American Journal of Infection Control 46 (2018) 1060-8

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Practice Forum

Rapid diagnostics for bloodstream infections: A primer for infection preventionists

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Key Words:
Antimicrobial susceptibility testing
Bloodstream infections
Rapid molecular diagnostics
Rapid diagnostics
Antimicrobial stewardship

Accurate and rapid antimicrobial susceptibility testing with pathogen identification in bloodstream infections is critical to life results for early sepsis intervention. Advancements in rapid diagnostics have shortened the time to results from days to hours and have had positive effects on clinical outcomes and on efforts to combat antimicrobial resistance when paired with robust antimicrobial stewardship programs. This article provides infection preventionists with a working knowledge of available rapid diagnostics for bloodstream infections.

The accurate and rapid determination of the identity and antimicrobial susceptibility of pathogens plays a critical role in the management of bloodstream infections (BSIs).[1-3] While organism identification (ID) is important and can provide direction in antimicrobial choice for some bacteria, antimicrobial susceptibility testing (AST) is required for effective management of BSIs caused by common pathogens, such as *Staphylococcus aureus*, *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. Antimicrobial resistance rates of these organisms represent a major public health concern. For example, 65% of Acinetobacter pneumonia infections in the United States and Europe are caused by carbapenem-resistant species; the rate of carbapenem-resistant enterobacteriaceae has risen 5-fold in community hospitals in the southeastern United States; and the latest methicillin-resistant *S. aureus* (MRSA) prevalence rates in the United States are reported to be as high as 66.4 per 1000 inpatients.[4-6] The prevalence of antibiotic-resistant organisms varies between communities, but collectively they are responsible for over 2 million infections and 23,000 deaths each year in the United States alone.[7] Antibiotic-resistant organisms have been implicated in a significant proportion of hospital-acquired BSIs, particularly among patients in intensive care units, where as many as half of isolates have been identified as multidrug resistant.[8-10]

The emergence of multidrug-resistant organisms (MDROs) has led to use of broad-spectrum, empiric antimicrobial therapy as the standard of care approach to managing patients with suspected BSIs, pending the ID and AST of the infecting bacteria. Traditionally, such testing typically takes 48-72 hours for the laboratory to perform. Decreasing the time patients are on broad-spectrum therapy through rapid diagnostics that include ID and AST information may have implications not only for ensuring appropriate treatment, whether it involves escalating or de-escalating antimicrobial therapy, but also for reducing *Clostridium difficile* infection (CDI) and reducing antimicrobial resistance incidence by mitigating the selective pressure placed on microorganisms.[11,12]

The pace at which new rapid diagnostic technologies, heralded as "game changers" by some in the infectious disease community,[13] are evolving presents a challenge to infection preventionists (IPs), whose role and responsibilities have already undergone a dramatic expansion.[14] Maintaining a working knowledge of the basic principles of the different rapid methods and the information they provide; determining which technology best meets the needs and goals of their antimicrobial stewardship program (ASP) and infection prevention programs; and learning how they can advocate for the technology in their institution often requires time and resources that IPs no longer have. This is substantiated by surveys of

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Conflicts of interest: None to report.

0196-6553/
https://doi.org/10.1016/j.ajic.2018.02.022

IPs reporting a lack of understanding of certain technologies and a desire for more education on laboratory diagnostics.[15,16] Furthermore, it has repeatedly been shown that rapid diagnostics rarely have an effect on antimicrobial use or patient outcomes unless they are paired with a robust ASP intervention; thus, it is imperative that IPs have a working knowledge of the available technologies.[3,17-21] The aim of this article is to provide a basic framework of available BSI rapid diagnostics for IPs.

BLOODSTREAM INFECTIONS: THE SEPSIS BURDEN

Sepsis presents the most substantial diagnostic and therapeutic challenge of all BSIs, although the term BSI can also refer to various grades of bacteremia. Bacteremia is defined as the presence of bacteria in the bloodstream and can be diagnosed as transient, intermittent, or continuous.[11] When these circulating bacteria and their toxins elicit a dysregulated host response, resulting in significant organ dysfunction, circulatory collapse, and metabolic deterioration, sepsis, a true medical crisis, occurs.[22] Understanding the burden placed on the healthcare system by sepsis is key to appreciating the need for rapid diagnosis of the causative organism(s) and their antimicrobial susceptibility. The most recent report on sepsis by the Agency for Healthcare Research and Quality revealed that sepsis-related hospital stays increased by 153% between 1993 and 2009, with an average annual increase of 6%.[23] Sepsis is also the single-most expensive reason for hospitalization, with an annual cost estimated in excess of $20 billion.[23,24] In-hospital mortality rates from sepsis are a staggering 16%, over 8 times higher than other diagnoses,[12] with as many as 600 deaths occurring per day in the United States alone.[25]

The critical value of rapid ID and AST in sepsis is perhaps best demonstrated by the work of Kumar et al., who documented a 7.6% drop in survival of patients with severe sepsis and septic shock for every hour of delay prior to administration of effective antimicrobial therapy.[26] Furthermore, studies have shown that as many as 20%-30% of septic patients receive inadequate empiric antimicrobial therapy, which is strongly associated with increased mortality.[1,27,28]

The use of broad-spectrum, empiric therapy in treating BSIs, including sepsis, has repeatedly been implicated as a contributor to antimicrobial resistance.[18,19,25,29-31] Despite this, empiric therapy remains a mainstay of BSI—and particularly sepsis—treatment for several valid reasons. In fact, the international Surviving Sepsis Campaign Guidelines recommend "empiric broad-spectrum therapy with one or more antimicrobials for patients presenting with sepsis or septic shock to cover all likely pathogens (including bacterial and potentially fungal or viral coverage)."[32]

This practice is based on the fact that, in many cases of primary BSIs, the clinical picture belies a specific microbiologic diagnosis, leading healthcare providers to initiate therapy that covers a broad range of potential pathogens.[1] Additionally, the acuity of BSIs and the knowledge that mortality directly correlates with time to effective therapy precludes waiting for ID and AST results.[1,18,19,26,31] Thus, the longer the turnaround time (TAT) for those results, the longer it takes to de-escalate therapy and the more likely the empiric therapy is to contribute to downstream resistance. A vicious cycle ensues in which suspicion of resistant organisms as causative pathogens in BSI leads to the use of increasingly broad-spectrum antibiotics.

Traditional approach to microbiology testing of patients with suspected bacteremia or sepsis

Standard of care for suspected bacteremia and sepsis has long included collection of blood cultures and concomitant administration of empiric antimicrobial therapy, along with other sepsis bundle interventions outlined by the Society of Critical Care Medicine and the European Society of Intensive Care Medicine's collaborative Surviving Sepsis Campaign.[33] When the blood culture bottle turns positive, a cascade of additional diagnostic testing begins, including the Gram stain, the results of which are phoned to the team caring for the patient, and subculturing of the blood to solid media so that the organism can be grown in pure culture, as shown in Figure 1. The following day, bacterial colonies are identified and AST is performed, using a suspension of the organism. AST is performed by exposing the bacteria to a panel of antibiotics and observing if growth is inhibited—a process performed in most North American laboratories using automated instrumentation. Additional manual techniques, such as gradient strips or disk diffusion, may be required to confirm results or to test antibiotics not available on the assay panels provided for these automated systems.[34] Although advancements in culture media and monitoring systems have improved the sensitivity and TAT of blood cultures over the past several decades,[24,35] they are inherently hampered by several limitations: 12 hours to 5 days before detection of bacteria, issues arise with contamination of skin flora, and limited efficacy is seen in the case of prior antibiotic exposure and/or infections caused by fastidious organisms.[11,12] This is compounded by the time required to subculture the bacteria from positive blood cultures, obtain a pure culture, and test on automated ASTs.

RAPID DIAGNOSTICS FOR BSI FROM POSITIVE BLOOD CULTURES: CURRENT TECHNOLOGIES AND THEIR DIAGNOSTIC CAPABILITIES

Rapid diagnostics represent a significant advance from traditional culture methods on the continuum of BSI diagnostic capabilities. Blood culture and traditional AST methods are still the core laboratory practice; however, they are increasingly being supplemented with novel diagnostics that yield information hours to days faster than the traditional techniques. Most of these rapid diagnostics dramatically improved the time-to-result associated with ID of the most common bacteria and yeast that cause BSIs. Significantly, until early 2017, advances in time-to-result in new AST methods have generally lagged behind those for ID and resistance marker testing.

One means of distinguishing among the commercially available fast diagnostic technologies is to categorize them by technology type and their diagnostic capabilities (e.g., ID and/or genotypic/phenotypic AST), as demonstrated in Table 1. Accurate bacterial ID, beginning with a Gram stain, is clearly the first step toward achieving appropriate antimicrobial therapy and is a critical step in providing initial information on targeting therapy (either through escalation or de-escalation) and potential contaminants. For example, identification of *Streptococcus pneumoniae* or Group A or B Streptococcus can facilitate antimicrobial de-escalation based on the high susceptibility profile of these organisms to penicillins.[36-38] Community, facility, unit, or specimen type (e.g., blood, sputum, or wound) antibiograms may then facilitate a more effective antibiotic selection. Unfortunately, even antibiograms updated annually, grouped by unit or specimen type, still represent a "best guess" for the susceptibility profile of organisms. It is not uncommon to find specific drug/bug combinations where 20%-30% of isolates are resistant to a common therapy choice (e.g., in 1 large, urban academic medical center's intensive care unit, 26% of *Klebsiella pneumoniae* isolates were resistant to cefazolin, which is an antimicrobial frequently used for *K. pneuominae* infections).[39] As such, in many cases, targeted therapy cannot be implemented until AST is performed. AST, in its current forms, still lags behind ID in TAT but is important in progressing from a "more effective" antimicrobial selection, in which the chosen antibiotic is generally known to cover the

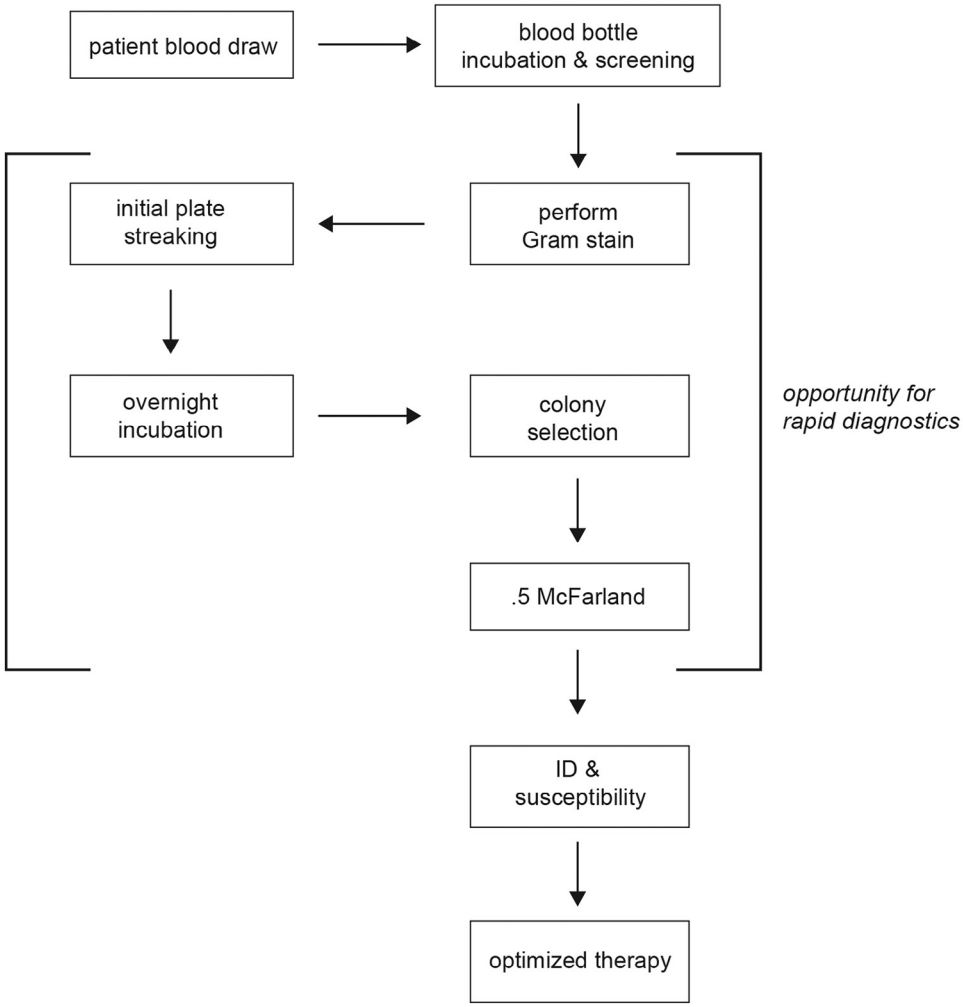

Fig 1. Current ID and AST workflow and opportunity for rapid diagnostics to expedite process.

Table 1
Diagnostic capabilities for commercially available rapid ID/AST technologies in bloodstream infections.

Technology	Identification (ID)	Detection of antibiotic resistance	Antimicrobial susceptibility testing (AST) with MIC	Turnaround time/time to result from positive blood culture
Singleplex PCR	Yes	No	No	1-3 hours
Multiplex PCR	Yes	Yes*	No	1-2 hours
Microarray	Yes	Yes	No	2.5 hours
MALDI-TOF	Yes	No	No	24 hours
PNA-FISH	Yes	No	No	1 hour
Integrated MCA w/FISH ID	Yes	Yes†	Yes	1.25 hours for ID and 5 hours for AST

*This capability is specific to certain assays only, targeted by genotypic resistance marker.
†Resistance detected through phenotypic methods, irrespective of genotype.
PNA-FISH, peptide nucleic acid fluorescence in situ hybridization; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MCA, morphokinetic cellular analysis; MIC, minimum inhibitory concentration; PCR, polymerase chain reaction.

identified bacteria, to optimal, targeted therapy, in which the antibiotic administered has demonstrated bactericidal activity/efficacy.

I. Nucleic acid-based molecular testing

Currently, 2 primary testing categories are used for rapid diagnostics directly from a positive blood culture specimen (i.e., no subculture needed): fluorescence in situ hybridization (FISH) and nucleic acid amplification tests (NAATs), which include polymerase chain reaction (PCR) and microarray. Although some of these methods, such as FISH or PCR, are no longer considered novel technologies, having been used in some form over the past 40 years, their capabilities have evolved over the last decade, and the technology has several new iterations.

FISH was one of the first rapid molecular diagnostic tests to become commercially available for positive blood cultures.[2] Original FISH assays involved the use of a single fluorescently labeled DNA probe used to hybridize with the RNA of target pathogens.[2,40] These fluorescent probes bind to the target pathogen's RNA, and ID is determined by identifying the fluorescence pattern via microscopy.[2] However, these standalone FISH tests for ID have largely become obsolete. Current commercial use of FISH methods is limited

to newer multiplex FISH ID combined with fast AST technology (Accelerate Pheno system, Accelerate Diagnostics, Tucson, Arizona).

The basic premise of all NAAT technology is the amplification of a specific pathogen's DNA when present. In PCR, DNA is extracted, exposed to short-complimentary gene sequences (primers) of known bacterial or fungal DNA, and amplified through the action of the enzyme DNA polymerase.[11] In the case of traditional or singleplex PCR, a targeted piece of a single pathogen's DNA is amplified, whereas multiplex PCR involves the use of multiple primers and allows for the ID and amplification of multiple organisms. Microarray based on direct DNA extraction is a more recently commercially available molecular testing method for rapid ID from positive blood cultures. Magnetic beads are used to extract nucleic acid sequences from a positive blood culture specimen. Nanoparticle probes are then added to capture targeted DNA in a "microarray" that is then analyzed for the presence or absence of specific bacterial nucleic acid sequence(s).[2,41] As with multiplex PCR, this technology performs species-level ID of certain Gram-positive and Gram-negative bacteria and some yeast, in addition to specific resistance markers.[2]

Modern iterations of traditional and multiplex PCR (examples include GeneXpert, Cepheid, Sunnyvale, California, and FilmArray, bioMerieux, Marcy l'Etoile, France) and microarray (Verigene, Luminex, Austin, Texas) technologies offer several advantages in BSI pathogen ID, including reduced TATs (to between 1 and 3 hours) and high sensitivities and specificities for targeted organisms.[1,11,12,42] While these automated systems have eliminated lengthy hands-on time,[8] they remain limited by the breadth of species they can detect and their inability to differentiate between viable and nonviable microorganisms.[1,11,43] A relatively newer advantage, however, to multiplex PCR or microarray testing is the detection of certain resistance markers within a predetermined set of species and markers (often referred to as genotypic AST), including *mecA* in staphylococci and *vanA/vanB* genes in enterococci, which has enhanced abilities to narrow antimicrobial therapy by predicting drug resistance.

It should be noted, though, that use of the term AST with current resistance marker testing is technically a misnomer, as the genotypic testing provided by PCR or microarray may provide information on drug resistance but not susceptibility, nor does it allow for quantification of susceptibility to specific antibiotics (i.e., determine minimum inhibitory concentration [MIC], which is the lowest concentration of an antibiotic that will inhibit the growth of a microorganism).[3] Variable expression levels of resistance genes or sequence variations within the genes can influence the antibiotic susceptibility phenotype of a bacteria, and, thus, the mere presence of the gene does not always predict resistance.[3] For example, while organisms containing extended spectrum beta lactamase (ESBL)/AmpC resistance markers are generally susceptible to carbapenems, overexpression of the genes, combined with cell permeability defects in some bacteria, can lead to resistant carbapenem MICs.[3] This cannot be determined unless phenotypic AST is performed, since the genotypic result of an ESBL is not predictive of carbapenem resistance.[3] Conversely, some bacteria harbor genes that would be predicted to confer resistance to carbapenems but test susceptible. In these cases, treatment outcome is associated with MIC and not resistance gene presence.[44] Furthermore, these assays are limited in their ability to detect certain acquired resistance genes and cannot detect new resistance genes, which is particularly problematic in Gram-negative BSIs.[3,42,45,46] Rates of false-positive resistance marker results can also be significant.[3,47] Blanc et al. found that a commercial methicillin-resistant/methicillin-sensitive *S. aureus* (MRSA/MSSA) PCR assay incorrectly identified MRSA in 12.9% of *S. aureus* isolates.[47] As a result, the Infectious Disease Society of America recommends "back-up in vitro susceptibility testing" for these genotypic tests in guiding sepsis treatment.[1]

II. Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a more recently developed diagnostic technology for pathogen ID from pathogen isolates cultured on solid media (examples include MALDI Biotype, Bruker Corporation, Billerica, Massachusetts, and Vitek MS, bioMerieux).[3,11,12] Target pathogen proteins are ionized (via the MALDI), and a mass profile of the resulting fragments is then created and compared to a large database of organism profiles for ID.[2,3,11] A comparably rapid TAT (1-2 hours from isolates from subculture plates) for species ID, broad species panel, high sensitivity and specificity, ability to run multiple analytes simultaneously, and minimal hands-on time for processing have made MALDI-TOF an increasingly popular method in microbiology for subcultured isolate identification, including those from positive blood cultures. However, unlike FISH and DNA-based testing, its dependence on subculture isolates requires an additional 24-36 hours from blood culture positivity before ID results can be generated.[1-3,11,48] While daily operation costs are low, the capital costs and unit maintenance costs are high and may serve as an obstacle for adoption of the technology by some institutions.[1,11,12,48] Additionally, some evidence suggests that the ability of MALDI-TOF to detect gram-positive organisms, most notably *S. pneumoniae*, is suboptimal.[11,12,49,50]

Arguably, the most notable limitation to MALDI-TOF in current commercial systems is its inability to provide AST. Commercially available MALDI-TOF systems provide only organism ID, and, thus, their effect on antimicrobial use is dependent on local susceptibility data and subsequent culture-based AST of positive blood cultures.[3,48] Software systems are available that allow laboratories to interface MALDI-TOF testing modules with certain automated AST systems after organism ID, although this still requires growth and colony selection from a subculture plate. These manually intensive steps add up to 12-24 additional hours to processing time before AST results are available.

IV. Morphokinetic cellular analysis

Antimicrobial susceptibility testing

One of the most recent developments in rapid diagnostics for BSIs is morphokinetic cellular analysis (MCA) technology, which provides fast phenotypic AST. Phenotypic AST is accomplished by measuring dynamic features, including morphology, division rate, mass, and growth rates of the bacterial cells as they respond to specific antimicrobials, using time-lapse microscopy.[3,40] MICs, interpreted using Clinical and Laboratory Standards Institute (CLSI) and Food and Drug Administration (FDA) breakpoints, are determined by analysis of these features.[3,40] MCA is commercially available in combination with multiplex FISH, such that ID and AST can be performed simultaneously on a single blood culture sample as soon as it flags positive (i.e., no need for subculture) (Accelerate Pheno system, Accelerate Diagnostics).[3,40,51] In this case, the FISH process is actually preceded by gel electrofiltration to clean the sample by removing antimicrobial agents, extracellular debris, and proteins before hybridization.[3,40] Thus, AST results can be generated with 1 sample, requiring a TAT of less than 90 minutes for ID and 5 hours for AST,[3] for a total TAT of less than 7 hours from a positive blood culture, without subculture steps or additional manual interventions. Clinical studies have demonstrated ID sensitivities that are similar to other rapid diagnostics and with even higher specificities, as well as high categorical agreement (i.e., same susceptible, intermediate, or resistant determination) with standard of care methods for both ID and AST.[40,51]

RAPID DIAGNOSTICS AND ANTIMICROBIAL STEWARDSHIP

Mounting evidence demonstrates the clinical value of rapid diagnostics in addressing the growing problem of antimicrobial resistance and the burden of CDI from antibiotic overuse and in improving patient outcomes.[1] As the Infectious Disease Society of America has stated, "there is an urgent need for tests that are easy to use, identify the microbe causing the infection, determine whether it is drug resistant, and provide results faster than current tests. Faster, more accurate tests would help ensure that patients are receiving the best treatment for a variety of infectious diseases, guide more effective infection control practices, and improve the tracking of outbreaks. Better tests would also help protect our dwindling supply of effective antibiotics by reducing their misuse."[52] Rapid diagnostics offer an opportunity to mitigate the problems of resistance and CDI while helping meet federal mandates for ASPs, but their success is *critically dependent* on concomitant ASP interventions.

Clinical value: tackling antimicrobial resistance and improving outcomes in BSIs by reducing the time to optimal therapy

With the advent of commercially available rapid diagnostics, actionable results can be in the hands of healthcare providers in as little as 90 minutes (ID) and 7 hours (AST) of a blood culture bottle turning positive.[3,40] Ideally, this would allow for swift de-escalation of coverage, but it also informs potentially life-saving escalation in rare cases. A robust body of evidence demonstrates the implications of this rapid TAT. Multiple studies have shown shorter time to optimal therapy along with reduced mortality rates, shorter hospital lengths of stay, and lower hospital costs when rapid diagnostics are combined with robust ASPs.[18,19,30,31,46]

In a study of rapid PCR-based ID (GeneXpert MRSA/SA BC, Cepheid), Parta et al. reported a 44.6-hour reduction in mean time to appropriate therapy, while Bauer et al. identified a reduction of 1.7 days and $21,387 in time to narrowed therapy and hospital costs, respectively, when the same rapid PCR-based ID combined with ASP interventions were compared with traditional blood culture testing.[53,54] Sango et al. demonstrated significant reductions in mean time (23.4 hours, $P = .0054$) to appropriate antimicrobial therapy, hospital length of stay (21.7 days, $P = .0484$), and hospital costs (mean savings of $60,729, $P = .02$) when microarray technology was implemented for BSI detection along with ASP interventions.[54] Similar results have been shown in studies assessing rapid ID with MALDI-TOF.[18,19,31,55] In a study of MALDI-TOF-based rapid ID (MALDI Biotyper, Bruker Corporation) for BSI and ASP interventions compared with conventional blood culture ID and no ASP intervention, Huang et al. demonstrated significantly reduced time to effective therapy (84.0 vs 55.9 hours, $P < .001$), mortality (20.3% vs 12.7%, $P = .021$), and length of intensive care unit stay (14.9 vs 8.3 days, $P = .014$) in the MALDI-TOF group.[31] In 2 separate studies, Perez et al. demonstrated similar results for the same MALDI-TOF ID system and ASP interventions in Gram-negative bacteremia along with significantly reduced hospital expenditures (mean reduction of $19,547).[18,19] However, these results are confounded by the fact that these investigators also performed susceptibility testing directly off positive blood culture broths, rather than from a subculture, resulting in a significantly shorter time to AST results, in addition to ID.

Patel et al. reported lower hospital costs per BSI ($42,580 vs $45,019) and reduced 30-day mortality rate (12% vs 21%, $P < .01$) when MALDI-TOF ID (MALDI Biotyper, Bruker Corporation) was combined with ASP interventions compared with traditional ID methods and limited ASP involvement.[55] While these results are indisputably impressive, Maurer et al. noted in their review of diagnostic advances in the clinical microbiology laboratory that the "exact contribution of rapid pathogen ID by MALDI-TOF remains difficult to assess," because many of these studies combine the technology with other ASP interventions.[3] It is important to note that these benefits can only be realized if the rapid ID results, both for organism and resistance markers, are correctly interpreted and acted on by clinicians, which, because of lack of standardization in reporting, remains a challenge.[56]

Achieving rapid ID, particularly when combined with ASP efforts, clearly contributes to improved outcomes and can help guide antibiotic therapy, including de-escalation, in cases such as infection with *S. pneumoniae* in areas with low resistance rates. However, in areas with higher resistance patterns, the missing piece to definitively optimize antibiotic therapy and reduce clinical variation for patients with BSIs is the AST result. Although no studies to date have evaluated the effect of rapid AST versus rapid ID alone, with or without ASP, on antibiotic use or clinical effect, it is arguably the AST result that allows the pharmacist or clinician to definitively identify the right drug, at the right dose, with the least collateral damage to the patient. This is true of AST results generated by any method, but studies of new diagnostics have shown that the AST TAT can be significantly reduced, allowing for this change of care far sooner, and further benefiting a patient's clinical course.[40,51,57,58]

Marschal et al. evaluated the effect of a fast phenotypic AST system (Accelerate Pheno system, Accelerate Diagnostics) using MCA on Gram negative BSIs and FISH for ID, compared with conventional culture-based methods, and found that the time-to-result was reduced by 27.5 hours and 40.4 hours for ID and AST, respectively ($P < .0001$).[51] In a study of the same system for rapid directed antibiotic treatment of sepsis, Burnham et al. demonstrated that time to AST result was reduced by 40.8 hours compared with conventional culture methods, affecting care for 70% of patients.[57] Mortality rates among patients receiving ineffective initial therapy was 45.2% compared with 11.8% among patients who received effective initial therapy, and mechanical ventilation was reduced by an average of 1 day for patients on effective initial therapy.[57] De Cardenas et al. studied combined FISH/MCA technology (Accelerate Pheno system, Accelerate Diagnostics) in a pediatric oncology hospital and found significant reductions in time-to-result for both ID and AST compared with MALDI-TOF ID and automated AST.[40] Their results demonstrated a mean time-to-result of 1.4 hours compared to 32.5 hours for ID and 6.6 hours compared to 46.7 hours for AST.[40] The study authors, noting the significance of these reductions in their immunocompromised pediatric population, concluded that "this technology may well afford a paradigm shift in how we are able to test and report ID and AST results for bloodstream infections."[40]

In a more recent study of *S. aureus* and *Enterococcus* spp BSIs by Sofjan et al., the same system was evaluated for diagnostic accuracy compared to a conventional automated biochemical system (Vitek 2, bioMerieux), and its effect on antimicrobial stewardship was assessed by retrospective audit of 231 patients.[58] In addition to finding high sensitivity and specificity (98.0% and 99.5%, respectively), the study authors noted that therapy interventions could have been expedited in 98% of patients.[58] Specifically, 60% of patients could have had unnecessary therapy discontinued earlier, 34% could have been de-escalated to targeted therapy sooner, and 4% could have been escalated to active therapy more quickly.[58]

Although the novelty of this fast AST diagnostic technology (only commercially available since February 2017) currently precludes a body of evidence commensurate with that of the existing rapid ID technologies, it is easy to assume that the clinical value seen with rapid ID can only be improved upon with reducing time-to=result for AST, as demonstrated by the evidence to date. Moving from empiric therapy to "best guess" therapy based on ID to narrowed, targeted therapy based on AST as expeditiously as possible holds great potential for both reducing antimicrobial resistance and improving patient outcomes.

An opportunity to reduce CDI

The use of broad-spectrum, empiric antibiotics in BSIs has another significant downstream repercussion in the form of CDI. With rates having tripled over the past 15 years and an estimated annual societal cost in excess of $5 billion, CDI places a tremendous burden on the healthcare system.[29,54] That burden has taken on new dimensions with the increasing incidence of multiply recurrent CDI and the emergence of highly virulent and transmissible strains.[29,59,60] Antimicrobial therapy has long been recognized as a major (yet modifiable) risk factor for CDI by causing disruption of the protective intestinal microbiota.[5] Research has shown that this risk increases with exposure to broad-spectrum antibiotics and multiple classes of antibiotics.[29,61,62] In a large-scale study of antibiotic use and CDI in a California healthcare system, Tartof et al. demonstrated a 5-fold increase in CDI incidence when 3 or more antibiotic classes were administered. Studies have also demonstrated that when broad-spectrum antibiotic use is monitored and restricted, CDI rates can be reduced.[61,63-67] The effect that fast phenotypic AST results could have on achieving early narrowed/targeted antimicrobial therapy, and thus cumulative antibiotic class exposure, is clearly significant. Furthermore, a growing body of research is stratifying specific antibiotic classes with CDI risk, suggesting that if accurate AST results can be rapidly obtained, prescribing practice can be modified to mitigate CDI risk by not only narrowing therapy but also by choosing certain "effective" antibiotics with a lower CDI risk profile over others.[29]

Advancing antimicrobial stewardship

ASPs are a requirement for accreditation by The Joint Commission and are already mandated in 2 states: California and Missouri. ASPs have also been endorsed by multiple professional organizations, including the Society for Healthcare Epidemiology of America, the Infectious Diseases Society of America, the Surgical Infection Society, the Association for Professionals in Infection Control and Epidemiology, and the American Hospital Association, as well as some state departments of public health. As a result, IPs are becoming increasingly familiar with the core elements mandated for these programs[68,69]:

- leadership commitment
- accountability
- drug expertise
- action
- tracking
- reporting
- education

The "action" element includes "interventions to improve antibiotic use," which could certainly be argued to encompass early ID and AST to guide optimal therapy. In fact, in their report, *The Core Elements of Hospital Antibiotic Stewardship Programs*, the Centers for Disease Control and Prevention (CDC) states that "rapid diagnostic tests have been successfully incorporated into some stewardship programs and may become important additions to stewardship efforts."[68]

A strong case can be made for rapid diagnostics representing a valuable "performance improvement plan," as outlined by The Joint Commission's Standard MM.09.01.01. Significant reductions in the time-to-result for pathogen ID and AST in BSIs, leading to shorter time to optimal therapy, can dramatically reduce the time a patient is placed on potentially unnecessary antibiotics.

For this benefit to be realized, however, facilities must have a strong implementation plan in place to ensure that results are effectively and efficiently communicated to the necessary individuals and acted upon within a reasonable timeframe. This is a point that cannot be overemphasized, since research has repeatedly shown that, in the absence of such a plan, these rapid molecular diagnostics do not significantly affect outcomes.[20,21] In a large systematic review and meta-analysis of 31 studies, Timbrook et al. demonstrated that molecular diagnostics were associated with significant reductions in mortality compared with conventional laboratory methods when used in conjunction with an ASP, but that effect was not seen without an ASP.[20]

Terp et al. studied the effect of using MRSA/MSSA PCR for patients with purulent skin and soft tissue infections admitted to the hospital from the emergency department and found no significant reduction in the use of "excessive empiric prescription of MRSA-active antibiotics despite the test's accuracy."[21] They concluded that introducing the rapid molecular diagnostic test without establishing a strong protocol for ensuring that the results were appropriately used undermined the intended outcome of the technology.[21]

Similarly, Donner et al. demonstrated that physician interpretation of rapid molecular diagnostics results is "suboptimal and can result in ineffective treatment or missed opportunity to narrow therapy."[56] They implemented a PCR-based system for blood culture ID (BCID) along with stewardship-based education on interpretation and then assessed physician result interpretation and prescribing practice by electronic survey. Their results were striking and perhaps most notable for indicating that "misinterpretation of BCID results may be occurring at rates approaching 50%."[56] Among all respondents, the most common prescribing practice error was failure to de-escalate antimicrobial therapy, which the authors noted has significant consequences given the association between de-escalation and improved outcomes.[56] In fact, only 60% of physicians reported adjusting antimicrobial therapy based on BCID results.[56] These researchers found no association between specialty or degree of training (e.g., resident vs attending) and higher score/correct interpretation and suggested that the wide variability in the ways ID results are reported from different testing methods undermines the potential benefit of rapidly obtaining those results.[56] Conversely, the broad standardization for reporting susceptibility results leaves less room for misinterpretation, and, thus, obtaining AST results faster offers significant promise to reduce clinical variation and improve the speed and accuracy with which physicians act on diagnostic results.

Developing a robust implementation plan undoubtedly requires multidisciplinary collaboration between all of the CDC's identified key players in the ASP: clinicians, IPs, quality improvement staff, laboratorians, information technology personnel, pharmacists, and nursing staff.[68] Establishing protocols for communicating easily interpretable microbiology results to clinicians and pharmacists, including through electronic medical records; educating all players on result interpretation; and tracking of results and prescribing practices should be fundamental elements of any ASP. Integrating rapid diagnostics into this workflow requires additional education on result interpretation and an accelerated response to results, but this can significantly advance patient care, with critical-to-life results delivered faster.

CONCLUSION

The body of evidence demonstrating that rapid ID and AST of BSIs can reduce the time to optimal antimicrobial therapy and improve clinical outcomes is robust. Although research is needed to establish a direct correlation between the use of rapid diagnostics for BSIs and a reduction in the prevalence of antimicrobial-resistant organisms or CDI rates, the connection is intuitive. The challenge is choosing a technology that best suits a facility's and/or

an ASP's needs and goals. For example, identifying diagnostic gaps, such as the need for rapid AST in sepsis, or limitations in staffing/laboratory hours can help direct a facility toward a technology that accelerates the time that AST results are delivered to clinicians. Assessing what diagnostic information is desired and what each technology provides is critical, since some technologies address ID only, some add a limited suite of resistance markers, and others require subculture steps prior to providing ID or AST results. Time-to-result also varies considerably between the array of options and should be another factor weighed when assessing the potential effect of a technology.

Additionally, establishing a strong business case for rapid diagnostics acquisition may be necessary to sway the hospital administration. This case should be built on identifying savings from shortened hospital stays that translate into increased patient throughput, reduced hospital-acquired adverse events (healthcare-associated CDI), avoided financial penalties for readmissions, reduced pharmacologic costs (by reducing overall antibiotic use and use of more expensive, broad-spectrum drugs), and better reported rates.[70] In their public policy statement on improved diagnostics for infectious diseases, the Infectious Diseases Society of America warns against taking a "siloed approach to budgeting that leads. . .to consider[ing] only laboratory costs and [therefore] see[ing] the novel tests as expensive compared to traditional methods."[1] Departments may need to share the burden of initial capital costs, potentially tapping into contingency funds, but the evidence for both a financial and clinical return on investment is strong.[18,19,55]

It may be that, as these returns on investment become increasingly evident, a shift in antimicrobial stewardship will occur. As the battle against the threat of antimicrobial resistance in the face of a dwindling armamentarium of effective antibiotics is waged, rapid diagnostics, particularly those that can generate AST results in a matter of hours, may prove to be one of antimicrobial stewardship's most potent and transformative weapons. Accordingly, ASPs may increasingly take a more "theragnostic" approach, as recently proposed by Dik et al., in which the most effective strategy is achieved through integrating antimicrobial, infection prevention, and diagnostic stewardship.[71] The IP's role in this evolving ASP paradigm should include involvement with early detection and close surveillance of bacteremias, CDI, and MDROs, and monitoring antibiograms for changes in resistance. By reviewing blood culture AST results along with the prescribed therapy, IPs can assist nurses and physicians in recognizing unnecessary, inadequate, or suboptimal antimicrobial therapy and help prevent antimicrobial resistance and opportunistic infections such as CDI.

GLOSSARY OF TERMS

FISH

FISH is a molecular technology for bacterial ID that uses synthetic, fluorescence-labeled probes designed to bind to species-specific bacterial ribosomal RNA (rRNA). After a blood culture turns positive and a Gram stain is performed, the probe is added to sample from the positive culture and allowed to hybridize with the rRNA of the target pathogen. The hybridized product is then visualized and identified with a fluorescence microscope. Currently, FISH technology is rarely used solely for organism ID; however, multiplex FISH technology is used in combination with MCA for concomitant ID/AST processing.

MALDI-TOF MS

MALDI-TOF MS is a multistep technology for pathogen ID based on a microbe's proteome, which is the component of proteins expressed by an organism's genetic material. Once a bacterial isolate from a positive blood culture has been obtained, it is combined with an organic matrix on a plate and allowed to dry or crystallize. A laser beam is then applied to the analyte, fragmenting (through desorption and ionization) the bacterial peptides into singly charged ions. These ions are then accelerated through a chamber at a fixed potential, allowing them to separate based on their mass-to-charge ratio. An analyzer detects the time each ion takes to travel through the chamber (hence, its "time of flight") and generates a peptide mass fingerprint for the analyte. This is then compared to a known database of bacterial fingerprints for ID.

Microarray from direct DNA extraction

In microarray testing, nucleic acid sequences are extracted from positive blood culture samples and hybridized with nanoparticle probes to capture targeted bacterial DNA sequences on a microarray. Automated optical imaging of the microarray determines the presence or absence of the specific sequence(s).

MCA

MCA is a novel technology, having just received FDA clearance in 2017. It provides fast phenotypic AST by exposing the identified organism to antibiotics in an automated system and measuring the dynamic features of the bacteria (morphology, mass, division rate, and growth patterns) as the bacteria respond to the antibiotics. Software analysis of these features generates MICs based on these features and on CLSI and FDA breakpoints.

Multiplex PCR

Multiplex PCR involves the use of multiple primer DNA sequence pairs to amplify more than 1 target sequence in a single reaction. Multiplex PCR allows for the ID of more than 1 pathogen as well as for the detection of specific resistance markers in those pathogens.

Phenotypic AST

Phenotypic AST is an assessment of how bacteria respond to an antibiotic. Traditional phenotypic AST methods required isolation of bacterial colonies from positive blood cultures, followed by incubation with antibiotics for standardized periods of time, and visible growth monitoring to measure susceptibility. Breakpoints (assessments of bacterial growth at breakpoint concentrations of antibiotics) calibrated to these incubation periods have been established by organizations such as the CLSI, the FDA, and the European Committee on Antimicrobial Susceptibility Testing. Phenotypic AST methods include automated systems that perform more sensitive biochemical detection of growth from the bacterial isolate samples and fast automated systems that use MCA to perform more sensitive microscopy-based detection of growth directly from samples such as positive blood cultures.

PCR

PCR is a molecular technology in which multiple copies of a segment of DNA can be produced. The technique involves the use of 2 short DNA sequences called primers, which are designed to bind to the beginning and end of a targeted DNA segment. The targeted DNA segment, the primers, free nucleotides, and the enzyme DNA polymerase are combined and placed into a PCR machine. The mixture is initially heated to denature and separate the double-stranded DNA into single strands. This is followed by cooling, which

facilitates binding of the primers to the single DNA strands. DNA polymerase then synthesizes new strands of DNA from the single-stranded templates beginning with the primers, resulting in a double-stranded DNA molecule consisting of 1 old DNA strand and 1 new DNA strand. Each new DNA molecule can serve as a template for repetitions of this cycle, such that millions of copies can be produced. The amplified DNA segment is then identified.

Resistance marker testing/genotypic AST

Resistance marker testing for antimicrobial resistance involves tests designed to detect the presence or absence of specific bacterial resistance gene sequences or markers. These markers are then used as a proxy for predicting how bacteria would respond to an antibiotic. Resistance marker testing does not allow for the measurement of susceptibility to antibiotics and is limited by variable expression of resistance markers (e.g., does the genotype accurately predict the phenotype) and inability to detect some acquired resistance markers. As such, the phrase "genotypic AST" is a misnomer, since resistance markers may effectively "rule out" antibiotic choices but cannot "rule in" the optimal choice for therapy. PCR and microarray testing are most commonly used for resistance marker testing, which do not provide AST results.

Singleplex PCR

In singleplex PCR, a single DNA target is amplified in 1 reaction using 1 set of primers. Singleplex PCR allows for the ID of a single pathogen or single resistance marker.

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