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BOOK REVIEW

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Stephen H. Gillespie (ed): Antibiotic resistance: methods and protocols

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In the 1930s, the war against disease was aimed mostly at infectious diseases such as pneumonia, meningitis, typhoid fever, syphilis and tuberculosis. Very often, the only thing that physicians could do was to alleviate their patients suffering and hope that they – the patients – would be strong enough to overcome infection. Nowadays cancer, heart disease, and hypertension are the three diseases responsible for the majority of patient admissions in hospitals. The development and use of antimicrobial agents has been an important measure leading to the control of bacterial diseases in the twentieth century. Antimicrobial therapy has provided the ability to prevent some infections, to cure others and to curtail the transmission of certain diseases. At first, with the flood of drug discoveries (chloramphenicol, tetracyclines, erythromycin, cephalosporins and aminoglycosides) resistance was not a problem. However, the emergence of multiple drug resistance in *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus* and *Shigella dysenteriae* has made many currently available antimicrobial drugs ineffective, and in certain instances this is already causing significant public health problems.

Antibiotic resistance is structured like the other volumes of the *Methods in Molecular Medicine* series (Fig. 1). Each chapter includes a brief informative introduction, a list of material, a detailed description of methods, special notes and, finally, a list of references. Some chapters also have photographs and diagrams.

This volume consists of five different sections: Part I (12 chapters) describes different methods to detect antibiotic resistance; Part II (3 chapters) discusses the response to antibiotic treatment; Part III (5 chapters) describes studies of molecular epidemiology, Part IV (7 chapters) assesses molecular mechanisms of resistance to antibiotics or chemotherapeutic compounds and, Part V (1 chapter) details the transmission of resistance. In addition, an introduction by the book's editor provides the historical background of anti-infective chemotherapy and the development of antibiotic resistance.

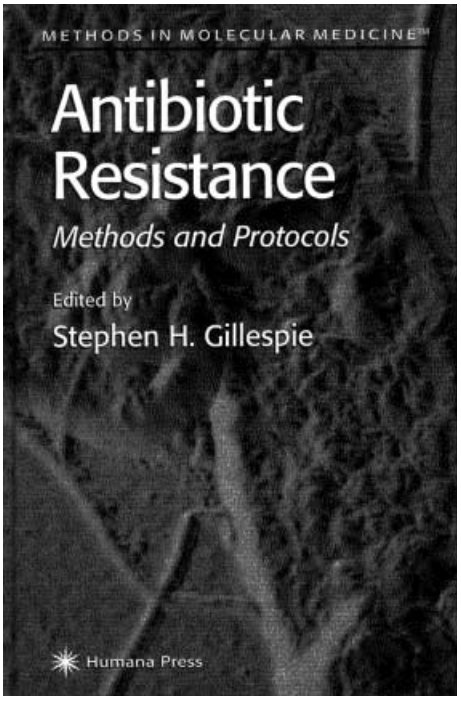

Fig. 1 Stephen H. Gillespie (ed): Antibiotic resistance: methods and protocols

The methods to determine antimicrobial drug susceptibility in microorganisms are based on an analysis of their growth on solid or liquid medium containing a specified concentration of a single drug. The development of susceptibility tests of slowly growing bacteria such as mycobacteria is of great importance to quickly and efficiently find the ideal patient treatment, and such tests will reduce the risk of transmission of resistant organisms. In Part I of *Antibiotic resistance*, many techniques that address rapid diagnosis are described, including polymerase chain reaction–restriction fragment length polymorphism

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(PCR-RFLP), an assay that can be performed directly on isolated colonies (Chaps. 1, 5); drug susceptibility of *M. tuberculosis* based on analysis of mycolic acid content (Chap. 2); phage replication assay for screening *Mycobacteria* for resistance to rifampicin and streptomycin (Chap. 3); polymerase chain reaction–single-stranded conformational polymorphism (PCR-SSPC) (Chap. 4); rapid rifampicin susceptibility testing of *M. tuberculosis* cultures by detection of precursor rRNA (Chap. 6); nucleotide sequence analysis of the pneumococcal *pbp* genes from penicillin-resistant strains compared with the *pbp* gene from susceptible ones (Chap. 7); a combination of PCR amplification and RFLP analysis of the *pbp* gene of *S. pneumoniae*, using the restriction enzyme *Hin*fI (Chap. 8); utilization of square plates of agar impregnated with continuously increasing concentrations of antibiotic to detect subtle differences in the resistance level (Chap. 9); quantitation of UDP-*N*-acetylmuramyl pentapeptide (MMP) to investigate how the cell wall synthesis system is altered in *Staphylococcus aureus* in association with glycopeptide resistance (Chap. 10); PCR to detect methicillin resistance genes and genes encoding toxin production in *S. aureus* (Chap. 11); chromogenic detection of aminoglycoside phosphotransferase (Chap. 12).

Part II deals with the *M. tuberculosis* response to antibiotic treatment. The most common laboratory measures of response to therapy for patients with pulmonary tuberculosis are conversion of acid-fast bacilli sputum smear to negative, or culture-positive sputum to negative. Note that a culture of *M. tuberculosis* can take weeks to be detectable, making it necessary to apply techniques that rapidly monitor treatment, such as quantification of *M. tuberculosis* DNA in sputum during the treatment (Chap. 13), quantification of mRNA in sputum taken before and after initiation of chemotherapy, and correlating the amount with the number of viable bacilli (Chap. 14), and detection of viable *M. tuberculosis* cells by reverse transcriptase strand displacement amplification of mRNA (Chap. 15).

Molecular epidemiology is discussed in Part III. The high incidence of pneumococcal infections and the increasing emergence of drug resistant isolates are major drives for epidemiological surveillance. Phenotypic and genotypic methods have been developed to assist in epidemiological investigations. These methods include serotyping, multilocus enzyme electrophoresis, penicillin binding protein (PBP) typing, pneumococcal surface protein A typing, and various DNA fingerprinting methods such as ribotyping, DNA fingerprinting of the PBP genes, pneumococcal BOX repetitive DNA element (Chap 16); restriction fragment end labeling (RFEL) analysis, which detects RFLPs of small-size DNA fragments (Chap. 17), and pulse-field gel electrophoresis of large size DNA restriction fragments (Chap. 18). Chapter 19 describes the isolation and analysis by SDS-PAGE of *Klebsiella pneumoniae* OMPs. When studying clonally-related multiresistant isolates, the initial strain usually expresses one porin, whereas the variant with increased resistance is deficient in this porin. And finally, atomic force microscopy (AFM) analyzes the tridimensional structure of the surface of biological specimens (Chap. 20).

The phenomenon of antibiotic resistance is an unusual aspect of microbial ecology and diversity which has evolved recently as a result of human activity. For the microbial world, the use of antibiotics originated a situation that should have been catastrophic. However, some bacteria were able to survive and even thrive in this hostile environment. Part IV describes "the biology of resistance." How microorganisms acquire resistance is studied in this section. Chapter 21 assesses the activity of bacterial multidrug efflux pumps measuring, by means of a fluorescence spectrophotometer, the accumulation of a fluorescent dye in the presence of an energy inhibitor. The use of a continuous culture system to study the antimicrobial susceptibility of bacteria in biofilm is discussed in Chap. 22. In Chap. 23, a method to estimate mutation rates in antibiotic research is presented. This method has been developed to investigate rifampicin resistance in *M. tuberculosis*, but can be readily adapted for other organisms in which resistance develops through point mutations in chromosomal genes, e.g., quinolone resistance. Chapter 24 explains how to assess the fitness of resistant *M. tuberculosis* bacteria by competition assay. The following chapter describes the purification of DNA topoisomerases and inhibition by fluoroquinolones. Site-directed mutagenesis is used in Chap. 26 to determine the relationships between structure and function in *S. pneumoniae* PBP genes. Finally, Chap. 27 presents a method to detect low-affinity PBP in *S. pneumoniae* using radioactive β-lactam antibiotic, followed SDS-PAGE and fluorography. The last section (Part V) focuses on the transmission of resistance. The ability to share genetic information with other bacteria is a major adaptive mechanism available to bacteria. The exchange of many different types of genetic information seems to occur frequently, and the exchange of determinants responsible for antibiotic resistance is the best studied, since the movements of resistance determinants are easy to follow. The most common vehicles by which bacteria exchange resistance determinants are plasmids and transposons (Chap. 28).

Antibiotic resistance is a compendium of useful techniques to study this subject. Although many of the methods applied were designed for *M. tuberculosis* or *S. pneumoniae*, they can be readily adaptable to other problem organisms, Thus, this volume may be of great value to investigators working in the field of antibiotic research.