The broad range of current PCR and DNA chip applications in clinical microbiology includes the detection of pathogens and the analysis of genomic alterations, such as sequence and copy number alterations in bacterial genes and single nucleotide polymorphisms. This article focuses on the possible application of modern molecular methods for the detection of bacterial resistance genes and mechanisms.

As many of the genetic mechanisms of antimicrobial resistance have become better understood, new molecular methods are proving to be useful for the confirmation of antimicrobial resistance in laboratory isolates and for the direct detection of such resistance in clinical specimens. Conventional culture and susceptibility test procedures for most pathogenic bacteria generally take 48–72 hours. The performance of these tests may be erratic because factors such as inoculum size or variability in culture conditions can affect phenotypic expression of resistance.

The recent popularity of new molecular methods, such as nucleic acid amplification techniques or DNA chip technology, has been fostered by the increasing demand for new diagnostic tools which allow quick nucleic acid hybridization experiments and simultaneous analyses of large numbers of PCR products. The development of DNA chip-based assays in particular has been strongly driven by modern approaches aimed at the comprehensive analysis of multiple gene mutations and expressed sequences.

Detection of genetic determinants using modern molecular techniques may therefore be used to confirm antimicrobial resistance based on the organism's genotype, rather than relying on the variability of phenotypic expression of the resistance. Moreover, these tests can be done within hours, providing clinically relevant information days before conventional susceptibility test results become available. Molecular assays to detect antimicrobial resistance directly from clinical samples have been developed.

Testing is not only required for therapy but also to monitor the spread of resistant organisms or resistance genes throughout the hospital and the community. However, the presence of a resistance gene does not necessarily lead to treatment failure because the level of expression may be too low. For example β-lactamase production among Enterobacteriaceae is common, but the development of resistance is dependent on the mode and amount of expression. The application of nucleic acid-based technology is particularly useful for slow-growing or non-culturable micro-organisms and the detection of point mutations or certain genotypes.

**Causes of antimicrobial resistance**

Antimicrobial resistance of bacterial isolates can be caused by a variety of mechanisms:

- The presence of an enzyme that inactivates the antimicrobial agent
- The presence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent
- A mutation in the target of the antimicrobial agent, which reduces the binding of the antimicrobial agent
- Modification of the target of the antimicrobial agent, which reduces binding of the antimicrobial agent
- Reduced uptake of the antimicrobial agent
- Active efflux of the antimicrobial agent
- Overproduction of the target of the antimicrobial agent

In addition, resistance may be caused by a previously unrecognized mechanism. As with any diagnostic test, the predictive value of molecular assays is dependent on the prevalence of the genes or mutations assayed. On the other hand, a gene, which is not expressed in vivo, may be expressed in vitro.

**Specific applications of molecular techniques**

The identification of methicillin resistance in Staphylococcus aureus and vancomycin resistance in Enterococcus spp. represent ideal applications of modern molecular methods.

Methicillin-resistant S. aureus (MRSA) is an important hospital-acquired pathogen capable of causing life-threatening infections and nosocomial outbreaks. The incidence of infections from this pathogen in European hospitals has increased dramatically in the past few years. The rapid and accurate identification of this pathogen is critical for patient management and for infection control programmes in hospitals. However, the reliable detection of MRSA using culture and susceptibility tests may be problematic because expression of resistance is usually heterogeneous and is influenced by culture conditions, especially in strains with low-level resistance. All strains of MRSA produce a unique penicillin-binding protein (PBP2) that is encoded by a chromosomal gene, mecA. The mecA gene is not present in susceptible strains. PCR has been used successfully to amplify and detect mecA gene sequences from clinical isolates within a few hours. These methods have also been used to detect MRSA directly from clinical specimens such as blood cultures and endotracheal aspirates.

Vancomycin-resistant enterococci have also emerged as important nosocomial pathogens in hospitals. Identification using culture and susceptibility tests is...
even more problematic than that of MRSA, primarily because of difficulties in detecting low-levels of resistance and because accurate identification using conventional laboratory procedures may take as long as 4–6 days. Vancomycin resistance in enterococci is mediated by one of several genes: vanA, vanB, vanC2, vanC1, vanC2, vanC3 or vanD. PCR assays have been developed to recognize the vanA, vanB and vanC genotypes and have demonstrated value in characterizing enterococci in the laboratory when conventional laboratory test results have been inconclusive. Another potential use of the assay is to assist in epidemiological studies when there is an outbreak.

PCR-based methods for the detection of antimicrobial resistance have also been applied to multidrug-resistant Mycobacterium tuberculosis. In the wake of the HIV epidemic and the breakdown of medical services in several Eastern European countries, the incidence of tuberculosis is rising rapidly, but treatment is threatened by the emergence of multidrug-resistant strains of M. tuberculosis. It can usually be treated with only a limited number of antimicrobial agents, the most important ones being rifampin, isoniazid, streptomycin and ethambutol. Because the organism is slow-growing, traditional diagnosis is time-consuming. Phenotypic determination of resistance may take up to 10 weeks after referral of a sample to the laboratory, but both commercial and in-house amplification assays can greatly improve detection time. It is not surprising therefore that within the past 10 years a multitude of different resistance assays based on molecular techniques have been specifically developed. However, many laboratories have trouble with the technical requirements imposed by these assays. Often isolates show multidrug resistance and with the number of potential genes and mutations involved, the number of assays needed to cover them all can be quite large. Therefore, a number of PCR assays have been developed which do not directly determine the presence or absence of resistance-causing genes and mutations, but identify either multidrug-resistant strains by other properties or monitor the effect of chemotherapy. One such completely different approach to determine resistance in M. tuberculosis was taken by developing a reverse transcriptase PCR-probe assay that was specific for M. tuberculosis precursor rRNA. Precursor rRNA carries terminal stems which are removed when mature rRNA subunits are formed. The number of these stems present in the bacterial cell is markedly affected by the inhibition of RNA synthesis.

Detection of resistance to antiviral agents by molecular methods has also been described for acyclovir-resistant herpesviruses and HIV resistance to reverse transcriptase inhibitors and protease inhibitors. These assays have been used in a number of reference and research laboratories.

**Limitations of the methods**

Despite the obvious advantages of these newer procedures for the detection of resistance, there are potential limitations to DNA amplification technology and chip technology in the diagnostic microbiology laboratory. The accuracy and reproducibility of PCR assays depend on the technical expertise and experience of the operator. Specificity of the test may be affected by contamination of the specimen. In addition, very often the detection of resistance genes for diagnostic purposes is restricted to research and epidemiology. This is due to the overwhelming number of different resistance mechanisms and genes and their variants. Furthermore, most bacteria can be easily cultured. This, coupled with the cost of most molecular assays, puts such assays in an unfavourable position compared with phenotypic assays for detection of resistance.

**Conclusions**

Molecular analysis of bacterial resistance has yielded a wealth of information during the last decade. With the aid of molecular amplification techniques, great progress has been made in the knowledge of the distribution and spread of resistance markers among species. However, the original expectation that molecular techniques would routinely surpass phenotypic susceptibility testing has not (yet) been realized. Challenges that remain include the variety of point mutations or genes leading to resistance, and the labour-intensive nature of current amplification methods. DNA-chip technology, combined with automated amplification techniques, has the potential to meet these challenges. However, the development of DNA chips containing a broad range of resistance markers usable for many different species remains a formidable challenge and requires a broader knowledge of resistance markers than is currently available.

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