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COMMENTARY

The Slow March toward Rapid Phenotypic Antimicrobial Susceptibility Testing: Are We There Yet?

Christopher D. Doern[a]

[a]Department of Pathology, Virginia Commonwealth University Health System, Richmond, Virginia, USA

ABSTRACT Antimicrobial susceptibility testing (AST) provides critical information for the management of patients with infections. The gold standard methods for assessing organism susceptibility are still based on growth and require incubation over relatively long periods of time. Until now, little progress has been made in developing rapid, growth-based, phenotypic AST systems. This commentary puts the recently FDA-cleared Accelerate PhenoTest (P. Pancholi et al., J Clin Microbiol 56:e01329-17, 2018, https://doi.org/10.1128/JCM.01329-17) in context by providing a historical perspective on attempts to accelerate phenotypic susceptibility results. In addition, some promising new innovations that promise to shorten the turnaround time for phenotypic AST will be briefly reviewed.

KEYWORDS rapid, blood culture, susceptibility testing, phenotypic

Clinical microbiology laboratories (CMLs) have always endeavored to produce timely results, but clinical microbiology has historically been one of the slower laboratory disciplines. Given the obstacles posed by the necessity of organism incubation, the path to impacting care requires that results be produced as quickly as possible. The laboratory is given the task of diagnosing infections, which in many cases are acute events that require the administration of empirical treatment long before definitive diagnostic microbiology results are provided by the laboratory. In fact, the very concept of empirical therapy is a testament to the reality that the CML is often unable to render information in a time frame that can inform initial treatment decisions. The microbiology community is not oblivious to this fact, but the practical reality is that the speed of our diagnostic methods is dependent on cultivating an organism, and that simply takes time.

The past decade or so has witnessed widespread adoption of game-changing technologies that have dramatically improved the speed with which some infections can be diagnosed. Among the most important advances has been the development of rapid multiplex PCR panels (RMPPs) and matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS). In the case of RMPPs, they have been applied primarily to the diagnosis of upper respiratory tract infections, but they have also been widely adopted for organism and resistance gene identification from positive blood cultures (1, 2). Numerous studies have now shown that RMPPs performed from positive blood cultures can lead to tremendous improvements in both patient outcomes and hospital costs (3, 4).

Mass spectrometry has had a similarly impressive impact on shortening time to identification, and some outcome-based studies have demonstrated the same impact for MALDI-TOF MS as they have for RMPPs (5) Interestingly, although susceptibility testing is not performed with MALDI-TOF MS, it does appear to have an indirect effect on the turnaround time of susceptibility results. In two large academic medical centers (one pediatric and one primarily adult), the implementation of MALDI-TOF MS led to significant improvements in the time to organism identification and time to definitive

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Editor Betty A. Forbes, Virginia Commonwealth University Medical Center

Address correspondence to Christopher.doern@vcuhealth.org.

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susceptibility results. The impact was most pronounced in cystic fibrosis cultures, but it was also observed for blood cultures (unpublished data).

Despite improvements offered by these technologies, there is still one advance that continues to elude the microbiology laboratory: rapid phenotypic antimicrobial susceptibility testing (AST). Due in large part to the fact that phenotypic AST requires growth in the presence of an antibiotic, the turnaround time for AST has remained virtually unchanged over the past several decades. Recognizing these limitations, microbiologists have looked for ways to accelerate AST using adaptations of currently available methods. A particular focus has been AST directly from positive blood culture. Here, a variety of approaches have been studied and generally show that using the positive blood culture medium as the inoculum for AST can produce reliable results (6–8). In the early 1980s, Doern et al. utilized positive blood culture broth as an inoculum for disk diffusion AST and estimated that this practice could yield results in 16 to 18 h, which was a 30-h improvement compared to the 48 h generally required for conventional AST methods (9). Fast forward more than 30 years, and not much has changed. Wattal and Oberoi recently published very similar findings in their study which evaluated direct inoculation of an automated AST system from positive blood culture broth (10). In their study, they found that automated AST results could be ready in approximately 10 h when using blood culture broth as an inoculum.

These methods are appealing in that they generate rapid results with reasonably reliable performance, but they require significant amounts of hands-on time and are cumbersome in high-volume laboratory settings. Consequently, despite the promise of improved turnaround times for the CML's most urgent result (blood culture AST), many laboratories have not implemented direct AST from positive blood cultures.

Although the methods described above shorten the reporting turnaround time for blood cultures, the methods themselves are not improvements in the time it takes to assess the antimicrobial susceptibility of an organism. Molecular biology and the detection of resistance genes can certainly play an important role in determining what antibiotics cannot be used to treat an infection, but these methods generally lack the ability to provide a comprehensive assessment of what an organism can be treated with. At this time, growth-based, phenotypic AST is still required to determine fully the susceptibility profile of an organism. As a result, a number of innovative approaches have been developed in an attempt to accelerate phenotypic AST, which has always taken at least 12 h.

The prospect of shortening growth-based AST is, biologically, a difficult task. Most, if not all, antibiotics exert their activity by targeting some aspect of organism viability; such as cell wall assembly, protein synthesis, replication of nucleic acids, or metabolic pathways. Therefore, in order for AST to assess the activity of an antibiotic, the organism in question must be actively replicating. One of the most fundamental elements of bacteriology is the three phases of an organism growth curve; lag phase, logarithmic (or exponential) phase, and stationary phase. Unfortunately, traditional AST methods require that an organism transition from lag phase to an actively growing phase, or logarithmic growth, in order to fully assess antimicrobial susceptibility. The length of time of lag phase growth varies, but it can take several hours for many organisms. This reality poses a significant challenge to those growth-based technologies which are attempting to shorten the turnaround time for phenotypic AST results.

A general area with great promise in improving AST turnaround times is nanotechnology partnered with microfluidics. A number of companies have developed very sophisticated nanoscale AST platforms that allow large numbers of organism/antibiotic combinations to be tested in a rapid time frame. One of the benefits of the scalability of nanotechnology is that it allows for replicate testing within a single assay, which can help to mitigate erroneous results. There are numerous mechanisms by which nanoscale methods detect organism growth (and AST) including optical density readings (11), fluorescence secondary to metabolic pathways (12), and many others. For an excellent review of emerging applications of nanotechnology for susceptibility testing, see Li et al. (13).

In addition, alternatives to nanotechnology are also being explored. For example, Entenza et al. (14) were able to use heat detection as a marker of growth to accurately determine whether methicillin-resistant *Staphylococcus aureus* (MRSA) strains were vancomycin susceptible, intermediate, or heterogeneous populations of intermediate resistance (hVISA). Their method was able to accurately classify vancomycin susceptibility in $<$8 h (14).

Of particular interest is the concept of phenotypic susceptibility testing directly from the specimen, where the specimen itself is used as the organism inoculum. While appealing, there are significant challenges to this approach. First, most growth-based susceptibility testing requires a standardized inoculum where a known concentration of organism is used for AST. In routine testing, the organism concentration is fixed, and it may be significantly higher than what is encountered in a clinical specimen which may be used for direct inoculation. An exception may be the urine culture, where patients with real infections commonly have more than 10^5 CFU/ml. Mezger et al. published a proof-of-concept study in which urine was used as an inoculum for rapid AST (15). This method employed a brief incubation period ($\sim$120 min) followed by quantitative PCR designed to quantify growth. Pilot experiments showed that the assay was able to accurately determine *Escherichia coli* susceptibility to ciprofloxacin and trimethoprim within 3.5 h.

All of these technologies have two things in common. First, they offer the potential to greatly improve AST turnaround times, and second, they are not commercially available and have not been approved for use in patient care by the Food and Drug Administration (FDA). Published in this issue of the *Journal of Clinical Microbiology* is the clinical trial data for the first commercially available product to obtain FDA approval for rapid, phenotypic AST (16). The data presented by Pancholi et al. (16) document the performance of the Accelerate PhenoTest blood culture kit which is designed to identify common causes of bacteremia ($\sim$90 min) as well as perform rapid antimicrobial susceptibility testing ($\sim$7 h) using positive blood culture broth as an inoculum. Both Gram-negative and Gram-positive organisms can be identified by the system, and when appropriate, the organisms can be tested against 15 and 8 antibiotics, respectively. The system utilizes fluorescent *in situ* hybridization along with microscopy-based, single-cell analysis to assess growth and ultimately establish organism susceptibility. A key breakthrough for this product is a completely automated workflow which requires very little hands-on-time to process. In general, the system performed quite well in the clinical trial, and all major criteria for acceptability in AST and organism identification were met.

The Accelerate PhenoTest BC kit represents an important breakthrough in the pursuit of rapid, phenotypic AST. Although an impressive achievement, there may be some significant hurtles to gaining widespread acceptance in clinical microbiology laboratories. Certainly, the Accelerate PhenoTest offers tremendous advantages over the manual, direct-AST methods discussed above. However, it is interesting to note that despite the supposedly critical nature of these results, the practice of performing manual, direct AST, is far from commonplace. Why is this? Perhaps it is simply a matter of a cumbersome workflow, and laboratories do not have the staffing to perform the testing. Or perhaps, it is the fact that phenotypic AST for blood cultures does not matter as much as one might like to believe. The findings of Munson et al. suggest that definitive AST had very little impact on how bacteremic patients were managed and they showed that most antibiotic decisions were made at the time of phlebotomy and Gram stain reporting (17). Empirical coverage for potentially septic patients is, by definition, designed to cover most possible etiologies. As a result, in many patient populations, physicians "get it right" most of the time. This is not true for all patient populations, however, and it may be the most vulnerable patients who are at greatest risk of developing bloodstream infections caused by drug-resistant organisms where empirical therapy will be inadequate (18).

On balance, de-escalation, rather than escalation in the face of resistance, will be the most commonly recommended antibiotic regimen change for bacteremic patients. A

significant body of evidence suggests that de-escalation of therapy is unlikely to occur in the absence of a stewardship intervention (19). Interestingly, the opposite does not appear to be true, and physicians are likely to escalate therapy in the face of antimicrobial resistance without antimicrobial stewardship intervention (19). These are the situations where lives are saved, and the patient is most likely to benefit from rapid AST results, such as those that would be provided by the Accelerate PhenoTest.

In summary, FDA approval of the Accelerate PhenoTest represents an important advance in the pursuit of more-rapid, phenotypic AST. The success or failure of such a product, as measured by improved patient outcomes, will depend largely on the local prevalence of resistance and the manner in which the system is integrated into an institution's practice. CMLs that pair the implementation of the Accelerate PhenoTest with their antimicrobial stewardship programs will undoubtedly have a significant impact on patient care. The literature is now replete with studies describing promising new technologies that can offer similar gains in AST performance. Hopefully, the success of the Accelerate PhenoTest system will spur the development of these technologies into commercially viable products.

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