Identification of difficult to detect MRSA/MSSA strains by two commercial AST methods

Justin Towne
+1-520-241-3778
jtowne@axdx.com

J. Towne, R. Humphries
Accelerate Diagnostics, Inc., Tucson, AZ

INTRODUCTION

Antimicrobial susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA) uses oxacillin and/or cefoxitin as the appropriate test since methicillin is no longer manufactured. *S. aureus* is a very common pathogen that is responsible for infections ranging from skin infections to life-threatening respiratory infections and sepsis. The *mecA* and *mecC* genes cause resistance to beta-lactams in MRSA and have become problematic for individual patient treatment and resistance surveillance.

The Accelerate PhenoTest™ BC kit (AXDX) provides antimicrobial susceptibility results in approximately 7 hours directly from positive blood cultures, which is 1-2 days faster than isolate-based methods such as the VITEK® 2 system. This study compared agreement of AXDX and VITEK® 2 system against reference broth microdilution (BMD).

METHODS

60 isolates of difficult to detect MRSA, including 51 that were positive for *mecA* with borderline oxacillin resistance (n=23) or *mecC* (n=30) (MRSA) and 9 that were negative for *mecA* or *mecC* with borderline susceptibility to oxacillin (MSSA) were evaluated. All isolates were obtained from Denmark (*mecC*) or the CDC AR Bank and had oxacillin and/or cefoxitin MICs near the clinical breakpoint.

Isolates were seeded into BD BACTEC™ Aerobic Plus bottles containing 10 mL of human blood at a concentration of 30-50 CFU/mL, and were incubated on the BD BACTEC™ FX blood culture system until they flagged positive. Seeded positive blood cultures were assessed with the Accelerate PhenoTest™ BC kit on the Accelerate Pheno™ system which evaluates two concentrations of cefoxitin to enhance MRSA detection. MICs were also determined by testing colonies obtained from subculture by reference BMD in cation-adjusted Mueller Hinton broth (Difco™, BD, Sparks MD) according to CLSI M07-A4 standard and the VITEK® 2 system (bioMérieux, Marcy L'Etoile, FR).

Antimicrobial susceptibility testing of the Accelerate PhenoTest™ BC kit using the Accelerate Pheno™ system utilizes morphokinetic cellular analysis (MCA), which optically records bacteria growing in the presence of an antibiotic. Bacterial cell response profiles are generated from time-lapse images and compared to algorithm models to report out MIC values. The AXDX software utilized v1.4.1 image analysis.

RESULTS

Table 1: *mecA* isolate results by method.

Isolate	AXDX	VITEK® 2 system	BMD
mecA-1	R	R	R
mecA-2	S	S	R
mecA-3	R	R	R
mecA-5	R	R	R
mecA-6	R	S	R
mecA-7	R	R	R
mecA-8	R	R	R
mecA-9	R	R	R
mecA-10	R	R	R
mecA-11	R	R	R
mecA-12	R	R	R
mecA-13	R	R	R
mecA-14	S	S	R
mecA-15	R	S	R
mecA-16	R	R	R
mecA-17	R	R	R
mecA-18	R	R	R
mecA-19	R	R	R
mecA-20	R	R	R
mecA-21	R	R	R
mecA-22	N/A	S	R
mecA-23	R	R	R
TOTAL	**19/21**	**17/22**	**22/22**

Table 3: MSSA isolate results by method.

Isolate	AXDX	VITEK® 2 system	BMD
MSSA-1	S	S	S
MSSA-2	S	S	S
MSSA-3	S	S	S
MSSA-4	S	S	S
MSSA-5	N/A	S	S
MSSA-6	S	S	S
MSSA-7	N/A	S	S
MSSA-8	N/A	S	S
MSSA-9	N/A	S	S
mecA-4*	S	S	S
TOTAL	**6/6**	**10/10**	**10/10**

*One isolate with *mecA* classified as MSSA by BMD comparator.

Table 2: *mecC* isolate results by method.

Isolate	AXDX	VITEK® 2 system	BMD
mecC-1	R	R	R
mecC-2	R	R	R
mecC-3	R	R	R
mecC-4	R	R	R
mecC-5	R	R	R
mecC-6	R	R	R
mecC-7	R	R	R
mecC-8	R	R	R
mecC-9	R	R	R
mecC-10	R	R	R
mecC-11	R	R	R
mecC-12	R	R	R
mecC-13	R	R	R
mecC-14	R	R	R
mecC-15	R	R	R
mecC-16	N/A	R	R
mecC-17	R	R	R
mecC-18	R	R	R
mecC-19	R	R	R
mecC-20	R	R	R
mecC-21	R	R	R
mecC-22	R	R	R
mecC-23	R	R	R
mecC-24	R	R	R
mecC-25	R	R	R
mecC-26	R	R	R
mecC-27	R	R	R
mecC-28	R	R	R
TOTAL	**27/27**	**28/28**	**28/28**

RESULTS

AXDX yielded identification results for 60/60 *S. aureus* isolates and AST results for 54/60 (90.0%). Six isolates did not yield AST results, due to poor confidence in the MRSA call by the system: 2 MRSA and 4 MSSA. For the isolates that yielded AST, AXDX had 95.8% (46/48) sensitivity and 100% (6/6) specificity for MRSA compared to BMD. Sensitivity for the *mecC* isolates was 100% (27/27), while sensitivity for the remaining MRSA isolates was 90.5% (19/21).

The VITEK® 2 system yielded AST results for all 60 isolates, and had 90% (45/50) sensitivity and 100% (10/10) specificity for MRSA compared to BMD. Sensitivity for the *mecC* isolates was 100% (28/28), while sensitivity for the remaining MRSA isolates was 77.3% (17/22). One isolate with *mecA* was consistently cefoxitin and oxacillin susceptible by all methods.

Table 4: Sensitivity of MRSA/MSSA detection by AXDX and the VITEK® 2 system compared to broth microdilution.

Resistance Profile		AXDX	VITEK® 2 system
MRSA	*mecA*	90.5% (19/21)	77.3% (17/22)
	mecC	100% (27/27)	100% (28/28)
	Total	95.8% (46/48)	90% (45/50)
MSSA*	Total	100% (6/6)	100% (10/10)

*One isolate with *mecA* classified as MSSA by BMD comparator.

CONCLUSIONS

This study demonstrated >90% and 77.3% detection of MRSA due to *mecA* by AXDX and the VITEK® 2 system, respectively, for a collection of difficult to detect MRSA/MSSA strains. MRSA detection due to *mecC* was 100% by both strains.

With gram-positive bacteria gaining increased resistance, minimizing the use of vancomycin has become necessary. Confidence in accurate methicillin- and oxacillin-resistance testing will allow for more effective therapies and use of narrow-spectrum antibiotics.

ACKNOWLEDGEMENTS