Current Pharmacology Reports (2023) 9:198–216
https://doi.org/10.1007/s40495-023-00323-1

Rapid Diagnostics to Enhance Therapy Selection for the Treatment of Bacterial Infections

HaYoung Ryu[1] · Ahmed Abdul Azim[2] · Pinki J. Bhatt[2,3] · Priyanka Uprety[4] · Sana Mohayya[5] · Deepali Dixit[3,5] · Thomas J. Kirn[2,6] · Navaneeth Narayanan[2,3,5,7]

Accepted: 20 May 2023 / Published online: 29 June 2023
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2023

Abstract

Purpose of Review Rapid diagnostic tests (RDTs) may reduce morbidity and mortality related to bacterial infections by reducing time to identification of pathogens and antibiotic resistance mechanisms. There has been a significant increase in the breadth and depth of available technology utilized by RDTs.

Recent Findings There are numerous Food and Drug Administration (FDA)-cleared assays for rapid detection of bacteria from various specimen types from sites including blood, stool, central nervous system, and respiratory tract. Most RDTs currently FDA-cleared are molecular tests designed as syndromic panels that provide identification of on-panel organisms and resistance genes. One FDA-cleared rapid phenotypic assay for antimicrobial susceptibility testing is currently available and others are in development. Studies of these technologies' clinical impact consistently demonstrate improvements in clinical care processes such as time to de-escalation and escalation of antibiotic therapy, particularly for blood and respiratory specimen tests. Other RDTs show inconsistent impact on antibiotic use. Antimicrobial stewardship programs are vital to ensure the greatest benefit from RDTs in clinical practice.

Summary The advancement and implementation of RDTs, in conjunction with antimicrobial stewardship, to enhance treatment selection for bacterial infections should be regarded as a core element to improve clinical outcomes for patients. Although challenges exist in the use of RDTs, there is a need for continued innovation in technology, implementation science, and collaboration across clinical professions to optimize care.

Keywords Bacterial infections · Rapid diagnostics · Antimicrobial stewardship · Clinical microbiology

Introduction

Clinicians are at the forefront of the post-antibiotic era where antimicrobial resistance (AMR) in bacterial infections are yielding a global mortality and morbidity burden on par with major infectious diseases such as HIV and malaria [1••, 2, 3••]. In the USA alone, over 2.8 million AMR infections and 35,000 associated deaths were estimated in 2019 [1••]. Novel and concerning forms of AMR have been observed across the globe and continue to emerge

✉ HaYoung Ryu
hayoungr@buffalo.edu

✉ Navaneeth Narayanan
navan12@pharmacy.rutgers.edu

1 Department of Pharmacy, Oregon Health & Sciences University Hospital and Clinics, Portland, OR, USA

2 Division of Infectious Diseases, Allergy and Immunology, Department of Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, USA

3 Department of Pharmacy Practice and Administration, Rutgers University Ernest Mario School of Pharmacy, Piscataway, NJ, USA

4 Becton, Dickinson and Company, Life Sciences - Integrated Diagnostic Solutions, Sparks, MD, USA

5 Department of Pharmacy, Robert Wood Johnson University Hospital, New Brunswick, NJ, USA

6 Department of Pathology & Laboratory Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, USA

7 Center of Excellence in Pharmaceutical Translational Research and Education, Rutgers University Ernest Mario School of Pharmacy, Piscataway, NJ, USA

[4–6]. The economic burden is substantial with estimates of $4.6 billion in US healthcare costs for treatment of hospitalized patients with AMR bacterial infections in 2017 [7]. The COVID-19 pandemic has exacerbated an already difficult-to-contain global health threat. In the USA, years of progress to reduce the burden of AMR infections and related deaths were reversed during the SARS-CoV-2 pandemic [8•].

Rapid diagnostic tests (RDTs) represent a core tool in the effort against AMR and, in turn, patient care optimization. Time to effective antibiotic therapy is a key mediator of clinical outcomes in septic patients as a delay in effective antibiotics is associated with decreasing survival [9]. Rapid identification of pathogens to tailor therapy is crucial but it is also paramount to have rapid detection of resistance genes or rapid phenotypic susceptibility to escalate therapy as needed [10••]. This is an unfortunate reality in the setting of growing AMR globally even to novel agents slated to treat AMR bacteria [11]. In contrast, RDTs can also facilitate de-escalation or discontinuation of unnecessary antibiotics within hours as opposed to days when using conventional identification and susceptibility testing methods [12•]. For these reasons, antimicrobial stewardship guidelines advocate for the use of RDTs to optimize antibiotic therapy, all in the context of a multidisciplinary antimicrobial stewardship team (physicians, pharmacists, clinical microbiologists) to interpret, apply, and optimize RDTs [13–15]. In this review, we will critically evaluate recent literature in this area and provide insight into the findings.

Rapid Diagnostic Tests for Bacterial Infections

Traditional growth-based phenotypic methods have been the main tools for bacterial identification and susceptibility testing in clinical microbiology laboratories [16–18]. While conventional phenotypic antimicrobial susceptibility test (AST) methods like broth microdilution (BMD) and disk diffusion are the reference AST methods, they are limited by long turnaround time (TAT) and significant hands-on time to perform the test by a well-trained microbiology laboratory technologist.

The evolution of RDTs that use genotypic or rapid phenotypic methods have revolutionized the field of clinical microbiology. Genotypic methods like multiplex polymerase chain reaction (PCR)-based syndromic panels have allowed for identification of organisms and resistance determinants within 2–4 h. Rapid phenotypic methods combined with automated fluorescent in situ hybridization (FISH) technology have allowed for rapid bacterial identification and antibiotic susceptibility results [19].

There are various Food and Drug Administration (FDA)-cleared assays that allow for rapid detection of microorganisms from blood, stool, and respiratory specimens. This section provides an overview of notable rapid diagnostic technologies currently available to detect pathogens using various clinical specimens. Tables 1 and 2 list available FDA-cleared RDTs used for bacterial infections and their key features, including the targeted bacteria, resistance determinants, and turnaround times.

Fluorescent In Situ Hybridization/Rapid Phenotypic AST

Combining FISH with time-lapse microscopy allows the Accelerate Pheno® system (Accelerate Diagnostics, Tucson, AZ, USA) to identify microorganisms through FISH probes and provide rapid phenotypic AST in a fully automated manner directly from positive blood cultures [19]. The assay is capable of detecting a number of bacteria (both Gram-positive and -negative) and yeast. In a study evaluating the use of the Accelerate Pheno® system in positive blood cultures for Gram-negative bacteria, this system correctly detected organisms in 88.7% of all bacteremia episodes, and 97.1% of the on-panel isolates were correctly identified using the system's panel; in addition, 91.3% of specimens containing on-panel organisms yielded AST results [19]. Compared to culture-based methods, the Accelerate Pheno® system decreased time to pathogen identification by 28 h, and for antimicrobial susceptibility by almost 41 h [19].

Multiplex Array Polymerase Chain Reaction

RDTs known as 'syndromic panels' are utilized in clinical practice to identify infections with pathogen(s) (e.g., meningoencephalitis; respiratory tract infections; gastroenteritis). For diagnostics, some panels utilize multiplex PCR technology to help identify multiple pathogens in a single test. In 2015, the FDA approved the FilmArray® Meningitis/Encephalitis panel (Biofire® Diagnostics, Salt Lake City, UT, USA) for use in cerebrospinal fluid (CSF) samples obtained from patients with meningitis or encephalitis, with a TAT of approximately 60 min. This test detects 6 bacteria, 7 viruses, and *Cryptococcus neoformans/gattii*. The FilmArray® Gastrointestinal (GI) panel (Biofire® Diagnostics, Salt Lake City, UT, USA) can be used on stool samples from patients with a diarrheal illness, with a TAT of 60 min. This test can detect 22 targets (bacterial, viral, and parasitic), including species of *Campylobacter, Vibrio, Escherichia coli, Yersinia, Salmonella*, and *Plesiomonas* (Table 2). As for respiratory syndromes, the FilmArray® Respiratory panel (Biofire® Diagnostics, Salt Lake City, UT, USA) detects up to 22 targets, including 4 bacterial species (*Bordetella parapertussis, B. pertussis, Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*), with a TAT of 45–60 min (Table 2). Another similar panel is GenMark ePlex® RP or RP2 panel

Table 1 US FDA-cleared rapid diagnostic assays for the identification of bacteria and/or bacterial resistance determinants from blood specimens

Assay (manufacturer)	Specimen type	Category	Targeted organism(s)	Genotypic resistance determinants	Phenotypic Identification	Run time (min)
FilmArray® BCID2 (BioFire®)	Positive blood culture	Gram-positive bacteria (molecular)	*Enterococcus faecalis* *Enterococcus faecium*	*vanA, vanB*	VRE, VSE	60
			Staphylococcus spp. *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus lugdunensis*	*mecA/C*, MREJ	MRSA, MSSA, MSSE, MRSE	
			Listeria monocytogenes *Streptococcus* spp. *Streptococcus agalactiae* *Streptococcus pneumoniae* *Streptococcus pyogenes*			
		Gram-negative bacteria (molecular)	*Acinetobacter calcoaceticus-baumannii* complex *Bacteroides fragilis* Enterobacterales *Enterobacter cloacae* complex *Escherichia coli* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae* group *Proteus* spp. *Salmonella* spp. *Serratia marcescens* *Haemophilus influenzae* *Neisseria meningitidis* *Pseudomonas aeruginosa* *Stenotrophomonas maltophilia*	CTX-M KPC NDM VIM OXA-48-like IMP *mcr-1*	NS to ESCs (exception: cefepime — may be S or NS) NS to carbapenems[1] (ertapenem [Enterobacterales only], meropenem, imipenem, doripenem) R to colistin	

Table 1 (continued)

Assay (manufacturer)	Specimen type	Category	Targeted organism(s)	Genotypic resistance determinants	Phenotypic Identification	Run time (min)
ePlex® BCID-GP Panel (GenMark Dx®)	Positive blood culture	Gram-positive bacteria (molecular)	*Enterococcus* *Enterococcus faecalis* *Enterococcus faecium*	*vanA, vanB*	VRE, VSE	90
			Staphylococcus *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus lugdunensis*	*mecA, mecC*	MRSA, MSSA, MSSE, MRSE	
			Bacillus cereus group *Bacillus subtilis* group *Corynebacterium* *Cutibacterium acnes* *Lactobacillus* *Listeria* *Listeria monocytogenes* *Micrococcus* *Streptococcus* *Streptococcus agalactiae* *Streptococcus anginosus* group *Streptococcus pneumoniae* *Streptococcus pyogenes*			
			Pan Gram-negative Pan *Candida*			

Table 1 (continued)

Assay (manufacturer)	Specimen type	Category	Targeted organism(s)	Genotypic resistance determinants	Phenotypic Identification	Run time (min)
ePlex® BCID-GN Panel (GenMark Dx®)	Positive blood culture	Gram-negative bacteria (molecular)	*Acinetobacter baumannii* *Bacillus fragilis* *Citrobacter* *Cronobacter sakazakii* Enterobacter (non-cloacae complex) *Enterobacter cloacae complex* *Escherichia coli* *Fusobacterium nucleatum* *Fusobacterium necrophorum* *Haemophilus influenzae* *Klebsiella oxytoca* *Klebsiella pneumoniae* group *Morganella morganii* *Neisseria meningitidis* *Proteus* *Proteus mirabilis* *Pseudomonas aeruginosa* *Salmonella* *Serratia* *Serratia marcescens* *Stenotrophomonas maltophilia*	CTX-M IMP KPC NDM OXA-23, OXA-48 VIM	NS to ESCs (exception: cefepime — may be S or NS) NS to carbapenems[1] (ertapenem [Enterobacterales only], meropenem, imipenem, doripenem)	90
			Pan Gram-positive Pan *Candida*			
T2Bacteria® Panel (T2 Biosystems®)	Whole blood	Bacteria (NMR/molecular)	*Staphylococcus aureus* *Enterococcus faecium* *Pseudomonas aeruginosa* *Escherichia coli* *Klebsiella pneumoniae*	N/A (separate panel)		180–300
Verigene® BC-GP (Luminex®)	Positive blood culture	Gram-positive bacteria (molecular)	*Enterococcus faecalis* *Enterococcus faecium*	*vanA, vanB*	VRE, VSE	150
			Staphylococcus *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus lugdunensis*	*mecA*	MRSA, MSSA, MSSE, MRSE	
			Streptococcus agalactiae *Streptococcus pneumoniae* *Streptococcus pyogenes* *Streptococcus anginosus*			

Table 1 (continued)

Assay (manufacturer)	Specimen type	Category	Targeted organism(s)	Genotypic resistance determinants	Phenotypic Identification	Run time (min)
Verigene® BC-GN (Luminex®)	Positive blood culture	Gram-negative bacteria (molecular)	*Escherichia coli* *Shigella* *Klebsiella pneumoniae* *Klebsiella oxytoca* *Pseudomonas aeruginosa* *Acinetobacter* spp. *Citrobacter* spp. *Enterobacter* spp. *Proteus* spp.	CTX-M IMP KPC NDM OXA VIM	NS to ESCs (exception: cefepime — may be S or NS) NS to carbapenems[1] (ertapenem [Enterobacterales only], meropenem, imipenem, doripenem)	150
Accelerate PhenoTest® BC Kit (Accelerate Diagnostics)	Positive blood culture	Gram-positive bacteria (phenotypic)	*Staphylococcus aureus* *Staphylococcus lugdunensis* Coagulase-negative *Staphylococcus* spp. *Enterococcus faecalis* *Enterococcus faecium* *Streptococcus* spp.	N/A	N/A	420
		Gram-negative bacteria (phenotypic)	*Escherichia coli* *Klebsiella* spp. *Enterobacter* spp. *Proteus* spp. *Citrobacter* spp. *Serratia marcescens* *Pseudomonas aeruginosa* *Acinetobacter baumannii*			
		Yeast (phenotypic)	*Candida albicans* *Candida glabrata*			
Xpert® MRSA/SA Blood Culture (Cepheid®)	Positive blood culture	Gram-positive bacteria	*Staphylococcus aureus*	*spa*, *mecA*, SCC_{mec}	MRSA, MSSA	50–60
Xpert® Carba-R (Cepheid®)	Culture isolates	Gram-negative bacteria	N/A[2]	KPC NDM VIM OXA-48 IMP	NS to carbapenems[1] (ertapenem [Enterobacterales only], meropenem, imipenem, doripenem)	60

MRSA, methicillin-susceptible *S. aureus*; *MSSA*, methicillin-resistant *S. aureus*; *MSSE*, methicillin-susceptible *S. epidermidis*; *MRSE*, methicillin-resistant *S. epidermidis*; *VRE*, vancomycin-resistant *Enterococcus*; *VSE*, vancomycin-susceptible *Enterococcus*; *NS*, non-susceptible (i.e., intermediate or resistant); *S*, susceptible; *R*, resistant; *ESC*, extended-spectrum cephalosporin; *NMR*, nuclear magnetic resonance; *N/A*, not applicable

[1]Intrinsic low-level resistance of *Proteus*, *Providencia*, and *Morganella* to imipenem

[2]Assay to be used for carbapenem-non-susceptible pure colonies of Enterobacterales, *Acinetobacter baumannii*, or *Pseudomonas aeruginosa*

Table 2 US FDA-cleared rapid diagnostic assays for the identification of bacteria and/or bacterial resistance determinants from non-blood specimens[1]

Assay (manufacturer)	Specimen type	Category	Targeted organism(s)/identification	Run time (min)
FilmArray® GI Panel (BioFire®)	Stool	Bacteria	*Campylobacter* (*C. jejuni, C. coli, C. upsaliensis*) *Clostridioides difficile* (toxin A/B) *Plesiomonas shigelloides* *Salmonella* *Yersinia enterocolitica* *Vibrio (V. parahaemolyticus, V. vulnificus, V. cholerae*) Diarrheagenic *Escherichia coli/Shigella*[2]	60
		Virus	Adenovirus F40/41 Astrovirus Norovirus GI/GII Rotavirus A Sapovirus (I, II, IV, and V)	
		Parasite	*Cryptosporidium* *Cyclospora cayetanensis* *Entamoeba histolytica* *Giardia lamblia*	
Verigene® Enteric Pathogens Test (Luminex®)	Stool	Bacteria	*Campylobacter* group *Salmonella* spp. *Shigella* spp. *Vibrio* group *Yersinia enterocolitica*	120
		Toxins	Shiga Toxin 1 Shiga Toxin 2	
		Virus	Norovirus Rotavirus	
Verigene® *Clostridium difficile* Test (Luminex®)	Stool	Bacteria	*Clostridioides difficile* (toxins A/B) PCR Ribotype 027 hypervirulent strain[3]	120
xTag® (Luminex®)	Stool	Bacteria	*Campylobacter* *Clostridioides difficile* toxin A/B *Escherichia coli* O157 Enterotoxigenic *E. coli* (ETEC) Shiga-like toxin producing *E. coli* (STEC) *Salmonella* *Shigella* *Vibrio cholerae* *Yersinia enterocolitica*	300
		Virus	Adenovirus 40/41 Norovirus GI/GII Rotavirus A	
		Parasite	*Cryptosporidium* *Entamoeba histolytica* *Giardia*	
BD Max™ Enteric Bacterial Panel (BD)	Stool	Bacteria	*Salmonella* spp. *Shigella* spp. Enteroinvasive *E. coli* (EIEC) *Campylobacter* (*C. jejuni, C. coli*) STEC *Shigella dysenteriae*	180
BD Max™ Extended Enteric Bacterial Panel (BD)	Stool	Bacteria	*Yersinia enterocolitica* ETEC *Plesiomonas shigelloides* *Vibrio* (*V. vulnuficus, V. parahaemolyticus, V. cholerae*)	180

Table 2 (continued)

Assay (manufacturer)	Specimen type	Category	Targeted organism(s)/identification	Run time (min)
Xpert® vanA (Cepheid®)	Rectal swab[4]	Gram-positive bacteria	N/A[5]	60
		Resistance determinants	*VanA, VanB*	
FilmArray® Respiratory Panel (BioFire®)	NP swab	Bacteria	*Bordetella* (*B. parapertussis. B. pertussis*) *Chlamydia pneumoniae* *Mycoplasma pneumoniae*	60
		Virus	Adenovirus Coronavirus (HKU1, NL63, 229E, OC43) SARS-CoV-2 Human Metapneumovirus Human Rhinovirus/Enterovirus Influenza virus (A, A/H1, A/H3, A/H1-2009, B) Parainfluenza virus (1,2,3,4) RSV	
Verigene® Respiratory Pathogen *Flex* Test (Luminex®)	NP swab	Bacteria	*Bordetella* (*B. pertussis, B. holmesii, B. parapertussis/bronchiseptica*)	<120
		Virus	Adenovirus Human Metapneumovirus Rhinovirus Influenza virus (A, A/H1, A/H3, B) Parainfluenza virus (1,2,3,4) RSV (A,B)	
NxTAG® Respiratory Pathogen Panel (Luminex®)	NP swab	Bacteria	*Mycoplasma pneumoniae* *Chlamydia pneumoniae* *Legionella pneumophila*	300
		Virus	Adenovirus Coronavirus (HKU1, NL63, 229E, OC43) Human Metapneumovirus Rhinovirus/Enterovirus Influenza virus (A, A/H1, A/H3, B) Human Bocavirus Parainfluenza virus (1,2,3,4) RSV (A,B)	
ePlex® RP/RP2 panel (GenMark Dx®, Inc.)	NP swab	Bacteria	*Chlamydia pneumoniae* *Mycoplasma pneumoniae*	90
		Virus	Adenovirus Coronavirus (HKU1, NL63, 229E, OC43) SARS-CoV-2 Human Metapneumovirus Human Rhinovirus/Enterovirus Influenza virus (A, A/H1, A/H3, A/H1-2009, B) Parainfluenza virus (1,2,3,4) RSV (A,B)	

Table 2 (continued)

Assay (manufacturer)	Specimen type	Category	Targeted organism(s)/identification	Run time (min)
Unyvero Lower Respiratory Tract Panel (Curetis GmbH)	ET aspirate	Bacteria	*Acinetobacter* spp. *Chlamydia pneumoniae* *Citrobacter freundii* *Enterobacter cloacae complex* *Escherichia coli* *Haemophilus influenzae* *Klebsiella* (*K. oxytoca, K. pneumoniae, K. variicola*) *Legionella pneumophila* *Moraxella catarrhalis* *Morganella morganii* *Mycoplasma pneumoniae* *Proteus* spp. *Pseudomonas aeruginosa* *Serratia marcescens* *Staphylococcus aureus* *Stenotrophomonas maltophilia* *Streptococcus pneumoniae*	<300
		Fungus	*Pneumocystis jirovecii*	
		Resistance determinants	KPC NDM OXA-23, OXA-24, OXA-48, OXA-58 VIM CTX-M *mecA* TEM	
Xpert® MRSA/SA SSTI (Cepheid®)	Skin and soft tissue swabs	Bacteria	MRSA, MSSA	60
		Resistance determinants	*mecA*	

GI, gastrointestinal; *RP*, respiratory pathogen; *PCR*, polymerase chain reaction; *RSV*, respiratory syncytial virus; *NP*, nasopharyngeal; *ET*, endotracheal; *SSTI*, skin and soft tissue infection; *MRSA*, methicillin-resistant *S. aureus*; *MSSA*, methicillin-sensitive *S. aureus*

[1]If an assay detects any non-bacterial pathogens, information is provided in the table for inclusiveness

[2]Diarrheagenic *E. coli*/*Shigella* for FilmArray® GI Panel include Enteroaggregative *E. coli* (EAEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2, *E. coli* O157, and *Shigella*/Enteroinvasive *E. coli* (EIEC)

[3]For epidemiological purposes only

[4]For surveillance purposes only

[5]Assay does not detect Enterococcus, only the resistance determinant

(GenMark Diagnostics, Inc., Carlsbad, CA, USA) which utilizes a nasopharyngeal swab sample to detect 2 bacterial species and a multitude of viruses. The Unyvero LRT panel (Curetis GmbH, Germany) utilizes multiplex PCR to detect 20 bacterial species and 10 resistance determinants in lower respiratory tract samples (Table 2).

Multiplex array PCR tests can also be utilized in positive blood culture results to reduce the time to identification of Gram-positive or Gram-negative bacteria, as well as testing for resistance genes. The FDA-cleared FilmArray® Blood Culture Identification (BCID) panel (Biofire® Diagnostics, Salt Lake City, UT, USA) can identify bacterial pathogens from positive blood cultures including *Staphylococcus* spp., Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*; moreover, it identifies resistance genes such as *Klebsiella pneumoniae* carbapenemase (KPC)-encoding genes, *mecA/C*, and *vanA/B* (Table 1). Other tests utilizing multiplex PCR methods in positive blood cultures include Cepheid® Xpert MRSA/SA panel (Cepheid®, Sunnyvale, CA, USA) and Xpert Carba-R, which can detect genes encoding carbapenemases (Table 1). Uniquely, the Cepheid® Xpert MRSA/SA SSTI test provides rapid identification of *S. aureus* and MRSA resistance determinants from skin and soft tissue swab specimens in approximately 1 h.

Nanosphere's FDA-approved Verigene® Gram-Positive Blood Culture Nucleic Acid Test (BC-GP) and Verigene® Gram-Negative Blood Culture Nucleic Acid Test (BC-GN) (Nanosphere, Northbrook, IL) detect multiple bacteria in positive blood cultures along with a multitude of resistance genes in 150 min (Table 1).

The FDA-cleared ePlex® BCID panels (GenMark Diagnostics, Carlsbad, CA, USA) use multiplex PCR platforms to identify pathogens and resistance genes from positive blood cultures. The ePlex BCID-GP panel detects 20 Gram-positive bacteria and 4 resistance genes (Table 1). The BCID-GN panel detects 21 Gram-negative bacteria and 7 resistance genes that confer either extended-spectrum beta-lactamases (ESBLs) or carbapenemases (Table 1). Both panels have a TAT of 90 min.

Nuclear Magnetic Resonance

There are currently 2 FDA-cleared, culture-independent tests that utilize nuclear magnetic resonance technology to detect bacterial (T2Bacteria®) and Candida (T2Candida®) species in whole blood (T2 Biosystems®, Lexington, MA, USA) [20]. The T2Bacteria® Panel can detect 5 common bacterial species implicated in bloodstream infections: *Staphylococcus aureus, Enterococcus faecium, Pseudomonas aeruginosa, Escherichia coli*, and *Klebsiella pneumoniae*. This test has a sensitivity of 83% and specificity of 98% for diagnosing bacteremia with the aforementioned species, with a TAT of 3–5 h [21].

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

Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) *Mass Spectrometry* (MS) technology has significantly helped to reduce time to accurate identification of bacteria, mycobacteria, and fungi with a rapid TAT of <15 min from bacterial growth isolated on plated culture media. There are 2 leading manufacturers in the field of diagnostic microbiology: Vitek MS and Bruker Microflex LT MALDI-TOF MS [22]. Bruker and Vitek have both FDA-cleared and research-use only databases that are regularly updated to incorporate new organisms and relevant taxonomic changes for identification of bacteria, mycobacteria, and fungal pathogens. Additionally, the MBT Sepsityper® Kit US IVD utilizes MALDI-TOF MS technology to identify microorganisms directly from signal positive blood cultures [23].

Clinical Applications of RDTs

Bloodstream Infections

Bloodstream infection (BSI), the primary etiology of sepsis, is the leading cause of inpatient mortality in the USA, with the prevalence rising over the last decade. It is responsible for over 270,000 deaths annually [24] and is the most expensive reason for hospitalization, with over $20 billion in annual cost [25]. In the setting of sepsis, early initiation of appropriate but broad antimicrobial therapy after blood culture collection is critical for improving patient's morbidity and survival [26] and is recommended by the international Surviving Sepsis Campaign Guidelines [27]. Swift de-escalation of antibiotics to targeted therapy is strongly encouraged; however, this can often be limited by the TAT of conventional testing as it can typically take 12 h to 5 days before an organism is detected [25, 28]. Additionally, patients initially diagnosed with sepsis but later found not to have an infection are often treated with unnecessary broad-spectrum antibiotics for long periods [29].

Advancement in rapid diagnostic tests for detection of organisms from positive blood cultures have shown to improve clinical outcomes. With organism identification being readily available to clinicians within 90 min and the AST results within 7 h, prompt de-escalation or escalation of antibiotics is possible. A randomized controlled trial evaluating outcomes associated with use of RDT for identification of microorganisms and resistance genes demonstrated significant reduction in the time to appropriate antimicrobial de-escalation and escalation and reduced the use of broad-spectrum antimicrobials [12•]. In an observational study performed by Satlin et al. looking at impact of RDT for KPC-encoding gene (bla_{KPC}) and the use of ceftazidime-avibactam on outcomes in patients with carbapenem-resistant Enterobacterales bacteremia, patients who had the rapid bla_{KPC} testing had a shorter time to active antibiotic (median: 24 vs 50 h; $p = 0.009$) and decreased 14-day (16% vs 37%; $p = .007$) and 30-day mortality (24% vs 47%; $p = 0.007$) when compared to patients without bla_{KPC} testing [30]. MALDI-TOF MS, for example, has become the most widely adapted RDT over the recent years because it has shown to overall improve patient outcomes by reduction in time to antibiotic optimization and effective therapy, decreased ICU length of stay (LOS), decreased hospital LOS, and decreased 30-day all-cause mortality [24, 31, 32].

Clinical outcomes in BSI are augmented when paired with antimicrobial stewardship programs (ASP). O'Donnell et al. demonstrated that when early ASP intervention was paired with MALDI-TOF, the time to definitive therapy was shortened by 30.3 h (71.6 vs 41.3 h, $p = 0.01$) with shorter hospital LOS following the first positive blood cultures (8.7 vs 11.2 days, $p = 0.049$) when compared to standard of care [33]. Moreover, Verroken et al. demonstrated a drastic reduction in time to optimal antibiotic from 14 h on standard management with MALDI-TOF MS to 4 h when FilmArray® BCID panel was additionally performed [34]. Studies have shown that limited target PCR panels, in combination with ASP, reduced time to optimal therapy as well as decreased hospital costs and shorter LOS in patients with *Staphylococcus aureus* bacteremia [31, 35]. Additionally, the use of microarray-based early identification and resistance marker detection system (such as Verigene BC-GN)

have shown a significant reduction in time to effective antibiotic therapy in ESBL-producing Enterobacterales and vancomycin-resistant *Enterococcus* BSI as well as high rate of antibiotic optimization in several studies [36–38]. This was particularly shown by Kunz Coyne et al., where the time to effective antimicrobial therapy was reduced (15.9 h [IQR 1.9 to 25.7 h] vs 28.0 h [IQR 9.5 to 56.7 h], $p < 0.001$) with the addition of Verigene BC-GN molecular RDT to existing ASP measures [39]. No statistically significant mortality benefit was seen [39]. Lastly, the utility of Accelerate Pheno® with ASP on clinical outcomes was also assessed in an observational study by Elliott et al., where they noted a potential impact to reduce the time to antibiotic optimization (estimated 18-h reduction from 54.7 to 36.6 h in a simulation-based analysis) [40].

Respiratory Infections

Respiratory tract infections (RTIs) are one of the leading reasons for urgent care and emergency department (ED) visits and account for approximately 4 million deaths per year in children and adults worldwide [41]. It is also one of the most common causes of antibiotic misuse. Though the majority of these infections are viral where treatment with antibiotics is not indicated, over half of acute RTIs are inappropriately treated with antibiotics [42–44] leading to secondary adverse effects, complications, and AMR. Thus, RDTs play a critical role not only in limiting unnecessary antibiotic use but also in improving patient care, aiding in therapeutic management, and preventing infection.

In the setting of suspected group A *Streptococcus* (GAS) infections, for example, the use of highly sensitive RDTs could prevent unnecessary antibiotic use. Some pediatric studies have shown that the use of rapid antigen detection test (RADT) with confirmatory culture was less specific and sensitive which led to increased rates of inappropriate antibiotic use. However, point-of-care (POC) PCR test, compared to RADT, for detection of GAS was highly sensitive and specific with a rapid TAT and led to higher use of targeted antibiotic therapy (97.1% vs 87.5%; $p = .0065$) [45]. Similarly, a prospective study by Ralph et al. showed that the use of molecular testing compared to traditional throat swab cultures was more sensitive and specific (100% and 79.3%, respectively) with positive and negative predictive values of 48.8% and 100%, respectively. The authors of the study felt that the use of molecular testing could improve antibiotic use [46]. In pediatric patients presenting to the ED with suspicion for streptococcal pharyngitis, it has also been shown that the use of RDTs is associated with decreased antibiotic use [47].

A retrospective analysis of ambulatory, urgent care, and ED encounters found that the use of RDTs aimed for primary diagnosis of upper or lower RTIs were infrequently used in the outpatient setting. Rapid GAS test was predominantly ordered to diagnose pharyngitis with direct implication of ordering antibiotics if the test was positive. However, antibiotics were also often inappropriately prescribed in the setting of a negative result. Similarly, Li et al. describe that of the 323 patients discharged from the ED with a viral diagnosis, 21.1% were inappropriately prescribed antibiotics [48]. The infrequent use of RDTs in the outpatient setting highlights the importance of clinician education on the utility of RDTs and implementation in settings of high volume of patient encounters.

For suspected community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), or ventilator-associated pneumonia (VAP), prompt initiation of empirical antibiotics based on the patient's risk factors is recommended, followed by de-escalation to targeted therapy based on microbiology data [49, 50]. However, this traditional method often causes delays in appropriate therapy given the long TAT. The use of RDTs with reverse transcription (RT)-PCR has played a remarkable role in improving patient care with its quick TAT. Several studies have highlighted that the use of RDTs compared to traditional methods directly correlates with increased documentation of a microbiological etiology [51]. The use of multiplex RDTs in respiratory samples has become particularly helpful during the SARS-CoV-2 pandemic as it can report significantly more viral pathogens than traditional culture techniques when trying to distinguish between a bacterial versus viral etiology for CAP. A prospective study of adult patients with CAP in Taiwan showed that bacterial pathogens were detected in 106 (50%) patients, viruses in 77 (36.3%) patients, and fungal pathogen in 1 patient (0.5%) with overall detection rate being 70.7% ($n = 150$) in culture and molecular testing method versus 36.7% ($n = 78$) in traditional microbial culture alone. The most common pathogens were influenza virus (16.1%), *Klebsiella pneumoniae* (14.1%), *P. aeruginosa* (13.6%), human rhinovirus (11.8%), and *Streptococcus pneumoniae* (9.9%) [52]. In addition, several studies have shown not only de-escalation or modification of empirical treatment for CAP/HAP/VAP, but reduction in LOS and hospital costs with the use of RDTs [53–60].

The development and implementation of multiplex RDTs [61] in respiratory infections play a significant role in prompt diagnosis that can affect patient care, possibly limiting the use of antibiotics in the setting of a viral infection. Thus, clinician education and targeted implementation of RDTs are important to enhance and optimize the management of both bacterial and non-bacterial respiratory infections.

Central Nervous System Infections

Accurate diagnosis of central nervous system (CNS) infections is often complicated and challenging due to the overlapping clinical manifestations of infectious and

non-infectious etiologies, limited number of RDTs available, and rapid initiation of empiric antimicrobial therapy prior to adequate sample collection for testing, which may decrease the likelihood of organism detection. Greater than 50% of acute meningitis and encephalitis cases do not have an etiology documented, demonstrating the ongoing challenges associated with accurate diagnosis of CNS infections [62, 63]. Syndromic RDTs may help with prompt diagnosis of CNS infections and provide opportunities for selecting a targeted therapy or alternatively allow early discontinuation of unnecessary antimicrobials [63].

The FilmArray® Meningitis/Encephalitis (ME) panel utilizes multiplex PCR technology to detect an array of common pathogens including 6 bacteria: *Escherichia coli* K1*, Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus agalactiae,* and *Streptococcus pneumoniae*. A recent systematic review and meta-analysis compared the diagnostic accuracy of the FilmArray® ME to both traditional culture results (CSF or blood) and clinician adjudicated diagnosis based on clinical presentation, CSF analysis, and/or alternative molecular test results compatible with bacterial infection [64]. This recent review specifically reported on the accuracy of the FilmArray® ME for bacterial pathogens, separate from viral and fungal pathogens also captured by the panel [64]. Across 16 studies ($n = 6183$), the reported sensitivity and specificity were 89.5% (95% CI 81.1–94.4), and 97.4% (95% CI 94–98.9), respectively, when compared to traditional culture-based diagnosis. When using clinician adjudicated diagnosis, sensitivity and specificity slightly increased to 92.1% (95% CI 86.8–95.3) and 99.2% (95% CI 98.3–99.6) across 15 studies ($n = 5524$). Moderate sensitivities (range: 64.9–87.5) and high specificities (range: 98.5–99.6) were reported for each individual bacteria, with lowest sensitivities reported for *L. monocytogenes*, *H. influenzae,* and *E. coli*. Therefore, the authors concluded that the FilmArray® ME can be a great tool for ruling in bacterial CNS infections but less reliable for ruling them out; the varying degrees of accuracy per pathogen type is an important consideration in result interpretation [64].

The impact of the FilmArray® ME panel on important clinical outcomes, such as reducing antibiotic use or shortening hospital LOS, remains uncertain. A prospective cohort study of pediatric patients by Posnakoglou et al. reported significant reductions in duration of antimicrobials (4 vs 7 days, $p < .001$) and the median hospital LOS (5 vs 8 days, $p < .001$) in patients randomized to the FilmArray® ME panel versus standard of care [65]. However, other studies have shown negligible differences in length of hospitalization and inconsistent effect on reducing antimicrobial use [66]. One study reported that 78% of patients with negative results continued on empiric therapy, demonstrating the reluctance of clinicians to use negative results to rule out an infectious source as contributory to CNS manifestations [67]. Such outcome is in line with the limited utility of the FilmArray® ME to rule out an infection as aforementioned study suggests [64]. Most importantly, all patients should undergo standard diagnostic testing (Gram stain, culture, etc.) for CNS infections as the FilmArray® ME is an adjunct tool that screens a very limited number of bacterial species.

Gastrointestinal Infections

Acute diarrheal illness accounts for approximately 500,000 hospitalizations annually and may be a manifestation of a gastrointestinal (GI) infection [68]. Healthcare costs due to an acute diarrheal illness can be substantial given the need for ED visits, hospitalizations, and multiple diagnostic tests associated with diagnostic workup [69]. Standard diagnostic tests include stool cultures and/or pathogen-directed molecular panels specific to bacteria or viruses or toxins produced by a pathogen. A major challenge in diagnosing infectious diarrhea includes the low sensitivity of stool cultures. Furthermore, clinician knowledge and selection of an appropriate rapid molecular test is essential to minimize delay to accurate etiological diagnosis and treatment of GI infections [70, 71].

There are 11 commercially available multiplex panels for GI infections [72]. The currently available multiplex panels can target varying numbers of pathogens including bacteria, viruses, and parasites. In particular, the BioFire FilmArray® GI panel has the broadest target (13 bacterial, 5 viral, 4 parasitic) and provides a result within 2 h of testing [73]. With high sensitivity and specificity and significant reduction in TAT compared to culture-dependent methods (which may take several days to result), these panels can potentially reduce the number of diagnostics such as imaging studies and additional stool tests [73]. Ultimately, they may affect clinically important patient outcomes such as antibiotic duration, isolation needs, and hospital LOS. A study comparing the FilmArray® GI panel to conventional stool cultures showed that patients tested using the panel had a lower number of stool tests (0.58 vs 3.02 tests per patient, $p = .0001$) and imaging studies (0.18 vs 0.39, $p = .0002$) compared to the control group [74]. A cost analysis showed healthcare cost savings of $293.61 per patient [74]. Similarly, Machiels et al. tested the FilmArray® GI panel by running it in conjunction with routine pathogen-directed molecular panels of providers' choice ($n = 182$) [70]. The FilmArray® GI panel had a shorter TAT (16 h) compared to standard of care (53 h) and a higher likelihood of a positive finding (39.6% vs 28.6%) in 20 additional patients that had a negative result on standard testing. It was estimated that the FilmArray® GI panel could have resulted in earlier discontinuation of isolation orders by a median of 29 h for 26 patients, 3.6 antibiotic-days saved, and additional imaging prevented in 5 patients. However, these potential benefits may be an overestimate as providers

may not be able to review and act upon the results immediately in the real-world setting. Several studies have shown a positive impact of multiplex GI PCR panels on judicious antimicrobial use [68, 71, 75, 76]. A retrospective review of antibiotic use pre- and post-implementation of syndromic GI panel ($n = 300$) found decreased time to appropriate therapy (1 vs 2 days, $p < .0001$), increased discharges without antibiotics (14.0% vs 4.5%, $p < .001$), and shorter hospital LOS (3 vs 7.5 days, $p = .0002$) compared to a historical cohort that utilized standard stool cultures [68]. Other studies reported an increase in appropriate antibiotic use in the setting of positive result on syndromic GI panel, which is best explained by the higher diagnostic yield compared to that of stool cultures [71, 75]. A study evaluating diagnostic yield and agreement of results of a multiplex GI panel in pediatric patients ($n = 125$) demonstrated that the use of the panel increased likelihood of microbiological diagnosis compared to conventional microbiology methods (68.8% vs 35.2%, $p < .001$) [75]. Furthermore, multiplex panel results led to a change in antimicrobial agent for 18 patients with a positive finding (11 new starts, 5 switches to targeted antimicrobial, 2 discontinuations of empiric antimicrobial). Authors concluded that use of the multiplex GI panel increased the judicious use of antimicrobials in hospitalized children [75]. Similarly, a prospective, single-center, randomized controlled trial in the ED ($n = 74$) showed that the use of multiplex GI panel increased appropriate antibiotic use for bacterial and protozoal diarrheal illnesses compared to culture-dependent testing [71].

Overall, recent studies have similarly shown that multiplex GI panels can shorten time to diagnosis, improve antimicrobial therapy optimization, lead to possible cost-savings from shortened hospitalization, reduce the number of diagnostic tests, and result in early discontinuation of isolation orders. However, like any other PCR-based molecular assays, multiplex GI panels are culture-independent tests and are unable to provide susceptibility data. In addition, clinician interpretation is crucial in the setting of a positive result as detected enteric organisms may not necessarily represent an infection (e.g., *C. difficile* or *E. coli* colonization). Lastly, cost-effectiveness data are limited and the use of less broad, pathogen-directed tests should be considered over multiplex assays in the setting of high pretest probability for specific infection such as *C. difficile* infection.

Interplay Between Rapid Diagnostic Tests and Antimicrobial Stewardship

Between 2014 and 2019, national resistance trends in a cohort of 890 hospitals in the USA demonstrated increasing incidences of ESBL- or carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* phenotypes [33]. Using only conventional methods for pathogen detection and identification may result in delayed detection of the resistant phenotype and consequently delay effective therapy compared to RDTs [39]. In the setting of Gram-negative bacteremia, ineffective antimicrobial therapy is a predictor for mortality, second only to intensive care unit (ICU) status [33]. RDTs have demonstrated the potential to reduce time to microbiological data, increase diagnostic yield, and subsequently allow for timely de-escalation or escalation of empiric regimens and lead to improved clinical outcomes [77].

RDTs have demonstrated faster identification of certain pathogens and resistance genes resulting in shorter time to effective treatment and reduction in antibiotic misuse [78•]. However, the majority of studies evaluate the impact of RDTs in conjunction with effective ASP measures. To date, no RDT has been shown to be effective and accurate as the sole method in the diagnosis of bacterial infections [77]. ASPs involve interdisciplinary practices which design and implement strategies to promote the timely evaluation of microbiology data with the objective to optimize antimicrobial use and, subsequently, decrease the risk of unintended adverse effects of suboptimal or ineffective therapy. Because RDTs shorten the time to microbiology results, ASP interventions can be implemented earlier in therapy and allow for earlier optimization of therapeutics. A single-center, prospective cohort study demonstrated that while the use of MALDI-TOF decreased the time to pathogen identification by up to 24 h, only the study cohort with additional ASP measure, in the form of prospective review and feedback by pharmacists, had a significantly decreased time to definitive therapy (41.3 vs 71.6 h, $p = 0.01$) and decreased hospital LOS (8.7 vs 11.2 days, $p = 0.049$) [33]. Numerous studies continue to support ASP interventions in conjunction with RDTs for reducing inappropriate antibiotic use and antibiotic-related adverse effects, resulting in lower healthcare costs [24, 30, 33, 39, 66, 77, 78•].

RDT results may not be as effective in improving patient outcomes without ASPs, which may be related to incorrect interpretation of test results by providers [24]. Interpretation of novel RDTs may be complex and requires educational interventions [78•]. For example, the FilmArray® ME panel was not as effective in reducing inappropriate treatment when diagnostic guidance was not available. This was attributed to the lack of provider knowledge regarding test availability and interpretation of the results [66]. Another consideration is that despite the benefits of RDTs, conventional BMD for AST continues to be the gold standard. For RDTs to be appropriately utilized, providers must understand that results may still require confirmation by conventional methods [24]. In the case of molecular RDTs, genotype detection may not equate to phenotypic susceptibility (i.e., minimum inhibitory concentration [MIC] results), especially in cases of more complex resistance mechanisms. For example, the lack of detection of beta-lactamase genes in Gram-negative bacteria may not exclude

phenotypic resistance given other possible resistance mechanisms not included on the panel (e.g., porin mutations, efflux upregulation) [78•]. A mixed method study by Burrowes et al. investigated trends in provider knowledge, behavior, and attitudes towards RDTs, which showed that most clinicians often rely on their clinical judgment over prescribing guidelines. Their survey also found that many clinicians had limited understanding or familiarity with comprehensive respiratory PCR panel and procalcitonin which can help differentiate RTIs of viral or bacterial etiology [79]. In order to maximize the impact of an RDT, the institution must emphasize clinician buy-in and education before, during, and after implementation. The continued education and assessment of provider knowledge can be achieved with an effective ASP in place.

Role of Procalcitonin in the Diagnosis of Bacterial Infections

ASPs are increasingly imperative to ensure optimal utilization of antimicrobials and minimize AMR and healthcare costs. Diagnostic uncertainty and provider reluctance to discontinue antibiotics hinder improvement of ASP outcomes. Procalcitonin (PCT) is a serum biomarker that has shown promise in distinguishing between viral and bacterial infections [80]. PCT is undetectable in healthy conditions but increases rapidly in response to proinflammatory mediators and endotoxins; it has been shown to correlate with the extent and severity of bacterial infections [81]. PCT demonstrates favorable kinetics; levels increase within 3 to 6 h of exposure to bacteria or bacterial endotoxin and peak at 6 to 13 h with a half-life of approximately 22 to 36 h [80, 82–84]. Normal PCT serum levels are <0.1 ng/L, whereas PCT levels >0.25 ng/mL may be indicative of bacterial infection; PCT levels decline with clinical improvement [85]. Owing to these characteristics, PCT can be considered a reliable marker of bacterial infections, with a possible role in trending clinical improvement and de-escalation of antibiotics [86]. As such, PCT has been evaluated as a diagnostic tool for bacterial infections to reduce antibiotic use; however, the results have been conflicting [81, 87–89].

PCT has been evaluated in various populations and infections, but RTIs have been represented in most of the randomized controlled trials. As a result, in 2017, the FDA cleared the use of the PCT assay to guide the initiation and discontinuation of antimicrobials for suspected lower RTIs and discontinuation in patients with sepsis [90]. Furthermore, studies to date have demonstrated that PCT-based algorithms for antibiotic discontinuation are safe and offer a cost-effective means of reducing antibiotic exposure [91, 92]. More recently, PCT has gained recognition in clinical guidelines to help guide the discontinuation of antimicrobials and initiate antimicrobials independent of PCT level [27, 49].

Although PCT assays have shown promising results over the years, several limitations require consideration before implementing in everyday clinical practice. For example, PCT reliability for guiding antimicrobials has been questioned in various conditions, such as renal dysfunction, cardiac compromise, or immunosuppression. In these situations, PCT levels are elevated at baseline with a further increase in the presence of infection. Additionally, PCT production is particularly elevated in nonbacterial inflammatory processes such as trauma, burns, carcinomas, and immunomodulatory therapy, which can all increase proinflammatory cytokines, making PCT interpretation challenging. While PCT testing can produce rapid results within 1 to 2 h if performed on-site, factors such as intermittent batching or sending to an outside laboratory can delay the availability of results to several days. Such delay can render ineffective the clinical utility of PCT in guiding timely de-escalation of antibiotics.

As with any diagnostic tool, PCT could be part of a clinical algorithm to assist clinicians in evaluating potential infection. However, clinical decisions should not be made exclusively on PCT but in concert with clinical context and evaluation of other diagnostic results. Definitive recommendations remain elusive owing to conflicting data, but PCT's usefulness in guiding antibiotic discontinuation in the absence of bacterial infections appear promising.

Challenges and Limitations of RDTs

Despite the advances in rapid diagnostic platforms and enhancements in patient care, currently available RDTs are not without challenges and limitations. When introducing new technology into practice, clinician education should be a key component of the implementation process as inadequate dissemination of knowledge of the RDT can lead to incorrect or suboptimal use and interpretation of the test results [79, 93, 94]. While syndromic tests may be useful in quickly identifying a pathogen for early diagnosis and transition to appropriate antibiotic treatment, these platforms are limited to a specific set of common organisms and thus infections by less common organisms cannot be ruled out. This is especially important to consider in patients with different infectious disease risk factors, such as those with compromised immunity. Thus, a negative result may not necessarily rule out an infection. Conversely, false positives as a result of detecting colonizers or contaminants can occur, which is an inherent limitation of molecular-based RDTs. Clinicians are responsible for interpretation of the results and must distinguish colonization (or contamination) from true infection. Overuse of RDTs in cases of low pretest probability not only

inappropriately deplete resources, but can also increase likelihood of false negative or positive results; false positive results may mislead clinicians to overtreat which can lead to AMR, antibiotic-associated adverse events, and increased healthcare costs [66, 93–95]. For these reasons and more, diagnostic stewardship, ordering the right tests for the right patient at the right time to inform optimal clinical care, continues to emerge as a core part of ASPs to optimize antibiotic use and improve patient outcomes [96•].

In an era of AMR, rapid diagnostics have shown to be useful in providing essential information on drug resistance by capturing genes that encode for certain resistance mechanisms. However, it is prudent to remember the continued evolution of AMR and the development of new and complex resistance mechanisms as this can further challenge treatment decisions [78•, 97]. The utility of a molecular panel to rapidly rule out potential AMR can be limited, especially in areas with high baseline resistance rates, for drug and bacteria combinations with heterogenous resistance mechanisms, and in patients susceptible to resistant organisms (e.g., bone marrow transplant recipients). In contrast, confounding factors such as polymicrobial samples or specific bacterial species may trigger RDT panels to inaccurately report drug resistance [98]. Such errors can misinform clinicians and prompt the use of broad-spectrum antibiotics and delay the opportunity to use a targeted therapy for a given infection. Lastly, there are still limited data to demonstrate the cost-effectiveness of the available RDT platforms. RDTs can be expensive and providing an accurate cost analysis remains challenging as cost savings from improved outcomes and decreased overall healthcare costs are difficult to capture [74, 94, 99].

Emerging Technologies

While there are a number of rapid diagnostic technologies currently available, there remain gaps in which novel or expanded diagnostics would yield benefits in clinical practice. This includes technologies that address novel infection sites or specimen types, detection of clinically important bacteria, and broader susceptibility testing or resistance detection. Such advancements would enhance the impact of RDTs on therapeutic optimization. We note select emerging technologies that address these gaps.

Rapid phenotypic assays have been a welcome advancement in rapid diagnostics given the complexity of resistance mechanisms and associated phenotypic susceptibility prediction. However, expansion in the number of identified organisms and tested antibiotics has the potential to improve patient care. An example is the Next-Generation Phenotyping™ platform (Selux Diagnostics Inc., Charlestown, MA, USA), which can provide rapid susceptibility results within 5 h. This is a novel method that uses signal amplification of bacterial surfaces to measure bacterial surface area and allows for the differentiation of bacterial morphologies in response to antibiotics (e.g., filamentation versus septation) to delineate susceptible versus resistant isolates by predicting phenotypic MICs. Bacterial surface area-determined MICs had high essential agreement with MICs by Clinical and Laboratory Standards Institute (CLSI) reference method [100]. Most notably, it provides testing capacity for 5 times as many antibiotics in parallel as compared to current automated AST systems [101]. Various other rapid phenotypic AST methods have been developed with other types of technologies [102–104].

Bone and joint infections are an often encountered infectious syndrome in clinical practice; however, the yield of conventional culture from synovial fluid to make a microbiologic diagnosis is suboptimal [105]. The BioFire® Joint Infection Panel (BioFire® Diagnostics, Salt Lake City, UT, USA) is a rapid molecular test (1 h) that provides a syndromic panel of 38 target organisms, including fastidious bacteria, and 8 AMR genes using synovial fluid specimen. The broad panel includes targets for aerobic and anaerobic Gram-positive and -negative bacterial pathogens associated with joint infections. The performance characteristics (>90% sensitivity and >99% specificity) also provide a high negative predictive value allowing for potential discontinuation of empiric antibiotics [106]. Clinical data are currently limited and will be necessary to understand real-world utility and impact.

Conclusion

When treating patients for bacterial infections, time to effective therapy influences clinical outcomes. The advancement and implementation of RDTs, in conjunction with antimicrobial stewardship, to enhance treatment selection for bacterial infections should be regarded as a core element to improve clinical outcomes for patients in various healthcare settings. Furthermore, it is crucial to use RDTs in the appropriate clinical context to maximize benefit while ensuring cost-effectiveness. Data suggests that RDTs, particularly combined with ASPs, are cost-effective and can reduce healthcare costs [99]. Although challenges exist in the use of rapid diagnostics, there is a need for continued innovation in technology, implementation science, and collaboration across clinical professions — microbiologists, physicians, pharmacists, nursing, and many others — to optimize care for patients suffering from bacterial infections. High-quality studies will continue to be necessary for current and emerging technologies to understand their clinical role and impact.

Funding NN is supported by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) under Award Number K23AI159396.

Declarations

Conflict of Interest The authors received no financial support in the writing of this manuscript. P. U. is an employee of Becton, Dickinson and Company, Life Sciences - Integrated Diagnostic Solutions. T. J. K. has received personal fees from Accelerate Diagnostics, Roche Diagnostics, Selux Diagnostics, Opgen, and FirstLight Diagnostics, all outside the submitted work. N. N. has received research grant funding from Merck, outside the submitted work. The opinions expressed in this publication are those of the authors and do not necessarily reflect those of the institutions/company who employs them.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1.•• CDC. Antibiotic resistance threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019. **This article is an updated major report from the US Centers for Disease Control and Prevention (CDC) that provides national death and infection estimates for 18 antimicrobial-resistant bacteria and fungi**.

2. Kwon JH, Powderly WG. The post-antibiotic era is here. Science. 2021;373(6554):471.

3.•• Antimicrobial RC. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629–55. **This article is the first and most comprehensive study of the estimates of antimicrobial-resistance burden across the world**.

4. Eyre DW, Town K, Street T, Barker L, Sanderson N, Cole MJ, et al. Detection in the United Kingdom of the *Neisseria gonorrhoeae* FC428 clone, with ceftriaxone resistance and intermediate resistance to azithromycin, October to December 2018. Euro Surveill. 2019;24(10)

5. Lahra MM, Ryder N, Whiley DM. A new multidrug-resistant strain of *Neisseria gonorrhoeae* in Australia. N Engl J Med. 2014;371(19):1850–1.

6. McGann P, Snesrud E, Maybank R, Corey B, Ong AC, Clifford R, et al. *Escherichia coli* harboring mcr-1 and blaCTX-M on a novel IncF plasmid: first report of mcr-1 in the United States. Antimicrob Agents Chemother. 2016;60(7):4420–1.

7. Nelson RE, Hatfield KM, Wolford H, Samore MH, Scott RD, Reddy SC, et al. National estimates of healthcare costs associated with multidrug-resistant bacterial infections among hospitalized patients in the United States. Clin Infect Dis. 2021;72(Suppl 1):S17–26.

8.• Centers for Disease Control and Prevention. COVID-19 & antimicrobial resistance 2022 [updated February 25, 2022. Available from: https://www.cdc.gov/drugresistance/covid19.html. **This article is a special report by the CDC demonstrating the substantial impact the COVID-19 pandemic has inflicted on the progress in controlling antimicrobial-resistant infections**].

9. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med. 2006;34(6):1589–96.

10.•• Banerjee R, Komarow L, Virk A, Rajapakse N, Schuetz AN, Dylla B, et al. Randomized trial evaluating clinical impact of RAPid IDentification and Susceptibility Testing for Gram-negative Bacteremia: RAPIDS-GN. Clin Infect Dis. 2021;73(1):e39–46. **This article presents the results of a pivotal multicenter randomized controlled trial that evaluated rapid organism identification and phenotypic antimicrobial susceptibility testing using the Accelerate Pheno System**.

11. Humphries RM, Yang S, Hemarajata P, Ward KW, Hindler JA, Miller SA, et al. First report of ceftazidime-avibactam resistance in a KPC-3-expressing *Klebsiella pneumoniae* isolate. Antimicrob Agents Chemother. 2015;59(10):6605–7.

12.• Banerjee R, Teng CB, Cunningham SA, Ihde SM, Steckelberg JM, Moriarty JP, et al. Randomized trial of rapid multiplex polymerase chain reaction-based blood culture identification and susceptibility testing. Clin Infect Dis. 2015;61(7):1071–80. **This article is the first prospective randomized controlled trial to demonstrate benefit of a rapid molecular PCR-based blood culture diagnostic test for more appropirate antibiotic use**.

13. Barlam TF, Cosgrove SE, Abbo LM, MacDougall C, Schuetz AN, Septimus EJ, et al. Implementing an Antibiotic Stewardship Program: guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. Clin Infect Dis. 2016;62(10):e51–77.

14. Hill B, Narayanan N, Palavecino E, Perez KK, Premraj S, Streifel A, et al. The role of an antimicrobial stewardship team in the use of rapid diagnostic testing in acute care: an official position statement of the Society of Infectious Diseases Pharmacists. Infect Control Hosp Epidemiol. 2018;39(4):473–5.

15. Zeitler K, Narayanan N. The present and future state of antimicrobial stewardship and rapid diagnostic testing: can one ideally succeed without the other? Curr Treat Options Infect Dis. 2019;11(2):177–87.

16. Humphries RM. Update on susceptibility testing: genotypic and phenotypic methods. Clin Lab Med. 2020;40(4):433–46.

17. Yee R, Dien Bard J, Simner PJ. The genotype-to-phenotype dilemma: how should laboratories approach discordant susceptibility results? J Clin Microbiol. 2021;59(6)

18. Datar R, Orenga S, Pogorelcnik R, Rochas O, Simner PJ, van Belkum A. Recent advances in rapid antimicrobial susceptibility testing. Clin Chem. 2021;68(1):91–8.

19. Marschal M, Bachmaier J, Autenrieth I, Oberhettinger P, Willmann M, Peter S. Evaluation of the Accelerate Pheno system for fast identification and antimicrobial susceptibility testing from positive blood cultures in bloodstream infections caused by Gram-negative pathogens. J Clin Microbiol. 2017;55(7):2116–26.

20. Nguyen MH, Clancy CJ, Pasculle AW, Pappas PG, Alangaden G, Pankey GA, et al. Performance of the T2Bacteria panel for diagnosing bloodstream infections: a diagnostic accuracy study. Ann Intern Med. 2019;170(12):845–52.

21. De Angelis G, Posteraro B, De Carolis E, Menchinelli G, Franceschi F, Tumbarello M, et al. T2Bacteria magnetic resonance assay for the rapid detection of ESKAPEc pathogens directly in whole blood. J Antimicrob Chemother. 2018;73(suppl_4):iv20–iv6.

22. Deak E, Charlton CL, Bobenchik AM, Miller SA, Pollett S, McHardy IH, et al. Comparison of the Vitek MS and Bruker Microflex LT MALDI-TOF MS platforms for routine

identification of commonly isolated bacteria and yeast in the clinical microbiology laboratory. Diagn Microbiol Infect Dis. 2015;81(1):27–33.
23. Buchan BW, Riebe KM, Ledeboer NA. Comparison of the MALDI Biotyper system using Sepsityper specimen processing to routine microbiological methods for identification of bacteria from positive blood culture bottles. J Clin Microbiol. 2012;50(2):346–52.
24. Briggs N, Campbell S, Gupta S. Advances in rapid diagnostics for bloodstream infections. Diagn Microbiol Infect Dis. 2021;99(1):115219.
25. Edmiston CE, Garcia R, Barnden M, DeBaun B, Johnson HB. Rapid diagnostics for bloodstream infections: a primer for infection preventionists. Am J Infect Control. 2018;46(9):1060–8.
26. Levy MM, Evans LE, Rhodes A. The Surviving Sepsis Campaign Bundle: 2018 update. Intensive Care Med. 2018;44(6):925–8.
27. Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Intensive Care Med. 2021;47(11):1181–247.
28. Scerbo MH, Kaplan HB, Dua A, Litwin DB, Ambrose CG, Moore LJ, et al. Beyond blood culture and Gram stain analysis: a review of molecular techniques for the early detection of bacteremia in surgical patients. Surg Infect (Larchmt). 2016;17(3):294–302.
29. Klein Klouwenberg PM, Cremer OL, van Vught LA, Ong DS, Frencken JF, Schultz MJ, et al. Likelihood of infection in patients with presumed sepsis at the time of intensive care unit admission: a cohort study. Crit Care. 2015;19:319.
30. Satlin MJ, Chen L, Gomez-Simmonds A, Marino J, Weston G, Bhowmick T, et al. Impact of a rapid molecular test for *Klebsiella pneumoniae* carbapenemase and ceftazidime-avibactam use on outcomes after bacteremia caused by carbapenem-resistant Enterobacterales. Clin Infect Dis. 2022:ciac354.
31. Huang AM, Newton D, Kunapuli A, Gandhi TN, Washer LL, Isip J, et al. Impact of rapid organism identification via matrix-assisted laser desorption/ionization time-of-flight combined with antimicrobial stewardship team intervention in adult patients with bacteremia and candidemia. Clin Infect Dis. 2013;57(9):1237–45.
32. Perez KK, Olsen RJ, Musick WL, Cernoch PL, Davis JR, Peterson LE, et al. Integrating rapid diagnostics and antimicrobial stewardship improves outcomes in patients with antibiotic-resistant Gram-negative bacteremia. J Infect. 2014;69(3):216–25.
33. O'Donnell JN, Rhodes NJ, Miglis CM, Zembower TR, Qi C, Hoff BM, et al. Impact of early antimicrobial stewardship intervention in patients with positive blood cultures: results from a randomized comparative study. Int J Antimicrob Agents. 2022;59(2):106490.
34. Verroken A, Despas N, Rodriguez-Villalobos H, Laterre PF. The impact of a rapid molecular identification test on positive blood cultures from critically ill with bacteremia: a pre-post intervention study. PLoS One. 2019;14(9):e0223122.
35. Perez KK, Olsen RJ, Musick WL, Cernoch PL, Davis JR, Land GA, et al. Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs. Arch Pathol Lab Med. 2013;137(9):1247–54.
36. Walker T, Dumadag S, Lee CJ, Lee SH, Bender JM, Cupo Abbott J, et al. Clinical impact of laboratory implementation of Verigene BC-GN microarray-based assay for detection of Gram-negative bacteria in positive blood cultures. J Clin Microbiol. 2016;54(7):1789–96.
37. Rivard KR, Athans V, Lam SW, Gordon SM, Procop GW, Richter SS, et al. Impact of antimicrobial stewardship and rapid microarray testing on patients with Gram-negative bacteremia. Eur J Clin Microbiol Infect Dis. 2017;36(10):1879–87.
38. Hayakawa K, Mezaki K, Kobayakawa M, Yamamoto K, Mutoh Y, Tsuboi M, et al. Impact of rapid identification of positive blood cultures using the Verigene system on antibiotic prescriptions: a prospective study of community-onset bacteremia in a tertiary hospital in Japan. PLoS One. 2017;12(7):e0181548.
39. Kunz Coyne AJ, Casapao AM, Isache C, Morales J, McCarter YS, Jankowski CA. Influence of antimicrobial stewardship and molecular rapid diagnostic tests on antimicrobial prescribing for extended-spectrum beta-lactamase- and carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in bloodstream infection. Microbiol Spectr. 2021;9(2):e0046421.
40. Elliott G, Malczynski M, Barr VO, Aljefri D, Martin D, Sutton S, et al. Evaluation of the impact of the Accelerate Pheno system on time to result for differing antimicrobial stewardship intervention models in patients with gram-negative bloodstream infections. BMC Infect Dis. 2019;19(1):942.
41. Chen AP, Chuang C, Huang YC, Wu PF, Huang SF, Cheng NC, et al. The epidemiology and etiologies of respiratory tract infection in Northern Taiwan during the early phase of coronavirus disease 2019 (COVID-19) outbreak. J Microbiol Immunol Infect. 2021;54(5):801–7.
42. Barlam TF, Morgan JR, Wetzler LM, Christiansen CL, Drainoni ML. Antibiotics for respiratory tract infections: a comparison of prescribing in an outpatient setting. Infect Control Hosp Epidemiol. 2015;36(2):153–9.
43. Chua KP, Fischer MA, Linder JA. Appropriateness of outpatient antibiotic prescribing among privately insured US patients: ICD-10-CM based cross sectional study. BMJ. 2019;364:k5092.
44. Shively NR, Buehrle DJ, Clancy CJ, Decker BK. Prevalence of inappropriate antibiotic prescribing in primary care clinics within a Veterans Affairs health care system. Antimicrob Agents Chemother. 2018;62(8)
45. Rao A, Berg B, Quezada T, Fader R, Walker K, Tang S, et al. Diagnosis and antibiotic treatment of group a streptococcal pharyngitis in children in a primary care setting: impact of point-of-care polymerase chain reaction. BMC Pediatr. 2019;19(1):24.
46. Ralph AP, Holt DC, Islam S, Osowicki J, Carroll DE, Tong SYC, et al. Potential for molecular testing for group A *Streptococcus* to improve diagnosis and management in a high-risk population: a prospective study. Open Forum Infect Dis. 2019;6(4):ofz097.
47. Bird C, Winzor G, Lemon K, Moffat A, Newton T, Gray J. A pragmatic study to evaluate the use of a rapid diagnostic test to detect group a streptococcal pharyngitis in children with the aim of reducing antibiotic use in a UK emergency department. Pediatr Emerg Care. 2021;37(5):e249–e51.
48. Li J, Kang-Birken SL, Mathews SK, Kenner CE, Fitzgibbons LN. Role of rapid diagnostics for viral respiratory infections in antibiotic prescribing decision in the emergency department. Infect Control Hosp Epidemiol. 2019;40(9):974–8.
49. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, et al. Diagnosis and treatment of adults with community-acquired pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. Am J Respir Crit Care Med. 2019;200(7):e45–67.
50. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis. 2016;63(5):e61–e111.
51. Martinez Sagasti F, Calle Romero M, Rodriguez Gomez M, Alonso Martinez P, Garcia-Perrote SC. Urgent need for a rapid microbiological diagnosis in critically ill pneumonia. Rev Esp Quimioter. 2022;35(Suppl 1):6–14.

52. Lin WH, Chiu HC, Chen KF, Tsao KC, Chen YY, Li TH, et al. Molecular detection of respiratory pathogens in community-acquired pneumonia involving adults. J Microbiol Immunol Infect. 2021;
53. Monard C, Pehlivan J, Auger G, Alviset S, Tran Dinh A, Duquaire P, et al. Multicenter evaluation of a syndromic rapid multiplex PCR test for early adaptation of antimicrobial therapy in adult patients with pneumonia. Crit Care. 2020;24(1):434.
54. Maataoui N, Chemali L, Patrier J, Tran Dinh A, Le Fevre L, Lortat-Jacob B, et al. Impact of rapid multiplex PCR on management of antibiotic therapy in COVID-19-positive patients hospitalized in intensive care unit. Eur J Clin Microbiol Infect Dis. 2021;40(10):2227–34.
55. Verroken A, Scohy A, Gerard L, Wittebole X, Collienne C, Laterre PF. Co-infections in COVID-19 critically ill and antibiotic management: a prospective cohort analysis. Crit Care. 2020;24(1):410.
56. Shengchen D, Gu X, Fan G, Sun R, Wang Y, Yu D, et al. Evaluation of a molecular point-of-care testing for viral and atypical pathogens on intravenous antibiotic duration in hospitalized adults with lower respiratory tract infection: a randomized clinical trial. Clin Microbiol Infect. 2019;25(11):1415–21.
57. Pham SN, Sturm AC, Jacoby JS, Egwuatu NE, Dumkow LE. Impact of a pharmacist-driven MRSA nasal PCR protocol on pneumonia therapy. Hosp Pharm. 2021;56(4):221–7.
58. Buchan BW, Windham S, Balada-Llasat JM, Leber A, Harrington A, Relich R, et al. Practical comparison of the BioFire FilmArray pneumonia panel to routine diagnostic methods and potential impact on antimicrobial stewardship in adult hospitalized patients with lower respiratory tract infections. J Clin Microbiol. 2020;58(7)
59. Peiffer-Smadja N, Bouadma L, Mathy V, Allouche K, Patrier J, Reboul M, et al. Performance and impact of a multiplex PCR in ICU patients with ventilator-associated pneumonia or ventilated hospital-acquired pneumonia. Crit Care. 2020;24(1):366.
60. Posteraro B, Cortazzo V, Liotti FM, Menchinelli G, Ippoliti C, De Angelis G, et al. Diagnosis and treatment of bacterial pneumonia in critically ill patients with COVID-19 using a multiplex PCR assay: a large Italian hospital's five-month experience. Microbiol Spectr. 2021;9(3):e0069521.
61. Gradisteanu Pircalabioru G, Iliescu FS, Mihaescu G, Cucu AI, Ionescu ON, Popescu M, et al. Advances in the rapid diagnostic of viral respiratory tract infections. Front Cell Infect Microbiol. 2022;12:807253.
62. Vila J, Bosch J, Munoz-Almagro C. Molecular diagnosis of the central nervous system (CNS) infections. Enferm Infecc Microbiol Clin (Engl Ed). 2020;
63. Broadhurst MJ, Dujari S, Budvytiene I, Pinsky BA, Gold CA, Banaei N. Utilization, yield, and accuracy of the FilmArray meningitis/encephalitis panel with diagnostic stewardship and testing algorithm. J Clin Microbiol. 2020;58(9)
64. Trujillo-Gomez J, Tsokani S, Arango-Ferreira C, Atehortua-Munoz S, Jimenez-Villegas MJ, Serrano-Tabares C, et al. Biofire FilmArray Meningitis/Encephalitis panel for the aetiological diagnosis of central nervous system infections: a systematic review and diagnostic test accuracy meta-analysis. EClinicalMedicine. 2022;44:101275.
65. Posnakoglou L, Siahanidou T, Syriopoulou V, Michos A. Impact of cerebrospinal fluid syndromic testing in the management of children with suspected central nervous system infection. Eur J Clin Microbiol Infect Dis. 2020;39(12):2379–86.
66. Goodlet KJ, Tan E, Knutson L, Nailor MD. Impact of the FilmArray meningitis/encephalitis panel on antimicrobial duration among patients with suspected central nervous system infection. Diagn Microbiol Infect Dis. 2021;100(4):115394.
67. Dack K, Pankow S, Ablah E, Zackula R, Assi M. Contribution of the BioFire® FilmArray® meningitis/encephalitis panel. Kans J Med. 2019;12(1):1–3.
68. Torres-Miranda D, Akselrod H, Karsner R, Secco A, Silva-Cantillo D, Siegel MO, et al. Use of BioFire FilmArray gastrointestinal PCR panel associated with reductions in antibiotic use, time to optimal antibiotics, and length of stay. BMC Gastroenterol. 2020;20(1):246.
69. Amjad M. An overview of the molecular methods in the diagnosis of gastrointestinal infectious diseases. Int J Microbiol. 2020;2020:8135724.
70. Machiels JD, Cremers AJH, van Bergen-Verkuyten M, Paardekoper-Strijbosch SJM, Frijns KCJ, Wertheim HFL, et al. Impact of the BioFire FilmArray gastrointestinal panel on patient care and infection control. PLoS One. 2020;15(2):e0228596.
71. Meltzer AC, Newton S, Lange J, Hall NC, Vargas NM, Huang Y, et al. A randomized control trial of a multiplex gastrointestinal PCR panel versus usual testing to assess antibiotics use for patients with infectious diarrhea in the emergency department. J Am Coll Emerg Physicians Open. 2022;3(1):e12616.
72. Sood N, Carbell G, Greenwald HS, Friedenberg FK. Is the medium still the message? Culture-independent diagnosis of gastrointestinal infections. Dig Dis Sci. 2022;67(1):16–25.
73. Chang LJ, Hsiao CJ, Chen B, Liu TY, Ding J, Hsu WT, et al. Accuracy and comparison of two rapid multiplex PCR tests for gastroenteritis pathogens: a systematic review and meta-analysis. BMJ Open Gastroenterol. 2021;8(1)
74. Beal SG, Tremblay EE, Toffel S, Velez L, Rand KH. A gastrointestinal PCR panel improves clinical management and lowers health care costs. J Clin Microbiol. 2018;56(1)
75. Castany-Feixas M, Simo S, Garcia-Garcia S, Fernandez de Sevilla M, Launes C, Kalkgruber M, et al. Rapid molecular syndromic testing for aetiological diagnosis of gastrointestinal infections and targeted antimicrobial prescription: experience from a reference paediatric hospital in Spain. Eur J Clin Microbiol Infect Dis. 2021;40(10):2153–60.
76. O'Neal M, Murray H, Dash S, Al-Hasan MN, Justo JA, Bookstaver PB. Evaluating appropriateness and diagnostic stewardship opportunities of multiplex polymerase chain reaction gastrointestinal testing within a hospital system. Ther Adv Infect Dis. 2020;7:2049936120959561.
77. Eubank TA, Long SW, Perez KK. Role of rapid diagnostics in diagnosis and management of patients with sepsis. J Infect Dis. 2020;222(Suppl 2):S103–S9.
78.• Giacobbe DR, Giani T, Bassetti M, Marchese A, Viscoli C, Rossolini GM. Rapid microbiological tests for bloodstream infections due to multidrug resistant Gram-negative bacteria: therapeutic implications. Clin Microbiol Infect. 2020;26(6):713–22. **This review article provides a focused summary of rapid diagnostic tests used for Gram-negative bacteria bloodstream infections, including key publications that evaluated their impact on therapeutic decisions, patient outcomes, and stewardship**.
79. Burrowes SAB, Barlam TF, Skinner A, Berger R, Ni P, Drainoni ML. Provider views on rapid diagnostic tests and antibiotic prescribing for respiratory tract infections: a mixed methods study. PLoS One. 2021;16(11):e0260598.
80. Gilbert DN. Procalcitonin as a biomarker in respiratory tract infection. Clin Infect Dis. 2011;52(Suppl 4):S346–50.
81. Schuetz P, Albrich W, Mueller B. Procalcitonin for diagnosis of infection and guide to antibiotic decisions: past, present and future. BMC Med. 2011;9:107.
82. Brunkhorst FM, Heinz U, Forycki ZF. Kinetics of procalcitonin in iatrogenic sepsis. Intensive Care Med. 1998;24(8):888–9.
83. Maruna P, Nedelnikova K, Gurlich R. Physiology and genetics of procalcitonin. Physiol Res. 2000;49(Suppl 1):S57–61.

84. Dandona P, Nix D, Wilson MF, Aljada A, Love J, Assicot M, et al. Procalcitonin increase after endotoxin injection in normal subjects. J Clin Endocrinol Metab. 1994;79(6):1605–8.
85. Soreng K, Levy HR. Procalcitonin: an emerging biomarker of bacterial sepsis. Clin Microbiol Newsl. 2011;33(22):171–8.
86. Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. Clin Infect Dis. 2004;39(2):206–17.
87. Branche AR, Walsh EE, Vargas R, Hulbert B, Formica MA, Baran A, et al. Serum procalcitonin measurement and viral testing to guide antibiotic use for respiratory infections in hospitalized adults: a randomized controlled trial. J Infect Dis. 2015;212(11):1692–700.
88. Musher DM, Bebko SP, Roig IL. Serum procalcitonin level, viral polymerase chain reaction analysis, and lower respiratory tract infection. J Infect Dis. 2014;209(4):631–3.
89. Kamat IS, Ramachandran V, Eswaran H, Guffey D, Musher DM. Procalcitonin to distinguish viral from bacterial pneumonia: a systematic review and meta-analysis. Clin Infect Dis. 2020;70(3):538–42.
90. US Food and Drug Administration. FDA clears test to help manage antibiotic treatment for lower respiratory tract infections and sepsis February 23, 2017 [Available from: https://www.fda.gov/news-events/press-announcements/fda-clears-test-help-manage-antibiotic-treatment-lower-respiratory-tract-infections-and-sepsis#:~:text=The%20U.S.%20Food%20and%20Drug,stopped%20in%20patients%20with%20sepsis].
91. Kip MM, Kusters R, MJ IJ, Steuten LM. A PCT algorithm for discontinuation of antibiotic therapy is a cost-effective way to reduce antibiotic exposure in adult intensive care patients with sepsis. J Med Econ. 2015;18(11):944–53.
92. Kip MMA, van Oers JA, Shajiei A, Beishuizen A, Berghuis AMS, Girbes AR, et al. Cost-effectiveness of procalcitonin testing to guide antibiotic treatment duration in critically ill patients: results from a randomised controlled multicentre trial in the Netherlands. Crit Care. 2018;22(1):293.
93. Donner LM, Campbell WS, Lyden E, Van Schooneveld TC. Assessment of rapid-blood-culture-identification result interpretation and antibiotic prescribing practices. J Clin Microbiol. 2017;55(5):1496–507.
94. Messacar K, Parker SK, Todd JK, Dominguez SR. Implementation of rapid molecular infectious disease diagnostics: the role of diagnostic and antimicrobial stewardship. J Clin Microbiol. 2017;55(3):715–23.
95. Liaquat S, Baccaglini L, Haynatzki G, Medcalf SJ, Rupp ME. Clinical consequences of contaminated blood cultures in adult hospitalized patients at an institution utilizing a rapid blood-culture identification system. Infect Control Hosp Epidemiol. 2021;42(8):978–84.
96.• Curren EJ, Lutgring JD, Kabbani S, Diekema DJ, Gitterman S, Lautenbach E, et al. Advancing diagnostic stewardship for healthcare-associated infections, antibiotic resistance, and sepsis. Clin Infect Dis. 2022;74(4):723–8. **This article provides an overview of the current state of diagnostic stewardship in infectious diseases, including its potentials and limitations, and suggests strategies for advancing diagnostic stewardship with a focus on improving patient safety and health outcomes**.
97. Evans SR, Tran TTT, Hujer AM, Hill CB, Hujer KM, Mediavilla JR, et al. Rapid molecular diagnostics to inform empiric use of ceftazidime/avibactam and ceftolozane/tazobactam against *Pseudomonas aeruginosa*: PRIMERS IV. Clin Infect Dis. 2019;68(11):1823–30.
98. Patel YA, Kirn TJ, Weinstein MP, Uprety P. Systematic evaluation of the Accelerate Pheno system for susceptibility testing of Gram-negative bacteria isolated from blood cultures. Microbiol Spectr. 2021;9(3):e0183621.
99. Pliakos EE, Andreatos N, Shehadeh F, Ziakas PD, Mylonakis E. The cost-effectiveness of rapid diagnostic testing for the diagnosis of bloodstream infections with or without antimicrobial stewardship. Clin Microbiol Rev. 2018;31(3)
100. Flentie K, Spears BR, Chen F, Purmort NB, DaPonte K, Viveiros E, et al. Microplate-based surface area assay for rapid phenotypic antibiotic susceptibility testing. Sci Rep. 2019;9(1):237.
101. SeluxDx. Next-generation phenotyping (NGP) has arrived: Selux Diagnostics; [Available from: seluxdx.com/technology].
102. Kallai A, Kelemen M, Molnar N, Tropotei A, Hauser B, Ivanyi Z, et al. MICy: a novel flow cytometric method for rapid determination of minimal inhibitory concentration. Microbiol Spectr. 2021;9(3):e0090121.
103. Mulroney K, Kopczyk M, Carson C, Paton T, Inglis T, Chakera A. Same-day confirmation of infection and antimicrobial susceptibility profiling using flow cytometry. EBioMedicine. 2022;82:104145.
104. Li X, Liu X, Yu Z, Luo Y, Hu Q, Xu Z, et al. Combinatorial screening SlipChip for rapid phenotypic antimicrobial susceptibility testing. Lab Chip. 2022;
105. Mathews CJ, Weston VC, Jones A, Field M, Coakley G. Bacterial septic arthritis in adults. Lancet. 2010;375(9717):846–55.
106. BioFire Diagnostics. The BioFire® Joint Infection (JI) Panel [Available from: biofiredx.com/products/the-filmarray-panels/ji].

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.