Case Study
Direct Detection of MRSA/MSSA From Positive Blood Cultures

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A 14-year-old female was brought to the Emergency Department with vomiting and fever. One week prior, the patient had been seen at an outpatient clinic and diagnosed with a viral respiratory illness that had gotten progressively worse. At presentation, physicians were concerned that the patient may have bacterial pneumonia and sepsis; therefore, blood and sputum cultures were obtained. After twelve hours of incubation, the first blood culture became positive and gram-positive cocci in clusters were observed on Gram stain. Per laboratory protocol, a multiplex molecular assay was performed directly from the positive blood culture bottle to provide early identification and antimicrobial susceptibility information. Results from the molecular test are shown in the preliminary report in Figure 1.

Figure 1: Initial Workup Directly From Positive Blood Culture Bottle.

Blood Culture
Obtained: 1/2/18 10:30 am
Received: 1/2/18 11:45 am

1/3/18 6:15 am
Preliminary Report:
Gram-positive cocci in clusters

1/3/18 9:00 am
Preliminary Report based on molecular test:
Methicillin-resistant *Staphylococcus aureus*
Further susceptibility results to follow

Molecular assay result:

Staphylococcus	Detected
Staphylococcus epidermidis	Not Detected
Staphylococcus aureus	Detected
Staphylococcus lugdunensis	Not Detected
Streptococcus	Not Detected
Streptococcus agalactiae	Not Detected
Streptococcus pneumoniae	Not Detected
Streptococcus pyogenes	Not Detected
Enterococcus faecalis	Not Detected
Enterococcus faecium	Not Detected
mecA	Detected
vanA/B	Not Detected

Figure 2: Confirmatory Workup from Solid Media.

1/4/18 7:18 am
Amended (Preliminary) Report:
1. Methicillin-resistant *Staphylococcus aureus*.
   Further susceptibility results to follow
2. *Staphylococcus haemolyticus*.
   Probable contaminant.

1/6/18 5:10 am
Amended (Final) Report:
1. Methicillin-susceptible *Staphylococcus aureus*.
2. *Staphylococcus haemolyticus*, methicillin-resistant.
   Probable contaminant.

1. *Staphylococcus aureus*

	MIC (µg/ml)	
Clindamycin	≤0.5	S
Daptomycin	≤0.5	S
Linezolid	≤0.5	S
Oxacillin	≤2	S
Trimeth-sulfa	≤1/20	S
Vancomycin	≤1	S

2. *Staphylococcus haemolyticus*

	MIC (µg/ml)	
Clindamycin	>4	R
Daptomycin	≤0.5	S
Linezolid	≤0.5	S
Oxacillin	>4	R
Trimeth-sulfa	≤1/20	S
Vancomycin	≤1	S

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Results from the molecular test **(see Figure 1)** indicated the presence of *Staphylococcus aureus, Staphylococcus* spp., and *mecA* gene. Given that *mecA* was detected, the laboratory reported the result as methicillin-resistant *Staphylococcus aureus* (MRSA). The following day, growth on solid media revealed two colony types that were identified by MALDI TOF MS as *S. aureus* and *S. haemolyticus*. Preliminary report was amended to include the coagulase-negative staphylococci (*S. haemolyticus*). Antimicrobial susceptibility testing (AST) by a commercial system was performed on the *S. aureus* isolate. About 18 hours later, the AST result of the *S. aureus* isolate revealed oxacillin minimum inhibitory concentration (MIC) of ≤ 2 µg/ml (S). Cefoxitin screen by disk diffusion confirmed the *S. aureus* isolate to be methicillin-susceptible. The isolate was also confirmed to be *S. aureus* by slide coagulase test. AST of the *S. haemolyticus* revealed oxacillin MIC of > 4 µg/ml (R). Report was again amended to reflect that the culture was growing a methicillin-susceptible *S. aureus* (MSSA) and a methicillin-resistant *S. haemolyticus* **(see Figure 2).** The physician was notified of the amended report and therapy was narrowed from vancomycin to cefazolin since antimicrobial coverage against solely the *S. aureus* was needed. The microbiology director requested that an investigation be conducted to determine how the error occurred. Results of the investigation are presented below.

The performances of blood culture molecular multiplex assays have high concordance compared to culture, especially in cases of monomicrobial infections.[1-3] In contrast, erroneous results and lower concordance are reported when the positive blood culture is polymicrobic.[1-3] The biggest limitations of such multiplex assays run on polymicrobial blood cultures is that the organism with the higher bacterial load may dominate and prevent the other target(s) from being detected, or one target may be present below the limit of detection of the assay. In the case described here, the discrepancy occurred because the blood culture was thought to be monomicrobial and the *mecA* was assumed to be expressed in the *S. aureus* isolate when it was actually expressed in the *S. haemolyticus* isolate. This is due to the fact that in the presence of *S. aureus,* not only would the *"Staphylococcus aureus"* target be detected, but the *"Staphylococcus"* target would also be detected. Hence, the results can be interpreted as a lone *S. aureus* or a mixture of *S. aureus* and a separate *Staphylococcus* sp. This also applies to the *S. epidermidis* and *S. lugdunensis* targets. Another reason for the discrepancy (if there had not been a mixture of staphylococcal species in the sample) could be the presence of an altered staphylococcal cassette chromosome resulting in the so-called "drop-out phenomenon" which would result in detection of *mecA* despite phenotypic susceptibility. In this case, if discrepancy analysis does not yield a resolution, then MRSA would have been reported as final.

If the case were reversed and *mecA* not detected from a blood culture grew MRSA and another *Staphylococcus* sp., one may reason that the false-negative report of the *mecA* gene may be due to high target expression of the two isolates.

S. aureus, both as a cause of sepsis and superinfection following a viral respiratory infection, primarily influenza, has a high mortality rate; therefore, rapid differentiation of MRSA from MSSA for appropriate treatment is critical for patient care. Laboratories must be aware of the limitations of any assay performed and possess the ability to quickly resolve testing issues, specifically when they affect antimicrobial therapy.

Best Practice Pearls:

- Phenotypic susceptibility testing is required to confirm the detection or absence of resistance genes from molecular assays performed on positive blood cultures.
- Identification of the isolate(s) must be confirmed in cases of discrepant susceptibility results.
- If discrepancy remains unresolved in a case such as this, report as MRSA.

CLSI provides a table **here** to assist laboratorians in investigating discrepant susceptibility results and guide them on how to report final results when there is discordance between molecular and phenotypic assays for MRSA. In cases where the presence or absence of *mecA* detection in *S. aureus* contradicts the cefoxitin and/or oxacillin result, identification and susceptibility should be repeated and bacterial growth on agar plates should be carefully screened to rule out mixed culture. Another option would be to perform an additional molecular test to screen for *mecA* from isolated colonies. If the discrepancy is not resolved, it is recommended that the isolate be reported as MRSA to ensure appropriate antimicrobial coverage. In the case presented here, the discrepancy was resolved by confirming methicillin resistance in the *S. haemolyticus* and hence the preliminary MRSA report was amended to MSSA. The strategy for handling discrepancies in this case also apply when screening for vancomycin resistance by detection of *vanA/B* gene in *Enterococcus* species.

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References

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3 Martinez RM, Bauerle ER, Fang FC, Butler-Wu SM. Evaluation of three rapid diagnostic methods for direct identification of microorganisms in positive blood cultures. *J Clin Microbiol.* 2014;52(7):2521-2529.