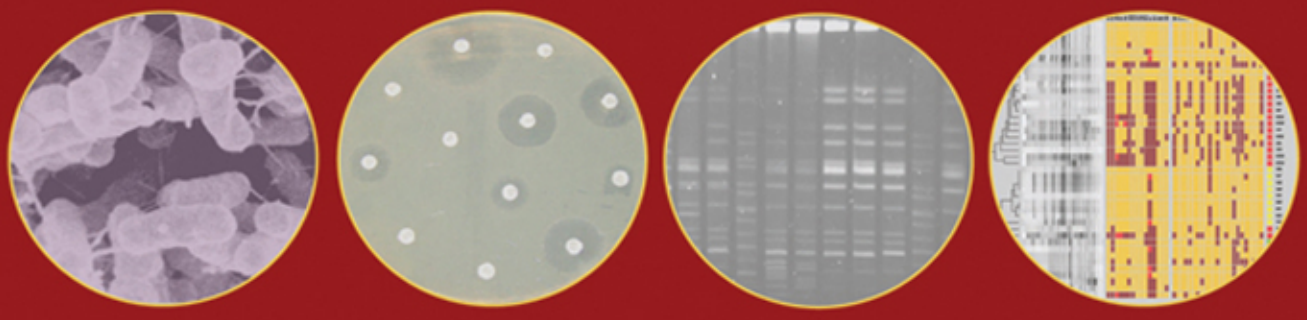
Molecular Techniques for the Study *of* Hospital Acquired Infection



Edited by
Steven L. Foley
Anne Y. Chen
Shabbir Simjee
Marcus J. Zervos

WILEY-BLACKWELL

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Steven L. Foley
National Farm Medicine Center, Marshfield Clinic Research Foundation, Marshfield, Wisconsin, USA

Anne Y. Chen
Division of Infectious Diseases, Henry Ford Hospital, Detroit, Michigan, USA

Shabbir Simjee
Elanco Animal Health, Eli Lilly and Company, Basingstoke, Hampshire, UK

Marcus J. Zervos
Division of Infectious Diseases, Henry Ford Hospital, Detroit, Michigan, USA

WILEY-BLACKWELL
A John Wiley & Sons, Inc., Publication

Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada

Library of Congress Cataloging-in-Publication Data:

ISBN: 978-0-470-42085-0

Printed in the United States of America

eISBN: 978-1-118-06382-8
oISBN: 978-1-118-06384-2
ePub ISBN: 978-1-118-06383-5

10 9 8 7 6 5 4 3 2 1

To Missy and our kids, Colin, Riley and Ava, and to my parents

Steven L. Foley

To my husband Merwin and our children, Brandon and Kathryn

Anne Y. Chen

To my mother Farida, wife Saida, and son Usman

Shabbir Simjee

To my wife Ellene and children Mary, John, and Tommy

Marcus J. Zervos

Contents

Contributors

Ngolela Esther Babady, M.D., Mayo Clinic, Rochester, Minnesota 55905

Faiqa Alam Cheema, M.D., Division of Infectious Diseases, Henry Ford Hospital, Detroit, Michigan 48202

Anne Y. Chen, M.D., Division of Infectious Diseases, Henry Ford Hospital, Detroit, Michigan 48202

Franklin R. Cockerill, M.D., Mayo Clinic, Rochester, Minnesota 55905

Louise-Marie Dembry, M.D., Division of Infectious Diseases, Yale University School of Medicine, New Haven, Connecticut 06519

Steven L. Foley, Ph.D., National Farm Medicine Center, Marshfield Clinic Research Foundation, Marshfield, Wisconsin 54449

Beilei Ge, Ph.D., Food Science Department, Louisiana State University, Baton Rouge, Louisiana 70803

Richard V. Goering, Ph.D., Department of Medical Microbiology and Immunology, Creighton University School of Medicine, Omaha, Nebraska 68178

Sonja Hansen, M.D., M.P.H., Institute of Hygiene and Environmental Medicine, Charité—University Medicine Berlin, Campus Benjamin Franklin, 12203 Berlin, Germany

Vanthida Huang, Phar M.D., Department of Pharmacy Practice, College of Pharmacy and Health Sciences, Mercer University, Atlanta, Georgia 30341

Aaron M. Lynne, Ph.D., Department of Biological Science, Sam Houston State University, Huntsville, Texas 77341

Robin Patel, M.D., Mayo Clinic, Rochester, Minnesota 55905

Eli N. Perencevich, M.D., Department of Internal Medicine, The University of Iowa, Iowa City, Iowa 52242

Hiren Pokharna, M.D., M.P.H., Division of Infectious Diseases, Henry Ford Hospital, Detroit, Michigan, 48202

Johann D. D. Pitout, M.D., Division of Microbiology, Calgary Laboratory Services, Calgary, Alberta, T2L 2K8, Canada

Carl M. Schroeder, Ph.D., Office of Public Health and Science, USDA Food Safety and Inspection Service, Washington, DC 20250

Sanjay K. Shukla, Ph.D., Clinical Research Center, Marshfield Clinic Research Foundation, Marshfield, Wisconsin 54449

Shabbir Simjee, Ph.D., Elanco Animal Health, Eli Lilly and Company Limited, Hampshire, RG24 9NL, United Kingdom

Mary E. Stemper, M.S., Division of Laboratory Medicine, Marshfield Clinic, Marshfield, Wisconsin 54449

Carlos Torres-Viera, M.D., Division of Infectious Diseases, Yale University School of Medicine, New Haven, Connecticut 06519

Jose A. Vazquez, M.D., Division of Infectious Diseases, Henry Ford Hospital, Detroit, Michigan 48202

Ralf-Peter Vonberg, M.D., Institute for Medical Microbiology and Hospital Epidemiology, Medical School Hannover, D-30625 Hannover, Germany

Marc-Oliver Wright, M.T. (ASCP), M.S., Department of Infection Control, Evanston Northwestern Healthcare, Evanston, Illinois 60201

Marcus J. Zervos, M.D., Wayne State University School of Medicine and Division of Infectious Diseases, Henry Ford Hospital, Detroit, Michigan 48202

Foreword

Nosocomial infections continue to be an issue of increasing importance, especially in light of the fact that many of these pathogens are becoming increasingly resistant to our effort to treat them, resulting in infections that have significant morbidity, mortality, and cost. With the emergence of strains with increasing levels of resistance to multiple antimicrobial agents, along with limited new drugs, we are faced with formidable diagnostic, prevention, and treatment challenges for nosocomial pathogens. Despite these challenges, our understanding of epidemiology, mechanisms for pathogen control, and measures for detection and characterization has also progressed.

Much has been written recently about the problems of healthcare-associated infections and the development of antimicrobial resistance in the causative organisms. *Techniques for the Study of Hospital-Acquired Infection* is, however, a unique and concise text providing state-of-the-art information. It provides the infection control practitioner, clinician, epidemiologist, and microbiologist a practical tool to understand the mechanism and implementation of a comprehensive program to study and control healthcare-associated infections.

The text of the book is divided into three parts. Part I is an introduction to healthcare-associated infections and their control, while Part II focuses on the techniques to characterize nosocomial pathogens; Part III examines the application of techniques to characterize predominant nosocomial pathogens focusing on representative Gram-positive, Gram-negative, and fungal pathogens.

The text is written by an internationally recognized team of contributors. Importantly, it is the clinical perspective that distinguishes this book from other publications, and it is the

coordination of infection control, laboratory methods, and exploration of clinical practices to control pathogens that will be of interest to readers and will help the healthcare practitioner develop improved strategies to minimize the impact of nosocomial infections on patients.

Barbara E. Murray, M.D.

J. Ralph Meadows Professor and Director,
Division of Infectious Diseases Houston, Texas
December 2010

Preface

Nosocomial infections are a significant cause of morbidity and mortality. Each year it is estimated that 2 million patients develop a healthcare-acquired infection in the United States. Put another way, this represents nearly 5% of all hospitalized patients, and these infections directly contribute to approximately 88,000 deaths and add an additional 4.5 billion dollars to healthcare costs. Because of the importance of these infections, we were invited to edit this book exploring the techniques for the study of hospital-acquired infections. The book is divided into three general parts: Part I is an introduction to healthcare-associated infections and their control, Part II discusses molecular techniques to characterize nosocomial pathogens, Part III describes the application of techniques to characterize predominant nosocomial pathogens focusing on representative Gram-positive, Gram-negative, and fungal pathogens.

Major themes that are examined in this book include the characteristics of healthcare settings that allow for the development and spread of nosocomial pathogens, the implementation of effective infection control programs, the epidemiological methods to study nosocomial disease, and the elimination of pathogens and the development of resistance to these treatments. These topics are followed up by sections that focus on the molecular techniques used to study hospital infections, as well as by an exploration of the characteristics of some of the major nosocomial pathogens that are currently plaguing healthcare settings in the context of best practices to deal with these pathogens.

Why is it important to examine the techniques for the study of hospital-acquired infections? There a number of good reasons; these include the fact that there are increasing numbers of healthcare-associated infections that are caused by multidrug-resistant pathogens, which, in addition to the infection-associated pathology, leads to difficulty in treating infected patients. These more commonly resistant pathogens include Gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), glycopeptide (vancomycin) intermediate, and resistant *S. aureus* and glycopeptide-resistant enterococci (VRE). Some of the primary concerns among the Gram-negatives include fluoroquinolones-resistant *Pseudomonas aeruginosa* and *Escherichia coli* and the extended spectrum beta-lactamase (ESBL)-producing strains of *E. coli* and *Klebsiella pneumoniae*. Because of their

importance in causing healthcare-associated infections, individual chapters have been dedicated to antimicrobial resistance, as well as to *S. aureus* and *E. coli.*

There are many known risk factors that associated with the development of nosocomial infections, including suffering from underlying conditions such as cancer, diabetes, or renal failure, prior antimicrobial therapy, the presence of indwelling catheters, surgical procedures, and having extended hospital stays. The examination of the transmission of pathogens from the healthcare environment and/or personnel to the patient is also very important to explore and as such has been explored thoroughly in the Part I of the book. The determination of the sources and relatedness among pathogens is important to understanding the epidemiology of nosocomial infections, which will aid development of rational pathogen control strategies. Many of the bacterial species that are prominent causes of nosocomial infections are also readily found as normal commensal organisms on the human body, including the staphylococci, enterococci, and *E. coli.* Because these commensal strains are not typically associated with human infection, it is important to be able to determine whether strain isolated from a patient is pathogenic and the likely source of the infection or part of the normal flora.

To assist in the determination of the strain type, infection control practitioners and molecular epidemiologists typically rely on pathogen subtyping to determine if strains are likely pathogens and to decipher whether isolates that are epidemiologically related are also genetically similar. Traditionally, much of the strain typing was done based on the phenotypic characteristics of isolates, including methods such as biochemical profiles, serotyping, phage typing, and antimicrobial susceptibility profiles. However, these techniques typically lack the specificity to more closely link isolates from patients to likely sources. Thus as molecular typing methods became more readily available, many hospitals and other healthcare organizations began to rely more on DNA-based technologies to characterize pathogens. These molecular methodologies include polymerase chain reaction (PCR)-based rapid identification and typing methods as well as restriction-enzyme-based methods such as pulsed-field gel electrophoresis (PFGE). Many of these methods are covered extensively in chapters in the Part II of the book. The molecular methods allow for a deeper assessment of strain interrelationship, which is important to establish genetic links that provide evidence which aids in determining the source of organisms that cause disease and distinguish them from commensal strains; this is important in the development of treatment strategies to minimize the health impact of the infections. Part III of this book explores some of the major groups of nosocomial pathogens with representative Gram-positive, Gram-negative, and fungal pathogens, examining their characteristics and methods for characterization and study of hospital-acquired infections caused by these organisms. We hope that you find this book helpful as you tackle the difficult problems associated with healthcare-associated infections.

Steven L. Foley

Anne Y. Chen

Shabbir Simjee

Marcus J. Zervos

PART ONE

Introduction to Healthcare-Associated Infections and their Control

CHAPTER ONE

The Hospital and Ambulatory Care Environment

HIREN POKHARNA AND ANNE Y. CHEN

Introduction

HEALTHCARE-ASSOCIATED INFECTIONS (HAIs): THE EVOLUTION

Although the modern-day concepts of prevention and control of hospital-associated infections originated in the middle of the nineteenth century, the history regarding knowledge about hospital-related infections dates back to the sixteenth century. Ambroise Pare (1517–1590), a surgeon at Hotel-Dieu in Paris, was one of the first physicians to describe increased frequency and severity of wound infections in hospitalized patients compared to nonhospitalized patients. The phrase "hospital disease" was first used in the eighteenth century. Hungarian physician Ignaz Philipp Semmelweiss (1818–1865) introduced the concept of hand washing while Sir Joseph Lister (1827–1912), a British surgeon, pioneered the concept of asepsis. Over the years, the Center for Disease Control and Prevention (CDC) has published several sets of definitions for Nosocomial Infections. Definitions used during the Comprehensive Hospital Infections Project (CHIP) (1969–1972) and in the National Nosocomial Infectious Study (NNIS) (1970–1974) were first used in the *Proceedings of the First International Conference on Nosocomial Infection* organized by CDC in 1970 (1). Definitions were further extended in 1974 for hospitals participating in NNIS (2). Definitions for nosocomial infections were again modified by CDC in 1988 (3). The term HAI (4) was officially introduced in 2008 to reflect infections

Molecular Techniques for the Study of Hospital-Acquired Infection, First Edition. Edited by Steven L. Foley, Anne Y. Chen, Shabbir Simjee, and Marcus J. Zervos.

acquired by patients while receiving treatment for any surgical or medical conditions. It was defined as a localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s) without any evidence that the infection was present or incubating at the time of admission to the acute care setting. HAIs can occur in acute care settings within hospitals or in ambulatory outpatient care settings, including same-day surgical centers or dialysis center. HAIs are increasingly associated with long-term facilities such as nursing homes and rehabilitation centers.

Healthcare-associated infections (HAIs) are a major cause of morbidity and mortality in the United States. It is estimated that there were 1.7 million HAI in 2002 which resulted in around 99,000 deaths, making it among the most common healthcare-associated adverse event (5). HAIs can occur in patients at any age and in any healthcare setting, but the most common infections are seen among adults and children in the non-ICU setting. According to National Nosocomial Infections Surveillance (NNIS) system data from 1990 to 2002, out of an estimated 98,987 deaths associated with HAI in the United States hospitals, 35,967 were due to pneumonia, 30,665 were related to bloodstream infections, 13,088 were related to UTIs, 8205 were due to for surgical site infections (SSI), and 11,062 due to infections from other sites.

HAIs have significant economic implications as well. They increase the healthcare burden on the society by $35.7–45 billion every year (6).

PATHOGENS

Bacteria remain the most common pathogens and source of HAIs (7, 8). HAI are typically associated with Gram-positive pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA) (9–13), coagulase-negative *Staphylococci* (14), and glycopeptide (vancomycin)-resistant *Enterococci* spp. (15–20). More recently, there are increasing reports of glycopeptide intermediate and glycopeptide-resistant *S. aureus* (21). *Clostridium difficile*, a normal intestinal flora in 3% of healthy adults and 20–30% of hospitalized adults (22), is responsible for 25–30% of antibiotic-associated diarrhea and is being increasingly recognized as a major nosocomial pathogen (23–27). There is increasing resistance among Gram-negative organisms. Among *Enterobacteriaceae* pathogenic isolates, resistance to fluoroquinolones, extended-spectrum cephalosporins, and carbapenems is increasing (14). There is also an increasing carbapenem resistance among *Acinetobacter* spp. (14, 28) and *Klebsiella pneumoniae* (14). MDR *Pseudomonas* spp., *Klebsiella* spp. *and Enterobacter* spp. are concerning as well. Emerging resistance to carbapenems conferred by New Delhi metallo-B-lactamase 1 (NDM-1) in countries such as India, Pakistan, and United Kingdom is a potential global health problem that will require coordinated international surveillance (29).

Candida species remain the most common healthcare-associated pathogens among fungi (14, 30); and although less common, viruses including Adenovirus, Rotavirus, Norovirus, and hepatitis B have been recognized as nosocomial pathogens.

Common HAIs

URINARY TRACT INFECTION (UTI)

UTIs are the most common HAIs in both acute care setting and long-term care facilities. A major cause of septicemia and mortality, rates are similar in adult and pediatric patients (31)

and account for 36% of all HAIs (5). Intrinsic risk factors associated with UTIs include: advanced age, female gender, and severity of underlying illness (e.g., diabetes mellitus (DM)) (32). Duration of indwelling catheterization is by far the most important extrinsic risk factor for UTIs (33). Indwelling urinary catheters are used in nearly all hospital nursing units, unlike ventilators and many other devices. Various studies have emphasized that catheter use is frequently inappropriate; inattention to both the proper indications for catheter use and catheter status in patients seems to be an important factor (34–39).

The most common etiologic agents for catheter-associated UTI (CAUTI) as reported to the NHSN at CDC, 2006–2007, are *E. coli* (21%), *Candida* spp. (20%) [*C. albicans* (14%)], *Enterococcus* spp. (15%), *P. aeruginosa* (10%), *K. pneumoniae* (8%), *Enterobacter* spp. (4%), coagulase-negative *Staphylococci* (3%), *S. aureus* (2%), *A. baumannii* (1%), and *K. oxytoca* (1%) (14).

Hospitals and Long-Term Care Facilities (LTCF) should develop, maintain, and propagate policies regarding indications for catheter insertion and removal. Education of staff, use of condom catheters where appropriate, and consideration of intermittent catheterization and suprapubic catheterization as an alternative to short-term or long-term indwelling urethral catheterization have all been recommended to reduce the risk of nosocomial UTIs (40).

PNEUMONIA

Pneumonia is the third most common HAI, the second most common in the ICU, and the most common cause of mortality among all HAIs (5). It is associated with considerably increased healthcare costs and hospitalization days (32, 41). Hospital-acquired pneumonia (HAP) is defined as pneumonia that occurs 48 hours or more after admission, which was not incubating at the time of admission (42, 43). Ventilator-associated pneumonia (VAP) refers to pneumonia that arises more than 48–72 hours after endotracheal intubation (44, 45). Healthcare-associated pneumonia (HCAP) includes any patient who was hospitalized in an acute care hospital for two or more days within 90 days of the infection; resided in a nursing home or long-term care facility; received recent intravenous antibiotic therapy, chemotherapy, or wound care within the past 30 days of the current infection; or attended a hospital or hemodialysis clinic (43, 45, 46). Most current data, including microbiological, have been collected from patients with VAP but can be extrapolated to HAP and HCAP patients as well (47). Tracheal intubation and mechanical ventilation are the strongest risk factors, with 3- to 21-fold increase in risk for nosocomial pneumonia (48).

Among etiologic agents, *S. aureus* (24%) is the most common pathogen, followed by Gram-negative organisms: *P. aeruginosa* (16%), *Enterobacter* spp. (8%), *A. baumannii* (8%), *K. pneumoniae* (7%), and *E. coli* (5%) (14).

Various infection control measures can help modify the risk factors for pneumonia. Intubations and reintubations should be avoided if possible, and noninvasive modes of ventilation should be used whenever possible. Orotracheal intubations and orogastric tubes, semirecumbent position rather than supine position, continuous aspiration of subglottic secretions, adequate endotracheal cuff pressures to prevent leakage of bacterial pathogens into the lower respiratory tract, and passive humidifiers or heat-moisture exchangers can all help decrease the risk for VAP (47).

SURGICAL SITE INFECTIONS (SSIs)

SSIs are second only to UTIs in frequency, accounting for 22% of all HAIs (5). It is estimated that SSI develop in 2–5% of the 27 million patients undergoing surgical procedures each year (49, 50). Healthcare personnel and operating room environment have been implicated as common sources of pathogens for SSIs. Prolonged preoperative stay, preoperative shaving, length of surgery, and skill of surgeons are well-documented risk factors for SSIs (51). Intrinsic host-related risk factors include: severity of underlying illness (e.g., high American Society for Anesthesiology score, DM), obesity, advanced age, malnutrition, trauma, loss of skin integrity (e.g., psoriasis), and presence of remote infections at time of surgery (32).

Most common etiologic agents are: *S. aureus* (30%), coagulase-negative *Staphylococcus* (14%), *Enterococcus* spp. (11%), *E. coli* (10%), *P. aeruginosa* (6%), *Enterobacter* spp. (4%), *K. pneumoniae* (3%), and *Candida* spp. (2%) (14).

Various pre-, intra- and postoperative measures will help minimize the risk of SSI (51). Preoperative bathing with an antimicrobial has been advocated to reduce skin colonization (52). Removing hair from the site of surgery and preoperative skin preparation reduces contamination of the operative site (53). Clipping with clippers or using cream to remove hair results in fewer surgical site infections than shaving (54). Intraoperatively, appropriate barrier devices, good skills, adequate hemostasis to prevent hematomas and seromas, and adequate debridement of dead tissue are some ways to reduce transmission of microorganisms (55). Postoperatively, adequate wound care will help prevent infections.

BLOODSTREAM INFECTIONS (BSIs)

Bloodstream infections (BSIs) are the fourth common cause of HAIs (5). Both the incidence and prevalence of BSIs have increased over the past several decades. An estimated 350,000 nosocomial BSIs are reported in the United States every year (56). Differentiating a clinically significant BSI from a blood culture contaminant remains a constant challenge for physicians (57). Because many patients receive home healthcare, including intravenous infusions and chemotherapy that until the recent past would have been administered in inpatient settings, the distinction between nosocomial and community-acquired BSIs has been difficult. Friedman et al. (58) and Siegman-Igra et al. (59) described 37% and 39% BSIs, respectively, that occurred in settings traditionally classified as community acquired and could be more accurately classified as healthcare-associated. The term "nosohusial" has been proposed to describe infections occurring in homecare subjects (60).

Various automated blood cultures systems that are reasonably comparable to each other are being used by most laboratories. To ensure appropriate identification of the pathogen, all efforts should be made to avoid contamination of the sample. Skin preparation plays a major role. Various methods have been used for skin preparation. This includes cleaning venipuncture site with alcohol followed by an iodophor or iodine tincture and povidone iodine. More recently, Mimoz et al. (61) showed that alcoholic chlorhexidine may be more efficacious in preventing skin contamination compared to povidone iodine. Reliability of blood culture results also depends on various other factors including amount of blood volume sampled, timing of blood cultures, and site from where blood cultures are obtained (57).

Bloodstream infections (BSIs) are associated with various risk factors. In the past, 75% of healthcare-associated (nosocomial) BSIs were secondary to SSIs, UTIs, intra-abdominal

infections, pneumonia, or skin and soft tissue infections (62, 63). Over the years, the proportion of primary nosocomial BSIs has increased and most episodes without an obvious source are thought to be related to intravascular catheters (57, 64). Age (<1 year and >65 years) is a known predisposing factor for BSI (32, 57, 65–67). Patients with underlying malignancies and/or neutropenia are long known to be at risk for BSI (68–70). Notably, patients with hematologic malignancies are at higher risk than those with solid tumors. Other risk factors include patient with chronic liver disease (71), hemodialysis patients (72), burn patients (73), spinal cord injury patients (74, 75), transplant patients (76), and patients admitted to the ICU (77).

The pathogens differ in patients with various risk factors. Based on most recent studies, the common pathogens associated with central line associated BSI are coagulase-negative *Staphylococcus* (34%), *S. aureus* (15%), *Enterococcus* spp. (12%), *Candida* spp. (11%), *E. coli* (10%), *P. aeruginosa* (8%), *K. pneumoniae* (6%), *Enterobacter* spp. (5%), and *A. baumannii* (3%)(14).

Various recommendations have been made to prevent catheter-related BSI (78). Use of an all-inclusive catheter cart kit and barrier devices, chlorhexidine-based antiseptic for skin preparations, disinfecting catheter hubs, needleless connectors, and injection ports before accessing the catheter and appropriate surveillance are a few of the recommendations made by the Society for Healthcare Epidemiology of America/Infectious Disease Society of America (SHEA/IDSA).

Epidemiology of Infectious Disease and the Hospital and Ambulatory Care Environment

Epidemiology is defined as the study of the determinants and distribution of health and disease in populations. It is well recognized that health and disease occur due to the complex interactions between an agent, the host that is the target of agent actions, and the environment. In relation to HAIs, agent refers to the various healthcare-associated microorganisms, the host comprises the patients or/and the healthcare workers, and the environment would include different healthcare components such as acute care hospital, intensive care units, hemodialysis centers, ambulatory clinics, and so on.

Various models and equations have been used to describe these multifarious interactions. The simplest epidemiologic model described is the triangle model (79) (Figure 1.1). It signifies, in the most simplified manner, the complex yet close interaction between the agent, host and environment. The Seesaw model (Figure 1.2) is another way by which the interplay between the three components (agent, host, environment) has been described (80). By introducing the Seesaw model, Fox et al. (80) has illustrated the role that environment plays to keep an equilibrium between the agent and the host. Conversely, any disequilibrium results in adverse events. Therefore, the environment provides platform upon which the interaction between host and agent takes place. An "equation of infection" has been used to determine the probability of a microbial agent to cause infection in the host (81):

$$IP = \frac{D \times S \times T \times V}{Hd}$$

where Ip is the probability of infection, D is the dose (number of microorganisms) transmitted to the host, S is the receptive host site of contact with the agent, T is the time

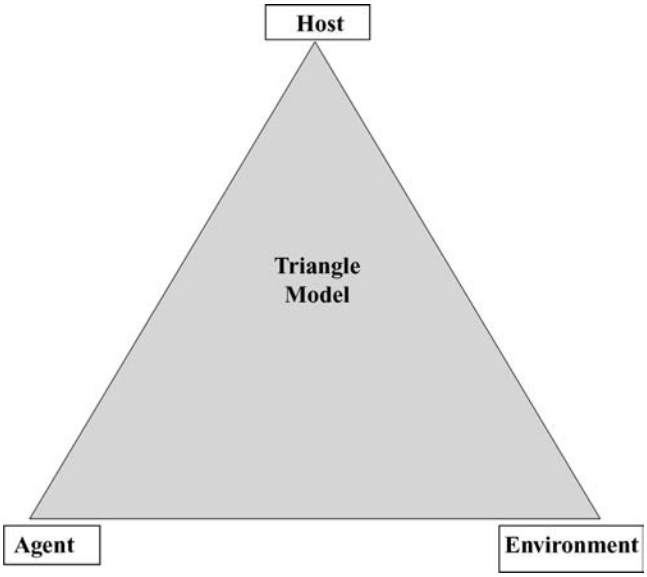

FIGURE 1.1 Triangle model for epidemiological relationships. The simplified model demonstrates the complex yet close interaction between the agent, host and environment.

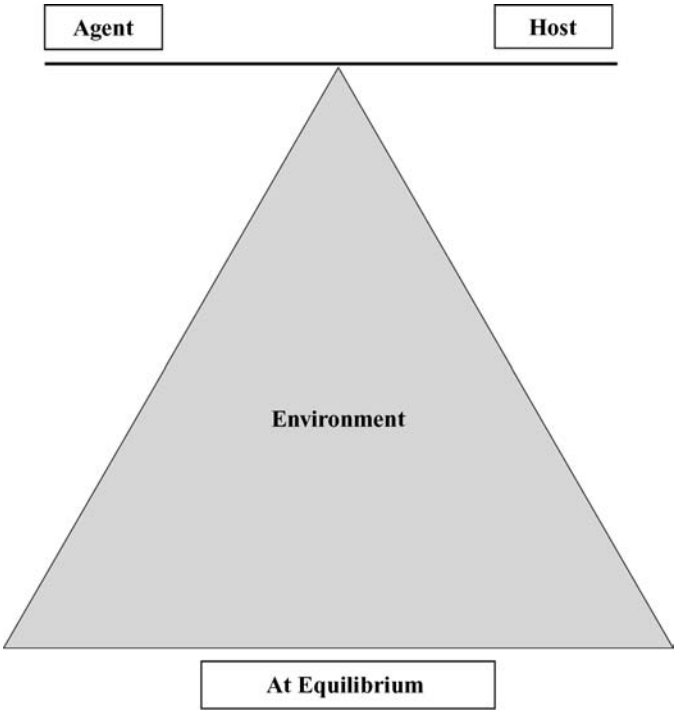

FIGURE 1.2 Seesaw model for epidemiological relationships. A second simple model to demonstrate the epidemiologic balance between the agent, host and environment.