JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2009, p. 823–826 Vol. 47, No. 3
0095-1137/09/$08.00+0 doi:10.1128/JCM.01884-08
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Rapid Detection of *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* (MRSA) in Wound Specimens and Blood Cultures: Multicenter Preclinical Evaluation of the Cepheid Xpert MRSA/SA Skin and Soft Tissue and Blood Culture Assays[▽]

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Received 30 September 2008/Returned for modification 19 November 2008/Accepted 5 January 2009

A multicenter preclinical evaluation was conducted to evaluate the performance of two Cepheid Xpert assays for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and *S. aureus*. Sensitivity was 97.1% and 98.3% for MRSA in wound and blood culture specimens, respectively. Sensitivity was 100% for *S. aureus* from both specimen types.

Staphylococcus aureus causes systemic infections (7, 10) and a range of skin and soft-tissue infections (SSTIs), such as surgical site infections, abscesses, carbuncles, and boils (8, 14). Mortalities of 33% and 16% are reported for methicillin-resistant *Staphylococcus aureus* (MRSA) and *S. aureus* bacteremia, respectively (15, 16).

Laboratory methods for detecting MRSA and *S. aureus* from wounds or blood cultures require incubation time and do not support rapid decisions for selection of the most appropriate procedural or therapeutic interventions. To address this limitation, two Cepheid Xpert MRSA/SA assays, used with the GeneXpert Dx system, were evaluated. The MRSA/SA SSTI assay is performed on wound swabs from SSTIs, and the MRSA/SA blood culture (BC) assay is performed on BC medium. A total of 114 wound specimens and 406 BC bottles were tested from study sites in the United States and Europe in order to characterize the performance of these assays in a clinical setting.

The study design was a preclinical comparison of Xpert MRSA/SA assays to the gold standard, broth-enriched culture methods. Subjects included individuals in hospitals and clinics whose routine care called for a wound or blood culture. Remnant specimens, from patients over the age of 18 years, were used in accordance with federal policy governing human subject protection and federal medical privacy standards (5).

Wound specimen swabs, collected on Copan Liquid Stuarts, Cepheid part no. 900-0370, and newly positive BC broths with Gram stains consistent with gram-positive cocci in clusters were tested. Broths from each of the following continuously monitoring BC instruments were included: Bactec Peds Plus/F medium (n = 27), Plus Aerobic/F medium (n = 166), Plus Anaerobic/F medium (n = 126), Standard/10 Aerobic/F medium (n = 18), Lytic/10 Anaerobic/F culture vials (n = 26), VersaTREK Redox 1 aerobic (n = 21), and Redox 2 anaerobic (n = 22).

Xpert MRSA/SA SSTI and BC assay testing was conducted according to the manufacturer's instructions for preclinical and subsequent FDA-approved versions of the assays. Samples were placed in elution buffer, vortexed for 10 s, and then transferred into Xpert MRSA/SA cartridges (Xpert MRSA package insert, 300-5188, revision C, 2007). The MRSA/SA assays, used on the GeneXpert system, automate sample purification, nucleic acid amplification, and target sequence detection. The primers and probes in the Xpert MRSA/SA assays detect sequences within the staphylococcal protein A (*spa*) gene, the gene for methicillin resistance (*mecA*), and the staphylococcal cassette chromosome (SCC*mec*) inserted into the *S. aureus* chromosomal *attB* insertion site (12, 13). The assays are the first commercial methods to simultaneously detect the *mecA* gene along with the *attB* insertion site. Inclusion of both the *attB* insertion site and the *mecA* gene targets enables the assays to identify the presence of SCC*mec* cassette variants with *mecA* gene deletions, thus reducing false-positive results which occur in molecular tests that target only the SCC*mec* cassette (6, 9). External calibrated controls consisted of MRSA SCC*mec* types I to V with CFU ranging from 4.3×10^3 to 13.3×10^3. Wound specimens (n = 114) and BC broths growing gram-positive cocci (n = 406) were collected and tested within 24 h at six healthcare sites in the United States and Europe. Wound specimens, analyzed upon receipt, and newly positive BC specimens, analyzed upon completion of Gram staining, generated PCR results within 1 hour.

All wound specimen swabs, routed at refrigerated temperature to one central laboratory (University of Arizona) for broth enrichment, were incubated overnight in 1.5 ml of tryptic soy

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▽ Published ahead of print on 14 January 2009.

broth with 6.5% NaCl, prior to subculture of 10 μl on BBL CHROMagar MRSA and CHROMagar SA (BD Diagnostics, Sparks, MD). Presumptive positive colonies were subcultured to tryptic soy agar with 5% sheep red blood cells and incubated aerobically at 35°C for up to 48 h. Confirmation of *S. aureus* was performed using a catalase test, a tube coagulase test, and Gram staining. Methicillin resistance was confirmed by the 30-μg disk diffusion method, as described in CLSI guidelines (17), as the historical gold standard method. Technologists performing both the routine culture and reference broth-enriched culture were blinded to the PCR results. Quality control was performed according to the manufacturer's instructions (Xpert MRSA package insert).

The analytical sensitivity and inclusivity of the Xpert MRSA/SA SSTI and BC assays were determined prior to preclinical testing, by adding known bacterial CFU to the assay's elution buffer. The analytical sensitivity (limit of detection [LoD]) was defined as the lowest number of CFU per sample that could be reproducibly distinguished from negative samples with 95% confidence. The LoD was determined by evaluating 20 replicates of dilution series of MRSA and *S. aureus*. The inclusivity was determined using 25 strains from a collection of USA100 through USA1200.

The LoD for *S. aureus* was 48 CFU/test (95% confidence interval, 42.4 to 57.2), and for MRSA, it was 109.4 CFU/test (95% confidence interval, 98.8 to 128.2). In the inclusivity studies, all MRSA strains were correctly identified as MRSA positive and *S. aureus* positive; each *S. aureus* strain was correctly identified as MRSA negative and *S. aureus* positive. Among U.S. strain types, positive results were documented for bacterial densities ranging from 43 to 417 CFU/assay.

For wounds, the culture-confirmed prevalence of MRSA and *S. aureus* in the study population was 30.0% and 18.2%, respectively, for a combined *S. aureus* prevalence of 48.2%. Of note, predictive values may vary in populations where prevalence varies. Under the prevalence conditions described here, the assay's performance characteristics are described in Table 1. During preclinical testing of quality control results, Xpert MRSA/SA, including the sample processing controls, performed as expected. No statistical difference in performance was noted among study sites, as determined by chi-square analysis; therefore, performance data were pooled for the final analysis. The MRSA/SA SSTI assay for MRSA detection performed with a sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 97.1%, 96.2%, 91.9%, and 98.7%, respectively. For *S. aureus* detection, the sensitivity, specificity, PPV, and NPV were 100%, 96.6%, 96.5%, and 100%, respectively. Overall agreement between the MRSA/SA assay and the culture standard was 96.5% and 98.2% for MRSA and *S. aureus*, respectively.

False-positive PCR results are known to occur in assays that measure the MRSA SCC*mec* signal alone (6, 8, 9). Testing for a combination of the *spa*, *mec*, and SCC*mec* targets allows for assessment of potential false-positive samples due to SCC*mec* variants, by examination of the assay's cycle threshold (C_T) values. In this sample set, three wound specimens exhibited PCR-positive/culture-negative results, which were noted and investigated. For two of the potential false-positive cultures, *S. aureus* was cultivated; the remaining culture was negative for *S. aureus*. All three samples exhibited *mecA* C_T signals that were essentially equivalent (within the expected experimental error) to the C_T values for the SCC*mec* target; this situation is not indicative of an *S. aureus* empty cassette variant (methicillin-resistant–methicillin-susceptible *S. aureus* [MSSA]), in which the SCC*mec* signal would be expected to be different than that of the *mecA* signal because of the dropout in the *mecA* gene region.

TABLE 1. Performance of Xpert MRSA-*S. aureus* assay for SSTIs and for BC bottles in comparison with broth-enriched routine culture

Source and organism	% (no. of positive samples/total no.)			
	Sensitivity	Specificity	PPV	NPV
SSTI				
MRSA	97.1 (34/35)	96.2 (76/79)	91.9 (34/37)	98.7 (76/77)
S. aureus	100 (55/55)	96.6 (57/59)	96.5 (55/57)	100 (57/57)
BC				
MRSA	98.3 (57/58)	99.4 (346/348)	96.6 (57/59)	99.7 (346/347)
S. aureus	100 (120/120)	98.6 (282/286)	96.7 (120/124)	100 (282/282)

While there is a noted oversampling of Bactec Plus Aerobic/F medium and Plus Anaerobic/F medium in the MRSA-*S. aureus* BC assay comparison, no statistical performance difference for sensitivity and specificity was observed between bottle types or testing sites for MRSA or MSSA, as determined by chi-square analysis; therefore, results were pooled for the final statistical analysis. For BCs, culture-confirmed prevalences of MRSA and *S. aureus* in the study population were 14.0% and 16.0%, respectively, for a combined *S. aureus* prevalence of 30%.

As depicted in Table 1, the MRSA/SA assay for MRSA detection from blood cultures performed with a sensitivity, specificity, PPV, and NPV of 98.3%, 99.4%, 96.6%, and 99.7%, respectively. For *S. aureus* detection, the sensitivity, specificity, PPV, and NPV were 100%, 98.6%, 96.7% and 100%, respectively.

In the first multicenter preclinical evaluation of the Xpert MRSA/SA SSTI and BC assays, performance characteristics were determined at study sites in the United States and Europe. We found that, across all sites, the MRSA/SA assays provided rapid, sensitive, and accurate identification of MRSA and *S. aureus* from wound specimen swabs and positive BCs. Sensitivity was 97.1% and 98.3% for MRSA in wound and blood cultures, respectively; sensitivity was 100% for *S. aureus* from both wound and blood cultures.

In addition, the Xpert assays have a high specificity due to the presence of three assay targets that limit the potential for false-positive results due to SCC*mec* variants with missing or incomplete *mecA* genes, which have been described with other commercial assays that do not target the *mecA* gene (6, 8, 9). In the MRSA/SA assays, all three signals (*spa*, *mecA*, and SCC*mec*) must be present in order for the assays to yield a positive result for MRSA (Xpert MRSA package insert).

While none of the isolates in this study were shown to be "false-positive" due to the "empty cassette" phenomenon (methicillin-resistant–MSSA), there is one scenario in which the Xpert assays will generate a false-positive MRSA result:

that is, when testing a mixed infection containing both a methicillin-resistant coagulase-negative staphylococcus species (MRCoNS), which provides the *mecA* target, and an SCC*mec* empty cassette variant, which provides *spa* and SCC*mec*. Although no data exist from wound specimens, data from nasal specimens provide a reasonable measure of the potential for cocolonization to occur. The highest reported global prevalence of SCC*mec* variants is 74% (6), but the reported cocolonization of MRCoNS and MSSA in nasal specimens is low (3.4%) (3); therefore, the current theoretical calculated risk of SCC*mec* variants and cocolonization with MRCoNS would be 2.5% of *S. aureus* strains. In the United States, the current predictions would be much lower; one U.S. report described a population in which an SCC*mec* variant was detected in 12% of all *S. aureus* strains (4); another U.S. report of potential SCC*mec* variants is lower, 6% of *S. aureus* strains and 1% of all samples (18). In the United States the predicted risk for false positives caused by cocolonization would be <0.4% of *S. aureus* strains. Examination of the C_T values, as described in our results, would allow the operator to discern a mixed bacterial population unless the sample contained both an SCC*mec* variant and an MRCoNS, in equivalent bacterial densities; this situation is very unlikely to occur.

The assay PPV for SSTI is 91.9%, as expected when PCR-positive/culture-negative results occur. Reasons for this phenomenon vary and may include the presence of nonviable organisms; a positive test result does not necessarily indicate the presence of viable organisms (4). In this study patients were screened in order to exclude those who had received antibiotics in the previous 3 weeks; however, errors in self-reporting are possible. Another common reason for low PPV is poor sensitivity of the gold standard culture method as the arbitrator for discrepant results, which is likely to play a role here, as previously described for other MRSA PCR methods. Chapin and Dickenson (4) reported arbitrative broth enrichment to be less sensitive than arbitration by another PCR method. In their report, individual *spa* and *mecA* PCR proved to be a more sensitive method to confirm PCR-positive/culture-negative samples; only 23 of 32 were identified by broth enrichment, and therefore, 28.1% of *spa*/*mec* PCR-positive samples were not detected by broth enrichment (4). Lastly, while the CLSI methods for 30-μg cefoxitin disk screening are noted for high accuracy, the method is not infallible; rare false-negative results have been reported elsewhere (20) and, if present, would lower the PCR PPV.

NPVs are very high, 98.7 and 99.7% for MRSA from wounds and blood cultures, respectively. With such a low number of false-negative samples, the most likely explanation is sampling variability for samples containing low bacterial densities, because *spa*, *mecA*, and SCC*mec* C_T signals were not detected and sample processing controls performed as expected for these culture-positive/PCR-negative samples.

The Xpert assays can provide rapid results to identify MRSA and *S. aureus* from wound specimens so that appropriate action related to drainage or therapy may be rapidly instituted. Furthermore, since studies have shown that rapid and targeted antimicrobial therapy can substantially reduce morbidity and mortality for bloodstream infections (1, 2, 11, 19), the Xpert assays, shown here to provide rapid detection and differentiation of MRSA and *S. aureus* from BC bottles, would support rapid administration of the most appropriate antibiotic for infections caused by *S. aureus*. We conclude that the Xpert MRSA/SA assays provide a useful tool for rapid intervention or targeted antimicrobial therapy for MRSA and *S. aureus* from wound swabs and positive BC specimens.

We thank Cepheid for financially supporting this study.

For editing, we thank Patricia Richards and Linda Hilbert. We thank Melanie de Boer for statistical review and Janice Prill for laboratory support.

REFERENCES

1. **Ahrens, T., and D. Tuggle.** 2004. Surviving severe sepsis: early recognition and treatment. Crit. Care Nurse *October*(Suppl.)**:**2–13.
2. **Annane, D., E. Bellissant, and J. M. Cavaillon.** 2005. Septic shock. Lancet **365:**63–78.
3. **Becker, K., I. Pagnier, B. Schuhen, F. Wenzelburger, A. W. Friedrich, F. Kipp, G. Peters, and C. von Eiff.** 2006. Does nasal cocolonization by methicillin-resistant coagulase-negative staphylococci and methicillin-susceptible *Staphylococcus aureus* strains occur frequently enough to represent a risk of false-positive methicillin-resistant *S. aureus* determinations by molecular methods? J. Clin. Microbiol. **44:**229–231.
4. **Chapin, K. C., and R. A. Dickenson.** 2006. Comparison of GeneOhm Sciences IDI-MRSA molecular assay on the Cepheid SmartCycler system and culture for detection of MRSA carriage from nasal swab specimens, abstr. C-075. Abstr. 106th Gen. Meet. Am. Soc. Microbiol.
5. **Department of Health and Human Services.** 14 August 2002. Standards for privacy of individually identifiable health information; final rule. 45 CFR parts 160 and 164. Department of Health and Human Service, Washington, DC.
6. **Donnio, P. Y., D. C. Oliveira, N. A. Faria, N. Wilhelm, A. Le Coustumier, and H. de Lencastre.** 2005. Partial excision of the chromosomal cassette containing the methicillin resistance determinant results in methicillin-susceptible *Staphylococcus aureus*. J. Clin. Microbiol. **43:**4191–4193.
7. **Edwards, J. R., K. D. Peterson, M. L. Andrus, J. S. Tolson, J. S. Goulding, M. A. Dudeck, R. B. Mincey, D. A. Pollock, and T. C. Horan.** 2007. National Healthcare Safety Network (NHSN) report, data summary for 2006, issued June 2007. Am. J. Infect. Control **35:**290–301.
8. **Farley, J. E.** 2008. Epidemiology, clinical manifestations, and treatment options for skin and soft tissue infection caused by community-acquired methicillin-resistant *Staphylococcus aureus*. J. Am. Acad. Nurse Pract. **20:** 85–92.
9. **Francois, P., M. Bento, G. Renzi, S. Harbarth, D. Pittet, and J. Schrenzel.** 2007. Evaluation of three molecular assays for rapid identification of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **45:**2011–2013.
10. **Griffin, F. A.** 2007. 5 Million Lives Campaign. Reducing methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Jt. Comm. J. Qual. Patient Saf. **33:**726–731.
11. **Ibrahim, E. H., G. Sherman, S. Ward, V. J. Fraser, and M. H. Kollef.** 2000. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest **118:**146–155.
12. **Ito, T., K. Kuwahara, K. Hisata, K. Okuma, L. Cui, and K. Hiramatsu.** 2004. Community-associated methicillin-resistant *Staphylococcus aureus*: current status and molecular epidemiological perspective. Kansenshogaku Zasshi **78:**459–469. (In Japanese.)
13. **Katayama, Y., H. Z. Zhang, and H. F. Chambers.** 2004. PBP 2a mutations producing very-high-level resistance to beta-lactams. Antimicrob. Agents Chemother. **48:**453–459.
14. **Klevens, R. M., M. A. Morrison, J. Nadle, S. Petit, K. Gershman, S. Ray, L. H. Harrison, R. Lynfield, G. Dumyati, J. M. Townes, A. S. Craig, E. R. Zell, G. E. Fosheim, L. K. McDougal, R. B. Carey, and S. K. Fridkin.** 2007. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA **298:**1763–1771.
15. **Miller, L. G., C. Quan, A. Shay, K. Mostafaie, K. Bharadwa, N. Tan, K. Matayoshi, J. Cronin, J. Tan, G. Tagudar, and A. S. Bayer.** 2007. A prospective investigation of outcomes after hospital discharge for endemic, community-acquired methicillin-resistant and -susceptible *Staphylococcus aureus* skin infection. Clin. Infect. Dis. **44:**483–492.
16. **Moran, G. J., A. Krishnadasan, R. J. Gorwitz, G. E. Fosheim, L. K. McDougal, R. B. Carey, and D. A. Talan.** 2006. Methicillin-resistant *S. aureus* infections among patients in the emergency department. N. Engl. J. Med. **355:**666–674.

17. **NCCLS.** 2000. Performance standards for antimicrobial disk susceptibility tests, 8th ed. NCCLS document M2-A7. Approved guidelines. NCCLS, Wayne, PA.
18. **Shepley, D., D. Sloane, J. Marx, D. Driscoll, G. Sanchez, E. Sabbagh, and D. M. Wolk.** 2007. Prospective clinical evaluation of CHROMagar MRSA and BD GeneOhm MRSA PCR as independent predictors of MRSA status, abstr. C-231. Abstr. 107th Gen. Meet. Am. Soc. Microbiol.
19. **Velasco, E., M. Soares, R. Byington, C. A. Martins, M. Schirmer, L. M. Dias, and V. M. Goncalves.** 2004. Prospective evaluation of the epidemiology, microbiology, and outcome of bloodstream infections in adult surgical cancer patients. Eur. J. Clin. Microbiol. Infect. Dis. **23:**596–602.
20. **Witte, W., B. Pasemann, and C. Cuny.** 2007. Detection of low-level oxacillin resistance in mecA-positive *Staphylococcus aureus*. Clin. Microbiol. Infect. **13:**408–412.