The Journal of Infectious Diseases

SUPPLEMENT ARTICLE

Role of Rapid Diagnostics in Diagnosis and Management of Patients With Sepsis

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Delayed administration of active anti-infective therapy is associated with increased rates of adverse events, mortality, and costs among sepsis patients. Inherent limitations of conventional culture identification methods and the lengthy turnaround time of antimicrobial susceptibility testing are significant barriers to the timely delivery of life-saving therapy, particularly among antibiotic-resistant infections. Culture-independent diagnostic techniques that detect pathogens and antimicrobial resistance genes within clinical samples present a tremendous benefit to timely diagnosis and management of patients. Improved outcomes for rapid intervention with rapid diagnostics have been documented and include decreased mortality rates, decreased health care delivery costs, and faster delivery of appropriate therapeutics.

Keywords. sepsis; rapid diagnostic testing; antimicrobial stewardship.

A global rise in antimicrobial-resistant infections has resulted in increased morbidity and mortality for patients of all ages and backgrounds [1, 2]. The Centers for Disease Control and Prevention estimate that more than 2.8 million antibiotic-resistant infections occur annually in the United States, accounting for more than 35 000 deaths [3]. These alarming trends occur against a backdrop of renewed global sepsis-related mortality estimates that have recently doubled [4].

Sepsis occurs when the body's immune response to an existing viral, fungal, or (most commonly) bacterial infection causes damage, dysfunction, or even failure of the host's own tissues and organs. Prompt administration of antimicrobials with activity against the causative pathogen is the cornerstone of sepsis management [5, 6]. However, when actionable microbiology results are pending, broad-spectrum, empiric antibiotics are prescribed initially [7–9]. Therefore, timely and critical assessment of available microbiology results is necessary to ensure septic patients receive prompt, effective, and targeted treatment to ensure adequate antimicrobial coverage and allow for de-escalation or cessation of the empiric regimen. Although conventional cultures remain the gold standard, the timeframe for pathogen recovery, identification, and antimicrobial susceptibility testing (AST) to detect drug resistance may take several days, which can delay time-sensitive, life-saving treatment. Indeed, delayed administration of optimized antibiotics is associated with progression from severe sepsis to septic shock, and increased rates of adverse events, mortality, and health care costs [10–18].

RAPID DIAGNOSTICS

Emerging rapid diagnostic testing methods have debuted in clinical microbiology laboratories and include a large variety of technologies that vary greatly in terms of complexity, price, speed, and the ability to identify single or multiple pathogens [16]. Advances in technology, such as matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS), combined with clinical best practices, like active antimicrobial stewardship, can lead to significant decreases in morbidity, mortality, length of hospitalization, and costs when compared to traditional culture techniques [10, 11, 19–21]. These multiple advantages underscore the clinical benefit of reducing the time to organism identification and AST determination as this critical information may be reliably translated into improved patient care.

In the setting of sepsis, the ideal rapid diagnostic test would provide advantages that include rapid and reliable results, low detection limits, high-throughput testing, and specific organism and/or resistance detection directly from a clinical specimen. Here, we summarize the benefits and limitations of several rapid diagnostic technologies that aim to improve clinical outcomes and reduce delays to life-saving treatments.

Nucleic Acid Amplification-Based Methods

Polymerase chain reaction (PCR)-based pathogen detection methods allow target pathogen DNA sequence to be amplified. Real-time PCR-based methods utilize a fluorescently labeled probe with 2 primers to amplify target DNA of a specific pathogen. Because the probe will bind only DNA fragments of interest, nonspecific products are not detected, which eliminates

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The Journal of Infectious Diseases® 2020;222(S2):S103–9

DOI: 10.1093/infdis/jiaa263

the need for the postamplification specificity step required in conventional PCR methods. Limitations of real-time PCR test options are that they are predetermined by the specific primers utilized within the test, offer a limited menu of test organisms, and may require costly reagents and instrumentation. Laboratory-developed tests may offer a degree of customization but, in general, the ready availability of commercially prepared reagents and panels makes them relatively advantageous.

Multiplex PCR differs from conventional or singleplex PCR in that it can detect and amplify multiple pathogens' genome sequences and/or antibiotic resistance gene markers in the same sample. Multiplex PCR allows for detection of species-specific, genus-specific, or serogroup-specific targets (eg, Gram stain result: gram-positive cocci in clusters to methicillin-resistant *Staphylococcus aureus*) with rapid assay run times of around 1 hour. The ability of the multiplex PCR assay to detect antibiotic resistance gene markers is beneficial to clinicians but several limitations exist. Namely, the numbers of detectable resistance genes on PCR-based panels are limited and do not always correlate with phenotypic AST results.

Syndromic panels consist of the most common causative pathogens specific to a clinical syndrome (eg, upper and lower respiratory, meningitis, and gastrointestinal). These syndromic panels make use of multiplex PCR technologies and can be performed directly on clinical specimens. In conjunction with other clinical, epidemiological, and laboratory data, these assays aid in the identification of agents causing specific disease states. A limitation of multiplex assays, particularly in polymicrobial infections, is that the menu panel may not contain all clinically relevant pathogens. Thus, clinical microbiologists should consider this possibility when single organisms are reported. Further, given the high sensitivity of these techniques, limitations to the practical utility of these diagnostics include the risk of contamination, availability of laboratory space for dedicated DNA/RNA-free areas, considerations for the sensitivity of assays to inhibitors present in clinical specimens that may lead to false-negative results, and variability in cost. Clinical utility is further limited by identification of insignificant pathogens representing colonization versus true infection, a limited menu of pathogen options, and an inability to correlate pathogen viability with treatment effect.

In Situ Hybridization-Based Methods

Instead of conventional DNA or RNA probes, in situ hybridization-based methods make use of nucleic acid mimics to detect microorganisms in clinical samples. Peptide nucleic acid (PNA) fluorescence in situ hybridization (FISH), for example, uses fluorescently labeled synthetic oligomers that mimic the DNA or RNA structure as hybridization probes to detect and bind to species-specific 16S ribosomal RNA directly from clinical specimens. Multiple species can be detected simultaneously by using 2 or more specific probes labeled with unique fluorescent dyes. As PNA probes allow visible detection of microorganisms without the need for amplification, they are less likely to be affected by contamination than PCR-based methods. Indeed, the sensitivity and specificity in studies utilizing PNA probes for the identification of *S. aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans* in blood cultures has been shown to be more than 98% [22–24]. Limitations to PNA FISH include the requirement for an organism concentration of at least 10^5 colony forming units/mL for detection [25]. This could be problematic in fastidious organisms; however, the utility of PNA FISH for rapidly identifying *C. albicans* has been demonstrated to significantly decrease antifungal therapy costs in patients with candidemia infections [26].

The PhenoTest BC (Accelerate Diagnostics) system uses a unique combination of growth media intended for simultaneous detection and identification of multiple microbial targets by FISH identification and provides quantitative AST results. The PhenoTest BC assay is performed directly on positive blood culture samples. The Food and Drug Administration (FDA)-cleared system and kit provide full automation and sample preparation steps to identify and report a pathogen in approximately 1 hour and provide phenotypic AST results in approximately 7 hours, directly from positive blood cultures. The current platform uses digital microscopy to acquire 20 AST results that are then reported as minimum inhibitory concentration values with categorical results (susceptible, intermediate, and resistant) in agreement with reference methods [26].

Mass Spectral Methods

MALDI-TOF MS is a desorption/ionization technique that can be used to characterize and identify microorganisms. This high-throughput method can accurately identify a large range of pathogens including bacteria, yeasts, filamentous fungi, and mycobacteria in minutes. Our lab has shown that rapid identification with MALDI-TOF can reduce time to appropriate therapy in 11%–44% of cases [11]. This technique provides rapid turnaround time (≤ 1–2 hours) and, although the instruments carry considerable capital acquisition costs, the costs per sample are very low. Briefly, samples are incorporated into a matrix and bombarded with a laser, which results in vaporization of a portion of the sample. The mass/charge ratio of the resulting molecular peptide fragments is then analyzed to produce a molecular signature (peptide fingerprint) for the unknown organism. This fingerprint is unique to individual microorganisms, with peaks specific to genera, species, and strains. The test isolate's signature is compared to a database of reference spectra to determine identification at the family, genus, and species levels. Reference database spectra are proprietary to each manufacturer and have recently expanded. However, spectra can only identify species that are present in the database, effectively resulting in "blind spots." This is a clear limitation of the method as misidentified

reference strains have been reported to cause downstream identification problems [27]. This methodology is FDA-approved for use on isolates grown in routine cultures, and protocols for testing specimens directly are commercially and widely available [28, 29]. Currently, MALDI-TOF MS cannot reliably detect antimicrobial resistance or resistance genes. Collaboration between the hospital epidemiologist and the clinical microbiology laboratory is crucial for determining empiric antimicrobial regimens based on local susceptibility patterns. Protocols that combine rapid identification results from MALDI-TOF MS with automated AST and PCR methods directly from a positive blood culture have been shown to substantially reduce the time to administration of targeted therapy in septic patients [29–31].

Phenotypic Methods of Detecting Antibiotic Resistance

Although sepsis guidelines recommend initiating broad-spectrum antibiotics at the onset of sepsis recognition, increasing identification of antibiotic-resistant organisms in clinical specimens is a concern. Organisms may manifest antibiotic resistance through different mechanisms, including the production of enzymes that inactivate commonly administered broad-spectrum antibiotics. Specifically, extended-spectrum β-lactamase or carbapenemase-producing gram-negative organisms both increase the likelihood of inactive initial antibiotic therapy and are associated with higher mortality [10]. The ability to triage patients quickly, assign risk factors for resistance, and flag identification of resistant microorganisms is paramount for targeted treatment in sepsis, particularly with gram-negative organisms. Phenotypic tests make use of bacteria cultured in clinical samples and therefore require 24–48 hours to obtain results. The rapid detection of extended-spectrum β-lactamase and carbapenemase-producing isolates is highly desired, and many techniques have been developed to address this, such as the modified Hodge test, the disk diffusion synergy test, and the CarbaNP (Nordmann Poirel) test. The modified Hodge test broadly detects the presence of carbapenemases, though it cannot differentiate between carbapenemase classes like serine β-lactamases and zinc metallo–β-lactamases. The disk diffusion synergy test can be utilized to detect carbapenem resistance using disk zone inhibition in agreement with reference methods as well as the production of carbapenemase using β-lactamase inhibitors. However, like the modified Hodge test, it does not differentiate between carbapenemase classes. These tests are typically slow and time-consuming, lack sensitivity, or include only a limited number of antibiotic targets. The CarbaNP biochemical test detects the hydrolysis of carbapenems in carbapenemase-producing organisms by monitoring the color change of a pH indicator. The CarbaNP test offers a simple, cost-effective benchtop test that does not require a significant investment in capital equipment to perform and may still have a valuable role to play in reducing the time to administration of appropriate antibiotics for sepsis patients [32–34].

Magnetic Resonance-Based Methods

The T2 platform (T2Biosystems), a miniaturized magnetic resonance technology that detects the behavior of water molecules within a magnetic field, was among the first platforms to offer direct primary specimen testing without the need for culture. Briefly, this instrument analyzes whole-blood specimens, mechanically lyses cells, and uses PCR primers to amplify target DNA sequences. This is followed by hybridization of the amplicons to probe-enriched superparamagnetic nanoparticles to provide species-level identification by measuring the magnetic resonance signal produced as a result of the agglomeration of these nanoparticles [35, 36]. Initial assays for *Candida* detection (T2Candida) and, more recently, bacterial detection (T2Bacteria) resulted in a 3- to 5-hour turnaround time [35, 37]. Importantly, conventional blood cultures are still necessary to recover organisms for AST. The T2Candida Panel is indicated for the presumptive diagnosis of candidemia. Although evidence suggests reasonably high sensitivity and specificity, routine clinical adoption has been stymied by a lack of clinical and economic outcomes data. Practical diagnostic stewardship strategies are needed for target populations as clinicians are often reluctant to remove antifungal therapy in patients presenting with sepsis with ongoing high risk for candidemia [38]. The T2Bacteria Panel identifies 6 common bacterial pathogens (*Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa,* and *E. coli*) and displayed specificity and sensitivity greater than 90% in a prospective multicenter study that analyzed 1427 patient samples [39]. While the pathogens identified contribute to a large portion of sepsis pathogens, not considering many other causative pathogens is a limitation. The same study that evaluated the diagnostic accuracy of T2Bacteria found that approximately half of the bloodstream infections in the study were caused by bacterial species undetectable by the assay. In addition to this limitation, the ability to test for antimicrobial resistance genes or markers is lacking in this method. Upcoming advancements include the ability to identify a broader range of organisms and resistance markers, addressing these limitations. Health care outcomes and cost-minimization analyses may help drive adoption of this new technology.

Metagenomic Shotgun Sequencing Methods

Metagenomic shotgun sequencing refers to a broad array of methods that rely on the sequencing of nucleic acids within a clinical sample in an attempt to identify pathogens of interest. These tests take a pathogen-agnostic approach and sequence all the nucleic acids present in a specimen with the hope of detecting the causative organism amongst any background contamination. This method differs from multiplex PCR methods in the utilization of a universal approach to sequence a wide variety of nucleic acid in the specimen rather than an amplification-based approach, which uses a limited set of pathogen-specific

Table 1. Summary of Available Rapid Molecular Diagnostic Tests for Sepsis Workflow Consideration

Method/Technology, Test (Manufacturer)	Organisms/Resistance Genes Detected[a]	Time, h	Costs	Automated	Testing Specimen
In situ hybridization					
PNA FISH *S. aureus*/CNS PNA FISH (OpGen) *E. faecalis*/OE PNA FISH (OpGen) Gram-Negative PNA FISH (OpGen) *Candida* PNA FISH (OpGen) AccuProbe (Hologic)	*Blastomyces dermatitidis, Candida* spp., *Coccidoides immitis,* CoNS, Enterococcus *faecalis, E. faecium, E. coli, Klebsiella pneumoniae, Listeria monocytogenes, Mycobacterium* spp., *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae*	1–2	$$	No	Positive culture
PNA FISH with digital microscopy for AST Accelerate PhenoTest BC (Accelerate Diagnostics)	*Acinetobacter baumannii, C. albicans, C. glabrata, Citrobacter* spp., CoNS spp., *E. faecalis, E. faecium, E. coli, Klebsiella* spp., *P. aeruginosa, Proteus* spp., *S. aureus, S. lugdunensis, Streptococcus* spp., *S. marcescens* AST results as MIC	1–7	$$	Yes	Clinical specimen
MALDI-TOF MS MALDI Biotyper (Bruker Corporation) VITEK MS (bioMérieux) IRIDICA BAC BSI assay (Abbott Diagnostics)[b]	Proprietary databases with multiple bacteria, fungi, and mycobacteria	0.2	$$$$	Yes	Positive culture
Nucleic acid amplification					
Real-time PCR LightCycler MRSA (Roche Diagnostics) LightCycler SeptiFast Test MGRADE (Roche Diagnostics) Magicplex Sepsis Real-Time Test (SeeGene)	*A. baumanni, Aspergillus fumigatus Candida* spp., CoNS, *E. coli, E. faecium, E. faecalis, E. gallinarum, Enterobacter* spp., *Klebsiella* spp., *P. aeruginosa, Proteus mirabillis, S. aureus, Serratia* spp., *Stenotrophomonas maltophilia,* MRSA, *S. pneumoniae, Streptococcus* spp. Resistance markers: *MecA, vanA,* and *vanB*	3–8	$$	Yes	Clinical specimen
Real-time multiplex PCR BD Max StaphSR Assay (BD Diagnostics) Xpert MRSA/SA BC (Cepheid) Xpert MRSA/SA SSTI (Cepheid) Xpert Carba-R (Cepheid)	MSSA, MRSA, CoNS Resistance markers: *MecA, MecC* and *KPC, IMP, NDM, OXA-48,* and *VIM*	1–2	$$–$$$	Yes	Positive culture
Multiplex PCR with and without DNA-microarray hybridization Verigene Gram-Positive Blood Culture test (Luminex) Verigene Gram-Negative Blood Culture test (Luminex) BioFire FilmArray system and panels (bioMérieux Diagnostics) ePlex Blood Culture Identification (GenMark Diagnostics) iC-GN Assay (iCubate)	*Staphylococcus* spp., *Streptococcus* spp., *E. faecalis*, *E. faecium*, *Micrococcus* spp., *Listeria* spp., *E. coli, K. pneumoniae, K. oxytoca, P. aeruginosa, Serratia marcescens, Acinetobacter* spp., *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp. Resistance markers: *MecA, vanA, vanB, CTX-M, IMP, KPC, NDM, OXA,* and *VIM*	1–2.5	$$	Yes	Positive culture
Magnetic resonance					
T2Candida, T2Bacteria, and T2Resistance Panels (T2Platform)	*Candida* spp., *E. coli, E. faecium, K. pneumoniae*, *P. aeruginosa, S. aureus* Resistance markers: *AmpC, CTX-M, IMP, KPC, OXA-48, NDM, mecA, mecC, vanA, vanB*	3–5	$$$$	Yes	Clinical specimen
Metagenomic shotgun sequencing methods					
SeptiTest (Molzym)[c] iDETECT Dx Blood assay (PathoQuest) Karius NGS Plasma Test (Karius) Microbiome analysis service (CosmosID) Microbiome analysis service (One Codex)	Extensive databases of many bacteria, viruses, and fungi	12–24	$$$$	Yes	Clinical specimen

Abbreviations: AST, antimicrobial susceptibility testing; CoNS, coagulase negative *Staphylococcus* spp.; FISH, fluorescence *in situ hybridization;* MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; MS, mass spectrometry; MSSA, methicillin-sensitive *S. aureus*; NGS, next-generation sequencing; PCR, polymerase chain reaction; PNA, peptide nucleic acid.

[a]Includes organisms and resistance genes for all tests within category.

[b]With the exception of *A. fumigatus* and *C. krusei.*

[c]With the exception of *C. krusei.*

primers. Metagenomic shotgun sequencing typically uses next-generation sequencing (NGS). NGS-based methods not only attempt to provide taxonomic resolution of every pathogen in the clinical sample but also can potentially detect markers associated with antimicrobial resistance. Limitations include the possibility of false positives in the sense that the organisms identified by sequenced nucleic acids may not be the pathogen driving the sepsis response, but rather could represent deceased organisms, commensals, nucleic acids from kits or reagents, or contamination. However, a single-center study in patients with septic shock found that a newly established relevance score, the sepsis indicating quantifier (SIQ) score, was able to show high diagnostic performance in reducing the nonrelevant contaminants based on statistical calculations [40]. The SIQ is being evaluated through the Next GeneSiS-Trial, which is a prospective, observational, noninterventional, multicenter study currently ongoing [41].

Available NGS-based tests for sepsis include SeptiTest (Molzym), iDETECT Dx Blood (PathoQuest), Karius NGS Plasma Test (Karius), and microbiome analysis services (CosmosID and One Codex) (Table 1). These test platforms require send out of the clinical sample with results being delivered the day after sample receipt, limiting their potential in septic patients due to a delayed turnaround time. In addition, these tests often suffer from high costs with uncertain utility, which further limits their routine use [42, 43].

IMPROVING SEPSIS MANAGEMENT

In accordance with best practices and strategies to combat antimicrobial resistance, sepsis guidelines recommend antimicrobial streamlining to optimize therapy choice, dose, and duration as more clinical and microbiologic data becomes available through pathogen identification, resistance gene presence, and AST results, in the full context of the patient's clinical status. Although few studies have assessed the clinical effect of de-escalation strategies in critically ill patients with sepsis, data from a 2014 prospective observational study that included patients with severe sepsis or septic shock found that de-escalation therapy was a protective factor for in-hospital and 90-day mortality [44]. Use of molecular rapid diagnostic testing in patients with bloodstream infections reduced the time to effective therapy and length of hospital stays. A systematic review and meta-analysis of 31 studies and 5920 patients with bloodstream infections found that molecular rapid diagnostic testing with various techniques, including PCR, MALDI-TOF MS, and PNA FISH, was associated with significant decreases in mortality risk in the presence of an antimicrobial stewardship program, but not in its absence, and yielded a number needed to treat of 20 to prevent 1 mortality event [45]. Utilization of rapid diagnostics as discussed within this article can be tools for the minimization of time to target therapy and improved clinical outcomes [11, 46].

CONCLUSION

Accelerated phenotypic methods, molecular techniques, MALDI-TOF MS, and NGS all hold tremendous promise or have been shown to optimize workflows within the laboratory, increase clinical diagnosis yield, decrease turnaround time, and improve patient outcomes when integrated into an effective antimicrobial stewardship program. Implementation of rapid diagnostic tests may be cost-neutral or even constitute a cost savings, especially when stewardship efforts streamline care by decreasing time to escalation or de-escalation of therapy [47, 48]. Clinical and economic outcomes and logistics strategies are scarce but will be necessary to deploy these technologies to the bedside, intensive care unit, emergency department, and hospital wide. The ideal rapid diagnostic test would provide advantages that include rapid results, low detection limits, high-throughput testing, and specific organism and/or resistance detection directly from a clinical specimen. Despite the ability of rapid diagnostic methods to identify key pathogens and resistance genes from clinical isolates, none of these methods in isolation has shown diagnostic accuracy to the extent that it may be used as the sole microbiological diagnostic powerhouse. Large multicenter trials will be needed to test the efficacy, added benefit, and practicability of these tests in clinical routine.

Notes

Supplement sponsorship. This supplement is sponsored by bioMérieux, the Gordon and Betty Moore Foundation and Beckman Coulter.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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