Reconstruction of the Phenotypes of Methicillin-Resistant *Staphylococcus aureus* by Replacement of the Staphylococcal Cassette Chromosome *mec* with a Plasmid-Borne Copy of *Staphylococcus sciuri* *pbpD* Gene

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Received 15 August 2008/Returned for modification 29 September 2008/Accepted 6 November 2008

The *mecA* gene, the central determinant of methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA), is not native to this bacterial species but may have originated in the animal commensal species *Staphylococcus sciuri*. All *S. sciuri* strains carry a close homologue of *mecA* in the form of *pbpD*, the genetic determinant of penicillin binding protein 4 (*PBP* 4) of *S. sciuri*. Here we describe an experimental system that could be used for additional tests for this proposition. The *S. sciuri* *pbpD* gene was cloned into a shuttle plasmid and introduced into methicillin-susceptible *S. aureus* strain COL-S derived from parental MRSA strain COL from which the resistance cassette staphylococcal cassette chromosome *mec* was excised. The *S. sciuri* *pbpD* determinant was transcribed and translated in the *S. aureus* transductants producing large amounts of the 84-kDa *S. sciuri* *PBP* 2A and was then deposited in the plasma membrane of the host bacterium. Transductants carrying the heterologous *S. sciuri* *pbpD* gene exhibited properties typical of those of parental MRSA strain COL, including broad-spectrum, high-level, and homogeneous resistance to structurally different β-lactams. Antibiotic resistance was dependent on the functioning of *S. aureus* *PBP* 2 and was suppressed by the specific regulatory genes *mecI* and *mecR* and by inhibitors of an early step in cell wall biosynthesis. *S. sciuri* *PBP* 4 was also able to replace the essential physiological function(s) of the native *PBP* 2 of *S. aureus* and produce peptidoglycan typical of that of parental MRSA strain COL. Our results provide further support for the proposition that the resistance determinant *mecA* of MRSA strains has evolved from *S. sciuri* *pbpD*.

*Staphylococcus aureus* is a major human pathogen responsible for several life-threatening infections, including septicemia, endocarditis, and toxic shock syndrome. Methicillin (meticillin)-resistant *S. aureus* (MRSA) strains were first reported in 1961, shortly after the introduction of methicillin in clinical practice. Since then, MRSA has become an increasing critical threat in hospital and community environments worldwide. The genetic determinant of β-lactam resistance, the *mecA* gene, is carried on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*), and encodes a low-affinity penicillin binding protein (*PBP* 2 and was suppressed by the specific regulatory genes *mecI* and *mecR* and by inhibitors of an early step in cell wall biosynthesis. *S. sciuri* *PBP* 4 was also able to replace the essential physiological function(s) of the native *PBP* 2 of *S. aureus* and produce peptidoglycan typical of that of parental MRSA strain COL. Our results provide further support for the proposition that the resistance determinant *mecA* of MRSA strains has evolved from *S. sciuri* *pbpD*.

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† Published ahead of print on 17 November 2008.

**MATERIALS AND METHODS**

Bacterial strains, plasmids, and growth conditions. The characteristics of the bacterial strains and plasmids used in this study are described in Table 1. Back-
TABLE 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Characteristics</th>
<th>Source or reference</th>
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<tbody>
<tr>
<td>S. aureus strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COL</td>
<td>Clinical isolate; Ox' Em' Cm' Te'</td>
<td>RU collection</td>
</tr>
<tr>
<td>COL-pbpB</td>
<td>COL derivative in which pbpB is under the control of spac promoter/pPBP2i; Em'</td>
<td>13</td>
</tr>
<tr>
<td>COL-S</td>
<td>COL with SCoMec excised; Ox' Em' Cm' Te'</td>
<td>11</td>
</tr>
<tr>
<td>COL-S(pbpB)</td>
<td>COL-S derivative in which pbpB is under the control of spac promoter/pPBP2i; Es'</td>
<td>This study</td>
</tr>
<tr>
<td>COL-S(pbpD)</td>
<td>COL-S carrying the plasmid-borne S. aureus mecA region/PSTSW2C; Te'</td>
<td>This study</td>
</tr>
<tr>
<td>COL-S(pbpD)</td>
<td>COL-S carrying the plasmid-borne S. sciuri pbpD region/pSS37MA; Te'</td>
<td>This study</td>
</tr>
<tr>
<td>COL-S(pbpD)</td>
<td>COL-S carrying the plasmid-borne S. aureus mecI-mecR1 region/pGC2::mecI-mecR1; Te' Cm'</td>
<td>This study</td>
</tr>
<tr>
<td>COL-S(pbpD)</td>
<td>COL-S carrying the plasmid-borne S. aureus mecI-mecR1 region/pGC2::mecI-mecR1; Te' Cm'</td>
<td>This study</td>
</tr>
<tr>
<td>COL-S(pbpD)</td>
<td>COL-S(pbpD) carrying the plasmid-borne S. aureus mecI-mecR1 region/pGC2::mecI-mecR1; Te' Cm'</td>
<td>This study</td>
</tr>
<tr>
<td>S. sciuri strain SS37</td>
<td>Clinical isolate recovered from the nasopharynx of a patient; Ox'</td>
<td>4</td>
</tr>
</tbody>
</table>

Plasmids

| pPB2i               | S. aureus integrational vector with pbpB ribosome-binding site and first 171 codons fused to P_pB promoter; Em' | 13                   |
| pSTSW2C             | pSTP181C with 3,737-bp PCR product of S. aureus COL mecA region; Te' | 19                   |
| pSS37MA             | pSTP181C with 4,130-bp PCR product of S. sciuri SS37 pbpD region; Te' | 20                   |
| pGC2::mecI-mecR1    | pGC2 with 2,466-bp PCR product of S. aureus N315 mecI-mecR1 region; Cm' | D. C. Oliveira       |

| Abbreviations: Ox', oxacillin susceptible; Ox', oxacillin resistant; Em', erythromycin susceptible; Em', erythromycin resistant; Cm', chloramphenicol susceptible; Cm', chloramphenicol resistant; Te', tetracycline susceptible; Te', tetracycline resistant; RU, The Rockefeller University. |

Antibiotic susceptibility testing. The susceptibilities of the S. aureus strains to β-lactam antibiotics were determined by population analysis, as described previously (17).

Peptidoglycan purification and analysis by HPLC. Peptidoglycan was purified from 1-liter cultures of bacteria grown at 30°C to mid-exponential phase, as described previously (5). Purified peptidoglycan was digested with mutanolysin (Sigma-Aldrich) and monomers were reduced with sodium borohydride (Sigma-Aldrich), separated by high-performance liquid chromatography (HPLC) on a C18 column (3 μm, 4.6 by 250 mm; ODS-Hypersil; Thermo Electron Corporation), and detected by measurement of the absorbance at 206 nm, as described previously (5).

Membrane purification and PBP assay. Membrane preparations were purified from cultures grown to the late exponential phase, as described previously (15). The detection of PBPs was performed by incubating the membrane preparations (150 μg of proteins) with a saturating concentration (20 μg/ml) of [14C]penicillin in 99.5% methanol (Sigma-Aldrich). The proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) in an 8% (wt/vol) acrylamide gel. Purified peptidoglycans were digested with mutanolysin (Sigma-Aldrich) and were used for PAGE as described previously (5).

Membrane preparations were separated by SDS-PAGE as described above. Transfer and blotting were done according to the instructions accompanying the ECL Western blotting analysis system (GE Healthcare). The ChromPure human immunoglobulin G Fe fragment (Jackson ImmunoResearch Laboratories) was used at 3 μg/ml to eliminate nonspecific hybridization with protein A. The primary antibody was a monoclonal antibody against S. aureus PBP 2A (dilution, 1:20,000) obtained by injecting a rabbit with the synthetic peptide sequence CDKNFKOVYKDSSYISKDNG conjugated to keyhole limpet hemocyanin (a gift from JoAnn Hoskins, Eli Lilly, Indianapolis, IN), and the secondary antibody was the anti-rabbit antibody included in the kit (dilution, 1:5,000). S. sciuri PBP 4 has previously been shown to react also with the monoclonal antibody raised against S. aureus PBP 2A (4, 19).

RNA isolation and Northern blot analysis. Total RNA was extracted from cultures grown up to an optical density at 602 nm (OD602) of 0.7. RNA (5 μg) was resolved by electrophoresis on 1.2% agarose–0.66 M formaldehyde gels in morpholinepropanesulfonic acid running buffer. RNA was blotted onto Hybond-N+ membranes (GE Healthcare) with a turbo blotter alkaline transfer system (Schleicher & Schuell) with 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The PCR-amplified DNA probes were labeled with [α-32P]dCTP (GE Healthcare) by using a Ready-To-Go labeling kit (GE Healthcare) and hybridized under high-stringency conditions. The blots were subsequently washed and autoradiographed.

Introduction of a plasmid-borne S. aureus COL mecA gene and S. sciuri SS37 pbpD gene into S. aureus strain COL-S and into a pbpB conditional mutant of COL-S. Plasmid pPB2i (13) was transformed into oxacillin-susceptible S. aureus strain COL-S (11) to produce strain COL-S(pbpB::pbpD), in which the expression of the chromosomal pbpD gene is under the control of the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible spac promoter. A PCR-amplified sequence of 3,737 bp from S. aureus COL mecA was ligated into shuttle plasmid pSTP181C to form pSTSW2C (19). A 4,130-bp fragment that included the promoter region of pbpB was cloned into pcDNA3/109 bp upstream of the start codon and the entire pbpB gene was amplified from S. sciuri SS37 by PCR and was cloned into shuttle plasmid pSTP181C and plasmid pSS37MA (20). Recombinant plasmids pSSP2WC and pSS37MA were subsequently introduced into S. aureus strain RN4200 by electroporation and were then transduced by phage 80α into S. aureus strain COL-S to yield the transductants COL-S(pbpB::pbpD::spac) and COL-S(pbpB::pbpD::spac), respectively.

Cloning of mecI and mecR1 regulatory genes and introduction into S. aureus. The 2,466-bp mecI-mecR1 region from S. aureus strain N315 was amplified with primers mecIP5 (5’-AGAGGGGAATCCCTAAGGAGATTTTCTG-3’) and mecRP5 (5’-GGGATCTCTACTTACACACATTCGCCG-3’) and was ligated into the BamHI and EcoRI sites of high-copy-number plasmid pGC2 (D. C. Oliveira, unpublished data). The resulting recombinant plasmid, pGC2::mecI-mecR1, was electroporated into RN4200 and then transduced into COL-S(mecI-mecR1), COL-S(mecA::spac), and COL-S(mecA::spac) to produce the transductants COL-S(mecI-mecR1), COL-S(mecA::spac), and COL-S(mecA::spac), respectively.

RESULTS

The experimental system: construction of MSSA strain COL-S and introduction of plasmid-borne copies of S. sciuri pbpD and S. aureus mecA genes. The chromosomal SCCmec type I cassette was removed from MRSA strain COL by pre-
cise excision, producing strain COL-S, which was fully susceptible to all beta-lactam antibiotics tested (Table 2) (11). A \( \text{pbpB} \) conditional mutant of strain COL-S (COL-\( \text{spac}::\text{pbpB} \)) was constructed by placing the genetic determinant of PBP 2 under the control of the IPTG-inducible \( \text{spac} \) promoter (13).

The source of the \( S. \text{sciuri} \) \( \text{pbpD} \) gene was clinical isolate \( S. \text{sciuri} \) SS37, which was recovered from the nasopharynx of a patient (3). Strain SS37 exhibited heterogeneous resistance to oxacillin and carried a \( \text{pbpD} \) gene that was overexpressed due to the insertion of \( \text{IS}_{256} \) upstream of the gene (3, 4). The upregulated \( \text{pbpD} \) gene was cloned into a shuttle plasmid (20) and introduced into \( S. \text{aureus} \) COL-S and COL-\( \text{spac}::\text{pbpB} \) to produce transductants COL-S SS\( \text{pbpD} \) and COL-\( \text{spac}::\text{pbpB}/\text{SSpbpD} \), respectively. As a control, the same shuttle plasmid carrying the \( \text{mecA} \) gene from MRSA strain COL (19) was also introduced into the same \( S. \text{aureus} \) backgrounds, yielding transductants COL-S SA\( \text{mecA} \) and COL-\( \text{spac}::\text{pbpB}/\text{SAmecA} \), respectively.

**Transcription and translation of plasmid-borne \( S. \text{aureus} \) \( \text{mecA} \) and \( S. \text{sciuri} \) \( \text{pbpD} \) in \( S. \text{aureus} \).** Figure 1 shows the results of Northern blot analysis, performed to estimate the degree of expression of \( S. \text{aureus} \) \( \text{mecA} \) and \( \text{pbpB} \) and \( S. \text{sciuri} \) \( \text{pbpD} \) in the different \( S. \text{aureus} \) backgrounds. No \( \text{mecA} \) transcript was detected in COL-S or its \( \text{pbpB} \) conditional mutant, COL-\( \text{spac}::\text{pbpB} \). \( S. \text{aureus} \) \( \text{mecA} \) and \( S. \text{sciuri} \) \( \text{pbpD} \) were both effectively and highly expressed in transductants COL-\( \text{spac}::\text{pbpB}/\text{SAmecA} \) and COL-\( \text{spac}::\text{pbpB}/\text{SSpbpD} \). The inhibition of \( \text{pbpB} \) transcription was confirmed in cultures of strains COL-

**Table 2. Susceptibility to \( \beta \)-lactam antibiotics**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>COL</th>
<th>COL-S</th>
<th>COL-( \text{spac}::\text{mecA} )</th>
<th>COL-( \text{spac}::\text{pbpB} )</th>
<th>COL-( \text{spac}::\text{mecA} ) ( \text{spac}::\text{mecI-mecR} )</th>
<th>COL-( \text{spac}::\text{pbpB} ) ( \text{spac}::\text{mecI-mecR} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>800</td>
<td>0.8</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>0.8</td>
</tr>
<tr>
<td>Ceftizoxime</td>
<td>800</td>
<td>0.8</td>
<td>800</td>
<td>400</td>
<td>400</td>
<td>3</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>800</td>
<td>0.8</td>
<td>800</td>
<td>200</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Cephradine</td>
<td>400</td>
<td>6</td>
<td>400</td>
<td>100</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>800</td>
<td>3</td>
<td>200</td>
<td>3</td>
<td>6</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**FIG. 1.** Transcription of \( S. \text{aureus} \) \( \text{mecA} \) and \( \text{pbpB} \) genes and \( S. \text{sciuri} \) \( \text{pbpD} \) gene. RNA was purified from cultures grown at 30°C to an OD\( \text{600} \) of 0.7. RNA (5 \( \mu \)g) was resolved by electrophoresis on agarose-formaldehyde gels. After transfer, the membranes were hybridized with \( { }^{32}\text{P}- \)labeled \( S. \text{aureus} \) \( \text{mecA} \) and \( \text{pbpB} \) and \( S. \text{sciuri} \) \( \text{pbpD} \) DNA probes.

**FIG. 2.** PBP patterns and detection of protein products of \( S. \text{aureus} \) \( \text{mecA} \) (PBP 2A) and \( S. \text{sciuri} \) \( \text{pbpD} \) (PBP 4). (A) Membrane preparations (150 \( \mu \)g of proteins) were incubated with a single saturating concentration of \( [\text{14C}]\text{benzylpenicillin} \). After SDS-PAGE, the gel was exposed to a tritium storage phosphor screen for 2 weeks. (B) Membrane preparations (80 \( \mu \)g of proteins) were tested by Western blot analysis for the production of proteins that react with a monoclonal antibody raised against \( S. \text{aureus} \) PBP 2A. The results for \( S. \text{aureus} \) COL and \( S. \text{sciuri} \) SS37 are provided as positive controls and size markers for PBP 2A and PBP 4, respectively. (C) Protein patterns on an SDS-polyacrylamide gel stained with Coomassie blue.
and a Western blot analysis was performed with an antibody raised against S. aureus PBP 2A (Fig. 2B), which has previously been shown to also react with S. sciuri PBP 4 (4, 19, 20). In accordance with the results of the Northern blot analysis, these experiments confirmed that PBP 2 was absent from the membrane preparation of COL-S pbpB/spac:ss/pbpD grown in the absence of IPTG and that PBP 2A was absent from COL-S and its derivative, COL-S pbpB/spac:ss/pbpD. Large amounts of PBP 2A were detected in the membrane preparations of COL-S pbpB/spac:ss/pbpD. The foreign S. sciuri PBP 4 was also correctly translocated and deposited in large amounts in the plasma membrane of S. aureus host cells. As shown in Fig. 2C, S. aureus PBP 2A (78 kDa) and S. sciuri PBP 4 (84 kDa) were already evident from the SDS-polyacrylamide gel stained with Coomassie blue.

Similarly, high levels of transcription of S. aureus mecA and S. sciuri pbpD and large amounts of PBP 2 and PBP 4 were also observed for transductants COL-S mecA and COL-S pbpD, respectively (data not shown).

Phenotypic expression of β-lactam resistance. Table 2 shows that the introduction of both the S. sciuri pbpD gene and the S. aureus mecA gene into MSSA strain COL-S was able to provide virtually identical levels of resistance to a group of β-lactam antibiotics which have considerably different degrees of selective binding to the four S. aureus PBPs. Transductants COL-S pbpD and COL-S mecA produced high-level and homogenous resistance to cefoxitin and cefotaxime (selective affinity for PBP 2), cephradine (PBP 3), cefoxitin (PBP 4), and oxacillin.

Inhibition of β-lactam resistance in transductants COL-S pbpD and COL-S mecA by subinhibitory concentrations of d-cycloserine and by the mecI-mecR1 regulatory genes. It has previously been shown that inhibitors of the early steps of cell wall synthesis can reduce the level of methicillin resistance and change the homogeneous resistance phenotype of strain COL to a heterogeneous one (16). Susceptibility to oxacillin in the presence of subinhibitory concentrations (0.25× MIC) of the cell wall synthesis inhibitor d-cycloserine was determined by population analysis. While the oxacillin MIC of strain COL-S remained unchanged, the resistance levels were drastically reduced in the presence of d-cycloserine, from MICs of 800 to 400 μg/ml for strains COL, COL-S mecA, and COL-S pbpD to MICs as low as 1, 8, and 0.75 μg/ml, respectively. In addition, the heterogeneous expression of oxacillin resistance in COL-S mecA and COL-S pbpD was converted to heterogeneous expression (data not shown), as previously described for COL (16).

The S. aureus mecA gene has been shown to be transcriptionally regulated in some clinical isolates by mecR1 and mecI, cotranscribed chromosomal genes that encode a signal transducer and a repressor, respectively (9). The mecI-mecR1 region from S. aureus strain N315 was cloned in a high-copy-number plasmid and introduced into COL, COL-S mecA, and COL-S pbpD. The mecI and mecR1 regulatory genes repressed the phenotypic expression of β-lactam resistance in COL, as described previously (10), and well as in COL-S mecA (Table 2). Most interestingly, the level of β-lactam resistance was also extensively repressed in strain COL-S pbpD, which expressed the plasmid-borne S. sciuri PBP 4 gene (Table 2), suggesting that the MecI repressor can bind to the promoter region of S. sciuri PBP 4 similar to the way in which it binds to the promoter region of S. aureus mecA (14).

Replacement of the normal (essential) physiological function(s) of the S. aureus host PBP 2 by S. sciuri PBP 4. Previous studies have shown that S. aureus PBP 2, the protein product of the pbpB gene, is essential for growth in β-lactam-susceptible S. aureus strains. However, the additional PBP 2A present in MRSA strains can replace the essential function(s) of PBP 2 (7, 13). In order to test if S. sciuri PBP 4 was also able to support growth in PBP 2-deprived S. aureus cells, the plasmid-borne S. sciuri pbpD gene was introduced into S. aureus strain COL-S pbpD, in which the pbpB gene was put under the control of an IPTG-inducible promoter. As a control, the plasmid-borne S. aureus mecA gene was also introduced in the same background. As previously described for a β-lactam-susceptible S. aureus strain, the absence of IPTG prevented the growth of COL-S pbpD but only slowed the growth of the pbpB conditional mutant of MRSA strain COL, COL-S pbpD.
The composition of transductants carrying the plasmid-borne *S. aureus* mecA or *S. sciuri* pbpD genes and suppression of pbpB transcription on the expression of oxacillin resistance in *S. aureus*. Aliquots of overnight cultures were plated on tryptic soy agar containing increasing concentrations of oxacillin. The numbers of CFU were counted after incubation for 72 h at 30°C. The oxacillin susceptibility profiles were determined for COL (●), COLspac::pbpD grown in the presence (▲) and absence (●) of IPTG, COLspac::pbpD grown in the presence of IPTG (▼), COLspac::pbpD/SamecA grown in the presence (▲) and absence (●) of IPTG, and COLspac::pbpD/SsphpD grown in the presence (●) and absence (▼) of IPTG.

Effect of suppression of pbpB transcription on peptidoglycan composition of transductants carrying the plasmid-borne *S. sciuri* pbpD or *S. aureus* mecA gene. Peptidoglycans were purified from 1-liter cultures grown at 30°C to an OD620 of 0.4, digested with mutanolysin, and separated by HPLC. The doglycans were purified from 1-liter cultures grown at 30°C to an absence of IPTG, and the growth of strain pbpB::spac gene into COL-S. sciuri PBpB/::SSspac gene into COL-S. aureus mecA (Fig. 3B) indicating transcription on peptidoglycan pbpB. These results indicate that *S. sciuri* PBP 4 was able to replace the essential enzymatic activity of *S. aureus* PBP 2 and catalyze the biosynthesis of peptidoglycan to produce an *S. aureus* type of cell wall with the cell wall precursors of the *S. aureus* host.

Effect of suppression of pbpB transcription on the oxacillin resistance level of transductants carrying the plasmid-borne *S. sciuri* pbpD or *S. aureus* mecA gene. High-level and homogeneous resistance to oxacillin in MRSA strain COL is known to depend on the level of transcription of the *S. aureus* pbpB gene (7). As shown in Fig. 5, the inhibition of pbpB transcription in the conditional mutant of strain COL (COLspac::pbpB) reduced the oxacillin resistance levels of the majority of the cells and converted the homogeneous phenotype to a heterogeneous one. In the presence of IPTG, the introduction of either the
plasmid-borne \textit{S. sciuri pbpD} gene or the \textit{S. aureus mecA} gene into the \textit{pbpB} conditional mutant COL-S\textsubscript{pac}:\textit{pbpB} produced high-level and homogeneous resistance that was close to that of \textit{S. aureus} strain COL (Fig. 5). However, in the absence of IPTG, the oxacillin MICs for the majority of the cells decreased 16-fold and the population analysis profiles became heterogeneous (Fig. 5). These results indicate that even in the presence of large amounts of either \textit{S. sciuri} PBP 4 or \textit{S. aureus} PBP 2A in the transductants, the host PBP 2 was still required for the optimal expression of antibiotic resistance in \textit{S. aureus}, as was already shown for MRSA strain COL (7, 12).

**DISCUSSION**

The genetic determinant of \(\beta\)-lactam resistance, \textit{mecA}, is not native to \textit{S. aureus} but was acquired from an extraspecies source (1). The \textit{S. sciuri pbpD} gene, which is uniformly present in both \(\beta\)-lactam-susceptible and -resistant isolates of this widely spread animal commensal species, was first identified on the basis of its high degree of structural similarity with the \textit{S. aureus mecA} gene (2, 18). Previous studies have proposed that \textit{S. sciuri pbpD} may represent the evolutionary precursor of the \textit{S. aureus mecA} gene, mainly on the basis of epidemiological and genetic evidence. The protein product of \textit{pbpD} was subsequently identified as PBP 4, one of the six PBPs detected in \textit{S. sciuri} (20). Recombinant \textit{S. sciuri} PBP 4 was purified and was shown to share several biochemical properties with \textit{S. aureus} PBP 2A (6).

In this report, a new experimental system was designed to further test the validity of the proposition that the \textit{mecA} resistance determinant present in all MRSA strains may have originated from the \textit{S. sciuri pbpD} gene. The resistance cassette \textit{SCCmec} was excised from extensively studied MRSA strain COL to generate MSSA strain COL\textsubscript{S}, which was subsequently used as the recipient of a plasmid-borne copy of an upregulated \textit{mec} gene, we were able to reconstruct a typical MRSA strain which was phenotypically indistinguishable from original MRSA strain COL. These results provide further support for the proposition that the wide-spectrum \(\beta\)-lactam resistance determinant \textit{mecA} carried by all MRSA strains has evolved from the \textit{S. sciuri pbpD} gene.

**REFERENCES**


