Molecular Characteristics of Rifampin-Sensitive and -Resistant Isolates and Characteristics of \( rpoB \) Gene Mutations in Methicillin-Resistant \textit{Staphylococcus aureus}

**Introduction:** Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) infections have become a leading cause of severe infections in both healthcare and community settings. Mutations in the \( rpoB \) gene cause resistance to rifampin (RIF\(^R\)), a critical antibiotic for the treatment of multidrug-resistant \textit{Staphylococcus aureus}. The aim of this study was to detect the molecular characteristics of RIF\(^R\) MRSA and analyze the \( rpoB \) gene mutations involved in RIF resistance.

**Methods:** A total of 49 RIF\(^R\) MRSA and 38 RIF\(^S\) MRSA isolates collected from seven cities in China were analyzed by multilocus sequence typing, staphylococcus chromosomal cassette mec (SCCmec) typing, \textit{spa} typing, and \( rpoB \) gene mutations.

**Results:** ST239-III-t030 (35/49, 71.4%), the major clone in RIF\(^R\) MRSA isolates; ST45-IV-t116 (16/38, 42.1%), the major clone in RIF\(^S\) MRSA isolates with \( rpoB \) mutations. RIF\(^R\) MRSA isolates were resistant to erythromycin, ciprofloxacin, tetracycline, gentamicin, and clindamycin. By contrast, RIF\(^R\) MRSA isolates with \( rpoB \) mutation were more susceptible to ciprofloxacin, tetracycline, and gentamicin. Forty-three (87.8%) isolates present the mutational change H481N and L466S, conferring 128–512 \( \mu \)g/mL RIF resistance. The four isolates with RIF MIC ≥ 1024 \( \mu \)g/mL had additional amino acid substitution: H481N, L466S, A473T (n=2); H481Y (n=2), associated with a high-level RIF resistance. Of 38 RIF\(^S\) MRSA isolates, two mutations were observed, including H481N (n=37) and A477D (n=1).

**Conclusion:** In conclusion, the predominant RIF\(^R\) MRSA clones in China were ST239-III-t030. Molecular characteristics, antibiotic-resistant profiles, and \( rpoB \) mutations between RIF\(^R\) MRSA and RIF\(^S\) MRSA were diverse. Antibiotics for treating patients with MRSA infections can be selected based on molecular characteristics.

**Keywords:** MRSA, rifampin, \( rpoB \) mutations, MLST, SCCmec, \textit{spa}

**Introduction**

\textit{Staphylococcus aureus} is a major human pathogen that causes a diversity of diseases ranging from relatively minor to invasive and systemic diseases with significant morbidity and mortality, which results in significant economic and societal costs.\(^1\) Since the first European isolate\(^2\) of methicillin-resistant \textit{Staphylococcus aureus} (MRSA) was detected in the 1960s, MRSA infections have become a leading cause of bacterial infections in both healthcare and community settings and a global concern.\(^3\) The spread of different clones from different geographic regions has been reported.\(^4\) Sequence type (ST239) clone was the most
important hospital-associated MRSA (HA-MRSA) around the world and disseminated in hospitals through Europe, North America, South America, and Asia. A previous study showed that MRSA ST239 and MRSA ST5 were also predominant in Chinese hospitals. However, ST228 was the predominant clone of RIF MRSA isolates in Spain.

MRSA was generated when methicillin-susceptible S. aureus (MSSA) acquires mecA gene encoding the penicillin-binding protein 2a (PBP2a) and acquired by horizontal transfer of a mobile genetic element designated staphylococcal cassette chromosome mec (SCCmec). To date, 13 SCCmec types have been identified among S. aureus in the world. Generally, HA-MRSA typically belongs to SCCmec I, II, and III, while CA-MRSA carries SCCmec IV or V. In addition, spa typing can be used for the investigation of both molecular evolution and hospital outbreaks.

Most MRSA isolates are resistant to multiple antibiotics. Glycopeptides such as vancomycin are the primary treatment option for severe infections caused by MRSA and most strains of multidrug-resistant S. aureus. Because of poor tissue diffusion and moderate bactericidal activity, vancomycin is often combined with rifampin for deep-seated infections. However, the efficacy of vancomycin has declined with the emergence of vancomycin-intermediate S. aureus (VISA) and heterogeneous VISA. A number of studies have revealed a worrying link between certain rpoB mutations and decreased susceptibility not only to rifampin but also other last line anti-MRSA antibiotics such as beta-lactams, imipenem, vancomycin, or daptomycin in S. aureus. One study reported that 86% of all resistance to rifampin isolates in their global sample carried the mutations promoting cross-resistance to vancomycin and 52% to both vancomycin and daptomycin.

Rifampin is a potent anti-staphylococcal agent and acts by interacting specifically with the β subunit of the bacterial RNA polymerase encoded by the rpoB gene. Rifampin is indicated in combination therapy for implant-associated S. aureus infections and to eradicate asymptomatic carriage of MRSA. However, the emergence and spread of rifampin-resistant MRSA during vancomycin–rifampin combination therapy in an intensive care unit has been reported. In China, the frequency of the RIF-R MRSA isolates decreased from 2017 to 2020 reported by the China Antimicrobial Surveillance Network (CHINET): 16.2% (986/6084) of all MRSA clinical isolates in 2017, 12.2% (894/7327) of all MRSA clinical isolates in 2018, and 8.2% (588/7170) of all MRSA clinical isolates in 2020.

Resistance to rifampin occurs through mutation in the rpoB gene that codes for the Beta subunit of RNA polymerase which inactivates the drug. Resistance to rifampin in M. tuberculosis is largely associated with mutations within an 81 bp RIF resistance determining region (RDRR) in the rpoB gene. In S. aureus, rifampin resistance is associated with mutations in particular regions (cluster I and cluster II) of the gene rpoB (462 to 488 and 515 to 530). Not all rpoB mutations have the same phenotypic consequences.

In this study, we aim to investigate the molecular profile and antimicrobial resistance associated with RIF R and RIF MRSA isolates and analyze mutations in rpoB gene related to rifampin resistance in MRSA and epidemiology.

**Materials and Methods**

**Bacterial Strains**

From 2011 to 2020, a total of 565 non-duplicate MRSA isolates were collected from the seven regions (Inner Mongolia, Wuhan, Chengdu, Guangzhou, Shanghai, Nanchang, Wenzhou) in China. Our team performed whole-genome sequencing on 565 isolates of MRSA, of which 49 (8.7%) isolates were resistant to rifampin, and 38 isolates of the remaining RIF-sensitive MRSA had mutations in rpoB gene, and 84 isolates were randomly selected from RIF-sensitive MRSA without rpoB mutations.

The clinical isolates were identified as S. aureus using Matrix-Assisted Laser Desorption/Ionization Time of Flight (MOLDI-TOF) by VITEK Mass Spectrometry. Escherichia coli ATCC8739 was used as a control strain for the identification of bacteria. MRSA was determined based on the minimal inhibitory concentrations (MICs) of oxacillin and cefoxitin and confirmed by detecting the presence of mecA gene. The proportions of MRSA isolates isolated from various specimens were as follows: 34.5% (30/87), sputum; 43.7% (38/87), pus; 34.5% (30/87), blood. This study was approved by the research ethics board at Shanghai Pulmonary Hospital.

**Whole-Genome Sequencing**

All of S. aureus isolates were sequenced using the HiSeq 2500 sequencing platform (Illumina Inc., San Diego, CA), with 150 base pair paired-end reads. The data generated
from the Illumina platform were analyzed after quality control was performed. De novo assembly of the genomes of all *S. aureus* isolates was performed using Spades v3.14\(^{26}\) and annotated using Prokka v1.12.\(^{27}\)

**Molecular Typing**

Molecular typing was performed using multi-locus sequence typing (MLST) as previously described. Staphylococcal cassette chromosome mec (SCCmec) type and spa type were performed using the web-based SCCmecFinder (https://cge.cbs.dtu.dk/services/SCCmecFinder/) and web-based spaFinder (https://cge.cbs.dtu.dk/services/spatyper/), respectively.

**Antibiotic Susceptibility Testing**

Antimicrobial susceptibility testing of 18 antimicrobial agents including ciprofloxacin (CIP), clindamycin (CLI), tetracycline (TET), erythromycin (ERY), quinupristin–dalfopristin (QD), ceftaroline (CPT), rifampin (RIF), sulfamethoxazole/trimethoprim (SXT), gentamicin (GEN), daptomycin (DAP), mupirocin (MOP), teicoplanin (TCL), linezolid (LN), fusidic acid (FA), vancomycin (VAN), dalbavancin (DAL), and cefoxitin (FOX) was determined in accordance with the protocols recommended by the Clinical and Laboratory Standards Institute (CLSI). Susceptibility testing of MRSA isolates was performed routinely by the disk diffusion method on Mueller–Hinton agar plates to the following antibiotics: CIP (5 μg), CLI (2 μg), TET (30 μg), ERY (15 μg), QD (15 μg), and CPT (30 μg). MICs of RIF, SXT, GEN, DAP, MOP, TCL, LN, FA, VAN, DAL, and FOX were determined in all strains by microdilution following CLSI recommendations. *S. aureus* ATCC 29213 and ATCC 25923 were used as quality controls.

**Data Analysis and Statistical Methods**

The statistical analyses were accomplished using SPSS software (SPSS, Chicago, IL, USA). Comparisons were made between RIF\(^R\) and RIF\(^S\) MRSA isolates using the chi-square test. P-value with <0.05 was considered statistical significance. The MIC distribution result was analyzed with Prism 8.0 software (GraphPad, San Diego, CA). The detailed information of MRSA isolates resistance to RIF was listed in the Supplementary Table 1 (Molecular characteristics and drug sensitivity results of MRSA (n=49) isolates resistance to RIF), and MRSA isolates sensitivity to RIF was listed in the Supplementary Table 2 (Molecular characteristics and drug sensitivity results of MRSA (n=38) isolates sensitivity to RIF).

**Results**

**Rifampin Resistance Levels and Associated rpoB Mutations**

The majority (n=40, 81.6%) of the 49 RIF\(^R\) MRSA isolates, showed RIF MICs of 256 μg/mL. The MIC values of RIF for remaining isolates were as followed: >1024 μg/mL, 3; 1024 μg/mL, 1; 512 μg/mL, 3; 128 μg/mL, 1; 8 μg/mL, 1. The mutations in the rifampin resistance-determining region of rpoB gene are shown in Tables 1 and 2. The MIC distributions for RIF in relation to mutations in rpoB are shown in Figure 1. Forty-three (87.8%) isolates present the mutational change H481N and L466S, conferring 128–512 μg/mL RIF resistance. The four isolates with MIC ≥1024 μg/mL had additional amino acid substitution: H481N, L466S, A473T (n=2); H481Y (n=2), associated with a high-level RIF resistance. Of 38 RIF\(^S\) MRSA isolates, two mutations were observed, including H481N (n=37) and A477D (n=1).

**SCCmec Typing, MLST, and spa Typing**

The evolution of MRSA isolates was analyzed by MLST (Tables 1 and 2). There were five distinct CCs (CC8, CC59, CC45, CC5, and CC398) identified within the 49 RIF\(^R\) MRSA isolates (Table 1). ST239 (CC8) was the most predominant ST (44/49, 89.8%) in RIF\(^R\) MRSA isolates, and was distributed in five cities. By spa typing, ST239 included spa types t030, t459, t037, t233, and t270 in RIF\(^R\) MRSA isolates. The most predominant spa type in ST239 RIF\(^R\) MRSA isolates was t030 (35/49, 71.4%), followed by t459 (5/49, 10.2%). In addition, three SCCmec types were found in RIF\(^R\) MRSA isolates: III, IV, and V. The most common type was type III, which was present in 43 (87.8%) RIF\(^R\) MRSA isolates.

However, 10 STs that could be clustered into 7 CCs (CC45, CC5, CC8, CC9, CC1, CC59, and CC121) were identified in 39 RIF\(^S\) MRSA isolates with mutations in rpoB gene (Table 2). ST45 (CC45) was the most common ST (22/38, 57.9%) in RIF\(^S\) MRSA isolates with mutations in rpoB gene, followed by ST5 (5/38, 13.2%), and ST239 (5/38, 13.2%). spa type t116 was the most common type (16/22, 72.7%) in ST45 RIF\(^S\) MRSA isolates with mutations in rpoB gene. SCCmec type IV was the most predominant type, present in 63.2% (24/38) of the RIF\(^S\) MRSA isolates and five provinces, being most prevalent in Guangzhou (15/38, 39.5%).

**Antimicrobial Susceptibility Profiles**

As shown in Tables 3 and 4, the results of antibiotic susceptibility testing showed that all the isolates were...
susceptible to DAP, TCL, LNZ, VAN, and DLA. Of 49 RIF MRSA isolates, 69.4% (34/49) with resistance to three or more classes of antimicrobial agents tested were identified as multidrug-resistant isolates. Excluding intermediate resistance, 71.4% of the RIF MRSA isolates were resistant to ERY and 69.4% to CLI. Similarly, 78.9% of the RIF MRSA isolates were resistant to ERY and CLI. The resistance rates of the 49 RIF MRSA isolates to TET (77.6%), CIP (89.8%), and GEN (83.7%) were relatively high. However, the resistance rates of 38 RIF MRSA isolates to TET, CIP, and GEN were 31.6%, 31.6%, and 23.7%, respectively, which were significantly lower than that of RIF MRSA isolates. The resistance rates to other antibiotics (FA, MOP, SXT, and CPT) were relatively low. Among 84 RIF MRSA without rpoB mutations isolates, except CIP (57.1%) and SXT (0%), the drug resistance rate of other agents was similar to that of RIF MRSA with rpoB mutations isolates.

**Resistance Genes**

As shown in Table 5, resistance genes (gyrA, erm (A), tet (M), and aac(6’)-Ie/aph(2”)-Ia) of RIF MRSA were significantly higher than those of RIF MRSA with rpoB mutations isolates.

**Discussion**

MRSA is an increasing problem and HA-MRSA infections have been found worldwide. The growing number of antibiotic-resistant pathogens is increasingly threatening the efficacy of healthcare institutions worldwide. Antibiotic discovery needs to be re-energized, to rival the threat of the post-antibiotic era. Although a steady decrease in the prevalence of RIF MRSA among Chinese hospitals within recent years has been already reported by the CHINET, and the relationship between RIF MICs and rpoB mutation of MRSA have been reported, there have been few reports, however, associating the decrease in the prevalence of RIF MRSA with molecular characteristics.

ST239-III is the predominant clone among HA-MRSA strains in Asia, Middle East, Africa, New Zealand, and Australia. The major pandemic clones are usually related to specific geographical locations. The ST5-I/II clone in the USA, Canada, Mexico, and South America, ST36-II in Europe. Evidence suggests that the CC8-ST239 subgroup (ST239-III) lineage from South Korea, Hong Kong, Taiwan, and Vietnam and CC5(ST5-II) from South Korea and Sri Lanka have traveled from hospitals into the community. Belgium is the only location where ST239
Table 2 Molecular Characteristics of Main Clones Among MRSA (n=38) 8 isolates Sensitivity to RIF.

| CC  | MLST | SCCmec Type | spa  | II  | III | IVa | Yb | VII | NO | Source          | Region | MIC  | Mutation |
|-----|------|-------------|------|-----|-----|-----|-----|-----|-----|-----------------|---------|-------|----------|
| CC45| ST45 (22, 57.9%) | t116 | 16  | I   | 16  | I   | 16  | Blood (7), Pus (6), Sputum (3) | B (2), C (1), D (11), F (2), | 2 (22) | H481N |
|     |      | t1714       | I    | I   | I   | 1   | 1   | Blood (1)                     | D (1)   | 2     | H481N   |
|     |      | t1823       | I    | I   | I   | 1   | 1   | Blood (1)                     | D (1)   | 2     | H481N   |
|     |      | t26         | I    | I   | I   | 1   | 1   | Pus (1)                       | F (1)   | 2     | H481N   |
|     |      | t466        | I    | I   | I   | 1   | 1   | Blood (1)                     | G (1)   | 2     | H481N   |
|     |      | t510        | I    | I   | I   | 1   | 1   | Sputum (1)                    | B (1)   | 2     | H481N   |
|     |      | N           | I    | I   | I   | 1   | 1   | Sputum (1)                    | G (1)   | 2     | H481N   |
| CC8 | ST239 (5, 13.2%) | t37  | 5   | I   | 5   | I   | 5   | Pus (2), Sputum (3)           | C (1), D (1), E (2), G (1) | 1 (1), 2 (4) | H481N |
| CC5 | ST5 (5, 13.2%)  | t2460 | 5   | I   | 5   | I   | 5   | Sputum (4), pus (1)           | G (5)   | <0.25 (2), 1 (1), 2 (2) | H481N |
| CC1 | ST1 (1, 2.6%)   | t2    | I   | I   | 1   | 1   | 1   | Blood (1)                     | E (1)   | 2     | H481N   |
|     |      | t27         | I    | I   | I   | 1   | 1   | Blood (1)                     | D (1)   | 1     | H481N   |
|     |      | t899        | I    | I   | I   | 1   | 1   | Pus (1)                       | D (1)   | 2     | H481N   |
| CC9 | ST59 (1, 2.6%)  | t437  | 1   | I   | 1   | 1   | 1   | Sputum (1)                    | D (1)   | 2     | A477D   |
| CC121| ST121 (1, 2.6%)| t2613 | 1   | I   | 1   | 1   | 1   | Pus (1)                       | F (1)   | 2     | H481N   |
| N   | N (1, 2.6%)     | t116  | 1   | I   | 1   | 1   | 1   | Sputum (1)                    | D (1)   | 2     | H481N   |

Notes: Region A: Inner Mongolia; B: Wuhan; C: Chengdu; D: Guangzhou; E: Shanghai; F: Nanchang; G: Wenzhou.
Abbreviation: N, no mutation.
has been detected in livestock so far.\(^5\) In China, ST239-III and ST5-II are both the major HA-MRSA clones.\(^30\) Similarly, 87.8\% (43/49) RIF\(^R\) MRSA ST239-III isolates were detected, while one ST5 MRSA isolate was detected in the present study. Li et al found ST239-t030 clone and ST239-t037 clone, which accounted for the large proportion of \textit{S. aureus}, were on the wane and progressively replaced by ST59-t2460 in China.\(^7\) However, ST239-III-t030, the major clone in RIF\(^R\) MRSA isolates, had a stronger survival advantage and could easily transmit in Chinese hospitals, which was in concordance with a previous study that reported the MRSA isolates of the ST239-III-t030 clone were more resistant to RIF.\(^30,31\)

Interestingly, ST45-IV-t116 MRSA was the predominant clone in RIF\(^S\) MRSA isolates with \textit{rpoB} mutation. CC45 is common in the United States (ST45-II) and Europe (ST45-IV/V).\(^5\) ST45-II is the hospital-associated clone and ST45-IV is community-associated clone.\(^5\) A previous study reported that a multicenter outbreak of ST45 MRSA containing deletions in the \textit{spa} gene in New South Wales, Australia.\(^32\) Of 131 ST45 MRSA clinical isolates, 72 (54.9\%) represented Australian Staphylococcal Sepsis Outcome Program bacteremia isolates.\(^32\) In the present study, 10 (10/22, 45.5\%) isolates were isolated from blood. However, ST239 and ST5, the second predominant clones in RIF\(^S\) MRSA isolates with \textit{rpoB} mutation, were isolated from pus and sputum.

In general, RIF\(^R\) MRSA isolates showed much higher resistance rates to all the tested antibiotics than RIF\(^S\) MRSA. The antibiotic testing results of this research revealed that RIF\(^R\) MRSA isolates were resistant to ERY, CIP, TET, GEN, and CLI. By contrast, RIF\(^S\) MRSA isolates with \textit{rpoB} mutation were more susceptible to CIP, TET, and GEN. The molecular characteristics of RIF\(^R\) and RIF\(^S\) MRSA with \textit{rpoB} gene mutation were different, so the drug resistance profiles were also different.

Almost all MRSA isolates showed the mutational change H481N. It has previously been reported that the RpoB H481Y mutation can be associated with a remarkably persistent \textit{S. aureus} infection.\(^33\) Forty-three (87.8\%) isolates present the mutational change H481N and L466S, conferring 128–512 μg/mL RIF resistance. High-level rifampicin resistance could be attributable to double mutations within \textit{rpoB}, as previously described.\(^24\) In addition, the single amino acid substitution H481Y also causes high-level resistance. In the present study, the two MRSA isolates with RIF MIC ≥ 1024 μg/mL had additional amino acid substitution: H481N, L466S, and A473T. Although H481N, L466S, and A473T have been described separately, they have not been detected in one clinical isolate. The two isolates with triple mutations, which belong to ST239-III-t037 clone, were from one region. Additionally, we also found two RIF\(^R\) isolates revealing no mutations.

In conclusion, ST239-III-t030, the major clone in RIF\(^R\) MRSA isolates; ST45-IV-t116, the major clone in RIF\(^S\) MRSA isolates with \textit{rpoB} mutations. RIF\(^R\) MRSA isolates showed much higher resistance rates to all the tested antibiotics than RIF\(^S\) MRSA. High-level rifampicin resistance was attributable to double mutations within \textit{rpoB}.

\[\text{Figure 1} \quad \text{Distribution of the MIC of rifampin for 87 MRSA in relation to mutations in rpoB.}\]
Table 3 The MIC Distribution of rpoB Gene Mutations in Rifampicin-Resistant and -Sensitive MRSA Isolates in China.

| Antimicrobial Agent | RIFR (n=49) | RIFS (n=38) |  |
|---------------------|-------------|-------------|---|
|                     | MIC<sub>50</sub> | MIC<sub>90</sub> | S (%) | I (%) | R (%) | MIC<sub>50</sub> | MIC<sub>90</sub> | S (%) | I (%) | R (%) | P   |
| CPT<sup>a</sup>     | 45 (91.8)   | 3 (6.1)     | 1 (2.0) |       |       | 38 (100.0)       |       |       |       |     | 0.376 |
| ERY<sup>a</sup>     | 14 (28.6)   | 1 (2.0)     | 35 (71.4) |       |       | 8 (21.1)        | 30 (78.9) |       |       | 0.424 |
| CLI<sup>a</sup>     | 14 (28.6)   | 1 (2.0)     | 34 (69.4) |       |       | 8 (21.1)        | 30 (78.9) |       |       | 0.316 |
| TET<sup>a</sup>     | 11 (22.4)   | 38 (77.6)   |       |       |       | 26 (68.4)       | 12 (31.6) |       |       | 0.000 |
| CIP<sup>a</sup>     | 3 (6.1)     | 2 (4.1)     | 44 (89.8) |       |       | 24 (63.2)       | 12 (31.6) |       |       | 0.000 |
| QD<sup>a</sup>      | 49 (100.0)  |           |     |       |       | 37 (97.4)       | 1 (2.6) |       |       | 0.253 |
| SXT                 | 0.25        | 1           | 45 (91.8) | 4 (8.2) | ≤0.125 | I            | 33 (86.8) | 5 (13.2) |       | 0.448 |
| GEN                 | >64         | >64         | 8 (16.3) | 41 (83.7) | ≤0.5 | >64 | 29 (76.3) | 9 (23.7) |       | 0.000 |
| DAP                 | 0.25        | 0.5         | 49 (100.0) | 0.25 | 0.5 | 38 (100.0) |       |       |       | – |
| MOP                 | ≤2          | ≤2          | 46 (93.9) | 3 (6.1) | ≤2 | ≤2 | 37 (97.4) | 1 (2.6) |       | 0.440 |
| TCL                 | 0.5         | 1           | 49 (100.0) | 0.5 | 0.5 | 38 (100.0) |       |       |       | – |
| LNZ                 | 1           | 2           | 49 (100.0) | 1 | 1 | 38 (100.0) |       |       |       | – |
| FA                  | ≤0.5        | ≤0.5        | 45 (91.8) | 4 (8.2) | ≤0.5 | ≤0.5 | 34 (89.5) | 4 (10.5) |       | 0.448 |
| VAN                 | 0.5         | 1           | 49 (100.0) | 0.5 | 1 | 38 (100.0) |       |       |       | – |
| DAL                 | 0.125       | 0.25        | 49 (100.0%) | 0.25 | 0.25 | 38 (100.0) |       |       |       | – |
| FOX                 | 256         | >256        | 49 (100.0) | 64 | 256 | 38 (100.0) |       |       |       | – |

Notes: *Susceptibility testing of MRSA isolates was performed routinely by the disk diffusion method on Mueller–Hinton agar plates according to CLSI. Bold fonts represent statistical differences between the two groups.

Abbreviations: CPT, ceftaroline; ERY, erythromycin; CLI, clindamycin; TET, tetracycline; CIP, ciprofloxacin; QD, quinupristin–dalfopristin; SXT, sulfamethoxazole/trimethoprim; GEN, gentamicin; DAP, daptomycin; MOP, mupirocin; TCL, teicoplanin; LNZ, linezolid; FA, fusidic acid; VAN, vancomycin; DAL, dalbavancin; FOX, cefoxitin.
Table 4 The MIC Distribution of Rifampicin-Sensitive MRSA Isolates in China.

| Antimicrobial Agent | RIF\(^a\) with rpoB Mutations (n=38) | RIF\(^a\) Without rpoB Mutations (n=84) |   |
|---------------------|---------------------------------|---------------------------------|---|
|                     | MIC\(_{50}\) MIC\(_{90}\) | S (%) | I (%) | R (%) | MIC\(_{50}\) MIC\(_{90}\) | S (%) | I (%) | R (%) | P |
| CPT\(^a\)           | 38 (100.0%)                   | 8 (21.1%)                     | 2 (5.3%) | 30 (78.9%) | 84 (100.0%)                   | 15 (17.9%) | 1 (1.2%) | 69 (82.1%) | 0.676 |
| ERY\(^a\)           | 8 (21.1%)                     | 30 (78.9%)                   | 0.676 |
| CLI\(^a\)           | 26 (68.4%)                    | 12 (31.6%)                   | 0.914 |
| TET\(^a\)           | 24 (63.2%)                    | 12 (31.6%)                   | 0.009 |
| CIP\(^a\)           | 37 (97.4%)                    | 1 (2.6%)                     | 1 (1.2%) | 8 (9.5%) | 48 (57.1%)                   | 0.135 |
| QD\(^a\)            | ≤0.125 1                      | 33 (86.8%)                   | 5 (13.2%) | ≤0.125 ≤0.125 | 84 (100.0%)                   | 6 (7.1%) | 0.321 |
| SXT                 | ≤0.5 >64                      | 29 (76.3%)                   | 9 (23.7%) | ≤0.5 >64 | 65 (77.4%)                   | 19 (22.6%) | 0.896 |
| GEN                 | ≤0.5 >64                      | 38 (100.0%)                  | 0.25 | 0.5 | 84 (100.0%)                   | 8 (1.2%) | 0.975 |
| DAP                 | ≤2 0.5                        | 37 (97.4%)                   | 1 (2.6%) | ≤2 ≤2 | 78 (92.9%)                   | 1 (1.2%) | 0.500 |
| MOP                 | ≤0.5 0.5                      | 38 (100.0%)                  | 0.25 | 0.5 | 84 (100.0%)                   | 84 (100.0%) | 0.500 |
| TCL                 | 1 1                           | 38 (100.0%)                  | 1 | 1 | 84 (100.0%)                   | 84 (100.0%) | 0.500 |
| LNZ                 | ≤0.5 ≤0.5                     | 34 (89.5%)                   | 1 (10.5%) | ≤0.5 ≤0.5 | 75 (89.3%)                   | 9 (10.7%) | 0.975 |
| FA                  | 0.5 1                         | 38 (100.0%)                  | 0.5 | 0.5 | 84 (100.0%)                   | 84 (100.0%) | 0.500 |
| VAN                 | 0.25 0.25                     | 38 (100.0%)                  | 0.125 | 0.25 | 84 (100.0%)                   | 1 (1.2%) | 0.500 |
| DAL                 | 64 256                        | 38 (100.0%)                  | 32 >256 | 1 (1.2%) | 83 (98.8%)                   | 0.500 |

Notes: *Susceptibility testing of MRSA isolates was performed routinely by the disk diffusion method on Mueller–Hinton agar plates according to CLSI. Bold fonts represent statistical differences between the two groups.
Table 5 Resistance Genes of Other Agents of rpoB Gene Mutations in Rifampicin-Resistant and -Sensitive MRSA Isolates.

| Genes   | RIF\(^R\) (n=49) | %   | RIF\(^S\) (n=38) | %   |
|---------|------------------|-----|------------------|-----|
| emr(A)  | 44               | 89.8| 10               | 26.3|
| emr(B)  | 2                | 4.1 | 1                | 2.6 |
| erm(C)  | 14               | 28.6| 20               | 52.6|
| tet(38) | 49               | 100 | 38               | 100 |
| tet(L)  | 1                | 2.0 | 1                | 2.6 |
| tet(M)  | 44               | 89.8| 10               | 26.3|
| aac(6\(^{II}\))-Ie/aph(2\(^{"}\))-Ia | 40 | 81.6 | 10 | 26.3 |
| aph(3\(^{III}\))-Ila | 3 | 6.1 | 6 | 15.8 |
| gyrA G106D | 42 | 85.7 | 0 | 0 |
| gyrA SB4L | 45 | 91.8 | 10 | 26.3 |
| gyrA SB4A | 0 | 0 | 1 | 2.6 |
| gyrA E88G | 0 | 0 | 1 | 2.6 |

Data Sharing Statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics Statement

The protocols applied in this study were also approved by the Ethics Committee of Shanghai Pulmonary Hospital, Tongji University School of Medicine Academy of Sciences, and informed consent was obtained from all patients whose specimens were used in scientific studies. This study was conducted in accordance with the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have no financial or non-financial conflicts of interest for this work.

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