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ORIGINAL ARTICLE

# Emergence of oxacillin-resistant *Staphylococcus lugdunensis* carrying staphylococcal cassette chromosome *mec* type V in central Taiwan



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## KEYWORDS

coagulase-negative  
staphylococcus;  
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SCC*mec* gene;  
*Staphylococcus*  
*lugdunensis*

**Background:** *Staphylococcus lugdunensis* has emerged as a key pathogen for clinical infection. It is sensitive to most antistaphylococcal agents, but it is increasingly resistant to  $\beta$ -lactam antibiotics. Oxacillin-resistant *S. lugdunensis* isolates carrying the *mecA* gene pose a major concern for therapy failure.

**Methods:** To assess the epidemiology and presence of *mecA* in *S. lugdunensis*, we gauged the prevalence and antibiotic resistance of *S. lugdunensis* in clinical specimens by using multiplex polymerase chain reaction (PCR) and pulsed-field gel electrophoresis.

**Results:** Thirty *S. lugdunensis* isolates were collected and examined between October 2009 and December 2010. The resistance to penicillin (87%) and oxacillin (20%) was noted. All oxacillin-resistant isolates (6/30) had type V or V<sub>T</sub> SCC*mec*. Most (67%, 4/6) isolates carried SCC*mec* type V. These organisms caused invasive infections such as peritonitis, osteomyelitis,

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and septic arthritis. Pulsed-field gel electrophoresis analyses showed most (83%, 5/6) isolates carrying *mecA* were pulsotype D with high similarity (93.8%).

**Conclusions:** The findings suggest oxacillin-resistant *S. lugdunensis* carrying SCCmec type V is emerging in central Taiwan.

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## Introduction

*Staphylococcus lugdunensis* is a member of the coagulase-negative staphylococci (CoNS). In 1988, it was first described by Freney et al<sup>1</sup> as a resident flora of the human skin, notably in the perineum and breast areas.<sup>2</sup> Similar to *Staphylococcus aureus*, it causes skin and soft tissue infection,<sup>3</sup> lymphadenitis,<sup>4</sup> osteomyelitis and prosthetic joint infection,<sup>5</sup> endophthalmitis,<sup>6</sup> peritonitis, brain abscess, urinary tract infection,<sup>7</sup> meningitis, pacemaker infections,<sup>8</sup> and endocarditis.<sup>2,9,10</sup> *S. lugdunensis* infection may be community-acquired or hospital-acquired.<sup>11</sup>

*S. lugdunensis* differs from other CoNS species because of its ornithine decarboxylase (ODC) activity. It is sensitive to most antistaphylococcal antibiotics, but 12–53% *S. lugdunensis* isolates are resistant to  $\beta$ -lactam antibiotics such as penicillin and oxacillin.<sup>9,11–14</sup> In 2003, oxacillin-resistant *S. lugdunensis* isolates manifested the *mecA* gene. The staphylococcal cassette chromosome *mec* (SCCmec), which is a movable genetic element, can be horizontally transmitted to different *Staphylococcus* species such as the CoNS and make them resistant to methicillin. This finding raised the importance of *S. lugdunensis* infection: therapy for the infection must change accordingly.<sup>15</sup> Few studies have previously demonstrated the clinical significance of *S. lugdunensis* carrying the SCCmec gene. In 2013, Tseng et al<sup>16</sup> reported that two oxacillin-resistant isolates contained SCCmec type V. In 2014 in Taiwan, Lin et al<sup>17</sup> demonstrated that eight of 10 isolates with SCCmec type V were resistant to oxacillin. Most strains were isolated from hospital-acquired or health care-associated sources. The type V strains were the most prevalent multiple-resistant, community-acquired, methicillin-resistant *Staphylococcus aureus* (MRSA) strains in Taiwan.<sup>18</sup> To clarify the importance of *S. lugdunensis* carrying SCCmec, we examined the prevalence and antibiotic resistance profile of *S. lugdunensis* among clinical specimens. We also used multiplex polymerase chain reaction (PCR) and pulsed-field gel electrophoresis (PFGE) to determine presence of SCCmec in *S. lugdunensis* isolates and epidemiology in Taiwan.

## Materials and methods

### Bacterial isolates and phenotypic identification

From October 2009 to December 2010, 30 *S. lugdunensis* isolates from 29 patients' specimens submitted for bacterial culture and confirmed as *S. lugdunensis* were collected from the Department of Laboratory Medicine at the China

Medical University Hospital (CMUH) in Taichung, Taiwan. All bacteria were cultured on 5% sheep blood plates for 24-hour growth at 37°C in 5% carbon dioxide (CO<sub>2</sub>) atmosphere. Gram stain, catalase, slide coagulase test, and clumping factor test (Staphaurex; Remel, Lenexa, KS, USA) were performed to confirm the colonies as CoNS. If the results were inconsistent, the tube coagulase test was performed as the gold standard identification method. The CoNS with  $\beta$ -hemolysis on blood agar were detected by the ornithine decarboxylase (ODC) test. The ODC-positive strains were identified using the BD Phoenix system (Becton Dickinson Diagnostic Systems, Sparks, MD, USA).

### Genotypic identification of *S. lugdunensis*

Chromosomal DNA of *S. lugdunensis* isolates was isolated by the UltraClean DNA extraction kit (MoBio, Solana Beach, CA) in accordance with the manufacturer's instructions. We used the *tanA* and *fbl* genes as the species-specific target for *S. lugdunensis* identification. These primers detected *tanA* and *fbl* genes by PCR: *tanA*-forward (5'-AGCATGGG CAATAACAGCAGTAA-3'), *tanA*-reverse (5'-GCTGCGCCA AT TTGTTCTAAATAT-3'); *fbl*-forward (5'-GAAGCAACAACGCA-GAAC AA-3') and *fbl*-reverse (5'-TGCT TGTGCCTCGCTAT TTA-3'). As previously described, PCR amplifies a 239-bp fragment of *tanA* and a 63-bp fragment of *fbl*.<sup>19,20</sup>

### Antimicrobial susceptibility testing

The Phoenix PMIC/ID-62 ID/AST combo panel (Becton Dickinson Diagnostic Systems, Sparks, MD, USA) was used for identification and antimicrobial susceptibility testing of the isolates. Antibiotics included erythromycin, penicillin, vancomycin, teicoplanin, tetracycline, clindamycin, linezolid, trimethoprim/sulfamethoxazole, and levofloxacin. Oxacillin susceptibility was tested by the BD Phoenix system (0.25–4  $\mu$ g/mL; Becton Dickinson Diagnostic Systems, Sparks, MD, USA) and by cefoxitin disk diffusion, based on the Clinical and Laboratory Standards Institute (CLSI) guidelines<sup>21</sup>; penicillin resistance was tested by beta-lactamase detection with the BD Phoenix system (Becton Dickinson Diagnostic Systems, Sparks, MD, USA).

### Multiplex PCR

Based on the CLSI guidelines, all isolates that had oxacillin minimum inhibitory concentration (MIC) greater than 4  $\mu$ g/mL or a cefoxitin inhibition zone less than or equal to 21 mm (an indicator of oxacillin-resistant strains) were

evaluated for presence of the *mecA* gene.<sup>21</sup> Multiplex PCR (M-PCR) for the detection and identification of SCCmec, which carries the *mecA* gene responsible for oxacillin resistance, was performed with genomic DNA from each MRSA isolate as a template, as previously described.<sup>16,22</sup> The SCCmec elements contain genes encoding cassette chromosome recombinase (*ccr*) and the *mec* complex. Fourteen primers were used for M-PCR: two primers for the detection of the *mecA* gene, four primers for the classification of the *mec* gene complex (i.e., classes A-D), and eight primers for the identification of the *ccr* types (types 1–5).<sup>22</sup> The SCCmec types V and V<sub>T</sub> of the MRSA strains were identified by comparing the M-PCR banding patterns of the isolates with the patterns of the reference strains [i.e., WIS (SCCmec type V, 325 bp)<sup>23</sup> and TSGH-17 (SCCmec type V<sub>T</sub>, 1600 bp)].<sup>18</sup>

### Pulsed-field gel electrophoresis

PulsNet standardized PFGE protocol for enterobacteria,<sup>24</sup> but with some modification,<sup>25</sup> was followed for *Sma*-digested genomic DNA samples of *S. lugdunensis*. Pulsed-field gel electrophoresis was performed on the CHEF-DR III System (Bio-Rad Laboratories, Hercules, CA) within the following parameters: switch time, 1–5 seconds for 8 hours and 3.5–45 seconds for 12 hours; 6 V/cm; 14°C; and 120° angle. Patterns were examined with Bionumerics software (Applied Maths, Kortrijk, Belgium). Isolates with sequence similarities of 80% or greater were clustered as highly genetically related.

### Clinical data of patients

The basic and clinical data of the patients were gleaned from their medical charts. *S. lugdunensis* isolated from two or more consecutive blood cultures or sterile body fluids of patients were considered clinically significant cases of *S. lugdunensis* infection. For patients with a monomicrobial surgical pus culture of *S. lugdunensis*, the clinical significance was determined in accordance with significant signs of skin or soft tissue infections. *S. lugdunensis* isolates from community-acquired infections were collected in outpatient or emergency departments or within the first two days of hospital admission; *S. lugdunensis* isolates from hospital-acquired infections were collected more than 2 days after admission.<sup>26</sup> This study was approved by the Institutional Review Board of the CMUH (Taichung, Taiwan; approval number, DMR100-IRB-188).

### Results

Between October 2009 and December 2010, a total of 633 CoNS were isolated at the CMUH. Thirty (4.7%) of 633 *S. lugdunensis* isolates from 29 patients were identified. Table 1 shows the clinical traits. Most patients were 19–65 years old. The most common comorbidities were diabetes mellitus (17%, 5/29 patients), malignancy (14%, 4/29 patients), surgery/trauma (10%, 3/29 patients), foreign body implant (21%, 6/29 patients), and hypertension (14%, 4/29 patients). *S. lugdunensis* was the only pathogen in 22 (76%) specimens and the predominant pathogen in eight

**Table 1** The characteristics of 29 patients infected with *Staphylococcus lugdunensis*

Characteristic	No. (%)
Age (y)	
0–18	3 (10%)
19–65	23 (80%)
>65	3 (10%)
Mean	42.6 y
Sex	
Male	13 (45%)
Female	16 (55%)
Comorbidities	
None	16 (55%)
Diabetes mellitus	5 (17%)
Foreign body implantation	6 (21%)
Malignancy	4 (14%)
Surgery/trauma	3 (10%)
Hypertension	4 (14%)
End-stage renal disease	2 (7%)
Chronic obstructive pulmonary disease	2 (7%)
Cirrhosis/hepatitis	1 (3%)
≥ 2 comorbidities	6 (21%)
Isolation	
Only pathogen	22 (76%)
Polymicrobial infection or colonization	7 (24%)
<i>Enterococcus faecalis</i>	3
<i>Citrobacter</i> species	2
<i>Stenotrophomonas maltophilia</i>	1
<i>Fusobacterium necrophorum</i>	1
<i>Candida albicans</i>	1
Isolation sites	
Skin and soft tissue pus	21 (72%)
Blood	3 (10%)
Urine	3 (10%)
Ascites	1 (3%)
Joint fluid	1 (3%)
Clinically significant infections	24 (83%)
Community-acquired	20 (83%)
Skin and soft tissue infection	14 (70%)
Arthritis/osteomyelitis	3 (15%)
Urine tract infection	3 (15%)
Mortality	0 (0%)
Hospital-acquired	4 (17%)
Skin and soft tissue infection	2 (50%)
Endocarditis	1 (25%)
Peritonitis	1 (25%)
Mortality	2 (50%)

specimens from seven patients with polymicrobial infections or colonization. The most common co-isolate was *Enterococcus faecalis* (3 patients). Other co-isolates included the *Citrobacter* species, *Stenotrophomonas maltophilia*, *Fusobacterium necrophorum*, and *Candida albicans*. Most isolates were from the skin and soft tissue pus (73%, 21/29 patients).

Twenty-four of 30 *S. lugdunensis* isolates caused clinical infections and were clinically significant. The remaining six isolates from routine cultures but without evidence of clinical infection were clinically insignificant. Most infections (83%, 20/24 isolates) were community-acquired;

skin and soft tissue infections (67%, 16/24 isolates) were the chief main clinical presentation. Eighty-five percent (17/20) of community-acquired *S. lugdunensis* infections were noninvasive infections and had a benign clinical course without mortality. However, one-half (50%, 2/4) of hospital-acquired infections had an invasive clinical course and the patients finally died because of persistent *S. lugdunensis* endocarditis or peritonitis.

The antimicrobial susceptibility profile of *S. lugdunensis* isolates showed no isolates were resistant to vancomycin, teicoplanin, linezolid, and levofloxacin. Twenty-six (87%) isolates were resistant to penicillin; five (17%) isolates, to erythromycin; six (20%) isolates, to tetracycline; five (17%) isolates, to clindamycin; and three (10%) isolates, to trimethoprim/sulfamethoxazole. The BD Phoenix system (Becton Dickinson Diagnostic Systems, Sparks, MD) was used to determine the MICs for oxacillin. Three isolates were 2 µg/mL and three isolates were greater than 4 µg/mL. All isolates were tested by cefoxitin disk diffusion with a cefoxitin inhibition zone less than or equal to 21 mm in diameter. The overall oxacillin resistance was 20% (6/30 isolates). Multiplex PCR assay for SCCmec typing demonstrated that all six oxacillin-resistant *S. lugdunensis* isolates (derived from five patients) had the *mecA* gene. Four isolates were SCCmec types V and two isolates were V<sub>T</sub>.

Table 2 lists the clinical characteristics of the six strains. Four of six strains were community-derived with diverse times and geographical distributions, and all patients visited different medical departments of the hospital during the therapeutic period. Oxacillin-resistant *S. lugdunensis* isolates carrying the SCCmec gene V (67%, 4/6 isolates) tended to cause invasive clinical infections (e.g., peritonitis, osteomyelitis, or septic arthritis). Fig. 1 shows the genetic linkages and *Sma*I PFGE patterns of 30 *S. lugdunensis* isolates. Among the six identified pulsotypes, two major PFGE pulsotypes—A (16 isolates) and C (6 isolates)—accounted for 22 (73%) of 30 isolates. Most (5/6) oxacillin-resistant *S. lugdunensis* isolates with *mecA* gene were pulsotype D (83%, 5/6 isolates) with similarity up to 93.8%. Most oxacillin-sensitive *S. lugdunensis* isolates were pulsotype A.

## Discussion

*S. lugdunensis* infections and *S. aureus* infections are clinically indistinguishable.<sup>14</sup> The frequency of *S. lugdunensis* isolates from CoNS varies with geographical area: 0.7% in China,<sup>27</sup> 0.8% in Korea,<sup>28</sup> 1.3% in Japan,<sup>29</sup> 3% in America,<sup>30</sup> and up to 6% in Argentina.<sup>31</sup> In 2014, Lin et al<sup>17</sup> reported a 0.87% incidence of *S. lugdunensis* in CoNS bacteremia. We

first demonstrated a 4.7% (30/633 isolates) prevalence of *S. lugdunensis* among routine clinical CoNS specimens in Taiwan; this is the highest rate in Asia. Previous experience shows it usually causes community-acquired and noninvasive infections,<sup>14</sup> but mortality is uncommon.<sup>11</sup> In our research, most (83%) community-acquired infections presented as a benign skin or soft tissue infection (70%) without mortality. However, hospital-acquired infections were more invasive and had a mortality of 50% (2/4 patients). *S. lugdunensis* should be carefully distinguished from clinical CoNS isolates to prevent underestimation; positive *S. lugdunensis* cultures should not be considered as merely a contaminant.

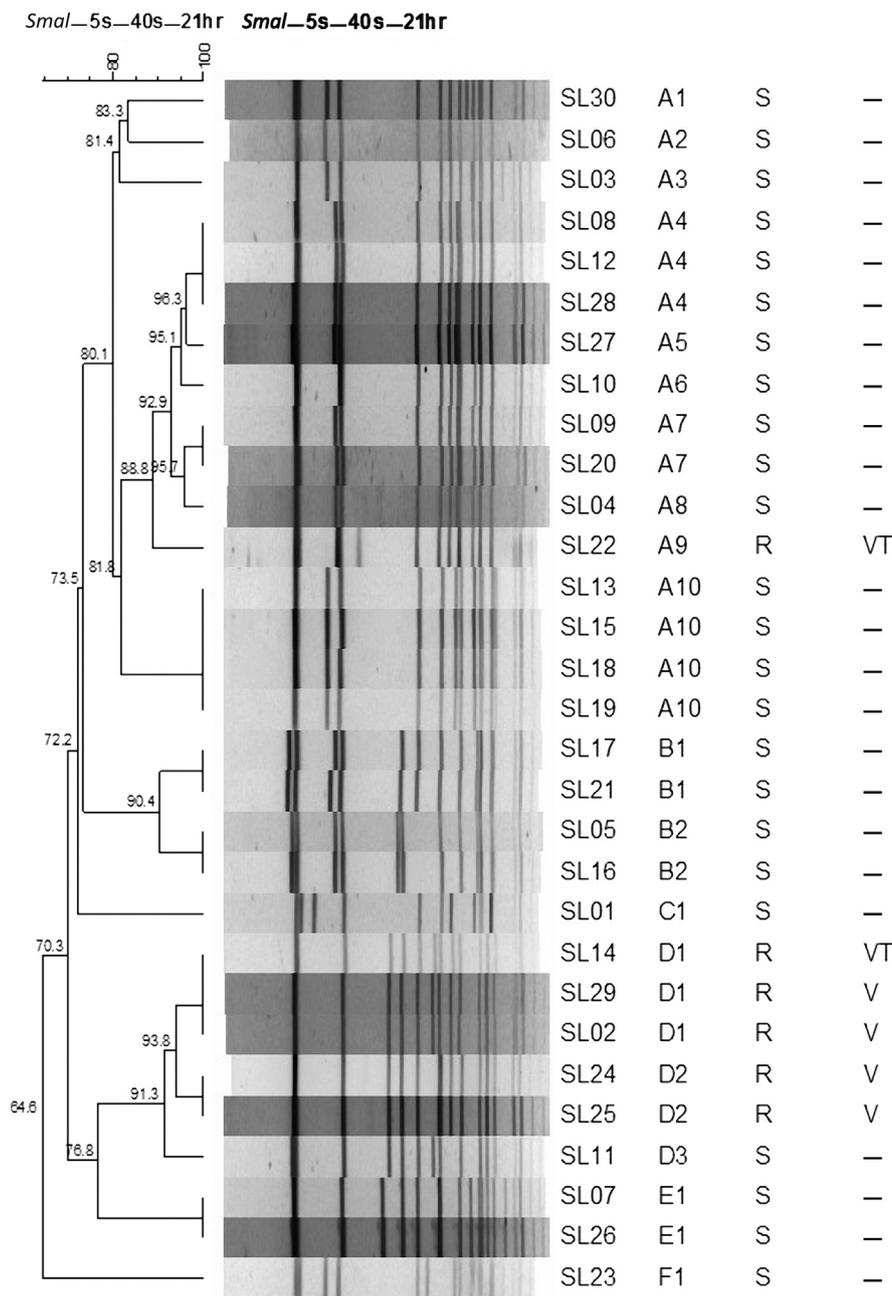
*S. lugdunensis* is generally susceptible to most antibiotics. Earlier studies recorded the penicillin resistance rate at less than 4%,<sup>32</sup> whereas more recent studies have recorded the rate at 12–27%.<sup>9,12–14</sup> Wu et al<sup>11</sup> tallied a penicillin resistance of 53% among common *S. lugdunensis* specimens. In addition, the overall oxacillin resistance rate is reportedly 0–5% in routine cultures.<sup>11,13,14,33</sup> In 2014, Lin et al<sup>17</sup> cited a penicillin resistance of 68.8%, and an oxacillin resistance of 20.8% among invasive *S. lugdunensis* infections in Taiwan.<sup>17</sup> We found 87% penicillin resistance versus 20% oxacillin resistance (6/30) from common *S. lugdunensis* specimens. Both of these resistance levels rank highest worldwide and highlight the importance of antibiotic resistance among *S. lugdunensis* infections. Therapy for infection must accordingly change.

Few studies demonstrate the clinical significance of *S. lugdunensis* carrying the SCCmec gene. In 2003, Tee et al<sup>15</sup> first reported oxacillin-resistant *S. lugdunensis* with the *mecA* gene; this microbe caused bloodstream infection in premature neonates. In 2008, Tan et al<sup>13</sup> found *S. lugdunensis* carrying the *mecA* gene from clinical isolates, clinical presentations not prescribed. In 2011, Wu et al<sup>11</sup> mentioned that nosocomial infections were caused by oxacillin-resistant *S. lugdunensis* with the *mecA* gene. In 2012, Liu et al<sup>27</sup> described one strain that caused neonatal pneumonia and septicemia in one case of infection by oxacillin-resistant *S. lugdunensis* with SCCmec type V; it resulted in fatal nosocomial bacteremia. In 2014, Lin et al<sup>17</sup> noted 10 oxacillin-resistant isolates with the *mecA* gene; eight (80%) of these isolates were type V.<sup>17</sup> All of our oxacillin-resistant isolates (20%, 6/30 isolates) had the *mecA* gene. Four isolates were type V and caused septic arthritis, osteomyelitis, or peritonitis, and one death. Two isolates were type V<sub>T</sub>. One isolate caused cellulitis. Our study showed a high incidence of oxacillin-resistant *S. lugdunensis* carrying the SCCmec gene, which emphasizes the importance of antibiotic therapies.

**Table 2** The characteristics of six oxacillin-resistant *S. lugdunensis* isolates

Patient No.	Lab ID	SCCmec type	Origin	Isolation site	Isolation date	Diagnosis	Prognosis
1	SL 14	V <sub>T</sub>	CA	Blood	10/11/2009	NS	—
2	SL 22	V <sub>T</sub>	CA	Skin pus	07/19/2010	Cellulitis	Cured
3	SL 25 and 29	V	HA	Ascites	2/23/2010 and 2/26/2010	Peritonitis	Mortality
4	SL 2	V	CA	Skin pus	03/10/2010	Osteomyelitis	Cured
5	SL 24	V	CA	Synovial fluid	04/01/2010	Septic arthritis	Cured

CA = community-acquired; HA = hospital-acquired; No. = number; NS = not significant; SL = *Staphylococcus lugdunensis*.



**Figure 1.** Pulsed-field gel electrophoresis of 30 *Staphylococcus lugdunensis* (SL 1–30) isolates; groups are designated by A–F. SL = *Staphylococcus lugdunensis*.

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing is essential for understanding the molecular epidemiology of MRSA. The SCC*mec* elements are currently classified as types I–VIII, based on the nature of the *mec* and *ccr* gene complexes; these types are further divided into subtypes, based on their junkyard region DNA segments.<sup>34</sup>

The SCC*mec* element is a movable genetic element that is horizontally transmitted to *Staphylococcus* species such as CoNS. This element makes *Staphylococcus* species resistant to methicillin. We believe the *mecA* gene is horizontally transmitted from *S. aureus* to other *S. lugdunensis* strains.

Our study detected six oxacillin-resistant *S. lugdunensis* strains that carried SCC*mec*; four strains belonged to type V. Pulsed-field gel electrophoresis showed most (83%, 5/6) isolates with *mecA* were pulsotype D with high similarity (93.8%) and one isolate was SCC*mec* type V<sub>T</sub>. All patients visited different medical departments of the hospital during the therapeutic period. The strains were isolated at diverse times and geographical distributions. Therefore, clonal spreading in hospital is less likely. Types V and V<sub>T</sub> were the most prevalent multiple-resistant community-acquired MRSA strains; furthermore, SCC*mec* V<sub>T</sub> was a specific SCC*mec* variant in Taiwan.<sup>18</sup> The high incidence of oxacillin-resistant *S. lugdunensis* with SCC*mec* type V noted by Lin et al<sup>17</sup> and in

our study indicate the regional spread of *S. lugdunensis* isolates with the *mecA* gene in Taiwan.

In conclusion, *S. lugdunensis* is a key organism in community and hospital-acquired infections with a 4.7% prevalence among routine clinical CoNS isolates in Taiwan. We tallied higher penicillin resistance (87%) and oxacillin resistance (20%). Six (20%, 6/30) oxacillin-resistant isolates had *mecA* gene of type V or type V<sub>T</sub> that caused invasive clinical infection (67%, 4/6 patients). The PFGE test indicated that most isolates with *mecA* were pulsotype D (83.3%, 5/6) with high similarity (93.8%). This suggests that oxacillin-resistant *S. lugdunensis* with SCCmec type V is emerging in central Taiwan.

## Conflicts of interest

The authors have no conflicts of interest to declare.

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