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1 # Very low prevalence of MRSA carrying the *mec*C gene in Switzerland

2 P. Basset[a], G. Prod'hom[b], L. Senn[a], G. Greub[b], and D.S. Blanc[ab]

3 [a] Service of Hospital Preventive Medicine, [b] Institute of Microbiology; Lausanne University Hospital,

4 Switzerland

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7 Corresponding address:

8 Dominique Blanc

9 Service of Hospital Preventive Medicine

10 Lausanne University Hospital

11 Rue du Bugnon 46

12 1011 Lausanne

13 Switzerland

14 Phone +41 021 314 02 59

15 e-mail: Dominique.Blanc@chuv.ch

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17

18 **Abstract.**

19 We report the first case of MRSA with the *mec*C gene in one patient in Western Switzerland. After

20 this first identification, a PCR protocol was setup to investigate the occurrence of this new *mec*C gene

21 in our population. We investigated enrichment broths of MRSA screenings from 1062 patients, MSSA

22 isolates of clinical samples from 475 patients, and 80 MRSA isolates (2005 to 2011) showing

23 discrepancies between genotypic and phenotypic methicillin resistance. None were found to be

24 positive for the *mec*C suggesting it is rarely present in our population of patients.

25

26

27 **Introduction**

28 Methicillin resistance in *Staphylococcus aureus* is explained by the acquisition of the *mec*A gene
29 which is located on the Staphylococcal Cassette Chromosome (SCC). Different types of SCC elements
30 have been identified since their first emergence in *S. aureus* in the 1960's (www.sccmec.org). So far,
31 the homology of the *mec*A gene sequence among SCC*mec* types was very high, allowing the setup of
32 molecular PCR tests detecting all types. The recent discovery of the SCC*mec* XI that harbours a new
33 *mec*C element with only 70% sequence homology with *mec*A genes [1-3], raised concern about the
34 detection of MRSA carrying this element. Moreover, it was reported that these new MRSA clones are
35 growing fastidiously on some commercial chromogenic agars[4]. In addition, standard laboratory
36 procedures (such as susceptibility testing with automated system, or PBP2' agglutination tests) might
37 not be able to recognize this new type of methicilin resistance. In this study, we report the first case
38 of a MRSA carrying the *mec*C gene in Western Switzerland and we show that the prevalence of this
39 gene in this region is currently very low.

40 **Methods**

41 The University Hospital of Lausanne is a 1000-bed tertiary care hospital where active surveillance
42 cultures or rapid PCR tests are part of the MRSA control program. It includes initial screening samples
43 obtained from the following patients: i) patients transferred from a foreign hospital or a nursing
44 home, ii) roommates of newly identified MRSA infected/colonized patients, iii) patients hospitalized
45 in a ward with active nosocomial transmissions, and iv) patients at admission and discharge from
46 adult intensive care units (ICUs).

47 Routine screening for MRSA consists in pooling nose, throat and groin swabs into m-Staphylococcus
48 broth (Difco, Basel, Switzerland). After overnight incubation at 35°C, the broth was inoculated on
49 chromogenic M-select medium (Bio-Rad, Marnes-la-Coquette, France), which was incubated
50 overnight at 35°C before reading. For the *mec*C investigation, 100 μl of each enrichment broth were

51 saved into 96-wells plates. The plates were kept frozen until full. The reference strain LGA251 and
52 our SCC*mec* XI strain were added as positive controls. A high throughput PCR protocol in 96-well
53 plates was developed to detect the new *mec*C . After several attempts to find the best procedure, we
54 used the following protocol: 100 μl of the broth were centrifuged at 3700 rpm for 5 min, the pellet
55 was re-suspended into 50 μl of $H_2O$, boiled at 95°C for 5 min, centrifuged again at 3700 rpm for 5
56 min, and the supernatant was used for the PCR reaction. Preliminary analyses showed that the *mec*C
57 PCR was as sensitive as culture to detect the new *mec*C gene from enrichment broths (data not
58 shown).

59 Specific amplification of the *mec*C gene was performed using the primers *mec*$A_{LGA251}$MultiFP
60 (GAAAAAAAGGCTTAGAACGCCTC) and *mec*$A_{LGA251}$MultiRP (GAAGATCTTTTCCGTTTTCAGC) as already
61 described [5]. To overcome PCR inhibition problems due to a high concentration of foreign DNA, PCRs
62 were performed with 3 μl of the DNA solution at two dilutions: 1/1 and 1/100. The PCR consisted of 3
63 min at 95°C, followed by 40 cycles starting with 30 sec at 94°C, 30 sec at 50°C, and 60 sec at 72°C;
64 and a final extension step of 5 min at 72°C. The expected 138 bp amplification product was separated
65 by gel electrophoresis (1.5% agarose, 300V/cm during 30 min), and visualized on a UV table after
66 GelRed™ (Biotium, Hayward, CA, USA) staining.

67 The prevalence of the *mec*C gene was investigated in several local populations: i) enrichment broth
68 cultures from routine screening of hospitalized patients, ii) enrichment broths from nursing home
69 residents from our area (one enrichment broth culture per resident was saved in 96-well plates and
70 stored frozen at -20°C), iii) frozen *S. aureus* isolates (one per patient) recovered from clinical
71 specimens of hospitalized patients were thawed and cultured in enrichment broth. In addition, we
72 tested all *S.aureus* isolates recovered in our hospital since 2005 and showing discrepancies between
73 genotypic and phenotypic methicillin resistance.

74 The MRSA isolate carrying the *mec*C gene was typed using the MLST[6] , *spa* typing [7] and Double Locus
75 Sequence Typing (DLST, sequencing of *clf*B and *spa* genes) [8, 9] methods as previously described.

76

77 **Results and discussion**

78 In December 2011, we identified by chance one strain harbouring the SCC*mec* XI in a screening
79 sample. This *S. aureus* isolate was negative by the Xpert MRSA, had a very low growth on M-select
80 chromogenic agar plate (Biorad, Marne-la-Coquette, France), and the PBP2' agglutination test was
81 negative. The presence of the *mec*C gene in this isolate was confirmed by the *mec*C gene specific
82 PCR [5]. This MRSA was recovered from a 59 year-old man admitted to the ICU for community
83 acquired septic shock secondary to a perforated duodenal ulcer. MRSA screening (nose, throat and
84 groin) was performed at ICU admission. The patient was not known for previous MRSA
85 colonisation/infection. He had not been hospitalised the last two years, did not travel and had no
86 contact with animals except his cat. He did not present any MRSA infection during his hospital stay
87 and the nine months thereafter. This represents the first report of *mec*C MRSA from Western
88 Switzerland. Molecular typing showed that this isolate belonged to ST 130 (CC130), a new spa-type
89 t11150 and DLST typing identified a new DLST type (714-663), which has never been encountered
90 previously in our area.

91 Following the identification of this strain, we investigated the prevalence of *mec*C in several local
92 populations, using the high through put protocol in 96-well plates previously set up (Table). First,
93 between February to March 2012, we screened 564 enrichment broth cultures from 486 patients
94 hospitalized in our hospital. Second, we screened 576 enrichment broths from a large MRSA survey
95 in nursing homes in 2010. Third, we analysed 475 frozen *S. aureus* isolates (one per patient)
96 recovered from clinical specimens of patients hospitalized between September and December 2011.
97 The new *mec*C gene was not found in these three populations (Table). Finally, among the more than
98 8000 isolates analyzed by molecular typing (i.e. double locus sequence typing, SCC*mec* typing, *mec*A
99 and PVL genes detection by PCR [8]), 80 showed discrepancies between a resistant phenotype to

100 methicilin and a negative result of the *mec*A PCR. The presence of the *mec*C was investigated by PCR

101 in these 80 isolates and none were positive.

102 Despite an extensive search for *S. aureus* with the new *mec*C, we did not find other patients

103 harbouring such strains in our hospital and in nursing home residents from our area. Thus, it seems

104 that currently, as reported in Germany[4, 10], these new MRSA do not constitute a risk for our

105 institution. Nevertheless, changes in local epidemiology can occur quickly. There is a need for

106 manufacturers of selective media, of automated susceptibility testing systems, and of rapid PCR tests

107 to provide solutions for this problem in the near future. However, while waiting for these solutions,

108 the detection procedure that we set up represents a useful alternative to evaluate the prevalence of

109 these new clones that may remain undetected with current routine approaches.

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156 **Table**. Population groups in Western Switzerland investigated for the presence of *mecC*

| | Periods | No of samples | No of patients | No of *mec*C |
|---|---|---|---|---|
| Routine MRSA screening at the tertiary care hospital | Feb to March 2012 | 564 | 486 | 0 |
| Nursing home residents | 2010 | 576 | 576 | 0 |
| MSSA isolates from hospitalized patients (2012) | Sep to Dec 2011 | 475 | 475 | 0 |
| *S. aureus* isolates with discrepancies between genotypic and phenotypic methicillin resistance | 2005-2011 | 80 | 80 | 0 |

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