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The Genotype—Phenotype Conundrum for Antimicrobial Susceptibility Testing

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Guests: Dr. Austin Ing is an infectious diseases pharmacist and a member of the antimicrobial stewardship program at Vanderbilt University Medical Center. Dr. Romney Humphries is the division director of laboratory medicine at Vanderbilt University Medical Center.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the American Association for Clinical Chemistry. I'm Bob Barrett. Bloodstream infections are a significant health concern that requires rapid initiation of effective antimicrobial therapy to reduce mortality. This task is complicated by the increasing prevalence of antimicrobial resistance leading to increased use of molecular rapid diagnostic tests that identify resistance genes and positive blood cultures. For some pathogens, detection of a given resistance gene provides enough information to select the appropriate antimicrobial treatment. In other cases, the actual antimicrobial susceptibility profile may be markedly different from that predicted by molecular diagnostic tests, making it difficult to apply this information to improve patient outcomes.

A perspective article appearing in the April 2023 issue of *Clinical Chemistry* discusses these challenges and highlights the central role of antimicrobial stewardship programs with a particular focus on the patient care benefits of active real-time involvement in the interpretation of rapid diagnostic tests. In this podcast, we are excited to talk with the authors of this perspective article. Dr. Austin Ing is an infectious disease pharmacist and a member of the Antimicrobial Stewardship Program at Vanderbilt University Medical Center. Dr. Romney Humphries is the division director of laboratory medicine at Vanderbilt University Medical Center and her research interests include antimicrobial resistance and diagnostic microbiology.

So, Dr. Humphries, let's start with you. Detection of antibiotic resistance genes doesn't always correlate with final susceptibility test results. Could you tell us about scenarios in which these molecular diagnostic tests work well and where they might fall short?

Romney Humphries: Yeah, absolutely. So, I think that when we look at the tests that we have available to us from a molecular testing perspective, these are usually single gene or maybe a handful of genes--resistance genes--that are being detected. And so, bacterial resistance is usually more complicated than driven by a single gene with a couple of key exceptions. And so,

these are really important ones. Detection of the *mecA* gene tells you whether or not an isolate of *Staph. aureus* is MRSA or if it's MSSA. And that's pretty much the only resistance mechanism that we worry about, so if *mecA* is there, you know what you're treating. If *mecA* is not there, you know what you're treating. Similarly for enterococci, *vanA* and *vanB*, which are two genes that regulate vancomycin resistance. If those are there, you know you're dealing with a vancomycin resistant strain. If they're not there, you know it's going to be susceptible. So, those worked really, really well.

On the flip side, when we talk about gram-negative bacteria, these bugs have just so many pathways that they can take to become resistant to some of our key antimicrobials. And so, typically with the gram-negative panel, what you're looking for is if you detect a gene then that's a pretty good indication it'll be resistant. If you don't detect a gene, it's kind of all bets are off. You don't know if it's going to be resistant or if it'll be susceptible. And the thing I think that makes it a little tricky is it's incredibly dependent on your local epidemiology and so some hospitals that don't see a lot of antibiotic resistance, maybe the gram-negative tests work great for them; but in other areas, like at our hospital where Dr. Ing and I practice, we actually see a lot of different resistance mechanisms and so we are less quick I'd say to trust the absence of a gene, meaning that the organism is going to be sensitive or susceptible to that drug.

Bob Barrett: So, how can laboratories handle discordant genotype-phenotype results in routine practice?

Romney Humphries: Absolutely. So, I think it comes down to sort of two buckets, right? One bucket is where we're trying to make sure that there's been no lab testing errors, and these happen in day-to-day practice. So, the first step is really to take a look and say, "Is this the same bacteria that I'm testing in both cases or was there a mix-up? Maybe, is there more than one bacteria present and I've just picked the wrong one for my testing and that's why it's not matching? Perhaps, is there a species misidentification or was it just a false positive on the test?" So, we want to make sure that we've ruled out all of these technical things that can happen throughout the testing process that can lead to discrepancies.

But once you've done all that and it looks like things are as they state, where it becomes really important is to partner with your antibiotic stewardship team to decide how those results are best reported. Most labs tend to err on the side of calling it on the more resistant end, because we don't want to be calling something sensitive to a drug and then having the patient be put on that drug only to find out that actually

it's the resistant result that we should have trusted. So, we do tend to err more on the side of resistance.

But there're definitely circumstances where that may not be the best solution, and so having those conversations with the stewardship pharmacist or the physician that's on the case is really helpful as well. I'd also say, while the live is doing all this troubleshooting, we should also be in communication with the treating team. So, we don't want to take a couple of days to do our troubleshooting while the clinical team is sort of in the dark--that we know that there's an issue with that patient's sample.

Bob Barrett: Okay, thank you so much for that. Let's go to you, Dr. Ing. In cases of gram-negative bacteremia, how can antimicrobial stewardship program interpretation and intervention provide value after molecular diagnostic testing has been performed?

Austin Ing: That's a great question. These tests are very useful in decreasing time to active therapy, thus improving mortality. However, getting the patient on active drug based on in vitro results is only half the picture. However, the whole picture, rapid diagnostics with standardized templated comments would be sufficient. Due to the complexities of both pathogens and patients, these comments cannot account for many factors that can lead to treatment failure. From pathogen standpoint, there's a multitude of resistance mechanisms that could accompany *CTX-M* or *KPC* that further limit our therapeutic options. Alternatively, these resistance mechanisms may still be present despite the absence of these beta-lactamases, per molecular results.

Rapid diagnostic interpretation by an antimicrobial stewardship program, or ASP, provides clinical context to in vitro results. For example, asking what risk factors does the patient have for resistance, 'what have they grown in the past?' and 'what are the common resistance patterns at the institution?' may lead to optimize therapy even when molecular results are negative. Additional analysis by ASP ensures reliable drug concentrations at the site of infection while avoiding drug toxicity. This was well illustrated by one of our colleagues at Vanderbilt Children's Hospital, Ritu Banerjee, in 2015. She conducted a study in which patients with positive blood cultures were randomly assigned to one of three different interventions.

Standard susceptibility testing, rapid multiplex PCR with templated comments, or rapid multiplex PCR with real-time ASP intervention. Her group found that rapid diagnostics reduce unnecessary antibiotic use, the addition of stewardship involvement improved appropriate de-escalation of antimicrobials, stewardship involvement also led to appropriate escalation of antimicrobials, ASP involvement

was associated with optimized doses and durations, and lastly, ASP involvement led to an increase in infectious diseases consultation. I think it is really important to note that ASPs are very strong proponents of escalation of therapy when it is appropriate, and that sort of involvement early can lead to optimization early in that disease state. In summary, in addition to rapid molecular results, an ASP provides accurate interpretation and active intervention.

Bob Barrett: Finally, Dr. Ing, given the many challenges in interpreting gram-negative genotypic results, would an antimicrobial stewardship program intervention that relies on standard susceptibility testing be equally effective? And might it be more cost efficient than relying on molecular testing?

Austin Ing: Great question. We just spent a few minutes discussing the value of an antimicrobial stewardship program and evaluating complexities of both the pathogen and the patient. So, it begs the question, do we see the same effect from an ASP without rapid diagnostics? A study published in 2021 from Microbiology Spectrum sought to answer this question. This multicenter, retrospective cohort assessed patients with *E. coli* or *Klebsiella pneumoniae* bloodstream infections determined to be *ESBL*- or *KPC*-producing by rapid diagnostics and/or conventional susceptibility methods. Both groups had antimicrobial stewardship interpretation and intervention, so one rapid diagnostic group, one standard conventional susceptibility method.

The rapid diagnostic group demonstrated a statistically significant decrease in time to effective antimicrobial at 16 hours, time to optimal therapy just under a day, and time to clearance at about one day earlier than the standard susceptibility testing. Early optimization and clearance was associated with that 5.6% decrease in mortality in the rapid diagnostic group, although in this particular trial, was not statistically significant. While physicians and pharmacists can evaluate institutional and patient-specific data to predict the likelihood of resistance, this trial does an excellent job displaying the value of molecular results in combination with ASP initiatives.

In summary, molecular testing and antimicrobial stewardship display a symbiotic relationship. The full benefit of molecular testing is not seen without antimicrobial stewardship involvement and the full potential of an antimicrobial stewardship program is not seen in the absence of rapid diagnostics.

Bob Barrett: That was Dr. Austin Ing and Dr. Romney Humphries from Vanderbilt University Medical Center. They shared their perspective on appropriate utilization of molecular rapid diagnostic tests in the management of patients with

bloodstream infections in the April 2023 issue of *Clinical Chemistry* and they've been our guests in this podcast on that topic. I'm Bob Barrett. Thanks for listening.