Bacterial Infections

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Abstract

Molecular techniques have revolutionized the detection and identification of microorganisms. Real-time PCR has allowed for the rapid and accurate detection of MRSA, VRE, and group B *Streptococcus*. The identification of difficult and slow-growing organisms has been expedited by sequence-based methods such as 16S rRNA gene sequencing. Rapid identification of organisms and detection of resistance markers directly from positive blood culture bottles has become a reality. Finally, a transformation is taking place with the introduction of MALDI-TOF into clinical laboratories that promises to improve the accuracy and speed of bacterial and fungal identifications by days. The advantages of these methodologies and their associated clinical applications, along with their inherent pitfalls and problems, are elucidated in this chapter.

Keywords

MRSA • *Staphylococcus aureus* • VRE • *Enterococcus* • Group B *Streptococcus* • Antimicrobial resistance • Mass spectrometry • MALDI-TOF • 16S rRNA • Tuberculosis • Sepsis • Molecular epidemiology

Introduction

As the general population ages, the incidence of chronic conditions rises, the prevalence of antimicrobial resistance increases, and emerging pathogens arise, the laboratory diagnosis of infectious diseases has evolved and become more complex. As the complexity of diagnostic microbiology has increased, so have the methods employed to detect infectious agents. The implementation of molecular technology in the clinical microbiology laboratory in some cases has augmented traditional methods, such as culture and serology, while in other circumstances it has completely replaced traditional methods. For routine bacteriology (i.e., blood cultures, urine cultures, and respiratory cultures), culture has remained the gold standard primarily based on a lower cost and the potential complex nature of infections. However, in situations where low quantities of the pathogen may be present, the patient may have received antibiotics prior to specimen collection, the etiologic agent may require unusual culture conditions, or a more rapid turnaround time is needed, molecular testing approaches are particularly beneficial.

Currently, the optimal use of molecular techniques in microbiology resides with specimens in which a limited number of pathogenic organisms are sought (i.e., detection of methicillin-resistant *Staphylococcus aureus* from nares or vancomycin-resistant *Enterococcus* from rectal swabs) and in cases where the enhanced sensitivity, decreased turnaround time, and/or patient impact of molecular methods outweighs the increased cost to the laboratory

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(i.e., molecular identification of organisms directly from positive blood cultures). A particularly exciting transition in clinical microbiology is the use of mass spectrometry (MS) for the identification of a wide spectrum of bacterial and fungal organisms as well as the detection of antimicrobial resistance. This chapter discusses the most common molecular methods and their applications in clinical bacteriology laboratories, including the associated advantages and disadvantages.

Bacterial Identification

Probe Hybridization

The molecular methods used for the identification of bacterial organisms cultured from patient specimens include direct probe hybridization and sequencing. Direct probe hybridization can be used for culture confirmation as well as direct detection of organisms from clinical material. Both nucleic acid probes and peptide nucleic acid (PNA) probes are commercially available.

Probes are single-stranded oligonucleotides that vary in size from 20 base pairs (bp) to a few kilobases, but are generally less than 50 bp. Probe specificity is defined by the nucleic acid sequence of the probe. Bacterial identification using probes to 16S rRNA or 23S rRNA are commonly used due to the relatively high copy number of rRNAs in bacteria which increases the sensitivity of direct detection. Further, rRNA sequences contain conserved regions in addition to hypervariable regions allowing for the level of identification to be varied depending on the probe sequence. Commercially available probes for culture confirmation include Group B *Streptococcus*, *Listeria monocytogenes*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and several mycobacteria including *M. tuberculosis* (Hologic, San Diego, CA). In addition, probes for direct detection of group A *Streptococcus* from throat swabs are available (Hologic). Although more expensive than conventional culture and identification methods, probe-based detection and identification methods have moderately increased sensitivity and specificity and decreased turnaround time [1]. However, both false-negative and false-positive results may occur. Bacterial strains may possess polymorphisms that prevent probe hybridization [2, 3] or different strains may contain similar sequences that result in cross-reactivity [4, 5]. Additional disadvantages of probe hybridization methods are the limited number of commercial probes and the inability to probe clinical specimens directly.

In situ hybridization (ISH) allows for the detection of nucleic acid sequences in cells or tissues fixed to glass slides. Probes, which can be DNA, RNA, or PNA, are typically short (15–30 bps) allowing for easier penetration and access to the target sequence. Both colorimetric and fluorescent ISH (FISH) probes are used in histopathology and clinical microbiology. Advantages of ISH in histopathology are the ability to evaluate the host tissue response and identification of the specific cells containing the infectious agent(s). In addition, "non-culturable" or difficult to culture organisms can be detected by ISH, i.e., *Tropheryma whipplei* for Whipple's disease. Disadvantages include autofluorescence by some microorganisms (including *Pseudomonas*, *Legionella*, many yeasts, and molds), specificity and reliability of certain probe sequences, insufficient probe penetration of sample material, secondary structure of target sequence, low target content, and photobleaching [6].

In clinical microbiology, the direct identification of microbial organisms in patient samples or cultures often is determined using commercial PNA-FISH probes (AdvanDx, Woburn, MA). PNA probes have a neutral peptide-like backbone, as opposed to the negatively charged sugar–phosphate backbone of DNA probes [7]. However, like DNA probes, PNA probes hybridize to DNA and RNA in a sequence-specific manner and can be fluorescently labeled for ease of detection. Reported advantages of PNA probes include stronger and faster hybridization, discrimination of one bp difference, resistance to nucleases and proteases, survival under stringent conditions (e.g., high temperature) that allow for access to regions with secondary structure, and increased hydrophobicity that allows for penetration of cell membranes during ISH [7]. Commercial PNA probes include many multi-labeled probe kits for the discrimination of morphologically similar organisms including *Staphylococcus aureus*/coagulase-negative *Staphylococcus*, *Enterococcus faecalis*/other enterococci, *Escherichia coli*/*Pseudomonas aeruginosa*, gram-negative rods (*E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae*), yeast (*C. albicans*/*parapsilosis*, *C. tropicalis*, and *C. glabrata*/*krusei*), and Group B *Streptococcus* (AdvanDx, Inc., Woburn, MA). PNA-FISH probes are used by clinical laboratories for the identification of organisms from positive blood culture bottles in less than 2.5 h, which has a significant positive impact on patient care and institutional cost savings [8–11].

Sequencing

In many larger laboratories, sequencing is used to rapidly and accurately identify organisms. Sequencing is more rapid than conventional methods, but initial growth of an isolate is still required prior to sequencing. Ideal applications of sequencing for organism identification include *Mycobacterium* spp., aerobic actinomycetes including *Nocardia* spp., select anaerobes and gram-positive bacteria, which are organisms that are typically slow-growing or dif-

ficult to identify by routine methods. Sequencing can also be used to identify organisms that cannot be cultured because they are inherently difficult to grow or as a result of antibiotic therapy. In this situation, sequencing would need to be performed directly from the clinical specimen, but this practice must be used with caution and only for specimens from sterile sites. A substantial body of evidence exists for direct sequencing from explanted heart valves for the identification of organisms causing endocarditis [12, 13].

Many bacterial genome regions are used for sequence-based identification in clinical laboratories, but the 16S rRNA gene is the most common target. The 16S rRNA gene encodes for the highly conserved rRNA associated with the small subunit of the ribosome and is often used for taxonomic purposes and species identification. While 16S rRNA is highly conserved among bacteria, nucleotide variations unique to each species are concentrated in specific regions. The entire gene is 1,550 bp including the conserved and variable regions, but discriminatory sequence can generally be obtained using 500 bp [14]. Universal primers complementary to the conserved regions on either side of the variable region permit amplification from all bacterial species and the resulting amplicon contains unique sequence for identification. Commercial research use only kits are available for sequencing of 16S rRNA (MicroSeq; Applied Biosystems) (Thermo Fisher Scientific, Waltham, MA), but many clinical laboratories use laboratory-developed protocols. Notably, some organisms are identical by 16S rDNA sequencing (e.g., *M. chelonae* and *M. abscessus*, *S. pneumoniae* and *S. mitis*). However, some of the identical organisms by 16S rDNA sequencing can be differentiated by sequencing other genes, such as those of the internal transcribed space (ITS) region, *rpoB*, *secA*, or *hsp65* [15–17].

Sequence results are more robust than conventional culture methods because they are less subjective if a comprehensive and accurate database is used for sequence comparison. Analysis of the sequence data involves evaluating the quality of the sequence obtained and subsequent comparison of the sequence with known sequences through public and/or commercial databases, such as NCBI GenBank, MicroSeq, Integrated Database Network System (SmartGene, Raleigh, NC), or RipSeq (Isentio, Palo Alto, CA). Once an isolate is growing in culture, the entire procedure can be done in about 1.5 work days. Beyond the relatively long time to result compared to mass spectrometry, other limitations are the quality of public databases or the expense of access to curated databases, and the similarity of 16S rRNA sequences of some organisms.

Mass Spectrometry

A major advancement in bacterial identification is the routine use of mass spectrometry (MS) in clinical microbiology laboratories [18]. Traditionally, bacterial identification is achieved by performing a number of biochemical reactions to identify the unique combination of phenotypic properties that are specific to a particular microorganism. Originally performed individually, these phenotypic tests have been streamlined and are now performed on automated instruments. Although automated systems have reduced the time to identification relative to traditional methods, there is still room for improvement.

The use of MS to identify organisms was first described in the mid-1970s [19], but not until the advent of using matrix assisted MS was the reliability and reproducibility sufficient for clinical applications [20]. The type of MS most commonly used in clinical microbiology is *M*atrix *A*ssisted *L*aser *D*esorption/*I*onization *T*ime *O*f *F*light (MALDI-TOF) MS. As illustrated in Fig. 49.1, the core of MALDI-TOF MS for bacterial identification is that differences in DNA lead to

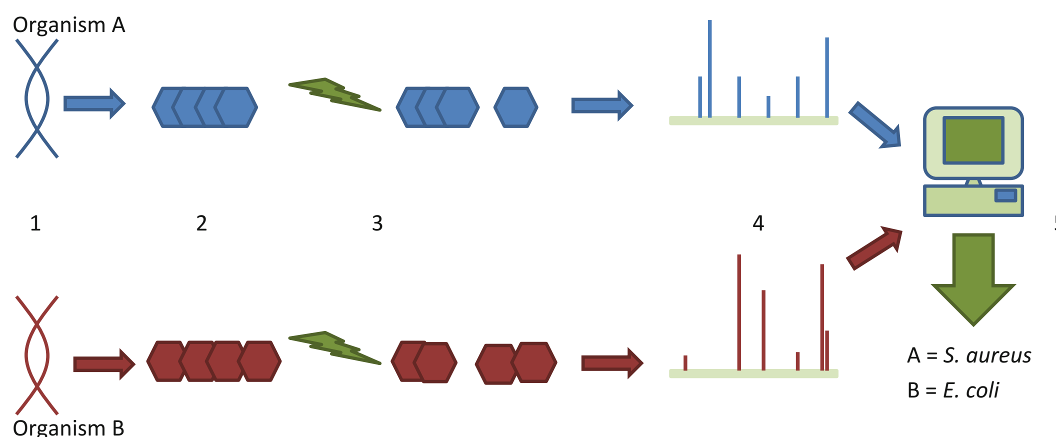

Figure 49.1 Schematic of MALDI-TOF mass spectrometry for differentiation of two organisms (A and B). Differences in DNA (*1*) encode for different protein products (*2*) which are differentially fragmented under laser excitation (*3*). These fragmented proteins produce organism-specific spectra (*4*), which are compared against a reference database of spectra (*5*) for organism identification

differences in the protein composition of an organism, and the differences in protein composition can be resolved by MALDI-TOF MS. The technology works by using the laser to create a cloud of ions to which a current is applied and released into a flight chamber. The ions are generated from the bacterial isolate that has been smeared onto a target slide and overlaid with a matrix solution (typically α-cyano-4-hydroxycinnamic acid). The matrix solution is critical for even distribution of the laser energy, generation of primarily singly charged ions, and reproducible results. The process of using smeared bacterial isolates is often referred to as whole cell or intact cell MS (WCMS or ICMS) [21, 22]. As the ions travel through the flight chamber they are separated according to their size and charge, with the smallest and most highly charged particles moving fastest through the chamber. The ions strike a detector at the end of the chamber, and a spectrum is generated that provides the relative quantity of ions of a particular mass–charge ratio. These spectra are algorithmically compared to a reference database with identity and confidence values assigned.

Much like 16S rRNA gene sequencing, the strength of this application relies on a robust reference database for comparison [18]. Several studies have shown that identification rates significantly increase after database augmentation [23–25]. Also, much as quality sequence reads are necessary for identification, high quality spectra are a must for good reference matching and identification. Ideal spectra for identification typically consist of proteins in the 2–20 kDa range, which is rich in ribosomal and other cytoplasmic proteins. Obtaining quality spectra using WCMS can be difficult with organisms such as mycobacteria, filamentous fungi, and yeasts, due to their rigid cell walls [18, 26]. Therefore, these organisms must undergo an additional extraction step to make the internal cellular proteins more accessible for ionization. The most basic extraction step is to apply a formic acid solution to the smeared spot and allow it to dry before adding the matrix solution. Higher order bacteria, such as mycobacteria and *Nocardia* spp., and filamentous fungi require a more rigorous extraction, typically involving bead beating, formic acid, and acetonitrile treatments [27]. In general, MALDI-TOF MS performs well, typically identifying >90 % of routine organisms to the correct species [18, 28, 29]. Two MALDI-TOF MS platforms currently are used in clinical microbiology laboratories: the MALDI Biotyper (Bruker Daltronics, Billerica, MA) and VITEK MS (bioMerieux, Durham, NC) each offering FDA-cleared databases. Additional developments in MALDI-TOF MS for the clinical microbiology laboratory include detection of antimicrobial resistance and direct pathogen detection from blood cultures (see below).

Another emerging technology for the identification of microorganisms is the use of PCR electrospray-ionization mass spectrometry (ESI/MS). This methodology uses conserved primers to generate PCR amplicons directly from a specimen source as well as MS to generate an approximation of the base content of the amplicons. This information is unique enough to develop spectral signatures for different organisms. These spectra are then compared to a database which provides likely identifications based on the primer sets used as well as the relative abundance of the organism(s) identified [30]. PCR ESI/MS has several advantages: (1) direct detection of a wide variety of potential pathogens (viruses, bacteria, and fungi) from specimens; (2) more rapid and cost-effective testing compared to sequencing technologies such as next-generation sequencing; and (3) to provide information outside the constraints of array-based technologies such as only being able to query a limited number of predefined organisms [30]. In fact, this technology can be used in pathogen discovery as new pathogens will not be identified but will group with similar known organisms. This was done successfully during the Sudden Acute Respiratory Syndrome (SARS) pandemic [31]. Still, as with all new technologies, work remains to be done to optimize the process for routine clinical use including further optimization of extraction methods as well as the development of additional primer sets.

Antimicrobial Resistance Detection

The increased emphasis on faster turnaround times for results combined with availability of more targeted therapeutics has created a niche for rapid molecular detection of resistance determinants in clinical microbiology laboratories. Antimicrobial resistance can be detected by probe hybridization, nucleic acid amplification (NAA) technologies such as PCR, and sequencing. However, the use of molecular methods to detect microbial resistance is not without its limitations. Multifactorial resistance mechanisms, polyclonal or polymicrobial infections, phenotypic synergism, and unknown genotype–phenotype relationships can prevent accurate determination of resistance using molecular methods.

Amplification Methods

Methicillin-Resistant *Staphylococcus aureus*

The most established application of molecular bacterial resistance testing is the detection of methicillin-resistant *Staphylococcus aureus* (MRSA). Resistance to methicillin in staphylococci is almost exclusively caused by a single mechanism, the alteration of the penicillin binding protein PBP2 to the conformer PBP2a. This change is mediated by a well-defined genetic component, the *mecA* gene. The altered PBP2a has a lower affinity for methicillin and other penicillinase-stable β-lactams such that resistance is conferred.

Traditional detection methods include chromogenic agars, oxacillin screening agars, and traditional disk diffusion for cefoxitin and minimum inhibitory concentration testing for oxacillin. These methods require 12–24 h of incubation. Decreasing the time to differentiate methicillin-susceptible staphylococcus aureus (MSSA) and MRSA by use of either protein-based methods (PBP2a latex; Oxoid, Cambridge, UK) or molecular methods for the detection of the *mecA* gene (see below) is associated with improved patient outcomes and institutional cost savings [9, 32].

Vancomycin-Resistant *Enterococcus*

First detected nearly 30 years after the introduction of vancomycin, vancomycin-resistant enterococci (VRE) developed in part due to increasing use of vancomycin for *Clostridium difficile* colitis and MRSA infections [33, 34]. Vancomycin acts by blocking the transglycosylation and transpeptidation steps of cell wall biosynthesis. The resistance phenotype is based on lowering the affinity of vancomycin for its target peptidoglycan precursors and is encoded by the *van* genes. High-level resistance (MIC, ≥64 μg/ml) is encoded *vanA* and *vanB* which are typically found on transposons, or the chromosomally associated *vanD*, and is generally found in *Enterococcus faecium* and *Enterococcus faecalis* [35–37]. Also chromosomally encoded are the *vanC* genes of *Enterococcus gallinarum*, *Enterococcus casseliflavus*, and *Enterococcus flavescens*, which are associated with low-level resistance (MIC, 2–32 μg/ml). Because *vanA* and *vanB* tend to reside on mobile elements and confer high-level resistance, detection of enterococci containing these resistance determinants is critical for effective infection control measures.

Although numerous laboratory-developed NAA assays and commercial analyte specific reagents (ASRs) are available, only a few assays for the molecular detection of VRE from rectal sites are cleared by the US Food and Drug Administration (FDA) (Xpert *vanA* test, Cepheid, Sunnyvale, CA; BD GeneOhm VanR, Becton Dickinson, Sparks, MD; and IMDX VanR, Intelligent Medical Devices, Beverly, MA). Although VRE in the USA and Europe most commonly contains *vanA*, *vanB* should also be considered due to its lower but significant prevalence. The main advantages to the molecular detection of VRE are increased sensitivity, increased specificity (exclusion of *vanC* mediated resistance), and decreased time-to-result [38–40].

Mycobacterium tuberculosis

Although resistance to antituberculosis drugs is not a new phenomenon, new methods have been developed to identify resistant strains. Due to the slow growth of *M. tuberculosis* (TB), molecular techniques are well suited to not only detect TB directly from patient specimens but also screen for resistance (see Chap. 53). Several test kits have been CE-marked for clinical use in Europe. These include the Genotype MTBDR system (Hain Lifescience, Germany), the Innogenetics INNO-LiPA Rif.TB (Gent, Belgium), and the Xpert MTB/RIF cartridge for the Cepheid GeneXpert platform, with the latter also receiving FDA clearance. All the systems detect rifampin resistance as it is the most common resistance found among the first-line TB drugs. In addition, rifampin resistance can be a marker for multidrug-resistant (MDR) TB in geographic regions with endemic MDR-TB [41]. Rifampin resistance is determined by analyzing the *rpoB* gene for specific mutations in the 81 bp rifampin resistance determining region using hybridization probes [42]. The Genotype MTBDR system also determines isoniazid resistance by screening the *katG* and *inhA* genes [43, 44]. An expanded Genotype MTBDRsl panel adds detection of resistance to fluoroquinolones, aminoglycosides, and ethambutol. Additional information on mycobacterial detection and resistance can be found in Chap. 53.

Mass Spectrometry

Much like the revolutionary impact on bacterial identifications, MS will likely impact resistance testing. Preliminary studies have demonstrated the rapid identification of MRSA, extended spectrum beta lactamase (ESBL) organisms, and carbapenemase-producing organisms by MALDI-TOF MS. Resistant organisms can be identified in two ways using MS. Similar to genetic approaches, resistant organisms can be identified by the presence or absence of characteristic mass peaks. This approach has been most widely used in the identification of MRSA by MS, though there are conflicting reports as to its effectiveness [45–47]. The other approach to identifying resistant organisms using MS is to apply a phenotypic approach such as measurement of substrate modification. For example, to determine the presence of a microbial carbapenemase, a carbapenem and test organism can be co-incubated followed by MS detection of native carbapenem drug peaks and/or peaks of its hydrolyzed products in the supernatant [48, 49]. Although this approach only detects resistance mechanisms that modify the substrate, it has the distinct advantage of looking for a phenotype instead of a particular resistance determinant. This can be especially useful in the cases of ESBLs and carbapenemases which have many genetic determinants that cause the same phenotype [50].

Specific Applications

Staphylococcus aureus/MRSA

Screening patients for MRSA nasal colonization is a central strategy for preventing the spread of this organism in health care settings. The reference method used to accurately detect

resistance due to altered PBP2 in *S. aureus* is NAA and detection of the *mecA* gene. Conventional and real-time PCR have been used to detect *mecA* both on bacterial isolates and directly on patient specimens. However, direct specimen testing has limitations, often including a lower positive predictive value than conventional methods based on the possible co-detection of MSSA and methicillin-resistant coagulase-negative staphylococci [51, 52]. Manufacturers have circumvented this problem through the detection of the SCC*mec-orf* junction in the *S. aureus* genome. However, strains that contain the SCC*mec* cassette but have a non-functional or deleted *mecA* (so-called "*mecA*-dropouts") will be falsely positive. In addition, MRSA strains that carry *mecC*, a *mecA* homologue, will be falsely negative in these assays, though the prevalence of these strains is still low [53]. Several FDA-cleared molecular assays are available for the detection of MRSA with or without MSSA detection from nasal swabs and clinical specimens, such as positive blood cultures and swabs obtained from skin and soft tissue infections (Table 49.1). NAA detection of MRSA is at least equal in sensitivity to culture-based methods, but has the advantage of offering a faster turnaround time, which, when combined with appropriate infection control interventions, may significantly decrease hospital costs by decreasing the number of health-care-associated MRSA infections [54].

Group B *Streptococcus* (*S. agalactiae*)

Although the incidence of Group B *Streptococcus* (GBS) neonatal disease has been declining since the 1990s due to enhanced prevention efforts, it is still the leading infectious cause of morbidity and mortality in neonates in the USA. In 2002, the Centers for Disease Control and Prevention (CDC), with the American College of Obstetricians and Gynecologists and the American Academy of Pediatrics, first published guidelines to perform vaginal–rectal screening of all pregnant women at 35–37 weeks gestation. Women who are colonized should be given intrapartum prophylactic treatment. Thus, accurate GBS results are critical to ensure appropriate antibiotic administration. Additionally, if a woman's GBS colonization status is not known due to lack of prenatal care or premature delivery, she should receive prophylactic antibiotics based on risk assessment, specifically for gestation less than 37 weeks, membrane rupture more than 18 h prior to delivery, or a fever of greater than 38 °C [55]. Since antibiotic administration is not without risks to the mother and newborn, intrapartum rapid molecular tests for GBS colonization are beneficial.

The first molecular technique used for routine GBS screening was direct probe hybridization either to colonies or swab-inoculated Lim broth. Although this provided the advantage of decreased turnaround time and reduced technologist time [56], it is not cost-effective for routine antepartum screening. Further development of molecular technologies in GBS detection has resulted in seven FDA-cleared molecular tests (Table 49.2) and numerous laboratory-developed tests (LDTs). Notably, the BD GeneOhm StrepB test (BD GeneOhm Sciences, San Diego, CA) and the Cepheid Smart GBS and Xpert GBS offer detection of GBS directly from rectovaginal swabs for antepartum or intrapartum detection of GBS colonization. FDA-cleared in 2006, Xpert GBS performed on the GeneXpert (Cepheid) is a moderate-complexity test that is self-contained from extraction to result. This technology makes random access testing for intrapartum screening feasible. Given that approximately 10 % of women with negative cultures at 35–37 weeks' gestation are GBS positive at the time of delivery [57],

Table 49.1 FDA-cleared molecular tests for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) directly from patient specimens

Test name	Manufacturer	Organism(s) detected	Specimen types	References
Screening tests				
GeneOhm MRSA ACP	BD Diagnostics	MRSA	Nasal swab	[77]
IDI-MRSA	BD Diagnostics	MRSA	Nasal swab	[78–81]
LightCycler MRSA Advanced	Roche Molecular Diagnostics	MRSA	Nasal swab	[82]
NucliSens EasyQ MRSA	bioMérieux	MRSA	Nasal swab	–
Xpert MRSA	Cepheid	MRSA	Nasal swab	[79, 83]
Xpert SA Nasal Complete	Cepheid	MRSA/SA	Nasal swab	[84]
Diagnostic tests				
GeneOhm StaphSR	BD Diagnostics	MRSA	Positive blood cultures	[85, 86]
Filmarray BCID	BioFire	MRSA/SA	Positive blood culture bottles	[101]
Verigene BC-GP Nucleic Acid	Nanosphere		Positive blood cultures	[87–89]
Xpert MRSA/SA BC	Cepheid	MRSA/SA	Positive blood cultures	[90–92]
Xpert MRSA/SA SSTI	Cepheid	MRSA/SA	Skin/soft tissue swabs	[92, 93]

Table 49.2 FDA-cleared molecular tests for detection of group B *Streptococcus*

Test name	Manufacturer	Methodology	Specimen tested	Sensitivity[a]	Specificity[a]	References
BD Max GBS	BD GeneOhm	Real-time PCR	Enrichment broth, antepartum swabs	95 %*	96.7 %*	[94]
Strep B (IDI-Strep B)	BD GeneOhm	Real-time PCR	Direct swab, antepartum and intrapartum	94 %* 86.8–95 %	96 %* 92.5–99.1 %	[59, 95–97]
Smart GBS	Cepheid	Real-time PCR	Direct swab, antepartum and intrapartum	81.6–98.7 %* 98.6–100 %	90.4–96.3 %* 90.4–100 %	[98, 99]
Xpert GBS	Cepheid	Real-time PCR	Direct swab, antepartum and intrapartum	88.6 %* 83.3–98.5 %	96.7 %* 64.5–99.6 %	[58, 100]
Illumigene GBS	Meridian Bioscience	Loop-mediated isothermal amplification	Enrichment broth of antepartum swabs	97.4 %*	92.3 %*	[102]
Group B AccuProbe	Gen-Probe	Hybridization Protection Assay	Enrichment broth or cultured isolate	97.7 %* 86.5–95.6 %	99.1 %* 97.5–100 %	[56, 97, 101]
GBS PNA FISH	AdvanDx	Fluorescent in situ hybridization	Enrichment broth of antepartum swabs	89.2 %* 98.4 %	98.1 %* 100 %	[99]

[a]Sensitivities and specificities were calculated using culture as the gold standard and vary depending on whether used for antepartum or intrapartum screening in the cited reference. Package insert data are indicated by an asterisk (*)

intrapartum testing is the most accurate test for colonization at the time of delivery. As an intrapartum screening test at one institution, the Xpert GBS had a sensitivity of 95.8 % and specificity of 64.5 %, whereas the antenatal culture was 83.3 % sensitive and 80.6 % specific, when intrapartum culture was used as the gold standard [58]. In a multicenter study of the IDI-StrepB assay (BD GeneOhm), when intrapartum culture was the gold standard, molecular detection at the time of labor was 94 % sensitive and 95.9 % specific [59]. Relative to either the sensitivity of antenatal cultures (54 %) or risk factor analysis (42 %), the sensitivity of the IDI-StrepB assay was superior [59]. The advantage in all these applications is the decreased turnaround time relative to culture in the intrapartum setting. Additional data regarding the sensitivity and specificity of molecular tests for GBS detection is shown in Table 49.2.

Sepsis

The use of molecular methods for the diagnosis of sepsis has been a challenging endeavor. Only one FDA-approved test is available for the identification of potential pathogens directly from blood obtained from septic patients, and this test is limited to candidemia. The gold standard remains automated blood cultures, and this reference method may be difficult to match owing to the large amount of blood that is cultured (typically 40 ml). Nonetheless, research use only products are available for direct testing of blood. Roche Molecular Systems SeptiFast (Branchburg, NJ) uses multiplex real-time PCR and melt curve analysis, while the Molzym SepsiTest (Bremen, Germany) uses multiplex PCR followed by sequencing, and the SIRS-Lab Vyoo (Jena, Germany) uses multiplex PCR followed by gel electrophoresis. These products vary in both the organisms and the resistance determinants detected, as well as analytical performance characteristics [60–64]. In general, these products suffer from both a lack of sensitivity and specificity, as well as requiring additional optimization before routine clinical use is possible.

Other shortcomings of NAA-based diagnosis of sepsis include the inconclusive clinical significance of the detection of pathogen DNA in the blood stream and the inability to obtain full antimicrobial susceptibility results [62]. However, blood culture is an imperfect reference method, suffering from a number of limitations including a prolonged time to pathogen identification, effects of variable blood volume, and lack of growth for fastidious pathogens or in the presence of prior antimicrobial therapy [62]. One limitation that can be addressed by molecular methods is the time to definitive identification.

A number of commercial molecular testing products are available for the identification of organisms and resistant determinants directly from positive blood culture bottles. This approach takes advantage of the culture amplification of bacteria from blood while adding molecular methods to lessen the time to identification. FDA-cleared tests for use directly with positive blood culture bottles include AdvanDx PNA-FISH (Woburn, MA), Cepheid (Sunnyvale, CA), Biofire FilmArray BC-ID (Salt Lake City, UT) and Nanosphere BC-GP and BC-GN panels (Northbrook, IL). The molecular targets for each of these products are listed in Table 49.3. The use of MALDI-TOF MS in direct pathogen detection directly from positive blood cultures also is being investigated [65, 66]. Recent data show identification rates of

Table 49.3 FDA-cleared molecular tests for identification of potential pathogens from positive blood culture bottles

Test name	Manufacturer	Methodology	Organisms detected	Resistance detected
AdvanDx	Separate tests as listed in Organisms Detected column	PNA-FISH	*Candida albicans*	Not directly; inferred from some species identifications
			C. albicans/glabrata	
			Yeast Traffic Light[a]	
			Enterococcus faecalis/OE[b]	
			E. coli/P. aeruginosa	
			EK/*P. aeruginosa*	
			GNR Traffic Light[c]	
Becton Dickinson	StaphSR	Real-time PCR	*Staphylococcus aureus*	*mecA* (MRSA)
Cepheid	Xpert MRSA/SA BC	Real-time PCR	*Staphylococcus aureus*	*mecA* (MRSA)
Nanosphere	Verigene BC-GP	Multiplex gold nanoparticle probes	*Staphylococcus* spp.	*mecA* (MRSA) *vanA* (VRE) *vanB* (VRE)
			Streptococcus spp.	
			Listeria spp.	
			Staphylococcus aureus	
			Staphylococcus epidermidis	
			Staphylococcus lugdunensis	
			Streptococcus pneumonia	
			Streptococcus anginosus group	
			Streptococcus agalactiae	
			Streptococcus pyogenes	
			Enterococcus faecalis	
			Enterococcus faecium	
Nanosphere	Verigene BC-GN		*Escherichia coli*	
			Klebsiella pneumoniae	
			Klebsiella oxytoca	
			Pseudomonas aeruginosa	
			Serratia marcescens	
BioFire	Filmarray BCID		*Escherichia coli K1*	
			Haemophilus influenzae	
			Listeria monocytogenes	
			Neisseria meningitidis	
			Streptococcus agalactiae	
			Streptococcus pneumoniae	

[a] *Candida albicans/parapsilosis*, *Candida tropicalis*, *Candida glabrata/krusei*
[b] Other enterococci
[c] *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*

approximately 85 %, while reducing time to identification by more than a day [67]. Several studies have demonstrated the cost-effectiveness of utilizing rapid detection of organisms from positive blood culture bottles [8, 9, 11].

Molecular Epidemiology

Pulsed field gel electrophoresis (PFGE) is the gold standard for molecular epidemiology studies of the majority of organisms [68]. In brief, bacterial cells are immobilized in agarose and subjected to proteolytic degradation followed by restriction endonuclease digestion. The resulting genomic fragments are separated by PFGE which allows for better resolution of high molecular weight products [69]. PFGE is a critical tool for infection control and public health specialties, as a proven reproducible method to show strain relatedness and identify outbreaks. Other common approaches to molecular epidemiology include amplified fragment length polymorphism (AFLP) analysis and multi locus sequence typing (MLST) [70]. AFLP analysis is based on the same theory as PFGE: differences in DNA sequence can be identified by differences in restriction endonuclease patterns. The major difference between AFLP analysis and PFGE is that PFGE looks at the entire genome of an organism, while AFLP analysis emphasizes regions of the genome known to have high rates of polymorphisms. MLST analysis is done by amplifying and sequencing a small set of known housekeeping genes (usually 7–14) that have a standard rate of genetic variability.

These labor-intensive, and often expensive, approaches to molecular epidemiology have their disadvantages. Although PFGE has high discriminatory power, it is not very reproducible even amongst members of the same laboratory. This variability makes longitudinal comparisons difficult and often requires the same strains to be run repeatedly and/or implementation of a standard strain to normalize banding patterns. Although MLST analysis is very reproducible, it lacks the discriminatory power that more genome wide approaches such as AFLP analysis and PFGE offer. AFLP analysis tries to combine the discriminatory power of PFGE with the reproducibility of MLST analysis, with some success, but this approach is not applicable to all organisms [68].

MALDI-TOF MS represents a promising development for the field of molecular epidemiology, providing a relatively fast and easy method of comparing strain relatedness, with minimal hands-on time. MALDI-TOF MS has the advantage of interrogating the entire proteome of a microorganism, although the protein size range analyzed is typically only 2–20 kDa. Researchers have looked at the ability of MALDI-TOF MS to distinguish bacterial subspecies of organisms such as *Salmonella* [71, 72]. Only a few studies have been performed that compare the ability of a MALDI-TOF MS system directly to PFGE and assess its ability to determine absolute strain relatedness [73]. MALDI-TOF MS has been used to identify clonal populations of MRSA with some success [74], but the accuracy of this has been debated [75]. It remains to be seen, however, if the level of resolution of MALDI-TOF MS fingerprinting is enough to allow this technology to replace PFGE in the epidemiologic investigations of microorganisms.

Future Perspectives

Advances in molecular biology in the last 10–15 years have made an astounding impact on clinical laboratory testing for infectious diseases. Notably, TB can be confirmed in 24 h as opposed to 6–8 weeks, sexually transmitted infections such as those caused by *C. trachomatis* and *N. gonorrhoeae* can be rapidly and accurately identified improving treatment and prevention of transmission, and organisms with important infection control implications such as *B. pertussis*, MRSA, and VRE, can be quickly identified leading to appropriate therapy and/or precautions. The applications of molecular technology in clinical microbiology are endless, but disadvantages also abound. A molecular infectious disease laboratory is established only with considerable cost and expertise. Further, we are still learning what many NAA test results mean in terms of infectious etiology and clinical significance [76]. Is the mere presence of a microorganism's DNA convincing evidence of disease causation? Additional clinical and scientific evidence may be needed to validate the clinical relevance molecular-based results.

Though there is still much to be learned about the appropriate application and interpretation of molecular infectious disease testing, numerous exciting opportunities are on the horizon. The use of real-time PCR testing in the clinical laboratory has revolutionized diagnostic microbiology. The expanding capacity of multiplex technologies is allowing the simultaneous detection of over 20 analytes in just over an hour (BioFire Diagnostics). Perhaps the technology with the greatest impact on clinical microbiology is the use of MALDI-TOF MS not only for the identification of organisms, but also their potential resistance profiles and strain typing. Lastly, as next-generation sequencing becomes more affordable and accessible to clinical laboratories, clinical investigators will be able to ask questions about pathogenesis and microbiome changes in real time. Never before has clinical microbiology changed at the rapid pace we are currently experiencing. We must remember that the power of molecular technologies should be coupled with well-controlled and clinically relevant diagnostic approaches to have the greatest impact on patient care.

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