Supplementary Material

# Methods

1.1. Antimicrobial susceptibility test

Minimum inhibitory concentrations (MICs) of antibiotics were determined using the VITEK2 Compact (Biomerieux, France) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (M100S, 30th edition)[1]. The six strains were tested by MIC determination for oxacillin using broth microdilution method. MIC results were interpreted according to the CLSI (susceptible ≤2μg/mL, resistant ≥4μg/mL) Cefoxitin disc diffusion (DD) tests were performed as described by the CLSI using Muller-Hinton (MH) agar from Becton Dickinson (BD, USA). Cefoxitin DD test results were interpreted according to the CLSI (inhibition zone diameter, susceptible ≥22 mm, resistant ≤21 mm) [1]. An in-house Muller-Hinton (BD, USA) agar supplemented with 2% sodium chloride and 2 μg/mL oxacillin was used. Absence and presence of growth (>1 colony or thin film) was interpreted as indicating susceptibility and resistance, respectively[1,2]. *S. aureus* ATCC 29213 and ATCC 43300 were included for quality control.

1.2. Genomic DNA extraction and sequencing

The strains were cultured on cation adjusted Mueller-Hinton Broth (BD, USA) for 6–8 h at 37°C, and genomic DNA was extracted using a Magnetic Universal Genomic DNA Kit (Tiangen DP705; Tiangen, China) with an additional incubation step with lysozyme (50 mg/mL) at 37°C for 1 h. The harvested DNA was detected using agarose gel electrophoresis and a Nanodrop instrument (Thermo Scientific, USA).

For next-generation sequencing, the sequencing library was prepared using a NEBNext Ultra DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer’s recommendations, and index codes were added to attribute sequences for each sample, with an average insert size of 350 bp. Paired-end reads of 150 bp were generated using the Illumina PE150 platform at Beijing Novagene Bioinformatics Technology Co., Ltd. Raw reads were filtered to remove low-quality reads (< Q20) using fastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). To complete the genome, we performed Oxford Nanopore sequencing on a MinION platform. The sequencing library was constructed using a rapid PCR barcoding kit (SQK-RPB004; ONT). The raw reads were filtered using Nanoplot software to obtain high-quality (≥Q7) reads. The genome sequence was obtained with a hybrid de novo assembly using Illumina short reads and ONT long reads with Unicycler software [3].

1.3. Genome annotations

Prokka was used for gene prediction and annotation [4]. Resistance genes were identified using the Comprehensive Antibiotic Resistance Database (CARD) 3.2.4, with parameters of perfect and strict hits only, exclude nudge, and high quality/coverage [5]. Secondary metabolism gene clusters were analyzed using antiSMASH, with relaxed strictness [6]. Multilocus sequence typing (MLST) [7] was performed by depositing the complete genome sequence of strains on the Center for Genomic Epidemiology (CGE) website with default parameters (<https://cge.cbs.dtu.dk/services/MLST/>). The BURST program (http://www.mlst.net) was used to define clonal complexes (CCs; groups in which every isolate shares at least five identical alleles with at least one other isolate)[8]. SCC*mec* typing of MRSL isolates was carried out using SCCmecFinder 1.2 [9,10] on the CGE website (https://cge.cbs.dtu.dk/services/SCCmecFinder/). We used EasyFig 2.2.5 [11] to perform collinearity analysis of the SCC*mec* gene clusters.

1.4. Identification of genes in the lugdunin operon

Comparisons of individual sequences of the lugdunin operon were performed on the genomes of *S. lugdunensis* from our laboratory and the GenBank database using Blastx and Blastp methods against proteins of the *lug* operon, as previously published [12]. According to the results of Blastp, no genome completely matched all 14 proteins; therefore, we chose nucleotide sequences of each lugdunin gene from the genomes of several strains as the reference gene sequences.

# Supplementary Figures and Tables

## Supplementary Figures



**Supplementary Figure 1. Genomic organization of the chromosome of *S. lugdunensis* strains.** The strain RMLUG1 is reference genome. The first innermost circle shows G + C contents, and the second circle shows GC-skew (values above zero are in blue, and those below zero are in green). The third and fourth circles show open reading frames on the minus and plus strands, respectively. The fifth to ninth circles show BLASTN alignment showing percent identity against RMLUG4, RMLUG3, RMLUG2, RMLUG5, and RMLUG6. Positions of SCC*mec*, *dnaA* gene, *lug* operon and two prophages of strain RMLUG1 are also indicated.

fig1-1

**Supplementary Figure 2: Structure of SCCmec compared with closely-related structures.** Arrows indicate open reading frames (orfs) and their directions.

1. SCC*mec* elements of RMLUG1 and RMLUG6 compared with SCC*mec* type Ⅴ (*S. aureus* WIS, AB121219).
2. SCC*mec* element of RMLUG3 compared with SCC*mec* type Ⅳi (*S. aureus* JCSC6668, AB425823) and a portion of SCC*mec* type Ⅳi (*S. aureus* pt259, CP049429).
3. SCC*mec* element of RMLUG2 compared with SCC*mec* type Ⅴ (*S. aureus* WIS, AB121219) and SCC*mec* type Ⅶ (*S. aureus* JCSC6082, AB373032).
4. SCCRMLUG4 compared with SCC*mec* type Ⅻ (*S. aureus* BA01611, KR187111) and a portion of SCC*mec* (*S. epidermidis* MB1143, MK784555). Sequences similar to other SCC*mec* elements are shaded in light blue with the similarity. DR, direct repeat.

## Supplementary Table

**Supplementary Table S1.** Patient characteristics

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Strains** | **Ward** | **Sex** | **Age** | **Diagnosis** | **Sample source** | **Geographical location** |
| **RMLUG1** | Thoracic surgery | Male | 63 | Lung adenocarcinoma | BALF | Fujian |
| **RMLUG2** | E.N.T. | Male | 29 | Obstructive sleep apnea syndrome | Blood | Fujian |
| **RMLUG3** | Burn and plastic surgery | Male | 55 | Skin defect with infection | Wound secretion | Fujian |
| **RMLUG4** | Gynecology | Female | 69 | Catheter associated infection | Abdominal drainage | Fujian |
| **RMLUG5** | ICU | Male | 73 | Subarachnoid hemorrhage | Sputum | Beijing |
| **RMLUG6** | Plastic surgery | Female | 26 | Hip sinus | Postoperative incision | Wuhan |

E.N.T., ear-nose-throat department; ICU, intensive care unit; BALF, bronchoalveolar lavage fluid.

**Supplementary Table S2.** Phenotypic and genotypic characterization of *mecA* positive and negative isolates.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Strains** | ***mecA* gene** | **VITEK2** | | **MIC(μg/mL)** | **Zone diameter(mm)** | **Oxacillin salt agar screen** |
| Cefoxitin | Oxacillin | Oxacillin | Cefoxitin |
| **RMLUG1** | + | R | R | R (≥16) | S (22) | R |
| **RMLUG2** | + | S | S | S (2) | S (27) | S |
| **RMLUG3** | + | R | R | R (≥16) | R (14) | R |
| **RMLUG4** | - | S | S | S (1) | S (28) | S |
| **RMLUG5** | - | S | S | S (0.5) | S (29) | S |
| **RMLUG6** | + | R | R | R (8) | S (23) | R |

S, susceptible; R, resistant.

**Supplementary Table S3.** Antimicrobial susceptibility for six isolates of *S. lugdunensis*.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Strain** | **RMLUG1** | | **RMLUG2** | | **RMLUG3** | | **RMLUG4** | | **RMLUG5** | | **RMLUG6** | |
| MIC(μg/mL) | Result | MIC(μg/mL) | Result | MIC(μg/mL) | Result | MIC(μg/mL) | Result | MIC(μg/mL) | Result | MIC(μg/mL) | Result |
| **Cefoxitin** | positive | R | negative | S | positive | R | negative | S | negative | S | positive | R |
| **Penicillin** | ≥0.5 | R | ≤0.03 | S | ≥0.5 | R | ≥0.5 | R | 0.06 | S | ≥0.5 | R |
| **Oxacillin** | ≥4 | R | 2 | S | ≥4 | R | 2 | S | 2 | S | ≥4 | R |
| **Gentamicin** | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S |
| **Ciprofloxacin** | 1 | I | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S | 1 | I |
| **Levofloxacin** | 1 | S | ≤0.12 | S | 0.25 | S | ≤0.12 | S | 0.25 | S | 1 | S |
| **Moxifloxacin** | 1 | I | ≤0.25 | S | ≤0.25 | S | ≤0.25 | S | ≤0.25 | S | 1 | I |
| **Erythromycin** | ≥8 | R | ≤0.25 | S | ≥8 | R | ≤0.25 | S | ≤0.25 | S | ≥8 | R |
| **Clindamycin** | ≥8 | R | ≤0.25 | S | ≥8 | R | ≤0.25 | S | ≤0.25 | S | ≥8 | R |
| **Inducible Clindamycin Resistance** | negative | S | negative | S | negative | S | negative | S | negative | S | negative | S |
| **Quinupristin/Dalfopristin** | ≤0.25 | S | ≤0.25 | S | ≤0.25 | S | ≤0.25 | S | ≤0.25 | S | ≤0.25 | S |
| **Linezolid** | 1 | S | 1 | S | 1 | S | 2 | S | 2 | S | 1 | S |
| **Vancomycin** | ≤0.5 | S | ≤0.5 | S | 1 | S | ≤0.5 | S | 1 | S | 1 | S |
| **Tetracycline** | ≤1 | S | ≤1 | S | ≥16 | R | ≤1 | S | ≤1 | S | ≤1 | S |
| **Tigecycline** | ≤0.12 | S | ≤0.12 | S | ≤0.12 | S | ≤0.12 | S | ≤0.12 | S | ≤0.12 | S |
| **Nitrofurantoin** | ≤16 | S | ≤16 | S | ≤16 | S | ≤16 | S | ≤16 | S | ≤16 | S |
| **Rifampicin** | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S | ≤0.5 | S |
| **Sulfamethoxazole** | ≤10 | S | ≤10 | S | ≤10 | S | ≤10 | S | ≤10 | S | ≤10 | S |
| **Teicoplanin (K-B method)** | 18 | S | 20 | S | 20 | S | 18 | S | 18 | S | 18 | S |

S, susceptible; I, intermediate; R, resistant; the reference range for teicoplanin is 11-13 millimeters.

**Supplementary Table S4.** Comparison of *lug* operon among *S. lugdunensis* strains.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| CC | ST | Strain | Accession number | Geographical location | Putative regulator | transporters | transporters | transporters | transporters | transporters | putative regulator | NRPS enzyme | NRPS enzyme | NRPS enzyme | modification enzyme | NRPS enzyme | modification enzyme | modification enzyme |
| *LugJ* | *LugI* | *LugE* | *LugF* | *LugG* | *LugH* | *LugR* | *LugA* | *LugB* | *LugC* | *LugT* | *LugD* | *LugZ* | *LugM* |
| CC1 | 1 | **HKU09-01** | CP001837.1 | Asia | MS | + | MS | + | + | MS | del,FS | MS | FS | MS | + | + | insert,FS | MS |
| 1 | **N920143** | FR870271.1 | Europe | MS | + | + | + | + | MS | + | MS | FS | MS | + | + | + | MS |
| 1 | **ACS-027-V-Sch2** | KB373322.1 | Not found | MS | + | + | + | + | MS | del | MS | MS | MS | FS | + | + | MS |
| 1 | **VISLISI\_37** | CP020761.1 | Europe | MS | + | + | + | + | MS | + | MS | MS | MS | + | + | + | MS |
| 1 | **SL29** | CP041723.1 | Europe | MS | + | + | + | + | MS | + | MS | MS | MS | + | + | + | MS |
| 1 | **SL117** | CP041725.1 | Europe | MS | + | + | + | + | MS | + | MS | MS | MS | + | + | + | MS |
| 1 | **SL122** | CP041727.1 | Europe | MS | + | + | + | + | MS | + | MS | MS | 2;FS;MS | + | 2;+;FS | + | 2;MS;MS |
| 6 | **RMLUG5** | CP084443 | Asia | MS | + | MS | + | + | MS | del | MS | FS | del,FS | + | + | insert | MS |
| 6 | **CGMH-SL118** | CP048008.1 | Asia | MS | + | del,FS,MS | + | + | MS | + | MS,FS | FS | FS,MS | + | + | insert,FS | MS |
| 6 | **VISLISI\_33** | CP020769.1 | Europe | MS | + | MS | + | + | MS | + | MS | FS | FS | + | + | insert | MS |
| 6 | **SL13** | CP041722.1 | Europe | MS | + | MS | + | + | MS | + | MS | FS | FS | + | + | insert | MS |
| 12 | **VISLISI\_27** | CP020735.1 | Europe | MS | + | MS | + | + | MS | + | FS | FS | MS | + | + | insert | MS |
| 34 | **RMLUG4** | CP084440 | Asia | MS | + | MS | + | + | MS | del, MS | FS | FS | del,FS | + | + | insert | MS |
| CC3 | 3 | **RMLUG1** | CP084480 | Asia | + | + | MS | MS | + | + | del | + | + | + | + | + | + | + |
| 3 | **RMLUG3** | CP084437 | Asia | + | + | MS | MS | + | + | del | MS | + | + | + | + | + | + |
| 3 | **RMLUG6** | CP084444 | Asia | + | + | MS | MS | + | + | del | + | FS | + | + | + | + | + |
| 3 | **VCU139** | AHLK01000029.1 | Not found | + | + | MS | MS | + | + | del,FS | + | FS | FS | + | + | + | + |
| 3 | **UCIM6116** | KI978384.1 | North American | + | + | MS | MS | + | + | del | + | FS | + | + | + | + | + |
| 3 | **VCU148** | JIBR01000035.1 | Not found | + | + | MS | MS | + | + | del,FS | part,FS | part,FS | FS | + | + | + | + |
| 3 | **FDAARGOS\_141** | CP014022.1 | North American | + | + | MS | MS | + | + | + | + | del | + | + | + | + | + |
| 3 | **VISLISI\_21** | CP020762.1 | Europe | + | + | MS | MS | + | + | + | + | + | MS | + | + | + | + |
| 3 | **Klug93G-4** | CP017069.2 | Asia | + | + | MS | MS | + | + | + | + | + | + | + | + | FS | + |
| 3 | **FDAARGOS\_377** | CP023539.1 | North American | + | + | MS | MS | + | + | + | + | + | + | + | + | + | + |
| 3 | **FDAARGOS\_381** | CP023970.1 | North American | + | + | MS | MS | + | + | + | FS | FS | FS | FS | + | + | + |
| 3 | **E7** | SCHB01000001.1 | North American | + | + | MS | MS | + | + | + | + | + | + | + | + | + | + |
| 3 | **APC 3758** | CP038807.1 | Europe | + | + | MS | MS | + | + | + | + | FS | + | + | + | + | + |
| 3 | **JICS135** | AP021848.1 | Asia | + | + | MS | MS | + | + | del | insert | FS | + | + | + | + | + |
| 3 | **IVK28** | CP063143.1 | Europe | + | + | MS | MS | + | + | + | + | + | + | + | + | + | + |
| 3 | **MBAZ2** | CP060160.1 | Europe | + | + | MS | MS | + | + | + | + | + | MS | + | + | + | + |
| 3 | **s38** | QVUS01000002.1 | North American | + | + | MS | MS | + | + | + | + | + | + | + | + | + | + |
| CC4 | 4 | **VCU150** | JIBS01000016.1 | Not found | - | - | - | - | - | - | - | - | - | - | - | - | - | MS |
| CC5 | 5 | **VISLISI\_25** | CP020763.1 | Europe | - | - | - | - | - | - | - | - | - | - | - | - | - | MS |
| CC6 | 10 | **SL55** | CP041724.1 | Europe | + | + | MS | MS | MS | MS | + | MS | del,MS | MS | + | + | + | MS |
| 10 | **SL118** | CP041726.1 | Europe | + | + | MS | MS | MS | MS | + | MS | FS | MS | + | + | + | MS |
| 24 | **FDAARGOS\_222** | CP020406.2 | Europe | + | + | MS | MS | MS | MS | + | MS | MS | MS | + | + | + | MS |
| 24 | **VISLISI\_22** | CP020764.1 | Europe | + | + | MS | MS | MS | MS | + | MS | MS | MS | + | + | + | MS |
| 24 | **HD104N11** | JACMRY010000003.1 | Europe | + | + | MS | MS | MS | MS | + | MS | MS | MS | + | + | + | MS |
| 24 | **NCTC12217** | LS483482.1 | Not found | + | + | MS | MS | MS | MS | del | MS | MS | FS | FS | + | + | MS |
| CC7 | 26 | **FDAARGOS\_143** | CP014023.2 | North American | + | + | MS | MS | + | MS | + | MS | MS | MS | MS | + | + | MS |
| 27 | **RMLUG2** | CP084434 | Asia | - | - | - | - | - | - | - | - | - | - | - | - | - | NS |
| 27 | **C\_33** | CP020768.1 | Europe | - | - | - | - | - | - | - | - | - | - | - | - | - | MS |
|  | ST28 | **M23590** | GL622352.1 | Not found | + | + | MS | MS | + | + | + | MS | FS | MS | + | NS | MS | MS |
| **MJR7738** | KQ957365.1 | North American | + | + | MS | NS | + | part | - | part | part,MS | part | part | part | MS | MS |
| **NCTC7990** | LS483312.1 | Europe | + | + | MS | MS | + | + | del | MS | FS | MS | + | + | NS | MS |

The presence of a *lug* operon gene is indicated by “+” while absence is indicated by “-”. CC, clonal complex; NRPS, non-ribosomal peptide synthetase; MS, missense mutation; FS, frameshift mutation; NS, nonsense mutation; del, deletion; “2” means 2 copies of the gene. “part” means incomplete identification of gene.

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