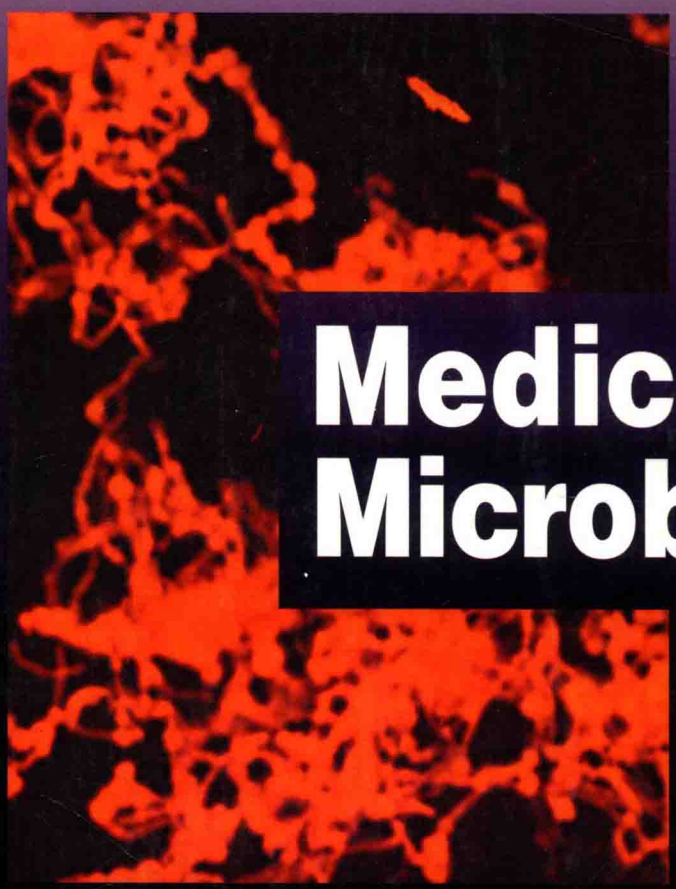

Medical Microbiology

FOURTH EDITION

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Medical Microbiology

To all who use
this textbook,
that they may benefit
from its use
as much as we did
in the preparation.

Preface

Medical microbiology can be a bewildering field to the novice. The student is faced with many questions when learning microbiology. How do I learn all the names? Which infectious agents cause which diseases? Why? When? Who is at risk? Is there a treatment? However, all these concerns can be reduced to one essential question: What information do I need to know which will help me understand how to diagnose and treat an infected patient?

Certainly, there are a number of theories about what a student needs to know and how to teach it, which supposedly validates the plethora of microbiology textbooks that have flooded the bookstores in recent years. Although we do not claim to have the one right approach to teaching medical microbiology (there is truly no one perfect approach to medical education), we have founded the revisions of this textbook on our experience gained through years of teaching medical students, residents, and infectious disease fellows as well as on the work devoted to the three previous editions. We have tried to present the basic concepts of medical microbiology clearly and succinctly in a manner that addresses different types of learners. The text is written in a straightforward manner with, it is hoped, uncomplicated explanations of difficult concepts. Details are summarized in tabular format rather than in lengthy text, and there are colorful illustrations for the visual learner. Important points are emphasized in boxes to aid the student, especially in their review; and the study questions address relevant aspects of each chapter, including clinical cases.

The material included in this text—and maybe more important, the material excluded—can be a subject for debate, but we have used our perception of the practical needs of the student as our guide. We are faced with the dilemma that new and exciting discoveries add not only to our foundation of knowledge but can also add to the length of the book. We used our experience as authors and teachers to choose the most important information and explanations for inclusion in this textbook. Each chapter has been carefully updated and expanded to include new, medically relevant discoveries. In each of these chapters, we have attempted to present the material that we feel will help the student gain a clear understanding of the significance of the individual microbes and their diseases.

To the Student

How can the student digest what appear to be innumerable facts? On first impression, success in medical microbiology would seem to depend on memorization. Although memorization is an important part of any medical discipline, understanding the basic principles and developing a system for storing this information plays an important role in mastering this science. We suggest that the student concentrate on learning what is important by **thinking like a physician.** Continue to ask seven basic questions as you approach this material: Who? Where? When? Why? Which? What? and How? For example: Who is at risk for disease? Where does this organism cause infections (both body site and geographic area)? When is isolation of this organism important? Why is this organism able to cause disease? Which species and genera are medically important? What diagnostic tests should be performed? How is this infection managed? Each organism that is encountered can be systematically examined. Know the specifics about how the organism grows, the virulence properties of the organism, and the diseases it causes; understand the epidemiology of infections; know what specimens should be collected and the basic identification tests that should be performed; and be familiar with preventive and therapeutic strategies. Learn three to five words or phrases that are associated with the microbe—words that will stimulate your memory **(trigger words)** and organize the diverse facts into a logical picture. Develop **alternative associations.** For example, this textbook presents organisms in the systematic taxonomic structure (frequently called a "bug parade," but which the authors think is the easiest way to introduce the organisms). Take a given virulence property (e.g., toxin production) or type of disease (e.g., meningitis) and list the organisms that share this property. Pretend that an imaginary patient is infected with a specific agent and create the case history. In other words, do not simply attempt to memorize page after page of facts; rather, use techniques that stimulate your mind and challenge your understanding of the facts presented throughout the text.

No textbook of this magnitude would be successful without the contributions of numerous individuals. We

are grateful for the valuable professional help and support provided by the staff at Harcourt Health Sciences, particularly William Schmitt, Antony Galbraith, Linda Grigg, and Peter Faber. We also want to thank the many students and professional colleagues who have offered their advice and constructive criticism throughout the development of this fourth edition of *Medical Microbiology*.

Patrick R. Murray
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Contents

CHAPTER 1

Introduction to Medical Microbiology

Since the last edition of this textbook, wondrous discoveries in outer space have been made—new planets, galaxies, black holes, and unimaginable details of our sun and the neighboring planets. On our planet Earth, we have seen the escalation of wars, the discovery of peace, and the human struggles to understand our neighbors' needs. We have also discovered new pathogens and their diseases, old pathogens causing new diseases, and the increased threat of biologic terrorism. Diseases such as smallpox, anthrax, and brucellosis have taken on renewed interest. Finally, the antibiotics that were so effective in the past are now impotent against some very common and important pathogens because these drugs have been freely prescribed to humans and farm animals alike. Thus, we find that the science of microbiology is in dynamic flux—an intellectually satisfying but socially alarming development.

The Microbial World

Imagine the excitement felt by the Dutch biologist Anton van Leeuwenhoek in 1674 as he peered through his carefully ground microscopic lenses at a drop of water and discovered a world of millions of tiny "animalcules." Almost 100 years later the Danish biologist Otto Müller extended van Leeuwenhoek's studies and organized bacteria into genera and species according to the classification methods of Linnaeus. This was the beginning of the taxonomic classification of microbes. In 1840, the German pathologist Friedrich Henle proposed criteria for proving that microorganisms were responsible for causing human disease (the "germ theory" of disease). Koch and Pasteur confirmed this theory in the 1870s and 1880s with a series of elegant experiments proving that microorganisms were responsible for causing anthrax, rabies, plague, cholera, and tuberculosis. Other brilliant scientists went on to prove that a diverse collection of microbes was responsible for causing human disease. The era of chemotherapy was begun in 1910 when the German chemist Paul Ehrlich discovered the first antibacterial agent, a compound effective against the spirochete that causes syphilis. This was followed by Alexander Fleming's discovery of penicillin in 1928, Gerhard Domagk's discovery of sulfanilamide in 1935, and Selman Waksman's discovery of streptomycin in 1943. In 1946, the American microbiologist John Enders was the first to cultivate viruses in cell cultures, leading the way to the large-scale production of virus cultures for vaccine development. Thousands of scientists have followed these pioneers, each building on the foundation established by his or her predecessors, and each adding an observation that expanded our understanding of microbes and their role in disease.

The world that van Leeuwenhoek discovered was complex, consisting of protozoa and bacteria of all shapes and sizes. However, the complexity of medical microbiology we know today rivals the limits of the imagination. We now know that there are thousands of different types of microbes that live in, on, and around us—and hundreds that cause serious human diseases. To understand this information and organize it in a useful manner, it is important to understand some of the basic aspects of medical microbiology. To start, the microbes can be subdivided into four groups: viruses, bacteria, fungi, and parasites, each having its own level of complexity.

Viruses

Viruses are the smallest infectious particles, ranging in diameter from 18 to almost 300 nm (particles less than 200 nm cannot be seen with a light microscope). More than 40 genera of viruses have been implicated in human disease, and, certainly, more will be discovered each year. Viruses consist of either DNA or RNA (but not both) and proteins required for replication and pathogenesis. These components are then enclosed in a protein coat with or without a lipid membrane coat. These organisms are true parasites, requiring host cells for replication. The cells they infect and the outcome of the infection dictate the nature of the clinical manifestation. Infection can lead either to rapid replication

of the organisms and destruction of the cell or to a long-term latent relationship with possible integration of the viral genetic information into the host genome. The factors that determine which of these takes place are only partially understood. For example, infection with the human immunodeficiency virus, the etiologic agent of the acquired immunodeficiency syndrome (AIDS), can result in the latent infection of CD4 lymphocytes or the active replication and destruction of these immunologically important cells. Likewise, infection can spread to other susceptible cells, such as the microglial cells of the brain, resulting in the neurologic manifestations of AIDS. Thus, the diseases caused by viruses can range from the common cold to gastroenteritis to fatal catastrophes such as rabies, smallpox, and AIDS.

Bacteria

Bacteria are relatively simple in structure. They are **prokaryotic** organisms—a simple unicellular organism with no nuclear membrane, mitochondria, Golgi bodies, or endoplasmic reticulum that reproduces by asexual division. Although the cell wall encircling bacteria is itself complex, there are two basic forms: a gram-positive cell wall with a thick peptidoglycan layer and a gram-negative cell wall with a thin peptidoglycan layer and an overlying outer membrane (additional information about this structure is presented in Chapter 3). Some bacteria lack this cell wall structure and compensate by surviving only inside host cells or in a hypertonic environment. The size (1 to 20 μm or longer), shape (spheres, rods, spirals), and spatial arrangement (single cells, chains, clusters) of the cells are used for the preliminary classification of bacteria, and the phenotypic and genotypic properties of the bacteria form the basis for the definitive classification. The human body is inhabited by thousands of different bacterial species—some living transiently, others in a permanent parasitic relationship. Likewise, the environment that surrounds us, including the air we breathe, water we drink, and food we eat, is inhabited by bacteria, many of which are relatively avirulent and some of which are capable of producing life-threatening disease.

Fungi

In contrast to bacteria, the cellular structure of fungi is more complex. These are **eukaryotic** organisms that contain a well-defined nucleus, mitochondria, Golgi bodies, and endoplasmic reticulum. Fungi can exist either in a unicellular form (**yeast**) that can replicate asexually or in a filamentous form (**mold**) that can replicate asexually and sexually. Most fungi exist as either yeasts or molds; some can assume either morphology, however. These are known as **dimorphic** fungi and consist of such organisms as *Histoplasma*, *Blastomyces*, and *Coccidioides*.

Parasites

Parasites are the most complex microbes. Although all parasites are classified as eukaryotic, some are unicellular and others are multicellular. They range in size from tiny protozoa as small as 1 to 2 μm in diameter (the size of many bacteria) to arthropods and tapeworms that can measure up to 10 m in length. Indeed, considering the size of some of these parasites, it is hard to imagine how these organisms came to be classified as microbes. Their life cycles are equally complex, with some parasites establishing a permanent relationship with humans and others going through a series of developmental stages in a progression of animal hosts. One of the difficulties confronting students is not only an understanding of the spectrum of diseases caused by parasites, but also an appreciation of the epidemiology of these infections, which is vital for an understanding of the control and prevention of these infections.

Microbial Disease

One of the most important reasons for studying microbes is to understand the diseases they cause and the ways to control them. Unfortunately, the relationship between many organisms and their diseases is not simple. Specifically, organisms rarely cause a single well-defined disease, although there are certainly ones that do (e.g., *Treponema pallidum*, syphilis; poliovirus, polio; *Plasmodium* species, malaria). Instead, it is more common for a particular organism to produce many manifestations of disease (e.g., *Staphylococcus aureus*—endocarditis, pneumonia, wound infections, food poisoning) or for many organisms to produce the same disease (e.g., meningitis caused by viruses, bacteria, fungi, and parasites). In addition, relatively few organisms can be classified as always pathogenic, though some do belong in this category (e.g., rabies virus, *Brucella* species, *Sporothrix schenckii*, *Plasmodium* species). Instead, most are able to establish disease only under well-defined circumstances (e.g., the introduction of an organism with a potential for causing disease into a normally sterile site, such as the brain, lungs, and peritoneal cavity). Some diseases arise when a person is exposed to organisms from external sources. These are known as **exogenous infections**, and examples include diseases caused by influenza virus, *Neisseria gonorrhoeae*, *Coccidioides immitis*, and *Entamoeba histolytica*. Most human diseases, however, are produced by organisms in the person's own microbial flora that spread to body sites where disease can ensue (**endogenous infections**).

The interaction between an organism and the hu-

man host is complex. The interaction can result in transient colonization, a long-term symbiotic relationship, or disease. The outcome of this interaction is determined by the virulence of the organism, the site of exposure, and the host's ability to respond to the organism. Thus, the manifestations of disease can range from mild symptoms to organ failure and death. The role of microbial virulence and the host's immunologic response is discussed in depth in subsequent chapters.

Although the human body is remarkably adapted to controlling exposure to pathogenic microbes, the physical barriers that prevent invasion by the organism and the immunologic response to infection are frequently inadequate. To improve the human body's ability to prevent infection, the immune system can be augmented either through the passive transfer of antibodies present in immune globulin preparations or through active immunization with microbial antigens. Infections can also be controlled with a variety of chemotherapeutic agents. Unfortunately, microbes can alter their antigenic complexion (**antigenic variation**) or develop resistance to even the most potent antibiotics. Thus, the battle for control between microbe and host continues, with neither side yet able to claim victory (although the microbes have demonstrated remarkable ingenuity).

Diagnostic Microbiology

The clinical microbiology laboratory plays an important role in the diagnosis and control of infectious diseases. However, the ability of the laboratory to perform these functions is limited by the quality of the specimen collected from the patient, the means by which it is transported from the patient to the laboratory, and the techniques used to demonstrate the microbe in the sample. Because most diagnostic tests are based on the ability of the organism to grow, transport conditions must ensure the viability of the pathogen. In addition, the most sophisticated testing protocols are of little value if the collected specimen is not representative of the site of infection. This seems obvious, but many specimens sent to laboratories for analysis are contaminated during collection with the organisms that colonize the mucosal surfaces. Because most infections are caused by endogenous organisms, it is virtually impossible to interpret the testing results with contaminated specimens.

The laboratory is also able to determine the antimicrobial activity of selected chemotherapeutic agents, although the value of these tests is limited. The laboratory must test only organisms capable of producing disease and only medically relevant antimicrobials. To test all isolated organisms or an indiscriminate selection of drugs can yield misleading results, with potentially dangerous consequences. Not only can a patient be treated inappropriately with unnecessary antibiotics, but the true pathogenic organism may not be recognized among the plethora of organisms isolated and tested. Finally, the in vitro determination of an organism's susceptibility to a variety of antibiotics is only one aspect of a complex picture. The virulence of the organism, site of infection, and patient's ability to respond to the infection influence the host-parasite interaction and must also be considered when planning treatment.

Summary

It is important to realize that, as was stated in the introduction of this chapter, our knowledge of the microbial world is evolving continually. Just as the early microbiologists built their discoveries on the foundations established by their predecessors, so, too, will we and future generations continue to discover new microbes, new diseases, and new therapies. The following chapters are intended as a foundation of knowledge that can be used to enrich your understanding of microbes and their diseases.

Basic Principles of Medical Microbiology

CHAPTER 2

Bacterial Classification

Understanding the relevance and complex nomenclature of literally hundreds of "important" bacteria can be challenging. The mastery of this exercise depends on the systematic organization of the bewildering array of different organisms into logical relationships (i.e., the taxonomic classification of the organisms).

Phenotypic Classification

The **microscopic** and **macroscopic morphologies** of bacteria were the first characteristics used to identify bacteria and form the cornerstones for most identification algorithms used today (Box 2–1). For example, bacteria can be classified by their ability to retain the Gram stain (gram-positive or gram-negative) and by the shape of the individual organisms (cocci, bacilli, curved, or spiral). The macroscopic appearance of colonies of bacteria can also be used to identify bacteria (e.g., hemolytic properties on agar containing blood, pigmentation of the colonies, size and shape of the colonies). Thus, *Streptococcus pyogenes* is a gram-positive bacterium that forms long chains of cocci and appears as small, white, hemolytic colonies on blood agar plates. Because many organisms can appear very similar on microscopic and macroscopic examination, morphologic characteristics are used to provide a tentative identification of the organism and to select more discriminating classification methods.

The most common methods that are still used to identify bacteria consist of measuring the presence or absence of specific biochemical markers (e.g., ability to ferment specific carbohydrates or use different compounds as a source of carbon for growth; presence of specific proteases, lipases, or nucleases; presence of various aminopeptidases). With the use of carefully selected biochemical tests, most clinically significant isolates can be identified with a high degree of precision. These methods have also been used for subdividing groups of organisms beyond the species level, primarily for epidemiologic purposes (e.g., to determine whether a group of organisms from the same genus and species is from a common source or from distinct sources). These techniques are referred to as **biotyping**.

Many bacteria possess antigens that are unique, and antibodies used to detect these antigens are powerful tools for their identification (**serotyping**). These serologic tests can be used to identify organisms that are inert in biochemical testing (e.g., *Francisella*, the organism that causes tularemia), difficult or impossible to grow (e.g., *Treponema pallidum*, the organism responsible for syphilis), associated with specific disease syndromes (e.g., *Escherichia coli* serotype O157, responsible for hemorrhagic colitis), or need to be identified rapidly (e.g., *S. pyogenes*, responsible for streptococcal pharyngitis). Serotyping is also used to subdivide bacteria below the species level for epidemiologic purposes.

Other examples of phenotypic methods used to classify bacteria include analysis of **antibiogram patterns** (patterns of susceptibility to different antibiotics) and **phage typing** (susceptibility to viruses that infect bacteria—bacteriophages). Assessment of antibiotic susceptibility patterns is commonly performed but has limited discriminatory power. Phage typing is technically cumbersome and has now been replaced by more sensitive genetic techniques.

Analytic Classification

Analysis of the analytic characteristics of bacteria has also been used to classify bacteria at the genus, species, or subspecies level (Box 2–2). The chromatographic

BOX 2–1. Phenotypic Classification of Bacteria

Microscopic morphology
Macroscopic morphology
Biotyping
Serotyping
Antibiogram patterns
Phage typing

BOX 2–2. Analytic Classification of Bacteria

Cell wall fatty acid analysis
Whole cell lipid analysis
Whole cell protein analysis
Multifocus locus enzyme electrophoresis

BOX 2–3. Genotypic Classification of Bacteria

Guanine plus cytosine ratio
DNA hybridization
Nucleic acid sequence analysis
Plasmid analysis
Ribotyping
Chromosomal DNA fragment

pattern of cell wall mycolic acids is unique for many of the individual species of mycobacteria and has been used for more than 25 years to identify the most commonly isolated species. Analysis of the lipids in the entire cell has also proved to be a useful method for characterizing many bacterial species, as well as yeasts. Analyses of the whole cell proteins and cellular enzymes **(multilocus enzyme electrophoresis)** are also techniques that have been used to characterize bacteria, most typically at the subspecies level for epidemiologic investigations. Although these analytic methods are accurate and reproducible, they are labor intensive, and the instrumentation is expensive. For these reasons the analyses are used primarily in reference laboratories.

Genotypic Classification

The most precise method for classifying bacteria is by analysis of their genetic material (Box 2–3). Organisms were initially classified by the **ratio of guanine to cytosine;** this procedure has largely been forsaken for more discriminating methods, however. **DNA hybridization** was used initially to determine the relationship among bacterial isolates (e.g., to determine whether two isolates were in the same genus or species). More recently, this technique has been exploited for the rapid identification of organisms by use of molecular probes. That is, DNA from an organism to be identified is extracted and exposed to species-specific molecular probes. If the probe binds to the DNA, then the organism's identity is confirmed. This technique has also been used to detect organisms directly in clinical specimens, thus avoiding the need to grow the organisms. DNA hybridization has proved to be a valuable tool for the rapid detection and identification of slow-growing organisms such as mycobacteria and fungi.

An extension of the hybridization method is **nucleic acid sequence analysis.** Probes are used to localize specific nucleic acid sequences that are unique to a genus, species, or subspecies. These sequences are amplified so that millions of copies are produced, and then the amplified genetic material is sequenced to define the precise identity of the isolate. This method primarily analyzes sequences of ribosomal DNA (because highly conserved [family- or genus-specific] sequences and highly variable [species- or subspecies-specific] sequences are present). It has also been used to define the evolutionary relationship among organisms and to identify organisms that are difficult or impossible to grow. Most of the recent changes in taxonomic nomenclature were determined by nucleic acid sequence analysis. An extension of this work is the complete sequencing of a bacterium's entire genome, a technique that has now become technically feasible.

Various other methods have been used, primarily to classify organisms at the subspecies level for epidemiologic investigations: **plasmid analysis, ribotyping,** and **analysis of chromosomal DNA fragments.** In recent years, the technical aspects of these methods have been simplified to the point that most clinical laboratories use variations of these methods in their day-to-day practice.

Boxes 2–4 through 2–8 provide a useful classification scheme for organizing the many bacteria that are discussed in subsequent chapters. It should be noted that the list of organisms is not exhaustive. Many genera that are recovered in clinical specimens are omitted for the sake of simplifying this presentation. The organisms that are included in these summary boxes are only those that are discussed in subsequent chapters. In addition, the precise arrangement of bacteria in families, genera, and species continues to change. In Section IV,

BOX 2–4. Aerobic, Gram-Positive Cocci

Catalase-Positive Cocci

Micrococcus
Staphylococcus
Stomatococcus

Catalase-Negative Cocci

Aerococcus
Alloiococcus
Enterococcus
Lactococcus
Leuconostoc
Pediococcus
Streptococcus