

A Common Variant of Staphylococcal Cassette Chromosome *mec* Type IVa in Isolates from Copenhagen, Denmark, Is Not Detected by the BD GeneOhm Methicillin-Resistant *Staphylococcus aureus* Assay[∇]

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Rapid tests for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage are important to limit the transmission of MRSA in the health care setting. We evaluated the performance of the BD GeneOhm MRSA real-time PCR assay using a diverse collection of MRSA isolates, mainly from Copenhagen, Denmark, but also including international isolates, e.g., USA100-1100. Pure cultures of 349 MRSA isolates representing variants of staphylococcal cassette chromosome *mec* (SCC*mec*) types I to V and 103 different staphylococcal protein A (*spa*) types were tested. In addition, 53 methicillin-susceptible *Staphylococcus aureus* isolates were included as negative controls. Forty-four MRSA isolates were undetectable; of these, 95% harbored SCC*mec* type IVa, and these included the most-common clone in Copenhagen, *spa* t024-sequence type 8-IVa. The false-negative MRSA isolates were tested with new primers (analyte-specific reagent [ASR] BD GeneOhm MRSA assay) supplied by Becton Dickinson (BD). The ASR BD GeneOhm MRSA assay detected 42 of the 44 isolates that were false negative in the BD GeneOhm MRSA assay. Combining the BD GeneOhm MRSA assay with the ASR BD GeneOhm MRSA assay greatly improved the results, with only two MRSA isolates being false negative. The BD GeneOhm MRSA assay alone is not adequate for MRSA detection in Copenhagen, Denmark, as more than one-third of our MRSA isolates would not be detected. We recommend that the BD GeneOhm MRSA assay be evaluated against the local MRSA diversity before being established as a standard assay, and due to the constant evolution of SCC*mec* cassettes, a continuous global surveillance is advisable in order to update the assay as necessary.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common nosocomial pathogen in countries all over the world. In recent years, community-associated MRSA (CA-MRSA) has become increasingly prevalent and has shown potential to cause health care-associated bloodstream infections (8, 26). Screening and isolation of MRSA-positive patients is essential to control the transmission of MRSA in hospitals (16, 24). However, conventional detection of MRSA by culture takes at least 48 h before a preliminary result is available, and as patients in many countries are only isolated when they are recognized as MRSA positive, the risk of having already transmitted MRSA is high. The real-time PCR BD GeneOhm MRSA assay (Becton Dickinson [BD] Diagnostics GeneOhm; San Diego, CA), formerly called IDI-MRSA, is one of a number of commercial kits for rapid MRSA detection directly from nasal swabs (7) and is based on primers developed by Huletsky et al. (18). The forward primers bind to the J3 region of the staphylococcal cassette chromosome *mec* (SCC*mec*), and the reverse primer binds in the *orfX* region that is specific for *Staphylococcus aureus*. At least seven SCC*mec* types are known (types I to VII) (3), and several subtypes, especially of type IV, have been described (21, 27).

The BD GeneOhm MRSA assay has been tested in a number of studies (4, 5, 10, 11, 13–15, 22, 23, 25, 29–31). Most studies screened hospitalized patients, but only two studies described the SCC*mec* types of their MRSA isolates (15, 25). Therefore, it is possible that only a few predominant hospital clones with the same SCC*mec* types were tested. In Denmark, different CA-MRSA clones dominate and MRSA isolates mainly harbor SCC*mec* types IV (85%) and V (6%) (2). In-house testing with the Huletsky primers (18) revealed that they did not amplify a PCR fragment from our most-common MRSA clone, *spa* t024-sequence type 8 (ST8)-IVa. Based on this finding and with the knowledge of the high number of type IV subtypes known, we were interested in finding out whether the BD GeneOhm MRSA assay could detect MRSA isolates from a collection that included mainly CA-MRSA strains. We tested 349 MRSA isolates representing variants of SCC*mec* types I to V. Furthermore, we chose MRSA isolates of different staphylococcal protein A (*spa*) types to have a broad range of genetic backgrounds, testing the hypothesis that the same SCC*mec* type might have minor differences in different MRSA lineages and that these differences could be in the primer regions of the assay.

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MATERIALS AND METHODS

Setting. Denmark is a country with 5.5 million inhabitants and has a low prevalence of MRSA (20). The Department of Clinical Microbiology at Hvidovre

TABLE 1. The 22 international MRSA strains

Strain ^a	<i>spa</i> type	SCCmec type	ST/CC ^b
Finland E1	t002	III	ST5/CC5
Finland E-5	t018	II	ST36/CC30
Finland E-7	t008	I	ST247/CC8
Finland E-10	t051	I	ST247/CC8
Finland E-24	t037	III	ST241/CC8
France A	t008	IVc	ST8/CC8
France B	t008	IVc	ST8/CC8
France C	t051	I	ST247/CC8
UK-EMRSA-1	t037	III	ST239/CC8
UK-EMRSA-3	t001	I	ST5/CC5
UK-EMRSA-15	t022	IVh	ST22/CC22
UK-EMRSA-16	t018	II	ST36/CC30
USA100	t002	II	ST5/CC5
USA200	t018	II	ST36/CC30
USA300	t008	IVa	ST8/CC8
USA400	t125	IVa	ST1/CC1
USA500	t064	IVh	ST8/CC8
USA600	t266	II	ST45/CC45
USA700	t126	IVa	ST72/CC8
USA800	t088	IVc	ST5/CC5
USA1000	t316	IVg	ST359/CC59
USA1100	t019	IVa	ST30/CC30

^a The strains from Finland, France, and the United Kingdom are from the Harmony collection (9).

^b ST, sequence type; CC, clonal complex. The ST and CC of the Harmony collection strains are from Cookson et al. (9), and those of the United States strains from the Ridom *spa* server and E-Burst on the MLST website (www.mlst.net).

Hospital services five hospitals and general practices in the Copenhagen and Frederiksberg municipalities (600,000 inhabitants). The Danish Reference Laboratory for Staphylococci at Statens Serum Institut, Copenhagen, receives all MRSA strains isolated in Denmark.

Isolates. Three hundred forty-nine MRSA isolates (327 Danish and 22 international reference isolates) (Table 1) were included. Isolates of 103 *spa* types harboring variants of SCCmec types I to V plus nontypeable (NT) isolates were chosen. Fifty-three methicillin-susceptible *S. aureus* (MSSA) isolates (23 *spa* types) were included as negative controls. All isolates were tested with a duplex PCR on a pure culture, detecting the *mecA* gene and the *spa* gene. MRSA isolates had both genes, and MSSA isolates only the *spa* gene.

Typing. Amplification and sequencing of the *spa* region were performed on MRSA and MSSA isolates as previously described (2, 17). Designation of *spa* type was conducted by using the Ridom StaphType program. The SCCmec type was determined with an in-house multiplex PCR (6). The SCCmec multiplex PCR was also used to screen MSSA isolates that were positive by the BD GeneOhm MRSA assay. NT isolates were tested in singleton mode with each primer set from the multiplex PCR. Isolates of SCCmec type IV were subtyped by the method of Milheirico et al. (21).

BD GeneOhm MRSA assay and ASR BD GeneOhm MRSA assay. Colonies from pure cultures of all MRSA and MSSA strains were resuspended in sterile saline (0.85%) to a turbidity of 0.5 McFarland. DNA extraction was performed by using a BD GeneOhm MRSA lysis kit (BD Diagnostics). The BD GeneOhm MRSA assay was performed as recommended by the manufacturer, and a positive and negative control were included in each run. All PCRs were run on a SmartCycler system (Cepheid, Sunnyvale, CA). Due to a high number of false-negative MRSA results with the BD GeneOhm MRSA assay, seven of the false-negative MRSA isolates along with two lysates of each strain, produced either by using the MRSA lysis kit or by boiling one colony in 250 µl of sterile water, were sent to BD. They determined that the MRSA isolates had other MREJ (SCCmec right-extremity junction) types than those detected by the BD GeneOhm MRSA assay (18). Therefore, BD developed an investigational real-time PCR assay (analyte-specific reagent [ASR] BD GeneOhm MRSA assay) containing new primers. The primer sequences are proprietary.

From the first collection, 139 MRSA and 25 MSSA isolates were selected for ASR BD GeneOhm MRSA testing, including all MRSA strains that were initially negative. The DNA lysates from the initial BD GeneOhm MRSA evaluation had been stored at minus 20°C and were reused. PCR amplification products were analyzed on a 1.5% agarose gel. The band sizes were compared to the band

TABLE 2. Results of BD GeneOhm MRSA assay

SCCmec type	No. tested	No. positive	% Detected
I	21	21	100
II	21	21	100
III	21	21	100
IV	8	8	100
IVa	105	63	60
IVc	80	79	99
IVd	1	1	100
IVg	4	4	100
IVh	23	23	100
V	46	45	98
NT	19	19	100
Total no. of MRSA isolates	349	305 ^a	87
No. of MSSA isolates	53	8	15

^a Ten isolates were detected only after repeated DNA extraction from the MRSA isolates.

sizes of the isolates examined by BD. DNA from MRSA isolates that were negative by both assays was reextracted with the lysis kit and retested with both assays.

RESULTS

Two hundred ninety-five of 349 MRSA isolates (84.5%) were positive with the BD GeneOhm MRSA assay, including all international reference isolates. Fifty-four (15.5%) of the MRSA strains gave false-negative results. BD examined seven of these isolates. They contained two MREJ types that are not detected by the BD GeneOhm MRSA assay and led to the development of the ASR BD GeneOhm MRSA assay. The ASR BD GeneOhm MRSA assay identified 42 of the 54 false-negative MRSA isolates as MRSA. After a new DNA extraction of the remaining 12 false-negative isolates, 10 isolates were positive in the BD GeneOhm MRSA assay; 1 of these was also positive by the ASR BD GeneOhm MRSA assay (with an aberrant gel band size), and 2 remained false negative. The SCCmec characteristics of all isolates and the results of the BD GeneOhm MRSA assay are shown in Table 2. The *spa* and SCCmec types of the 44 MRSA isolates that were false negative in the BD GeneOhm MRSA assay are presented in Table 3. The majority of the 44 false-negative MRSA isolates harbored SCCmec IVa. Only 5 of 33 t024-ST8-IVa isolates were detected with the BD GeneOhm MRSA assay, three of them after the DNA reextraction, and all were positive in a late PCR cycle.

Eighty-five MRSA isolates that were positive by the BD GeneOhm MRSA assay were also tested with the ASR BD GeneOhm MRSA assay to detect potential overlap of the assays. Only three isolates were positive, but these had gel bands of unexpected sizes. Because the primer sequences were proprietary, the significance of this finding is unknown.

Out of 53 MSSA isolates, 8 (15%) with different *spa* types were false positive by the BD GeneOhm MRSA assay, and none of the 25 MSSA isolates was false positive by the ASR BD GeneOhm MRSA assay. In one of the false-positive MSSA isolates (t127), which was only resistant to penicillin, we identified the insertion sequence-like element IS1272 (confirmed

TABLE 3. *spa* and SCC*mec* types of the 44 BD GeneOhm MRSA assay false-negative MRSA isolates

<i>spa</i> type-SCC <i>mec</i> type	No. not detected	No. positive	No. tested
t012-IVa	1	0	1
t024-IVa	28	5 ^a	33
t350-IVa	1	0	1
t351-IVa	1	0	1
t430-IVa	4	0	4
t648-IVa	1	0	1
t690-IVa	1	0	1
t842-IVa	4	0	4
t846-IVa	1	0	1
t019-IVc	1	15	16
t688-V	1	0	1
Total	44	20	64

^a Positive in late cycles.

by sequencing of the PCR product). One false-positive MSSA isolate (t843) was resistant to penicillin, erythromycin, and moxifloxacin, while the remaining six were only resistant to penicillin (five isolates) or were fully susceptible (one isolate).

DISCUSSION

A number of Danish MRSA isolates were not detected by the BD GeneOhm MRSA assay. Investigations at BD revealed that they harbored MREJ types different from the ones included in the BD GeneOhm MRSA assay. By using new primers, the investigational ASR BD GeneOhm MRSA assay detected 42 of the 44 MRSA isolates that gave false-negative results with the BD GeneOhm MRSA assay. The main issue with the BD GeneOhm MRSA assay when used for isolates from Copenhagen was the lack of sensitivity in detecting isolates of SCC*mec* type IVa. The type IVa cassette is common in CA-MRSA and is found in the most-abundant MRSA clone in Copenhagen—the t024-ST8-IVa clone. The t024-ST8-IVa clone has mainly affected people in nursing homes and has caused small outbreaks in local hospitals. In our area, it accounts for 32% of all MRSA isolates found between 2003 and 2007. While 5 of the 33 t024-ST8-IVa isolates were detected by the BD GeneOhm MRSA assay, this was only with weak signals near the limit of detection. Since the PCR was run on pure cultures, it is unlikely that they would have been detected from nasal swabs. In our selection of isolates, the BD GeneOhm MRSA assay detected 60% of the isolates harboring SCC*mec* IVa, including USA300, while 40% were undetectable. The subtyping of type IV is mainly based on differences in the J1 region (21), whereas the primers from BD GeneOhm MRSA amplify from the J3 region across the origin of replication (*orfX*). This shows that in type IVa, the J3 region exhibits some variability. Two isolates, t690-IVa and t688-V, were negative by both assays. Both patients from whom these isolates were obtained had recently traveled in Egypt, and it is currently unknown why these isolates were not detected.

Eight of 53 MSSA isolates (15%) gave false-positive results. This false-positive rate is much higher than the rate of 4.6% found by Huletsky et al. (18). However, relatively high frequencies of false-positive results have been reported by others (11,

23). High false-positive rates could be a problem in countries with a low prevalence of MRSA, as this would result in low positive predictive values. Remnants of the SCC*mec* cassette have been identified in some MSSA strains and could explain false-positive results by PCR (12, 28). It is of interest whether this is the cause in a country with a low prevalence of MRSA, and it needs to be addressed in further studies. In one MSSA isolate (t127), we found the insertion sequence-like element *IS1272* that is present in SCC*mec* type I and type IV and could indicate that this isolate has a remnant of an SCC*mec* cassette. However, *IS1272* has been found in coagulase-negative staphylococci, MRSA and MSSA, and may have been disseminated irrespective of the presence of *mec* DNA (19).

Though the BD GeneOhm lysis kit has been reported to be superior to five other extraction methods (1), 10 MRSA isolates (3%) were only detected by the BD GeneOhm MRSA assay after repeated DNA extraction. While this might be due to a handling error in our laboratory, the internal control (which is added together with the PCR reagents) was detected in all 10 cases after the first round of extraction. These samples would have been classified as MRSA negative in a clinical setting. False-negative results possibly due to extraction problems have been reported by others (25).

In a screening situation with the goal of preventing nosocomial MRSA infections, a high negative predictive value of a MRSA screening assay is important. In Denmark, the prevalence of MRSA is low and we do not expect to find many MRSA carriers. Hospitalized MRSA patients are always isolated, and patients at risk of being MRSA carriers are kept in isolation until a negative MRSA test result is obtained. A false-negative result would release these patients from isolation and could start a MRSA outbreak.

The local diversity of CA-MRSA strains makes the current version of the BD GeneOhm MRSA assay inadequate for screening in Copenhagen. The main problem with the test was that most of the type IVa cassettes of our major MRSA clone (t024-ST8-IVa) were undetected. New primers not yet commercialized can detect these strains, but the ASR BD GeneOhm MRSA assay would be an add-on assay to the BD GeneOhm MRSA assay, increasing the laboratory work and costs. Based on the findings in this study, we recommend that the BD GeneOhm MRSA assay be evaluated against the local MRSA diversity before being established as a standard assay. Due to the rapid evolution of SCC*mec* in CA-MRSA, we recommend that the assay's usefulness be continuously monitored.

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