Chapter 85
Genotypic Drug Resistance Assays

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1 Introduction

Antimicrobial resistance has become a major public health threat worldwide. Some hospital-acquired pathogens are becoming multiple resistant or totally resistant to antimicrobials. Known examples include vancomycin-resistant Enterococcus faecium and E. faecalis (VRE), methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-intermediate and -resistant S. aureus (VISA and VRSA), and extended-spectrum β-lactamases (ESBL)-producing Enterobacteriaceae (1). In the community, the prevalence of multidrug resistance in Streptococcus pneumoniae is increasing in some countries and includes resistance to β-lactams (intermediate and high-level resistance to penicillin and cross-resistance to cephalosporins), the macrolides and, more recently, the fluoroquinolones (2). Furthermore, virulent strains of MRSA that differ from the hospital strains have emerged in the communities of several countries (3). Another major public health problem is the increasing incidence of multidrug-resistant Mycobacterium tuberculosis (4). Physicians practicing in both hospitals and the community must treat infections caused by multiresistant organisms, and new, emerging antimicrobial resistances are becoming more complex to detect (5). With the limited number of antimicrobial agents available to treat the infections caused by multidrug-resistant organisms, the need for rapid and reliable susceptibility testing methods or alternative resistance testing methods for detection of antimicrobial resistance becomes increasingly important. Conventional phenotypic culture-based susceptibility test results are usually obtained in 24–48 h or more after a bacterial culture has been isolated. Moreover, susceptibility tests are not always accurate in difficult-to-detect emerging antimicrobial resistance and often more than one method is needed to obtain an accurate susceptibility profile. The lack of accurate and timely susceptibility data by the microbiology laboratory has consequences on antibiotic usage and prescription. Patients have to be treated empirically and often with broad-spectrum antibiotics, which results in increased resistance rates and healthcare costs (6). The advances in our understanding of the genetic mechanisms of antimicrobial resistance and the progress in sample preparation, nucleic acid–based amplification, and sensitive nucleic acid detection have allowed the development of genotypic methods for rapid detection of antimicrobial resistance. While most genotypic resistance tests are presently performed with pure bacterial culture which requires at least 18–24 h, it is now possible to identify a microorganism and its resistance to antimicrobial agents directly from clinical specimens in 1 h (7, 8). Some genotypic drug resistance assays are increasingly used in the clinical settings, providing more accurate and rapid resistance testing.

The purpose of this review is to describe the mechanisms of antimicrobial resistance and to present some genotypic drug resistance assays used to detect antimicrobial resistance. Genotypic drug resistance assays that are increasingly used in the clinical microbiology laboratory and their applications in the clinical settings will be further discussed.

2 Mechanisms of Resistance to Antimicrobial Agents

Different strategies have been developed by bacteria to evade the action of the antimicrobial agents. In general, antimicrobial resistance results from (a) production of enzymes that inactivate the antimicrobial agent, (b) acquisition of exogenous resistance genes that are not inhibited by the antimicrobial agent, (c) reduced uptake of the antimicrobial agent, (d) active efflux of the antimicrobial agent, (e) mutation of cellular target genes reducing the binding of the antimicrobial agent, or (f) overproduction of the target of the antimicrobial agent. The major resistance mechanisms for the important antimicrobial classes will be briefly described.
2.1 Resistance to Aminoglycosides

The aminoglycosides constitute a large family of antimicrobials that inhibit the translation process by binding to the bacterial 16S rRNA of the 30S ribosomal subunit. Four mechanisms of resistance to aminoglycosides have been described: (a) alterations in the ribosomal target site (rrs gene encoding 16S rRNA and rpsL gene encoding the S12 protein) that prevent binding, especially in streptomycin-resistant M. tuberculosis (9), (b) decreased cell membrane permeability, (c) expulsion by efflux pumps, and (d) enzymatic inactivation by aminoglycoside-modifying enzymes (AMEs). Inactivation by AMEs is the most important in terms of frequency and level of resistance (10). Aminoglycosides are modified by three types of enzymes, classified as aminoglycoside phosphotransferases (APHs), aminoglycoside adenylases (ANTs), and aminoglycoside acetyltransferases (AACs). These enzymes covalently modify specific amino or hydroxyl groups, resulting in aminoglycosides that bind poorly to the target ribosomes. Within each class, there are enzymes with different, specific sites of modification. More than 50 AMEs have been described (10).

2.2 Resistance to β-Lactams

The β-lactams are a structurally diverse group of antimicrobials that interfere with the synthesis of the bacterial cell wall as a result of their interaction with penicillin-binding proteins (PBPs). Resistance to β-lactam antibiotics can be caused by four different mechanisms: (a) acquisition or hyperexpression of β-lactamases, which is considered the most common resistance mechanism, (b) alteration, overexpression, or acquisition of PBPs, (c) permeability change in the outer membrane, and (d) active efflux of the antimicrobial (11). β-Lactamases can be grouped on the basis of either their molecular structure or function. Four different molecular classes of β-lactamases have been defined on the basis of the similarities in amino acid sequences. Classes A, B, and C are serine β-lactamases, whereas class B are metallo-β-lactamases (12).

2.3 Resistance to Glycopeptides

Glycopeptide antibiotics such as vancomycin and teicoplanin inhibit cell wall synthesis by binding to the terminal β-alanyl-β-alanine of the pentapeptide peptidoglycan precursor molecule. This binding prevents the cross-linking of peptidoglycan precursors necessary for the formation of cell wall. Acquired resistance to vancomycin in Gram-positive bacteria differs depending on the bacterial species in which they have been described: (a) altered precursor formation in enterococci and staphylococci, (b) mutational cell wall changes in staphylococci, and (c) tolerance in S. pneumoniae (1, 13, 14). To date, six gene clusters conferring different glycopeptide resistance phenotypes have been described in enterococci; five are acquired (vanA, vanB, vanD, vanE, and vanG), while the sixth (vanC) is intrinsic to E. gallinarum, E. casseliflavus, and E. flavescens (13). The vanA, vanB, and vanD genes encode β-alanine-β-lactate ligases, whereas the vanC, vanE, and vanG genes encode β-alanine-β-serine ligases. The vanA gene has been recently described in different glycopeptide-resistant S. aureus isolates in the United States (15–17).

2.4 Resistance to Macrolides, Lincosamides, and Streptogramins

Macrolides, lincosamides, and streptogramins (A and B) inhibit protein synthesis by reversibly binding to the peptidyl-tRNA binding region of the 50S ribosomal subunit, stimulating dissociation of the peptidyl–tRNA molecule from the ribosome during elongation (18). Three different mechanisms of macrolide, lincosamide, and streptogramin resistance have been described: (a) alterations in the ribosomal target site by mutations in chromosomal genes (e.g., rrl gene encoding 23S rRNA) or mediated by several different acquired erythromycin ribosomal methylases (erm) that methylate the same adenine residue in 23S rRNA resulting in resistance against macrolides, lincosamides, and streptogramin B antibiotics (MLS) (alteration in the target site has not been described for streptogramin A resistance), (b) active efflux of the antimicrobial (e.g., mef(A) conferring macrolide resistance, vga(A) conferring streptogramin A resistance, and msr(A) conferring both macrolide and streptogramin B resistance), and (c) drug inactivation by several different enzymes including esterases (ere), phosphorylases (mph), lyases (vgb), and transferases (var) (19, 20).

2.5 Resistance to Quinolones

Quinolones interact with two type-2 topoisomerases, DNA gyrase and topoisomerase IV, both of which are essential for bacterial DNA replication. Inhibition appears to occur by interaction of the drug with a complex composed of DNA and either of these target enzymes. The GyrA and GyrB subunits of DNA gyrase are respectively homologs with ParC and ParE subunits of topoisomerase IV. Quinolone resistance results mostly from chromosomal mutations in the drug