

ANTIBIOTIC RESISTANCE GENES: MOLECULAR EPIDEMIOLOGY AND FOLLOW-UP

Thierry NAAS

Service de Bactériologie-Virologie, Hôpital de Bicêtre, Assistance Publique-Hôpitaux de Paris, 94275 Le Kremlin-Bicêtre, Faculté de Médecine Paris-Sud, France

Mapping, and ultimately preventing, the dissemination of infectious agents is an important topic in public health. Newly developed molecular-microbiological methods have contributed significantly to advances in the efficient tracking of the nosocomial and environmental spread of microbial pathogens.

Identification and typing was routinely carried out on the basis of microbiological, biochemical, serological, and physiological characters, gathered by the so-called 'phenotypic' procedures which, even combined have limited discrimination power. The molecular typing methods (genotypic) have several advantages over conventional (phenotypic) testing in being faster and unambiguous, more accurate, and able to detect masked resistances. They are used to address many different problems such as the study of genomic organization and evolution, the identification of patterns of infection and sources of transmission, the epidemiological surveillance of infectious diseases and outbreak investigations. Currently, genetic identification of microbes is within the reach of clinical microbiology laboratory professionals including those without specialized technology research interests.

The emergence of antibiotic resistance is one of the most worrisome phenomena of the last 20 years. The spread of micro-organisms that have become resistant to many clinically important antibiotics and the various mechanisms of resistant-gene exchange (rapid diffusion of plasmid-mediated enzymes, transposons, mobile gene cassettes and integrons...), underscores the need of molecular techniques capable of distinguishing the clonality of antibiotic-resistant micro-organisms with respect to horizontal transfer of the determinants of resistance.

In order to discriminate bacterial strains as much as possible, the best approach would be to sequence the whole genome of each strain. That would however, be time consuming and not cost effective, and therefore only parts of the genome are examined. The most widely used molecular typing methods [for example, pulsed-field gel electrophoresis (PFGE) and arbitrarily primed PCR (AP-PCR)] rely on

comparisons of DNA fragment patterns on agarose gels. These methods are highly discriminatory and rely on uncharacterized genomic differences between isolates of bacterial species and the genetic variation indexed by these methods appears to accumulate relatively rapidly. This is important for short-term epidemiological studies, as it leads to extensive variation within the pathogen of interest. Multi-locus sequence typing (MLST) provides a new approach to molecular epidemiology that can identify and track the global spread of virulent or antibiotic resistant isolates of bacterial pathogens using the Internet. MLST databases, together with interrogation software, are available for *N. meningitidis*, *S. pneumoniae*, *S. pyogenes* and *S. aureus*. MLST is based on the well-tested principles of MLEE but assigns the alleles at each locus directly by nucleotide sequencing, rather than indirectly from the electrophoretic mobilities of their gene products on starch gels. The nucleotide sequence of 500-bp internal fragments of seven housekeeping genes, provides sufficient variation to identify many different alleles within a population.

Several molecular techniques are available for determining the genotypic drug-resistance and monitoring epidemic spread of a particular antimicrobial resistance gene in a hospital or patient population. These techniques, ranging from simple plasmid analysis, through Restriction Fragment Length Polymorphism (RFLP), Polymerase Chain Reaction (PCR), PCR coupled with RFLP (PCR-RFLP), PCR-single strand conformational polymorphism (SSCP) analysis up to sequencing, have been shown useful for epidemiological studies involving resistance genes. These molecular tests will be addressed in respect to their applications and limitations using several examples. Furthermore, the latest trends in the search for new approaches to improve understanding of the constantly evolving molecular epidemiology of antibiotic resistance in microorganisms will be discussed. New techniques for investigation will always need to be developed in order to keep pace with nature's inexhaustible resources for survival.