SUPPLEMENT ARTICLE

Review of Rapid Diagnostic Tests Used by Antimicrobial Stewardship Programs

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Rapid microbiologic tests provide opportunities for antimicrobial stewardship programs to improve antimicrobial use and clinical and economic outcomes. Standard techniques for identification of organisms require at least 48–72 hours for final results, compared with rapid diagnostic tests that provide final organism identification within hours of growth. Importantly, rapid microbiologic tests are considered "game changers" and represent a significant advancement in the management of infectious diseases. This review focuses on currently available rapid diagnostic tests and, importantly, the impact of rapid testing in combination with antimicrobial stewardship on patient outcomes.

Keywords. antimicrobial stewardship; rapid diagnostic test; outcomes; costs.

A key concept in the field of infectious diseases is organism identification with subsequent antimicrobial susceptibility testing. This information is critical in selecting appropriate antimicrobial therapy. The optimization of therapy remains a challenge, particularly in patients for whom timely antimicrobial administration within the first few hours of sepsis recognition is critical [1]. Kumar and colleagues demonstrated the critical nature of timely antibiotic administration in patients with septic shock, with each hour of delay (over the first 6 hours) resulting in a 7.6% decrease in survival [2]. This study illustrates the importance of the concept of "getting it right up front."

Standard techniques for identification of organisms are based on phenotypic methods, which require 48–72 hours to provide final results, compared with rapid diagnostic tests, which provide final results within hours of growth. Studies have demonstrated that rapid microbiologic tests benefit the individual patient by enabling timely antimicrobial optimization, which, in turn, may lead to decreased mortality, shortened hospital stay, and lower hospitalization costs. Rapid microbiologic tests are considered "game changers" and represent an important advancement in the management of infectious diseases [3].

This review focuses on currently available rapid diagnostic tests, and, importantly, the impact of rapid testing in combination with antimicrobial stewardship on patient outcomes.

TRADITIONAL MICROBIOLOGIC METHODS FOR ORGANISM IDENTIFICATION

Hospital microbiology laboratories provide organism identification through a variety of methods, including Gram stain and rapid biochemical tests in combination with culture-based techniques. Traditional microbiologic methods remain suboptimal in providing rapid identification and susceptibility testing. One study reported that the average time required for a microbiology laboratory to provide organism identification and antimicrobial susceptibility testing results to a clinician was 40 hours [4]. The need for rapid results is evident, and

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current rapid molecular identification methods can provide results within minutes to a few hours.

RAPID MOLECULAR METHODS FOR ORGANISM IDENTIFICATION

There are several commercially available rapid molecular assays for organism detection. A summary of the assays is presented in Table 1.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) uses a fluorescently labeled probe with 2 primers to amplify a piece of target DNA. This technique combines amplification and detection into 1 process. There are several commercially available, US Food and Drug Administration (FDA)–cleared assays that employ real-time PCR, including Roche Molecular System's LightCycler SeptiFast MecA, BD GeneOhm's Cdiff assay, Cepheid's Xpert *C. difficile* assay, and Gen-Probe Prodesse's ProGastro Cd.

Multiplex PCR

Multiplex PCR uses a fluorescently labeled probe but >1 set of primers. This technology can be used for simultaneous detection of multiple organisms and resistance markers. Currently, BD GeneOhm's Staph SR assay, Cepheid's Xpert MRSA/SA blood culture and *C. difficile*/Epi assays, and BioFire Diagnostics' FilmArray blood culture identification (BCID) panel uses multiplex PCR. The FilmArray BCID tests for 24 organisms, including *Staphylococcus* species, *Enterococcus* species, *Listeria monocytogenes*, *Streptococcus* species, *Acinetobacter baumannii*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Enterobacter cloacae* complex, *Escherichia coli*, *Klebsiella* species, *Proteus* species, *Serratia marcescens*, and *Candida* species. In addition, antimicrobial resistance genes are also detected, including *mec*A, *van*A/B, and carbapenem resistance.

Nanoparticle Probe Technology (Nucleic Acid Extraction and PCR Amplification)

Nanosphere's Verigene blood culture gram-positive (BC-GP) assay uses nucleic acid extraction and PCR amplification from a clinical sample followed by hybridization of target DNA to capture oligonucleotides on a microarray. After hybridization, signal amplification of hybridized probes provides an automated qualitative analysis of results.

This BC-GP assay can rapidly identify the presence of the following organisms: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, *Streptococcus anginosus* group, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Listeria* species. Additionally, the BC-GP test identifies 3 resistance markers, including *mecA*, *van*A, and *van*B.

Nanosphere's Verigene blood culture gram-negative test identifies genus, species, and genetic resistance determinants for a broad panel of gram-negative bacteria directly from positive blood culture bottles, including *E. coli*, *K. pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Acinetobacter* species, *Proteus* species, *Citrobacter* species, and *Enterobacter* species. The test can discern resistance markers for the KPC, NDM, CTX-M, VIM, IMP, and OXA genes. The test received FDA clearance in 2014, and to date there are no published studies demonstrating its impact on patient care.

Peptide Nucleic Acid Fluorescent In Situ Hybridization

Peptide nucleic acid fluorescent in situ hybridization (PNA FISH) (AdvanDx, Woburn, Massachusetts) was one of the first commercially available rapid diagnostic tests from blood cultures. PNA FISH technology uses synthetic oligonucleotide fluorescence-labeled probes. The neutral charge of the synthetic molecule allows rapid hybridization to species-specific ribosomal RNA after penetrating the cell membrane and cell wall of organisms. After hybridization, fluorescence is detected using a fluorescence microscope.

PNA FISH uses a variety of currently approved probes, including for *S. aureus*, coagulase-negative staphylococci (CoNS), *E. faecalis* and other enterococci, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei,* and *Candida tropicalis* (AdvanDx). Importantly, the methodology for PNA FISH has changed considerably since its initial introduction. The original turnaround time took 3 hours from a positive Gram stain to PNA FISH result. Recently, to decrease identification time, PNA FISH has been modified with the development of the QuickFISH testing platform. This has reduced the turnaround time from Gram stain to final result to 30 minutes [5]. Importantly, this allows for the simultaneous notification of the Gram stain and PNA FISH result.

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) provides rapid technology and is capable of analyzing thousands of samples per day from a variety of sources, including blood, respiratory, urine, and wound. Mass spectrometry results in ionization and disintegration of a target molecule. The mass/charge ratio of the resulting molecular fragments is analyzed to produce a molecular signature. The generated mass spectrum provides a profile or fingerprint of the organism that is compared with those of well-characterized organisms in a database. Currently, 2 MALDI-TOF MS platforms are available in the United States, MALDI Biotyper (Bruker Corporation, Billerica, Massachusetts) and Vitek MS System (bioMérieux, Durham, North Carolina).

Table 1. Rapid Molecular Assays for Detection of Various Organisms

Organism	Detection Time, h	Technology	Manufacturer	Batching	Need for Pure Colony	Automated	CLIA Designation	Trade Name
Gram-positive								
Staphylococcus aureus, CoNS	0.3	PNA QuickFISH	AdvanDx	No	No	No	High complexity	*S. aureus*/CoNS PNA QuickFISH
MRSA	0.1	Immunochromatography	Alere Scarborough, Inc	No	Yes	No	Moderate complexity	Alere PBP2a Culture Colony Test
S. aureus	0.2	Immunochromatography	Alere Scarborough, Inc	No	No	No	Not rated	BinaxNOW *S. aureus*
MSSA, MRSA	20–26	Chromogenic medium	BD	No	Yes	No	High complexity	BBL CHROMagar MRSA II
MRSA	2	PCR	Roche Diagnostics USA	Yes	No	Yes	High complexity	LightCycler MRSA
MSSA, MRSA, CoNS	2	Multiplex PCR	BD GeneOhm	Yes	No	Yes	High complexity	BD GeneOhm Staph SR
MSSA, MRSA, CoNS	1	Multiplex PCR	Cepheid	No	No	Yes	Moderate complexity	Xpert MRSA/SA BC
MSSA, MRSA	1	Multiplex PCR	Cepheid	No	No	Yes	Moderate complexity	Xpert MRSA/SA SSTI
S. aureus, *Staphylococcus epidermidis*	2.5	Multiplex PCR	Nanosphere	No	No	Yes	Moderate complexity	Verigene: BC-GP
Enterococcus faecalis, *Enterococcus faecium*	0.5	PNA QuickFISH	AdvanDx	No	No	No	High complexity	*Enterococcus faecalis*/OE PNA QuickFISH
Clostridium difficile	1	LAMP	Meridian Bioscience	Yes	No	Yes	Moderate complexity	Illumigene *C. difficile*
C. difficile	2	PCR	BD GeneOhm	Yes	No	Yes	Not rated	BD GeneOhm Cdiff Assay
C. difficile	0.5	Multiplex PCR	Cepheid	No	No	Yes	Moderate complexity	Xpert *C. difficile*
C. difficile	0.75	Multiplex PCR	Cepheid	No	No	Yes	Moderate complexity	Xpert *C. difficile*/Epi
C. difficile	3	PCR	Gen-Probe Prodesse	Yes	No	Yes	Not rated	ProGastro Cd Assay
Staphylococcus spp, *Streptococcus* spp, *E. faecalis*, *E. faecium*, *Micrococcus* spp, *Listeria* spp	2.5	Multiplex PCR	Nanosphere	No	No	Yes	Moderate complexity	Verigene: BC-GP

Table 1 continued.

Organism	Detection Time, h	Technology	Manufacturer	Batching	Need for Pure Colony	Automated	CLIA Designation	Trade Name
Gram-negative								
Escherichia coli, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*,	0.5	PNA QuickFISH	AdvanDx	No	No	No	High complexity	GNR Traffic Light PNA QuickFISH
E. coli, *K. pneumoniae*, *Klebsiella oxytoca*, *P. aeruginosa*, *Serratia marcescens*, *Acinetobacter* spp, *Proteus* spp, *Citrobacter* spp, *Enterobacter* spp	<2.0	Multiplex PCR	Nanosphere	No	No	Yes	Not rated	Verigene: gram-negative blood culture
Fungal pathogens								
Candida albicans, *Candida parapsilosis*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*	1.5	PNA FISH	AdvanDx	Yes	No	No	High complexity	Yeast Traffic Light PNA Fish
Other								
Multiple bacterial, fungal, and viral pathogens	1	Multiplex PCR	BioFire Diagnostics	Yes	No	Yes	Moderate complexity	FilmArray System and panels
Multiple bacterial and fungal pathogens	6 (direct from blood prior to culture)	PCR	Roche Molecular Systems[a]	Yes	No	Yes	Not rated	LightCycler SeptiFast Test MGRADE
Multiple bacterial and fungal pathogens	0.2	MALDI-TOF MS	Bruker Corporation	No	Yes	Yes	High complexity	MALDI Biotyper CA
Multiple bacterial and fungal pathogens	0.25–1	MALDI-TOF MS	bioMérieux	No	Yes	Yes	High complexity	VITEK MS
Multiple bacterial and fungal pathogens	6–24	Optical	bioMérieux	No	Yes	No	High complexity	VITEK 2

Abbreviations: BC-GP, blood culture gram-positive; CLIA, Clinical Laboratory Improvement Amendments; CoNS, coagulase-negative staphylococci; FISH, fluorescence in situ hybridization; LAMP, loop-mediated isothermal amplification; MALDI-TOF MS, matrix-assisted laser desorption/ionization–time of flight mass spectrometry; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; PCR, polymerase chain reaction; PNA, peptide nucleic acid; SSTI, skin and soft tissue infection.

[a] All tests are cleared by the US Food and Drug Administration except the Roche Molecular PCR system.

MICROBIOLOGIC TESTS AND ANTIMICROBIAL STEWARDSHIP

Staphylococcus aureus

Staphylococcus aureus infections constitute a tremendous burden on hospitals in the United States. Importantly, *S. aureus* bacteremia requires prompt microbiological diagnosis and antibiotic administration. Vancomycin is considered to be standard treatment for methicillin-resistant *S. aureus* (MRSA) bacteremia; however, studies have demonstrated that vancomycin is associated with significant limitations [6, 7]. If the organism is identified as methicillin-sensitive *S. aureus* (MSSA), vancomycin has been shown to be less active against MSSA than antistaphylococcal β-lactams, including nafcillin and cefazolin [8].

Several published studies have evaluated the role of rapid microbiologic technologies combined with antimicrobial stewardship in patients with *S. aureus* infections (Table 2). Carver and colleagues evaluated the use of PCR testing for the *mec*A gene combined with infectious disease pharmacist interventions in patients with *S. aureus* bacteremia. Using their microbiology laboratory's own PCR test, the authors demonstrated a 25.4-hour reduction in the time to optimal antibiotic therapy (64.7 hours ± 36.8 hours to 39.3 hours ± 15.5 hours; $P = .002$) with the combination of a rapid test and intervention vs the rapid test alone [25].

Parta et al evaluated the Xpert MRSA/SA BC assay with a result notification protocol in comparison to traditional methods. More patients in the rapid diagnostic and result notification protocol group who did not have *S. aureus* bacteremia had a significant decrease in antibiotic therapy (76% vs 55%; $P < .01$). Additionally, a 44.6-hour reduction in the mean time to appropriate therapy was also demonstrated, favoring the Xpert MRSA/SA BC assay with result notification protocol group [14].

Bauer and colleagues conducted an additional study using the Xpert MRSA/SA BC assay in combination with infectious disease pharmacist interventions in patients with *S. aureus* bacteremia. The mean time to switch from empiric vancomycin to either nafcillin or cefazolin in patients with MSSA bacteremia was 1.7 days shorter postimplementation ($P = .002$). In addition, the mean length of stay was 6.2 days shorter ($P = .07$) and the mean hospital costs were $21 387 less per patient ($P = .02$) in the postimplementation group. The authors concluded that the Xpert MRSA/SA BC assay in conjunction with infectious disease pharmacist interventions enables timely effective therapy and is associated with decreased length of stay and healthcare costs [15].

There are several studies demonstrating improved antibiotic use and patient outcomes using the PNA FISH probe for *S. aureus*. Schweizer et al reviewed patients with *S. aureus* bacteremia from 2003 to 2007. The authors reported decreased time to appropriate therapy after the PNA FISH assay was instituted, compared with pre-PNA FISH implementation (0.34 days vs 0.56 days; $P = .06$) [19].

Ly and colleagues performed a randomized study at a community medical center that compared the PNA FISH probe for *S. aureus* and pharmacist interventions with routine standard reporting to the treatment team. They documented a significant reduction in mortality in the intervention group compared with the standard management group (7.9% vs 16.8%; $P = .05$). In addition, hospitalization charges were decreased by $20 000 in the intervention group [22].

Importantly, one study failed to demonstrate improved patient outcomes or a decrease in antibiotic use with utilization of a rapid microbiologic test in patients with *S. aureus* skin and soft tissue infections (SSTIs). Terp and colleagues introduced the Xpert MRSA/SA SSTI assay in combination with physician education and pharmacist guidance. The authors were unable to significantly reduce excessive empiric MRSA-active antibiotics despite the test's accuracy. They concluded that introducing a rapid diagnostic test in the absence of an effective implementation strategy might be insufficient to produce intended results [17].

Coagulase-negative staphylococci are often considered a blood culture contaminant in the setting of only 1 positive blood culture bottle out of multiple bottles in a patient without fever, chills, or hypotension. CoNS that are determined to be a contaminant do not require antimicrobial therapy. Conversely, CoNS in multiple positive blood cultures require antimicrobial therapy, but often clinicians may erroneously determine the positive blood cultures to represent contamination and not initiate therapy. The combination of rapid diagnostic testing and antimicrobial stewardship intervention has demonstrated a positive impact in reducing unnecessary antibiotic use and also ensuring the treatment of patients with CoNS bacteremia.

Wong and colleagues evaluated the value of Xpert MRSA/SA BC assay and infectious disease pharmacist interventions in patients with a positive blood culture for CoNS. The authors reported several notable findings, including that antistaphylococcal antibiotics were discontinued 32.0 hours sooner ($P < .005$) from the time of PCR result, total antibiotic exposure decreased by 43.5 hours ($P < .011$), infection-related length of stay decreased by 4.5 days ($P < .018$), and infection-related costs decreased by $8338 ($P = .144$) in the postintervention group. Importantly, the pharmacist initiated vancomycin in 7 (21.9%) patients with CoNS bacteremia [16].

A retrospective study by Forrest et al showed that PNA FISH reduced vancomycin use for CoNS bacteremia, with subsequent reductions in median length of stay from 6 to 4 days ($P < .05$) and cost reductions of $4000 per patient in 2004 in non–intensive care unit (ICU) patients only [18]. In contrast, Holtzman and colleagues were unable to demonstrate an impact in hospital length of stay or vancomycin duration with PNA FISH in patients with CoNS bacteremia. The authors concluded that active notification or antimicrobial stewardship combined with rapid diagnostic testing is necessary to improve outcomes and antibiotic use [20].

Enterococci are gram-positive cocci represent the third most common type of healthcare-associated pathogen in the United States. Enterococci are intrinsically resistant to many antibiotics and also may acquire additional resistance determinants, including for vancomycin resistance. Vancomycin-resistant *Enterococcus* bacteremia is associated with suboptimal patient outcomes, including increased mortality; therefore, time to appropriate antimicrobial therapy is critical [26]. Empiric therapy for enterococcal bacteremia includes vancomycin, daptomycin, or linezolid. With rapid organism identification, including resistance determinants, antimicrobial stewardship programs can initiate and optimize therapy sooner, resulting in improved patient outcomes.

Sango and colleagues evaluated the impact of rapid organism identification and resistance detection with the Verigene BC-GP assay in patients with enterococcal bacteremia [9]. An infectious disease or critical care pharmacist was contacted with the assay results and subsequently recommended effective therapy to the treating clinician. The mean time to appropriate antimicrobial therapy was 23.4 hours shorter in the postintervention group than in the preintervention group ($P = .0054$). In the postintervention group, the hospital length of stay was significantly shorter (21.7 days; $P = .0484$), and mean hospital costs were $60 729 lower ($P = .02$) than in the preintervention group [9].

Forrest and colleagues demonstrated the benefits of the PNA FISH *E. faecalis* and other enterococci probe with antimicrobial stewardship on patient outcomes [21]. They evaluated >200 patients with enterococcal bacteremia. All *E. faecalis* cultures were ampicillin sensitive, but 20% were vancomycin resistant whereas the other enterococci, which were all *E. faecium*, were 100% resistant to ampicillin and 85% resistant to vancomycin. Based on these results, the stewardship program would recommend daptomycin or linezolid to the prescribing clinician if the result was other enterococci. PNA FISH identified *E. faecalis* a median of 3 days earlier and other enterococci 2.3 days earlier compared with standard microbiology ($P < .001$). The other enterococci also had significantly shorter time to initiation of effective therapy (1.3 days vs 3.1 days; $P < .001$) and decreased 30-day mortality (26% vs 45%; $P = .04$) [21]. Gamage et al reported similar findings, with a time to appropriate therapy decrease from 4.4 days to 1.1 days ($P < .001$) and significant reductions in mortality in ICU patients ($P = .04$) [27].

Gram-Negative Organisms

Infections caused by gram-negative organisms have become a top healthcare priority. Gram-negative infections are commonly associated with suboptimal patient outcomes, including increased length of stay, mortality, and healthcare costs. Unfortunately, an increased number of gram-negative organisms have become multidrug resistant, and the antibiotic pipeline is limited for the treatment of these infections [10, 12]. This has led to the use of combinations of antibiotics and relatively toxic antibiotics. There is an urgent need to combine rapid microbiologic tests with stewardship interventions in the treatment of gram-negative infections.

The PNA FISH "traffic light" can identify a variety of gram-negative organisms from positive blood cultures. The assay can identify *E. coli*, *P. aeruginosa*, and *K. pneumoniae* (where *E. coli* fluoresces green, *P. aeruginosa* red, and *K. pneumoniae* yellow). No clinical studies have evaluated the impact of this assay on patient outcomes, which reflects the limitation of PNA FISH in its inability to identify resistance genes in gram-negative organisms, forcing stewardship members and providers to rely on the hospital's antibiogram to direct empiric therapy.

A limited number of studies have evaluated the benefits of MALDI-TOF MS combined with stewardship interventions in patients with bacterial and fungal infections. Clerc et al found that the addition of MALDI-TOF MS for rapid identification of gram-negative isolates from positive blood cultures following gram-stain results impacted antibiotic choice for a greater percentage of patients with ID consultation, compared with Gram stain results alone (35.1% vs 20.8%; P not reported) [11].

Perez and colleagues found that in patients with gram-negative bacteremia, MALDI-TOF MS and antimicrobial susceptibility testing performed directly on positive blood cultures, combined with real-time notification and infectious disease pharmacist intervention, resulted in a 46-hour reduction ($P = .004$) in time to antibiotic optimization, compared with conventional methods [13]. Additionally, rapid results and intervention improved the time to active treatment by 36.7 hours in patients with inactive empiric therapy ($P < .001$). Importantly, a decreased length of stay (11.9 days vs 9.3 days; $P = .01$) and reductions in hospital costs per patient ($45 709 vs $26 126; $P = .009$) were also observed [13].

Huang and colleagues [28] evaluated the impact of MALDI-TOF MS and stewardship intervention in patients with bacteremia or candidemia. Compared with traditional methods, the impact of MALDI-TOF MS combined with real-time notification to a member of the stewardship team yielded 43-hour ($P < .001$) and 9.7-hour ($P = .021$) reductions in time to antibiotic optimization and active therapy, respectively. In addition, a 2.8-day decrease in mean length of stay ($P = .066$) and reduced mortality (20.3% vs 14.5%; $P = .02$) was observed, favoring the intervention group [28].

Perez et al evaluated the clinical and economic effects of rapid identification with MALDI-TOF MS and susceptibility testing coupled with active antimicrobial stewardship on patients with bloodstream infections caused by multidrug-resistant and/or ESBL-producing gram-negative bacteria [29]. Integrating rapid diagnostics with antimicrobial stewardship improved time to optimal antibiotic therapy (80.9 hours in the preintervention period vs 23 hours in the intervention period; $P < .001$). Importantly, mortality among patients during the intervention period was lower (21% vs 8.9%; $P = .01$) [29].

Table 2. Summary of Rapid Diagnostic Studies and Antimicrobial Stewardship

Diagnostic Test/ Assay	Study	Organisms	Population	Findings
Verigene: BC-GP	Sango et al [9]	*Enterococcus* spp	74 patients with documented enterococcal bacteremia (46 pre–BC-GP, 28 post–BC-GP)	Mean time to appropriate antimicrobial therapy was 23.4 h shorter in the postintervention group than in the preintervention group ($P = .0054$). In the postintervention group, the hospital LOS was significantly shorter (21.7 d; $P = .0484$) and mean hospital costs were $60 729 lower ($P = .02$) than in the preintervention group.
MALDI-TOF MS	Perez et al [13]	Gram-negative	201 patients with gram-negative bacteremia surviving to hospital discharge (100 preintervention, 101 intervention)	MALDI-TOF MS and antimicrobial susceptibility testing performed directly on positive blood cultures combined with real-time notification and ID pharmacist intervention resulted in a 46-hour reduction ($P = .004$) in time to antibiotic optimization compared with conventional methods; rapid results and intervention improved time to active treatment by 36.7 h in patients with inactive empiric therapy ($P < .001$). Decreased LOS (11.9 d vs 9.3 d; $P = .01$) and reductions in hospital costs per patient ($45 709 vs $26 126; $P = .009$) were also observed.
	Huang et al [28]	Aerobic gram-positive and gram-negative organisms and yeast isolates	501 patients with bacteremia or candidemia (256 preintervention, 245 intervention)	Intervention group: decreased time to organism identification of 84.0 vs 55.9 h, ($P < .001$), improved time to effective antibiotic therapy of 30.1 vs 20.4 h ($P = .021$), and optimal antibiotic therapy (90.3 vs 47.3 h; $P < .001$), 2.8-day decrease in mean LOS ($P = .07$) and reduced mortality (20.3% vs 14.5%; $P = .02$).
	Clerc et al [11]	Gram-negative	202 patients with a first episode of gram-negative bacteremia leading to an ID consultation	Addition of MALDI-TOF MS for rapid identification of gram-negative isolates from positive blood cultures following Gram stain results impacted choice of antibiotic for a greater percentage of patients with ID consultation compared with Gram stain results alone (35.1% vs 20.8%; P = NR).
	Wenzler et al [24]	*Acinetobacter baumannii*	109 patients with pneumonia and/or bacteremia (66 preintervention, 53 intervention)	MALDI-TOF MS combined with stewardship interventions resulted in a significant reduction in time to effective therapy (77.7 h vs 36.6 h; $P < .0001$) and increase in clinical cure (15% vs 34%; $P = .016$).
	Perez [29]	Gram-negative	265 patients with antibiotic-resistant gram-negative bacteremia (112 preintervention, 153 intervention)	Rapid diagnostic testing with stewardship improved time to optimal antibiotic therapy (80.9 h vs 23 h; $P < .001$). Mortality among patients during the intervention period was lower (21% vs 8.9%; $P = .01$).
Xpert MRSA/ SA—blood culture	Parta et al [14]	*Staphylococcus* spp	212 patients with GPCC (89 in group 1, whose physicians were notified of results by use of Xpert MRSA/SA BC, 123 patients in group 2, with delayed reporting after traditional microbiological studies)	Patients in rapid diagnostic and result notification protocol group who did not have *S. aureus* bacteremia had a significant decrease in treatment for *S. aureus* infection (76% vs 55%; $P < .01$). Patients with MSSA had significantly reduced mean time to initiation of β-lactam therapy (44.6-h reduction).
	Bauer et al [15]	*Staphylococcus* spp	156 patients with *Staphylococcus aureus* (74 pre-rPCR, 82 post-rPCR)	Mean time to switch from empiric to targeted antimicrobial therapy in patients with MSSA was 1.7 d shorter after rPCR ($P = .002$). In the post-rPCR MSSA, and MRSA groups, mean LOS was reduced by 6.2 d ($P = .07$). Mean hospital costs were reduced by $21 387 ($P = .02$) for the post-rPCR group.
	Wong et al [16]	CoNS	53 patients (31 preintervention, 22 intervention)	In postintervention group: antistaphylococcal antibiotics were discontinued 32.0 h sooner from time of rPCR result (median, 57.7 vs 25.7 h; $P = .005$), total antibiotic exposure was decreased by 43.5 h (97.6 vs 54.1 h; $P = .011$), infection-related LOS was decreased by 4.5 d (10 vs 5.5 d; $P = .018$), infection-related costs were decreased by $8338 ($28 973 vs $20 635; $P = .144$). Vancomycin was initiated in 7 (21.9%) patients with CoNS bacteremia.

Table 2 continued.

Diagnostic Test/ Assay	Study	Organisms	Population	Findings
Xpert MRSA/SA SSTI—PCR assay	Terp et al [17]	MRSA	165 patients with purulent SSTI	No significant reduction in excessive empiric prescription of MRSA-active antibiotics in the absence of an effective stewardship implementation strategy.
PNA FISH	Forrest et al 2006 [18]	CoNS	87 patients (53 with CoNS, 34 with positive blood cultures with GPCC not tested in same time period in control group)	Case patients: significant reduction in median LOS from 6 to 4 d in PNA FISH group ($P < .05$; CI, .95–1.87); decrease in costs of approximately $4000 per patient.
	Schweizer et al [19]	*S. aureus*	814 patients with bacteremia admitted between 2001 and 2007	Of 774 patients who received appropriate antimicrobial therapy, the time to appropriate therapy was shorter among patients who were admitted after the PNA FISH assay was instituted compared to pre–PNA FISH implementation (0.34 d vs 0.56 d; $P = .06$).
	Holtzman et al [20]	*S. aureus*, CoNS	199 patients (100 pre–PNA FISH, 99 post–PNA FISH)	No reduction in LOS or vancomycin use. Study did not include active notification or antimicrobial stewardship intervention.
	Forrest et al [21]	*Enterococcus* spp	224 patients with hospital-acquired enterococcal bacteremia (129 preintervention period, 95 PNA FISH period)	PNA FISH identified *E. faecalis* a median of 3 d earlier and OE 2.3 d earlier compared with standard microbiology ($P < .001$). The OE had significantly shorter time to initiation of effective therapy (1.3 d vs 3.1 d; $P < .001$) and decreased 30-day mortality (26% vs 45%; $P = .04$).
	Ly et al 2008 [22]	*S. aureus*	202 patients with gram-positive cocci in clusters and blood cultures	Significant reduction in mortality in the intervention group compared with the standard management group (7.9% vs 16.8%; $P = .05$); hospitalization charges were less by approximately $20 000 in the intervention group.
Yeast Traffic Light PNA FISH	Heil et al [36]	*Candida* spp	82 patients with blood cultures testing positive for yeast (61 preimplementation of PNA FISH assay, 21 postimplementation)	Postimplementation group: mean time to targeted therapy of 0.6 d vs 2.3 d preimplementation ($P = .0016$), median time to culture clearance of 4 vs 5 d ($P = .01$). PNA FISH test reduced pharmacy costs by >$400 per patient.
CAG PNA FISH	Forrest et al [33]	*C. albicans*	72 patients with candidemia	PNA FISH facilitated the rapid identification of *C. albicans* in 31 of 72 patients and resulted in a significant decrease in the use of caspofungin. Cost savings of $1729 per patient were realized.

Abbreviations: BC-GP, blood culture gram-positive; CAG, *Candida albicans/glabrata*; CI, confidence interval; CoNS, coagulase-negative staphylococci; GPCC, gram-positive cocci in clusters; ID, infectious disease; LOS, length of stay; MALDI-TOF MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; NR, not reported; OE, other enterococci; PBP2a, penicillin-binding protein 2a; PNA FISH, peptide nucleic acid fluorescence in situ hybridization; rPCR, random polymerase chain reaction; SSTI, skin and soft tissue infection.

Tamma and colleagues evaluated 222 hospitalized patients whose clinical isolates were tested using standard methods and MALDI-TOF MS. The authors determined that MALDI-TOF MS could have reduced time to appropriate therapy for 28.8% and 44.6% patients based on the treating physician's choices and stewardship team recommendations, respectively [30].

Finally, Wenzler et al conducted a study to evaluate the impact of MALDI-TOF MS and stewardship intervention in patients with *Acinetobacter baumannii* pneumonia and/or bacteremia. The authors demonstrated a significant reduction in time to effective therapy (77.7 hours vs 36.6 hours; $P < .0001$) and increase in clinical cure (15% vs 34%; $P = .016$) with the use of MALDI-TOF MS combined with stewardship interventions. This study demonstrated similar benefits observed in previous studies, but, importantly, in patients infected with a multidrug resistant-organism associated with pneumonia and/or bacteremia [24].

Candida Species

Candidemia represents the fourth most common cause of nosocomial bloodstream infection in the United States [31]. Time to positive blood culture and species identification can take several days from the time the blood culture is drawn. This can increase the amount of time to effective antifungal therapy and hospital mortality if antifungal therapy is not administered until candidemia is confirmed [23, 32].

Currently, 2 PNA FISH probes are available: the *C. albicans/glabrata* (CAG) probe and the yeast traffic light (YTL). The YTL can detect *C. albicans*/*C. parapsilosis* as green fluorescence, *C. glabrata*/*krusei* as red fluorescence, and *C. tropicalis* as yellow fluorescence. A study showed that PNA FISH in combination with antimicrobial stewardship resulted in a significant reduction in caspofungin usage and a cost savings of $1729 per patient [33]. A second institution implemented the CAG FISH probe with direct fluconazole susceptibility, but could not demonstrate any benefits, as the providers did not use the results and instead waited for the susceptibility results [34]. This study illustrates the importance of combining a rapid diagnostic test with stewardship intervention. In contrast, another institution reported a 3-day improvement in time to appropriate therapy, which resulted in antifungal savings of $1500 per patient and overall savings of nearly $340 000 when CAG FISH was combined with stewardship intervention [35]. These results were replicated at a different institution that not only demonstrated cost savings of almost $2 million, but also decreased mortality from 26% before implementation of PNA FISH to 4.5% after implementation ($P < .001$) in the ICU setting [27]. The YTL probe offers a treatment-directed approach with the ability to identify the 5 most common *Candida* species. Heil et al [36] evaluated the YTL PNA FISH probe with stewardship intervention. The YTL probe identified *Candida* species 4 days sooner, which resulted in a decreased time to targeted therapy ($P = .0016$) and time to candidemia clearance ($P = .01$), and significantly positively impacted the pharmacy budget with >$400 savings per patient [36].

Clostridium difficile

Clostridium difficile has become an increasingly challenging infection with increases in severity of disease, clinical failure, and recurrences [37–39]. In addition, in recent years the epidemiology has markedly changed with the emergence of a previously rare strain (North American pulsed-field gel electrophoresis type 1/restriction endonuclease analysis BI/PCR ribotype 027 [NAP1/BI/027]). This strain demonstrates increased toxin production, contributing to a higher proportion of severe disease and reduced response to traditional therapy [40].

Historically, the in vitro cytotoxin neutralization assay was considered the gold standard for diagnosis. However, many laboratories have relied on the rapid enzyme immunoassay, detecting toxin A, toxin B, or both. Both methodologies lack sensitivity and the ability to provide rapid results [41]. Currently, there are 4 available PCR-based tests for the detection of *C. difficile*. An additional technology, loop-mediated isothermal amplification, is also available.

Of note, PCR technology is the most sensitive test for *C. difficile*; therefore, the rate of positive tests can more than double in the hospital setting [42]. For this reason, it is imperative that antimicrobial stewardship programs provide education to providers and hospital administration prior to the implementation of PCR testing. In addition, a stewardship-initiated protocol should also prohibit providers from ordering daily tests, as the positive predictive value decreases significantly with repeat testing. Currently, there are no published studies evaluating the impact of *C. difficile* assays in combination with antimicrobial stewardship.

CONSIDERATIONS FOR ANTIMICROBIAL STEWARDSHIP PROGRAMS

Rapid microbiologic methods provide collaborative opportunities for antimicrobial stewardship programs to work with administration, physicians, and microbiology laboratory personnel to improve patient outcomes and decrease antimicrobial use. It is imperative that antimicrobial stewardship programs have an active role in the implementation and incorporation of rapid microbiologic tests (Table 3). Rapid diagnostic tests are of little value if an antimicrobial stewardship program does not have a role as an active messenger and educator of the results. Notably, studies have demonstrated that rapid results are of little value if the tests are not acted on in a timely manner. It is important to consider which microbiologic tests will be implemented and should be based on prevalent or problematic organisms within the hospital setting. Consideration should also be given to the sensitivity and specificity of each test, as variability exists among the various rapid diagnostic

Table 3 Antimicrobial Stewardship Program Checklist for Rapid Diagnostic Tests

Preimplementation
- Identify most useful RDT based on hospital pathogen prevalence
  - Example: Number of *Staphylococcus aureus* bacteremias, number of coagulase-negative staphylococci, number of *Pseudomonas aeruginosa*, number of *Candida* species
- Identify hospital cost of infection
  - Example:
    - Utilize information warehouse personnel to pull cost by *ICD-9* code mortality data
    - Obtain time to ID specialist consult
    - Length of stay
    - 30-day readmission
- Time to effective therapy

Implementation
- Microbiologist-validated RDT instrument
- Determine if test is done in real time 24/7 or batch
- Communication of RDT results from microbiologist to physician and ASP pharmacist is established
- ASP pharmacist-physician educates medical staff
- ASP documents interventions and acceptance rate

Postimplementation
- Time to effective therapy
- Time to discontinuation or de-escalation
- Time to ID consult
- Documented negative blood culture prior to hospital discharge
- 30-day readmission
- Mortality

Abbreviations: ASP, antimicrobial stewardship program; *ICD-9, International Classification of Diseases, Ninth Revision*; ID, infectious diseases; RDT, rapid diagnostic test.

technologies and organisms. Microbiology personnel and stewardship members should also consider purchase price or lease agreement of the instrument, cost of a fluorescence microscope for PNA FISH, test supplies, laboratory space, and complexity of the test. A specific consideration for the implementation of MALDI-TOF MS is the interface with various susceptibility platforms. Currently, MALDI-TOF MS only has an interface with the Vitek platform. It is mandatory that microbiology laboratory personnel and antimicrobial stewardship members work together to determine the best approach to justify the overall institutional costs of the tests. The potential for a 3- or 4-month pilot study should also be considered. Antimicrobial stewardship programs must provide appropriate documentation, including avoidance of unnecessary antimicrobial use, additional testing, and infection-control savings associated with decreased infections owing to reductions in transmission of organisms. Importantly, antimicrobial stewardship programs must document the clinical and economic outcomes associated with each test to appropriately demonstrate the benefit to improved patient care.

Finally, several pharmaceutical companies have recently collaborated with rapid diagnostic companies in an effort to help provide transformational change in the field of infectious diseases [43, 44]. Cepheid has partnered with Cubist Pharmaceuticals, AstraZeneca, and GlaxoSmithKline to expand development of the Xpert Carba-R rapid molecular test to identify carbapenemase-producing gram-negative bacteria. Merck Global Health Innovation is funding the expansion of AdvanDx's rapid diagnostic tests. The collaboration between these companies will help to provide additional tools in the antimicrobial steward's toolbox.

CONCLUSIONS

In the treatment of infections, timely administration of antimicrobial therapy is critical in optimizing patient outcomes. Rapid microbiologic technologies in combination with antimicrobial stewardship have demonstrated significant decreases in time to appropriate therapy and optimization of clinical and economic outcomes, including hospital length of stay, mortality, and healthcare costs. It is likely that the combination of rapid microbiologic tests and antimicrobial stewardship will continue to demonstrate significant improvements in patient outcomes and antimicrobial use.

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