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# Evaluation of the BD GeneOhm Methicillin-Resistant *Staphylococcus aureus* (MRSA) Assay as a Method for Detection of MRSA Isolates, Using a Large Collection of European and North African Isolates

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**Using a large collection of European and North African methicillin-resistant *Staphylococcus aureus* (MRSA) isolates with a variety of genetic backgrounds and staphylococcal cassette chromosome *mec* (SCC*mec*) types, we evaluated the reliability of the BD GeneOhm MRSA assay. Results revealed high performance of this test for detecting MRSA strains provided from Europe and North Africa (98.3%).**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major pathogens responsible for nosocomial infections (1). Early screening for MRSA carriage in patients is essential for limiting transmission (2). To reduce the long response delay with conventional cultures (approximately 48 to 72 h), real-time PCR assays have been developed to ensure the rapid and reliable detection of MRSA. The BD GeneOhm MRSA assay (BD Diagnostics GeneOhm, Quebec City, QC, Canada) is a molecular test that detects a specific genetic fragment in the staphylococcal cassette chromosome *mec* (SCC*mec*) right extremity junction (MREJ), as described by Huletsky et al. (3). MRSA strains are not only derived from a variety of genetic backgrounds but they also present a high diversity of SCC*mec* elements which are characterized by the *mec* class, *ccr* recombinase complex, and three "joining regions." Eleven SCC*mec* types have been described based on the different combinations of these genetic structures, and they are referenced by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) (http://www.sccmec.org/). Several studies have endeavored to assess the reliability of this test for detecting MRSA strains by using collections of genotypically diverse MRSA strains. Notably, Boyle-Vavra and Daum have tested 914 MRSA isolates from the United States and Taiwan (4). The genetic backgrounds of MRSA strains are different between continents because of the geographical spread of specific clones (5); therefore, the aim of this study was to assess the reliability of the BD GeneOhm MRSA assay using a large collection of genetically diverse MRSA isolates from Europe and North Africa.

A total of 1,000 MRSA isolates collected between 2002 and 2010 that originated from Western, Central, and Eastern Europe and North Africa were tested. Isolates from the collections stored in the National Center of Staphylococci (Lyon, France) were selected to maximize the diversity of the genetic backgrounds and SSC*mec* types. The isolates were distributed as follows: Algeria, $n = 42$; Germany, $n = 60$; Austria, $n = 41$; Belgium, $n = 51$; Bulgaria, $n = 10$; Croatia, $n = 10$; Denmark, $n = 47$; Spain, $n = 48$; Finland, $n = 9$; France, $n = 122$; Greece, $n = 18$; Netherlands, $n = 29$; Hungary, $n = 6$; Ireland, $n = 26$; Israel, $n = 29$; Italy, $n = 61$; Poland, $n = 26$; Portugal, $n = 16$; Russia, $n = 40$; Senegal, $n = 29$; Slovenia, $n = 28$; Sweden, $n = 4$; Switzerland, $n = 68$; Czech Republic, $n = 12$; Togo, $n = 3$; Tunisia, $n = 48$; Turkey, $n = 21$; and the United Kingdom, $n = 42$. In addition, 54 strains isolated from pigs in France, Denmark, and Belgium were also tested. SCC*mec* typing was performed using Kondo's method and DNA microarrays (6, 7). MRSA suspensions were grown to a turbidity of a 0.5 McFarland standard. Fifty microliters of suspension was added to a lysis tube containing small glass beads, and the suspension was vortexed to lyse the bacteria. The BD GeneOhm MRSA assay was then performed using a SmartCycler (Cepheid, Sunnyvale, CA, USA) according to the manufacturer's instructions, and both a positive control and a negative control were included in each run. Three additional replicates using the same lysate were performed to confirm isolates that had tested negative using the BD GeneOhm MRSA assay. Furthermore, to confirm the integrity of these lysates, an in-house PCR (specific to *S. aureus* and the *mecA* gene) was performed as previously described (8). Isolates that tested negative using the BD GeneOhm MRSA assay and positive using the in-house PCR test were considered to be false negatives for the commercial assay. In addition, all of the false negatives from the BD GeneOhm MRSA assay were extensively characterized using an *S. aureus*-specific diagnostic DNA microarray (StaphyType; Clondiag, Jena, Germany) that covers 330 *S. aureus*-specific sequences (6, 7). The affiliation of isolates with clonal complexes (CCs) was determined by comparing their hybridization profiles with the reference strains included in the StaphyType database. *spa* typing and based upon repeat pattern (BURP) analysis were also performed as previously described (http://spaserver.ridom.de).

Of the 1,000 genotypically diverse isolates from Europe and North Africa used in this study, 983 tested positive (98.3%) using the BD GeneOhm MRSA assay. These results are consistent with those obtained by Boyle-Vavra and Daum (99.7%) using 914 iso-

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**TABLE 1** Characteristics of MRSA isolates not detected by the BD GeneOhm MRSA assay

| Isolate no. | Country of origin | *agr* | *spa* type | *ccr* identification by microarray | SCC*mec* type (by Kondo) | Clone assignment |
|---|---|---|---|---|---|---|
| 70 | France | I | 8[a] | *ccrA2*, *ccrB2*, *ccrC* | NT[b] | CC8 |
| 261 | France | I | 91 | *ccrC* | V | CC7 |
| 262 | Denmark | I | 34 | *ccrA2*, *ccrB2* | NT | CC398 |
| 360 | France | II | 45 | *ccrA2*, *ccrB2* | IV | CC5 |
| 591 | Switzerland | I | NT | *ccrA2*, *ccrB2*, *ccrC* | NT | CC45 |
| 683 | the Netherlands | II | 657 | *ccrA2*, *ccrB2*, *ccrC* | V | CC1 |
| 684 | the Netherlands | II | 2958 | *ccrA2*, *ccrB2*, *ccrC* | V | CC5 |
| 685 | Denmark | I | 24[a] | *ccrA2*, *ccrB2* | IV | CC8 |
| 729 | Belgium | I | 8a | *ccrA2*, *ccrB2*, *ccrC* | NT | CC8 |
| 730 | Belgium | I | 8a | *ccrA2*, *ccrB2*, *ccrC* | NT | CC8 |
| 731 | Belgium | I | 8a | *ccrA2*, *ccrB2*, *ccrC* | NT | CC8 |
| 732 | Belgium | III | 318 | *ccrC* | NT | CC30 |
| 733 | Belgium | I | 68[a] | *ccrA2*, *ccrB2*, *ccrC* | NT | CC8 |
| 841 | Portugal | II | 535 | *ccrA2*, *ccrB2*, *ccrA4*, *ccrB4* | NT | CC5 |
| 876 | Finland | I | 630 | *ccrA2*, *ccrB2*, *ccrC*, *ccrB/4* | V | CC45 |
| 877 | Finland | I | 2099 | *ccrB4* | NT | CC45 |
| 878 | England | I | 20 | *ccrA2*, *ccrB2* | IV | CC22 |

[a] NT, nontypeable by Kondo's method.
[b] Isolates clustered into the same *spa* group (*spa* CC024).

lates from a large collection of genetically diverse isolates from the United States and Taiwan (4). Taken together, these results confirm that the BD GeneOhm MRSA assay has a high capacity to detect MRSA clones from diverse genetic backgrounds, including geographically diverse strains from Europe and North Africa.

Only 17 of the isolates (1.7%) were not detected as MRSA (Table 1), and all of these strains were confirmed to be positive for *mecA* using an in-house PCR. All of these nondetected isolates originated in Western Europe (Belgium, Denmark, France, Finland, Netherlands, Portugal, and Switzerland). The BURP analysis revealed that 35% (6/17) of these isolates clustered into the same *spa* group (*spa* CC024), which includes the *spa* types t008, t024, and t068. Interestingly, Bartels et al. evaluated the performance of the BD GeneOhm MRSA assay using a Danish collection of MRSA isolates that were primarily from Copenhagen and reported that 12.6% (44/349) were false negatives (9). The most common clone among the false negatives in the Danish study harbored the *spa* type t024 (sequence type [ST8]-SCC*mec* IVa), which belongs to the *spa* group CC024 (data not shown). Taken together, these results suggest that a subset of the strains misidentified by the BD GeneOhm MRSA assay are phylogenetically related. Nevertheless, certain true-positive isolates (harboring *spa* types t051, t112, t121, t190, t451, t622, t801, t1635, t1677, t2054, t2206, t2942, t3060, and t4146) also belonged to the *spa* group CC024. These data suggest that the *spa* type or *spa* CC should not be used as the sole determinant for characterizing the genetic backgrounds of the false-negative MRSA strains by the BD GeneOhm MRSA assay.

Zhang et al. characterized the SCC*mec* elements in the BD GeneOhm MRSA assay-negative strains and suggested that this type of misidentification can be due to the insertion of non-*mec*-containing SCC elements downstream of the *orfX* gene and carriage of SCC*mec* in the left extremity, which is not detectable using this molecular MRSA test (10). In our study, most of the false-negative MRSA isolates (10/17; 59%) harbored a composite SCC*mec* type according to the microarray analysis of additional *ccr* genes (Table 1). For the remaining strains, the presence of other insertional cassettes (for which *ccr* genes are not detected by microarrays) or SCC*mec* in an atypical extremity is highly likely.

García-Álvarez et al. have recently reported the emergence of a highly divergent *mecA* homologue, *mecC*, which is located in a novel SCC*mec* element, named SCC*mec* type XI (11). These strains combine an unrecognized *mec* gene with an unrecognized SCC*mec* cassette, and *mecC* has only a 70% identity at the DNA level with other known *mecA* homologues (11, 12). Genome analysis revealed that three final open reading frames (ORFs) are located downstream of SCC*mec* XI and are considered to be remnants of an SCC element. Such MRSA strains would most likely be falsely identified as methicillin-susceptible *Staphylococcus aureus* (MSSA) strains because the primers supplied in the MRSA detection kit are not able to recognize the new divergent target sequences of the SCC*mec* cassette.

Overall, our results demonstrate that the BD GeneOhm MRSA assay provides accurate detection of most of the MRSA strains circulating in Europe and North Africa (98.3%). Nevertheless, our study highlights the importance of performing regular sentinel studies in different parts of the world to rapidly identify the emergence and dissemination of new MRSA clones and incorporate new oligonucleotide sequences into this test to keep up with the dynamic epidemiology of *Staphylococcus aureus* species.

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