Resistance targets: blood culture ID panel pitfalls

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May 2021—Most of the time, bloodstream infection antimicrobial resistance results achieved with blood culture molecular ID panels will be accurate. When and why they might not be was the focus of an AMP 2020 virtual session.

"I don't want to lead anyone to believe that these are not good, accurate, and important types of tests," Richard E. Davis, PhD, D(ABMM), MLS(ASCP)^{CM}, director of microbiology, Providence Healthcare, Spokane, Wash., said of the panels. "But as laboratorians and people thinking of bringing on tests, we should be aware of what the limitations might be so we can resolve discrepancies and inform clinicians about how exactly these tests operate."

When it comes to antimicrobial resistance, he said, false-negatives are "far and away the most important. The overall risk is low, but we should be aware that this is not impossible."

The SENTRY Antimicrobial Surveillance Program data for drug-resistant organisms reveal that rates of methicillin-resistant *Staphylococcus aureus* (MRSA) isolation from bloodstream infections have stayed relatively stable but vancomycin-resistant *Enterococcus* (VRE) rates have risen over the years, Dr. Davis said (Diekema DJ, et al. *Antimicrob Agents Chemother.* 2019;63[7]:e00355–19). "In the Gram-negatives, multidrug-resistant *Enterobacterales* from either community- or hospital-acquired sources have increased," as has the prevalence of extended-spectrum beta-lactamases among *E. coli* and *Klebsiella* spp., and carbapenem-resistant *Enterobacterales* (CRE).

Methicillin-resistant *S. aureus* is defined by the presence of *mecA* (or *mecC*), which encodes a penicillin-binding protein resistant to beta-lactams, so it's resistant to penicillins and some cephalosporins, Dr. Davis said. "Vancomycin-resistant *Enterococcus* has *vanA* or *vanB* genes that encode a peptidoglycan that has low affinity for vancomycin, so it's resistant to vancomycin."

For the Gram-negative determinants, "we're looking for targets that will confer the extended-spectrum beta-lactamase-type resistance, or the carbapenem-resistant *Enterobacterales* carbapenemases," he said. "The ESBL targets are beta-lactamases, like the CTX-M family, and the carbapenemase targets"—bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, or bla_{OXA}.

Importantly, not all Gram-negative rod beta-lactamases or other resistance determinants have discrete gene targets, he added.

Dr. Davis

Dr. Davis categorizes the root causes of false results with rapid diagnostics for bloodstream infections into external (specimen related) and biological (organism- or gene-specific) root causes. (See boxes, next page.) His co-presenter, Susan Butler-Wu, PhD, D(ABMM), SM(ASCP), of LAC+USC Medical Center, addressed false-positive results caused by blood culture bottle contamination (see CAP TODAY, April 2021).

A hypothetical biological root cause of a false-positive result could be, "This organism has a gene that is detected by your PCR, for example, but it can't encode a functional downstream actual resistance mechanism, so it looks

resistant but it's not," Dr. Davis said.

For false-negative results, an external root cause could be an assay design that doesn't detect a known version or variant of a resistance gene. However, a biological root cause could be a novel mutation in the gene target sequence that prevents detection by the PCR—though with functional expression. "These external or biological root causes of false results can be seen in the organism with the most published and reported cases of false antimicrobial resistance detection—MRSA," Dr. Davis said.

In MRSA, the *Staphylococcus* genome encodes a number of penicillin-binding proteins, and those contribute to the characteristic thick peptidoglycan layer of Gram-positive organisms. "Those penicillin-binding proteins can be targeted by beta-lactam antibiotics like penicillins, hence the name penicillin-binding protein."

MRSA develops when a mobile cassette called the staphylococcal cassette chromosome *mec* (*SCCmec*) gets inserted into the *Staphylococcus aureus* genome. "That *mecA* encodes a PBP2a, a protein that is resistant to being bound by beta-lactam antibiotics."

When it comes to blood culture panels, some false-negative MRSA results may be due to *mecC*, which is a rare *mecA* homologue primarily found in Europe. "It was first identified in livestock and very rarely in bloodstream infections," Dr. Davis said, adding it can appear sensitive but then become resistant over time or with treatment (Ford BA. *J Clin Microbiol.* 2017;56[1]:e01549–17).

While most cases of MRSA are caused by *mecA*, successful risk mitigation of possible false-negatives comes from improved *mec* targets for blood culture panels, he said. "Newer panels—like the BCID2, Xpert MRSA/SA, ePlex Gram-positive panels, and BD Max StaphSR—have a *mec* target that detects both *mecA* and *mecC.* So we don't need to worry about a *mecC* not being detected if our panel doesn't specifically target mecC." Without one of those panels, "there is a chance you might miss a *mecC*, but it's still very rare in the United States and outside of Europe," he said. Screening for phenotypic resistance, via cefoxitin disk or cefoxitin-chromogenic agar, would also be possible if a new panel is not available and *mecC* is of concern.

A biological root cause resulting in false-negatives, he said, are mutations, insertions, or deletions in the MRSA targets that cause rapid PCR detection tests to fail. In a survey of 252 methicillin-susceptible *Staphylococcus aureus* blood isolates from the United States and Europe, determined via the Cepheid Xpert MRSA/SA test cleared in 2013, only two isolates (0.8 percent) were phenotypically resistant. Tenover, et al., found that "results for all the isolates were correct" when tested with the updated Xpert MRSA/SA BC assay, which received FDA clearance in 2019 (Tenover FC, et al. *J Clin Microbiol.* 2019;57[11]:e01195–19).

The authors wrote, "These data suggest that genetic variations that may interfere with Xpert MRSA/SA BC test results remain rare."

A target-based change to help account for these rare mutations came when Cepheid changed its MRSA-calling rule-based algorithm. The previous algorithm was MRSA = *spa* and *mec* and *SCCmec*. The new algorithm: *spa* and *mec* or *mec* and *SCCmec*.

"This is a rare event," Dr. Davis said of false-negatives with biological causes, "but the altered algorithm does help mitigate that potential risk." He and Dr. Butler-Wu wrote in their 2020 ASM report titled "Genotypic False Detections from Blood Culture Bottles—Are We Only Seeing the Tip of the Iceberg?": "Surveillance and investigation of discordant genotypic and phenotypic resistance results will be necessary to identify sequence variants not detected by current assays. Manufacturers will hopefully continue to update panels to detect such variants."

Newer panels with better targets have mitigated much of the risk of false-positive resistance results, Dr. Davis said. In their 2020 report, he and Dr. Butler-Wu wrote that the most common scenario of false-positive resistance detection comes from a mixed blood culture bottle positive for both methicillin-susceptible *S. aureus* and *mecA*-containing coagulase-negative *Staphylococcus spp.* (CoNS), commonly *mecA*-positive *Staphylococcus epidermidis*.

“If we look at our *Staphylococcus* genome,” Dr. Davis said, “if we have a target in our assay that detects *mecA* and a target that detects a specific *Staph aureus* gene, such as *spa* or *nuc*, both targets are going to be amplified. You would think that indicates MRSA, when in fact it’s two different *Staphylococcus* species giving positive results for those two different targets.”

Most at risk for false-positive results are the FilmArray BCID—“not the BCID2, which is the most updated version”—the Verigene BC-GP, and the ePlex BCID-GP panels, he said, because they have only *mec* and *S. aureus*-specific targets.

There is no clear data on *S. aureus* CoNS co-isolation rates for blood culture, Dr. Davis said. “We know this can happen with other specimen types; in MRSA nose swabs, we can get both types.”

CoNS, including *S. epidermidis*, is the most common blood bottle contaminant, so the risk exists, though reports are scarce, he said.

Newer design panels such as the FilmArray BCID2, or standalone MRSA-detection tests like the Cepheid Xpert MRSA/SA or the BD Max StaphSR, have an additional target that mitigates this potential risk, he said. This additional target is the MREJ, short for *mec(SSCmec-orfX)* right-extremity junction. “When this target is positive, it not only shows that *mecA* is present but that it has been inserted specifically into the *S. aureus* genome,” he said.

When are bloodstream infection resistance determinants, however accurate the results, not as meaningful? “That is usually when they can’t describe or rule out resistance,” Dr. Davis said, noting this relates a lot to Gram-negative antibiotic resistance mechanisms.

There are several diverse mechanisms that contribute to Gram-negative resistance, and few definitive genetic determinants, he said. “So you could not have one but could have other types. Practically speaking, if a Gram-negative resistance gene is detected, it is worthwhile to assume the isolate is resistant. However, if those genes are not detected, can you be sure the isolate is not resistant? Not so much.”

On existing rapid bloodstream infection tests, most of the targets are for genes encoding beta-lactamase enzymes (ESBL or carbapenemase enzymes). (The FilmArray BCID2 also has a target for colistin resistance, *mcr-1*, which is not beta-lactamase mediated, he said.)

When bloodstream infection resistance results are not accurate

	False-positive	False-negative
External root causes	Example: Culture bottle contamination	Example: Assay target misses known subset of resistance gene
Biological root causes	Example: Organism w/ nonfunctional, detectable gene target	Example: Mutation in gene target sequence but functional expression

Causes of MRSA blood culture RDT false detections

	False-positive	False-negative
External root causes	■ MSSA and MRSE in the same culture bottle	■ *mecC* MRSA
Biological root cause		■ Target mutations

In reviewing Gram-negative resistance mechanisms, Dr. Davis pointed out that it is possible to get a beta-lactamase, *bla*$_x$ gene—with a different family of a beta-lactamase—either on a plasmid or inserted into a chromosome. “The plasmid-based are usually ESBLs or CREs,” he said, while chromosomes are usually AmpC types. “And when those beta-lactamases are expressed, they can destroy the beta-lactam antibiotics.”

However, Gram-negatives also have porins and efflux pumps that can physically remove or block entry of different

antibiotics, he said. “So you have multiple things going on, and no single bloodstream infection test is going to catch everything. The idea is to catch most of it.”

Dr. Davis shared a 2019 meta-analysis of the sensitivity and specificity of rapid diagnostic tests for antibiotic resistance (De Angelis G, et al. *Clin Microbiol Infect.* 2020;26[3]:271–280). The authors examined 20 studies (3,310 isolates, 2006 to 2019) that compared the Verigene and FilmArray systems with phenotypic and/or genotypic comparator methods. Overall, Dr. Davis said, the rapid tests had high specificity but less sensitivity compared with phenotypic testing.

“Unsurprisingly, when you have a separate comparative test looking at those genes, the rapid detection test targets—like the CTX-M and the carbapenemase genes—perform very well, so there’s very good correlation,” Dr. Davis said.

“The issue then is when you compare the performance of phenotypic tests, meaning you incubate your organism, your Gram-negative rod, and you see what classes of antibiotics it’s resistant to, that’s when you start to see there’s not as great a correlation. It detects a lot of them, but the sensitivity is somewhat low.”

“And when you look at what the overall sensitivity and specificity is, pooled sensitivity only comes to be about 85 percent in terms of capturing all of the potential extended-spectrum beta-lactamase resistance, whereas the specificity is very good.” If you find those genes then, it’s almost certainly going to be resistant, he said. “If you don’t find those genes, you’re only 85 percent sure you’ve ruled it out.”

Acting on bloodstream infection antimicrobial resistance determinants is the key. A Vanderbilt Children’s Hospital retrospective review of children with bloodstream infections and Verigene testing yielded two interesting findings, Dr. Davis said. “They looked at 301 positive blood cultures and found that of those, looking at the chart of what they could have done, in 57 percent [171] of the cases, the Verigene results revealed a chance to change antibiotics.” In 18 percent (30) of the 171 cases, antibiotics could be avoided altogether. In 36 percent (61) of cases, antibiotics could be deescalated. In 16 percent (28) of cases, antibiotics would be escalated (Juttukonda LJ, et al. *J Clin Microbiol.* 2020;58[4]:e01400–19).

Of the 171 cases in which there was the potential to change antibiotics, change occurred in 119 cases (70 percent). Why no change in 30 percent of cases? “There seems to be a reluctance or an inability to change antibiotics,” Dr. Davis said.

Clinicians were significantly slower to deescalate antibiotics rather than escalate antibiotics, he said, and day shift results led to significantly faster change in antibiotics than night shift results. The worst time for antibiotic changes was between 12 and 6 AM.

“Acting on and performing these tests is a human-based endeavor,” Dr. Davis said, “and you need humans to act on it and decide to make the change when you can.”

Without good genotypic methods to rapidly rule out antibiotic determinants, “we ideally can get faster phenotypic susceptibility testing,” Dr. Davis said. “The gold standard—the best and top-shelf option—is the Accelerate PhenoTest BC system that does identification and susceptibility testing at once.”

But there are also opportunities to use existing technologies, like rapid ESBL screens for Gram-negative rod blood cultures, or direct inoculation of blood culture broth into automated microbroth instruments, he said. His laboratory at Providence Sacred Heart Medical Center uses the BD Phoenix for direct susceptibility and reduced its time to AST report from 44.3 hours to 17.6 hours.

New assays in the pipeline include the T2 system, which has a direct-from-blood (not blood culture) resistance panel. “Whether that makes sense depends on how results are going to be used,” Dr. Davis noted. On the market are broader panels of multidrug resistant genes, he said, citing the Check-MDR Microarray panel (Powell EA, et al. *Microb Drug Resist.* 2020;26[7]:825–830) and Acuitas MDRO Gene Test (for urine isolates). But those mean extensive testing on all bloodstream infections, which Dr. Davis described as “questionable” in terms of

cost/benefit and “challenging to bring on board.”

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