ORIGINAL ARTICLE

Genotypes and phenotypes of *Staphylococcus lugdunensis* isolates recovered from bacteremia

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**KEYWORDS**

Accessory gene regulator typing; Bacteremia; Biofilm; SCCmec; *Staphylococcus lugdunensis*

**Background:** *Staphylococcus lugdunensis* is a member of coagulase-negative staphylococci, which has the potential to cause serious infections, such as endocarditis, bone and joint infections, and septicemia. Differences in phenotypic/genotypic characterization may be linked to different diseases.

**Methods:** Genotypes of 11 *S. lugdunensis* isolates from bacteremia were determined by pulsed field gel electrophoresis and accessory gene regulator (*agr*) typing. The SCCmec elements in two oxacillin-resistant isolates were sequenced. Phenotypes were tested by antimicrobial susceptibility testing, biofilm formation assessments, and virulence factor analysis (hemolytic and protease activities).

**Results:** Among the 11 isolates, six pulsotypes were found, and seven isolates belonged to two major pulsotypes. Two *agr* types (*agr-1* or *agr-2*) were found. The 11 isolates were susceptible to most antimicrobial agents tested. The SCCmec elements in two oxacillin-resistant...
isolates belonged to the SCCmec type V, but with additional ccrAB2 genes. The agr-2sl isolates (n = 7) displayed higher hemolytic and protease activities than the agr-1sl isolates. All isolates contained the icaA gene but with variable biofilm activities. The results suggest that protein might play an important part in S. lugdunensis biofilms, possibly through an ica-independent pathway. Of the 11 patients with S. lugdunensis bacteremia, one patient had a community-onset infection, and others had a hospital-acquired infection, which were mostly central venous catheter-related infections.

Conclusion: The 11 S. lugdunensis bacteremia isolates displayed various genotypes and phenotypes. Two oxacillin-resistant isolates contained SCCmec type V and carried additional ccrAB2 genes. Correlation of genotypes and phenotypes with infections needs further studies.

Materials and methods

Bacterial isolates and patients

Eleven S. lugdunensis isolates causing bacteremia were recovered from blood cultures between 2007 and 2008 in the Bacteriology Laboratory of the National Taiwan University Hospital, a university hospital with 2500 beds that is located in Northern Taiwan. S. lugdunensis was identified by BD Phoenix automated microbiology system (Becton-Dickinson Diagnostic Systems, Sparks, MD, USA) and 16S ribosomal RNA gene sequencing. Clinically significant bacteremia was defined as two or more sets of positive blood cultures or the coexistence of positive blood and central venous catheter (CVC) cultures.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by both disk diffusion and agar dilution according to the guidelines of the Clinical and Laboratory Standards Institute. The antimicrobial agents tested were oxacillin, fusidic acid, gentamicin, clindamycin, erythromycin, teicoplanin, minocycline, trimethoprim/sulfamethoxazole, and vancomycin.

agr typing

The agr types were determined by polymerase chain reaction (PCR; 3’ end of agrB to 5’ end of agrC) and sequencing. Because the primers of AGR-1 and AGR-2, which were designed for S. aureus, did not completely match with the agr sequence of S. lugdunensis, we redesigned the primers SLagrF (5’-TGTGGCATTCATCTGTC-3’) and SLagrR (5’-CTTGGATGGCGACCACTT-3’), based on the sequence from GenBank accession number AF173933.1, to obtain a 1333-bp amplification product.

Hemolytic activity

The hemolytic activity assay used in this study was slightly modified from the method reported by Majerczyk et al. Bacteria were grown in 20-mL tryptic soy broth (TSB) medium to an initial optical density at a wavelength of 600 (OD600) of 0.05 and incubated at 37°C in a shaker at 240 rpm. After 3, 6, 7, 8, and 10 hours, 0.5 mL of each

Introduction

Staphylococcus lugdunensis, first described in 1988, is a member of the coagulase-negative staphylococci (CoNS) and has been recognized as an important human pathogen. In contrast to other CoNS, this microorganism is very similar to S. aureus and causes serious infections such as endocarditis, bone and joint infections, and septicemia. Unlike other CoNS, S. lugdunensis is generally susceptible to many antimicrobial agents. Although the resistance of S. lugdunensis to meticillin (mecA positive) has been reported previously, only limited reports on the SCCmec structure of S. lugdunensis are available. The staphylococcal accessory gene regulator (agr) locus is a quorum-sensing system that modulates virulence factors in S. aureus. This locus encodes two divergent transcripts (RNAlI and RNAlII), which are regulated by the promoters P2 and P3, respectively. Two agr types (agr-1sl and agr-2sl) have been identified in S. lugdunensis, and the agr-like locus (which has been named agr-1sl) was correlated with hemolytic activity. However, the correlation between the agr type and other virulence factors remains unknown.

Biofilm activity has been studied in S. lugdunensis isolates. Although the icaABCD-encoded poly-N-acetylglucosamine (PNAG) is a major component of biofilm development in S. aureus and S. epidermidis, several studies revealed that the biofilm formation of S. lugdunensis was mediated through the ica-independent pathway. Previous studies indicated that the S. lugdunensis biofilm consists of proteins, extracellular teichoic acids, and glucosamine but lacks dominant PNAG. The biofilms were sensitive to proteinase K but not degraded by metaperiodate (a PNAG-degrading agent), which suggests that proteins may be the major component of biofilm formation in S. lugdunensis.

In this study, we examined the genotypes [pulsed field gel electrophoresis (PFGE) and agr typing] and phenotypes of 11 S. lugdunensis isolates causing bacteremia. The phenotypic characterizations were antimicrobial susceptibility testing, biofilm formation assessments, and virulence factor analyses (hemolytic and protease activities). The agr-2sl isolates displayed higher hemolytic and protease activities than the agr-1sl isolates. The SCCmec structure of two oxacillin-resistant S. lugdunensis isolates was also determined.
bacterial culture was collected. An equal volume of 2% rabbit RBCs (rRBCs) in phosphate-buffered saline (PBS) was added to each supernatant in 96-well V-bottom plates (Costar, Corning, Inc.) and incubated at 37°C for 15 minutes. The rRBCs were pelleted at 1000 rpm for 5 minutes at 4°C, and the supernatant fluid was measured by OD_{540}. The PBS plus 0.2% bovine serum albumin was used as a negative control, and 1% Triton X-100 was used as a positive control. The percentage of hemolytic activity was calculated using the linear interpolation method \((\text{test}_{OD540} - \text{negative control}_{OD540}) / (\text{positive control}_{OD540} - \text{negative control}_{OD540})\). Experiments were performed in triplicate.

Protease and lipase activities

The protease and lipase activities were determined by the plate methods.\(^{23,24}\) In brief, wells were dug into skim milk and salt-Tween agar plates with a 6-mm tip for a 200-μL pipette tip. Thirty microliters of bacterial suspension (10⁸ colony forming units/mL) from clinical isolates were placed into each well. The plates were incubated at 37°C for 48 hours. The diameters of the hydrolyzed zone were measured and verified for each well. Experiments were performed in triplicate.

Biofilm assay

Biofilm formation was assayed by microtiter plate assay.\(^{15}\) The crystal violet assay was used for the detection of PNAG production. Bacterial isolates were incubated on the Congo red plate, incubated at 37°C for 24 hours, and kept at room temperature overnight. Black colonies indicate the production of PNAG, whereas red colonies indicated no PNAG production. \(S.\) epidermidis RP62A was used as a positive control and experiments were performed in triplicate.

Determination of the SCCmec structure

The SCCmec types of the two oxacillin-resistant isolates were initially determined by examination of the mec complex and type of ccr genes using previously described methods.\(^{25}\) The entire sequence of SCCmec cassette was determined using a long and accurate PCR in vitro cloning strategy (Takara Shuzo Co. Ltd., Japan), which has been previously described.\(^{24}\)

PFGE

The PFGE typing of Smal (New England Biolabs, Ipswich, MA, USA)-digested DNA was prepared in accordance with previously described methods.\(^{23,25}\) The PFGE typing was performed by applying an electric voltage of 200 V at 13°C for 20 hours, with pulse times ranging from 5.3 to 34.9 seconds at 6 V/cm.\(^{26}\) Dice similarity indices were used to construct the dendrogram of pulsotype relationships through the unweighted pair group method with arithmetic mean. Pulsotypes were assigned to the same clusters if they exhibited 85% similarity in the dendrogram.

Southern blot hybridization assay

The DNA of an oxacillin-resistant isolate, NTUH-4179, was digested with Smal, Sall, PstI, and XbaI before the PFGE analysis. The PFGE gel was then subjected to Southern blotting and was hybridized with three digoxigenin-labeled probes, meCA, ccrA2B2, or 16S recombinant DNA. The hybridization and detection were performed in accordance with previously described methods.\(^{24,26}\)

Statistical analysis

The SPSS 12.0 program was used for the statistical analysis. A nonparametric test (Mann–Whitney U test) was used to test the difference between the two \(agr\) types with respect to virulence factors and biofilm formation under environmental stress. A \(p\) value < 0.05 was taken to be significant.

Nucleotide sequence accession numbers

The SCCmec type V sequence from \(S.\) lugdunensis NTUH-4179 was deposited in GenBank under the accession number JX914566.

Ethics statement

This study was approved by the Institutional Review Board of National Taiwan University Hospital, Taipei, Taiwan (201308048RIN).

Results

Genotyping

By PFGE analysis, 11 isolates were grouped into six pulsotypes. Seven isolates belonged to two major pulsotypes (D and F; Fig. 1). Two subtypes were found in pulsotypes D and F. By \(agr\) typing, \(agr\)-I, \(N = 4\) and \(agr\)-II, \(N = 7\) were identified. Isolates with the same \(agr\) types were clustered together by PFGE analysis (Fig. 1).

Antimicrobial susceptibility

All of the 11 isolates were susceptible to clindamycin, erythromycin, minocycline, teicoplanin, trimethoprim/sulfamethoxazole, and vancomycin. Three isolates within pulsotype E displayed different susceptibilities to oxacillin and gentamicin. NTUH-1748-3 (F1) showed susceptibility to all of the tested antibiotics, whereas NTUH-4179 (F1) and NTUH-6767 (F2) were resistant to both oxacillin [minimum
inhibitory concentration (MIC) = 8 μg/mL and gentamicin (MIC = 32 μg/mL). One isolate (NTUH-5183) was resistant to fusidic acid (MIC > 128 μg/mL).

Hemolytic and protease activities

Hemolytic and protease activities represent bacterial virulence factors and are known to be regulated by the agr quorum-sensing system in S. aureus.12 The 11 isolates of S. lugdunensis showed different levels of hemolytic and protease activities. The hemolytic activities were low at 3 hours of incubation, but increased with longer culture time (8–10 hours; Fig. 2). At 7, 8, and 10 hours of incubation, the hemolytic activities were higher in isolates of the agr-II type (7 isolates) than in agr-I type (p < 0.05 by the Mann–Whitney U test). For protease activities, nine isolates (2 agr-I and 7 agr-II) showed stronger protease activities (Fig. 2). Two isolates, NTUH-1901 and NTUH-7271 (both of which were agr-I), had very low protease activities.

Biofilm formation

By microtiter plate assay, the isolates displayed variable biofilm activities (Fig. 3A). Among the isolates, NTUH-2013 exhibited the highest biofilm formation (OD570: 3.03 ± 0.18), whereas NTUH-6767 and NTUH-4219 had the lowest biofilm formation (OD570: 1.10 ± 0.47 and 1.20 ± 0.56, respectively). All isolates contained the icaA gene, but none produced PNAG, which was confirmed by the Congo red agar plate method.

Furthermore, the impact of environmental factors on biofilm formation with different concentrations of glucose and sodium chloride was also tested. Biofilm formation in agr-I type isolates of S. lugdunensis was reduced when the medium contained increased concentration of sodium chloride, whereas the agr-II type isolates did not follow this trend (Fig. 3B). Increased concentrations of glucose (from 0.5 to 4%) did not affect the biofilm formation (data not shown).
To gain additional insight into the formation of biofilms in S. lugdunensis, detachment assay with sodium metaperiodate and proteinase K was performed. The degradation of PNAG-producing biofilms by sodium metaperiodate has been reported previously. A PNAG-producing S. epidermidis RP62A was used as a control. Treatment with metaperiodate showed few effects on the biofilm formations of S. lugdunensis isolates (Fig. 3C), whereas the detachment assay with proteinase K indicated that the S. lugdunensis biofilm contained proteins (Fig. 3D) (*p < 0.05 by the Mann-Whitney U test). Each assay was performed in triplicate.

The SCCmec structures in two oxacillin-resistant S. lugdunensis isolates

The SCCmec types in two oxacillin-resistant S. lugdunensis isolates (NTUH-4179 and NTUH-6767) were determined by PCR and sequencing. These two isolates had a class 2 mec gene complex (meca-ΔmecRF1-15431) and two ccr genes, ccrC, which were most closely related to the pseudo ccrC gene (open reading frames (ORFs) numbers CG106 and CG107) of SCCmec III (GenBank accession number AB047089) and the ccrAB2 locus (Fig. 4). Sequence analysis of the SCCmec element revealed that the structure is very similar to SCCmec type V (GenBank accession number AB121219.1) in S. aureus (Fig. 4). However, ORF V013 (372 bp) and ORF V014 (1620 bp) were only 72% and 79% identical to those of SCCmec type V in S. aureus, respectively. A database search indicated that ORF V013 and ORF V014 showed highest identity to HMPREF0789_1700 (99%) and HMPREF0789_1701 (99%) in the shotgun library of S. epidermidis BCM-HMP0060.

To further identify the location of ccrAB2, DNA digested with SmaI, SalI, PstI, and XbaI was subjected to PFGE and hybridized with meca, ccrAB2, and 16S rRNA gene probes, respectively (Fig. 5). The meca and ccrAB2 probes were hybridized to different bands, whereas the ccrAB2 was hybridized to a band also by using 16S rRNA as probe (Fig. 5). This result suggests that ccrAB2 was not located inside the structure of the SCCmec V cassette (NTUH-4179 SCCmec). Thus, the SCCmec type of these two isolates was still identified as type V.

Clinical characterization of S. lugdunensis bacteremia

The clinical characteristics of 11 patients with S. lugdunensis bacteremia are shown in Table 1. Among 11 patients, only one had a community-onset infection. The sex
distribution of these patients was nearly equal. Patients were all older than 45 years of age, and all had fever during *S. lugdunensis* bacteremia. The most possible source of infection was CVC, either with an implanted port or hemolysis catheter. Malignancy and end-stage renal disease (ESRD) were the two leading underlying diseases. Among the four ESRD patients, three were associated with double-lumen CVC. An examination of the echocardiography results showed that one patient (number 2013) had vegetation, who was diagnosed as having infective endocarditis according to the Duke criteria. Patients were receiving various antibiotics for treatment. Among them, vancomycin was the commonly used antibiotic. Six of the 11 patients recovered.

**Figure 4.** Comparison of the SCCmec cassette structures from *S. lugdunensis* NTUH-4179 with SCCmec type V (Accession no. AB121219.1) and SCCmec type IV (Accession no. AB063172). Black rectangle indicates IS431; gray arrows indicate orfX, mecA, and ccrC genes; and white arrows show hypothetical genes.

**Figure 5.** Hybridization of *S. lugdunensis* NTUH-4179 DNA with mecA, ccrA2B2, and 16S rRNA gene probes after PFGE. (A) PFGE of Smal-, SalI-, PstI-, and XbaI-digested chromosomal DNA of *S. lugdunensis* NTUH-4179. Lane M, DNA size marker (lambda DNA ladder). (B) Hybridization with a mecA probe. (C) Hybridization with a ccrA2B2 probe. (D) Hybridization with a *S. lugdunensis* 16S rRNA gene probe.
Table 1  Clinical characteristics of 11 patients with *Staphylococcus lugdunensis* bacteremia

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age/sex</th>
<th>Acquisition of infection</th>
<th>Possible source of entry</th>
<th>Major medical history</th>
<th>Echocardiography: vegetation</th>
<th>Treatment (duration, days)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>7271</td>
<td>62/F</td>
<td>Hospital</td>
<td>CVC (implanted port)</td>
<td>Diffuse large B-cell lymphoma ESRD</td>
<td>No vegetation</td>
<td>VA (3); OX (8); GM (8); CAZ (4); VA (4)</td>
<td>Recovered (30 hosp. days)</td>
</tr>
<tr>
<td>2894</td>
<td>45/F</td>
<td>Hospital</td>
<td>CVC (hemodialysis catheter)</td>
<td>ESRD</td>
<td>ND</td>
<td>AMO/CLA (3)</td>
<td>Death (3 hosp. days, acute heart failure, bacteremia by <em>S. lugdunensis</em>)</td>
</tr>
<tr>
<td>6749</td>
<td>49/F</td>
<td>Health care</td>
<td>CVC (implanted port)</td>
<td>Cervical cancer, hepatitis B carrier, Esophageal cancer, multiple liver, and lymph node metastasis</td>
<td>ND</td>
<td>VA (14); FEP (12); AMK (8); FLC (3); AmB (4)</td>
<td>Death (88 hosp. days, fungemia by <em>Candida tropicalis</em>)</td>
</tr>
<tr>
<td>1708</td>
<td>50/M</td>
<td>Hospital</td>
<td>CVC (implanted port)</td>
<td>Probable left ventricular diastolic dysfunction, MR and PR mild</td>
<td>VA (14); FEP (12); AMK (8); FLC (3); AmB (4)</td>
<td>Recovered (34 hosp. days)</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>49/M</td>
<td>Community</td>
<td>Infective endocarditis</td>
<td>Mitral valve prolapsed ESRD</td>
<td>Vegetation (+), MR moderate</td>
<td>Ox (31); GM (14); VA (4)</td>
<td>Recovered (34 hosp. days)</td>
</tr>
<tr>
<td>4219</td>
<td>54/F</td>
<td>Hospital</td>
<td>CVC (hemodialysis catheter)</td>
<td>AR and MR mild, TR moderate</td>
<td>VA (14); CAZ (7); FEP (4); LNZ (4)</td>
<td>Death (291 hosp. days, bacteremia by <em>P. aeruginosa</em>)</td>
<td></td>
</tr>
<tr>
<td>1901</td>
<td>81/F</td>
<td>Hospital</td>
<td>CVC (hemodialysis catheter)</td>
<td>ESRD, congestive heart failure</td>
<td>VA (14); CAZ (7); FEP (4); LNZ (4)</td>
<td>Death (291 hosp. days, bacteremia by <em>P. aeruginosa</em>)</td>
<td></td>
</tr>
<tr>
<td>1748-3</td>
<td>65/M</td>
<td>Hospital</td>
<td>CVC (implanted port)</td>
<td>Liver cirrhosis, pancreatic cancer CAD (2-vessel disease)</td>
<td>No vegetation</td>
<td>CAZ (3); CIP (14); VA (10)</td>
<td>Recovered (61 hosp. days)</td>
</tr>
<tr>
<td>6767</td>
<td>85/M</td>
<td>Health-care</td>
<td>CVC (hemodialysis catheter)</td>
<td>CAD, ESRD, hepatitis B carrier</td>
<td>No vegetation</td>
<td>VA (9)</td>
<td>Recovered (9 hosp. days)</td>
</tr>
<tr>
<td>4179</td>
<td>73/M</td>
<td>Hospital</td>
<td>Unknown</td>
<td>Hepatocellular carcinoma, hepatitis C carrier</td>
<td>No vegetation</td>
<td>VA (14); CMZ (10); CTX (6)</td>
<td>Death (34 hosp. days, multiple organ failure)</td>
</tr>
<tr>
<td>5183</td>
<td>64/M</td>
<td>Hospital</td>
<td>CVC (hemodialysis catheter)</td>
<td>Diabetes mellitus, hepatocellular carcinoma, hepatitis C carrier</td>
<td>ND</td>
<td>CAZ (5); PIP/TAZ (8); VA (8)</td>
<td>Death (126 hosp. days, acute renal failure)</td>
</tr>
</tbody>
</table>

AmB = Amphotericin B; AMK = amikacin; AMO/CLA = amoxicillin/clavulanic acid; AR = aortic regurgitation; CAD = coronary artery disease; CAZ = ceftazidime; CIP = ciprofloxacin; CMZ = cefmetazole; CTX = ceftriaxone; CVC = central venous catheter; ESRD = end-stage renal disease; FEP = cefepime; FLC = fluconazole; GM = gentamicin; hosp. days = hospitalization days; LNZ = linezolid; MR = mitral regurgitation; ND = not done; OX = oxacillin; PIP/TAZ = piperacillin/tazobactam; PR = pulmonic regurgitation; TR = tricuspid regurgitation; VA = vancomycin.
Discussion

Genotyping of 11 S. lugdunensis isolates by PFGE and agr type revealed low level of genetic heterogeneity, which is consistent with previous reports. In addition, the PFGE analysis revealed the clustering of isolates with the same agr type. It is well known that many virulence factors in S. aureus are regulated by the cell-density-sensing agr system. In S. lugdunensis, it has been reported that delta-like hemo-
lysin (S. lugdunensis synergetic hemolysin) was produced and regulated by the agr locus, causing a narrow but complete zone of hemolysis on blood agar. 

In this study, isolates with agr-II exhibited higher overall hemo-
lytic activity than isolates with agr-I (p < 0.05 by the Mann–Whitney U test). However, further study is needed to obtain a clearer understanding about the regulation of hemolytic activity by agr in S. lugdunensis.

Biofilm formation is considered as one of the virulence factors of bacteria. Our results showed that high concentrations of sodium chloride decreased the biofilm formation by an ica-independent pathway (Fig. 3B), which is in agreement with the findings for ica-independent isolates of S. aureus, S. epidermidis, and S. lugdunensis. Besides, biofilm formation in S. lugdunensis was sensitive to proteinase K but not degraded by metaperiodate, a PNAG-
degrading agent. Similar results have been reported in S. haemolyticus isolates, showing that the detachment of biofilms was 98% by proteinase K, 100% by DNase, and 38% by metaperiodate. These data indicate that protein, DNA, and teichoic acid may all possibly contribute to ica-
dependent biofilm formation in Staphylococcus species.

In agreement with other reports, our S. lugdunensis isolates were found to be susceptible to most antimicrobial agents. However, two isolates were resistant to oxacillin (containing mecA) and gentamicin, and one isolate was resistant to fusidic acid (Fig. 1). The mecA-positive S. lugdunensis and their SCCmec type have previously been reported, but the entire SCCmec structure has not been reported yet. In the present study, the nucleotide sequence of ccr (1678 bp) in NTUH-4179 showed highest identity (99.3%) to a pseudo ccrC gene (ORFs numbers CG106 and CG107) of SCCmec III (GenBank accession number AB047089), 98.5% identity to ccrC10 of SCCmec V (GenBank accession number GQ902038), and 97.3% identity to ccrC (GenBank accession number AB478780, SCCmec V), respectively. Based on the structure of mecA complex and ccrC gene sequence, the SCCmec elements in two methicillin-resistant S. lugdunensis (NTUH-4179 and 6767) were classified as SCCmec V, but differed from other typical type V by containing an additional ccrAB2 locus located on an unknown site (Fig. 4). Staphyloccocal species carrying multiple ccr genes and/or SCC elements have been previously reported. Different combinations of ccrAB2 and mec complex C1 variants (including ccrC3, ccrC6, or ccrC7) have been detected in S. haemolyticus. Eight S. epidermidis isolates from Algeria, Moldova, and Cambodia contained two ccr loci (ccrAB2 and ccrC) and mec complex C2. In addition, a community-acquired methicillin-resis-
tant S. aureus isolate that was recovered from a skin infection in Taiwan contained SCCmec IV with an additional ccrC2 locus. Although these reports suggested the presence of new SCCmec types, the exact location of additional ccr loci in the chromosome remains undetermined.

Similar to other reports, S. lugdunensis bacteremia in this study includes both community-onset and hospital-
acquired cases. Most (8/9) patients with clinically signifi-
cant S. lugdunensis bacteremia had hospital-acquired infection. Only one patient with endocarditis had community-onset infection. However, in contrast to our finding, a recent report from Southern Taiwan revealed that 84% of isolates (41/49) were community acquired. The reason for this discrepancy is unclear, but may partly be due to different sources of specimens that were analyzed (only blood culture isolates were analyzed in our study, compared with analysis of all specimens in the previous study). In this study, eight patients were all associated with CVC-related infection, suggesting that we should devote more attention to the appropriate treatment of catheter-insertion sites.

In conclusion, the 11 S. lugdunensis isolates displayed various genotypes and phenotypes. The seven agr-II isolates had stronger hemolytic and protease activities than agr-I isolates. All isolates showed biofilm formation, possibly by ica-independent pathway. Two oxacillin-resistant isolates (NTUH-4179 and 6767) contained SCCmec V structure, but carried an additional ccrAB2 locus. Because of the limited cases analyzed, the correlation of genotypes and pheno-
types with infections needs further study.

Conflicts of interest

All authors report no conflicts of interest relevant to this article.

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