Molecular detection of antibiotic resistance: when and where?

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Antibiotic resistance is a key issue affecting public health, and diagnostic bacteriology laboratories are essential for prompt recognition of resistant isolates. Determination of susceptibility or resistance using phenotypic tests is a 'gold standard' against which newer technologies are compared in terms of performance, cost and ease of use. Molecular methods for detecting resistance are myriad, and are used widely in academia and in reference laboratories, but gaining a significant foothold in diagnostic laboratories is proving more difficult. However, if used widely in a diagnostic setting, these techniques would impact more directly on patient care and would be valuable infection control tools, e.g. by rapidly confirming patients colonized by resistant bacteria. The cost of molecular assays may be considered prohibitive, and this is compounded by the daunting variety of proprietary platforms available; most diagnostic laboratories would prefer to invest their capital and to train their staff in a single versatile technology. In a market that has no clear leader, many laboratories are understandably reluctant to gamble on making the correct choice. If molecular detection of resistance is to achieve wide acceptance, manufacturers must broaden the repertoires of their technologies, develop more off-the-shelf applications with in-built quality control, and make them suitable for laboratory personnel with no specialist expertise in molecular biology.

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Descriptions of new molecular methods for the detection, typing and characterization of microbial pathogens or their resistance genes appear in most issues of all relevant scientific journals. These methods include, but are not limited to, simple and multiplex PCR, real-time PCR, DNA sequencing, and a plethora of hybridization-based techniques, which include macro- and microarrays. Many have become established ‘workhorse’ techniques in academic departments and reference laboratories, but they are used increasingly in larger diagnostic laboratories, especially those associated with teaching hospitals. Significant inroad into smaller diagnostic laboratories has been somewhat slower.

The emergence and spread of antibiotic resistance remains a global public health concern. This continuing problem reflects the adaptability of bacteria, and all newly introduced agents have only a limited ‘virginity’ before the spectre of resistance emerges. The mechanisms responsible for this resistance are diverse, and hundreds of resistance genes have been characterized in both Gram-negative and Gram-positive species.1,2 A core function of all bacteriology laboratories is to determine the antibiotic susceptibilities of bacterial isolates, i.e. to detect the resistance phenotypes conferred by these resistance genes. If a panel of agents in a particular antibiotic class is tested, interpretative reading of the resulting patterns of resistance or susceptibility often suggests the underlying resistance mechanisms, predicts second-line drugs to be tested and provides extended knowledge for decisions in antimicrobial therapy as well as infection control.3,4 Recently, this interpretation has been facilitated by automated systems that use in-built algorithms or sets of ‘expert rules’, such as the VITEK 2 (bioMerieux, Marcy l’Étoile, France)5,6 and Phoenix (BD Biosciences, Sparks, MD, USA)7,8 systems. Antibiotic resistance genes provide important targets for molecular detection techniques,9,10 but what advantages do such tests offer over accurate phenotypic susceptibility testing combined with visual or computer-assisted interpretative reading of antibiograms?

Clearly, rapid detection of a specific resistance mechanism in a molecular test would allow clinicians initially to avoid potentially inappropriate treatment options. However, bacteria that give positive results in genotypic tests may remain phenotypically susceptible to relevant antibiotics; the result may be a false-positive, or the test may have detected an unexpressed or partial gene. If phenotypic tests subsequently confirm susceptibility despite a positive molecular test, microbiologists and clinicians can then decide whether the risk of resistance developing in vivo is sufficiently likely to warrant continuation of alternative therapy. Conversely, in an increasingly litigious society, it would be unwise (even reckless) to treat a patient with an antibiotic based solely on molecular...
tests that had failed to detect relevant resistance genes. Therapeutic decisions are preferably based on detection of susceptibility. Molecular tests can only detect known mechanisms of resistance, or unknown mechanisms that share sufficient DNA homology to allow annealing of primers or probes; genuinely novel mechanisms or highly divergent resistance genes will be missed. Because of this fundamental short-coming it is highly unlikely that molecular tests for resistance will ever replace traditional phenotypic tests; the latter are cheaper, flexible and give the reassurance of confirming whether an isolate is phenotypically susceptible, which is a better criterion when considering treatment options. There is greater potential for molecular methods to augment susceptibility tests, by detecting resistant isolates faster, by defining the precise genetic basis of phenotypes that could be conferred by several possible mechanisms and by providing explanations for unusual, complex antibiograms that are composites of multiple mechanisms. These complex antibiograms are encountered increasingly, particularly among Gram-negative species, and can frustrate interpretative reading. Is it unreasonable to expect that molecular tests can be developed that are suitable for use in all diagnostic laboratories?

The need for tests to detect particular resistance genes will be influenced by the local prevalence of relevant resistance phenotypes and on the prevalence of the resistance genes themselves, if known. Molecular tests would be less cost-effective in areas of low prevalence but, conversely, it is more important to confirm unusual phenotypes in such areas as a basis for infection control measures. Ultimately, whether such confirmatory tests are performed locally or whether isolates are referred to a regional or national laboratory will depend on their ease of use, on local interest in rigorous investigation of resistance and on the perceived need for knowledge of underlying resistance mechanisms. The latter may impact on individual patient management, but also has broader benefits, by informing infection prevention and control efforts at a local level, and by adding depth to local, regional and national surveillance programmes. Key questions for any diagnostic laboratory wishing to introduce molecular detection methods must be, ‘What resistances should we target in our setting, and how should we detect them?’

There are many different technologies available and this multitude contributes to the reluctance of many laboratories to invest their capital and the time needed for staff training.11 Arguably, no single resistance phenotype should have sufficient impact to sell an entire platform. However, concerns about the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in many countries, together with its high political profile, make this resistance a likely exception. Rapid detection methods for use either directly in clinical samples and/or in the clarification of enrichment broths have been long desired aims in the prevention and control of MRSA,12,13 and there is urgent need for methods suitable for rapidly confirming carriage.14 Indeed, such methods are essential to sustain the demanding ‘search and destroy’ policy in low-prevalence countries. The IDI-MRSA kit (GeneOhm Sciences, San Diego, CA, USA), which allows detection of MRSA directly from nasal swabs in less than 2 h,12,15 has been commercially developed exclusively for use with the SmartCycler real-time PCR system (Cepheid, Sunnyvale, CA, USA). The potential for automation and avoidance of gel-based analysis are prerequisites for wide uptake of molecular methods. Real-time PCR fulfils this, and potentially is available to every diagnostic laboratory.16,17 However, colorimetric detection of amplification products via solid-phase immunoassays18 or ELISA-style assays19 would also be suitable for wide application.

Resistance in Mycobacterium tuberculosis arises via chromosomal mutations,20 and is another major public health issue for which molecular tests are already available. These include, for example, a line probe assay for detecting mutations responsible for rifampicin resistance (INNO-LiPA-Rif-TB; Innogenetics NV, Ghent, Belgium).21 However, the complexity in the mutational resistance mechanisms for other important antituberculosis drugs underlines the importance of conventional phenotypic tests in the diagnosis of drug-resistant tuberculosis.

For the foreseeable future the characterization of new resistance genes and mechanisms will remain beyond the resources of most diagnostic laboratories. Such investigations invariably require full gene sequencing and are best undertaken in reference laboratories or in academic departments with the necessary equipment and expertise. Such centres may also utilize this expertise to design, evaluate and customize novel molecular assays for detecting antibiotic resistance genes. Recent advances for use in these laboratories include pyrosequencing (Biotage AB, Uppsala, Sweden), denaturing high performance liquid chromatography (dHPLC; WAVE system; Transgenomic, Inc., Omaha, NE, USA) and nucleic acid analysis by mass spectrometry.22 None of these technologies is yet suitable for most routine diagnostic laboratories, although larger departments with access to some molecular expertise may benefit from them. These high-throughput technologies allow rapid detection of known single nucleotide polymorphisms (SNPs), including those associated with antibiotic resistance phenotypes, and can also be used for novel SNP discovery: pyrosequencing23–26 determines the actual sequence of short DNA fragments (ideally <50 bp, although ‘reads’ of ~100 bp are possible), and can therefore define mutations precisely, including novel SNPs if they are clustered in short, defined regions or ‘hotspots’; dHPLC27–30 allows longer DNA fragments to be investigated and can also identify novel SNPs, but the base changes are not defined precisely and must be investigated subsequently by sequencing.

It is unrealistic to have the same expectations of diagnostic laboratories, which have fundamentally different remits and therefore different requirements of molecular tests. Manufacturers should realize that most diagnostic laboratories have no interest in designing their own assays, requiring instead a more ‘black box’ approach (i.e. sample in, answer out) with rigorous in-built quality standards, no need for assay optimization and with no absolute requirement to understand the detail of the molecular principles that underpin the assay. There is potentially a significant niche in the market for the provision of such tests. The success or failure of any molecular platform in the routine diagnostic setting will depend on its versatility, and particularly on the continued development of relevant new applications by the manufacturer. In the diagnostic setting the local benefits of any test must outweigh the cost implications, e.g. by informing more rapidly appropriate therapy and by allowing prompt intervention in infection control issues.14 The closer to the bedside tests can be performed, the faster the turnaround time. Since the introduction of PCR, routine run times have decreased from a few hours to often less than 1 h with current real-time technology. Further technological advances, which use microfluidics and nanolitre volumes, promise amplification and detection of resistance genes (or other targets) in minutes.31,32
The challenge for manufacturers is to harness these exciting developments and to market off-the-shelf applications that have been developed specifically for use in any diagnostic laboratory. Molecular platforms must be refined and simplified so that they are taken up widely, thereby ensuring that local rapid detection and confirmation of key resistances, such as methicillin resistance in *S. aureus*, becomes a reality. This will allow reference and academic laboratories to investigate genuinely novel mechanisms, unusual combinations of mechanisms or emerging mechanisms of potential public health importance, while empowering diagnostic laboratories with molecular technologies that, if used locally, can impact more directly on patient care.

References