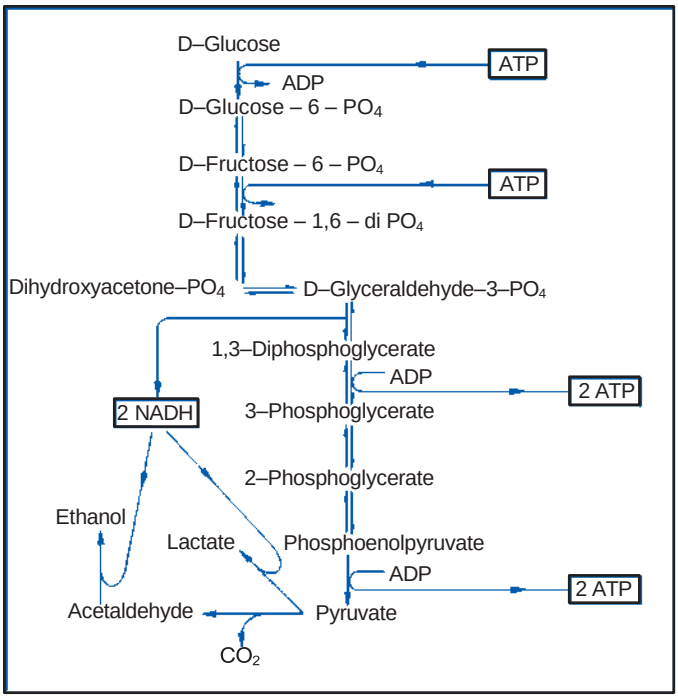

FIGURE 1-9 The Embden-Meyerhof-Parnas glycolytic pathway. (From Joklik WK et al: *Zinsser microbiology*, ed 20, Norwalk, Conn, 1992, Appleton & Lange.)

The three major metabolic pathways and their key characteristics are described in Box 1-1.

Anaerobic Utilization of Pyruvic Acid (Fermentation)

Pyruvic acid is a key metabolic intermediate. Bacteria process pyruvic acid further using a variety of fermentation pathways. Each pathway yields different end products, which can be analyzed and used as phenotypic markers (see Figure 1-8). Some of the fermentation pathways used by the microbes that inhabit the human body are as follows:

- *Alcoholic fermentation:* The major end product is ethanol. This is the pathway used by yeasts when they ferment glucose to produce ethanol.
- *Homolactic fermentation:* The end product is almost exclusively lactic acid. All members of the *Streptococcus* genus and many members of the *Lactobacillus* genus ferment pyruvate using this pathway.
- *Heterolactic fermentation:* Some lactobacilli use this mixed fermentation pathway, of which, in addition to lactic acid, the end products include carbon dioxide, alcohols, formic acid, and acetic acid.
- *Propionic acid fermentation:* Propionic acid is the major end product of fermentations carried out by *Propionibacterium acnes* and some anaerobic non–spore-forming gram-positive bacilli.

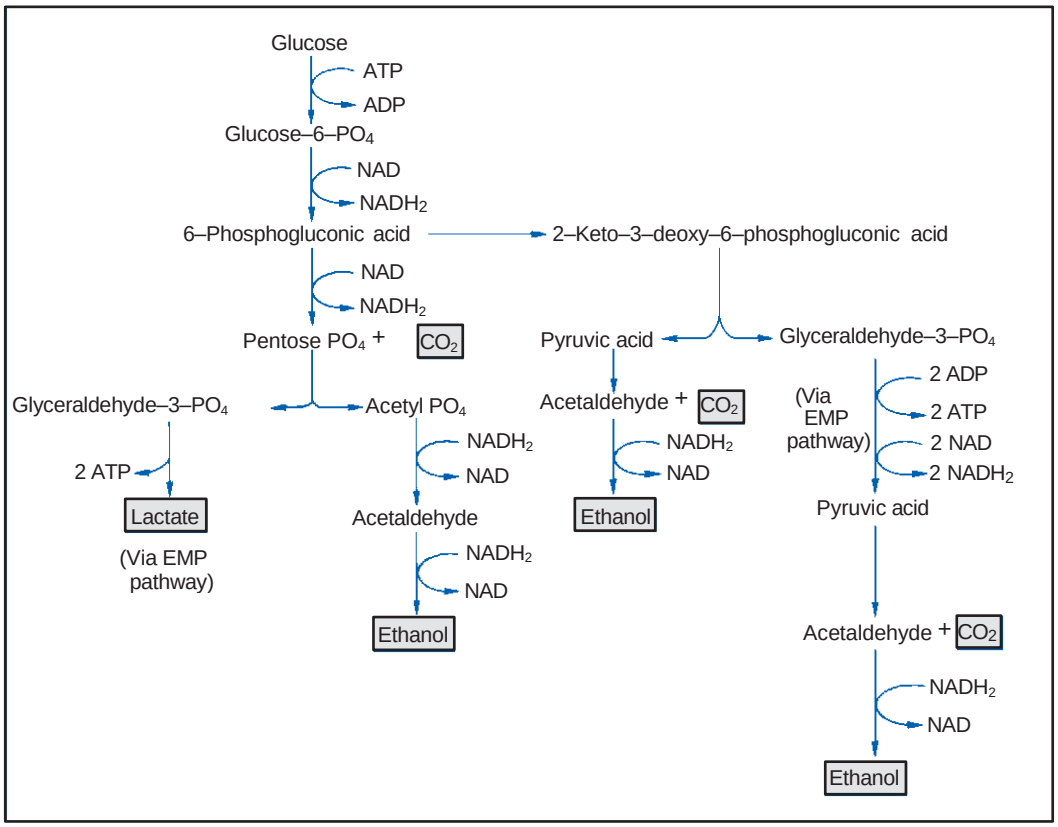

FIGURE 1-10 Alternative microbial pathways to the Embden-Meyerhof-Parnas pathway for glucose fermentation. The pentose phosphate pathway is on the left, and the Entner-Doudoroff pathway is on the right. (From Joklik WK et al: *Zinsser microbiology*, ed 20, Norwalk, Conn, 1992, Appleton & Lange.)

BOX 1-1 The Three Major Metabolic Pathways

EMP Glycolytic Pathway

- The major pathway in conversion of glucose to pyruvate
- Generates reducing power in the form of $NADH_2$
- Generates energy in the form of ATP
- Anaerobic; does not require oxygen
- Used by many bacteria, including all members of Enterobacteriaceae

Pentose Phosphate (Phosphogluconate) Pathway

- An alternative to EMP pathway for carbohydrate metabolism
- Conversion of glucose to ribulose-5-phosphate, which is then rearranged into other 3-, 4-, 5-, 6-, and 7-carbon sugars
- Provides pentoses for nucleotide synthesis
- Produces glyceraldehyde-3-phosphate, which can be converted to pyruvate
- Generates NADPH, which provides reducing power for biosynthetic reactions
- May be used to generate ATP (yield is less than with the EMP pathway)
- Used by heterolactic fermenting bacteria, such as lactobacilli, and by *Brucella abortus,* which lacks some of the enzymes required in the EMP pathway

Entner-Doudoroff Pathway

- Converts glucose-6-phosphate (rather than glucose) to pyruvate and glyceraldehyde phosphate, which can then be funneled into other pathways
- Generates one NADPH per molecule of glucose but uses one ATP
- Aerobic process used by *Pseudomonas, Alcaligenes, Enterococcus faecalis,* and other bacteria lacking certain glycolytic enzymes

ATP, Adenosine triphosphate; *EMP,* Embden-Meyerhof-Parnas; *$NADH_2$,* nicotinamide adenine dinucleotide dehydrogenase; *NADPH,* nicotinamide adenine dinucleotide phosphate.

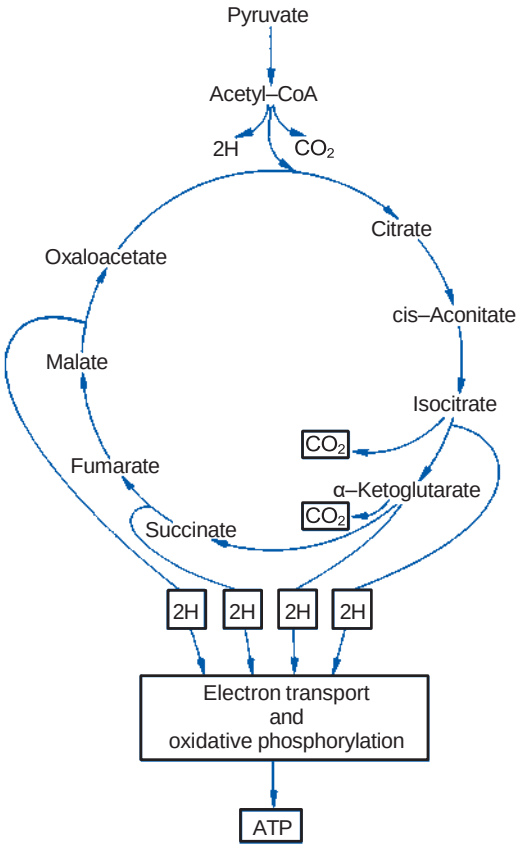

FIGURE 1-11 The Krebs tricarboxylic acid cycle allowing the complete oxidation of a substrate. (From Joklik WK et al: *Zinsser microbiology*, ed 20, Norwalk, Conn, 1992, Appleton & Lange.)

- *Mixed acid fermentation:* Members of the genera *Escherichia, Salmonella,* and *Shigella* within the Enterobacteriaceae use this pathway for sugar fermentation and produce a number of acids as end products—lactic, acetic, succinic, and formic acids. The strong acid produced is the basis for the positive reaction on the methyl red test exhibited by these organisms.
- *Butanediol fermentation:* Members of the genera *Klebsiella, Enterobacter,* and *Serratia* within the Enterobacteriaceae use this pathway for sugar fermentation. The end products are acetoin (acetyl methyl carbinol) and 2,3-butanediol. Detection of acetoin is the basis for the positive VP reaction characteristic of these microorganisms. Little acid is produced by this pathway. Thus organisms that have a positive VP reaction usually have a negative reaction on the methyl red test, and vice versa.
- *Butyric acid fermentation:* Certain obligate anaerobes, including many *Clostridium* species, *Fusobacterium,* and *Eubacterium,* produce butyric acid as their primary end product along with acetic acid, carbon dioxide, and hydrogen.

Aerobic Utilization of Pyruvate (Oxidation)

The most important pathway for the complete oxidation of a substrate under aerobic conditions is the Krebs or TCA (tricarboxylic acid) cycle. In this cycle, pyruvate is oxidized, carbon skeletons for biosynthetic reactions are created, and the electrons donated by pyruvate are passed through an electron transport chain and used to generate energy in the form of ATP. This cycle results in the production of acid and the evolution of carbon dioxide (Figure 1-11).

Carbohydrate Utilization and Lactose Fermentation

The ability of microorganisms to use various sugars (carbohydrates) for growth is an integral part of most diagnostic identification schemes. The fermentation of the sugar is usually detected by acid production and a concomitant change of color resulting from a pH indicator present in the culture medium. In general, bacteria ferment glucose preferentially over other sugars, and therefore glucose must not be present if the ability to ferment another sugar is being tested.

One of the important steps in classifying members of the Enterobacteriaceae family is the determination of the microorganism's ability to ferment lactose. These bacteria are classified as either lactose fermenters or lactose nonfermenters.

Lactose is a disaccharide consisting of one molecule of glucose and one molecule of galactose linked together by a galactoside bond. Two steps are involved in the utilization of lactose by a bacterium. The first step requires an enzyme,

β-galactoside permease, for the transport of lactose across the cell wall into the bacterial cytoplasm. The second step occurs inside the cell and requires the enzyme β-galactosidase to break the galactoside bond, releasing glucose, which can then be fermented. Thus all organisms that can ferment lactose can also ferment glucose.

BACTERIAL GENETICS

No discussion of bacterial genetics is complete without first describing deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Historically, DNA was first discovered by Frederick Miescher in 1869. In the 1920s, Phoebus A.T. Levine discovered that DNA contained phosphates, five-carbon sugars (cyclic pentose), and nitrogen-containing bases. Later, Rosalind Franklin discovered the helical structure by x-ray crystallography. Most everyone is familiar with James Watson and Francis Crick, who both described the three-dimensional structure of the DNA molecule in the 1950s.

Anatomy of a DNA and RNA Molecule

DNA is a double helical chain of nucleotides. The helix is a double strand twisted together, which many scientists refer to as a "spiral staircase" (resembling the handrail, sides, and steps of a spiral staircase). Others refer to it as a "zipper with teeth."

A nucleotide is a complex combination of the following:

- A phosphate group (PO_4).
- A cyclic five-carbon pentose (the carbons in the pentose are numbered 1′ through 5′) sugar (deoxyribose), which makes up the "handrails and sides."
- A nitrogen-containing base, or the "steps," either a purine or a pyrimidine. A purine consists of a fused ring of nine carbon atoms and nitrogen. There are two purines in the molecule: adenine *(A)* and guanine *(G)*. A pyrimidine consists of a single ring of six atoms of carbon and nitrogen. There are two pyrimidines in the molecule: thymine *(T)* and cytosine *(C)*. A nucleotide is formed when the 5′ carbon of the sugar and one of the nitrogenous bases attaches to the 1′ carbon of the pentose sugar. These are the basic building blocks of DNA (Figure 1-12).

In the chain of nucleotides, bonds form between the phosphate group of one nucleotide and the 3′ sugar of the next nucleotide. The base extends out of the sugar. Adenine of one chain always pairs with thymine of the other chain, and cytosine of one chain pairs with guanine of the other chain. The bases are held together by hydrogen bonds. The information contained in DNA is determined primarily by the sequence of letters along the "staircase" or "zipper." The sequence *ACGCT* represents different information than the sequence *AGTCC*. This would be like taking the word "stops" and using the same letters to form the word "spots" or "posts," which have different meanings but all the same letters. The two complementary sugar phosphate strands run in opposite directions (antiparallel), 3′ to 5′ and 5′ to 3′, like one train with its engine going one way alongside a caboose of a train going the opposite direction (Figure 1-13). The direction is based on what is found at the ends of the strands; for example, phosphate attaches to the 5′ carbon of the sugar and OH group is attached to the 3′ carbon of the sugar.

DNA is also involved in the production of RNA. In RNA, the nitrogenous base thymine is replaced by uracil, another pyrimidine. Unlike DNA, RNA is single stranded and short, not double stranded and long, and contains the sugar ribose, not deoxyribose.

An interesting aspect is introduced. Human beings are 99.9% identical. In a human genome of 3 billion "letters," even one tenth of 1% translates into 3 million separate lettering differences, an important characteristic useful in forensic science, but with related importance in diagnostic microbiology using the bacterial genome. Bacterial genetics is increasingly important in the diagnostic microbiology laboratory. New diagnostic tests have been developed that are based on identifying unique RNA or DNA sequences present in each bacterial species. The polymerase chain reaction (PCR) technique is a means of amplifying specific DNA sequences and thus detecting very small numbers of bacteria present in a specimen. Genetic tests circumvent the need to culture bacteria, providing a more rapid method of identifying pathogens.

An understanding of bacterial genetics is also necessary to understand the development and transfer of antimicrobial resistance by bacteria. The occurrence of mutations can result

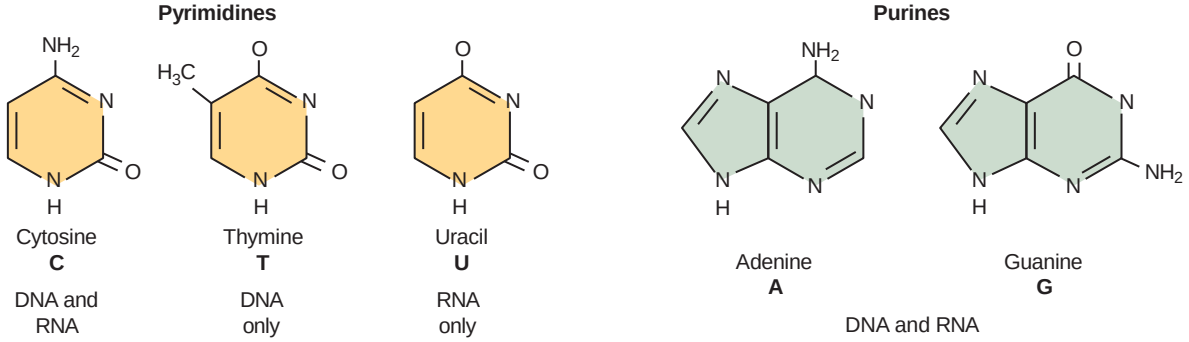

FIGURE 1-12 Molecular structure of nucleic acid bases. Pyrimidines: cytosine, thymine, and uracil. Purines: adenine and guanine.

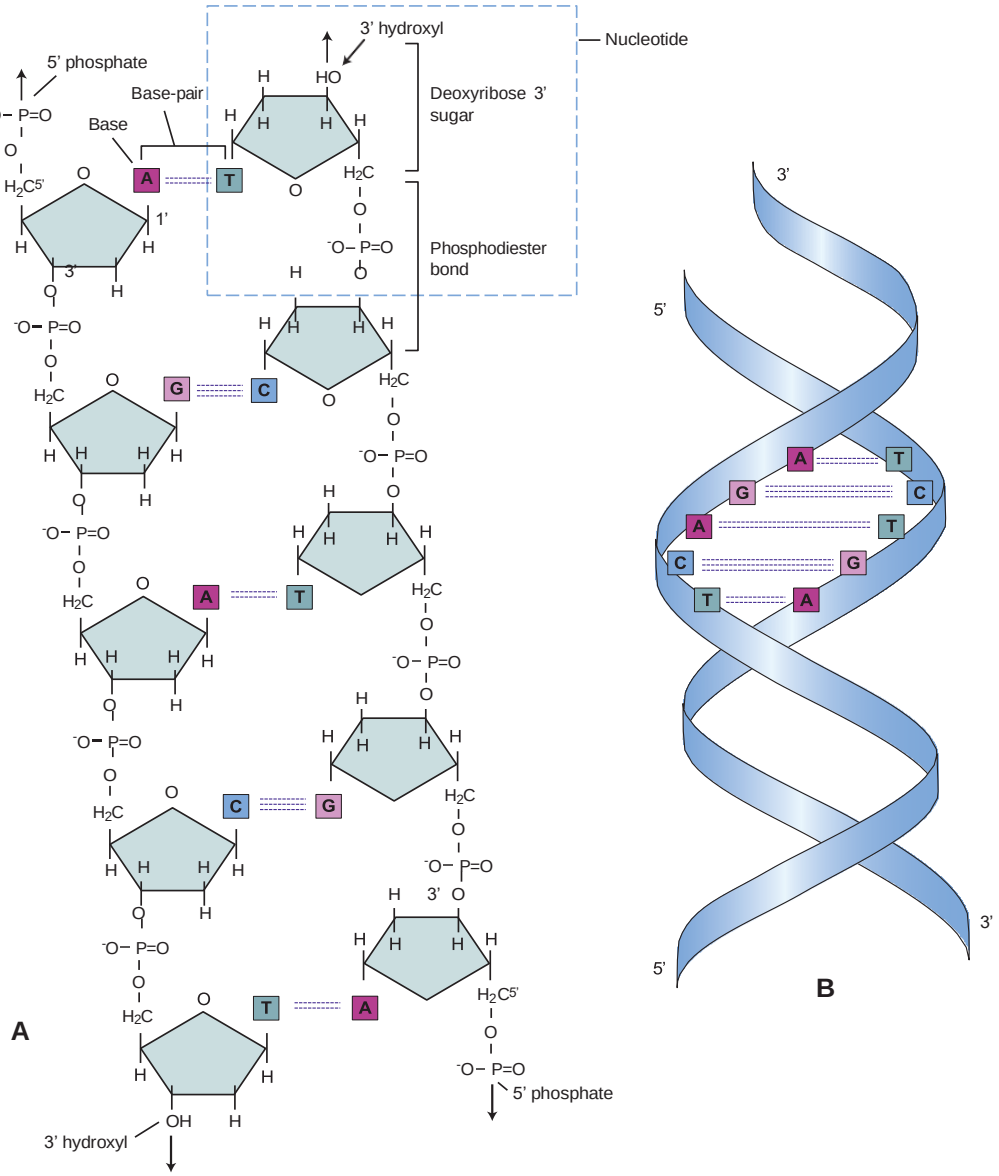

FIGURE 1-13 **A,** Molecular structure of DNA showing nucleotide structure, phosphodiester bond connecting nucleotides, and complementary pairing of bases (*A*, adenine; *T*, thymine; *G*, guanine; *C*, cytosine) between antiparallel nucleic acid strands. **B,** 5′ and 3′ antiparallel polarity and "twisted ladder" configuration of DNA.

in a change in the expected phenotypic characteristics of an organism and provides an explanation for atypical results sometimes encountered on diagnostic biochemical tests. This section briefly reviews some of the basic terminology and concepts of bacterial genetics. For a detailed discussion of DNA and molecular diagnostics, see Chapter 11.

Terminology

The genotype of a cell is the genetic potential of the DNA of an organism. It includes all the characteristics that are coded for in the DNA of a bacterium and that have the potential to be expressed. Some genes are silent genes, expressed only under certain conditions. Genes that are always expressed are constitutive. Genes that are expressed only under certain conditions are inducible. The phenotype of a cell consists of the genetic characteristics of a cell that actually are expressed and can be observed. The ultimate aim of a cell is to produce the proteins that are responsible for cellular structure and function and to transmit the information for accomplishing this to the next generation of cells. Information for protein synthesis is encoded in the bacterial DNA and transmitted in the chromosome to each generation. The general flow of information in a bacterial cell is from DNA (which contains the genetic information) to messenger RNA (mRNA) (which acts

as a blueprint for protein construction) to the actual protein itself. Replication is the duplication of chromosomal DNA for insertion into a daughter cell. Transcription is the synthesis of single-stranded RNA (with the aid of the enzyme RNA polymerase) using one strand of the DNA as a template. Translation is the actual synthesis of a specific protein from the mRNA code. The term **protein expression** also refers to the synthesis (i.e., translation) of a protein. Proteins are polypeptides composed of amino acids. The number and sequence of amino acids in a polypeptide, and thus the character of that particular protein, are determined by sequence of codons in the mRNA molecule. A **codon** is a group of three nucleotides in an mRNA molecule that signifies a specific amino acid. During translation, ribosomes containing ribosomal RNA (rRNA) sequentially add amino acids to the growing polypeptide chain. These amino acids are brought to the ribosome by transfer RNA (tRNA) molecules that "translate" the codons. Transfer RNA molecules temporarily attach to the mRNA using their complementary anticodon regions. An **anticodon** is the triplet of bases on the tRNA that bind the triplet of bases on the mRNA. It identifies which amino acid will be in a specific location in the protein.

Genetic Elements and Alterations

Bacterial Genome

The bacterial chromosome (also called the *genome*) consists of a single, closed, circular piece of double-stranded DNA that is supercoiled in order to fit inside the cell. It contains all the information needed for cell growth and replication. Genes are specific DNA sequences that code for the amino acid sequence in one protein (e.g., one gene equals one polypeptide), but this may be sliced up and/or combined with other polypeptides to form more than one protein. In front of each gene on the DNA strand is an untranscribed area containing a promoter region, which the RNA polymerase recognizes for transcription initiation. This area may also contain regulatory regions to which molecules may attach and cause either a decrease or an increase in transcription.

Extrachromosomal Elements

In addition to the genetic information encoded in the bacterial chromosome, many bacteria contain extra information on small circular pieces of *extrachromosomal*, double-stranded DNA called **plasmids.** They are *not* essential for bacterial growth, so they can be gained or lost. Genes that code for antibiotic resistance (and sometimes toxins or other virulence factors) are often located on plasmids. Antibiotic therapy selects for bacterial strains containing plasmids encoding antibiotic resistance genes; this is one reason antibiotics should not be overprescribed. The number of plasmids present in a bacterial cell may vary from one (low copy number) to hundreds (high copy number). Plasmids are located in the cytoplasm of the cell and are self-replicating and passed to daughter cells, just like chromosomal DNA. They may also sometimes be passed (nonsexually) from one bacterial species to another through **conjugation** (horizontal transfer of genetic material by cell-to-cell contact). This is one way resistance to antibiotics is acquired.

Mobile Genetic Elements

Certain pieces of DNA are mobile and may jump from one place in the chromosome to another place. These are sometimes referred to as *jumping genes*. The simplest mobile piece of DNA is an insertion sequence (IS) element. It is approximately 1000 base pairs long with inverted repeats on each end. Each IS element codes for only one gene, a transposase enzyme that allows the IS element to pop into and out of DNA. Bacterial genomes contain many IS elements. The main effect of IS elements in bacteria is that when an IS element inserts itself into the middle of a gene, it disrupts and inactivates the gene. This can result in loss of an observable characteristic, such as the ability to ferment a particular sugar. Transposons are related mobile elements that contain additional genes. Transposons often carry antibiotic-resistance genes and are usually located in plasmids.

Mutations

A gene sequence must be read in the right "frame" for the correct protein to be produced. This is because every set of three bases (known as a *codon*) specifies a particular amino acid, and when the reading frame is askew, the codons are interpreted incorrectly. Mutations are changes that occur in the DNA code and often (not always; "silent mutations" do not make a change in the protein) results in a change in the coded protein or in the prevention of its synthesis. A mutation may be the result of a change in one nucleotide base (a point mutation) that leads to a change in a single amino acid within a protein or may be the result of insertions or deletions in the genome that lead to disruption of the gene and/or a frameshift mutation. Incomplete, inactive proteins are often the result. Spontaneous mutations occur in bacteria at a rate of about one in 10^9 cells. Mutations also occur as the result of error during DNA replication at a rate of about one in 10^7 cells. Exposure to certain chemical and physical agents can greatly increase the mutation rate.

Genetic Recombination

Genetic recombination is a method by which genes are transferred or exchanged between homologous (similar) regions on two DNA molecules. This method provides a way for organisms to obtain new combinations of biochemical pathways and copy with changes in their environment.

Mechanisms of Gene Transfer

Genetic material may be transferred from one bacterium to another in three basic ways:

- Transformation
- Transduction
- Conjugation

Transformation

Transformation is the uptake and incorporation of naked DNA into a bacterial cell (Figure 1-14, *A*). Once the DNA has been taken up, it can be incorporated into the bacterial genome by recombination. If the DNA is a circular plasmid and the recipient cell is compatible, the plasmid can replicate in the cytoplasm and be transferred to daughter cells. Cells

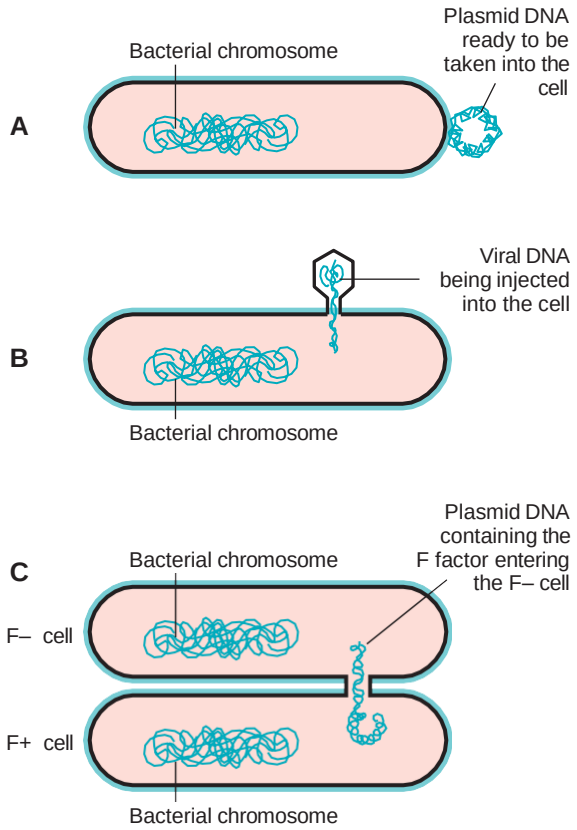

FIGURE 1-14 Diagram of methods of gene transfer into bacterial cells. **A,** Bacterial transformation. Free or "naked" DNA is taken up by a competent bacterial cell. After uptake, the DNA may take one of three courses: (1) it is integrated into existing bacterial genetic material, (2) it is degraded, or (3) if it is a compatible plasmid, it may replicate in the cytoplasm. **B,** Bacterial transduction. A phage injects DNA into the bacterial cell. The phage tail combines with a receptor of the bacterial cell wall and injects the DNA into the bacterium. One of two courses may then be taken. In the lytic cycle, replication of the bacterial chromosome is disrupted; phage components are formed and assembled into phage particles. The bacterial cell is lysed, releasing mature phage. In the lysogenic cycle, the phage DNA is incorporated into the bacterial genetic material, and genes encoded on the phage DNA may be expressed from this site. At a later time the phage may be "induced," and a lytic cycle will then ensue. **C,** Bacterial conjugation. An F+ cell connects with an F- cell via sex pili. DNA is then transferred from one cell to the other.

that can take up naked DNA are referred to as being **competent.** Only a few bacterial species, such as *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, and *H. influenzae*, do this naturally. Bacteria can be made competent in the laboratory, and transformation is the main method used to introduce genetically manipulated plasmids into bacteria, such as *E. coli*, during cloning procedures.

Transduction

Transduction is the transfer of bacterial genes by a **bacteriophage** (virus-infected bacteria) from one cell to another (see Figure 1-14, *B*). A bacteriophage consists of a chromosome (DNA or RNA) surrounded by a protein coat. When a phage infects a bacterial cell, it injects its genome into the bacterial cell, leaving the protein coat outside. The phage may then take a lytic pathway, in which the bacteriophage DNA directs the bacterial cell to synthesize phage DNA and phage protein and package it into new phage particles. The bacterial cell then lyses (lytic phase), releasing new phage, which can infect other bacterial cells. In some instances, the phage DNA instead becomes incorporated into the bacterial genome, where it is replicated along with the bacterial chromosomal DNA; this state is known as **lysogeny,** and the phage is referred to as being **temperate.** During lysogeny, genes present in the phage DNA may be expressed by the bacterial cell. An example of this in the clinical laboratory is *Corynebacterium diphtheriae*. Strains of *C. diphtheriae* that are lysogenized with a temperate phage carrying the gene for diphtheria toxin cause disease. Strains lacking the phage do not produce the toxin and do not cause disease. Under certain conditions a temperate phage can be induced, the phage DNA is excised from the bacterial genome, and a lytic state occurs. During this process, adjacent bacterial genes may be excised with the phage DNA and packaged into the new phage. The bacterial genes may then be transferred when the phage infects a new bacterium. In the field of biotechnology, phages are often used to insert cloned genes into bacteria for analysis.

Conjugation

Conjugation is the transfer of genetic material from a donor bacterial strain to a recipient strain (see Figure 1-14, *C*). It requires close contact between the two cells. In the *E. coli* system, the donor strain (F+) possesses a fertility factor (F factor) on a plasmid that carries the genes for conjugative transfer. The donor strain produces a hollow surface appendage called a *sex pilus*, which binds to the recipient F–cell and brings the two cells in close contact. Transfer of DNA then occurs. Both plasmids and chromosomal genes can be transferred by this method. When the F factor is integrated into the bacterial chromosome rather than a plasmid, there is a higher frequency of transfer of adjacent bacterial chromosomal genes. These strains are known as *high-frequency* (Hfr) *strains.*

Restriction Enzymes

Bacteria have evolved a system to restrict the incorporation of foreign DNA into their genomes. Specific **restriction enzymes** are produced that cut incoming, foreign DNA at specific DNA sequences. The bacteria methylate their own DNA at these same sequences so that the restriction enzymes do not cut the DNA in their own cell. Many restriction enzymes with a variety of recognition sequences have now been isolated from various microorganisms. The first three letters in the restriction endonuclease name indicate the bacterial source of the enzyme. For instance, the enzyme *Eco*RI was isolated from *E. coli*, and the enzyme *Hind*III was isolated from *H. influenzae* type d. These enzymes are used in the field of biotechnology to create sites for insertion of new genes. In clinical microbiology, epidemiologists use restriction enzyme fragment analysis to determine whether strains of bacteria have identical restriction sites in their genomic DNA.

BIBLIOGRAPHY

Baumann RW: *Microbiology*, San Francisco, 2004, Pearson Benjamin Cummings.

Campbell NA, Reece JB: *Essential biology*, San Francisco, 2001, Pearson Benjamin Cummings.

Forbes BA et al: *Bailey and Scott's diagnostic microbiology*, ed 12, St Louis, 2007, Mosby.

Holt JG et al: *Bergey's manual of determinative bacteriology*, ed 9, Baltimore, 1993, Williams & Wilkins.

Howard BJ: *Clinical and pathogenic microbiology*, ed 2, St Louis, 1994, Mosby.

Marieb EN: *Essentials of human anatomy and physiology*, ed 8, San Francisco, 2006, Pearson Benjamin Cummings.

Murray PR et al: *Medical microbiology*, ed 5, St Louis, 2005, Mosby.

Murray PR et al: *Manual of clinical microbiology*, ed 8, vol 1, Washington, DC, 2003, ASM.

Tortora GJ et al: *Microbiology: an introduction*, ed 7, San Francisco, 2002, Benjamin Cummings.

Points to Remember

- There are many organisms that inhabit our environment. The majority of these microorganisms are nonpathogenic.
- Prokaryotes, including bacteria, do not have membrane-enclosed nuclei and organelles.
- Eukaryotes differ from prokaryotes in that they have membrane-enclosed nuclei and organelles.
- Viruses cannot be seen under an ordinary light microscope, although their cytopathic effects on cell lines are visible. They are obligate parasites, and antibiotics are ineffective for their treatment. Viruses have DNA or RNA, but never both, in contrast to the prokaryotes and eukaryotes.
- Bacteria utilize two biochemical pathways, fermentation and respiration (oxidation) to catabolize carbohydrates to produce energy.
- The major way bacteria are classified in the diagnostic microbiology laboratory is by the Gram stain reaction. Whether an organism is gram-positive (blue or purple) or gram-negative (pink or red) is an important first step in identifying bacteria and in determining appropriate antimicrobial therapy.
- Bacterial spores are formed due to harsh environments. They are a means of survival, not reproduction.

Learning Assessment Questions

1. Explain the reason why the technologist should repeat the Gram stain procedure on the exudate.
2. What may have occurred to make the results invalid?
3. Differentiate the role of the pili from that of the flagella.
4. What is the role of the capsule in the pathogenesis of infectious diseases?
5. If the technologist forgets to add the Gram's iodine to the staining procedure, what will most likely occur?
6. Why is lipopolysaccharide (LPS) a significant outer-membrane structure in gram-negative bacteria?
7. Fimbriae present on the outer surface of bacteria are used for:
   a. Sexual reproduction
   b. Bacterial motility
   c. Adherence to surfaces
   d. Antibiotic resistance
8. Why are older bacterial cells more easily decolorized than younger colonies?
9. All of the following are characteristic of fermentation **except:**
   a. It follows glycolysis and produces NAD.
   b. It produces acids, alcohols, and gases.
   c. It occurs in the presence of oxygen.
   d. It begins with the breakdown of pyruvic acid.
10. Why are spore-forming organisms more resistant than non–spore-forming species?
11. Explain the three ways in which genetic material may be transferred from one bacterium to another.
12. For the following DNA, write the complementary sequence. Include labeling the 3′ and 5′ end.

    TTACGGACAAC

    _____
13. In RNA, thymine is replaced by_____.

CHAPTER 2

Host-Parasite Interaction

Connie R. Mahon, Maribeth L. Flaws*

A. Role of the Usual Microbial Flora

- ORIGIN OF MICROBIAL FLORA
  - Characteristics of Indigenous Microbial Flora
  - Factors That Determine the Composition of the Usual Microbial Flora
- COMPOSITION OF THE MICROBIAL FLORA AT DIFFERENT BODY SITES
  - Usual Flora of the Skin
  - Usual Flora of the Mouth
  - Usual Flora of the Respiratory Tract
  - Usual Flora of the Gastrointestinal Tract
  - Usual Flora of the Genitourinary Tract
- ROLE OF THE MICROBIAL FLORA IN THE PATHOGENESIS OF INFECTIOUS DISEASE
- ROLE OF THE MICROBIAL FLORA IN THE HOST DEFENSE AGAINST INFECTIOUS DISEASE

OBJECTIVES

After reading and studying this chapter, you should be able to:

1. Define the following terms: *parasitism, indigenous flora, commensal, symbiont, opportunist, resident flora, transient flora,* and *carrier.*
2. Explain how the following factors determine the composition of the microbial flora at various body sites:
   - Amounts and types of nutrients available in the environment
   - pH
   - Oxidation-reduction potential
   - Resistance to antibacterial substances
3. List the predominant flora of various body sites in a healthy individual.
4. Describe the role of the usual microbial flora in the pathogenesis of infectious disease.
5. Describe the role of the indigenous flora in host defense against infectious diseases.

Case in Point

A 48-year-old woman had a complete hysterectomy. After 3 days of uneventful hospitalization, the patient was discharged from the hospital with a 10-day regimen of prophylactic antimicrobial. On the fifth day after her discharge, she noticed the presence of creamy-white, cheesy material on her tongue and buccal cavity. Wet mount preparations of the material showed budding yeast cells and hyphal elements.

Issues to Consider

After reading the patient's case history, consider:

- Factors that predisposed this patient to her current condition
- Clues that indicate the source of the infection
- Significance of the microbial flora in protecting the host against pathogenic organisms

Key Terms

Carrier
Carrier state
Commensalism
Indigenous flora
Opportunists
Parasite
Parasitism
Pathogen
Resident microbial flora
Symbiosis
Transient flora

*This chapter was prepared by C.R. Mahon in her private capacity. No official support or endorsement by the FDA is intended or implied.

The outcome from the interactions between host and **pathogen** is influenced by numerous factors. The status of the host's immune system and ability of the host to defend itself from microbial invasion, combined with microbial factors inherent to the invading organism, often determine whether disease will occur. To appreciate and understand the concepts involved in the pathogenesis of infectious diseases, knowledge and understanding of the host-pathogen relationship is important.

This chapter describes the interactions between the host and infectious agent in the pathogenesis of disease. The first section of the chapter describes the origin of the indigenous microbial flora and the composition at different body sites. It presents the role of the microbial flora at each body site in the host immune defense and as a source of opportunistic infections. Factors that determine the composition of the flora at different body sites are described. The second section of the chapter discusses the virulence factors that contribute to the invasiveness of organisms, protective mechanisms the host employs, and how microbes are able to evade the host's defenses. Lastly, this chapter describes factors that may make the host more susceptible to microbial infections and how microbes are transmitted and acquired.

ORIGIN OF MICROBIAL FLORA

The fetus is in a sterile environment until birth. During the first few days of life, the newborn is introduced to the many and varied microorganisms present in the environment. Each organism has the opportunity to find an area on or in the infant into which it is to adapt. Those that find their niche colonize various anatomic sites and become the predominant organisms. Others are transient or fail to establish themselves at all. As the infant grows, the microbial flora eventually becomes similar to that seen in older individuals.

Once established onto or into a particular body site of the host, microorganisms develop a particular relationship with that host. The host-microbe relationship, depending on the circumstance, may be one of **symbiosis, commensalism,** or **parasitism.** Symbiosis is defined as the association of two organisms living together. The organisms are called *symbionts*. Symbiosis as a biological relationship between two or more organisms where both (host and organism) benefit from one another may be described as *mutualism*. Lactobacilli in the urogenital tract of women offer a mutual association, providing the host protection by preventing colonization of pathogenic species at that site while the lactobacilli derive nutrients from the host. In the relationship where the organism benefits, but there is no beneficial or harmful effect to the host, the association between organisms is called *commensalism*. *Proteus mirabilis* is a commensal species in the gastrointestinal tract of humans. In parasitism, one species (microbe) benefits at the expense of the other (host). The parasite *Entamoeba histolytica* is a pathogenic intestinal amoeba that derives nutrients from the host at the expense of the host, causing intestinal ulcers and amebic dysentery.

Characteristics of Indigenous Microbial Flora

Microorganisms that are commonly found on or in body sites of healthy persons are called *normal, usual,* or **indigenous flora.** The different body sites may have the same or different flora, depending on local conditions. Local conditions select for those organisms that are suited for growth in a particular area. For example, the environment found on the dry skin surface is different from that found on the moist surfaces of the mouth and so the flora is different at the two sites.

Microorganisms that colonize an area for months or years are called **resident microbial flora,** whereas **transient flora** are those that are present at a site temporarily. Transient flora comes to "visit" but does not usually live or stay. They are eliminated by either the host inherent immune defenses or by competition with the resident flora. Some pathogenic organisms may establish themselves in a host without manifesting symptoms. However, these hosts, called **carriers,** are capable of transmitting the infection. These hosts' condition is called the **carrier state.** The carrier state may be acute (short-lived or transient) or chronic (lasting for months, years, or permanently). An example of a chronic carrier state is found in post-*Salmonella typhi* infection. This organism may establish itself in the bile duct and be excreted in the stool over years. In contrast, *Neisseria meningitidis* may be found in the nasopharynx of asymptomatic individuals during an outbreak of meningitis. After a few days or weeks at most, these individuals may no longer harbor the organism, in which case the carrier state would be termed *acute*. The most transient of carrier states is the inoculation of a person's hands or fingers with an organism (e.g., with *Staphylococcus aureus* that has colonized the person's anterior nares) that is carried only until the hands are washed.

The organisms colonizing different body sites play a significant role in providing host resistance to infections. The efficiency of the microbial flora in providing protection to the human host is indicated by the relatively small number of infections caused by these organisms in immune-competent individuals. Nevertheless, these organisms may cause significant, often serious, infections or may exacerbate existing infections in individuals lacking a fully responsive immune system.

Factors That Determine the Composition of the Usual Microbial Flora

Which organisms are present at a particular body site is influenced by the nutritional and environmental factors such as the amount and types of nutrients available at the site. For example, more organisms inhabit moist areas than dry areas; these areas are dominated by diphtheroids. Although lipids and fatty acids are bactericidal to most bacteria, *Propionibacterium* spp. colonize the ducts of hair follicles because these bacteria are able to break down the skin lipids to fatty acids. *Propionibacterium acnes* cannot be removed by washing or scrubbing. The affinity of microorganisms for a specific site depends on the ability of those organisms to resist the anti-

bacterial effects of substances such as bile, lysozyme, or fatty acids. The composition of the microbial flora is also affected by pH. For example, the female genital tract flora depends on the pH of that environment, which in women of child-bearing age is approximately 4.0 to 5.0. Most bacteria do not survive at these extreme pH ranges. Another example is that of the fecal flora found in babies who are breast-fed, which differs from those in babies fed with cow's milk. Human milk has a high lactose concentration and maintains a pH of 5.0 to 5.5, an environment supportive of *Bifidobacterium* spp. Cow's milk, on the other hand, has a greater buffering capacity and is therefore less acidic. Infants fed with cow's milk do not have the high colonization by *Bifidobacterium* spp. found in breast-fed babies, but have instead colon flora similar to that seen in older children and adults (see Box 2-5). In areas of the body that have a low oxidation-reduction potential, the environment will support only organisms capable of fermentation, such as is seen in the gingival crevices colonized with *Bacteroides* and *Fusobacterium*.

The environmental conditions described here certainly may change with age, nutritional status, disease states, and drug or antibiotic effects. These changes may predispose an individual to infection by the indigenous flora, a type of infection referred to as an *opportunistic infection*. For example, two groups at increased risk for gram-negative rod pneumonia are diabetics and alcoholics. As described in the introductory case study, antibiotics may reduce a particular population of bacteria, allowing the proliferation of other organisms such as *Candida albicans*. An increase in age brings with it a decrease in the effectiveness of the immune response. As a result, the incidence of infection caused by opportunistic organisms increases.

COMPOSITION OF THE MICROBIAL FLORA AT DIFFERENT BODY SITES

The human host is colonized by approximately 100 different species of microorganisms and has a total organism load of about 100 trillion. The effectiveness of the various host defenses is evidenced by the relatively low incidence of infection in immune-competent individuals by members of the usual or indigenous flora. However, infections caused by members of the microbial flora are frequently encountered among immune-compromised patients. Because of this, the clinical microbiologist must be able to recognize and identify the types of microorganisms found at the various body sites.

Usual Flora of the Skin

Normal skin has a number of mechanisms to prevent infection and protect the underlying tissue from invasion by potential pathogens. These include the mechanical separation of microorganisms from the tissues, presence of fatty acids that inhibit many microorganisms, excretion of lysozyme by sweat glands, and the desquamation of the epithelium. The skin contains a wide variety of microorganisms, most of which are found on the most superficial layers of cells and the upper parts of hair follicles. The number of bacteria present on the skin can be reduced by about 90% by scrubbing and washing, but they are not completely eliminated and their numbers return to normal within a few hours.

The composition of the flora on the skin depends on the activity of the sebaceous or sweat glands. Organisms concentrate the most in areas that are moist such as the armpit, groin, and perineum. The apocrine sweat glands in these areas secrete substances metabolized by the skin bacteria, releasing odorous amines. Aerobic diphtheroids are usually found in moist areas such as the axillae and between toes. *Staphylococcus epidermidis* and *Propionibacterium* spp. reside in hair follicles and colonize the sebaceous glands because they are resistant to skin lipids and fatty acids as well as to superficial antiseptic agents commonly used to cleanse the skin. The presence of skin bacteria inhibits the growth of more pathogenic bacterial species, thus providing benefits to the host.

Microorganisms such as *P. acnes* colonize the deep sebaceous glands. Superficial antisepsis of the skin does not eliminate this organism, which may be found as a contaminant in those culture specimens obtained by invasive procedures (e.g., blood, cerebrospinal fluid), as a result of contamination of the needle.

Box 2-1 lists the microorganisms most commonly found on the skin. Other organisms have been isolated from the skin but are found only occasionally or rarely and are therefore not listed.

Usual Flora of the Mouth

The mouth contains large numbers of bacteria, with *Streptococcus* being the predominant genus. Many organisms bind to the buccal mucosa and tooth surfaces. Bacterial plaques that develop on teeth may contain as many as 10^{11} streptococci per gram. Plaque also results in a low oxidation-reduction potential at the tooth surface; this supports the growth of strict anaerobes, particularly in crevices and in the areas between the teeth. Box 2-2 gives a partial list of microorganisms found in the mouth.

Usual Flora of the Respiratory Tract

The respiratory tract, commonly divided into upper and lower tracts, is responsible for the exchange of oxygen and carbon dioxide as well as for the delivery of air from the outside of the body to the pulmonary tissues responsible for that exchange. The upper respiratory tract is composed of the mouth, nasopharynx, oropharynx, and larynx; the lower respiratory tract is composed of the trachea, bronchi, and pulmonary parenchyma. The trachea, bronchi, and lungs are protected by the action of ciliary epithelial cells and by the

BOX 2-1 Microorganisms Found on the Skin

Common	Less Common
Candida spp.	*Streptococcus* spp.
Micrococcus spp.	*Acinetobacter* spp.
Staphylococcus spp.	*Bacteroides* spp.
Clostridium spp.	Gram-negative rods (fermenters and nonfermenters)
Propionibacterium spp.	
Diphtheroids	*Moraxella* spp.

BOX 2-2 Microorganisms Found in the Mouth

Common

Staphylococcus epidermidis
Streptococcus mitis
Streptococcus sanguis
Streptococcus salivarius
Streptococcus mutans
Peptostreptococcus spp.
Veillonella spp.
Lactobacillus spp.
Actinomyces israelii
Bacteroides spp.
Prevotella/Porphyromonas
Bacteroides oralis
Treponema denticola
Treponema refringens

Less Common

Staphylococcus aureus
Enterococcus spp.
Eikenella corrodens
Fusobacterium nucleatum
Candida albicans

BOX 2-3 Microorganisms Found in the Nose and Nasopharynx

Common

Staphylococcus aureus
Staphylococcus epidermidis
Diphtheroids
Haemophilus parainfluenzae
Streptococcus spp.

Less Common

Streptococcus pneumoniae
Moraxella catarrhalis
Haemophilus influenzae
Neisseria meningitidis
Moraxella spp.

movement of mucus. The tissues of these structures are normally sterile as a result of this protective action.

The mouth, nasopharynx, and oropharynx are colonized predominantly with viridans streptococci such as *Streptococcus mitis, Streptococcus mutans, Streptococcus milleri, Streptococcus sanguis*, and *Moraxella catarrhalis, Neisseria* spp., and diphtheroids. Obligate anaerobes reside in the gingival crevices where the anaerobic environment supports these organisms. The organisms found in the mouth, nasopharynx, oropharynx, and nose, although similar show some differences. Box 2-3 lists common microorganisms encountered in the nose and nasopharynx. Opportunistic pathogens such as *S. aureus*, found in approximately 30% of healthy individuals, colonize the anterior nares. The population of the nasopharynx mirrors that of the nose, even if the environment is different enough from that of the nose to select for several additional organisms. *Haemophilus influenzae, Streptococcus pneumoniae*, and *N. meningitidis*, all potential pathogens, are also found in the nasopharynx of healthy individuals. Individuals who are hospitalized for several days may become colonized in the upper respiratory tract by gram-negative bacteria, particularly members of the Enterobacteriaceae.

The oropharynx contains a mixture of streptococci. A number of species of the viridans group can be isolated, including *S. mitis, S. mutans, S. milleri, S. sanguis*, and *S. salivarius*. In addition, diphtheroids and *M. catarrhalis* can be readily isolated. Hospitalized patients often show colonization with gram-negative rods. The usual flora of the oropharynx is listed in Box 2-4.

Usual Flora of the Gastrointestinal Tract

The gastrointestinal tract comprises the esophagus, stomach, small intestine, and colon. The gastrointestinal tract is

BOX 2-4 Microorganisms Found in the Oropharynx

Common

α-Hemolytic and nonhemolytic streptococci
Diphtheroids
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus pneumoniae
Streptococcus mutans
Streptococcus milleri
Streptococcus mitis
Streptococcus sanguis
Streptococcus salivarius
Moraxella catarrhalis
Haemophilus parainfluenzae
Anaerobic streptococci
Bacteroides spp.
Prevotella/Porphyromonas
Bacteroides oralis
Fusobacterium necrophorum

Less Common

Streptococcus pyogenes
Neisseria meningitidis
Haemophilus influenzae
Gram-negative rods

equipped with numerous defenses and effective antimicrobial factors. Because intestinal pathogens are usually acquired by ingesting organisms contained in contaminated food or drink, host defenses against infections are present throughout the intestinal tract.

Microorganisms usually do not multiply in the esophagus and stomach, but are present in ingested food and as transient flora. The stomach is usually sterile, especially after ingestion of a meal, when gastric juices, acids, and enzymes, which help to protect the stomach from microbial attack, are produced. Most microorganisms are susceptible to the acid pH of the stomach and are destroyed, with the exception of spore-forming bacterial species in their spore phase, the cysts of **parasites,** and *Helicobacter pylori*. Organisms that are pH-susceptible and survive are generally protected by being enmeshed in food, and they move to the small intestine. The stomach acidity greatly reduces the number of organisms that reach the small intestine.

The small intestine contains few microorganisms; organisms that are present are usually from the colon. Microorganisms prevalent in the colon may produce a count between 10^8 and 10^{11} bacteria per gram of solid material. Although we usually think of the facultative anaerobes as being the predominant organisms, anaerobes far outnumber the facultative gram-negative rods, making up more than 90% of the microbial flora of the large intestine. Gram-positive cocci, yeasts, and *Pseudomonas aeruginosa* are also usually present in the large intestine.

The gastrointestinal tract population may be altered by antibiotics. In some cases, certain populations or organisms are eradicated or suppressed and other members of the indigenous flora are able to proliferate. This can be the cause of a severe necrotizing enterocolitis *(Clostridium difficile),* diarrhea *(C. albicans, S. aureus),* or other superinfection. The bacteria constituting the usual intestinal flora also carry out a variety of metabolic degradations and syntheses that appear to play a role in the health of the host. A summary of the organisms found in the gastrointestinal tract is shown in Box 2-5.

Usual Flora of the Genitourinary Tract

The kidneys, bladder, and fallopian tubes are normally free of microorganisms, although there are a few organisms origi-

BOX 2-5 Common Microorganisms Found in the Gastrointestinal Tract

Bacteroides spp.
Clostridium spp.
Enterobacteriaceae
Eubacterium spp.
Fusobacterium spp.
Peptostreptococcus spp.
Peptococcus spp.
Staphylococcus aureus
Enterococcus spp.

BOX 2-6 Microorganisms Found in the Genitourinary Tract

Common	Less Common
Lactobacillus spp.	Group B streptococci
Bacteroides spp.	Enterobacteriaceae
Clostridium spp.	*Acinetobacter* spp.
Peptostreptococcus spp.	*Candida albicans*
Staphylococcus aureus	
Staphylococcus epidermidis	
Enterococcus spp.	
Diphtheroids	

nating from the perineum that may be found in the distal centimeter of the urethra, particularly in women. The urethra is colonized in its outermost segment by those organisms found on the skin. The composition of the vaginal flora is consistent with hormonal changes and age. Before puberty and in postmenopausal women, vaginal flora primarily consists of yeasts, gram-negative bacilli, and gram-positive cocci. During child-bearing years, high estrogen levels promote the deposition of glycogen in vaginal epithelial cells. Lactobacilli metabolize glycogen in vaginal epithelial cells to maintain a low pH, creating an environment that is inhibitory to many organisms. The low pH, however, encourages colonization of the vagina with lactobacilli, anaerobic gram-negative bacilli, and gram-positive cocci. Microorganisms expected to be isolated from the genitourinary tract are listed in Box 2-6.

ROLE OF THE MICROBIAL FLORA IN THE PATHOGENESIS OF INFECTIOUS DISEASE

Some organisms that comprise the usual microbial flora are actually parasites that live off the host's nutrients, but in most cases provide some benefit to the host, creating a symbiotic relationship with the host, as mentioned earlier. However, certain members of the usual flora are **opportunists**; they cause disease when their habitat is damaged, disturbed, or changed, or when the host's immune system is weakened or compromised. In the case of trauma, either accidental or surgical, enough of the usual microbial flora found in the traumatized area may reach other areas in the body where these organisms are not part of the usual microbial flora. For example, patients who undergo surgery become susceptible to infections caused by organisms that colonize the particular surgical site (e.g., abdominal cavity); leakage following perforation of the colon spills the contents of the colon into the peritoneal cavity, leading to an overwhelming infection by the colon flora.

The host's immune response may be reduced or altered due to suppression by immunosuppressive drugs, chemotherapy, or radiation. Individuals with lymphoma, leukemia, or other blood disorders in which there is a functional defect in phagocytic activity or a decrease in the number of functioning cells, or in which chemotactic activity is impaired, also may have a reduced immune response. Members of the usual microbial flora also may initiate an infection or make an infection more serious in patients with chronic illnesses such as diabetes or severe hepatic disease such as cirrhosis.

ROLE OF THE MICROBIAL FLORA IN THE HOST DEFENSE AGAINST INFECTIOUS DISEASE

The microbial flora provides beneficial effects. The development of immunologic competence depends on this flora. The immune system is constantly primed by contact with the microorganisms. Animals born and raised in a germ-free environment have a poorly functioning immune system. Exposure to otherwise innocuous organisms can be fatal to such animals. Likewise, consider how a sterile environment might affect a newborn. Antibody production would not be stimulated and the reticuloendothelial system would remain undeveloped. Serum IgG and other antibodies effective against microorganisms would be suppressed that would make the individual more susceptible to pathogenic species. Without activation by microorganisms and the supporting action of antigen-presenting cells and cytokines, cell-mediated immunity would not develop normally.

The microbial flora produces conditions at the microenvironmental level that block colonization by extraneous pathogens. When the composition of the indigenous flora is altered (e.g., by antibiotic therapy with broad-spectrum antibiotics), other organisms capable of causing disease may fill the void. For example, gastroenteritis due to *Salmonella* is generally not treated with antibiotics and is better eliminated by natural exclusion by the colon flora. If the microbial flora is eliminated, such as in patients receiving antimicrobial therapy or some types of chemotherapy, resistant or more pathogenic species may be able to establish infection. *C. albicans* may multiply and cause diarrhea or possibly infections in the mouth or vagina as illustrated in the Case in Point presented at the beginning of this chapter.

The microbial flora plays an important role in both health and disease. Eradication of the usual flora may have profound negative effects, yet many common infections are caused by members of the usual flora. Knowledge of the role of these organisms in the pathogenesis of an infectious disease is helpful in assessing their significance when isolated from clinical samples.

BIBLIOGRAPHY

Hentschel U, Steinert M, Hacker J: Common molecular mechanisms of symbiosis and pathogenesis, *Trends Microbiol* 8:226, 2000.

Kumar V, Fausto N, Abbas A, editors: *Robbins & Cotran pathologic basis of disease*, ed 7, Philadelphia, 2004, Saunders.

Mandell G, Bennett J, Dolin R: *Mandell, Douglas and Bennett's principles and practice of infectious diseases*, ed 6, New York, 2005, Churchill Livingstone.
McKenzie SB: *Clinical laboratory hematology*, Upper Saddle River, NJ, 2009, Pearson Prentice Hall.
Mims C, Nash A, Stephen J: *Mim's pathogenesis of infectious disease*, ed 5, San Diego, 2001, Academic Press.
Moran NA, Baumann P: Bacterial endosymbionts in animals, *Curr Opin Microbiol* 3:270, 2000.
Nelson KE, Masters Williams CF: *Infectious disease epidemiology: theory and practice*, ed 2, 2006, Jones & Bartlett.
Sheldon GF: *Boyd's introduction to the study of disease*, ed 11, Philadelphia, 1992, Lea & Febiger.
Sharp S: Commensal and pathogenic microorganisms of humans. In Murray PR et al, editors: *Manual of clinical microbiology*, ed 9, Washington DC, 2007, ASM Press.
Winn W et al, editors: *Koneman's color atlas and textbook of diagnostic microbiology*, ed 6, Philadelphia, 2005, Lippincott Williams & Wilkins.

Points to Remember

- Humans do not exist in a sterile environment. Colonization of the body by microorganisms begins at birth.
- The usual microbial flora present at each site in the body is dictated by nutritional and environmental factors.
- Some species of the usual microbial flora may be opportunists, capable of causing disease in an immunocompromised host.
- The usual microbial flora benefits the normal host by priming the immune system, out-competing potential pathogens for nutrients, and creating a hostile environment for other microbes.

Learning Assessment Questions

1. How did the patient in the case study develop the yeast infection in her mouth?
2. What is the difference between resident and transient flora?
3. What is a carrier?
4. What is the significance of the carrier in the pathogenesis of disease?
5. What determines the composition of the indigenous flora at the different body sites?
6. A long-term resident species of bacteria in the gastrointestinal tract produces vitamin K, which is required for blood clotting in mammals. Of what type of host-parasite relationship is this an example?
7. What are the functions of the microbial flora as a host protective mechanism?

B. Pathogenesis of Infection

- MICROBIAL FACTORS CONTRIBUTING TO PATHOGENESIS AND VIRULENCE
  - Pathogenesis
  - Virulence
  - Ability to Resist Phagocytosis
  - Surface Structures That Promote Adhesion to Host Cells and Tissues
  - Ability to Survive Intracellularly and Proliferate
  - Ability to Produce Extracellular Toxins and Enzymes
- HOST RESISTANCE FACTORS
  - Physical Barriers
  - Cleansing Mechanisms
  - Antimicrobial Substances
  - Indigenous Microbial Flora
  - Phagocytosis
  - Inflammation
  - Immune Responses
- MECHANISMS BY WHICH MICROBES MAY OVERCOME THE HOST DEFENSES
- ROUTES OF TRANSMISSION
  - Airborne Transmission
  - Transmission by Food and Water
  - Close Contact
  - Cuts and Bites
  - Arthropods
  - Zoonoses

OBJECTIVES

After reading and studying this chapter, you should be able to:

1. Define the following terms: *true pathogen, opportunistic pathogen,* and *virulence*.
2. Differentiate the mechanisms of infections caused by true pathogens from those caused by opportunistic pathogens.
3. Discuss the conditions that must be present or events that must occur for a microorganism to cause disease.
4. Name the characteristics of infectious agents that enable them to cause disease in the host.
5. Describe the factors and mechanisms by which the human host is protected from microbial invasion.
6. Discuss the sequence of events in the phagocytosis and killing of an infectious agent.
7. Name the routes of transmission that microorganisms use to initiate infection in a host and give examples of each.

Case in Point

A 52-year-old man was taken to the emergency department in a disoriented and unresponsive state, experiencing shortness of breath. The patient had a history of poorly controlled diabetes, chronic obstructive pulmonary disease, and Kaposi's sarcoma of the right leg. He had a history of homosexual activities and for several years had received steroid therapy. The patient had a diagnosis of acquired immunodeficiency syndrome.

On admission, physical examination showed that the patient was slightly febrile and in respiratory failure. A few days later he became progressively anemic, his mental status deteriorated, and a diagnosis of meningitis was made. Microbiologic studies performed on the cerebrospinal fluid identified *Cryptococcus neoformans*.

Issues to Consider

After reading the case history, consider:

- Factors that predisposed this patient to his current condition
- Role of the host innate and acquired immunity in protecting the host from infection
- Significance of microbial virulence factors in promoting infection

Key Terms

Adaptive immunity
Adhesin
Anamnestic immune response
Antibody
Antigen
Bacteriocidal effect
Bacteriocin
Chemotactic agent
Chemotaxis
Complement
Diapedesis
Dissemination
Endotoxin
Exotoxin
Fimbriae
Glycolysis
Humoral immune response
Iatrogenic infection
Immune response
Immune system
Immunoglobulin A (IgA)
Immunoglobulin E (IgE)
Immunoglobulin G (IgG)
Immunoglobulin M (IgM)
Immunoglobulins
Innate immunity
Interferon
Lactoferrin

Leukocidin
Lymphocyte
Lymphokine
Lysozyme
Opsonin
Opsonization
Panton-Valentine
Pathogenicity
Phagocyte
Phagocytosis
Pili
Respiratory burst
True pathogen
Virulence
Zoonoses

The relationship that exists between the human host and the microbial world is exceedingly complex. Each component of this relationship is in constant interaction with the other. The relationship is an equilibrium that is in constant motion and subject to change. Nutrition, stress, genetic background, and the presence of other diseases are additional influences that have an effect on the outcome of the meeting of human and microbe. This part of Chapter 2 discusses the role of each of the factors in host-pathogen interaction and how the host interacts with the microbe to prevent the onset of a disease process.

MICROBIAL FACTORS CONTRIBUTING TO PATHOGENESIS AND VIRULENCE

Pathogenesis

Pathogenicity is the ability of a microbe to produce disease in a susceptible individual. An organism may be described as a frank or true pathogen or as an opportunistic pathogen. **True pathogens** are organisms recognized to cause disease in healthy immune-competent individuals. Bacterial species such as *Yersinia pestis* and *Bacillus anthracis* are pathogenic in nearly all situations; when recovered in clinical samples taken from a body site, the clinical significance of these species is well established.

Over the last several years, patient populations have changed. Patients are living longer with their disease and are more likely to undergo highly invasive medical procedures, organ transplantation, and insertion of prosthetic devices, making them more susceptible to infections. As a result, organisms that are found as normal biota (normal flora) are being seen with increasing frequency in clinical infections among immunosuppressed and immunocompromised individuals. *Haemophilus influenzae* colonizes the upper respiratory tract of healthy individuals without causing disease, but given the opportunity, it may rapidly produce a life-threatening infection. Organisms such as *Staphylococcus epidermidis* under usual conditions do not cause disease, but can induce an infectious process in patients with prosthetic devices. *Haemophilus influenzae* and *S. epidermidis* are called *opportunistic pathogens* and the infections they cause are called *opportunistic infections*. Table 2-1 lists some of the common opportunistic microorganisms and the conditions with which they are most commonly associated.

Because of these situations, our definition of a pathogen must be expanded to apply to virtually any microorganism when conditions for infection are met. In deciding whether the isolation of a particular organism is a pathogen or not, we also must consider the human host from whom the organism was isolated and whether that host has underlying disease that may affect susceptibility to infection. For example, the potential pathogen list for a healthy 20-year-old college student is much shorter than for a healthy 90-year-old person, a transplant recipient, or a 20-year-old college student with AIDS. Because almost any organism in the right place can cause an infection, this needs to be considered when performing invasive procedures, because an inadvertent result of intervention can be the transfer of an organism from where it is present as part of the indigenous flora to a place where it can replicate and cause infection.

An **iatrogenic infection** is an infection that occurs as the result of medical treatment or procedures. For example, many patients who have indwelling urinary catheters develop a urinary tract infection. Although the catheter was a necessary procedure in the medical treatment of the individual, its use may result in an infection. Patients who are given immunosuppressive drugs because they have received a transplant are more susceptible to infection. Because any infection in such a patient would probably be the result of the physician-ordered drug therapy, it would be an iatrogenic infection.

TABLE 2-1 Abbreviated List of Opportunistic Microorganisms

Conditions Compromising Host Defenses	Organism(s)
Foreign bodies (catheters, shunts, prosthetic heart valves)	*Staphylococcus epidermidis* *Propionibacterium acnes* *Aspergillus* spp. *Candida albicans* Viridans streptococci *Serratia marcescens* *Pseudomonas aeruginosa*
Alcoholism	*Streptococcus pneumoniae* *Klebsiella pneumoniae*
Burns	*Pseudomonas aeruginosa*
Hematoproliferative disorders	*Cryptococcus neoformans* Varicella-zoster virus
Cystic fibrosis	*Pseudomonas aeruginosa* *Burkholderia cepacia*
Immunosuppression (drugs, congenital disease)	*Candida albicans* *Pneumocystis jiroveci* (*carinii*) Herpes simplex virus *Aspergillus* spp. Diphtheroids Cytomegalovirus *Staphylococcus* spp. *Pseudomonas* spp.

Virulence

Virulence is the relative ability of a microorganism to cause disease or the degree of pathogenicity. It is usually measured by the numbers of microorganisms necessary to cause infection in the host. Those organisms that can establish infection

with a relatively low infective dose are considered more virulent than those that require high numbers for infection. For example, because *Shigella* spp. causes disease with a relatively low infective dose, as few as 100 organisms, it is considered more virulent than *Salmonella* spp., wherein 10^5 colony-forming units (CFU) per milliliter of organisms are necessary to cause symptoms. This generalization is somewhat misleading because the severity of disease caused by different organisms varies from one to another. If a microorganism requires a relatively high infective dose but the disease it causes is often fatal, we tend to think of the microorganism as highly virulent. On the other hand, a different organism may require a low infective dose but produces a relatively mild disease.

Microbial Virulence Factors

Infectious organisms have a wide variety of mechanisms or virulence factors that allow them to persist in a host and cause disease. Some virulence factors, such as capsules and toxins, are used by many organisms. Other virulence factors tend to be specialized and specific to one particular organism, such as the tissue tropism of the gonococcus. Virulence factors allow the pathogen to evade or overcome host defenses and cause disease and encompass functions such as inhibiting phagocytosis, facilitating adhesion to host cells or tissues, enhancing intracellular survival after phagocytosis, and damaging tissue through the production of toxins and extracellular enzymes. Many virulence factors are well defined, such as the diphtheria and cholera toxins, the capsule of *Streptococcus pneumoniae*, and the fimbriae of *Neisseria gonorrhoeae*. Certain microorganisms produce extracellular factors that appear to aid in infection, but the exact role of these factors is unknown.

Ability to Resist Phagocytosis

Phagocytic cells such as macrophages and polymorphonuclear cells play a major role in defending the host from microbial invasion. In fact, the lack of phagocytic cells leaves the host susceptible to overwhelming infection. Therefore an extremely important event in the life of an invading pathogen that wants to survive in the host is avoiding phagocytosis. There are many ways by which microbial species evade phagocytosis. Table 2-2 lists some of the ways microorganisms interfere with phagocytic activities.

The most common mechanism for evading phagocytosis that is used by many different microorganisms is that of having a polysaccharide capsule on the surface. Many of those possessing a capsule are highly virulent (as in the Case in Point) until its removal, at which point their virulence becomes extremely low. Encapsulated strains of *S. pneumoniae* and *H. influenzae* are associated with highly invasive infections and are known to be more virulent than nonencapsulated strains. The capsule is composed usually of polysaccharides, but can also be made of proteins or a combination of protein and carbohydrate. The capsule inhibits phagocytosis primarily by masking the cell surface structures that are recognized by receptors on the surface of the phagocytic cell and in the same manner, inhibits the activation of complement by masking structures to which complement proteins bind.

TABLE 2-2 Microbial Interference with Phagocytic Activities

Microorganisms*	Type of Interference†	Mechanism (or Responsible Factor)
Streptococci	Kill phagocyte	Streptolysin induces lysosomal discharge into cell cytoplasm
	Inhibit chemotaxis	Streptolysin
	Resist phagocytosis	M substance
	Resist digestion	
Staphylococci	Kill phagocyte	Leucocidin induces lysosomal discharge into cell cytoplasm
	Inhibit opsonization	Protein A blocks Fc portion of Ab
	Resist killing	Cell wall mucopeptide
Bacillus anthracis	Kill phagocyte	Toxic complex
	Resist killing	Capsular polyglutamic acid
Haemophilus influenzae *Streptococcus pneumoniae*	Resist phagocytosis (unless Ab present)	Polysaccharide capsule
Klebsiella pneumoniae	Resist digestion	
Pseudomonas aeruginosa	Resist phagocytosis (unless Ab present) Resist digestion	"Surface slime" (polysaccharide)
Escherichia coli	Resist phagocytosis (unless Ab present)	O antigen (smooth strains) K antigen (acid polysaccharide)
	Resist killing	K antigen
Salmonella typhi	Resist phagocytosis (unless Ab present) Resist killing	Vi antigen
Cryptococcus neoformans	Resist phagocytosis	Polysaccharide capsule
Treponema pallidum	Resist phagocytosis	Cell wall
Yersinia pestis	Resist killing	Protein-carbohydrate cell wall
Mycobacteria	Resist killing and digestion	Cell wall structure
	Inhibit lysosomal fusion	?
Brucella abortus	Resist killing	Cell wall substance
Toxoplasma gondii	Inhibit attachment to PMN	?
	Inhibit lysosomal fusion	?

Adapted from Mims CA: *The pathogenesis of infectious disease,* ed 5, New York, 2001, Academic Press.
PMN, Polymorphonuclear neutrophil.
*Often only the virulent strains show the type of interference listed.
†Sometimes the type of interference listed has been described only in a particular type of phagocyte (polymorph or macrophage) from a particular host, but it generally bears a relationship to pathogenicity in that host.

Another bacterial structure that protects organisms from phagocytosis is Protein A. Protein A in the cell wall of *Staphylococcus aureus* helps the organism avoid phagocytosis by interfering with the binding of the host's antibodies to the surface of the organism. Antibodies bind to antigens via their

Fab or antigen-binding portion. Protein A binds to the Fc portion of IgG (at the opposite end of the Fab), preventing opsonization and phagocytosis by turning the antibody around on the surface.

Some organisms evade phagocytic cell killing by releasing potent materials in tissues that kill **phagocytes.** Streptococci produce hemolysins that lyse red blood cells but also induce toxic effects on white blood cells and macrophages. Pathogenic staphylococci release **leukocidins** that cause lysosomal discharge into cell cytoplasm. Staphylococcal leukocidin, called **Panton-Valentine,** is lethal to leukocytes and contributes to the invasiveness of the organism. Others inhibit chemotaxis, and thus the host is less able to direct polymorphonuclear neutrophils (PMNs) and macrophages into the site of infection.

Surface Structures That Promote Adhesion to Host Cells and Tissues

Most infectious agents must attach to host cells before infection occurs. In some diseases caused by exotoxins (e.g., botulism, staphylococcal food poisoning), adherence is not important. In virtually all other cases, however, the bacterium, virus, or fungus requires adherence to the host cell before infection and disease can progress. The cell surface structures that mediate attachment are called **adhesins.** The host cells must possess the necessary receptors for the adhesins. If the host or the infectious agent undergoes a mutation that changes the structure of the adhesin or the host receptor, adherence likely will not take place, and the virulence of the infectious agent is affected. Figure 2-1 illustrates surface bacterial structures that are involved in the pathogenesis of disease.

Virus infections depend on the cell being able to maintain an appropriate receptor for the virus particle. Infection of the cell occurs only if attachment is the initial event. The main adhesins in bacteria are the **fimbriae (pili)** and surface polysaccharides. Fimbriae enable bacteria to adhere to host cell surfaces, offering resistance by attachment to target cells, thereby increasing the organism's colonizing ability. Once attached, phagocytosis is less likely to occur. For example, the strains of *Escherichia coli* that cause traveler's diarrhea use their fimbriae to adhere to cells of the small intestine, where they secrete a toxin that causes the disease symptoms. Similarly, fimbriae are essential for gonococci to infect the epithelial cells of the genitourinary tract. Antibodies to the fimbriae of *N. gonorrhoeae* are protective by preventing the organism from attaching to the epithelial cells.

Ability to Survive Intracellularly and Proliferate

Some pathogens are able to survive within the phagocytic cell after they have been engulfed. These organisms have developed methods to prevent being killed intracellularly. Some prevent fusion of phagosomes and lysosomes, others have a resistance to the effects of the lysosomal contents, and still others escape from the phagosome into the cytoplasm.

To establish itself and cause disease, a pathogen must be able to replicate after attachment to host cells. Numerous host factors work to prevent proliferation. Secretory antibody, **lactoferrin,** and **lysozyme** are all produced by the host as a way to protect against infection. To be successful in establishing infection, infectious agents must be able to avoid or overcome these local factors. For example, lactoferrin competes with bacteria for free iron; meningococci can use lactoferrin as a source of iron. They are not inhibited by the presence of lactoferrin and, in fact, are able to use it for growth. On the other hand, the nonpathogenic *Neisseria* spp. are usually unable to use the iron in lactoferrin and thereby are inhibited by its presence.

Several pathogens (*H. influenzae*, *N. gonorrhoeae*, and *N. meningitidis*) produce an IgA protease that degrades the IgA found at mucosal surfaces. Other pathogens (influenza virus, *Borrelia* spp.) circumvent host antibodies by shifting key cell surface antigens. The host produces antibodies against the "old" antigens, which are no longer effective because the organism now has "new" antigens that do not bind to antibodies made against the old antigens.

In most situations when an organism is engulfed by macrophages, lysosomal contents are released into the phagocytic vacuoles and the organism is killed. If the engulfed organism is not exposed to intracellular killing and digestive processes, however, it is able to survive and multiply inside the macro-

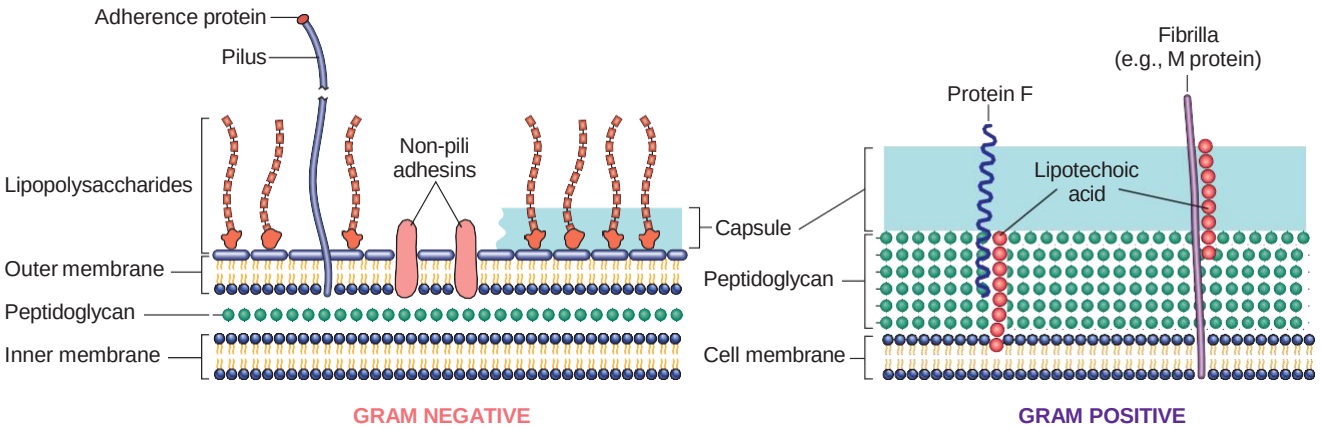

FIGURE 2-1 Surface bacterial structures that are involved in the pathogenesis of disease. (From Kumar V, Abbas AK, Fausto M: *Robbins and Cotran pathologic basis of disease*, ed 7, Philadelphia, 2005, Saunders.)

phage. Bacterial species such as *Chlamydia*, *Mycobacterium*, *Brucella*, and *Listeria* species are easily engulfed by macrophages and phagocytes; however, these species are not only able to survive inside the macrophages—protected from the host's other immune defenses—but are also able to multiply intracellularly.

Pathogens exhibit an ability to penetrate and grow in tissues. This process is called *invasion*. With some organisms, the invasion is localized and involves only a few layers of cells. With others, it involves deep tissues; for example, the gonococcus organism is invasive and may infect the fallopian tubes. With some organisms, such as *Salmonella* spp., the disease or organisms spread to distant sites (organs and tissues). This is called **dissemination.** Other organisms such as *Corynebacterium diphtheriae* do not spread beyond their initial site of infection, yet the disease they produce is serious and often fatal. Certain organisms that survive phagocytosis may be disseminated rapidly to many body sites, but the organisms themselves are not invasive. The phagocyte simply carries the organism, but the bacterium itself is incapable of penetrating tissues. *Clostridium perfringens* is an example of a highly invasive organism that may not necessarily disseminate.

Ability to Produce Extracellular Toxins and Enzymes

Generally, disease from infection is noticeable only if tissue damage occurs. This damage may be from toxins, either **exotoxins** or **endotoxins,** or from inflammatory substances that cause host-driven, immunologically mediated damage. The ability of organisms to produce exotoxins and extracellular enzymes is another major factor that contributes to the virulence and invasiveness of organisms. Toxins are poisonous substances produced by organisms that interact with host cells, disrupting normal metabolism and causing harm. Exotoxins are produced by both gram-negative and gram-positive bacteria and are secreted by the organism into the extracellular environment, or they are released upon lysis of the organism. Exotoxins can mediate direct spread of the microorganisms through the matrix of connective tissues and can cause cell and tissue damage. Some organisms produce soluble substances, such as proteases and hyaluronidases that liquefy the hyaluronic acid of the connective tissue matrix, helping to spread bacteria in tissues, thereby promoting the dissemination of infection. Endotoxins, on the other hand, are a constituent, the lipopolysaccharide (LPS), of the outer cell membrane of gram-negative bacteria exclusively. Endotoxins, in contrast to exotoxins, do not have enzyme activity, are secreted in only very small amounts, do not have specificity in their activity on host cells, are not very potent, and are not destroyed by heating.

Exotoxins

Many of the bacterial exotoxins are highly characterized. Most are composed of two subunits: the first is nontoxic and binds the toxin to the host cells; the second is toxic. The toxin gene is commonly encoded by phages, plasmids, or transposons. Therefore only those organisms that carry the extrachromosomal DNA coding for the toxin gene produce toxin. Isolates of *Clostridium difficile*, for example, have to be tested for toxin production. Other pathogenic bacteria show similarities. Diphtheria toxin inhibits protein synthesis and affects the heart, nerve tissue, and liver. Botulinum toxin is a neurotoxin that blocks nerve impulse transmission, causing flaccid paralysis, especially in infants. *Streptococcus pyogenes* and *S. aureus* both produce exfoliatin, which causes rash and massive skin peeling or exfoliation. Table 2-3 lists many of the bacterial exotoxins that are important in disease production as examples.

Endotoxins

Endotoxins are composed of the LPS portion of the outer membrane on the cell wall of gram-negative bacteria. The cell wall of gram-negative microorganisms is composed of two layers, the inner peptidoglycan layer and an outer membrane. The LPS is contained in the outer membrane along with proteins and phospholipids (see Figure 2-1). LPS contains three regions: an antigenic O–specific polysaccharide, a core polysaccharide, and an inner lipid A (also called *endotoxin*). The toxic activity of endotoxin is found in the lipid A portion of the LPS. The effects of endotoxin consist of dramatic changes in blood pressure, clotting, body temperature, circulating blood cells, metabolism, humoral immunity, cellular immunity, and resistance to infection. Endotoxin stimulates the fever centers in the hypothalamus. An increase in body temperature occurs within an hour after exposure. Exposure to endotoxin also causes hypotension. Severe hypotension occurs within 30 minutes after exposure. Septic or endotoxic shock is a serious and potentially life-threatening problem. Unlike shock caused by fluid loss, such as that seen in severe bleeding, septic shock is unaffected by fluid administration. The endotoxin also initiates coagulation, which can result in intravascular coagulation. This process depletes clotting factors and activates fibrinolysis so that fibrin-split products accumulate in the blood. These fragments are anticoagulants and can cause serious bleeding. Another feature of patients with endotoxic shock is severe neutropenia, which can occur within minutes after exposure. It results from sequestration of neutrophils in capillaries of the lung and other organs. Leukocytosis follows neutropenia because neutrophils are released from the bone marrow.

Endotoxin also produces a wide variety of effects on the immune system. It stimulates proliferation of B lymphocytes in some animal species, activates macrophages, activates complement, and has an adjuvant effect with protein antigens. It also stimulates interferon production and causes changes in carbohydrates, lipids, iron, and sensitivity to epinephrine. A severe infection with gram-negative bacteria therefore can lead to serious and often life-threatening situations. A comparison of bacterial exotoxins and endotoxins is given in Table 2-4.

HOST RESISTANCE FACTORS

Physical Barriers

Humans have evolved a complex system of defense mechanisms to prevent infectious agents from gaining access to and

TABLE 2-3 Examples of Exotoxins of Pathogenic Bacteria

Bacterium	Disease Caused in Humans	Toxins
Bacillus anthracis	Anthrax	Complex, lethal, edema-producing toxin
Bordetella pertussis	Whooping cough	Lethal, dermonecrotizing toxin
Clostridium botulinum	Botulism	6 type-specific lethal neurotoxins*
Clostridium oedematiens	Gas gangrene	Alpha, lethal, dermonecrotizing Beta, lethal, dermonecrotizing, hemolytic Gamma, lethal, dermonecrotizing, hemolytic Delta, hemolytic Epsilon, lethal, hemolytic Zeta, hemolytic
Clostridium perfringens	Gas gangrene and enteritis necroticans	Alpha, lethal, dermonecrotizing, hemolytic* Beta, lethal Gamma, lethal Delta, lethal Epsilon, lethal, dermonecrotizing Eta, lethal (?) Iota, lethal, dermonecrotizing Theta, lethal, cardiotoxic, hemolytic Kappa, lethal, proteolytic Enterotoxin*
Clostridium septicum	Gas gangrene	Alpha, letal, hemolytic
Clostridium sordellii	Gas gangrene	Edema-producing toxin Hemorrhagic toxin
Clostridium tetani	Tetanus	Tetanospasmin, lethal, neurotoxic* Neurotoxin, nonspasmogenic Tetanolysin, lethal, cardiotoxic, hemolytic
Corynebacterium diphtheriae	Diphtheria	Diphtheria toxin, lethal, dermonecrotizing*
Escherichia coli	Diarrhea	Heat-labile enterotoxin* Heat-stable enterotoxin Shiga toxin
Pseudomonas aeruginosa	Pyogenic infections	Exotoxin A
Staphylococcus aureus	Pyogenic infections, enterotoxemia	Alpha, lethal, dermonecrotizing, hemolytic Beta, lethal, hemolytic Gamma, lethal, hemolytic Delta, hemolytic Exfoliating toxin* Enterotoxin*
Streptococcus pyogenes	Pyogenic infections, scarlet fever, rheumatic fever	Dick toxin, erythrogenic, nonlethal Streptolysin O, lethal, hemolytic, cardiotoxic Streptolysin S, lethal, hemolytic
Vibrio cholerae	Cholera	Cholera toxin, lethal, enterotoxic*
Salmonella typhimurium	Enteritis	Enterotoxin?*
Shigella sp.	Dysentery	Enterotoxin*
Yersinia pestis	Plague	Murine toxin

From Braude AI, Davis CE, Fierer J, editors: *Infectious diseases and medical microbiology,* Philadelphia, 1986, Saunders.
*Indicates toxins that produce harmful effects of infectious disease.

replicating in the body. Healthy skin is an effective barrier against infection. The stratified and cornified epithelium presents a mechanical barrier to penetration by most microorganisms. Organisms that can cause infection by penetrating the mucous membrane epithelium usually cannot penetrate unbroken skin. Only a few microorganisms are capable of entering the body by way of intact skin. Some of these microorganisms, and others that normally enter when the skin barrier is compromised, are listed in Table 2-5. Most of the organisms listed in Table 2-5 require help in breaking the skin barrier (e.g., animal or arthropod bite). Those capable of penetrating normal, healthy skin are few and include *Leptospira* spp., *Francisella tularensis*, *Treponema* spp., and some fungi. Even these organisms probably require microscopic breaks in the skin surface. Healthy, intact skin is clearly the primary mechanical barrier to infection. The skin also has substantial numbers of microbial flora that are usually not pathogens, organisms that contribute to a low pH, compete for nutrients, and produce bactericidal substances. In addition, the low pH resulting from long-chain fatty acids secreted by sebaceous glands ensures that relatively few organisms can survive and prosper in the acid environment of the skin. These conditions prevent colonization by transient, possibly pathogenic organisms.

TABLE 2-4 Differences Between Bacterial Exotoxins and Endotoxins

Characteristic	Exotoxins	Endotoxins
Organism type	Gram-positive and gram-negative	Gram-negative
Part of or secreted from organism	Part of and secreted from organism	Part of organism
Chemical nature	Simple protein	Protein-lipid-polysaccharide
Stability to heating (100° C)	Labile	Stable
Detoxification by formaldehyde	Detoxified	Not detoxified
Neutralization by homologous antibody	Complete	Partial
Biologic activity	Individual to toxin	Same for all toxins
Toxicity compared with strychnine as 1	100 to 1,000,000	0.1

Modified from Braude AI, Davis CE, Fierer J, editors: *Infectious diseases and medical microbiology*, Philadelphia, 1986, Saunders.

TABLE 2-5 Microorganisms That Infect Skin or Enter the Body via the Skin

Microorganisms	Disease	Comments
Arthropod-borne viruses	Various fevers, encephalitides	150 distinct viruses, transmitted by infected arthropod bites
Rabies virus	Rabies	Bites from infected animals
Vaccinia virus	Skin lesion	Vaccination against smallpox
Rickettsieae	Typhus, spotted fevers	Infestation with infected arthropods
Leptospira	Leptospirosis	Contact with water containing infected animal urine
Staphylococci	Boils, impetigo	Most common skin invaders
Streptococci	Impetigo, erysipelas	
Bacillus anthracis	Cutaneous anthrax	Systemic disease following local lesion at inoculation site
Treponema pallidum and *pertenue*	Syphilis, yaws	Warm, moist skin is more susceptible
Yersinia pestis	Plague	Bite from infected rat flea
Plasmodium spp.	Malaria	Bite from infected mosquito
Dermatophytes	Ringworm, athlete's foot	Infection restricted to skin, nails, and hair

From Mims CA: *The pathogenesis of infectious disease*, ed 5, San Diego, 2001, Academic Press.

Cleansing Mechanisms

Normally the term *cleansing* brings to mind a liquid. One of the most effective cleansing mechanisms humans have, however, is the desquamation of the skin surface. The keratinized squamous epithelium or outer layer of skin is being shed continuously. Many of the microorganisms colonizing the skin are disposed of with the sloughing of the epithelium.

More obvious is the cleansing action of the fluids of the eye and the respiratory, digestive, urinary, and genital tracts. The eye is continually exposed to microorganisms, which means this organ has some highly developed antimicrobial mechanisms. Tears bathe the cornea and sclera. This not only lubricates the eye but also washes foreign matter and infectious agents away from the surface. Additionally, tears contain IgA and lysozyme.

The respiratory tract is also continuously exposed to microorganisms and is protected by nasal hairs, ciliary epithelium, and mucous membranes. A continuous flow of mucus emanates from the membranes lining the nasopharynx, which traps particles and microbes and sweeps them to the oropharynx, where they are either expectorated or swallowed. The trachea is lined with ciliary epithelium. These cells have hair-like extensions (cilia) that sweep particles and organisms upward toward the oropharynx. This material is then expectorated or swallowed. Heavy smokers have a significant reduction in ciliated epithelial cells and therefore are more susceptible to respiratory infections. The purpose of these mechanisms is to prevent infectious agents and other particles from reaching the bronchioles and lungs. Under normal conditions, they are very effective, and the air moving into and out of the lungs is sterile.

Bacteria are swallowed into the gastrointestinal tract either as part of the mouth flora and upper respiratory tract or in liquids and food. Most bacteria are easily destroyed by the low pH found in the stomach. Some bacteria, however, are able to survive and pass into the small intestine. The number of bacteria in the intestine increases as the distance from the stomach increases. The distal portion of the colon contains the highest number of organisms. The gastrointestinal tract is protected by mucous secretions and peristalsis that prevent the organisms from attaching to the intestinal epithelium. Additionally, secretory antibody and phagocytic cells lining the mucosa defend the gastrointestinal tract against infection.

The genitourinary tract is cleansed by voiding urine. Consequently, only the outermost portions of the urethra have a microbial population. The vagina contains a large population of organisms as part of the indigenous flora. The acidity of the vagina, resulting from the breakdown of glycogen by the resident flora, tends to inhibit transient organisms from colonizing.

Antimicrobial Substances

A variety of substances produced in the human host have antimicrobial activity. Some are produced as part of the phagocytic defense and are discussed later. Others, such as fatty acids, hydrogen chloride (HCl) in the stomach, and secretory IgA have already been mentioned. A substance that plays a major role in resistance to infection is lysozyme, a low–molecular-weight (approximately 20,000 daltons) enzyme that hydrolyzes the peptidoglycan layer of bacterial cell walls. In some bacteria, the peptidoglycan layer is directly accessible to lysozyme. These bacteria are killed by the enzyme alone. In other bacteria, the peptidoglycan layer is exposed after

other agents have damaged the cell wall (e.g., antibody and complement, hydrogen peroxide). In these cases, lysozyme acts with the other agents to cause death of the infecting bacteria. Lysozyme is found in serum, tissue fluids, tears, breast milk, saliva, and sweat.

Antibodies, especially secretory IgA, are found in mucous secretions of the respiratory, genital, and digestive tracts. They may serve as opsonins, thereby enhancing phagocytosis, or they may fix complement and neutralize the infecting organism.

Serum also contains low–molecular-weight cationic proteins, termed *β-lysins*. These proteins are lethal against gram-positive bacteria and are released from platelets during coagulation. The site of action is the cytoplasmic membrane.

These antimicrobial substances and systems work best together. A combination of antibody, complement, lysozyme, and β-lysin is significantly more effective in killing bacteria than each alone or than any combination in which one or more are missing.

Proliferation of viruses is inhibited by **interferon.** The interferons are a group of cellular proteins induced in eukaryotic cells in response to virus infection or other inducers. Uninfected cells that have been exposed to interferon are refractory to virus infection. A number of bacteria, viruses, and their products induce interferon production. The interferon produced binds to the surface receptors on noninfected cells. This binding stimulates the cell to synthesize enzymes that inhibit viral replication over several days. The antiviral effect of interferon is only one action it exhibits.

One type of interferon, interferon-gamma, plays an especially important role in the immune response. It inhibits cell proliferation and tumor growth, and enhances phagocytosis by macrophages, the activity of natural killer cells, and the generation of cytotoxic T cells.

Indigenous Microbial Flora

Nonpathogenic microorganisms compete with pathogens for nutrients and space. This competition lessens the chance that the pathogen will colonize the host. Some normal flora species produce **bacteriocins,** substances that inhibit the growth of closely related bacteria. These proteins are produced by a variety of gram-positive and gram-negative bacteria and appear to give the secreting bacterium an advantage, because they can eliminate other bacteria that would compete for nutrients and space. Some species of bacteria produce metabolic by-products that result in a microenvironment hostile to potential pathogens. Vitamins and other essential nutrients are synthesized by certain bacteria in the intestine and appear to contribute to the overall health of the host.

Phagocytosis

Phagocytosis is an essential component in the resistance of the host to infectious agents. It is the primary mechanism in the host defense against extracellular bacteria and a number of viruses and fungi. The PMNs and macrophages (monocytes in the peripheral blood) are the body's first line of defense.

The stem cells for neutrophils arise in the bone marrow, where they differentiate to form mature neutrophils. During this maturation, the cells synthesize myeloperoxidase, proteases, cathepsin, lactoferrin, lysozyme, and elastase. These products are incorporated into membrane-bound vesicles called *lysosomes*. The lysosomes contain the enzymes and other substances necessary for the killing and digestion of the engulfed particles. They show up as azurophilic granules on a Wright's stain. The PMN also has receptors on the cell membrane for some complement components that stimulate cell motion, the metabolic burst, and secretion of the lysosome contents into a phagosome. The PMN is an end-stage cell and has a circulating half-life of 2 to 7 hours. It may migrate to the tissues, where its half-life is less than 1 week.

Macrophages also originate in the bone marrow. They circulate as monocytes for 1 to 2 days and then migrate through the blood vessel walls into the tissues and reside in specific tissues as part of the reticuloendothelial system. These cells are widely distributed in the body and play a central role in specific immunity and nonspecific phagocytosis (Table 2-6).

Four activities must occur for phagocytosis to take place and be effective in host defense: (1) migration of the phagocyte to the area of infection (chemotaxis), (2) attachment of the particle to the phagocyte, (3) ingestion, and (4) killing.

Chemotaxis

The PMNs circulate through the body, followed by movement into the tissues by an action called **diapedesis,** which is movement of the neutrophils between the endothelial cells of the blood vessels into the tissues. The body is under constant surveillance by these and other phagocytic cells. When an infection occurs, massive numbers of PMNs accumulate at the site. This accumulation is not a random event; rather it is a directed migration of PMNs into the area needing their services. This migration is called **chemotaxis** (a chemical "taxi" or chemically caused movement). Several substances serve as **chemotactic agents.** These are certain components of complement, a number of bacterial products, products from damaged tissue cells, and products from responding immune cells. The initial contact of the PMN with an invading organism may be random. As the organism causes the body's defense mechanisms to respond via inflammation, directed

TABLE 2-6 Tissue Distribution of Monocytes/Macrophages

Cell Name	Tissue Distribution
Monocyte	Blood
Kupffer cell	Liver
Alveolar macrophage	Lung
Histiocyte	Connective tissue
Peritoneal macrophage	Peritoneum
Microglial cell	Central nervous system
Mesanglial cell	Kidney
Macrophage	Spleen, lymph nodes

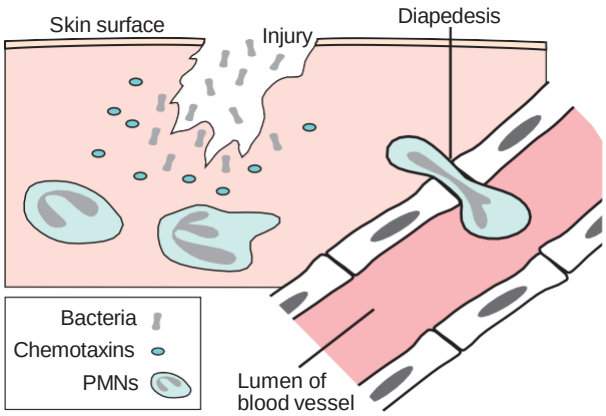

FIGURE 2-2 Phagocytosis: chemotaxis migration of phagocytes.

migration of phagocytes occurs (chemotaxis; Figure 2-2). The speed and magnitude of this response are easily visualized by recalling how quickly a splinter or similar injury becomes infected and how much pus is produced.

Attachment

One of the most effective defenses bacteria have against phagocytosis is the capsule. This structure prevents attachment of the organism to the neutrophil's membrane, which must occur before ingestion can take place. Attachment is facilitated by the binding of specific antibodies to the microorganism. The neutrophil membrane has a variety of receptors, including receptors for the Fc portion of IgG1, IgG3, and the C3b component of complement. These three factors can and do bind to the invading microorganism resulting in the invading microorganisms being coated with one or more of these factors. The receptor on the PMN for the particular factor coating the bacterium binds to the factor and forms a bridge that brings the bacterium into close physical contact with the leukocyte membrane. The coating of the bacterium with antibody or complement components results in enhanced phagocytosis by the PMN. This process or phenomenon is called **opsonization.**

The antibody and complement components are termed **opsonins.** Opsonization can be accomplished by three different types of responses: (1) IgG1 or IgG3 binding to the organism, (2) the antibody response is insufficient for opsonization, but complement is fixed on the surface of the organism, or (3) the alternative complement pathway is activated by the endotoxin or polysaccharides of the organism.

Ingestion

The next step of phagocytosis is ingestion. This process occurs rapidly following attachment. The cell membrane of the phagocytic cell invaginates and surrounds the attached particle. The particle is taken into the cytoplasm and enclosed within a vacuole called a *phagosome* (Figure 2-3). The phagosome fuses with lysosomes, which are vacuoles containing hydrolytic enzymes. The lysosomes release their contents into the phagosome, a process called *degranulation*. The list of enzymes found within the lysosomes is long—more than 20

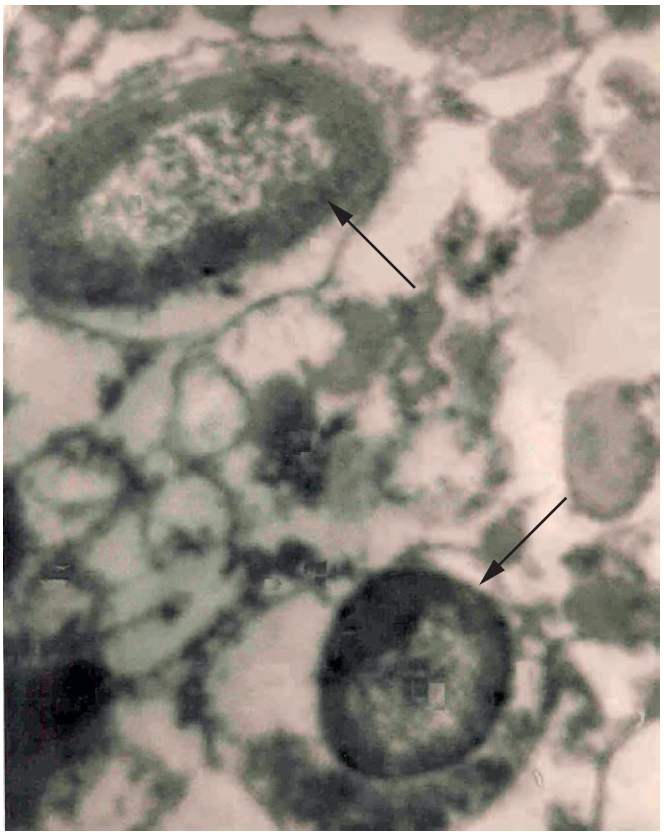

FIGURE 2-3 Engulfed bacterial cells inside phagosomes.

enzymes, including proteases, lipases, RNase, DNase, peroxidase, and acid phosphatase. Several of these are important in the killing and digestion of the engulfed bacterial cell.

Killing

The phagocytosis of a particle triggers a significant increase in the metabolic activity of the neutrophil or macrophage. This increase is termed a *metabolic* or **respiratory burst.** The cell demonstrates increases in **glycolysis,** the hexose monophosphate shunt pathway, oxygen use, and production of lactic acid and hydrogen peroxide. The hydrogen peroxide produced at this time diffuses from the cytoplasm into the phagosome. It acts in conjunction with other compounds to exert a **bactericidal effect.** In addition, other enzymes from the lysosome have antimicrobial action. They include lactoferrin, which chelates iron and prevents bacterial growth, lysozyme, and several basic proteins. The usual result is that a phagocytosed organism is quickly engulfed, killed, and digested.

Organisms that are "intracellular" (e.g., *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Brucella* spp.) are able to survive phagocytosis and, in fact, may actually multiply within the phagocyte. Obviously, other defense mechanisms must play a major role in immunity to these intracellular organisms.

The importance of phagocytosis is seen in patients with defects in the numbers or function of phagocytic cells. Such patients have frequent infections despite possessing high levels of serum antibody.

Many of the organisms listed in Table 2-2 are common isolates, which is not surprising, because they have developed a means to interfere with phagocytosis, thereby increasing their pathogenicity.

Inflammation

Inflammation is the body's response to injury or foreign body. Figure 2-4 illustrates the components involved in acute and chronic inflammatory responses. A hallmark of inflammation is the accumulation of large numbers of phagocytic cells. These leukocytes release mediators or cause other cell types to release mediators. The mediators cause erythema as a result of greater blood flow, edema from an increase in vascular permeability, and continued phagocyte accumulation, resulting in pus. The enzymes released by the phagocytes digest the foreign particles, injured cells, and cell debris. After the removal of the invading object, the injured tissue is repaired.

Immune Responses

This chapter gives a brief discussion on the immune system response to infection to provide the reader with an appreciation of its role and complexity. The balance between health and infectious disease is complex and mediated by humoral and cellular factors. As illustrated in the Case in Point at the beginning of this section, the relative importance of each factor depends on the microbe, route of infection, condition and genetic makeup of the host, and other factors yet to be clearly characterized. In this case, this AIDS patient would become susceptible to opportunistic organisms as his immune system continues to deteriorate.

Table 2-7 summarizes the defenses used by the human host against infection. Classically, the term *immunity* has been defined as a complex mechanism whereby the body is able to protect itself from invasion by disease-causing organisms. This mechanism, known as the **immune system,** consists of numerous cells and protein molecules that are responsible for recognizing and removing these foreign substances. This general definition has been broadened over the years to mean a reaction to any foreign substance, including proteins and polysaccharides, as well as invading microorganisms. Research advances over the years have dramatically increased our understanding of the basic **immune response** and given us an appreciation of the cellular interrelationships. The immune response can be divided into two broad categories. The first is known as *innate* or *natural immunity* with little or no specificity, and the second, called *adaptive* or *specific*, is highly specialized.

Innate, or Natural, Immunity

Innate immunity, also referred to as *natural* or *nonspecific immunity*, consists of several components. These include (1) physical and chemical barriers such as the skin and mucous membranes, (2) blood proteins that act as mediators of infection, and (3) a cellular mechanism capable of phagocytosis such as neutrophils and macrophages and other leukocytes such as natural killer cells. Figure 2-5 shows examples of the innate immune defenses located at different body sites. This first line of defense has a limited capacity to distinguish one organism from another; however, previous exposure to a particular foreign substance is not required. Physical barriers,

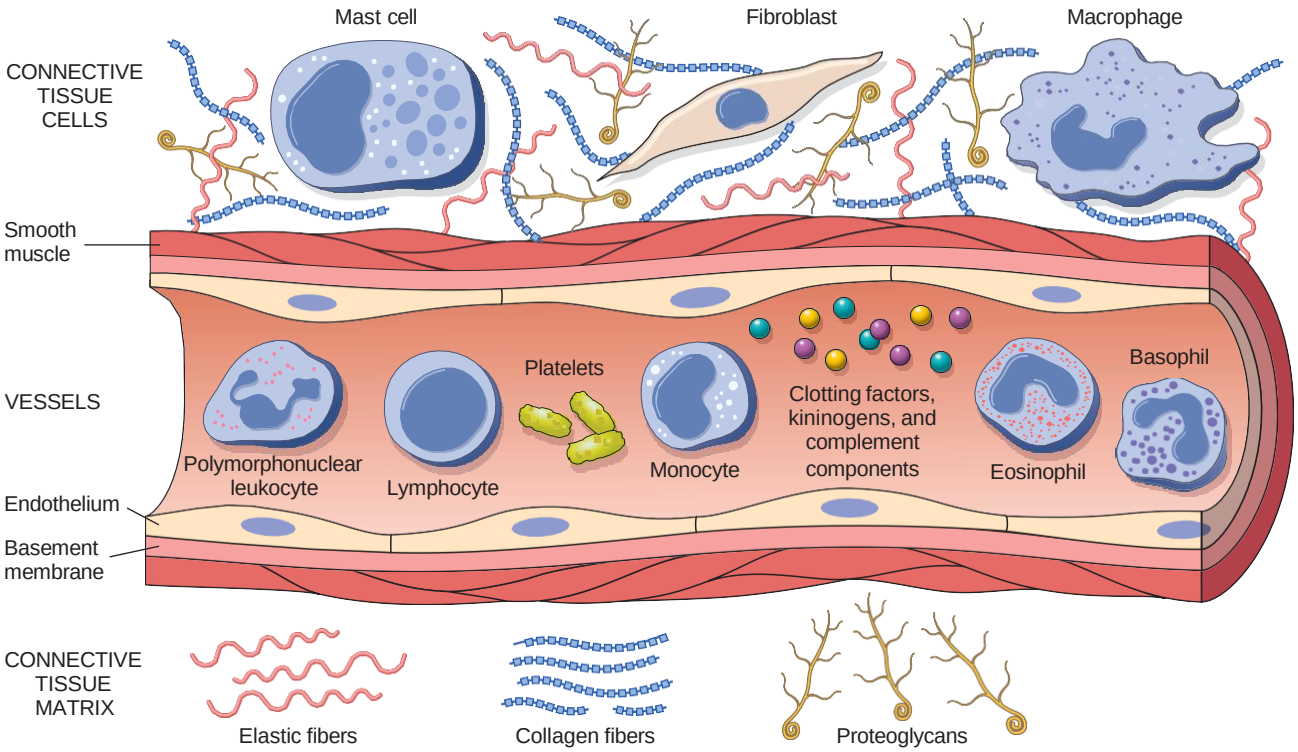

FIGURE 2-4 Components involved in acute and chronic inflammatory responses. (From Kumar V, Abbas AK, Fausto M: *Robbins and Cotran pathologic basis of disease*, ed 7, Philadelphia, 2005, Saunders.)

TABLE 2-7 Summary of Defenses of the Human or Animal Host to Infection and Evasion Mechanisms Attributed to Various Microorganisms

Host Defense	Mechanism of Evasion	Example
Hydrodynamic flow	Attachment	Fimbriae, surface proteins, lipoteichoic acid pseudomembrane of diphtheria
Mucus barrier	Attachment Penetration	Mannose-sensitive fimbriae
Deprivation of essential nutrients	Systems of high-affinity uptake	Iron metabolism
Lysozyme in secretions	Resistance to lysis	Substitution of peptidoglycan
Surface immunoglobulins	Absent or low immunogenicity	Hyaluronic acid, capsules
	Antigenic heterogeneity	Fimbriae, capsules, LPS, M protein, etc.
	Masking of antigens	Capsules, IgA-binding proteins
	Destruction	IgA protease
Unbroken surface (epithelial cell surface)	Penetration	*Neisseria gonorrhoeae*, *Shigella* spp.
Unknown defenses in lymphatics (intercellular space)		*N. gonorrhoeae*, *Shigella* spp.
Serum defenses		
Recognition by antibody	Antigenic heterogeneity	Fimbriae, capsules, LPS, M protein
	Masking of antigen	Capsules, Ig-binding proteins
	Destruction of antibody	*Borrelia*
	Antigenic variation	
Complement system	Failure to activate alternative pathway	Sialic acid capsules
	Inactivation of complement components	Cleavage of C3b in empyema fluids
	Resistance to bacteriolysis	CoIV plasmid
	Formation of abscess	*Bacteroides fragilis* capsule
Localization		
Fibrin tapping	Fibrinolysis	*Streptococcus* spp.
Abscess formation	Collagenase, elastase	*Pseudomonas*, *Clostridium*
Secondary immune response	Nonspecific B-cell activation	LPS, lipoprotein
	Inhibition of delayed hypersensitivity	Anergy of miliary tuberculosis
	Rapidly fatal (toxin)	Anthrax, plague, *Clostridium*
Phagocytosis	Inhibition of chemotaxis	*Brucella, Salmonella, Neisseria, Staphylococcus, Pseudomonas*
	Inhibition of attachment and ingestion	Capsules, M protein, Ig-binding proteins, gonococcal pili
	Inhibition of metabolic burst	*Salmonella typhi*
	Inhibition of degranulation	Mycobacteria
	Resistance to permeability inducing cationic protein	Gram-positive cell wall, smooth LPS, polyanionic capsules
	Resistance to oxidative attack	Catalase, superoxide dismutase, carotenoid pigments
	Escape from phagosome	*Mycobacterium bovis*, *Legionella pneumophila*
	Destruction of phagocyte	*Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa*

Adapted from Gotschlich EC: Thoughts on the evolution of strategies used by bacteria for evasion of host defenses, *Rev Infect Dis* 5:S779, 1983.
LPS, Lipopolysaccharide.

mentioned earlier, may be as simple as the keratinized outer layer of the skin. Also, the secretions of the mucous membranes and the ciliated epithelial cells of the respiratory tract promote trapping and removal of microorganisms. In addition, many secretions provide a chemical barrier, such as the acidic pH of the stomach and vagina. Saliva and tears contain enzymes such as lysozyme, and the sebaceous glands of the skin contain oils capable of inhibiting invasion by pathogenic organisms. The normal biota of all of these sites adds another dimension to the host's ability to resist invading pathogens.

Once the physical and chemical barriers to infection have been penetrated, nonspecific mechanisms of innate immunity become operational. Phagocytic cells ingest and kill microorganisms, whereas activated complement components contribute to a wide variety of immunologic events, including promotion of attachment and engulfment of bacteria by neutrophils (opsonization) and attraction of neutrophils to sites of infection (chemotaxis). Collectively, these immunologic defense mechanisms, along with host tissue damage caused by the invading organisms, combine to produce an acute inflammatory response in the host. The importance of natural immune mechanisms rests in their rapid response to invading organisms. However, these mechanisms are effective primarily against extracellular bacterial pathogens, playing only a minor role by themselves in immunity to intracellular bacterial pathogens, viruses, and fungi.

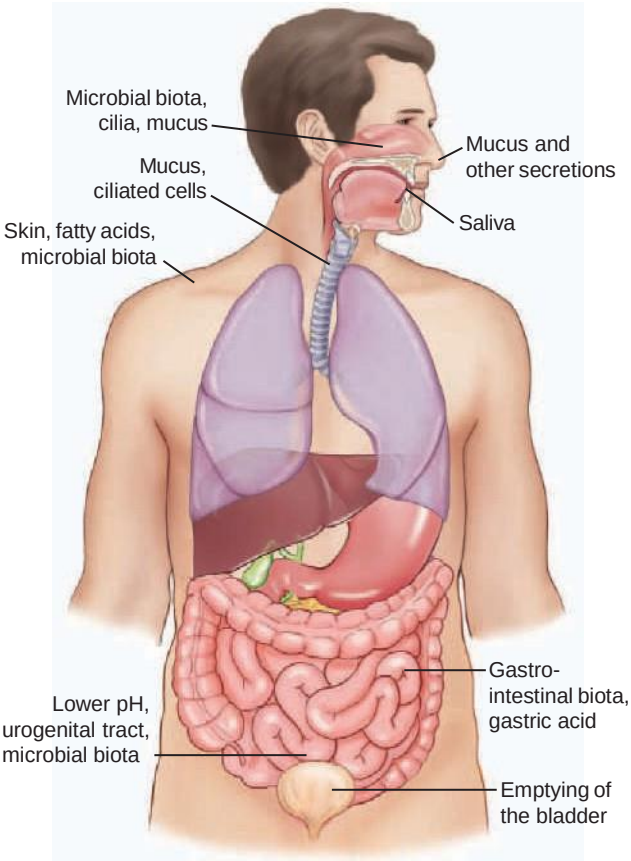

FIGURE 2-5 Innate immune defenses located at different body sites.

Adaptive, or Specific, Immunity

Adaptive immunity is much more highly evolved and complex than innate immunity and enhances the protective capability of the innate defense. This arm of the immune response is capable of being specific for distinct molecules, responding in particular ways to different types of foreign substances, and developing memory, which allows for a more vigorous response to repeated exposures to the same foreign invader. Lymphocytes and their products, known as **antibodies,** are the major constituents of the adaptive or specific immune response. Antibodies are produced in response to antigens, substances that are capable of inducing the immune response.

An interrelationship exists between the mechanisms of the innate and the adaptive. For instance, inflammation, a nonspecific response of the innate system, provides a signal that triggers an adaptive type of immune response. The adaptive immune system is able to enhance the protective mechanisms of the innate system, as previously mentioned. For instance, the activation of **complement** (a component of innate immunity) by invading bacteria is enhanced by the presence of specific antibodies (components of adaptive immunity). This activation leads to phagocytic clearance and elimination of the bacteria. The adaptive immune response adds a high degree of specialization to the passive mechanisms of the innate response. The nature of the adaptive immune response varies according to the type of organism and is designed to eliminate it efficiently. For example, antibodies are produced by B lymphocytes in response to blood-borne organisms and aid in their elimination. However, the response to phagocytosed antigens is primarily by T lymphocytes that produce chemicals that enhance the activities of the phagocytic cells. The nature and origin of these cells are described in the following paragraph. Most importantly, the specific response is able to remember each time it encounters a particular foreign antigen, called *immunologic memory*. Subsequent exposure to that antigen stimulates an increasingly effective and specific defense. Ultimately, the adaptive or specific immune response is the second line of defense and is able to significantly improve upon the first.

Lymphocytes originate in the bone marrow from stem and progenitor cells. Lymphocytes mature and take up residence in various body tissues and organs, including the thymus, lymph nodes, and spleen. They are a diverse group of cells that can be classified into two major types—T (thymus-derived) cells and B (bone marrow–derived) cells—on the basis of cell surface markers. The uniqueness of these cells lies in the presence of specific cell surface receptor molecules that recognize and bind a unique antigen, activating the cell to divide, differentiate, and secrete a number of effector substances. The millions of lymphocytes found in the body have been preengineered during embryogenesis to recognize a vast array of substances as foreign while learning which substances constitute self. Thus the result of encounter with the antigen is an expanded clone or clones of activated lymphocytes.

Nature of the Immune Response to Infectious Agents

Although both humoral immunity and cell-mediated immunity are important in protecting the human from a wide variety of infectious agents, each contributes differently, in terms of overall importance, according to the type of pathogen and virulence mechanisms. Whereas B lymphocytes play the predominant role in the **humoral immune response,** T lymphocytes mediate cellular immunity. Each B lymphocyte has surface receptors that recognize only one type of antigen. After antigen binding, the B lymphocyte undergoes multiple divisions, and the resulting cells, known as *plasma cells,* actively secrete proteins known as **immunoglobulins** or antibodies. All of the antibody molecules derived from a single clone of B cells are of a single specificity (recognize a unique antigen), identical to the receptor molecule on the original activated B cell. These antibody molecules circulate in the bloodstream and lymphatics, bathe body tissues, and bind to infectious agents or substances to aid the host in eliminating them from the body. The detection and quantification of these antibody molecules, obtained from a patient's serum, constitute the primary goal of diagnosing infectious diseases through serologic methods.

Classification and Characteristics of Antibodies

Antibody molecules, found in serum and other body fluids and secretions, may be classified into one of five distinct

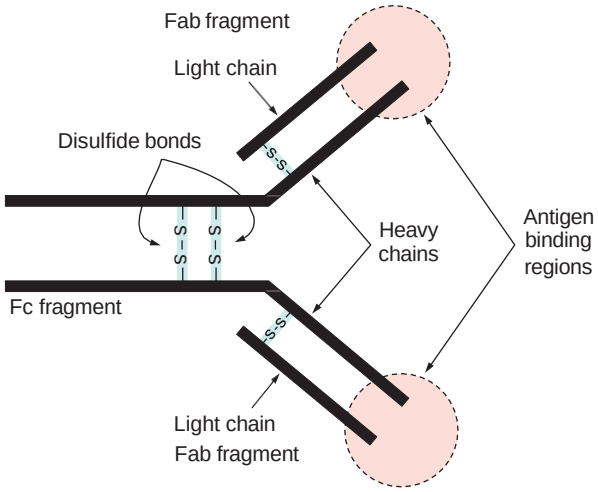

FIGURE 2-6 Immunoglobulin G (IgG).

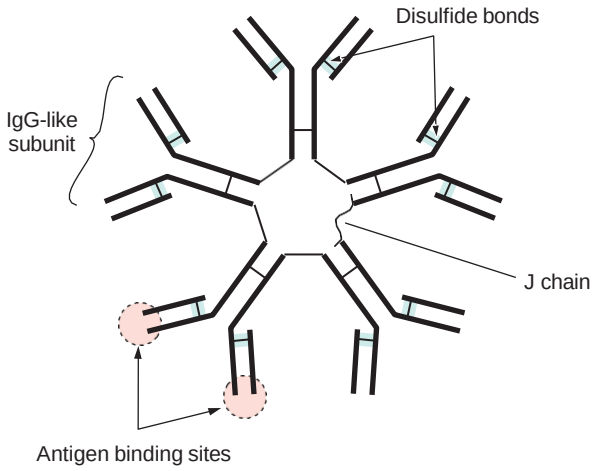

FIGURE 2-7 Immunoglobulin M (IgM).

immunoglobulin groups or classes. The classes differ from one another in several ways, including chemical structure, serum concentration, half-life, and functional activity.

Immunoglobulin G (IgG) class antibodies constitute about 70% to 75% of the total serum immunoglobulin pool. Their half-life in serum is about 3 to 4 weeks, and IgG can cross the maternal placenta to the fetus, possibly conferring some protection in both the prenatal and postnatal periods. Structurally, IgG is a protein with a molecular weight of about 150,000 daltons consisting of four polypeptides (two identical light chains and two identical heavy chains) bridged by several disulfide bonds (Figure 2-6). Although the amino acid sequence of some regions of the polypeptides is nearly identical among all IgG molecules (conserved regions), one of the ends of each polypeptide is highly variable. These variable regions create two active sites for antigen binding on each IgG molecule (see Figure 2-6). Thus IgG antibodies are said to be bivalent—capable of binding two antigen molecules.

Antibodies of the **immunoglobulin M (IgM)** class account for 10% to 15% of serum immunoglobulins. Their half-life in serum is about 5 days, and IgM cannot cross the placenta. A developing fetus in the second or third trimester, as well as a newborn, may respond to an infectious agent with an IgM antibody response of its own. The IgM antibody molecules are large; the whole molecule has a molecular weight of about 900,000 daltons and consists of five basic subunits—each composed of two heavy chains and two light chains (similar to an IgG molecule) and linked to another polypeptide chain (J chain) by disulfide bonds (Figure 2-7). Thus the IgM molecule has up to 10 antigen-binding sites available. Both IgG and IgM antibodies are commonly assayed in a variety of serologic tests. The differences in size and configuration of IgG and IgM molecules result in differences in functional activity of the molecules in serologic tests (Table 2-8).

TABLE 2-8 IgG versus IgM

Property	Immunoglobulin Class IgG	IgM
Molecular weight (daltons)	150,000	900,000
Number of 4-polypeptide subunits	1	5
Number of antigen-binding sites	2	10
Serum concentration (mg/dL)	800-1600	50-200
Percentage of total immunoglobulin	75	10
Ability to cross placenta	+	–
Half-life (days)	23-25	5-8

Immunoglobulin A (IgA) antibodies represent 15% to 20% of the total serum immunoglobulin pool. Immunoglobulin A constitutes the predominant immunoglobulin class in certain body secretions, such as saliva, tears, and intestinal secretions. Because of this association of IgA with mucosal surfaces, it provides protection against microorganisms invading at those sites. Serum IgA occurs primarily as two subunits (each similar to an IgG molecule) linked together by a J chain. When found in secretions, however, the molecule also contains a secretory component that stabilizes the molecule. Although significant increases in serum IgA may occur in association with certain infections, the function of serum IgA is unclear, and few serologic tests for the diagnosis of infectious disease are designed to specifically detect IgA antibody.

The remaining two immunoglobulin classes, IgD and IgE, are found in very low concentrations in serum (less than 1%). **Immunoglobulin E (IgE)** antibody levels rise during infection by a number of parasites and may play a role in eliminating these infectious agents from the host. Total serum IgE levels may rise during parasitic infection, and IgE-specific serologic tests for the diagnosis of a few parasitic agents have been developed. The role of serum IgD antibodies during infection is not known.

Primary and Secondary Antibody Responses

After exposure to an infectious agent, the host's acquired humoral immunity may respond through the production of various classes of antibody directed to one or more antigens associated with the agent. If the host has not been previously

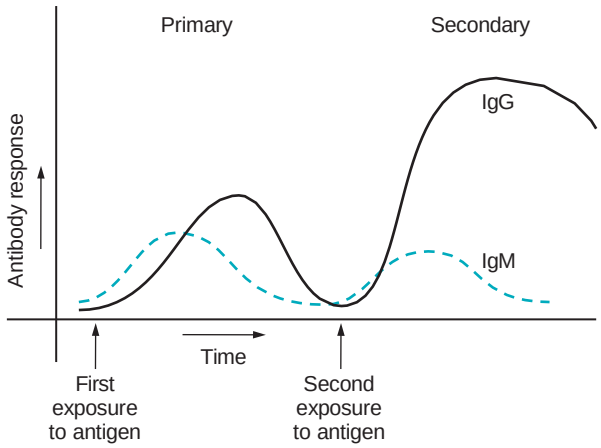

FIGURE 2-8 Primary versus secondary response.

exposed to the antigen, a primary immune response, characterized by the relatively rapid appearance of IgM antibodies, occurs. IM antibody levels usually peak in 1 to 2 weeks followed by a gradual decline to undetectable levels over the next few months. At the time when IgM levels have nearly peaked, IgG (and in some cases IgA) antibodies become detectable and continue to increase for about 1 month, surpassing peak IgM levels. IgG levels remain elevated for months and then decline slowly, often persisting at low but detectable levels for years (Figure 2-8).

A subsequent exposure to the same antigen elicits a secondary or **anamnestic immune response,** characterized by a rapid increase in IgG antibody associated with higher levels, a prolonged elevation, and a more gradual decline (see Figure 2-8). IgM antibody synthesis plays a minor role in a secondary immune response. Serologic tests that are designed to separately detect IgG and IgM antibodies take advantage of the differences in IgM production between a primary and a secondary immune response. Thus a positive test result for IgM antibody is considered indicative of a primary current or very recent infection, whereas the presence of IgG antibody alone suggests a previous infection or exposure. Similarly, the presence of significant levels of IgM antibody (with or without IgG) in a newborn suggests in utero infection (IgM can be synthesized by the fetus and cannot cross the placenta), whereas IgG antibody only in the newborn is indicative of passive maternal transfer of IgG across the placenta, not in utero infection.

Cell-Mediated Immune Response

In contrast to humoral immunity, the primary effector cell in cell-mediated immunity is the T lymphocyte. The T cell does not secrete antibody molecules; however, the result of antigen binding, activation, cell division, and differentiation of the T cell is the production of a number of low–molecular-weight proteins known as **lymphokines.** Lymphocytes affect their immunologic function through direct cell-to-cell contact or through the activity of the lymphokines on other cells, such as macrophages. The measurement and diagnostic significance of cell-mediated immunity are beyond the scope of this book, and cellular immune function tests generally are not performed in microbiology or microbial serology laboratories.

In essence, the wide variety and complexity of infectious agents necessitate flexibility in the immune mechanisms of the host. Immunity to extracellular bacterial pathogens, such as *S. aureus* and *S. pyogenes*, is mediated primarily by antibody functioning either alone (neutralization of toxins and extracellular bacterial enzymes) or with complement and neutrophils (chemotaxis and phagocytosis of bacterial cells). Immunity to intracellular bacterial pathogens, such as *M. tuberculosis*, is primarily cell mediated, through the activities of T lymphocytes, lymphokines, and macrophages. If antibody is produced, it plays little role in eliminating this pathogen, because the pathogenis sequestered(hidden) intracellularly where antibody cannot reach.

Meanwhile, viral infections often elicit both humoral and cell-mediated immune responses. Antibody may bind directly to and neutralize viral particles (render the virus noninfectious—unable to infect other cells) when they are found free in the bloodstream or other body fluids. For example, central nervous system infection by arboviruses (which cause encephalitis) or by certain enteroviruses (which cause meningitis) may be prevented if neutralizing antibody against these organisms is present when the virus reaches the bloodstream, and before it enters the central nervous system. Some viruses, however, cause infections that spread by cell-to-cell transmission (e.g., herpes simplex virus, which causes cold sores); they would not be subject to the neutralizing effect of antibodies. In these cases, cell-mediated immunity plays a predominant role in eliminating the agent. Immunity to both fungal and parasitic infections is also primarily cell mediated; antibody plays little or no role in prevention of or recovery from infection resulting from these agents. Although antibodies may not have protective value for certain infectious agents, they may nonetheless have diagnostic and prognostic value in serologic tests. Methods for detecting the presence and significance of antibodies for diagnostic purposes are discussed in Chapter 10.

MECHANISMS BY WHICH MICROBES MAY OVERCOME THE HOST DEFENSES

Because organisms can use a variety of antihost mechanisms, infectious agents are able to establish disease despite the host's defenses. The strategies that microbes employ to counter the host's defenses consist of bringing about tolerance, immune suppression, change in the appropriate target for the immune response, and antigenic variation.

Sometimes the host immune system fails to respond to specific antigens of the infecting microorganisms. This failure to respond is not necessarily due to immune suppression. The inability to induce an immune response to a microbial antigen, referred to as *tolerance*, may be due to a "feeble antigen"; that is, an antigen or antigenic component of an organism that is incapable of stimulating an immune response from the host. The host therefore fails to initiate a response or is sometimes slow in responding. This lack of response suggests tolerance to this antigen. Tolerance to an organism may also develop when the infection occurs during fetal life or in a neonate. For

example, it is believed that although the fetus, when infected by the rubella virus, responds and makes its own antibodies, the antibodies are often weak and unable to contain the infection. Because the T-cell response is also poor, the virus is able to persist in the fetus and during the neonatal period. Hence microorganisms can persist if they are able to survive in the host during prenatal infections without producing an overt form of the disease.

Certain microorganisms do cause immune suppression in the infected individual. The decreased immune response is often more far reaching than simply to the antigen of the involved microorganism. Viruses, certain bacteria, and protozoans are examples of microorganisms likely to cause immune suppression in the infected host. These infectious agents multiply in macrophages or in lymphoid tissues. The exact mechanisms of immunosuppression used by the infecting organism have not been defined for all organisms. However, individuals infected with viruses such as Epstein-Barr virus and cytomegalovirus show depressed T-cell or antibody responses to other unassociated antigens. Reduced immune reactivity caused by an infectious agent is exemplified by that caused by the human immunodeficiency virus (HIV), which targets CD4+ T cells. Because the virus destroys the major cells that defend the host against viral, fungal, and protozoan infections, the infected person becomes susceptible to opportunistic infections caused by these organisms.

Certain organisms have the ability to systematically change their surface antigens during the course of a single infection, even while inside the host, thereby evading the host immune defenses. This occurs in relapsing or recurring fever infections with *Borrelia recurrentis*. After an initial incubation period of 2 to 15 days following transmission of the spirochetes from a tick or louse, high numbers of the organism are found in the blood. The infected individual experiences high fever, rigors, severe headache, muscle pains, and weakness. The febrile period lasts for about 3 to 7 days but ends quickly with the induction of an immune response. However, a similar but less severe course of symptoms recurs several days to weeks later. The relapses are caused by antigen variation by the borreliae. Spirochetemia worsens during febrile periods and diminishes between recurrences.

Certain organisms such as *Brucella* spp., *Listeria* spp., and mycobacteria avoid the host's immune response by surviving inside infected cells. This is another evasion strategy used by microorganisms, making themselves unavailable as targets to the host's immune system. Macrophages that engulfed these microbial species protect them from antibacterial substances and support their growth inside the macrophage. For example, during the exoerythrogenic cycle in liver cells, the parasite *Plasmodium* spp. avoids being a target for the immune response. Malarial parasites can therefore infect red blood cells and cause disease while being protected from the host's defense mechanisms.

Hosts also produce antibodies against specific antigenic stimuli as an immune defense. However, if the antibodies produced against an infecting organism are of low avidity or have a weak antimicrobial effect on the infecting organisms, the ability of the infected host to control the infection is decreased. Therefore in certain microbial infections, antibodies, although produced, provide little or no protection to the host.

Similarly, interferons play a significant role in the host defense against foreign invaders. The main function of these cytokines is to stimulate the expression of major histocompatibility complex (MHC) proteins by T cells. Interferons (IFNs) are also antiviral; IFN-alpha and IFN-beta work against double-stranded RNA viruses, whereas IFN-gamma is produced following the activation of T cells. There are instances when viruses escape the effects of interferons, either because they are resistant to the antiviral effects or because the induction of interferon in the host does not take place. For example, vaccinia virus is able to resist the effects of interferons by inactivating IFN-gamma. Other viruses may produce persistent infections because these viruses do not induce interferon production.

ROUTES OF TRANSMISSION

The last portion of this chapter is devoted to the routes by which a pathogen may be transmitted to a susceptible host—an important factor in the establishment of infection, which is often explained by the characteristics of the pathogen. Although some organisms may be naturally transmitted by more than one route, most have a limited number of routes. These routes can be characterized as in the air (inhalation), via food and water (ingestion), through close contact (includes sexual transmission), through cuts and bites, and via arthropods; animal diseases that can infect humans are transmitted through animal contact **(zoonoses).** A summary of the routes of transmission is given in Table 2-9. Figure 2-9 shows the routes of entry to and exit of microbes from the body.

Airborne Transmission

Respiratory spread of infectious disease is common. Often, the respiratory secretions are aerosolized by coughing, sneezing, and talking. Very small particles, referred to as *droplet nuclei*, are the residue from the evaporation of fluid from larger droplets and are light enough to remain airborne for long periods. Pathogens that are spread through the air generally must be resistant to drying and inactivation by ultraviolet light. Some infectious agents may be transmitted by dust particles that have become airborne. As discussed earlier in this chapter, the body has a number of defenses against airborne infectious agents. The nasal turbinates, oropharynx, and larynx provide a twisting, mucus-lined passageway that makes direct access to the lower respiratory tract mechanically difficult. In addition, the lower portions of the respiratory tract contain ciliary epithelium that sweeps organisms upward. For a microorganism to cause disease, it must circumvent these defenses, penetrate the mucous layer, and attach to the epithelium. The host also produces secretory IgA, lysozyme, alveolar macrophages, and other factors that act on the pathogen that manages to get beyond the physical defenses.

Respiratory tract infections are the most common reason that patients of all ages seek medical attention. Although most upper respiratory tract infections are self-limiting and can be treated with over-the-counter medications, some are

TABLE 2-9 Common Routes of Transmission*

Route of Exit	Route of Transmission	Example
Respiratory	Aerosol droplet inhalation	Influenza virus; tuberculosis
	Nose or mouth → hand or object → nose	Common cold (rhinovirus)
Salivary	Direct salivary transfer (e.g., kissing)	Oral-labial herpes; infectious mononucleosis
	Animal bite	Rabies
Gastrointestinal	Stool → hand → mouth and/or stool → object → mouth	Enterovirus; hepatitis A
	Stool → water or food → mouth	Salmonellosis; shigellosis
Skin	Skin discharge → air → respiratory tract	Varicella; poxvirus infection
	Skin to skin	Human papillomavirus (warts); syphilis
Blood	Transfusion or needle prick	Hepatitis B; cytomegalovirus infection; malaria; human immunodeficiency virus
	Insect bite	Malaria; relapsing fever; West Nile Virus
Genital secretions	Urethral or cervical secretions	Gonorrhea; herpes simplex; *Chlamydia* infection
	Semen	Cytomegalovirus infection
Urine	Urine → hand → catheter	Hospital-acquired urinary tract infection
	Urine → aerosol (rare)	Tuberculosis
Eye	Conjunctival	Adenovirus
Zoonotic	Animal bite	Rabies
	Contact with carcasses	Tularemia
	Arthropod	Plague; Rocky Mountain spotted fever; Lyme disease

From Sherris JC, editor: *Medical microbiology: an introduction to infection diseases,* ed 4, New York, 2003, McGraw-Hill.
*The examples cited are incomplete, and in some cases more than one route of transmission exists.

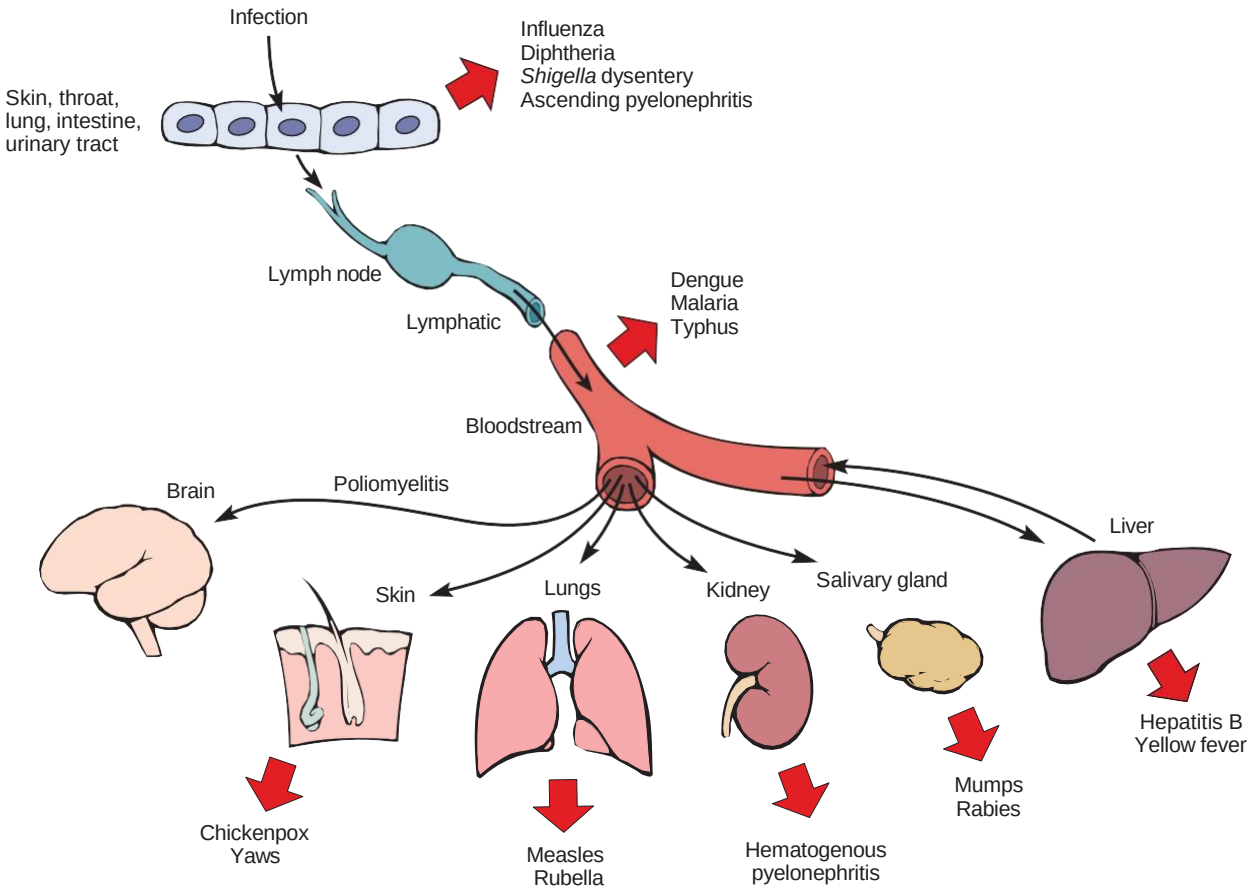

FIGURE 2-9 Routes of entry and exit. (Reprinted from Mims CA, Nash A, Stephen J: *Mims pathogenesis of infectious disease*, ed 5, San Diego, 2001, Elsevier Ltd, permission from Elsevier.)

more serious. Streptococcal sore throat, sinusitis, otitis media, acute epiglottitis, and diphtheria can be serious and even life threatening. Viral diseases causing the common cold and infectious mononucleosis are usually not life threatening but can result in much discomfort and absenteeism from work or school.

Although all the diseases mentioned can be spread via aerosols, some may also be transmitted via the fingers and hands; this is especially true of the common cold–causing rhinovirus. The fingers and hands are contaminated with infectious nasal secretions because of hand-to-nose contact. The infectious viral particles are passed from the infected individual to a susceptible recipient via hand-to-hand or hand-to-face contact. The recipient transmits the virus picked up from the hands of the infected individual by touching the face and nose. In this case, the disease is transmitted via the respiratory route, but not in the normal, classic manner of respiratory transmission.

Transmission may also result from contact with inanimate objects contaminated with the infectious agent. For example, a doorknob is contaminated by the hand and fingers of an infected individual, and the virus is transmitted to a susceptible person's hand and fingers when that person opens the door. Control of such transmission is often as simple as frequent handwashing.

Infections of the lower respiratory tract are less common but more serious than those of the upper respiratory tract. The organisms causing these infections have managed to bypass host defenses, or the host defenses have been compromised (e.g., by alcoholism, heavy smoking), allowing the pathogen access to the deeper portions of the respiratory tract.

The most common microorganism causing lower respiratory tract infection of individuals older than 30 years of age is *S. pneumoniae*. Although the pneumococcus is the most common community-acquired pneumonia, it is also often seen in aspiration pneumonia, a common type of hospital-acquired pneumonia. Pneumococcal pneumonia begins suddenly and is a serious, life-threatening disease, particularly in older patients. Among younger people, the common causes of pneumonia are *Mycoplasma* organisms and viruses. The onset of these pneumonias is more gradual than with pneumococcal pneumonia and the outcome is far more favorable.

In chronic lower respiratory tract infections, the survival of the infecting agent within phagocytes plays a role in the pathogenic mechanism. As the agent of tuberculosis, a chronic debilitating infection, *M. tuberculosis* is the classic example of an intracellular pathogen. This organism is highly virulent, is invasive, survives well, and multiplies within phagocytes.

Transmission by Food and Water

Transmission of gastrointestinal infections is usually a result of ingestion of contaminated food or water. In some situations, infection occurs via the fecal-oral route.

The digestive tract is colonized with vast numbers of different microorganisms. Under usual conditions, the gut flora maintains a harmless relationship with the host. Gastric enzymes and juices in the stomach prevent survival of most organisms, but many survive and colonize the small intestine and colon.

Gastrointestinal infections result from organisms that survive the harsh conditions of the stomach and competition with the microbial flora, and then produce damage to the tissues of the gastrointestinal tract. This damage is a result of either a preformed toxin or disruption of the normal functioning of the intestinal cells by invasion of the pathogen or production of a toxin within the intestine.

Organisms that can cause disease by means of a preformed toxin include *Clostridium botulinum*, *Bacillus cereus*, and *S. aureus*. The severity of disease ranges from a mild diarrhea to a rapidly fatal intoxication. Food poisoning by *B. cereus* and *S. aureus* is relatively common and is self-limiting. Botulism, caused by *C. botulinum*, although rare, can be life threatening.

Other bacteria produce a toxin after infection of the intestinal tract. Generally, to be effective as a disease producer, an organism must survive, adhere to, and colonize the intestinal mucosa and either produce a toxin or invade deeper tissues. A commonly seen cause of diarrhea and intestinal infection is *E. coli*. This organism is a member of the intestinal flora; however, some strains of *E. coli* produce cytotoxins that cause alterations in the biochemical activity of the intestinal epithelial cells, resulting in problems with fluid and electrolyte control by the intestinal cells. These strains of *E. coli*, referred to as *enterotoxigenic*, are a common cause of traveler's diarrhea and other intestinal problems.

Probably the classic intestinal pathogen is *Vibrio cholerae*, the cause of cholera. This organism produces an enterotoxin that causes the outpouring of fluid from the cells into the lumen of the intestine. Massive amounts (up to 20 L per day) of fluid can be lost. Other intestinal pathogens are *C. difficile*, *Shigella* spp., *Aeromonas hydrophila*, *Campylobacter jejuni*, and *Salmonella* spp. The infective dose, severity, and incidence of disease vary with the agent.

A number of viruses also cause diarrheal disease. They multiply within the cells of the intestinal mucosa and affect the normal functioning of the cells. Viral agents in this category include hepatitis A and E, rotavirus, adenovirus, coxsackievirus, and *Norovirus* spp. The incidence of diarrhea caused by these agents is high, especially when people are in close contact (e.g., in daycare centers, nursing homes, military camps). Numerous parasites, such as *Fasciolopsis buski*, *Giardia lamblia*, *Entamoeba histolytica*, and *Balantidium coli*, also infect the gastrointestinal tract.

Close Contact

All of the routes of transmission of infectious diseases require close contact. For a respiratory pathogen to be transmitted via aerosols, the susceptible host must be relatively close. For this discussion, however, *close contact* refers to passage of organisms by salivary, skin, and genital contact. Two prominent infections passed by direct transfer of saliva (e.g., kissing) are herpes simplex virus and Epstein-Barr virus. Skin-to-skin transfer of infectious disease is not as common as for some of the other routes, but diseases such as warts (human papillomavirus), syphilis, and impetigo result when material from infectious lesions inoculates a susceptible host's skin. The list

TABLE 2-10 Zoonoses

Disease	Organism
Anthrax	*Bacillus anthracis*
Brucellosis	*Brucella* spp.
Erysipeloid	*Erysipelothrix rhusiopathiae*
Leptospirosis	*Leptospira interrogans*
Tularemia	*Francisella tularensis*
Ringworm	*Trichophyton* spp.
	Microsporum spp.
Lyme disease	*Borrelia burgdorferi*
Plague	*Yersinia pestis*
Rocky Mountain spotted fever	*Rickettsia rickettsii*
Yellow fever	Flavivirus
Encephalitis	Alphavirus
Colorado tick fever	Orbivirus
Leishmaniasis	*Leishmania* spp.
Rabies	Rhabdovirus
Blastomycosis	*Blastomyces dermatitidis*
Tuberculosis	*Mycobacterium bovis*
Q fever	*Coxiella burnetii*
Ornithosis	*Chlamydia psittaci*
Gastroenteritis	*Campylobacter* spp.
	Salmonella spp.
Listeriosis	*Listeria monocytogenes*
Giardiasis	*Giardia lamblia*
Toxoplasmosis	*Toxoplasma gondii*
Tapeworms	*Taenia saginata*
	Taenia solium
	Diphyllobothrium latum
	Echinococcus spp.
Trichinosis	*Trichinella spiralis*

of sexually transmitted diseases is a long one. In North America, the most commonly transmitted venereal diseases or agents are gonorrhea, herpes simplex virus, hepatitis B and C, *Chlamydia* spp., syphilis, *Trichomonas* spp., and HIV.

Cuts and Bites

The classic example of a bite-wound infection is rabies. Human rabies though is relatively rare. Of more concern with animal bites, and especially human bites, is infection by the mouth flora. Dog-bite and cat-bite infections often yield *Pasteurella multocida*, but the possibilities are extensive. Human bites are extremely dangerous because they are difficult to treat and because the human oral flora comprises many different organisms in extremely high numbers.

Arthropods

Infection as a result of a tick, flea, or mite bite is a common occurrence in many parts of the world. The diseases spread by arthropods include malaria, relapsing fever, plague, Rocky Mountain spotted fever, Lyme disease, typhus, and untold numbers of regional hemorrhagic fevers. In most cases, the infectious agent multiplies in the arthropod, which then feeds off a human host and transmits the microorganism.

Zoonoses

The route of transmission known as *zoonosis* depends on contact with animals or animal products. Certain organisms causing disease in animals may also infect humans who have contact with them. These diseases may be passed by animal bites (rabies), arthropod vectors (plague), contact with secretions (brucellosis), and contact with animal carcasses and products (tularemia, listeriosis). The diseases are transmitted by routes already discussed. The common factor is that, regardless of the route, the disease is a disease of animals that is transmitted to humans. A partial list of zoonotic diseases and infecting organisms is given in Table 2-10.

BIBLIOGRAPHY

Ceciliani F, Giordano A, Spagnolo V: The systemic reaction during inflammation: the acute-phase proteins, *Protein Pept Lett* 9:211, 2002.

Chapel H et al: *Essentials of clinical immunology*, ed 5, Malden, Mass, 2006, Wiley Blackwell.

Fu YX, Chaplin DD: Development and maturation of secondary lymphoid tissues, *Annu Rev Immunol* 17:399, 1999.

Gabay C, Kushner I: Acute-phase proteins and other systemic responses to inflammation, *N Engl J Med* 340:448, 1999.

Grewal IS, Flavell RA: CD40 and CD154 in cell-mediated immunity, *Annu Rev Immunol* 16:111, 1998.

Hiemstra PS, Bals R: Series introduction: innate host defense of the respiratory epithelium, *J Leukoc Biol* 75:3-4, 2004.

Johnson HL, Chiou CC, Cho CT: Applications of acute phase reactant in infectious diseases, *J Microbiol Immunol Infect* 32:73, 1999.

Kamradt T, Mitchison NA: Tolerance and autoimmunity, *N Engl J Med* 344:655, 2001.

Kumar V, Abbas A, Aster J, editors: *Robbins and Cotran pathologic basis of disease*, ed 8, Philadelphia, 2010, Saunders.

Lanzavecchia A, Sallusto F: Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells, *Science* 290:92, 2000.

Mandell G, Bennett J, Dolin R: *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*, ed 7, New York, 2010, Churchill Livingstone.

McKenzie SB: *Clinical laboratory hematology*, ed 2, Upper Saddle River, NJ, 2010, Prentice Hall.

Medzhitov R, Janeway C Jr: Innate immunity, *N Engl J Med* 343:338, 2000.

Mims C, Nash A, Stephen J: *Mim's pathogenesis of infectious disease*, ed 5, San Diego, 2001, Academic Press.

Nemazee D: Receptor selection in B and T lymphocytes, *Annu Rev Immunol* 18:19, 2000.

Sheldon H: *Boyd's introduction to the study of disease*, ed 11, Philadelphia, 1992, Lea & Febiger.

Sompayac LM: *How the immune system works*, ed 3, Malden, Mass, 2008, Wiley-Blackwell.

Wilson JW, et al: Mechanism of bacterial pathogenicity, *Postgrad Med J* 78:216, 2002.

Winn W et al, editors: *Koneman's color atlas and textbook of diagnostic microbiology*, ed 6, Philadelphia, 2006, Lippincott Williams & Wilkins.

Points to Remember

- Some species of the usual microbial flora may be opportunists, capable of causing disease in an immunocompromised host.
- True pathogens cause disease in all individuals.

- The host defense system against microorganisms includes physical, mechanical, and chemical barriers; components of the innate immune system such as phagocytes, complement, cytokines, and the products of inflammation; and the components of the adaptive immune response.
- Microbes have mechanisms to evade the host's defenses, including the ability to evade phagocytosis, production of enzymes and exotoxins, the ability to induce tolerance in the adaptive immune system or to suppress the adaptive immune system, and the ability to avoid recognition by the adaptive immune system by varying the antigens present on the surface of the microorganism.

Learning Assessment Questions

1. What host resistance factor is compromised in the patient described in the Case in Point?
2. What microbial factor contributes to the virulence of the infecting organism?
3. What is the difference between true pathogens and opportunistic pathogens?
4. How does inflammation play a role as a host immune defense?
5. What is the difference between exotoxins and endotoxins?
6. What cells and soluble mediators are involved in the innate immune response versus the adaptive immune response? Humoral versus cellular?
7. How can microorganisms evade the immune response?
8. How are organisms transmitted?
9. Name the major immune response that is responsible for eliminating extracellular and intracellular bacteria and viruses.
10. What are the steps involved in phagocytosis, and how can microorganisms evade each step?
11. What are the differences between and functions of IgG and IgM?
12. What are zoonoses, and what organisms are considered zoonotic agents?

CHAPTER 3

Laboratory Role in Infection Control

David L. Taylor

- GENERAL CONCEPTS IN INFECTION CONTROL PRACTICE
  - Infection Control in Health Care Settings
  - Infection Control Surveillance
  - Frequently Identified Microbes
- OUTBREAK INVESTIGATION
  - Local
  - Widespread
  - Steps of Outbreak Investigation
  - Investigation Support from the Laboratory
  - Environmental Culturing
  - Reporting
- EDUCATION
  - Technologists and ICPs
  - Safety
- EMERGING AND REEMERGING PATHOGENS
  - Emerging Pathogens
  - Reemerging Pathogens
  - Response Plans

OBJECTIVES

After reading and studying this chapter, you should be able to:

1. Delineate the various roles the laboratory and the laboratory technologist may play in an infection control program.
2. List the facilities/settings in which an infection control program is important.
3. Define *surveillance* and the ways in which surveillance is conducted.
4. Describe outbreak investigation and the steps followed in an outbreak investigation.
5. List the ways in which a microbiology laboratory may support an outbreak investigation.
6. Define when and how environmental culturing is appropriate in an infection control program.
7. Describe agencies/entities to which an infection control program would provide reports.
8. Discuss educational activities that encompass an infection control program.
9. Correlate the activities of the laboratory with the safety and prevention activities of an infection control program.
10. Describe the roles of the microbiology laboratory and the epidemiology program in preparing for potential bioterrorism activities.

Case in Point

An 82-year-old man was admitted to an intensive care unit (ICU) from an **extended care facility (ECF);** he was confused and short-of-breath. His chest radiograph revealed consolidation in the left lower lobe. A bronchoalveolar lavage was performed and the specimen was sent to the laboratory for culture and antibiotic susceptibilities. An **endotracheal** tube was placed and the patient was attached to a ventilator for respiratory support. The cultures grew a multidrug-resistant strain of *Acinetobacter baumannii*. Within 3 days, two more patients in the ICU had respiratory cultures positive for *A. baumannii.* Over the next 4 days, three additional ventilated patients had tracheal aspirate cultures that grew the same microbe.

Infection control was notified by the microbiology technologist of the unusual occurrence of several isolates of the resistant *Acinetobacter*. The infection control practitioner, the microbiology technologist, the ICU nurse manager, and the physician in charge of the ICU met to review the situation. The patients were placed into Contact Precautions, education was initiated that highlighted hand hygiene practices, and environmental cultures were taken. Data were collected and analyzed. The occurrence of the multidrug-resistant strain of *Acinetobacter* decreased and eventually no new cases were observed.

Issues to Consider

After reading the patient's case history, consider:

- The role of the laboratory worker and the microbiology laboratory in an infection control program
- The surveillance of health care–associated infections and the required laboratory support
- The information needed in an outbreak investigation
- The role of the laboratorian as an educator in infection control
- Bioterrorism and emerging pathogens

Key Terms

Antibiograms
Antibiotic pressures
Baseline data
Case definition
Central line–associated bloodstream infections (CLA-BSIs)
Communal living
Community-acquired infections
Data mining
Emergency response plans
Emerging pathogens
Endotracheal
Environmental cultures
Epidemiologic curve
Extended care facility (ECF)
Hand hygiene
Health care–associated infections
Iatrogenic
Index case
Infection control practitioner (ICP)
Infection control risk assessment
Infection rate
Interventions
Intravascular device
Outbreaks
Outbreak investigation
Prevalence
Public health
Pulsed-field gel electrophoresis
Reemerging pathogens
Standard Precautions
Surgical site infections (SSIs)
Surveillance
Targeted surveillance
Total surveillance
Urinary tract infections (UTIs)
Ventilator-associated pneumonias (VAPs)

Every year, more than 2 million health care–associated infections occur that cost the health care system countless dollars and impact millions of lives. An effective infection control program must be established in a health care setting to recognize and prevent these health care–associated infections. This chapter covers the settings, the activities, and the analytic practices involved in implementing an effective infection control program, specifically from the laboratory technologist's perspective.

GENERAL CONCEPTS IN INFECTION CONTROL PRACTICE

The clinical laboratory and the laboratory technologists play a vital role in the functioning of an effective infection control program. In some settings, the technologists provide supportive data; in other settings, the technologist may function as a part-time **infection control practitioner (ICP)** as well as a part-time laboratorian. Not infrequently, the technologist may leave the laboratory bench and become the ICP or work as a member of an infection control department.

As the term implies, *infection control* involves activities aimed at preventing infections in persons in broad and varied settings. The activities sweep the gamut from surveillance activities to outbreak investigation to education. They require an inquisitive and analytic mind, both of which are characteristics seen in clinical laboratory scientists and technologists.

TABLE 3-1 Health Care Settings Involving Infection Control

Setting	Description
Public health	Community settings (e.g., daycare centers, schools)
Acute care	Hospitals
Ambulatory care	Outpatient surgery, chronic dialysis and infusion centers, physicians' offices, emergency care facilities
Extended care	Skilled nursing facilities, nursing homes, assisted living centers
Home care	Skilled nursing provided in the home
Communal living	Prisons and jails, behavioral health facilities

Infection Control in Health Care Settings

Infection control practices are important in many health care settings. Table 3-1 lists different health care environments wherein infection control practices play a vital role. In all of the settings, the goal is to prevent the spread of infectious agents. In **public health** or community-at-large settings, the transmission of microorganisms occurs through many events in daily living. Microbes are spread at home, in daycare centers, in schools, in crowds (malls), and by individuals. There are sexually transmitted diseases, diseases spread by respiratory routes, diseases spread by contact, food-borne diseases, and waterborne diseases, all of which occur in the public arena. In acute care hospitals, infections occur as **surgical site infections (SSIs), central line–associated bloodstream infections (CLA-BSIs), urinary tract infections (UTIs),** and **ventilator-associated pneumonias (VAPs).** They are found in adults, teenagers, children, and neonates, as well as in healthy individuals, and immunocompromised patients. These infections occur as **community-acquired infections**, health care–associated infections, and **iatrogenic** infections. They occur because of instrumentation, increased use of antimicrobial agents, breaks in aseptic techniques, and lack of **hand hygiene.** Ambulatory care settings include outpatient surgical, chronic dialysis, and infusion centers as well as physician offices and emergency care facilities. Patients are seen, treated, and released. Infection control must be practiced in these settings even though the patients may not be seen again. In extended care facilities (ECFs), patients are frequently immunosuppressed by disease, age, or therapy. Extended care settings include skilled nursing facilities, nursing homes, assisted living centers, rehabilitation centers, and hospice care settings. Because of their suppressed defenses, these patients are prime candidates to acquire infections. Patients who are at home and receive home care from family or from professional home care providers sometimes acquire infections. Most often, home care involves intravascular-related or some other device-related care. As in extended care settings, the immune defenses of these patients are often suppressed by their disease or by their therapy.

Communal living programs can also be settings in which infections occur. These community living programs might include prisons and behavioral health facilities. In these facilities, infections might be found that are spread by contact (illicit tattooing) or by intimate contact with blood and body fluids.

The microbiology laboratory may receive specimens from any of these settings, all of which represent opportunities for infections to spread and opportunities for infection control to be practiced. Microbiology technologists must know who their customers are and be poised to insert themselves into infection control activities for the varied customer base.

Infection Control Surveillance

Infection control programs include many activities aimed at preventing the spread of infections. To determine where to most efficiently direct these preventive activities, an effective infection control program must collect statistics on existing infections. By comparing baseline figures with periodic numbers, the ICP can recognize **outbreaks**, upward trends, and positive effects of **interventions.** Ongoing, systematic collection of these data and the analysis and interpretation of the details surrounding a disease or event is termed **surveillance.** The laboratory contributes on a daily basis to these records.

Surveillance Definitions

Surveillance data can best be done and the data compared internally, locally, and nationally if standard definitions are used. Table 3-2 lists the common terms used in surveillance and their definitions. The Centers for Disease Control and Prevention (CDC) have recommended definitions that are generally used in the infection control profession. Most infection control programs are concerned about infections that are acquired within the setting. These infections occur after the patient arrives (generally not within the first 48 hours) and were not incubating in the community before the patient arrived. These acquired infections are generally called **health care–associated infections**. These health care–associated infections can be followed within a setting to determine when infection numbers increase above baseline, thereby requiring some investigation and intervention.

TABLE 3-2 Surveillance Definitions

Term	Definition
Primary infection	An infection related to a single, specific site, not from multiple sites.
Bloodstream infection	An infection found in the bloodstream.
Central line–associated bloodstream infection	A bloodstream infection related to the presence of an intravascular device such as a central venous catheter.
Surgical site infection	An infection at a site where a surgical procedure was performed. Usually risk is stratified by length of surgery, site of infection, and degree of anticipated contamination.
Urinary tract infection	Infection of the urinary tract, frequently associated with a urinary catheter.
Ventilator-associated pneumonia	A pneumonia in a patient associated with a ventilator device such as an endotracheal tube or a tracheotomy.

Infections are generally defined by site, risk factors, and procedures as shown in Table 3-2; primary infections are those infections that occur at one site, for example, a primary bacteremia. A primary bacteremia would not have another site that might be the source of the infection, as would be seen in urosepsis, in which the primary site would most likely be the urinary tract. Using primary bacteremia as an example, the laboratorian recognizes a bloodstream infection by the recovery of clinically significant organisms from blood cultures, whereas the ICP determines whether the infection was health care associated and primary. If the infection control program was monitoring CLA-BSIs, then the ICP would determine whether the bloodstream infection was related to an **intravascular device.** For this reason, the site of the blood culture draw (e.g., peripheral internal jugular catheter, femoral catheter) is important to include with the specimen description on the microbiology laboratory report.

The incidence of SSIs is frequently reviewed by the ICP. To the laboratorian, the isolation of pathogens from the surgical site is indicative of postsurgical wound infection; the ICP determines whether the infection was superficial, deep, or an organ-space infection. Therefore the site of the wound culture is an important component of the specimen description on the laboratory report. The ICP relates the infection to other risk factors, such as the length of surgery, the degree of contamination of the surgical site (gunshot wound to the abdomen versus a hernia repair), and whether any breaks in surgical technique occurred.

Because UTIs are determined by microbial growth from a urine specimen, the method of urine specimen collection should be described and differentiated between a voided clean-catch urine specimen and a specimen collected by catheterization. The definition of a health care–associated UTI includes the presence or absence of a urinary catheter. Health care–associated pneumonias are difficult to assess from the perspectives of both the laboratory and the ICP. From the ICP perspective, the criteria to define a hospital-acquired pneumonia include the presence of an endotracheal tube or some other respiratory device, and whether the pneumonia was incubating or present when the patient arrived. In the laboratory, the microbiologist should include in the specimen description on the laboratory report the type and source of the respiratory specimens (bronchoalveolar lavage, tracheal aspirate, expectorated sputum). With sputum specimens, the quality of the specimen as evaluated by the presence of white blood cells and single bacterial morphotypes, and the absence of squamous epithelial cells, is an important adjunct. The use of these definitions is important in guiding the ICP and in allowing the comparison of data.

General or Targeted Surveillance. Infection control programs may have all the health care–associated infections within the setting under surveillance or, because of budget or personnel restraints, they may be observant for only specific infections. In **total surveillance** programs, all infections are

recorded and analyzed to determine whether the infections are health care associated. Risk assessments determine whether the situation is a high or low risk and whether the infections are occurring in an unanticipated number. The **infection rate** is determined and analyzed to establish whether the number of infections has increased or decreased. These rates can be compared with previous rates in the setting or with rates in similar local or national health care facilities. There are several ways of calculating infection rates. For example, the SSI rate is defined as the number of infections per number of procedures expressed as percentages. Infection rates of CLA-BSIs and VAPs are calculated as number of infections per 1000 device-days. If an unexpected change in rates is seen, the ICP may conduct further investigations to determine, if possible, the cause of the change and to propose the course of action to reverse the situation. Based on the results of the investigation, the ICP would recommend the appropriate intervention such as a change in procedure, education, or increased emphasis on hand hygiene. The ICP would continue to monitor the infection to determine whether the rate of infection decreases.

Unlike in total surveillance, **targeted surveillance** involves a close watch of only specific, high-risk, high-volume procedures. For example, the program may follow CLA-BSIs in the ICU only and SSI in gynecologic surgical procedures, whereas another program may conduct surveillance on VAP in the neonatal intensive care unit and in the adult intensive care unit. Which infections to target would largely be based on review of previously ascertained data on infection rates **(baseline data),** recognition of high-volume and high-risk procedures within the setting, and sometimes on requests by others (e.g., insurance companies, physicians). This process is often termed a *risk assessment.* The targeted surveillance may change from year to year depending on recognized outbreaks or changes in the number of procedures performed. All of the surveillance data must be carefully collected, must use high-quality laboratory data, and should be protected from legal discovery as determined by the risk management team for the setting.

Baseline Data. To recognize when infections constitute an outbreak or when an upward trend is occurring, the infection control program must have established baseline data; that is, the historical occurrence of infections over time. Data at the national level that can be derived from the National Healthcare Safety Network (NHSN) may also serve as the baseline data. However, baseline data from within the specific health care setting more accurately reflects happenings within that setting. The baseline data are collected and analyzed by numbers, by percentages, or by rates per 1000 device-days. Baseline data are an important component of the decision-making process regarding which types of infections to target.

Data Gathering

Culture Review. Many infection control programs rely heavily on review of culture results, which is initiated informally by the laboratory technologist who may then inform the ICP of what appears to be an increased number of specific isolates. For example, the technologist may notice the isolation of an increased number of *Serratia marcescens* or *Salmonella* spp. isolates, which may be infrequently seen in this particular health care facility. Or, the technologist may think that there is an unusual number of isolated methicillin-resistant *Staphylococcus aureus* (MRSA) from skin sites in a prison setting. The appearance of an increase in the number of surgical site specimens with various microorganisms may trigger a call to the ICP. These are informal data gathering activities that are significant to the infection control program. To report data, laboratorians use their experience and sense of what is usual regarding types of specimens and microbes encountered.

A more formal data review involves the daily review of actual culture results done either by the technologist or by the ICP. Such a review requires laboratory-generated results, generally from a laboratory information system (LIS). For example, the ICP or microbiologist reviews positive cultures and categorizes them into groups based on sites, units, organisms, or procedures. From this categorization, the ICP may recognize a trend, such as an upward trend in the number of *Pseudomonas aeruginosa* isolates from a skilled nursing facility or an increased number of positive cultures in laminectomy patients. Even more important is when the ICP determines that there are more *S. aureus* isolates than usual in the home health care setting. In this instance, an initiation of detailed investigations may be indicated. These fact-gathering activities are more formal and specific than the informal monitoring done by the technologist based on experience.

Recent advances in statistical correlation interface the LIS with a more sophisticated data review system for the purpose of **data mining.** In this type of data presentation, a multitude of events are analyzed and reported to the ICP. Data mining removes much of the day-to-day detail of the culture result review. It also adds other health care parameters to the analysis that are frequently not available to the ICP without detailed examination. This type of analysis may require the LIS and other hospital information systems to interface with the data mining system.

No matter how the culture results are screened and analyzed, a timely review is an integral component of an infection control program. Not only are the patients and their health care providers dependent on high-quality microbiology results, but the effectiveness of an infection control program is also dependent on such results.

Cases. The laboratory must be attuned to what is happening in the community and in the various settings within the community in regard to infection control issues. Infection control in the public health arena impacts the local microbiology laboratory. For instance, there may be an increase in the number of cases of whooping cough in surrounding counties. Even though the laboratory in the immediate county may not have seen any *Bordetella pertussis* isolates, they need to be aware of these other cases. Similarly, the anticipated appearance of influenza cases is a seasonal occurrence that influences the activities of a microbiology laboratory as well. The laboratory can be proactive in educating health care providers on specimen collection and transportation if those are unique to a specific public health concern. Awareness of infection control activities within the public health setting allows the laboratory to acquire the necessary media or reagents to meet

emerging needs. Potential infection control issues that might become apparent within a setting may drive activities within the microbiology laboratory. For example, a product used in the health care setting is recalled because of suspected bacterial contamination. Effective communication between the infection control program and the microbiology laboratory is needed to establish screening techniques in anticipation of cases involving contaminated products that might arise in the health care setting. The technologists need to increase their knowledge of the specific product, the types of specimens to anticipate, and the expected isolates that may be unique or previously unrecognized by the laboratory. Collaboration among the microbiology laboratory, the infection control program, and the health care customer becomes paramount. Reporting of cases, both to the laboratory and from the laboratory to the infection control community, is an important tool in surveillance activities.

Laboratory Support and Data Gathering. In addition to providing culture results, the microbiology laboratory also provides the ICP with other details. These may include specimen contamination rates, the numbers of isolates per site, or the number of isolates per unit within the health care facility. Knowledge of specimen contamination rates helps the ICP with the interpretation of culture results and provides guidance in developing educational activities to improve collection of quality specimens. For example, if respiratory specimens are frequently contaminated with upper respiratory flora, interpretation of the results for the health care provider as well as for the ICP becomes more difficult. Contaminated specimens also prove costly for the patient and the payer because they do not provide useful or appropriate information. Attempts to determine VAP become futile if the specimens are contaminated with upper respiratory flora. A similar situation is encountered in blood culture contamination, which lessens the likelihood of high-level interpretive abilities. Blood culture contamination rates greater than 3% are generally considered high and may indicate educational opportunities for the microbiology laboratory and the infection control program.

The types of pathogens isolated from given specimens represent important information that can be generated by the laboratory to support the infection control program. By knowing what organisms would most likely be recovered from a given body site, the ICP can guide the health care provider in considering the appropriate empirical therapy. For example, if *S. aureus* is frequently isolated from skin infections in a jail setting, then the health care provider can anticipate the success of anti-staphylococcal antimicrobials in routinely treating those infections. If MRSA, however, is more frequently isolated, the health care provider would change the empirical therapy.

The **prevalence** of a particular pathogen is another piece of information that the microbiology laboratory can provide to the ICP. Prevalence is the number of cases of disease that occur in a given moment in time or specific time period in a given population. Therefore not only knowing what pathogens are isolated from a given body site but also being familiar with what pathogens are frequently isolated from a given location within a health care facility is important to the ICP. For instance, a skilled nursing facility may be reassured that the lack of negative airflow rooms is acceptable if *Mycobacterium tuberculosis* has not been isolated from any of their patients over the past 7 years.

Being able to recognize what pathogens are isolated from patients in a medical intensive care unit may provide the opportunity for the ICP to inform health care providers the effects of **antibiotic pressures**. For example, if extended-spectrum β-lactamase (ESBL)–producing *Klebsiella pneumoniae* isolates were seen in that medical intensive care unit, then the physicians may be advised to limit the use of antimicrobial agents that tend to induce the formation of ESBLs. The microbiology laboratory then serves as an important adjunct to an infection control program by providing a multiplicity of types of data. The technologist must be aware of the customer needs and, in some cases, anticipate the customer needs in regard to these types of information.

Frequently Identified Microbes

Although the types of microorganisms seen in health care–associated infections vary from setting to setting, there are common pathogens that are frequently encountered. This discussion of the role of the laboratory in infection control addresses some of these common microbes to focus the attention of the laboratory technologist to the health care setting, the specimens, and the likely isolates. Table 3-3 delineates some of the most common organisms identified.

Public Health and Community Setting

The laboratory serving a public health or community setting is likely to identify infectious diseases of infection control importance that are less frequently seen in other health care situations. Some of the microbes frequently associated in water- or food-borne community outbreaks include *Giardia lamblia*, *Salmonella, Shigella, Campylobacter,* and *Cryptosporidium* species. Sexually transmitted diseases (STDs) such as syphilis, gonorrhea, and chlamydial infections are

TABLE 3-3 Health Care Settings and Common Microbes of Infection Control Significance

Health Care Setting	Microorganisms/Infectious Disease
Public health	*Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., syphilis, gonorrhea, *Chlamydia* spp., HIV, *Neisseria meningitidis,* encephalitis viruses, hepatitis B, hepatitis C
Acute care	*Staphylococcus aureus,* MRSA, VRE, *Escherichia coli, Pseudomonas aeruginosa, Clostridium difficile*
Ambulatory care	Hepatitis B, hepatitis C, HIV, *S. aureus,* MRSA, *P. aeruginosa,* VRE
Extended care facilities, home care	Opportunistic pathogens, *P. aeruginosa, Candida albicans, S. aureus,* MRSA, *Acinetobacter, C. difficile,* VRE
Communal living	*S. aureus,* MRSA, hepatitis C, lice

HIV, Human immunodeficiency virus; *MRSA,* methicillin-resistant *S. aureus*; *VRE,* vancomycin-resistant enterococci.

community-acquired infectious diseases that are identified in public health laboratories as well as laboratories serving acute care facilities. Some organisms that are important in public health settings may be more likely to be recovered in an acute care hospital but reported to a public health jurisdiction for the latter to follow up as a potential outbreak. Organisms such as *Neisseria meningitidis*, encephalitis viruses, coronaviruses (severe acute respiratory syndrome [SARS]), and West Nile virus are examples.

Acute Care Setting

Although a great variety of infectious agents can cause concern as health care–associated infections in acute care settings, some are more frequently seen than others. These are listed in Table 3-3. *S. aureus*, especially MRSA, is an important health care–associated pathogen, causing BSIs, SSIs, VAPs, and other infections. Although community-acquired MRSA is becoming more prevalent, health care–associated MRSA is worrisome because of its resistance to multiple antimicrobial agents. Similarly, enterococci are pathogens of concern in health care–associated infections because of their possible resistance to vancomycin and their potential ability to pass that resistance on to other microbes. *Escherichia coli* is a common fecal organism and is seen in a variety of health care–associated infections in a variety of sites including BSIs, SSIs, VAPs, and UTIs. In patients who are immunosuppressed by disease or by therapy, *P. aeruginosa* is seen as a cause of health care–associated infections. *Clostridium difficile* is an organism whose toxic products cause diarrhea associated with health care infections. Recovery of the organism is not the significant finding, but rather demonstration of the toxin. Although many other microbes are implicated in health care–associated infections, these are the top six in most acute care facilities.

Ambulatory Care Setting

Ambulatory care settings include a variety of different locations. However, the commonly encountered microorganisms of infection control importance do not vary. Most often, the patients have chronic illnesses, are immunosuppressed, and have infectious diseases caused by opportunistic pathogens. Other patients are likely to acquire community-acquired infections such as hepatitis B, hepatitis C, and human immunodeficiency virus (HIV). Other bacterial isolates include pathogens seen in other settings such as *S. aureus*, MRSA, vancomycin-resistant enterococci (VRE), and *P. aeruginosa*.

Extended Care Facility and Home Care Settings

Patients in both ECF and home care settings are frequently immunosuppressed by disease or therapy and often need intravascular or other device-related care. The microbes identified in these patients are often opportunistic pathogens. Infectious etiologic agents of infection control significance identified in these patients include *P. aeruginosa, Candida, S. aureus*, MRSA, VRE, *Acinetobacter* spp., and *C. difficile.*

Communal Living

People who are housed together in some form of communal living, such as prisons or behavioral health facilities, have infection control–related pathogens similar to those in the other settings previously described. The infectious diseases are more likely related to the activities of the persons within the facility. For example, *S. aureus* and MRSA are recovered from prisoners who practice illicit tattooing with nonsterile, shared equipment, whereas lice and hepatitis C are more frequently seen in behavioral health settings because of the community source of the clients and their intimate contact with blood and body fluids.

OUTBREAK INVESTIGATION

When numbers of isolates or infection rates increase above the baseline, or when an isolate of a rare or potential bioterrorism agent is recovered, an outbreak may have occurred. The microbiology laboratory may be the first to recognize the event and will likely participate in the **outbreak investigation.**

Local

In a given setting, an outbreak may be suspected when an unanticipated increase in infections occurs. Figure 3-1 shows infection rates that might trigger an outbreak investigation. In that figure, the baseline infection rate is seen for January through August as 3.3. In addition, the NHSN benchmark of 5.0 is also plotted. In September, the infection rate rose above the baseline and the NHSN benchmark. This might indicate that an outbreak has taken place, or the change in the rate of infections may have been due to other reasons. A more detailed investigation would reveal more.

As represented by the Case in Point at the beginning of this chapter, an outbreak may also involve the unexpected isolation of a microorganism. During the investigation and introduction of infection control interventions, an epidemiology curve is created. Figure 3-2 shows the **epidemiologic curve**

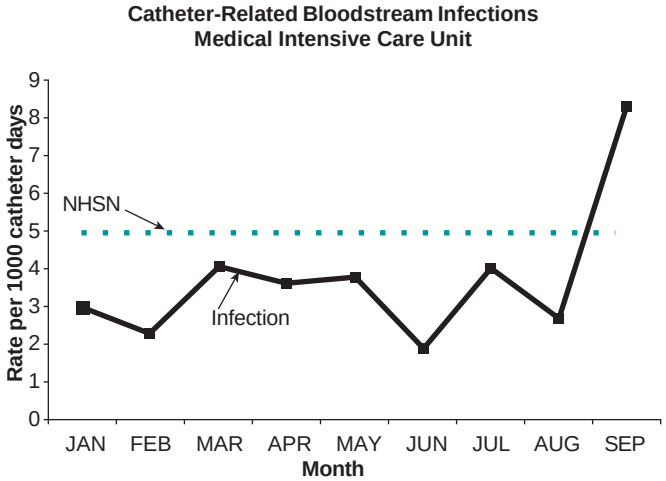

FIGURE 3-1 Plot of the rate of central line-associated bloodstream infections in a medical intensive care unit. The figure shows the National Healthcare Safety Network (NHSN) benchmark and the calculated monthly infection rate. The increase in the rate above the baseline and above the NHSN benchmark in September would trigger an investigation of a possible outbreak.

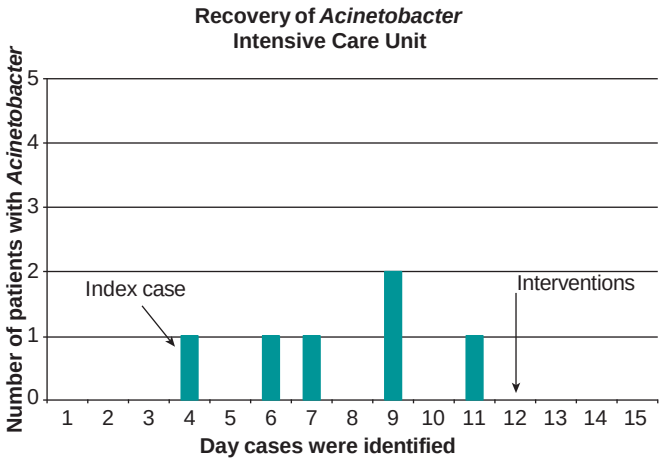

FIGURE 3-2 An epidemiologic curve of the occurrence of *Acinetobacter* spp. in respiratory specimens in an intensive care unit. Data were gathered as the result of a suspected outbreak. The graph plots the number of patients with *Acinetobacter* spp. infection by the day on which the cases were identified. Both the index case and the point when infection control interventions were implemented are indicated on the graph.

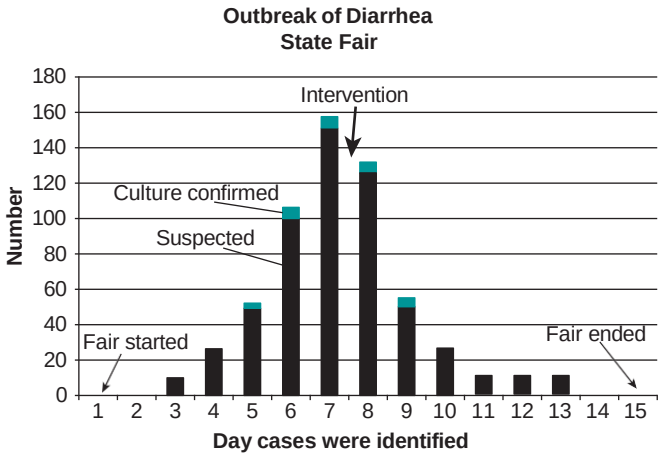

FIGURE 3-3 The epidemiologic curve of an outbreak investigation of diarrhea over a 15-day period at a state fair. The number of cases includes both suspected cases *(black bars)* and culture-proven cases *(colored bars)*. Infection control interventions were implemented on day 8 of the outbreak, and the number of cases decreased.

and the recovery of the *Acinetobacter* sp. The case first described on the fourth of the month is termed the **index case,** and it was to be determined whether the other infections that followed were related to that case. The investigation used the laboratory results provided by microbiologists.

Widespread

Widespread outbreaks occur outside the confines of a given health care setting. These outbreaks might be found in a statewide outbreak or a worldwide outbreak. An example of a statewide outbreak is the occurrence of diarrheal disease in persons attending a state fair. Figure 3-3 depicts an epidemiologic curve from such an outbreak. The outbreak involved reported cases in 565 fair attendees. A total of 47 cultures were obtained, and 26 of those cultures were positive for *Salmonella* spp. Contaminated well water at the fairgrounds was suspected as the source, and increased hand hygiene and use of bottled water were instituted 5 days after the first case was reported. After the interventions, the numbers of suspected cases decreased. Cases involved individuals from seven states and Canada. Some of the cases were in food handlers at the fair as well as fair attendees. Many suspected cases (more than 500) were reported but not cultured. The *Salmonella* spp. isolates recovered were all the same serogroup. Well water, food, and food preparation sites were cultured.

Another example involves the investigation of a potential measles outbreak involving an international flight from Russia to the United States. The patient involved in the suspected index case flew from Russia to London, where she changed planes for a flight to New York. She then changed planes in New York for a flight to Chicago, where she lived. The patient was a college student who visited her parents in Russia for the summer. While in Russia, she had contact with several young children who had measles. On her return flight to the United States, she complained of a fever but did not develop a rash until she had been home in Chicago for a day.

Imagine the complexity of an investigation of this potential outbreak. This scenario was recognized as a potential outbreak and not an actual outbreak when the investigation was undertaken. The recognition of measles by the microbiology laboratory set the investigation into motion, not the occurrence of any additional cases. Several issues must be examined. Did the student have measles? Most, but not all, people in the United States are immune. The people on the flight from Russia to London were the first exposed persons, followed by the people on the flight from New York to Chicago. Were they immune? Where did they go after their flight landed? How does the health department follow up with them? The incubation period for measles is approximately 10 days (8 to 13 days to the fever, 14 days to the rash). People are contagious before the development of the fever and the rash. Persons who are not immune are susceptible.

All three of these examples (*Acinetobacter* spp., *Salmonella* spp., measles) represent scenarios of potential infectious disease outbreaks. The investigation of these outbreaks depends heavily on the support of the microbiology laboratory to assist in ruling in or ruling out the infections and in identifying cases and sources. Laboratory support is described in detail later.

Steps of Outbreak Investigation

When an outbreak is suspected, steps are taken to investigate the event. The laboratory is integral in several of the steps. Table 3-4 lists the steps that are followed in an outbreak investigation. The first step is to establish a **case definition**. This step ensures that all of the remaining investigation is based on a single definition. This may involve the microbiology laboratory in the search for a specific pathogen or the recovery of several pathogens.

TABLE 3-4 Steps of an Outbreak Investigation

Steps	Description
1. Verify diagnosis of suspected cases	Establish a case definition.
2. Confirm that an outbreak exists	Be certain that all suspected cases meet the definition.
3. Find additional cases	Investigate to determine whether additional cases exist.
4. Characterize cases	Collect as much information as possible about the cases, including people, place, and time elements. Develop an epidemiologic curve.
5. Form hypothesis	Establish a "best guess" hypothesis about the outbreak.
6. Test hypothesis	Test the hypothesis with control groups and data collected.
7. Institute control measures	Implement intervention activities to control the outbreak.
8. Evaluate effectiveness of control measures	Determine whether the implemented activities have an impact on the outbreak. Does the number of cases diminish or disappear?
9. Communicate the findings	Document the investigation and communicate with all involved parties.

The second step is to confirm that an outbreak exists. One needs to be certain that all of the suspected cases match the definition and that there is more than an expected number of cases. At this point, the investigator seeks as much consultative assistance as possible. The laboratory is frequently asked for additional input. The third step is to find additional cases that may be added to the initial number of cases. Additional suspected cases may be discovered by more detailed investigation or by the new occurrence of cases. The laboratory may be asked to review microbiology data over a previous period of time to determine whether unrecognized cases have occurred.

The fourth step is to gather as much information as possible about the cases with respect to person, place, and time. An epidemiologic curve may be drawn to assist in the visualization of the outbreak numbers over time.

The fifth and sixth steps are to form a hypothesis about the event and then to test that hypothesis. In the fifth step, a tentative hypothesis is established as a "best guess" of what are the likely reservoir, source, and means of transmission. In testing that hypothesis, a control group is established; then the event is compared in both the incident and the control groups. Again, the microbiology laboratory may provide insight into the hypothesis and the relationship to the control group.

The seventh step in the investigation may actually occur at any point along the investigation time line. The establishment of interventions to stop the outbreak probably results from the initial recognition and the heightened awareness of a problem. The formal steps of the intervention process may not be developed until after the hypothesis is developed and tested. Undoubtedly, interventions of some type (e.g., increased hand hygiene) are introduced early in the investigation. The eighth step, which comes after the development of interventional strategies, is to evaluate the effectiveness of the interventions. Did the outbreak cease or at least decrease in its intensity? Step nine, the final step and one of extreme and often overlooked importance, is to communicate the findings of the investigation. This communication step must include a written report that is kept on file and provided to all responsible individuals.

It is not unusual for an outbreak to end before all data are collected and analyzed. This is probably due to early intervention. An early end to the outbreak does not ameliorate the need for communication and a written report.

Investigation Support from the Laboratory

The microbiology laboratory plays a crucial role in providing investigative support both in an outbreak investigation and in the creation of routine surveillance information. The availability of culture reviews, which may result in the initiation of an outbreak investigation, was discussed earlier. Other types of laboratory support are often important as well.

Cultures and Serology

In an outbreak investigation and in the collection of routine surveillance data, the collection, processing, reporting, and reviewing of pertinent cultures becomes critical. In the *Salmonella* spp. outbreak at the state fair, consider the number of fecal specimens that the health department laboratory processed, although cultures from patients are only one component of the investigative activity. Cultures from other sites may provide additional significant information. Well water and food may have been cultured in addition to specimens from the patients with diarrhea and the food handlers. In the outbreak of infections owing to *Acinetobacter* spp., the laboratory may have cultured respiratory therapy equipment and water samples.

One of the major difficulties in a large outbreak (e.g., the state fair outbreak) is the reduced ability to collect and transport specimens from persons from out of state. Individuals may have cultures processed in their home state, but those results may be difficult to retrieve. In the state fair example, only a few of the affected individuals had cultures processed and results included in the investigation. In the example of 565 suspected cases, only 47 cultures were processed. Reasons for this low number of cultures include the following: (1) the diarrhea lasts 24 to 48 hours and people may not seek medical help, (2) people not from the immediate area may not know of the potential outbreak and the need to provide culture material, (3) people do not want to be bothered with the expense and the time spent to collect cultures, and (4) the specimens may be collected too late in the infection or not transported properly, so no organisms are recovered.

In addition to the actual culture results, the laboratory may be asked to determine the serologic relationship of the isolates. Were all the *Salmonella* spp. of the same serogroup?

What was the epidemiologic profile of the serogroup? Both the isolate identification and the serologic relatedness may be important determinants in the outbreak investigation.

Antibiograms

Antibiograms can often be used in the investigation of an outbreak. As discussed in Chapter 13, there are times when antibiograms must be viewed with suspicion. Although these laboratory figures are not always as precise as other results, they may give guidance to the microbial relatedness of the isolates. Table 3-5 demonstrates comparisons of two antibiograms of isolates with the antibiogram of the index case. If the isolates all had identical antibiograms (such as isolate #1) or if some of the isolates were quite different in their susceptibility patterns (isolate #2), the inclusion or exclusion from the case definitions might have been affected. For example, relatively susceptible *S. aureus* can be distinguished from MRSA in an outbreak of skin infections in prisoners.

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis is a strain typing technique that can be an important adjunct to epidemiologic investigations. In this methodology, electrophoretic separation of enzyme-digested chromosomal fragments of bacteria is accomplished. The patterns of the fragments are compared among strains of microbes recovered in a possible outbreak. Strains with dissimilar patterns would be determined to be unrelated. However, if the patterns are similar, the strains can be identified as possibly related. This potential relatedness is an additional epidemiologic tool that can be incorporated into the investigation of an outbreak.

Environmental Culturing

As part of an effective infection control program, the microbiology laboratory may be called upon to perform cultures of various environmental sites. Recommendations surrounding environmental infection control have been extensively discussed in a CDC document, *Guidelines for Environmental Control in Health Care Facilities.* The environment is rarely implicated in disease transmission except with immunosuppressed patients. Although **environmental cultures** are generally to be avoided, there are times when they become an important and often required element of an infection control program that impacts the microbiology laboratory.

Air

Most often, infections traced to air quality occur during construction activities in a health care setting. Because microbes in the air can be incriminated in health care–associated infections, cultures of the air can be a component of air quality investigations. Initially, an **infection control risk assessment** should be conducted to determine whether the air is a likely source of infectious particles. Such an assessment is necessary in construction activities and must be done before any decision to culture the air can be made. The CDC makes no recommendation regarding "routine microbiologic air sampling" before, during, or after construction. If a fungal infection, such as aspergillosis, occurs during or immediately after construction, an outbreak investigation may be initiated and control measures implemented. Such an investigation may involve collecting environmental samples (e.g., searching for sources of airborne fungi). High-volume air samplers are the preferred method for collection, although settle plates may also be used. The results of these microbiologic cultures must be reported to the infection control team and evaluated.

Water

Water is incriminated in outbreaks in many of the settings for which microbiology laboratories provide service. Outbreaks may occur in various environmental situations such as those associated with contaminated drinking water (e.g., hospitals, extended care facilities, prisons) or recreation water (e.g., swimming pools, whirlpools, lakes, streams). They may take place in homes, aboard a plane or ship, in a city or state, or in a foreign country. In the United States alone, 15 to 20 outbreaks owing to waterborne pathogens cause diarrheal illness and affect several thousand people annually. Other waterborne diseases include respiratory illnesses (e.g., legionellosis), hepatitis (hepatitis A or hepatitis E), skin infections (from *Pseudomonas* spp. or mycobacteria), and central nervous system infections (*Naegleria* spp.). Because of these infection control implications, the laboratory must be prepared to offer diagnostic services or recommend laboratories that do offer those services for waterborne pathogens.

TABLE 3-5 Comparison of Antibiograms from Microbial Isolates

Antimicrobial Agent	Index Case	Isolate #1	Isolate #2
Ampicillin-sulbactam	R	R	S
Piperacillin	R	R	S
Cefepime	R	R	S
Imipenem	S	S	S
Ciprofloxacin	R	R	S
Gentamicin	R	R	S
Tobramycin	R	R	R
Amikacin	R	R	I

R, Resistant; *S*, sensitive; *I*, intermediate.

TABLE 3-6 Pathogens Related to Waterborne Infections

Viruses	Bacteria	Parasites
Noroviruses	*Salmonella* spp.	*Entamoeba histolytica*
Rotavirus	*Campylobacter* spp.	*Giardia enterolitica*
Hepatitis A	*Yersinia lamblia*	*Cryptosporidium* spp.
Hepatitis E	*Escherichia coli* (O157:H7)	Amoeba (*Naegleria* spp.)
	Legionella spp.	
	Pseudomonas spp.	
	Mycobacterium spp.	
	Aeromonas spp.	

Table 3-6 lists examples of waterborne pathogens. Some of the waterborne agents can be recovered by routine microbiology procedures such as cultures, but others may require specialized techniques. When asked to do environmental cultures of water, the microbiology laboratory must determine what specific pathogens are sought, if that information is known. If legionellosis is suspected, for example, the laboratory needs to have standard procedures for recovering this microbe. If the outbreak involves diarrheal diseases due to unknown etiology or etiologies, the recovery techniques must be broader and may require the use of a specialized laboratory. Consultation with the ICPs involved in the outbreak investigation must be obtained before routine culturing of environmental water is undertaken.

In some settings, routine water cultures must be performed because of specific guidelines. For example, for chronic dialysis centers, the CDC recommendations include performing bacteriologic assays of water and dialysis fluids at least once a month using standard methods. The infection control program or the managers of the specific area of concern must be familiar with regulations and guidelines addressing water cultures. The laboratory must be aware of the standard methods to ensure that proper procedures and proper media are used. The laboratory technologist, the ICP, and the manager should maintain a close working relationship to ensure compliance with culturing requirements.

Surfaces

The culturing of environmental surfaces should be performed only under the combined direction of the laboratory and the infection control program. In an outbreak investigation, surface culturing would be needed; however, such cultures should not be routinely obtained. The laboratory must be consulted before environmental surface cultures are undertaken to ensure that proper procedures and media are used. The laboratory technologist should be instrumental in the interpretation of the results.

Reporting

The role of the microbiology laboratory does not stop with providing culture results. Depending on statutory requirements, the laboratory may also be responsible for the reporting of certain infectious diseases to public health jurisdictions. Other groups may expect reports as well, such as committees, persons managing specific programs, and the news media.

Reporting to Public Health

There is the requirement to report the identification or suspicion of certain infectious diseases to the local, state, or federal public health entities. As shown in Table 3-7, diseases designated as Class A1 are considered public health concerns and are to be reported as soon as they are suspected or identified. Some of these requirements are federally mandated by the CDC, whereas some may be designated by the state. It is imperative that the laboratory technologist knows what infectious diseases are reportable, to what agency they are to be reported, and in what time frame they are to be reported.

Reporting to Committees and Programs

Depending on the setting in which the microbiology laboratory serves, there may be expectations of reports of infectious diseases to committees within that setting. For example, in an acute care hospital, infection control committees may desire reports of various microbiologic information. Periodic antibiograms, lists of reportable diseases, pathogens recovered in certain hospital units, isolates recovered from certain sites, and blood culture contamination rates are examples of reports that might be requested.

In other settings, physicians may expect periodic updates including antibiograms and pathogen prevalence. They may expect these to be delineated by office practice, by physician, by site, or by patient type. The settings making these requests may be home health care, extended care, or communal living.

Schools and businesses may request updated reports especially related to outbreaks that affect their operation. These reports must be tempered by public health needs and individual confidentiality restrictions. Recently, insurance companies and community advocacy groups have expressed interest in knowing about infection control rates. Some states require periodic reporting of these rates. The microbiology laboratory may be involved in providing data for these reports.

TABLE 3-7 Examples of Reportable Diseases*

Class	Description	Examples
Class A1	Diseases of major public health concern—reported immediately on recognition of a case, a suspected case, or positive laboratory results	Anthrax, botulism (food-borne), diphtheria, plague, rabies, smallpox
Class A2	Diseases of public health concern needing timely response reported by the end of the next business day after the recognition of a case, a suspected case, or positive laboratory results	Encephalitis (viral), food-borne disease outbreaks, hepatitis A, Legionnaires' disease, pertussis, syphilis, tuberculosis, typhoid fever
Class A3	Diseases of significant public health concern—reported by the end of the work week	Brucellosis, giardiasis, hepatitis B, hepatitis C, Lyme disease, Rocky Mountain spotted fever, trichinosis
Class B	Diseases reported only by number of cases—reported by the end of the work week	Chickenpox, influenza
Class C	Report of outbreak, unusual incidence, or epidemic—reported by the end of next working day	Blastomycosis, histoplasmosis, scabies, staphylococcal skin infections, toxoplasmosis

*These are reporting regulations from the Ohio Administrative Code and may vary from state to state.

Reporting to the Media

Among the activities of a microbiology laboratory, discussing microbiology and infection control activities with the media (e.g., television, radio, newspapers) may become necessary. Media relations for the laboratory technologist should be discussed with the risk management area associated with the laboratory. Media relations represent an educational opportunity that might be investigated before the laboratory technologist speaks to media personnel. One must balance the public's need for knowledge as perceived by the news media with privacy restraints for the patient, the laboratory, and the setting.

EDUCATION

Technologists and ICPs

The role of the microbiology laboratory in education is a further extension of its activities related to infection control. The laboratory technologists must not only keep themselves educated in their contribution to the infection control team, but also keep the infection control personnel educated regarding the laboratory's contribution to the team. Seminars, scientific articles and books, computer-based learning, and discussion with the infection control personnel are ways in which technologists can maintain their knowledge of infection control and laboratory techniques that can aid the infection control program. The technologist should continuously educate the ICP regarding the abilities of the laboratory in contributing meaningful information to the infection control program. As new techniques become available or old techniques are replaced, the technologist needs to relay that information to the ICP.

Similar knowledge needs to be provided for other people associated with the health care setting and the infection control program. Ancillary personnel, such as housekeeping and maintenance personnel, benefit from knowing the laboratory perspective of an infection control program. What cleaning and disinfecting agents work against viruses? How long does the environment need to be in contact with the agent to kill the virus? When do the maintenance personnel need to worry about fungi? What does mold growing on wet drywall look like? These and other questions arise among ancillary health care personnel to which the laboratory technologist needs to be prepared to respond.

Consultation with the laboratory technologist often is sought when construction is anticipated. Sometimes that education needs to be provided even when it is not sought. What microbes may be harbored in standing water? When should high-efficiency particulate air (HEPA) filters be used? What might spread through a facility if proper barriers are not used to control demolition dust and debris? These are samples of questions that might be addressed by the microbiology laboratory technologist while acting as a consultant to the infection control program.

Safety

The infection control program impacts the microbiology laboratory by emphasizing the need for laboratory safety. As discussed in Chapter 4, Section C, Microbiology Safety, safety within the microbiology laboratory embraces the infection control program. Hand hygiene is a critical part of laboratory safety. Hand hygiene involves handwashing when hands are soiled or the use of alcohol hand rubs when hands are not soiled. The practice of **Standard Precautions** further extends infection control to the microbiology laboratory. Gloves are always to be worn when handling blood or body fluids. Proper disposal of microbiologic waste, according to state or local regulations and national guidelines, is another critical component of the infection control program in the microbiology laboratory. Receiving available vaccines (for hepatitis B, chickenpox, influenza) is one step to protect susceptible laboratory personnel. All of these functions connect the microbiology laboratory safety program and the infection control program.

EMERGING AND REEMERGING PATHOGENS

With the advent of terroristic activities in the world, the microbiology laboratory has become an integral part of that area of the infection control program. Whether dealing with emerging diseases, such as SARS, or reemerging diseases, such as smallpox and anthrax, the laboratory must stay closely aligned with the infection control activities in the setting that the laboratory serves. Box 3-1 lists examples of **emerging pathogens** and **reemerging pathogens.**

Emerging Pathogens

Sometimes infectious agents that have not been previously recognized appear. Agents such as the West Nile virus, the coronavirus associated with SARS, and the avian influenza virus are three examples of emerging diseases. The laboratory must not only quickly learn how to identify these agents in human infections but also collaborate with the infection control program in dealing with the agents in the human population. How are these microbes spread? What reservoirs may harbor them? What is their incubation period? What antimicrobial agents can successfully treat them? How can the environment be disinfected? These are questions that the

BOX 3-1 Examples of Emerging and Reemerging Pathogens

Emerging Pathogens
- Avian influenza virus
- Coronavirus (SARS)
- Viral hemorrhagic fever viruses
- West Nile virus

Reemerging Pathogens
- Anthrax *(Bacillus anthracis)*
- Botulism *(Clostridium botulinum)*
- Plague *(Yersinia pestis)*
- Smallpox virus
- Tularemia *(Francisella tularensis)*

infection control program must ask. The laboratory technologist, the ICP, and the infectious disease physicians all must combine their knowledge to address these issues. The educational role of the laboratory technologist once again becomes evident in teaching specimen collection, specimen transport, disinfection, and safety methods.

Reemerging Pathogens

The reemergence or potential reemergence of pathogens once thought to be eliminated demands collaboration with the infection control program. Smallpox, anthrax, and plague are examples of agents that have been learned about but have not been identified by the average microbiology laboratory in many years. The laboratory technologist must relearn information once thought to be out of date. Media selection, identification techniques, and safety precautions must all be reexamined and implemented. Interaction with the infection control program strengthens the establishment of prevention and control strategies.

Response Plans

With the potential use of biologic agents in terrorism, the development of **emergency response plans** is paramount. The microbiology laboratory is an integral part of that infection control activity. Safety, specimen collection, agent identification, and agent control are components of the response plan to which the laboratory can contribute its input.

Whether in everyday activities or in activities in response to an outbreak or emerging diseases, the microbiology laboratory and the laboratory technologist constitute a critical component of an efficient and successful infection control program. The lessons of the chapter must be carefully learned and implemented.

BIBLIOGRAPHY

Arias KM: *Quick reference to outbreak investigation and control in health care facilities*, Gaithersburg, Md, 2000, Aspen Publishers.

Association for the Advancement of Medical Instrumentation: *Water treatment equipment for hemodialysis applications*, ANSI/AAMI RD62-2001, Arlington, Va, 2001, American National Standards Institute.

Carrico R et al: *APIC text of infection control and epidemiology*, ed 2, Washington, DC, 2005, Association for Professionals in Infection Control and Epidemiology.

Emori TG, Gaynes RP: An overview of nosocomial infections, including the role of the microbiology laboratory, *Clin Microbiol Rev* 6:428, 1993.

Garner JS et al: CDC definitions for nosocomial infections, *Am J Infect Cont* 16:28, 1988.

Guidelines for environmental infection control in health-care facilities, recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC), *MMWR Morbid Mortal Wkly Rep* 52(RR-10), 2000.

Guidelines for hand hygiene in health-care settings, recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene task force, *MMWR Morbid Mortal Wkly Rep* 51(RR-16), 2002.

Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings, June 2007. Available at: www.cdc.gov/ncidod/dhgp/pdf/isolation 2007.pdf.

National Healthcare Safety Network (NHSN) Report, data summary for 2006, issued June 2007, *Am J Infect Cont* 35:290-301, 2007.

Schiffman RB et al: Blood culture contamination: a College of American Pathologists Q-Probe study involving 640 institutions and 497,134 specimens from adults, *Arch Pathol Lab Med* 122:216, 1998.

Points to Remember

- The microbiology laboratory interacts with the infection control program in many different health care settings.
- Surveillance is important to establish baseline data and to recognize the need to investigate potential outbreaks.
- The microbiology laboratory supports outbreak investigations by providing consultative services including epidemiologic correlation of isolates.
- Although infrequently performed, environmental cultures may play a role in outbreak investigation.
- Microbiology technologists must recognize their role in providing reports to health departments, to committees, to the infection control program, and, on occasion, to the public.
- The microbiology laboratory must maintain competencies in new techniques.
- In anticipation of potential bioterrorism events, the microbiology laboratory must be equipped to participate in emergency preparedness programs.

Learning Assessment Questions

1. Surveillance is defined as:
   a. The systematic collection and analysis of data
   b. The review of health care–associated infections in laboratory personnel
   c. The recognition of emerging pathogens
   d. The development of an infection control risk assessment
2. Microbes commonly encountered in health care–associated infections in hospitals are:
   a. *Salmonella* spp., *Shigella* spp., hepatitis C, *Neisseria meningitides*
   b. *Staphylococcus aureus, Pseudomonas aeruginosa,* MRSA, *Escherichia coli*
   c. *Pseudomonas aeruginosa,* hepatitis C, lice, *Giardia* spp.
   d. All of the above
3. Pulsed-field gel electrophoresis might be performed to:
   a. Identify staphylococcal species
   b. Assist in an outbreak investigation
   c. Develop a new isolation precaution
   d. All of the above

4. The occurrence of surgical site infections (SSIs) is generally calculated as:
   a. A rate of infections in 1000 device-related events
   b. A percent of infections in 100 device-related events
   c. A percent of infections in surgical sites or procedures
   d. All of the above
5. Health departments frequently require the reporting by the laboratory of:
   a. Diseases of major health concerns (e.g., smallpox)
   b. Diseases needing timely response (e.g., food-borne outbreaks)
   c. Outbreaks of public health concern (e.g., scabies)
   d. All of the above
6. Microbial pathogens of potential bioterrorism activity include:
   a. *Bacillus anthracis, Staphylococcus aureus,* West Nile virus
   b. *Yersinia pestis, Staphylococcus aureus,* hepatitis C
   c. *Bacillus anthracis, Yersinia pestis, Francisella tularensis*
   d. *Bacillus anthracis, Escherichia coli,* coronaviruses, *Giardia* spp.
7. Environmental cultures are usually to be avoided except in:
   a. An outbreak investigation
   b. The occurrence of infections following construction
   c. Compliance with specific regulatory requirements
   d. All of the above
8. The formal steps in an outbreak investigation include:
   a. Establishing a case definition and culturing air and water
   b. Establishing a case definition, forming and testing a hypothesis, and communicating findings
   c. Forming and testing a hypothesis, performing PFGE, and calculating an infection rate
   d. Confirming an outbreak exists, calculating an infection rate, and performing serology and culture tests
9. Infection control programs rely on microbiology laboratory support in:
   a. Public health settings
   b. Acute care facilities
   c. Home care settings
   d. All of the above
10. The microbiology laboratory interacts with the infection control program by providing:
    a. Culture results
    b. Antibiograms and pathogen prevalence reports
    c. Environmental cultures when appropriate
    d. All of the above