The *pls* Gene Found in Methicillin-Resistant *Staphylococcus aureus* Strains Is Common in Clinical Isolates of *Staphylococcus sciuri*

Katri Juuti,1* Salha Ibrahim,2 Anni Virolainen-Julkunen,2 Jaana Vuopio-Varkila,2,3,4 and Pentti Kuusela3,4

General Microbiology, Faculty of Biosciences,1 and Department of Bacteriology and Immunology,2 Haartman Institute, University of Helsinki, Division of Clinical Microbiology, Helsinki University Central Hospital,4 and Department of Microbiology, National Public Health Institute,2 Helsinki, Finland

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*pls*, a gene found in type I staphylococcal cassette chromosome *mec* (SCC*mec*) regions of methicillin-resistant *Staphylococcus aureus* strains, was present in 12 of the 15 human clinical *Staphylococcus sciuri* isolates studied. Pls was expressed in the *S. sciuri* isolates, although at a lower level than in *S. aureus*. Other parts of SCC*mec* could also be found in the *S. sciuri* genome.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasing problem in hospital and community environments worldwide. Resistance towards methicillin is encoded by the *mecA* gene, carried by a mobile genetic element, staphylococcal cassette chromosome *mec* (SCC*mec*). Five types of SCC*mec* elements, containing slightly differing combinations of *mecA*, its regulators, and site-specific recombinase (*ccr*) genes, which enable a site-specific integration into the chromosome, as well as additional type-specific DNA, have so far been characterized (4, 11–13, 20). New MRSA strains are thought to emerge by acquisition of SCC*mec* by sensitive clones. The origin of *mecA*, however, has been suggested to be another staphylococcal species, possibly *Staphylococcus sciuri* or a close evolutionary relative, since a *mecA* homologue with 88% amino acid sequence similarity (32) is uniformly found in *S. sciuri* strains (2). *S. sciuri* is a taxonomically primitive staphylococcal species found on the skins of several animals as well as environmental sources (16, 17). It has occasionally been isolated from humans but has only rarely been associated with infections (6, 9, 21, 28–30).

*pls* is a part of the type I SCC*mec* element (11). *Pls* is a large surface protein with an LPXTG peptidoglycan-anchoring sequence and a so-far-uncharacterized carbohydrate-containing portion (7, 18, 27). Its expression results in reduced adherence to host proteins (27) and decreased cellular invasiveness (14). On the other hand, it mediates bacterial aggregation and binding to glycolipids (10) and to human desquamated nasal epithelial cells (25). It acts as a virulence factor in a mouse model of septic arthritis and sepsis (13a).

**The *pls* gene is common in *S. sciuri* isolates.** Fifteen *S. sciuri* clinical human isolates of three subspecies, isolated from various sites (Table 1) (21), were studied for the presence of the *pls* and *mecA* genes by Southern hybridization. SmaI-digested DNA separated by pulsed-field gel electrophoresis (PFGE) (26, 27) was hybridized with a digoxigenin-labeled *pls* probe (Table 1) at 68°C and a *mecA* probe (Table 1) at 55°C to allow hybridization to both the *S. aureus* and *S. sciuri* *mec* genes. All the strains differed from each other by their PFGE patterns (Fig. 1A). *pls* was present in 12 out of 15 *S. sciuri* strains. Three strains, N900165, N900228, and S A8b, all of which are *S. sciuri* subsp. *rodentium*, lacked the gene (Fig. 1B). *mecA* hybridization resulted in a single weak band in 7 of 15 *S. sciuri* strains (Fig. 1C). All of these strains are oxacillin and methicillin sensitive (Table 1), and primers specific for *S. aureus mecA* are unable to amplify their DNA or the amplification is weak (21). Eight of 15 of the strains showed either a single strong band (4 of the 8 strains) or a strong and a weak band (4 of the 8 strains). Seven of these eight strains are oxacillin resistant, and their *S. aureus* mecA genes can be detected by PCR (21). A *mecA* probe has been shown to hybridize with a large and a smaller fragment in methicillin-resistant *S. sciuri* strains (2). Based on these data and the better homology of our probe with *S. aureus mec* than *S. sciuri mec*, we conclude that the strong bands represent *S. aureus mecA* and that the weak bands represent its *S. sciuri* homologue, which is slightly different in sequence. In 10 strains, a *mecA*-hybridizing fragment, usually the weak one, also hybridized with the *pls* probe (Fig. 1B and C). The sizes of these fragments were at least 400 kb, and consequently, it is difficult to estimate how near to each other the two genes are in the *S. sciuri* genome.

The *pls* genes of five *S. sciuri* strains (N960509, N960546, N970234, N970555, and S 29) were characterized in more detail by hybridization and PCR analyses and found to be similar but not identical to *S. aureus pls*. DNA of each strain, isolated as described previously for *S. aureus* (27) and digested with Stul and PstI, hybridized with enhanced-chemiluminescence-labeled probes for repeat regions R1, R2, and R3 and that the weak bands represent identical *S. sciuri mecA* and that the weak bands represent *S. sciuri mec*. Other parts of SCC*mec* could also be found in the *S. sciuri* genome.

The *pls* genes of five *S. sciuri* strains (N960509, N960546, N970234, N970555, and S 29) were characterized in more detail by hybridization and PCR analyses and found to be similar but not identical to *S. aureus pls*. DNA of each strain, isolated as described previously for *S. aureus* (27) and digested with Stul and PstI, hybridized with enhanced-chemiluminescence-labeled probes for repeat regions R1, R2, and R3 and that the nonrepeat region A of *S. aureus* strain 1061 Pls (27; results not shown). However, only the R2 region of all five strains and the A region of strain N970555 were amplifiable by PCR with the primers used to prepare these probes (27; data not shown).

**Pls is expressed by *S. sciuri*.** To study the expression of *Pls* in these 15 *S. sciuri* strains, surface proteins were solubilized by incubating stationary-phase cells with lysostaphin in the presence of raffinose essentially as described previously for *S. au-
TABLE 1. Probes used for dot blot and Southern hybridizations and results of the dot blot hybridization

<table>
<thead>
<tr>
<th>Probe</th>
<th>Probe sequence (^c)</th>
<th>Primer(s) used for probe amplification (^b)</th>
<th>Template DNA in probe amplification</th>
<th>SCORI type(s) in which the primer sequence(s) is found (^d)</th>
<th>Oxa(^e) with (S. aureus) mecA (^f)</th>
<th>With Oxa (S. aureus) mecA (^g)</th>
<th>Oxa(^h) without (S. aureus) mecA (^i)</th>
<th>Controls (^j)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Upstream of pls gene</td>
<td>Loci A to H</td>
<td>IA I</td>
<td>– – – – – – – – – –</td>
<td>– – – – – – – – +/-</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
</tr>
<tr>
<td>B</td>
<td>(kdp) operon</td>
<td>Loci A to H</td>
<td>II II</td>
<td>+ – + + + + + + +</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
</tr>
<tr>
<td>C</td>
<td>mec1</td>
<td>Loci A to H</td>
<td>II II, III</td>
<td>– – – – – – + + + +</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
</tr>
<tr>
<td>D</td>
<td>(ORF) 5' to (orfX)</td>
<td>Loci A to H</td>
<td>IA I, II, IV</td>
<td>– – – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
</tr>
<tr>
<td>E</td>
<td>Between integrated pl1258 and Tn554</td>
<td>Loci A to H</td>
<td>IIIA III (not IIIIB)</td>
<td>– – – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
</tr>
<tr>
<td>F</td>
<td>Between Tn554 and (orfX)</td>
<td>Loci A to H</td>
<td>IIIA III (not IIIIB)</td>
<td>– – – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
</tr>
<tr>
<td>G</td>
<td>Left junction between (IS431) and pUB110</td>
<td>Loci A to H</td>
<td>II, IA</td>
<td>+ + + + + + + +</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
</tr>
<tr>
<td>H</td>
<td>Left junction between (IS431) and (pT181)</td>
<td>Loci A to H</td>
<td>III (not IIIA/B)</td>
<td>+ + + + + + + +</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
</tr>
<tr>
<td>ccr1</td>
<td>Part of (ccrA1) and (-B)</td>
<td>(\beta2)</td>
<td>IA I</td>
<td>– – – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
</tr>
<tr>
<td>ccr2</td>
<td>Part of (ccrA2) and (-B)</td>
<td>(\beta2)</td>
<td>II II</td>
<td>+ + + + + + + +</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
</tr>
<tr>
<td>ccr3</td>
<td>Part of (ccrA3) and (-B)</td>
<td>(\beta2)</td>
<td>IIIA III</td>
<td>+ + + + + + + +</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
</tr>
<tr>
<td>(pls)</td>
<td>Nonrepetitive A</td>
<td>27</td>
<td>1061 I</td>
<td>+ – + – – + + + +</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
<td>+ – – – – – + + + +/</td>
</tr>
<tr>
<td>meca</td>
<td>Part of (S. aureus) (meca)</td>
<td>477 nt</td>
<td>1061 All types</td>
<td>+ – + + + + + +</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
<td>– – – – – – – – –</td>
</tr>
</tbody>
</table>

\(^a\) \(ORF\), open reading frame.
\(^b\) Loci A to H, primers to amplify loci A to H in a multiplex PCR (24); \(\beta2\), primers \(\beta2\), specific for the \(ccrB\) gene, used together with either primer \(\alpha2\), primer \(\alpha3\), or primer \(\alpha4\), specific for the \(ccrA1\), -2, and -3 genes, respectively (11); 27, primers reported in reference 27; 477 nt, primers 5’ AAGATGGCAAAGATATTCAAC3’ and 5’TTCCTACTGCCTAATTCGAG3’ to amplify an internal 477-nt region of \(S. aureus\) meca.
\(^c\) 1A, Iberian; II, UK-EMRSA-16; IIA, Brazilian; III, Helsinki IV; 1061, clinical MRSA isolate (7, 27).
\(^d\) The strains of these types produce an amplification product in a multiplex PCR (24).
\(^e\) \(sc\.), \(S. sciuri\) subsp. \(sciuri\); \(rod\.), \(S. sciuri\) subsp. \(rodentium\); \(car\.), \(S. sciuri\) subsp. \(carnaticas\).
\(^f\) Strains for which the MIC was \(\geq 2\) \(\mu g/mL\) were considered to be resistant to oxacillin (21).
\(^g\) \(IA\), Iberian; II, UK-EMRSA-16; IIA, Brazilian; III, Helsinki IV; 1061, clinical MRSA isolate (7, 27).
\(^h\) MRSA DNAs positive and negative for the primer sequences were used as the controls. The symbol to the left side of the slash shows the hybridization signal by the strain used as a positive control, and that to the right side of the slash shows the signal by a negative control.

FIG. 1. Small-digested DNA of \(S. sciuri\) strains separated by PFGE (A) was capillary blotted onto a nylon membrane (Roche) and probed with the digoxigenin-labeled MRSA strain 1061 \(pls\) gene A region (B) and an internal region of MRSA meca (C). The lambda ladder PFG (Biolabs) was used as a marker. Molecular size standards (std) were run in three lanes, one of which by mistake was combined with a sample lane (N970234). Marker sizes are shown on the left.
strains S106j and 1061 were used. 

VOL. 43, 2005 NOTES 1417

1061) of a stationary-phase culture. (B) Twenty microliters of the peak detection. (A) The samples originate from 100 Antibodies (Dako) and alkaline phosphatase substrate were used for detections (14) followed by alkaline phosphatase conjugated rabbit anti-mouse antibodies (Dako) and alkaline phosphatase substrate were used for detection. (A) The samples originate from 100 μl (40 μl for strain 1061) of a stationary-phase culture. (B) Twenty microliters of the peak detection. (A) The samples originate from 100

membranes were blocked with 2% (wt/vol) bovine serum albumin–phosphate-buffered saline. Pooled mouse monoclonal anti-Pls antibodies (14) followed by alkaline phosphatase conjugated rabbit anti-mouse antibodies (Dako) and alkaline phosphatase substrate were used for detection. (A) The samples originate from 100 μl (40 μl for strain 1061) of a stationary-phase culture. (B) Twenty microliters of the peak fractions that eluted from strains N950282 and N970234 and 3 μl from strains S 106j and 1061 were used.

FIG. 2. Western analysis of lysostaphin-extracted surface proteins (A) or WGA-purified Pls proteins (B) of S. sciuri and S. aureus strains separated in a sodium dodecyl sulfate–8% polyacrylamide gel and blotted on 0.2-μm-pore-size nitrocellulose membranes (Protran). The membranes were blocked with 2% (wt/vol) bovine serum albumin–phosphate-buffered saline. Pooled mouse monoclonal anti-Pls antibodies (14) followed by alkaline phosphatase conjugated rabbit anti-mouse antibodies (Dako) and alkaline phosphatase substrate were used for detection. (A) The samples originate from 100 μl (40 μl for strain 1061) of a stationary-phase culture. (B) Twenty microliters of the peak fractions that eluted from strains N950282 and N970234 and 3 μl from strains S 106j and 1061 were used.

Pls proteins of three S. sciuri strains and S. aureus strain 1061 were affinity purified from lysostaphin digests by using wheat germ agglutinin (WGA) Sepharose and elution with 100 mM N-acetylglucosamine (7) and then analyzed by Western blotting for Pls (Fig. 2B). Pls of S. sciuri, like that of S. aureus, bound to WGA and thus seems to have a carbohydrate moiety containing N-acetylglucosamine.

Analysis of the “SCCmec region” in S. sciuri. The presence of areas homologous to different types of S. aureus SCCmec regions in S. sciuri DNA was examined. Dot blot hybridization was used for all 15 strains, and additionally, Southern hybridization was used for strains N920212, N950120, and N960546. Thirteen digoxigenin-labeled probes designed to differentiate among type I to IV SCCmec regions were PCR amplified (Table 1). Four micrograms of total DNA was dot blotted, or Smal-digested DNA separated by PFGE (5, 23) was capillary blotted, onto positively charged nylon membranes. The dot blot hybridizations were performed at 68°C (Table 1), and the Southern hybridizations were performed at 55°C (results not shown). To make sure that hybridization to S. aureus SCCmec regions was not examined, it was safest to look closely only at the S. sciuri strains not containing the S. aureus mecA gene. All the S. sciuri strains, even if positive for pls, gave negative results with probe A binding to a region upstream of pls in type I SCCmec regions. At 68°C, the strains without S. aureus mecA bound the mecA probe very weakly and not at all probe C, containing the mecI sequence. In a PCR analysis of 28 human S. sciuri isolates by Couto et al., the presence of mecI was always connected to the presence of a copy of S. aureus mecA (3). Similarly, mecA regions sequenced from four S. sciuri strains by Wu et al. revealed mecR2 and mecI adjacent to mecA genes of the S. aureus type but not adjacent to those of the S. sciuri type (31). It seems that mecI is not a part of the native S. sciuri mec region. Probes G, H, and ccr3 bound to negative-control DNA and were thus unable to differentiate between the SCCmec types. There were, however, three to four strains that did not bind to these probes at all, suggesting that IS431 or plasmids pUB101 and pT181 sometimes but not always are a part of the S. sciuri genome. All the other strains except the four S. sciuri strains that did not bind to any of the ccr probes probably have some kind of site-specific recombinease genes. Probes B, D, and F did not bind to the DNA of any strains having only the S. sciuri copy of mecA, suggesting that these regions of SCCmec were not a part of the S. sciuri genome.

The Southern analysis (data not shown) of strain N920212 at a low stringency gave a positive signal with pls, mecA, and the C and A probes, all of which hybridized with the largest fragment. One fragment of strain N950120 DNA hybridized with mecA and probe C, and another fragment hybridized with pls. This strain was one of the few strains having pls and mecA in different Smal fragments (Fig. 1). One fragment of strain
N960546 DNA hybridized with pls, mecA, and C, another one hybridized with the ccr probes and G, another one hybridized with the ccr probes and A, and yet another one hybridized with the H probe. Thus, sequences related to mecA and the upstream region of pls seem to exist in \textit{S. sciuri}.

The idea that pls, like mecA, might originate from \textit{S. sciuri} is supported by the much greater frequency of pls in \textit{S. sciuri} strains than in \textit{S. aureus} strains. The strains used in this study were human clinical isolates and may not represent the \textit{S. sciuri} populations in their natural habitats. There is a possibility that pls was a part of an \textit{S. aureus SCCmec} region in some of the \textit{S. aureus} mecA+ \textit{S. sciuri} strains. Half of the strains, however, did not contain \textit{S. aureus mecA} gene. We do not know what the function of Pls in \textit{S. sciuri} is, but the low level of expression suggests that the effects may be different from those in \textit{S. aureus}.

Some of the SCCmec sequences were present in \textit{S. sciuri} strains and even localized to the same chromosomal areas. Whether mec genes are a part of an SCCmec element also in species other than \textit{S. aureus} is not known. SCC elements without mecA have recently been found in \textit{S. aureus} as well as some other staphylococcal species, and they are thought to act as mobile genetic elements transferring any useful genetic information between species (8, 15, 19, 22). The presence of pls in \textit{S. sciuri} as well as \textit{S. aureus} is yet another piece of evidence that there is constant genetic exchange between staphylococcal species.


Shittu, A., J. Lin, D. Morrison, and D. Kolawole. 2004. Isolation and mo-


ERRATUM

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Volume 43, no. 3, p. 1415–1419, 2005. Page 1416: Table 1 should appear as shown below.

<table>
<thead>
<tr>
<th>Probe</th>
<th>Probe sequence</th>
<th>Primer(s) used for probe amplification</th>
<th>Template DNA in probe amplification</th>
<th>5SCcore type(s) in which the primer sequence(s) is found</th>
<th>Oxa±, with S. aureus mecA</th>
<th>Oxa±, with S. aureus mecA</th>
<th>Oxa±, without S. aureus mecA</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Upstream of pls gene</td>
<td>Loci A to H</td>
<td>IA</td>
<td>I</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>B</td>
<td>kdp operon</td>
<td>Loci A to H</td>
<td>II</td>
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<td>Loci A to H</td>
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<td>II</td>
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<td>D</td>
<td>ORF 5° to orfX</td>
<td>Loci A to H</td>
<td>IA</td>
<td>I, II, IV</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
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<tr>
<td>E</td>
<td>Between integrated pl258 and Tn554</td>
<td>Loci A to H</td>
<td>IIIA</td>
<td>III (not IIIB)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>F</td>
<td>Between Tn554 and orfX</td>
<td>Loci A to H</td>
<td>IIIA</td>
<td>III (not IIIB)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>G</td>
<td>Left junction between IS431 and pUB110</td>
<td>Loci A to H</td>
<td>II</td>
<td>II, IA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>H</td>
<td>Left junction between IS431 and pT181</td>
<td>Loci A to H</td>
<td>III</td>
<td>III (not IIIA/B)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

| ccr1 | Part of ccrA1 and -B | β2 | IA | I | - | - | - | - | - | - | - | - | - | - | +/-- |
| ccr2 | Part of ccrA2 and -B | β2 | II | II | + | + | - | - | - | - | - | - | - | - | +/-- |
| ccr3 | Part of ccrA3 and -B | β2 | IIIA | III | + | + | + | ++ | + | - | - | - | - | - | +/++ |
| pls Nonrepetitive A | 27 | 1061 | I | + | - | - | - | + | + | + | + | + | + | - | +/++ |
| mecA | Part of S. aureus mecA | 477 nt | 1061 | All types | + | - | - | - | + | + | - | - | - | - | +/++ |

* a ORF, open reading frame.
* b Loci A to H, primers to amplify loci A to H in a multiplex PCR (24); β2, primers β2, specific for the ccrB gene, used together with either primer α2, primer α3, or primer α4, specific for the ccrA1, -2, and -3 genes, respectively (11); 27, primers reported in reference 27; 477 nt, primers 5’TCTTACTGCTAATTCGAG3’ and 5’TCTTACTGCTAATTCGAG3’ to amplify an internal 477-nt region of S. aureus mecA.
* c IA, Iberian; II, UK-EMRSA-16; IIIA, Brazilian; IIII, Helsinki IV; 1061, clinical MRSA isolate (7, 27).
* d The strains of these types produce a amplification product in a multiplex PCR (24).
* e sc., S. sciuri subsp. sciuri; rod., S. sciuri subsp. rodentium; car., S. sciuri subsp. carnaticas.
* f Strains for which the MIC was >2 μg/ml were considered to be resistant to oxacillin (21).
* g MRSA DNAs positive and negative for the primer sequences were used as the controls. The symbol to the left side of the slash shows the hybridization signal by the strain used as a positive control, and that to the right side of the slash shows the signal by a negative control.