High proportion of wrongly identified methicillin-resistant *Staphylococcus aureus* carriers when using a rapid commercial PCR assay due to the presence of SCC element lacking the *mecA* gene

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During a 9-month period, 217 patients were newly diagnosed as MRSA carriers with 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%) patients harbored *S. aureus* isolates with a SCC element lacking the *mecA* gene and were thus wrongly considered as 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 hours. PCR detection of MRSA from clinical specimens requires primers specific to the different staphylococcal cassette chromosome *mec* (*SCCmec*) elements at their 3’ extremity sequence and a primer specific to the *S. aureus* chromosomal sequence located at the 3’ of the *SCCmec* 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 MSSA were PCR positive with a PCR targeting the *SCCmec* 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 this 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 beds 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 with 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. They 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 already 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. Thus, 28 of the 217 (12.9%) newly identified MRSA carriers by rapid commercial PCR test harbored a S. aureus strain which did not contain the mecA gene.

Most patients harboring a MSSA strain 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 lower patient care in several studies. In one case, the patient was cohorted with other MRSA positive patients, and subsequently became colonized with the roommates' 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 SCCmec 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 doesn't contain the mecA gene might be due to the loss of
this gene. In this case, we would expect that most of the false positive isolates 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 c.a. 500 bp of clfB and spa genes (13)) and MLST methods (4) as previously
described. A great diversity of genotypes was observed suggesting the non clonal
dissemination of one strain (Table). An excision of the mecA gene could be suspected in 4
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-SCCmec IV) in French hospitals (2,3). Partial excision of SCCmec was
suggested since SCCmec associated elements were still present in these trains and their
genotype 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),
suggesting that these MSSA with partial SCCmec element did not emerged from local MRSA.
Further studies should be done to investigate if these isolates harbored non-mec-containing
SCC elements, as was described in MSSA and other staphylococcal species (12,14).

In conclusion, this work identified a high proportion (12.9%) of patients wrongly considered
as MRSA carriers using a rapid commercial test for MRSA screening. This was due to the
presence of S. aureus with SCC element lacking the mecA gene. These false positive results
lead to inappropriate patient care (unnecessary decolonization treatment, additional
precautions measures and possibly 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.

Acknowledgments
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References


Table. Site of sampling, resistance profile, and genotypes (Double Locus Sequence Typing and MLST Sequence Type) of MSSA isolates from 28 patients that were positive by the GeneXpert MRSA assay.

<table>
<thead>
<tr>
<th>Pt #</th>
<th>Site</th>
<th>Resistance profile$^d$</th>
<th>DLST</th>
<th>ST$^b$</th>
<th>(CC)$^c$</th>
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<tbody>
<tr>
<td>1</td>
<td>Nose</td>
<td>Pen, Cip, Ery</td>
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<td>(8)</td>
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<td>Nose</td>
<td>Pen, Gm, Fu</td>
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<td>(8)</td>
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<tr>
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<td>Nose</td>
<td>Pen, Fu</td>
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<td>828</td>
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<tr>
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<td>288-19</td>
<td>SLV8</td>
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<td>(8)</td>
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<td>492-231</td>
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<tr>
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<td>(59)</td>
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<td>(398)</td>
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</table>

$^a$, Antibiogram was performed with oxacillin (Ox), ceftriaxone (Cef), penicillin (Pen), gentamycin (Gm), ciprofloxacin (Cip), clindamycin (Clin), erythromycin (Ery), co-trimoxazole (SxT), fucidin (Fu), and rifampin (Rif).

$^b$, SLV, new allele which is a single locus variant of the mentioned ST.

$^c$, CC, Clonal Complex.

$^d$, In 6 patients, the same MSSA was found in two samples.