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High Proportion of Wrongly Identified Methicillin-Resistant *Staphylococcus aureus* Carriers by Use of a Rapid Commercial PCR Assay Due to Presence of Staphylococcal Cassette Chromosome Element Lacking the *mecA* Gene[▽]

Dominique S. Blanc,[1]* Patrick Basset,[1] Immaculée Nahimana-Tessemo,[1] Katia Jaton,[2] Gilbert Greub,[2] and Giorgio Zanetti[1]

Hospital Preventive Medicine Service[1] and Institute of Microbiology,[2] Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland

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During a 9-month period, 217 patients were newly diagnosed as methicillin-resistant *Staphylococcus aureus* (MRSA) carriers by using a commercial rapid PCR-based test (GeneXpert). However, no MRSA was recovered by culturing the second swab in 61 of these patients. Further analyses showed that 28 (12.9%) of the patients harbored *S. aureus* isolates with a staphylococcal cassette chromosome element lacking the *mecA* gene and were thus incorrectly determined to be MRSA carriers.

Rapid and accurate detection of methicillin-resistant *Staphylococcus aureus* (MRSA) is a key element for early therapy and implementation of control measures to prevent onward transmission from carriers (5–7, 15, 16). Recently developed PCR-based methods have the potential to confirm or refute MRSA carriage in individual patients within 2 h. PCR detection of MRSA from clinical specimens requires primers specific to the different staphylococcal cassette chromosome *mec* (SCC*mec*) elements at their 3′ extremity sequences and a primer specific to the *S. aureus* chromosomal sequence located at the 3′ of the SCC*mec* integration site (9). However, the rapid PCR test will generate a false-positive result in the presence of SCC elements lacking the *mecA* gene (10, 11). For example, it was reported that 4.6% of 569 methicillin-susceptible *Staphylococcus aureus* (MSSA) were PCR positive with a PCR targeting the SCC*mec* element (8). Such false-positive results may lead to several unjustified actions such as (i) the empirical use of glycopeptide compounds instead of beta-lactam antibiotics, (ii) decolonization treatments, and (iii) isolation of patients and other constraining infection control measures. The purpose of the present study was to evaluate the proportion of patients wrongly identified as MRSA carriers with a rapid commercial PCR test.

The University Hospital of Lausanne is a 900-bed tertiary care hospital where active surveillance cultures are part of its MRSA control program. The rapid PCR-based test (GeneXpert system; Cepheid, Sunnyvale, CA) was introduced in June 2009 for screening MRSA in nose, throat, and groin swabs in addition to screening performed by culture. Samples were obtained using a double-swab Transystem (Copan, Brescia, Italy). In order to isolate the MRSA strain for further molecular typing, all second swabs were cultured when ≥1 sample was found positive in a screening set (nose, throat, and groin). Culture included an overnight incubation in an enrichment broth (m-Staphylococcus broth; Difco, Basel, Switzerland), followed by inoculation onto a chromogenic agar medium (MRSA-select; Bio-Rad, Marnes-la-Coquette, France). During the study period, a 1-ml aliquot of all enrichment broths was stored frozen for further analyses.

Between August 2009 and April 2010, 267 patients were newly diagnosed as MRSA carriers using the rapid MRSA PCR test. Fifty were excluded from the analysis because culture was not done. Among the remaining 217 patients, 156 (72%) had positive cultures for MRSA, whereas 61 (28%) had negative cultures. Enrichment broths were available for 58 of these 61 patients with negative cultures. The cultures were thawed and plated onto chromogenic *S. aureus* agar plates (SA-ID; bioMérieux, Marcy l'Etoile, France). For 28 of these patients, we retrieved isolates of *S. aureus* that were positive by the rapid PCR test. Antibiotic susceptibility testing was performed on these isolates with the Kirby-Bauer method, as previously described (2). All showed a methicillin-susceptible phenotype (oxacillin-S and cefoxitine-S). A PCR that amplified the *mecA* gene was also performed as previously described (6) and confirmed the absence of this gene. Characteristics of these isolates are given in Table 1. Thus, 28 of the 217 (12.9%) newly identified MRSA carriers by rapid commercial PCR test harbored a *S. aureus* strain that did not contain the *mecA* gene.

Most patients harboring an MSSA strain determined to be positive with the rapid MRSA test were subsequently screened several times for MRSA by culture, and no MRSA was recovered. The consequences for these patients were unnecessary decolonization procedures, which are time- and labor-consuming, and isolation with contact precautions, which has been associated with less patient care in several studies. In one case,

* Corresponding author. Mailing address: Service de Médecine Préventive Hospitalière, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland. Phone: 41 21 314 02 59. Fax: 41 21 314 02 62. E-mail: dominique.blanc@chuv.ch.

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TABLE 1. Site of sampling, resistance profile, and genotypes (DLST and MLST) of MSSA isolates from 28 patients that were determined to be positive by the GeneXpert MRSA assay

Patient no.	Site[a]	Resistance profile[b]	DLST	ST[c]	CC[d]
1	Nose	Pen, Cip, Ery	2-2	105	5
2	Nose and inguinal*	Pen, Clin, Cip, Ery, Fu	3-3	8	8
3	Nose	Pen, Gm, Fu	288-3	8	8
4	Nose	Pen, Fu	288-3	828	8
5	Nose	Pen, Fu	288-19	SLV8	8
6	Nose and throat*	Pen, Fu	288-231	8	8
7	Nose	Pen, Fu	492-231	8	8
8	Nose	Pen, Fu	534-122	8	8
9	Nose	Pen	533-353	72	8
10	Nose	No resistance	538-520	10	10
11	Nose and throat*	Pen	5-46	1	15
12	Inguinal	Pen	5-46	1	15
13	Throat	No resistance	503-489	1	15
14	Nose	Pen	5-46	852	15
15	Inguinal	No resistance	496-490	3	15
16	Throat and inguinal*	No resistance	532-228	3	15
17	Nose	Cip	527-72	15	15
18	Throat	Pen	537-152	22	22
19	Nose and throat*	Pen	122-109	34	30
20	Nose	Pen	122-516	34	30
21	Nose	Pen	489-485	45	45
22	Throat	Pen	86-76	59	59
23	Throat	Pen, Ery	86-76	59	59
24	Nose	Pen	86-76	59	59
25	Nose	Cip	536-41	78	88
26	Throat	Pen	217-519	88	88
27	Nose	No resistance	371-184	97	97
28	Nose and throat*	No resistance	162-486	SLV291	398

[a] In six patients, indicated by an asterisk, the same MSSA was found in two samples.
[b] An antibiogram was performed with oxacillin (Ox), ceftriaxone (Cef), penicillin (Pen), gentamicin (Gm), ciprofloxacin (Cip), clindamycin (Clin), erythromycin (Ery), cotrimoxazole (SxT), fucidin (Fu), and rifampin (Rif).
[c] SLV, new ST which is a single locus variant of the mentioned ST.
[d] CC, clonal complex.

a patient was grouped with other MRSA-positive patients in the same room (cohorted) and subsequently became colonized with the roommate's strain.

Most of the commercially available rapid tests (GeneXpert MRSA, GeneOhm MRSA [BD, Franklin Lakes, NJ], and LightCycler MRSA [Roche, Basel, Switzerland]) are based on the detection of a sequence indicating the integration of the SCC*mec* within the chromosome and do not specifically target the *mecA* gene. By adding the amplification of the *mecA* gene, as what is done in the new MRSA Nuclisens EasyQ from bioMérieux, one would expect that most of these false-positive results would be identified. However, the presence of coagulase-negative *Staphylococcus* carrying the *mecA* gene could still hide some of the false positives.

The presence of a SCC element that does not contain the *mecA* gene might be due to the loss of this gene. In this case, we would expect most of the false-positive isolates to be genetically related to predominant MRSA clones in the area. To investigate this hypothesis, all MSSA isolates of the present study were genotyped by the double-locus sequence typing (sequencing of ca. 500 bp of *clfB* and *spa* genes [13]) and multilocus sequence typing methods (4) as previously described. A great diversity of genotypes was observed, suggesting the nonclonal dissemination of one strain (Table 1). An excision of the *mecA* gene could be suspected in four cases since these strains showed a genotype related to local epidemic MRSA (Lyon clone [DLST 3-3, ST 8-IV] and a variant of the New York/Japan clone [DLST 2-2, ST 105-II]) (1). Such loss of the *mecA* gene was previously described during the emergence and spread of the Lyon clone (ST 8-SCC*mec* IV) in French hospitals (2, 3). Partial excision of SCC*mec* was suggested since SCC*mec* associated elements were still present in these trains, and their genotypes were related to the epidemic MRSA. Nevertheless, the majority of genotypes observed in our MSSA isolates were not related to local predominant MRSA clones (Table 1), suggesting that these MSSA with partial SCC*mec* elements did not emerge from local MRSA. Further studies should be done to investigate whether these isolates harbored non-*mec*-containing SCC elements, as was described for MSSA and other staphylococcal species (12, 14).

In conclusion, we identified here a high proportion (12.9%) of patients wrongly determined to be MRSA carriers using a rapid commercial test for MRSA screening. This was due to the presence of *S. aureus* with an SCC element lacking the *mecA* gene. These false-positive results led to inappropriate patient care (unnecessary decolonization treatment, additional precautions measures, and possibly the unjustified use of glycopeptides). In the future, more insight is needed on the performance of these molecular tests and, ideally, new generation tests should circumvent the current limitations.

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