horizontal gene transfer of plasmids and mobile genetic elements within and even between species, the task of not only defining all current but also predicting all future mechanisms at a genetic level is daunting or perhaps impossible.

Despite these challenges, in a few select cases where the genotypic basis for resistance has been well defined, genotypic assays for antibiotic resistance are already being introduced into clinical practice. One important example is the detection of methicillin-resistant *Staphylococcus aureus* (MRSA). *S. aureus* is one of the most common and serious bacterial pathogens of humans, particularly in health care settings. Resistance to methicillin, the most effective antistaphylococcal antibiotic, has become very common even in community-acquired strains. The alternative to methicillin, vancomycin, is effective against MRSA but measurably inferior to methicillin against methicillin-susceptible *S. aureus* (MSSA). Analysis of clinical MRSA isolates has demonstrated that the molecular basis for resistance to methicillin in essentially all cases stems from the expression of an alternative penicillin-binding protein (PBP2A) encoded by the gene *mecA*, which is found within a transferable genetic element called *mec*. This mobile cassette has spread rapidly through the *S. aureus* population via horizontal gene transfer and selection from widespread antibiotic use. Because resistance is essentially always due to the presence of the *mec* cassette, MRSA is amenable to molecular detection. In recent years, a PCR test for the presence of the *mec* cassette, which saves hours to days compared with standard culture-based methods, has been approved by the U.S. Food and Drug Administration.

Additional molecular diagnostics are being implemented in the evaluation of bacterial antibiotic resistance. Vancomycin-resistant enterococci (VRE) harbor one of a limited number of *van* genes responsible for resistance to this important antibiotic by altering the mechanism for cell wall cross-linking that vancomycin inhibits. Detection of one of these genes by PCR indicates resistance. Identification of two carbapenemase-encoding plasmids—NDM-1 and KPC—responsible for a significant fraction of carbapenem resistance (though not for all such resistance) has led to the development of a multiplexed PCR assay to detect these important resistance elements. Because carbapenems are broad-spectrum antibiotics frequently reserved for multidrug-resistant bacteria (particularly enteric gram-negative bacilli) and are often used as antibiotics of last resort, the initial appearance of resistance and the subsequent increase in its prevalence have caused considerable concern. Therefore, even though other mechanisms for carbapenem resistance exist, a rapid PCR test for the two plasmids encoding these two carbapenemases has been developed to aid in both diagnostic and infection control efforts. Efforts are under way to extend this multiplexed PCR assay to other plasmid-borne carbapenemases and thus to make it more comprehensive.

The power and application of molecular genetic tests are not limited to high-income settings. With the increasing burden of drug-resistant tuberculosis in the developing world, a molecular diagnostic test has now been developed to detect rifampin-resistant tuberculosis. The genetic basis for rifampin resistance has been well defined by targeted sequencing: characteristic mutations in the molecular target of rifampin, RNA polymerase, account for the vast majority of rifampin-resistant strains of *M. tuberculosis*. A PCR assay that can detect both the *M. tuberculosis* organism and a rifampin-resistant allele of RNA polymerase from clinical samples has recently been approved. Since rifampin resistance frequently accompanies resistance to other antibiotics, this test can suggest the possible presence of multidrug-resistant *M. tuberculosis* within hours instead of weeks.

Despite differences in relative genome complexity, HIV genotypic screening for antiretroviral resistance offers one framework for broadening efforts at genotypic assays for nonviral antibiotic resistance. As whole-genome pathogen sequencing has become increasingly feasible and inexpensive (Fig. 146-1), significant efforts have been launched to sequence hundreds to thousands of antibiotic-sensitive and -resistant isolates of a given pathogen in order to more comprehensively define resistance-conferring genetic elements. In parallel with advancing sequencing technologies, progress in computational techniques, bioinformatics and statistics, and data storage as well as experimental confirmatory testing of hypotheses will be needed to move toward the ambitious goal of a comprehensive compendium of antibiotic resistance determinants. Open sharing and careful curation of new sequence information will be of paramount importance.

Yet no matter how thorough and carefully curated such a genotype-phenotype database is, history suggests that comprehensively cataloguing resistance in nonviral pathogens, with new mechanisms continuously emerging, will be challenging at best. Even identifying and itemizing current resistance mutations is a daunting prospect: nonviral genomes are much larger than viral ones, and their abundance and diversity are such that hundreds to thousands of genetic differences often exist between clinical isolates, of which perhaps only one may cause resistance. For example, increasing resistance to artemisinin, one of the most effective new agents for malaria, has prompted recent large-scale efforts to identify the basis for resistance. While such studies have identified promising leads, no clear mechanism has emerged; in fact, a single genetic lesion alone may not fully account for resistance. Especially with multiple possible resistance mechanisms for a given antibiotic as well as ongoing evolutionary pressure resulting in the development and acquisition of new modes of resistance, a genotypic approach to diagnosing antibiotic resistance is likely to be imperfect.

We have already observed the accumulation of new or unanticipated modes of resistance from ongoing evolutionary pressure caused by the widespread clinical use of antibiotics. Even with MRSA, perhaps the best-studied case of antibiotic resistance and a model of relative simplicity with a single known monogenic resistance determinant (*mecA*), a genotype-based approach to resistance detection proved flawed. One limitation was a recall of the initial commercial genotypic resistance assay that was deployed for the identification of MRSA. A clinical isolate of *S. aureus* emerged in Belgium that expressed a variant of the *mec* cassette not detected by the assay's PCR primers. New primers were added to detect this new variant, and the assay was re-approved for use. More recently, an even more divergent but functionally analogous gene called *mecC*, which confers methicillin resistance but evades PCR detection by this assay, was found. This series of events exemplifies the need for ongoing monitoring of any genotypic resistance assay. A second limitation is that a contradiction can occur between genotypic and phenotypic evidence for resistance. Up to 5% of MSSA strains carry a copy of the *mecA* gene that is either nonfunctional or not expressed. Thus, the erroneous identification of these strains as MRSA by genotypic detection would lead to administration of the inferior antibiotic vancomycin rather than the preferred β-lactam therapy.

These examples illustrate one of the prime challenges of moving beyond growth-based assays: genotype is merely a proxy for the resistance phenotype that directly informs patient care. One alternative approach currently under development attempts to circumvent the limitations of genotypic resistance testing by returning to a phenotypic approach, albeit one informed by genomic methods: transcriptional profiles serve as a rapid phenotypic signature for antibiotic response. Conceptually, since dying cells are transcriptionally distinct from cells fated to survive, susceptible bacteria enact different transcriptional profiles after antibiotic exposure that are different from the profiles of resistant strains, independent of the mechanism of resistance. These differences can be measured and, since transcription is one of the most rapid responses to cell stress (minutes to hours), can be used to determine whether cells are resistant or susceptible much more rapidly than is possible if one waits for growth in the presence of antibiotics (days). Like DNA, RNA can be readily detected through predictable rules governing base pairing via either amplification or hybridization-based methods. Changes in a carefully selected set of transcripts form an expression signature that can represent the total cellular response to antibiotic without requiring full characterization of the entire transcriptome. Preliminary proof-of-concept studies suggest that this approach may identify antibiotic susceptibility on the basis of transcriptional phenotype much more quickly than is possible with growth-based assays.

Because of its sensitivity in detecting even very rare nucleic acid fragments, sequencing is now permitting studies of unprecedented depth into complex populations of cells and tissues. The strength of