Failure of the BD GeneOhm StaphSR Assay for Direct Detection of Methicillin-Resistant and Methicillin-Susceptible Staphylococcus aureus Isolates in Positive Blood Cultures Collected in the United States

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Fifty-nine Staphylococcus aureus isolates from one hundred blood cultures containing gram-positive cocci in clusters were identified by conventional methods and the BD GeneOhm StaphSR assay (SR). The SR misidentified three methicillin (meticillin)-resistant S. aureus (MRSA) isolates as methicillin-susceptible S. aureus (MSSA), while one MSSA isolate tested negative for S. aureus. The three MRSA isolates were strains with MREJ types that cannot be detected by the currently available SR.

Both the methicillin (meticillin)-susceptible (MSSA) and methicillin-resistant (MRSA) forms of Staphylococcus aureus are significant causes of bacteremia. Several studies have reported that patients with MRSA bacteremia have higher rates of mortality than patients infected with MSSA (2, 7).

The observation of gram-positive cocci in clusters from positive blood cultures provides only a presumptive identification of staphylococci. Confirmatory identification and susceptibility testing of staphylococci recovered from blood cultures require a minimum of two days (one day for culture and one day for identification and results of susceptibility testing).

This time dependency delays the administration of pathogen-directed therapy, promotes the unnecessary use of vancomycin, and increases the pressure to select resistant organisms.

To provide same-day definitive identification, nucleic acid amplification methods for the differentiation of MSSA and MRSA isolates are now commercially available (5, 8). The objective of this study was to evaluate the performance of the BD GeneOhm StaphSR assay (SR; BD GeneOhm, San Diego, CA) for the direct detection and differentiation of MSSA and MRSA isolates in positive blood cultures.

Blood cultures (aerobic/anaerobic bottles) submitted to the Clinical Microbiology Laboratory of the University of Louisville Hospital for routine culture were eligible for inclusion in this study. Positive blood cultures (in the Bactee 9240 system; BD Systems) containing gram-positive cocci in clusters visible by microscopy were subcultured (blood and chocolate agar) and identified by standard methods (catalase test, tube coagulase test, Staphaurex test [Remel, Lenexa, KS], mecA analysis [Oxoid, Ltd.], and testing for susceptibility to cefoxitin). Confirmatory identification to the species level and susceptibility testing were performed on the MicroScan Walkaway PC29 Combo panel (Siemens Healthcare Diagnostics, Sacramento, CA). Also, the SR was performed on these cultures in accordance with the manufacturer’s instructions.

Of the 100 blood cultures which contained gram-positive cocci in clusters, conventional methods identified 41 as non-S. aureus, 23 as MSSA, and 36 as MRSA. The SR produced four discrepant results for the 59 S. aureus isolates. Three MRSA isolates, 8.3% of all MRSA isolates, were misidentified as MSSA by the SR, while one MSSA isolate, 4.3% of all MSSA isolates, tested negative for S. aureus.

Isolates that yielded discordant PCR results were tested in duplicate (standard concentration and at a 1:100 dilution) and with repeat culture confirmation. The discordant isolates were referred as unknowns to the Technical Support Center, BD Diagnostics, GeneOhm, Ottawa, Ontario, Canada, which performed the following tests: the SR according to the package insert, conventional testing (catalase testing, Staphaurex test, use of CHROMagar MSSA medium and Mueller-Hinton agar plus oxacillin plates, and use of the Phoenix automated microbiology system), a PCR assay specific to the mecA gene, and an in-house PCR assay specific for variants of MRSA (MREJ typing; MRSA isolates only). MREJ refers to the staphylococcal cassette chromosome mec (SCmec) right-extremity junction. MREJ comprises the right extremity of SCmec, the SCmec integration site, and the orfX gene (4). It is the MREJ region which serves as the basis of the BD GeneOhm MRSA screening assay and the MRSA component of the SR.

BD GeneOhm’s commercially available SR results and conventional identification test results were identical to those obtained at the University of Louisville. The mecA gene and in-house MREJ-typing PCR results agreed with the conventional identification results.

The isolates with discrepant results were also shipped overnight to the Lexington Veterans Affairs Medical Center on BD transport swabs with liquid Stuart medium. The swabs were inoculated onto sheep blood agar plates as a purity check. Colonies were suspended in Trypticase soy broth and adjusted to the turbidity of a 0.5 McFarland barium sulfate standard.
Three milliliters of the standardized suspension was inoculated into BacT/Alert standard aerobic blood culture bottles (medium previously validated and verified by the Veterans Affairs Medical Center, Lexington, KY) without blood. The bottles were incubated in a stationary position overnight at 35°C in an offline ambient atmosphere incubator. The culture bottle were then tested using the Xpert MRSA-SA BC (Cepheid, Sunnyvale, CA) assay as specified by the manufacturer. The Xpert MRSA-SA BC assay correctly identified the MSSA isolate and two of the MRSA isolates. The third MRSA isolate was identified as MSSA.

The initial clinical study of the SR reported a sensitivity and specificity of 100% and 98.6%, respectively, for MRSA isolates and a sensitivity and specificity of 98.9% and 96.7%, respectively, for MSSA (5). A seeded blood culture study found that the SR had a sensitivity and specificity of 95.6% and 95.3%, respectively, for MRSA (3). No sensitivity and specificity data for MSSA were stated, but the sensitivity and specificity have been calculated to be 95.5% and 95.6%, respectively. These studies suggest that SR misidentifications of MSSA and MRSA strains are uncommon.

However, an Australian study reported MRSA detection sensitivity for the SR of 50%, based on the prevailing SCCmec types (6). The same has been found to be true of the BD GeneOhm MRSA assay used to screen for nasal colonization with MRSA, which uses the same MRSA target DNA. A Danish study found that the BD GeneOhm MRSA screening assay had a sensitivity of 87.4% and a specificity of 86.8% (1). Again, the basis for the low sensitivity was the prevailing SCCmec types.

The failure of the SR to detect the one MSSA strain is unexplained at this time. A possible explanation is the strain contains a spa variant that is not detected by the SR. However, further investigation is required to elucidate the true cause of the failure of the SR to detect this MSSA strain.

The original description of the BD GeneOhm MRSA screening test described seven MREJ types, designated i to vii (4). There are now at least 20 known MREJ types, designated i to xx (A. Huletsky and R. Giroux, U.S. patent application 20080227087). The results of this study demonstrate that undetectable MRSA MREJ variants are present in the United States.

Until future generations of the SR include these MREJ variants, the user must be aware of the limitations of the assay in its current format when applied to the detection and differentiation of MRSA and MSSA isolates in positive blood cultures.

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REFERENCES