

Acinetobacter is a uniquely successful organism that is now presenting unusual challenges in terms of its detection and treatment in human disease. New techniques for molecular typing, such as ECI-MS and pyrosequencing, are highly definitive, but their applicability to the clinical microbiology laboratory remains to be determined (Table 26.2). Application of various molecular typing methods has been instrumental in the definition of the 31 described species of *Acinetobacter*, as phenotypic methods may not achieve adequate levels of speciation. PCR has been widely applied for detection of specific genes associated with antibiotic resistance in *Acinetobacter*, and as an adjunct technique in other strain typing methodologies. This is no doubt partially due to the ability of *Acinetobacter* to incorporate genes that encode a variety of resistance mechanisms through horizontal transfer of plasmids and integrons. RAPD methods have been demonstrated to successfully type genomic species of *Acinetobacter* but may not be applicable to higher levels of strain discrimination. Classification of species within this genus has historically presented difficulty owing to the genetic diversity of this organism. A wide variety of β -lactamases, aminoglycoside-modifying enzymes, and porin modifications may be transferred via these mechanisms. Because of the issues of accurate determination of identification and susceptibility testing in *Acinetobacter*, a variety of molecular methods have been employed. MLST combines PCR amplification of housekeeping genes with sequence analysis of amplicon. Rep-PCR has demonstrated utility in both subspecies identification and strain typing analyses. The number and expression of multiple antimicrobial resistance mechanisms can present a challenge to conventional methods of MIC determination. Rep-PCR compares well with PFGE, it is rapid and technically simple to perform, and data are easily exchanged in an electronic format. Of all the molecular typing methods discussed, only ribotyping, RAPD, and rep-PCR are commercially available. A significant development is the emergence of *Acinetobacter* strains with resistance to multiple classes of antibiotics. Ribotyping has been successfully used to characterize *Acinetobacter* to group level, although it is somewhat less discriminatory than PFGE. AFLP uses selective amplification of DNA fragments secondary to restriction and nuclease digestion. DNA sequencing, while definitive, may only be applied to discrete segments of the *Acinetobacter* genome. *A. baumannii* has been isolated from extremely diverse environments and is well adapted to multiple sites on the human host. Pneumonia, bacteremia, and wound infections are the most common manifestations of *Acinetobacter* in the ICU. Use of specific target regions, such as 16S RNA, *rpoB*, or ITS, has allowed for subgroup differentiation of *Acinetobacter calcoaceticus* complex consists of four members: *A. baumannii*, *A. calcoaceticus* sensu stricto, *A. calcoaceticus* ATCC 29624, and *A. calcoaceticus* ATCC 29624. Its discriminatory power is high, and digitalization of data lends itself to computerized analysis. MLST has been shown to be highly concordant with PFGE and is reproducible between laboratories. Generation of data, which may be stored electronically, is advantageous for use in *Acinetobacter* surveillance programs. Advancements in sequencing technology have made this technique more readily available to many laboratories. *A. lwoffii* is also a significant cause of nosocomial infections. PFGE is a well-established gold standard method, although it is labor intensive and may take several days to complete. Based on DNA sequence homology, the *A. baumannii*-*A. calcoaceticus* complex is most clinically significant in human disease. This has allowed issues in assay standardization to be addressed. *Acinetobacter* spp.