21.4 Rapid Serological Methods

Few rapid serological methods are available for detecting antibiotic resistance. Commercially available latex agglutination and immunochromatographic assays for the detection of MRSA use latex particles and nitrocellulose membranes, respectively, which are coated with an antibody against protein PBP2a, encoded by the methicillin resistance gene *mecA*. In a *latex agglutination test* for MRSA, the antibody binds to PBP2a, resulting in the agglutination of MRSA cells with the latex particles (Cavassini et al. 1999; Alipour et al. 2014). In an *immunochromatographic assay* for MRSA, the appearance of both a sample line and a control line on a nitrocellulose membrane indicates the interaction of PBP2a from MRSA cells with an antibody fixed on the membrane (Trienski et al. 2013).

Commercial kits are available that use *polymerase chain reaction (PCR)* amplification (see next paragraph) with an *enzyme-linked immunosorbent assay* (*ELISA*); they have been used to detect *mecA* in blood cultures containing *S. aureus* (Wellinghausen et al. 2004) or different carbapenemase resistance genes in cultures of the *Enterobacteriaceae* (Ambretti et al. 2013). Another ELISA method detects the growth of *Helicobacter pylori* in cultures from biopsy fragments in the presence of clarithromycin or metronidazole in 24 hours (Perna and Vaira 2010).

21.5 Rapid Molecular (Genetic) Methods

PCR methods for the amplification of DNA sequences have been used to detect specific antimicrobial resistance genes or identify pathogens in clinical samples. In the standard PCR method (van Pelt-Verkuil et al. 2008), samples containing DNA are added to a reagent tube containing forward and reverse primers specific for the gene to be amplified, a deoxyribonucleotide triphosphate mix, and Taq polymerase. Tubes containing the reaction mixtures are inserted into the thermal block of a thermocycler, which is programmed to raise and lower the temperature during each cycle. The DNA is denatured first and then the gene located between the forward and reverse primers is synthesized by Taq polymerase. Since the DNA is amplified exponentially, there should be enough copies of the gene after 25–30 cycles to be detected. The amplified DNA is either visualized on an agarose gel after staining or else detected using labeled probes. If the genetic basis of resistance is known, PCR can be used to detect the genes whose expression may decrease the susceptibility of bacteria to particular antibiotics, such as the *mecA* gene for methicillin resistance in MRSA (Cuny and Witte 2005). Because resistance to antibiotics depends not only on the gene but also on the conditions for its expression, the amplification of a resistance gene