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Why Can't We Just Use PCR? The Role of Genotypic versus Phenotypic Testing for Antimicrobial Resistance Testing

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Abstract

There is a need for phenotypic susceptibility testing that is expeditious and that can be performed directly from clinical specimens. While rapid pathogen identification is important, it is the susceptibility result that is essential for antimicrobial optimization. The options for rapid susceptibility testing are limited, with the majority of commercial tests available offering genotypic resistance detection only. In this article, a laboratorian and a clinician discuss the benefits and limitations of genotypic and phenotypic susceptibility testing and provide examples of how results should be interpreted to maximize the clinical utility.

Introduction

Historically, a "fast" result from the clinical microbiology laboratory is one that is reported within 24 hours of specimen receipt. Recently, there has been a radical movement in the clinical microbiology laboratory to provide expeditious results. One of the most noteworthy developments was the introduction of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), which has successfully replaced traditional biochemical identification methods at many institutions [1]. Other identification-focused approaches are multiplexed PCR [2] and microarray-based technologies [3]. Technological advancements have focused primarily on rapid identification of microorganisms directly from clinical specimens, including syndromic panels that are cleared by the Food and Drug Administration for *in vitro* diagnostic detection of a variety of organisms within 1 to 3 hours. These rapid identification tests have been remarkable in significantly shortening time to identification and, in some cases, antimicrobial optimization.

Despite the advances in identification technologies, the development of faster antimicrobial susceptibility testing (AST) has remained stagnant, limiting the potential impact of early pathogen identification. Efforts to provide antimicrobial susceptibility results in a timely manner have been most evident in bloodstream infection syndromic panels [2,3], which may detect resistance markers expressed by specific Gram-positive and Gram-negative organisms. Nonetheless, full antimicrobial susceptibility profile testing is required in many instances to allow more targeted therapy. This article describes the approaches to performing AST from clinical specimens/isolates, discusses the benefits and limitations of genotypic and phenotypic testing, and highlights current and emerging rapid susceptibility approaches.

Antimicrobial Optimization

Choosing the correct antibiotic is a cornerstone of treating infections. While this seems intuitive, the definition of "correct" is challenging. Multiple studies have shown increased morbidity and mortality, delayed bacterial clearance, and

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negative economic outcomes across a variety of scenarios, including extended-spectrum beta-lactamases (ESBL), Gram-negative bacteremia [4], urinary tract infection [5], methicillin susceptible *Staphylococcus aureus* (MSSA) bacteremia [6], pneumonia [7], and sepsis [8]. When choosing an empiric antimicrobial regimen, the clinician must weigh the risk of poor patient outcome from potentially under-treating a drug-resistant pathogen against the societal importance of antimicrobial stewardship [9]. Complicating this are patient-specific factors, including medication allergies, renal or hepatic disease, and drug interactions (Table 1). Formulary restrictions add another layer of intricacy to the situation. While it is difficult to choose the best empiric agent, clinicians depend on the results from the microbiology laboratory to select the most appropriate, targeted therapy. Traditionally, this has been dependent on culture and susceptibility results, although that paradigm may be shifting.

MIC, Breakpoints, Pharmacokinetics/Pharmacodynamics, and Other Terminologies

The MIC is the smallest amount of drug required to inhibit visible growth of a microorganism. Clinicians may not always appreciate that the MIC does not necessarily reflect efficacy; rather, it is simply a laboratory value reflecting a relationship between drug concentration and organism killing. Determining whether a given medication can be used for a particular microbe or infection type requires knowledge of the medication's pharmacokinetic (PK) and pharmacodynamic (PD) parameters [10]. PK describes the interaction between the drug and the host: absorption, distribution, and elimination. PD details the effect of the drug on the organism. The combination of PK/PD parameters, along with the MIC and several other factors, such as clinical outcome data and MICs for isolates with known mechanisms of resistance, leads to the creation of clinical breakpoints and guides the clinical laboratory's reporting strategies for susceptibility.

Table 1. Clinician considerations when choosing empiric antibiotics

- Has the patient been exposed to antibiotics recently?
- Does the patient reside in a long-term care facility or have other multi-drug-resistant-organism risk factors?
- Does the patient have prior culture data showing antimicrobial resistance?
- Can the patient take medications orally?
- If intravenous antibiotics are needed, does the patient have stable venous access?
- What is the patient's renal function?
- What other medications is the patient receiving that might interact with what would be typically used for therapy?
- Where is the infection? Which antibiotics will penetrate that location?
- What antibiotics are on the institution (or, for outpatients, insurance) formulary?

If a "drug-bug" combination is reported as "susceptible," this indicates that the organism should be adequately responsive to therapy using normal recommended doses. "Resistant" indicates that this goal is not achievable. Sometimes, an "intermediate" result is reported, which generally indicates that the MIC or zone diameter may be more difficult to overcome than for "susceptible" isolates, but clinical efficacy might still be achieved, depending on the dosage or site of infection. Adding to the complexity is the more recent category of "susceptible-dose dependent," used when there is evidence that a more aggressive dosing regimen can be used to treat an infection with an MIC above susceptible but below resistant. This is different from intermediate. Occasionally, a report of "nonsusceptible" may be seen. In this case, only a breakpoint for susceptible exists because resistance is exceedingly rare. Nonsusceptible does not mean that the isolate has a resistance mechanism. Instead, it indicates that there is only evidence to support a definition for susceptibility. Finally, there are some drugs (i.e., colistin) that only have epidemiological cutoff values (ECVs), defined as the upper limit of susceptibility for the wild-type population of isolates. It is important to emphasize that ECVs are not clinical breakpoints and are provided for informational purposes only [11].

Phenotypic Antimicrobial Susceptibility Testing

Traditional phenotypic susceptibility testing is performed by disk diffusion, broth microdilution, and agar dilution. Disk diffusion depends upon the ability of antibiotic molecules to diffuse out from a disk into the agar, forming a circular zone. The zone diameter is measured after 18 to 24 hours of incubation and compared to standardized charts, allowing qualitative susceptibility interpretations [10,12]. In addition to disk diffusion, the Clinical and Laboratory Standards Institute (CLSI) recognizes two other reference standard AST methods: broth microdilution and agar dilution [13]. These two methods offer MIC results alongside clinical breakpoint interpretation. In contrast, a limitation of the disk diffusion method is the absence of an MIC result, and only the breakpoint interpretation is reported to clinicians.

Agar dilution requires the preparation of individual agar plates pre-mixed with known antibiotic concentrations, allowing MIC assessment. An organism is then dissolved into solution and inoculated on the plate. The plate with the lowest drug concentration at which there is no growth is determined to represent the MIC. Similarly cumbersome, the preparation of reference broth microdilution is rarely offered outside of reference or research laboratories. Therefore, MICs are generally obtained via automated systems such as Microscan Walkaway, BD Phoenix, Vitek 2, or Sensititre ARIS 2X [14]. Each system has strengths and limitations, but all are unified in the low requirement for hands-on time; use of intrinsic interpretation rules that are built into the software; and the ability to provide quantitative MIC values, albeit only in doubling concentrations (e.g., 0.5, 1, 2, 4, 8, etc). Additionally, gradient diffusion (i.e., Etest and Liofilchem) combines the

methodology of disc diffusion with the quantitative MIC results. This method utilizes an antibiotic-impregnated strip with decreasing gradients. The shape of the zone of inhibition is elliptical; the MIC is the point at which the tips of the ellipse touch the strip.

Why Phenotypic Results Are Needed

Standardized phenotypic testing and result interpretation are neither easy nor straightforward. How to best report results to provide optimal information to clinicians is an ongoing challenge for CLSI and other agencies [15]. An example of clinician confusion is the surprisingly common question, "Should I choose the antibiotic with the lowest MIC?" This leads to a teaching moment about the drug-bug relationship and the PK/PD aspects that should be considered. Other examples of difficulties are understanding the susceptibility pattern of methicillin-susceptible staphylococci (e.g., requests for cefazolin results) and requests for tetracycline susceptibility results for enterococci from sites other than urine.

Despite these limitations, phenotypic testing is available for bacteria regardless of the location of infection (with appropriate reporting in mind). The microbiology laboratory can provide susceptibility results for *Escherichia coli* from cerebral spinal fluid, blood, urine, and bone, all with reasonable reliability. Phenotypic testing also allows nuance in interpretation. For example, a carbapenemase-producing *Klebsiella* isolate with a detectable enzymatic mutation may have an MIC of 4 or of 16. The decision to use meropenem as part of the treatment regimen may, in part, be determined by that MIC (and knowledge of this MIC could not be represented simply by detection of a carbapenemase gene).

Phenotypic testing is the gold standard method for guiding antimicrobial treatment decisions. While molecular assays may change this, the inherent flexibility of phenotypic testing makes it a powerful tool in the treatment of infections. This, along with decades of experience basing therapeutic decisions on phenotypic results, as well as cost and ease, ensures that phenotypic testing will still be a part of the clinical microbiology laboratory's practice, at least for the near future.

Genotypic Antimicrobial Susceptibility Testing

Genotypic susceptibility testing is the detection of resistance genes expressed in a specific organism by molecular methods. An example in the clinical laboratory is the screening for methicillin-resistant *Staphylococcus aureus* (MRSA) colonization by detection of the *mecA* gene [16]. The combination of bacterial targets coupled with resistance determinants is a valuable component of the commercially available blood culture panels [2,3]. Table 2 summarizes the current FDA-cleared panels, as well as panels undergoing clinical trials that incorporate genotypic resistance detection alongside pathogen identification. The two primary Gram-positive resistance targets are *mecA* for the detection of MRSA and *vanA* and *vanB* for the detection of vancomycin-resistant enterococci (VRE). Resistance genes specific to Gram-negative organisms include CTX-M for detection of ESBL and a range of carbapenemase-producing genes (i.e., bla_{KPC}, bla_{NDM}, and bla_{VIM}) [2,17].

Rapid detection of the presence (or absence) of a resistance gene may allow improved antimicrobial therapy independent of phenotypic susceptibility results. This is most evident in the case of infections by Gram-positive bacteria (Table 3). Ruling out methicillin resistance in an *S. aureus* bloodstream infection based on the absence of *mecA* expression allows de-escalation from vancomycin to an anti-staphylococcal beta-lactam agent (cefazolin, oxacillin, or nafcillin) within a few hours rather than days. On the other hand, early detection of vancomycin resistance in *Enterococcus* species by detection of *vanA* or *vanB* ensures escalation of antimicrobial therapy if the patient is on vancomycin empiric therapy. Several studies have supported positive patient outcomes when rapid genotypic susceptibility results were available within hours of blood culture positivity [18, 19]. In one, there was a decrease in the treatment of coagulase-negative staphylococcal bacteremia and a more rapid change to appropriate therapy for VRE bacteremia [20]. Another group demonstrated faster initiation of appropriate ESBL bacteremia therapy and a reduction in intensive care unit length of stay [21].

Although not widely available for clinical use, genotypic screening for the *gyrA* gene in *Neisseria gonorrhoeae has* proven to be highly predictive of ciprofloxacin resistance [22]. Additional screening for extended-spectrum cephalosporins by targeting *penA* mosaic XXXIV yielded high sensitivity and specificity (97% and 100%, respectively) [23]. This is particularly relevant, as a majority of gonococcal infections are diagnosed by molecular methods, and recovery of organisms for AST is uncommon. An additional benefit of genotypic testing is that it is not dependent on live cells for detection, which may be beneficial in patients with prior exposure to antimicrobial agents.

Currently, rather than replacing phenotypic susceptibility testing, genotypic testing supplements it. An important concept to emphasize is that, in many incidences, the absence of a resistance gene does not necessarily predict susceptibility to a particular drug. This is most evident for the Gram-negative resistance markers included in the bloodstream infection panels (Table 4). For instance, molecular detection of *E. coli* from a positive blood culture in the absence of $bla_{CTX\text{-}M}$ or bla_{KPC} does not equal susceptibility to cephalosporins and carbapenems. There are many other resistance mechanisms to be considered, such as porin loss and efflux pumps, as well as numerous additional ESBL and carbapenemase genes that may be produced by an *E. coli* isolate [24].

In line with this issue is the modest number of resistance genes included in each panel, hindering antimicrobial stewardship even in the absence of targeted resistance genes. As mentioned above, this limitation is more relevant for the Gram-negative organisms but is also noteworthy for some Gram-positive organisms. For instance, a MRSA isolate that harbors the *mecC* gene, a divergent *mecA* gene, would be misidentified as MSSA by both the Verigene Gram-positive blood culture panel and Filmarray Blood Culture Identification (BCID) panel. Unfortunately, additional phenotypic susceptibility testing using commercial systems may also interpret these isolates as falsely susceptible [25]. In an attempt to avoid potential false negatives caused by *mecC*-harboring MRSA, the

Table 2. Commercial diagnostic panels with genotypic resistance detection[a]

Assay	Company	FDA cleared	Specimen type	Method	Target	Resistance gene	Run time (h)
Xpert MRSA/SA Gen 3	Cepheid	Yes	Blood culture	RT-PCR	*Staphylococcus aureus*	*mecA* *mecC*	1
Xpert MTB/RIF	Cepheid	Yes	Sputum	RT-PCR	*Mycobacterium tuberculosis* complex	*rpoB*	
Xpert MRSA/SA SSTI	Cepheid	Yes	Swab	RT-PCR	*Staphylococcus aureus*	*mecA*	
BD Max StaphSR	BD	Yes	Blood culture	RT-PCR	*Staphylococcus aureus*	*mecA*	~1.5
mecA *Xpress*FISH	AdvanDx	Yes	Blood culture	PNA-FISH	*Staphylococcus aureus*	*mecA*	0.5
Verigene Blood Culture-Gram Positive	Luminex	Yes	Blood culture	Microarray	*Staphylococcus aureus, Staphylococcus lugdunensis, Staphylococcus epidermidis, Staphylococcus* spp., *Streptococcus anginosus* group, *Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus* spp., *Enterococcus faecalis, Enterococcus faecium Listeria* spp.	*mecA, vanA, vanB*	2.5
Verigene Blood Culture-Gram Negative	Luminex	Yes	Blood culture	Microarray	*Escherichia coli/Shigella* spp., *Klebsiella pneumoniae, Klebsiella oxytoca, Pseudomonas aeruginosa, Serratia marcescens, Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., *Proteus* spp.	CTX-M, IMI, VIM, KPC, NDM, OXA	2.5
FilmArray Blood Culture Identification	BioFire Diagnostics	Yes	Blood culture	Nested PCR	**Gram-negative bacteria** *Acinetobacter baumannii, Haemophilus influenzae*, *Neisseria meningitidis, Pseudomonas aeruginosa, Enterobacteriaceae, Enterobacter cloacae* complex, *Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus* spp., *Serratia marcescens* **Gram-positive bacteria** *Enterococcus* spp., *Listeria monocytogenes, Staphylococcus* spp., *Staphylococcus aureus, Streptococcus* spp., *Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes* **Yeasts** *Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis, Candida tropicalis*	*mecA, vanA, vanB*, KPC	1
ePlex Blood Culture Identification-Gram Positive	GenMark	No	Blood culture	DNA hybridization and electrochemical detection	*Bacillus cereus* group, *Bacillus subtilis* group, *Corynebacterium, Cutibacterium acnes, Enterococcus* spp., *Enterococcus faecalis, Enterococcus faecium, Lactobacillus, Listeria monocytogenes, Micrococcus, Staphylococcus aureus, Staphylococcus lugdunensis, Staphylococcus epidermidis, Staphylococcus* spp., *Streptococcus anginosus* group, *Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus* spp. **Pan-Gram-negative** Pan-*Candida*	*mecA* *mecC* *vanA, vanB*	1.5

Table 2. Commercial diagnostic panels with genotypic resistance detection[a] (Continued)

Assay	Company	FDA cleared	Specimen type	Method	Target	Resistance gene	Run time (h)
ePlex Blood Culture Identification-Gram Negative	GenMark	No	Blood culture	DNA hybridization and electrochemical detection	*Acinetobacter baumannii, Citrobacter* spp., *Citrobacter sakazakii, Enterobacter cloacae* complex, *Enterobacter* (non-*cloacae* complex), *Escherichia coli, Haemophilus influenzae*, *Klebsiella oxytoca, Klebsiella pneumoniae, Morganella morganii, Neisseria meningitidis, Proteus* spp., *Proteus mirabilis, Pseudomonas aeruginosa, Salmonella* spp., *Serratia marcescens, Serratia* spp., *Stenotrophomonas maltophilia* **Obligate anaerobes** *Bacteroides fragilis, Fusobacterium nucleatum, Fusobacterium necrophorum* **Pan Gram positive** Pan *Candida*	CTX-M, IMI, VIM, KPC, NDM, OXA	1.5
FilmArray Pneumonia Panel	BioFire Diagnostics	No (submitted)	Lower respiratory tract specimens (BAL, mini-BAL, sputum, ETT aspirates)		**Bacteria (semi-quantitative)** *Acinetobacter calcoaceticus-baumannii* complex, *Serratia marcescens, Proteus* spp., *Klebsiella pneumoniae* group, *Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Haemophilus influenzae, Moraxella catarrhalis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella oxytoca, Streptococcus pyogenes, Streptococcus agalactiae* **Atypical bacteria (qualitative)** *Legionella pneumophila, Mycoplasma pneumoniae, Chlamydia pneumoniae* **Viruses (qualitative)** Influenza A virus, influenza B virus, respiratory syncytial virus, human rhinovirus/enterovirus, human metapneumovirus, parainfuenza virus, adenovirus, coronavirus, Middle East respiratory syndrome coronavirus	*mecA/, mecC,* CTX-M, IMI, VIM, KPC, NDM, OXA48-like	1
Unyvero Lower Respiratory Tract Panel	Curetis	No (submitted)	Lower respiratory tract specimens (ETT aspirates, BAL, mini-BAL)	Endpoint PCR and hybridization	**Gram-negative bacteria** *Acinetobacter baumannii* complex, *Citrobacter freundii, Escherichia coli, Enterobacter cloacae* complex, *Haemophilus influenzae, Klebsiella oxytoca Klebsiella pneumoniae, Klebsiella variicola, Moraxella catarrhalis, Morganella morganii, Proteus* spp., *Pseudomonas aeruginosa, Serratia marcescens, Stenotrophomonas maltophilia* **Gram-positive bacteria** *Staphylococcus aureus, Streptococcus pneumoniae* **Atypical bacteria** *Chlamydophila pneumoniae, Mycoplasma pneumoniae, Legionella pneumoniae* **Fungi** *Pneumocystis jirovecii*	CTX-M gyrA48 gyrA87 gyrA83 gyrA87 KPC mecA NDM OXA (23, 24/40, 48, 58) SHV sul1 TEM VIM	1

[a] SA, *Staphylococcus aureus*; RT-PCR, real-time polymerase chain reaction; PCR, polymerase chain reaction; PNA-FISH, peptide nucleic acid-fluorescence in situ hybridization; BAL, bronchoalveolar lavage; ETT, endotracheal tube.

Xpert MRSA Gen 3 (Cepheid, Sunnyvale, CA), was recently FDA cleared as a molecular test to screen for MRSA using both *mecA* and *mecC* [26]. Nonetheless, it is imperative that commercial companies continue to improve molecular panels by incorporating new and relevant resistance targets that maximize the utility of the test with respect to antimicrobial stewardship and patient management.

Finally, detection of Gram-negative resistance genes does not necessarily predict therapeutic failure. The CLSI does not require routine screening for ESBL and carbapenemase production, recommending testing only for epidemiological and infection control purposes [11]. To predict resistance solely based on the detection of these specific resistance genes may result in overcalling of resistance and unnecessary utilization of broader antimicrobial agents.

What Are Current Testing Options for Rapid AST?

As mentioned above, technological advances primarily offer genotypic markers as a susceptibility testing solution. There are a number of FDA-cleared assays that detect *S. aureus*, as well as the *mecA* gene, directly from positive blood cultures (Table 2). The Verigene blood culture panels (Luminex Corporation, Austin, TX) and the FilmArray BCID panel (BioFire Diagnostics, Salt Lake City, UT) offer the most comprehensive panels, targeting Gram-positive and Gram-negative organisms, as well as the respective resistance markers. The FilmArray BCID panel also includes targets for five *Candida* species. Numerous studies on the performance of these panels reported high agreement for both identification and resistance genes compared to conventional identification and AST methods [2,27]. The *mecA* and *vanA vanB* genes have high predictive value for *S. aureus* susceptibility to oxacillin and/or cefoxitin and enterococcal susceptibility to vancomycin, respectively [2,27]. Discrepancies between molecular and phenotypic results may be seen. The CLSI provides a guide to assist laboratorians in investigating these situations, which includes recommendations for reporting when there is discordance between molecular and phenotypic assays for *S. aureus* (oxacillin), *Enterococcus* spp. (vancomycin), and *Enterobacteriaceae* (extended-spectrum beta-lactams). The take home message is as follows: if discrepancy remains unresolved after

Table 3. Case example demonstrating the role of a Gram-positive resistance marker in optimizing antimicrobial therapy

Case presentation

A 35-year-old male with a history of intravenous drug use was admitted with signs and symptoms of infective endocarditis (fever, new right sternal systolic heart murmur, acute renal failure, and leukocytosis). He met the hospital's sepsis protocol criteria and received empiric vancomycin plus piperacillin-tazobactam.

Laboratory workup

Workup of positive blood cultures by molecular identification plus genotypic AST panel revealed MSSA. Results were reported within 3 hours from the time of blood culture positivity.

What was useful

Identification of MSSA from blood cultures confirmed that this was a case of endocarditis caused by a Gram-positive organism, and piperacillin-tazobactam may be discontinued.

Absence of the *mecA* gene by molecular methods indicated that the pathogen could be treated with a beta-lactam agent.

What was not useful

There was still a small (but non-zero) possibility of polymicrobial bacteremia, particularly if the source was a contaminated needle.

Addition of clindamycin or rifampin (in some patients) would still require phenotypic susceptibility results.

Response from clinician

The clinician agreed to narrow therapy to only nafcillin.

Table 4. Case example demonstrating the role of Gram-negative resistance markers in optimizing antimicrobial therapy

Case presentation

An 8-year-old female was admitted with chemotherapy-induced neutropenic fever. She was empirically started on meropenem and vancomycin according to the hospital's protocol, and 1 set of blood cultures was collected.

Laboratory workup

Workup of positive blood cultures by molecular identification plus a genotypic AST panel revealed *Klebsiella pneumoniae* with no KPC gene detected. Results were reported within 3 hours from the time of blood culture positivity.

What was useful

Identification of *K. pneumoniae* from blood cultures confirmed that this was a case of Gram-negative bloodstream infection, and vancomycin might be discontinued.

A caveat to this would be in patients with high risk for polymicrobial infections that might justify maintaining Gram-positive coverage.

What was not useful

Absence of a KPC gene does not mean absence of other carbapenemase genes expressed by the organism, and de-escalation of therapy might not be warranted.

Absence of KPC does not mean that meropenem has appropriate coverage. There might be other resistance mechanisms involved that might result in resistance to carbapenems. There is no way to determine this based on a molecular result.

Response from clinician

The clinician declined to narrow therapy, because he lacked data showing that the organism was susceptible to an alternate agent, such as cefepime.

additional testing, the organism should be reported as resistant to the respective antimicrobial agent [28].

Genotypic susceptibility is also applicable to other clinical sources. An excellent example of this is the Xpert platform (Cepheid), which couples pathogen identification and resistance detection from sputum for the detection of the *Mycobacterium tuberculosis* complex and the *rpoB* gene to predict resistance to rifampin. A multi-center study of 1,730 patients with suspected pulmonary tuberculosis reported correct prediction in 97.6% (200/205) of rifampin-resistant *M. tuberculosis* complex isolates and 98.1% (504/514) of rifampin-susceptible *M. tuberculosis* complex isolates [29]. A recent study reported high predictive value for rifampin-resistant bacteria but a mean agreement of drug regimen between genotypic and phenotypic susceptibility of only 49% [30]. This reiterates the need to complement genotypic susceptibility testing with phenotypic testing to avoid ineffective treatment regimens.

The lone FDA-cleared panel that offers expedited phenotypic susceptibility testing of positive blood cultures is the Accelerate PhenoTest BC (Accelerate Diagnostics, Tucson, AZ). This panel offers detection of 14 Gram-positive and Gram-negative bacteria, as well as two yeast targets, by fluorescent *in situ* hybridization (FISH). The *pièce de résistance* is the generation of MIC values using a morphokinetic cellular analysis of individual immobilized bacterial cells every 10 minutes in the presence or absence of antimicrobial agents. For each drug-specific test, a single concentration of antibiotic is used to provide MICs and categorical interpretations of susceptible, intermediate, or resistant in approximately 7 hours. This represents an innovative first step in the quest for rapid phenotypic susceptibility tests. The clinical trial was conducted across 13 U.S. institutions on 872 prospective clinical samples and 1,068 seeded samples [31]. The essential agreement (EA) and categorical agreement (CA) ranged from 94.3% to 97.9%, with ≤1.0 major error (ME) or very major error (VME). Similarly, a single-center U.S. study of 232 positive blood cultures reported an EA and CA of 95%, with 1 VME and 3 MEs. The investigators reported a substantial decrease in time to susceptibility results of almost 42 hours compared to standard-of-care testing, with a reduction in hands-on time of about 30 minutes [32]. The test does not eliminate the need to subculture positive blood cultures to ensure detection of other organisms not included in the panel. However, in the majority of cases, the PhenoTest BC can function as a standalone test in the sense that subsequent AST from culture is not required.

In view of the limited options available for rapid phenotypic AST, clinical laboratories have taken it upon themselves to provide solutions to the clinicians. Doern and colleagues [33] reported promising disk diffusion AST results from 555 blood cultures using a "quick and dirty" approach of 6 drops of blood culture broth on Mueller-Hinton agar. Numerous studies have also reported high agreement compared to conventional AST using such approaches as generating a bacterial cell pellet to test on commercial automated AST systems [34, 35]. Unfortunately, these approaches are not widespread in clinical laboratories for reasons including limited resources and expertise, regulatory concerns over using laboratory-developed tests, and a low sense of urgency due to the perception that patients are on appropriate empiric therapy. Therefore, in order to ensure widespread dissemination in clinical laboratories, development of direct-from-specimen AST systems at a reasonable/low cost needs to be a priority. For instance, a recent proof-of-concept study from the CLSI AST Subcommittee ad hoc working group on direct AST from blood cultures was conducted with the goal to develop and standardize a direct from blood culture AST that can be performed in all laboratories. Preliminary data indicate CAs between 94.7% and 96.2% after an 18-hour incubation [36].

New Rapid AST Technologies in the Pipeline

Development of panels with genotypic resistance detection is ongoing. The ePlex BCID panels (GenMark Diagnostics, Carlsbad, CA) are currently undergoing clinical trials and offer resistance markers identical to those in the Verigene blood culture panels, with the exception of *mecC* (Table 2). The FilmArray pneumonia panel will be the next test offered by BioFire Diagnostics, offering the first semi-quantitative multiplex panel for detection of bacteria, as well as qualitative detection of viruses and atypical bacteria. The resistance markers included in the panel are in line with those in the ePlex assay. Likewise, the Unyvero platform (Curetis, San Diego, CA), with lower respiratory tract application, was recently FDA cleared and consists of 36 pathogen targets and resistance markers. For additional commercial genotypic AST assays that will be coming down the pipeline, refer to a review by Dunne et al. [37].

There is certainly enthusiasm for faster phenotypic AST from a variety of clinical specimens, as demonstrated by an influx of innovative strategies. Several approaches have been explored, including digital real-time loop-mediated isothermal amplification (dLAMP) [38] and partnering microfluidics with nanotechnology [39]. The dLAMP assay proposes phenotypic AST results within 30 min directly from clinical urine samples [38]. A recent proof-of-principle study introduced a microscopy-based AST platform consisting of a solid-phase microwell growth surface that allows restriction of cells to a microfluidic channel. The proposed time to AST is approximately 2 hours, although a live culture of the bacteria is required for testing [40]. Refer to a review by Li et al. for an excellent summary of emerging technologies for phenotypic AST [41].

Despite many proof-of-concept systems in development, only a few, such as mariAST (ArcDia, Finland) [42], are commercially available, none of which are available in the U.S. One system with commercial prospects is the LifeScale-AST system (LifeScale, Santa Barbara, CA), which employs ultra-high -solution mass measurement to determine individual microbe mass. The initial panel will offer testing on Gram-negative bacilli from positive blood cultures using a customized Sensititre plate that allows MIC determination and breakpoint interpretation in approximately 3 hours. Since there is no identification feature, a drawback is that the system is reliant on the adoption of a rapid identification test in the clinical laboratory to maximize the utility of the test. Another upcoming assay is the Smarticle (Roche, Pleasanton, CA), which uses recombinant bacteriophages with DNA probes that bind to specific bacterial targets to deliver a custom-designed DNA molecule that causes viable bacteria to express luciferase. For example, the absence of

luciferase expression in a sample mixed with a defined concentration of antibiotic would be interpreted as susceptible. There are no publications on the two systems to date.

Another promising system is the 216Dx UTI System (BacterioScan, St. Louis, MO). The system utilizes forward laser light-scattering technology to rapidly screen urine for the presence of bacteria at a limit of detection of 10^3 CFU/ml. The technology has been proven to perform AST directly from urine samples with promising results [43,44]. An innovative approach from Accelerate Diagnostics is the development of a lower respiratory tract panel on the Pheno System (described above). This will offer both identification and phenotypic AST, a feature that continues to be unique among the FDA-cleared systems, including the lower respiratory tract panels that will be offered by BioFire Diagnostics and Curetis. A potential downside of these methods, however, is increased cost compared to traditional susceptibility testing methods.

Challenges of New Technologies

Despite the enthusiasm for new and emerging AST technologies, regulatory policies pose a continuous challenge for clinical laboratories and diagnostic manufacturers. An excellent review by Humphries and Hindler highlights the challenges encountered, including frequently modified clinical breakpoints, new antimicrobial agents that are not available for testing on commercial systems, and breakpoint restrictions for organisms not included in clinical trials. The authors offer potential solutions to the problems via collaborative efforts among multiple entities, including the FDA, CDC, diagnostic manufacturers, and pharmaceutical manufacturers [45].

Other challenges are the very practical considerations of cost and staff training. As health care costs continue to be closely scrutinized, clinical microbiology laboratories and health care systems will need to consider the cost of these assays in relation to the clinical outcomes.

As mentioned above, the assays add additional workflow complexity, rather than replacing traditional microbiology. Outcome data are also mixed. Positive results from rapid blood culture molecular AST detection were described above, but the data supporting de-escalation are much less robust, reflecting the limitations of the kits themselves, as well as lack of physician confidence in and understanding of molecular results [21,46].

Lastly, it is of paramount importance that clinical laboratories assess the performance of new technologies prior to implementation to ensure that discrepancies in antimicrobial susceptibilities compared to a reference method are at a minimum. For example, if drug-bug combinations are prone to ME or VME, the laboratory should consider withholding results and performing secondary AST prior to reporting.

Conclusion

Rapid detection of organism names and susceptibility patterns has the potential to profoundly alter treatment plans and patient outcomes. Novel methods, which will be accessible to clinical microbiology laboratories, are being designed to identify resistance markers and quickly determine susceptibility profiles. Many challenges remain, including the need to validate the assays and to ensure that results are understandable to clinicians and that the cost does not exceed the benefit. It is an exciting time to work in the fields of clinical microbiology and infectious diseases.

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