

# Comparison of the BD GeneOhm Methicillin-Resistant *Staphylococcus aureus* (MRSA) PCR Assay to Culture by Use of BBL CHROMagar MRSA for Detection of MRSA in Nasal Surveillance Cultures from an At-Risk Community Population<sup>∇</sup>

Jason E. Farley,<sup>1,2\*</sup> Paul D. Stamper,<sup>4</sup> Tracy Ross,<sup>4</sup> Mian Cai,<sup>4</sup>  
 Sharon Speser,<sup>4</sup> and Karen C. Carroll<sup>2,3,4</sup>

*The Johns Hopkins University School of Nursing,<sup>1</sup> Division of Infectious Disease<sup>2</sup> and Division of Medical Microbiology, Department of Pathology,<sup>3</sup> The Johns Hopkins University School of Medicine, and Microbiology Laboratory, Johns Hopkins Hospital,<sup>4</sup> Baltimore, Maryland*

Received 24 October 2007/Accepted 19 November 2007

**We compared the BD GeneOhm methicillin-resistant *Staphylococcus aureus* (MRSA) PCR assay to culture with BBL CHROMagar MRSA for nasal surveillance among 602 arrestees from the Baltimore City Jail. The sensitivity and specificity were 88.5% and 91.0%, respectively, and after secondary analysis using enrichment broth, they were 89.0% and 91.7%, respectively. Twenty-three of 42 false-positive PCR lysates contained methicillin-susceptible *S. aureus*.**

Recent community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) outbreaks among correctional institution populations have demonstrated the need for improved surveillance (3–5, 17). MRSA surveillance is essential to limit transmission and stem future outbreaks in this setting. While rapid diagnostic testing has been successfully implemented within the health care setting (1, 6, 10, 22), such testing will likely present technical and logistical difficulties for correctional facilities.

Trypticase soy broth (TSB) with 6.5% NaCl is considered the reference method for recovery of MRSA from nasal surveillance cultures (20). This method is not timely for programs that wish to implement rapid screening. Selective and differential media such as BBL CHROMagar MRSA (CHROM-MRSA) (BD Diagnostics, Sparks, MD) decrease the time to identification of MRSA from 2 or 3 days to 24 to 48 h, require limited training, and are relatively inexpensive compared to molecular methods (11; BBL CHROMagar MRSA package insert, <http://www.bd.com/ds/productCenter/215084.asp>). In a multicenter study comparing CHROM-MRSA to conventional culture using 5% sheep blood agar and five methods of susceptibility testing, CHROM-MRSA detected an additional 8% positive samples (11). The performance characteristics of chromogenic media compared with PCR have not been well studied. The BD GeneOhm MRSA PCR assay (BD GeneOhm, San Diego, CA) has proven effective for MRSA surveillance (2, 9, 11, 18) but is currently more costly than CHROM-MRSA.

An epidemiologic study of MRSA nasal colonization among newly arrested men provided the opportunity to compare the

performance characteristics of these methods for MRSA surveillance.

(This study was presented in part at the 107th General Meeting of the American Society for Microbiology, Toronto, Canada, 21 to 25 May 2007.)

**Subject selection and collection of clinical specimens.** The Maryland Department of Corrections and the Institutional Review Board of The Johns Hopkins University School of Medicine approved the study. Enrollment criteria included (i) arrested <24 h previously, (ii) male, (iii) age 21 years and older, and (iv) processed at the Central Booking Intake Facility in Baltimore, MD. Anterior nasal specimens were obtained using BactiSwab II dual-headed culturettes (Remel, Lenexa, KS) from 602 men. Both swabs were inserted into each nare simultaneously. The swabs were transported at room temperature and stored at 5°C until processing within 24 h of collection. The two swabs were randomly separated in the laboratory. One swab was used for culture and the other for the PCR. The conventional microbiology testing and the PCR were conducted independently by laboratory staff.

**Bacterial culture and susceptibility testing.** One swab was streaked onto CHROM-MRSA and then placed in TSB containing 6.5% NaCl (11, 16; BBL CHROMagar MRSA package insert) for enrichment for validation of discrepant results between CHROM-MRSA and the PCR. CHROM-MRSA plates were incubated and read according to the manufacturer's instructions (23). Mauve colonies growing on CHROM-MRSA that were confirmed as *S. aureus* by Gram staining and slide coagulation were considered MRSA. Each TSB culture was subcultured after overnight incubation to 5% sheep blood agar plates (BBL, BD Diagnostics, Sparks, MD). Presumptive *S. aureus* colonies were subcultured to oxacillin screening agar (OSA). Isolates positive on OSA but not growing on the CHROM-MRSA were reconfirmed as MRSA using the BD Phoenix automated microbiology system (Phoenix) (BD Diagnostics, Sparks, MD) (2). The second swab was processed and

\* Corresponding author. Mailing address: Johns Hopkins University, School of Nursing, 600 N. Wolfe Street, Carnegie 346, Baltimore, MD 21287. Phone: (410) 614-5458. Fax: (410) 614-0888. E-mail: jfarley@son.jhmi.edu.

<sup>∇</sup> Published ahead of print on 5 December 2007.

TABLE 1. Comparison of BD GeneOhm MRSA assay to CHROMagar MRSA before and after secondary analysis using broth enrichment

| BD GeneOhm MRSA PCR assay result | No. with CHROMagar MRSA result <sup>a</sup> : |          |       | % (95% CI) <sup>b</sup> |                  |                  |                  |
|----------------------------------|-----------------------------------------------|----------|-------|-------------------------|------------------|------------------|------------------|
|                                  | Positive                                      | Negative | Total | Sensitivity             | Specificity      | PPV              | NPV              |
| Before secondary analysis        |                                               |          |       |                         |                  |                  |                  |
| Positive                         | 77                                            | 46       | 123   | 88.5 (0.82–0.95)        | 91.0 (0.89–0.93) | 62.6 (0.54–0.71) | 97.9 (0.96–0.99) |
| Negative                         | 10                                            | 466      | 476   |                         |                  |                  |                  |
| Total                            | 87                                            | 512      | 599   |                         |                  |                  |                  |
| After secondary analysis         |                                               |          |       |                         |                  |                  |                  |
| Positive                         | 81                                            | 42       | 123   | 89.0 (0.82–0.95)        | 91.7 (0.89–0.94) | 65.9 (0.57–0.74) | 97.9 (0.97–0.99) |
| Negative                         | 10                                            | 466      | 476   |                         |                  |                  |                  |
| Total                            | 91                                            | 508      | 599   |                         |                  |                  |                  |

<sup>a</sup> Three PCR-inhibited specimens were excluded from data analysis, for a total of 599 evaluable samples.

<sup>b</sup> CI, confidence interval.

tested using the BD GeneOhm MRSA assay according to the manufacturer's instructions.

**Analysis of discrepant results.** For PCR-negative, CHROMagar-positive samples, if the enrichment broth also contained confirmed MRSA, no additional tests were performed and the PCR test was deemed a false-negative result. If the enrichment broth was negative, then the isolate from the CHROMagar plate was verified as described above under "Bacterial culture and susceptibility testing." For PCR-positive, CHROMagar-negative samples, if the enrichment broth was also positive, the result was considered a true positive by PCR. For PCR-positive samples that were negative by either culture method for MRSA, the lysates were retested by PCR. For all discrepant results, the isolates and lysates were sent to the research laboratories at BD GeneOhm, Quebec City, Canada, for separate analysis. At GeneOhm, the isolates were analyzed with the PBP 2' assay (Denka Seiken Co., Tokyo, Japan) and by PCR for the *mecA* gene (standard end detection) (15). In addition, both the isolates and the original lysates were tested using the BD GeneOhm MRSA assay.

**Data analysis.** Statistical analysis was completed using STATA version 9.0 (STATA Corp, LP, College Station, TX). The sensitivity and specificity of the PCR assay were calculated and compared to CHROM-MRSA.

**MRSA detection by culture.** Nasal swabs were collected from 602 arrestees who consented to participate. A total of 87 MRSA isolates were recovered from CHROM-MRSA and an additional eight MRSA isolates were recovered from enrichment broth only, for an overall prevalence of MRSA in this population of 15.8%.

**Performance of GeneOhm MRSA assay compared to CHROMagar.** Thirteen of the original 602 nasal specimens (2.2%) were inhibited in the PCR assay; 10 of these were resolved after a freeze-thaw. Three unresolved PCR samples were excluded from data analysis, for a total of 599 evaluable samples. One hundred twenty-three nasal swabs were positive for MRSA by the PCR assay. Among these, 77 were culture positive by CHROMagar. The overall agreement between PCR and CHROM-MRSA was 90.7%. Comparing the PCR assay to CHROM-MRSA, the sensitivity and specificity were 88.5% (77/87) and 91.0% (466/512), respectively. Table 1 provides a summary comparison, including positive predictive value (PPV) and negative predictive value (NPV).

**Discrepant analysis and enrichment broth resolution.** There were 56 samples for which there were discrepant results between the PCR assay and CHROM-MRSA (Table 2). Further laboratory analysis was completed with these discrepant samples as described above.

Forty-two of the 56 discrepant samples were PCR-positive specimens that did not grow MRSA on CHROM-MRSA. Twenty-three of these 42 PCR-positive samples grew methicillin-susceptible *S. aureus* in the enrichment broth (41% of the discrepant results; 3.8% of all specimens). Among these 23 false-positive results, 18 of the original lysate buffers were positive for MRSA on repeat testing by the PCR assay; 15 of the recovered isolates also gave a positive result when tested by the PCR assay. *mecA* PCR analysis was performed on all 23 *S. aureus* isolates, and only one isolate was positive for the presence of the *mecA* gene. Nineteen of the 42 samples were negative by culture for any *S. aureus*. On repeat testing of the lysate buffers, 9 (47.4%) were again positive for MRSA.

Ten samples were originally negative for MRSA by PCR but grew MRSA in culture (Table 2). Three PCR-negative, CHROM-MRSA-positive specimens were also negative by broth culture and were resolved as PCR-negative specimens. Seven specimens were noted to be MRSA positive in both CHROM-MRSA and broth but negative in PCR. These specimens were considered false-negative PCR specimens. All 10 isolates were positive upon testing with the PCR assay; these 10 were phenotypically MRSA when tested by Phoenix, OSA, and the PBP 2' assay.

TABLE 2. Resolution of discrepant isolates

| MRSA PCR | Result in:            |          | No. of discrepant samples | Comment            |
|----------|-----------------------|----------|---------------------------|--------------------|
|          | CHROM-MRSA            | Broth    |                           |                    |
| Positive | Negative              | Negative | 42                        | False-positive PCR |
| Positive | Negative              | Positive | 4                         | True-positive PCR  |
| Negative | Positive <sup>a</sup> | Negative | 3                         | False-negative PCR |
| Negative | Positive              | Positive | 7                         | False-negative PCR |

<sup>a</sup> Isolates verified as MRSA.

Four PCR-positive but CHROM-MRSA-negative samples grew MRSA in the enrichment broth. These were considered resolved samples and true positives by the PCR assay. Recalculation of the assay's sensitivity, specificity, PPV, and NPV after secondary analysis is presented in Table 1.

**Discussion.** In the context of a larger epidemiologic study within a correctional institution, we compared the BD GeneOhm MRSA assay to CHROMagar MRSA for identifying MRSA nasal colonization in this high-risk community population. The prevalence of *S. aureus* (40.4%) and MRSA (15.8%) among nasal isolates is substantially greater than estimates from larger community analyses of MRSA colonization to date, which noted *S. aureus* and MRSA colonization prevalences of 31% and 0.84%, respectively (12). Our findings are similar to the prevalence noted upon hospitalization of prisoners originating from Baltimore City Jail facilities (15.8% versus 17%) (24) and demonstrate a high community prevalence of community-associated MRSA.

We found the BD GeneOhm MRSA assay to have an initial sensitivity of 88.5% compared to CHROM-MRSA, which improved to 89.0% after secondary analysis. The high NPV in this study (97.9%) and those reported by others (7, 15, 18, 21) suggests that this assay provides a rapid method for the identification of persons who are not colonized with MRSA and in that context is likely to be useful for epidemiologic or surveillance activities, whether in a community setting as described in this evaluation or in a health care environment.

The specificity and PPV of the BD GeneOhm MRSA assay (91.7% and 65.9%, respectively) in our study are similar to the results noted by others. Several recent studies (14, 15, 19) report PPVs ranging from 63% to 94% for nasal specimens in comparison to selective chromogenic media in hospital settings. Using rectal and nasal specimens pooled in selective enrichment broth, Desjardins et al. noted a higher sensitivity (96%) than other authors (8); however, the investigators noted a significant decline in PPV, from 90% to 65% postimplantation.

One possible explanation for the weak specificity is the assay's potential to amplify retained segments of the right-junction sequence of the SCCmec by *S. aureus* strains that are missing the *mecA* gene (14). Our discrepant analysis provides some insight into this hypothesis and the evolving heterogeneity of MRSA. Interestingly, 23/42 (55%) false-positive PCR specimens contained methicillin-susceptible *S. aureus*. In addition, among the 19 PCR-positive, culture-negative samples, 9 lysates were again positive on repeat PCR testing despite negative enrichment broth results. Two possible scenarios may apply to this situation. Either these specimens contained isolates that have an incomplete *mecA* gene, or upon deletion, the gene left behind a residual fragment containing the target SCCmec region detected with the PCR assay. In our own discrepant analysis, 22 of the 23 false-positive isolates were found by separate *mecA* gene PCR to be missing the *mecA* gene. Donnio et al. (9) have hypothesized that this incomplete *mecA* gene may alter the diagnostic reliability of molecular assays that target the SCCmec (9), and we believe that the problem in specificity (91.7%) in the present study may be an example of this phenomenon. A noted limitation of this study is that resolution of the PCR-positive, culture-negative sam-

ples was impaired because the broth enrichment method did not use the PCR swab.

In conducting this study, the rapid turnover and release of newly arrested individuals resulted in our inability to conduct follow-up testing in subjects with discrepant PCR and culture results. This setting, however, provided an excellent location for evaluation of these assays outside of a clinical environment in persons in a high-risk community setting. Specimen collection procedures were standardized and conducted by the same individual for all samples, limiting biases in sample collection. However, variability in samples, especially with low levels of colonization, could be responsible for some of the PCR-negative, culture-positive specimens such that the inoculum was below the limit of detection for the PCR assay but enough to be grown in culture. In the study by Rossney et al., investigators noted a high limit of detection ( $10^3$  CFU/specimen), which was likely a large contributor to the poor sensitivity of the assay in their study (19). The authors concluded that the assay should not be used as a stand-alone test but should be verified by concomitant culture (19). Such a strategy leads to excessive costs. Since the NPV is high in most studies, an alternative scenario might be to provide culture verification of only PCR-positive samples, but this adds complexity to testing algorithms. Laboratories will have to evaluate the performance of this and similar assays in their environments to determine suitability for infection control practices or epidemiological studies.

Finally, during the course of the study, the poor hygienic state of some individuals and the presence of foreign material such as dirt or intranasal drugs may have influenced the performance of the assay. However, there was no way to assess the impact of these factors on either method. Fortunately, only a few ( $n = 3$ ) samples were eliminated because of overt PCR inhibition.

The evaluation of rapid methods for detection of MRSA outside of health care environments is currently limited, although it is increasingly necessary due to a growing prevalence and increasing reports of MRSA transmission in the community. Despite previous analysis demonstrating adequate sensitivity and specificity of the BD GeneOhm MRSA assay in settings where MRSA is endemic, our study in a high-prevalence community setting found the assay to be less sensitive and less specific than selective culture media. In our environment the use of the BD GeneOhm MRSA assay in clinical settings requires confirmation, yet its practical application for surveillance and isolation decision analysis is plausible.

We thank the correctional officers at the Baltimore City Central Booking Intake Facility for their assistance and professionalism during this study.

This research received support from a National Research Service Award (NRSA) funded by the National Institute of Nursing Research (NINR), National Institutes of Health (NIH) (grant 1 F31 NR009334-01) as well as an NIH-funded T-32 Institutional Training grant in Health Disparities. The laboratory work was supported in part by a grant from BD Diagnostics (Sparks, MD) and BD GeneOhm (San Diego, CA).

#### REFERENCES

1. Bishop, E. J., E. A. Grabsch, S. A. Ballard, B. Mayall, S. Xie, R. Martin, and M. L. Grayson. 2006. Concurrent analysis of nose and groin swab specimens by the IDI-MRSA PCR assay is comparable to analysis by individual-spec-

- imen PCR and routine culture assays for detection of colonization by methicillin-resistant *Staphylococcus aureus*. *J. Clin. Microbiol.* **44**:2904–2908.
2. **Carroll, K. C., A. P. Borek, C. Burger, B. Glanz, H. Bhally, S. Henciak, and D. C. Flayhart.** 2006. Evaluation of the BD Phoenix automated microbiology system for identification and antimicrobial susceptibility testing of staphylococci and enterococci. *J. Clin. Microbiol.* **44**:2072–2077.
  3. **Centers for Disease Control and Prevention.** 2003. Methicillin-resistant *Staphylococcus aureus* infections in correctional facilities—Georgia, California and Texas, 2001–2003. *MMWR Morb. Mortal Wkly. Rep.* **52**:992–996.
  4. **Centers for Disease Control and Prevention.** 2003. Outbreaks of community-associated MRSA skin infections—Los Angeles County, California, 2002–2003. *MMWR Morb. Mortal Wkly. Rep.* **52**:793–795.
  5. **Centers for Disease Control and Prevention.** 2001. Methicillin-resistant *Staphylococcus aureus* skin or soft tissue infections in a state prison—Mississippi, 2000. *MMWR Morb. Mortal Wkly. Rep.* **50**:919–922.
  6. **Cunningham, R., P. Jenks, J. Northwood, M. Wallis, S. Ferguson, and S. Hunt.** 2007. Effect on MRSA transmission of rapid PCR testing of patients admitted to critical care. *J. Hosp. Infect.* **65**:24–28.
  7. **de San, N., O. Denis, M. F. Gasasira, R. De Mondonca, C. Nonhoff, and M. J. Struelens.** 2007. Controlled evaluation of the IDI-MRSA assay for detection of colonization by *Staphylococcus aureus* in diverse mucocutaneous specimens. *J. Clin. Microbiol.* **45**:1098–1101.
  8. **Desjardins, M., C. Guibord, B. Lalonde, B. Toye, and K. Ramotar.** 2006. Evaluation of the IDI-MRSA assay for detection of methicillin-resistant *Staphylococcus aureus* from nasal and rectal specimens pooled in a selective broth. *J. Clin. Microbiol.* **44**:1219–1223.
  9. **Donnio, P., D. C. Oliveria, N. A. Faria, N. Wilhelm, A. Le Coustumier, and H. 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.
  10. **Drews, S. J., B. M. Willey, N. Kreiswirth, M. Wang, T. Janes, J. Mitchell, M. Latchford, A. J. McGeer, and K. C. Katz.** 2006. Verification of the IDI-MRSA assay for detecting methicillin-resistant *Staphylococcus aureus* in diverse specimen types in a core clinical laboratory setting. *J. Clin. Microbiol.* **44**:3794–3796.
  11. **Flayhart, D., J. F. Hindler, D. A. Bruckner, G. Hall, R. K. Shrestha, S. A. Vogel, S. S. Richter, W. Howard, R. Walther, and K. C. Carroll.** 2005. Multicenter evaluation of BBL CHROMagar MRSA medium for direct detection of methicillin-resistant *Staphylococcus aureus* from surveillance cultures of the anterior nares. *J. Clin. Microbiol.* **44**:5536–5540.
  12. **Graham, P. L., S. X. Lin, and E. L. Larson.** 2006. A U.S. population-based survey of *Staphylococcus aureus* colonization. *Ann. Intern. Med.* **144**:318–325.
  13. Reference deleted.
  14. **Huletsky, A., R. Giroux, M. Rossbach, M. Gagnon, M. Vaillancourt, M. Bernier, F. Gagnon, K. Truchon, M. Bastien, F. J. Picard, A. van Belkum, M. Ouellette, P. H. Roy, and M. G. Bergeron.** 2004. New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. *J. Clin. Microbiol.* **42**:1875–1884.
  15. **Martineau, F., F. J. Picard, N. Lansac, C. Ménard, P. H. Roy, M. Ouellette, and M. G. Bergeron.** 2000. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob. Agents Chemother.* **44**:231–238.
  16. **NCCLS/CLSI.** 2006. Performance standards for antimicrobial susceptibility testing; 16th informational supplement, 7th ed. M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA.
  17. **Pan, E. S., B. A. Diep, H. A. Carleton, and E. D. Charlebois.** 2003. Increased prevalence of methicillin-resistant *Staphylococcus aureus* infection in California jails. *Clin. Infect. Dis.* **37**:1384–1388.
  18. **Paule, S. M., D. M. Hacek, B. Kufner, K. Truchon, R. B. Thompson, K. L. Kaul, A. Robicsek, and L. R. Peterson.** 2007. Performance of the BD GeneOhm methicillin-resistant *Staphylococcus aureus* test before and during high-volume clinical use. *J. Clin. Microbiol.* **45**:2993–2998.
  19. **Rossney, A. S., C. M. Herra, M. Fitzgibbon, P. M. Morgan, M. J. Lawrence, and B. O'Connell.** 2007. Evaluation of the IDI-MRSA assay on the Smart-Cycler real-time PCR platform for rapid detection of MRSA from screening specimens. *Eur. J. Clin. Microbiol. Infect. Dis.* **26**:459–466.
  20. **Safdar, N., L. Narans, B. Gordon, and D. G. Maki.** 2003. Comparison of culture screening methods for detection of nasal carriage of methicillin-resistant *Staphylococcus aureus*: a prospective study comparing 32 methods. *J. Clin. Microbiol.* **41**:3163–3166.
  21. **van Hal, S. J., D. Stark, B. Lockwood, D. Marriott, and J. Harkness.** 2007. Methicillin-resistant *Staphylococcus aureus* (MRSA) detection: comparison of two molecular methods (IDI-MRSA PCR assay and GenoType MRSA Direct PCR assay) with three selective MRSA agars (MRSA ID, MRSASelect, and CHROMagar MRSA) for infection-control swabs. *J. Clin. Microbiol.* **45**:2486–2490.
  22. **Warren, D. K., R. S. Liao, L. R. Merz, M. Eveland, and W. M. Dunne, Jr.** 2004. Detection of methicillin-resistant *Staphylococcus aureus* directly from nasal swab specimens by a real-time PCR assay. *J. Clin. Microbiol.* **42**:5578–5581.
  23. **Weist, K., A. K. Cimbal, C. Lecke, G. Kampf, H. Ruden, and R. P. Vonberg.** 2006. Evaluation of six agglutination tests for *Staphylococcus aureus* identification depending upon local prevalence of methicillin-resistant *S. aureus* (MRSA). *J. Med. Microbiol.* **55**:283–290.
  24. **Wright, M. O., J. P. Furuno, R. A. Venezia, J. K. Johnson, H. C. Standiford, J. N. Hebden, J. Hill, D. M. Hartley, A. D. Harris, and E. N. Perencevich.** 2007. Methicillin-resistant *Staphylococcus aureus* infection and colonization among hospitalized prisoners. *Infect. Control Hosp. Epidemiol.* **28**:877–879.