Molecular Characteristics of Rifampin-Sensitive and -Resistant Isolates and Characteristics of *rpoB* Gene Mutations in Methicillin-Resistant *Staphylococcus aureus*

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Introduction: Methicillin-resistant *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 *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* (SCC*mec*) typing, *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^S 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 µg/mL RIF resistance. The four isolates with RIF 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).

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, SCC*mec*, *spa*

Introduction

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 isolate² of methicillin-resistant *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.⁵ A previous study showed that MRSA ST239 and MRSA ST5 were also predominant in Chinese hospitals.^{6,7} However, ST228 was the predominant clone of RIF^R MRSA isolates in Spain.⁸

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* (SCC*mec*).⁹ To date, 13 SCC*mec* types have been identified among *S. aureus* in the world.⁵ Generally, HA-MRSA typically belongs to SCC*mec* I, II, and III, while CA-MRSA carries SCC*mec* IV or V.⁵ In addition, *spa* typing can be used for the investigation of both molecular evolution and hospital outbreaks.¹⁰

Most MRSA isolates are resistant to multiple antibiotics.⁵ Glycopeptides such as vancomycin are the primary treatment option for severe infections caused by MRSA and most strains of multidrug-resistant *S. aureus*.¹¹ Because of poor tissue diffusion and moderate bactericidal activity, vancomycin is often combined with rifampin for deep-seated infections.¹² However, the efficacy of vancomycin has declined with the emergence of vancomycin-intermediate *S. aureus* (VISA) and heterogeneous VISA.¹³ 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*.^{14–17} 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.¹⁸

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.¹⁹ Rifampin is indicated in combination therapy for implant-associated *S. aureus* infections and to eradicate asymptomatic carriage of MRSA.^{20–22} However, the emergence and spread of rifampin-resistant MRSA during vancomycin–rifampin combination therapy in an intensive care unit has been reported.²³ 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, 11.5% (834/7251) of all MRSA clinical isolates in 2019,

and 8.2% (588/7170) of all MRSA clinical isolates in 2020 (<http://www.chinets.com>).

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 (RRDR) 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).^{24,25} 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^S 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 rifampicin, 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 (MALDI-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 ceftazidime 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 v3.14²⁶ 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* (SCC*mec*) type and *spa* type were performed using the web-based SCC*mec*Finder (<https://cge.cbs.dtu.dk/services/SCCmecFinder/>) and web-based *spa*Finder (<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 per the CLSI breakpoints.

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).

SCC*mec* 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 t2270 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 SCC*mec* 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. SCC*mec* 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

Table 1 Molecular Characteristics of Main Clones Among MRSA (n=49) Isolates Resistance to RIF.

CC	MLST	SCCmec Type						Source	PVL	Region	MIC	Mutation
		spa	II	III	IVa	IVi	Vb	NO				
CC8	ST239 (44.89.8%)	t030		35				35	-	A (15), B (10), C (5), D (2), E (2), F (1)	128 (1), 256 (32), 512 (2)	H48IN, L466S
		t459		4				5	-	B (2), C (1), D (1), E (1)	256 (5)	H48IN, L466S
		t037		2				2	-	D (2)	>1024	H48IN, L466S, A473T
		t233		1				1	-	F (1)	256	H48IN, L466S,
		t2270		1				1	-	D (1)	256	H48IN, L466S,
CC59	ST59 (2.4.1%)	t437						1	-	C (1)	256	N
CC45	ST508 (1.2.0%)	t172						1	-	C (1)	1024	H48IY
CC5	ST5 (1.2.0%)	t586						1	-	D (1)	>1024	H48IY
CC398	ST398 (1.2.0%)	t34						1	-	D (1)	512	H48IN, L466S
								1	-	C (1)	8	N

Notes: Region A: Inner Mongolia; B: Wuhan; C: Chengdu; D: Guangzhou; E: Shanghai; F: Nanchang; G: Wenzhou.
Abbreviation: N, no mutation.

susceptible to DAP, TCL, LNZ, VAN, and DLA. Of 49 RIF^R 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^R MRSA isolates were resistant to ERY and 69.4% to CLI. Similarly, 78.9% of the RIF^S MRSA isolates were resistant to ERY and CLI. The resistance rates of the 49 RIF^R MRSA isolates to TET (77.6%), CIP (89.8%), and GEN (83.7%) were relatively high. However, the resistance rates of 38 RIF^S MRSA isolates to TET, CIP, and GEN were 31.6%, 31.6%, and 23.7%, respectively, which were significantly lower than that of RIF^R MRSA isolates. The resistance rates to other antibiotics (FA, MOP, SXT, and CPT) were relatively low. Among 84 RIF^S 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^S 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^R MRSA were significantly higher than those of RIF^S 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.²⁸ Although a steady decrease in the prevalence of RIF^R 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^R MRSA with molecular characteristics.

ST239-III is the predominant clone among HA-MRSA strains in Asia, Middle East, Africa, New Zealand, and Australia.⁵ 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.⁵ 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.²⁹ 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						Source	Region	MIC	Mutation
		spa	II	III	IVa	Vb	VII	NO			
CC45	ST45 (22, 57.9%)	tl16			16			16	B (2), C (1), D (1), F (2),	2 (22)	H481N
		tl714			I			I	D (1)	2	H481N
		tl823			I			I	D (1)	2	H481N
		t26			I			I	F (1)	2	H481N
		t466			I			I	G (1)	2	H481N
		tl510			I			I	B (1)	2	H481N
CC8	ST239 (5, 13.2%)	N			I			I	G (1)	2	H481N
		t37		5				5	C (1), D (1), E (2), G (1)	1 (1), 2 (4)	H481N
		t2460	5					5	G (5)	<0.25 (2), 1 (1), 2 (2)	H481N
CC5	ST5 (5, 13.2%)	t2	I					I	E (1)	2	H481N
		tl27			I			I	D (1)	1	H481N
		t899				I		I	D (1)	2	H481N
CC59	ST59 (1, 2.6%)	t437				I		I	D (1)	2	A477D
		t2613				I		I	F (1)	2	H481N
N	N (1, 2.6%)	tl16			I			I	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.

Figure I Distribution of the MIC of rifampin for 87 MRSA in relation to mutations in *rpoB*.

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 *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 that 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 *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 *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 *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 *rpoB* mutation were more susceptible to CIP, TET, and GEN. The molecular characteristics of RIF^R and RIF^S MRSA with *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 *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 *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 *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 *rpoB*.

Table 3 The MIC Distribution of *rpmB* Gene Mutations in Rifampicin-Resistant and -Sensitive MRSA Isolates in China.

Antimicrobial Agent	RIFR (n=49)				RIFS (n=38)						P
	MIC ₅₀	MIC ₉₀	S (%)	I (%)	R (%)	MIC ₅₀	MIC ₉₀	S (%)	I (%)	R (%)	
CPT ^a			45 (91.8)	3 (6.1)	1 (2.0)			38 (100.0)			0.376
ERY ^a			14 (28.6)		35 (71.4)			8 (21.1)		30 (78.9)	0.424
CLI ^a			14 (28.6)	1 (2.0)	34 (69.4)			8 (21.1)		30 (78.9)	0.316
TET ^a			11(22.4)		38 (77.6)			26 (68.4)		12 (31.6)	0.000
CIP ^a			3 (6.1)	2 (4.1)	44 (89.8)			24 (63.2)	2 (5.3)	12 (31.6)	0.000
QD ^a			49 (100.0)					37 (97.4)		1 (2.6)	0.253
SXT	0.25	1	45 (91.8)		4 (8.2)	≤0.125	1	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)		38 (100.0)	–

Notes: ^aSusceptibility 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–dalbavancin; SXT, sulfamethoxazole/trimethoprim; GEN, gentamicin; DAP, daptomycin; MOP, mupirocin; TCL, teicoplanin; LNZ, linezolid; FA, fusidic acid; VAN, vancomycin; DAL, dalbavancin; FOX, ceftiofur.

Table 4 The MIC Distribution of Rifampicin-Sensitive MRSA Isolates in China.

Antimicrobial Agent	RIF ^S with <i>rpoB</i> Mutations (n=38)					RIF ^S Without <i>rpoB</i> Mutations(n=84)					P
	MIC ₅₀	MIC ₉₀	S (%)	I (%)	R (%)	MIC ₅₀	MIC ₉₀	S (%)	I (%)	R (%)	
CPT ^a			38 (100.0%)					84 (100.0%)			
ERY ^a			8 (21.1%)		30 (78.9%)			15 (17.9%)		69 (82.1%)	0.676
CLI ^a			8 (21.1%)		30 (78.9%)			16 (19.0%)	1 (1.2%)	67 (79.8%)	0.914
TET ^a			26 (68.4%)		12 (31.6%)			53 (63.1%)	1 (1.2%)	30 (35.7%)	0.656
CIP ^a			24 (63.2%)	2 (5.3%)	12 (31.6%)			28 (33.3%)	8 (9.5%)	48 (57.1%)	0.009
QD ^a			37 (97.4%)		1 (2.6%)			84 (100.0%)			0.135
SXT	≤0.125	1	33 (86.8%)		5 (13.2%)	≤0.125	≤0.125	84 (100.0%)			0.001
GEN	≤0.5	>64	29 (76.3%)		9 (23.7%)	≤0.5	>64	65 (77.4%)		19 (22.6%)	0.896
DAP	0.25	0.5	38 (100.0%)			0.25	0.5	84 (100.0%)			
MOP	≤2	≤2	37 (97.4%)		1 (2.6%)	≤2	≤2	78 (92.9%)		6 (7.1%)	0.321
TCL	0.5	0.5	38 (100.0%)			0.25	0.5	84 (100.0%)			
LNZ	1	1	38 (100.0%)			1	1	84 (100.0%)			
FA	≤0.5	≤0.5	34 (89.5%)		4 (10.5%)	≤0.5	≤0.5	75 (89.3%)		9 (10.7%)	0.975
VAN	0.5	1	38 (100.0%)			0.5	0.5	84 (100.0%)			
DAL	0.25	0.25	38 (100.0%)			0.125	0.25	84 (100.0%)			
FOX	64	256	38 (100.0%)		38 (100.0%)	32	>256	1 (1.2%)		83 (98.8%)	0.500

Notes: ^aSusceptibility 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)	%
<i>erm</i> (A)	44	89.8	10	26.3
<i>erm</i> (B)	2	4.1	1	2.6
<i>erm</i> (C)	14	28.6	20	52.6
<i>tet</i> (38)	49	100	38	100
<i>tet</i> (L)	1	2.0	1	2.6
<i>tet</i> (M)	44	89.8	10	26.3
<i>aac</i> (6')-Ie/aph(2'')-Ia	40	81.6	10	26.3
<i>aph</i> (3')-IIIa	3	6.1	6	15.8
<i>gyrA</i> G106D	42	85.7	0	0
<i>gyrA</i> S84L	45	91.8	10	26.3
<i>gyrA</i> S84A	0	0	1	2.6
<i>gyrA</i> 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.

References

- Gould IM. Costs of hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and its control. *Int J Antimicrob Agents*. 2006;28:379–384. doi:10.1016/j.ijantimicag.2006.09.001
- Gajdacs M, Urban E. Epidemiology and resistance trends of *Staphylococcus aureus* isolated from vaginal samples: a 10-year retrospective study in Hungary. *Acta Dermatovenol Alp Pannonica Adriat*. 2019;28:143–147.
- Lowy FD. *Staphylococcus aureus* infections. *N Engl J Med*. 1998;339:520–532. doi:10.1056/NEJM199808203390806
- Turner NA, Sharma-Kuinkel BK, Maskarinec SA, et al. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. *Nat Rev Microbiol*. 2019;17:203–218. doi:10.1038/s41579-018-0147-4
- Lakhundi S, Zhang K. Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. *Clin Microbiol Rev*. 2018;31. doi:10.1128/CMR.00020-18.
- Liu Y, Wang H, Du N, et al. Molecular evidence for spread of two major methicillin-resistant *Staphylococcus aureus* clones with a unique geographic distribution in Chinese hospitals. *Antimicrob Agents Chemother*. 2009;53:512–518. doi:10.1128/AAC.00804-08
- Dai Y, Liu J, Guo W, et al. Decreasing methicillin-resistant *Staphylococcus aureus* (MRSA) infections is attributable to the disappearance of predominant MRSA ST239 clones, Shanghai, 2008–2017. *Emerg Microbes Infect*. 2019;8:471–478. doi:10.1080/22221751.2019.1595161
- Mick V, Domínguez MA, Tubau F, et al. Molecular characterization of resistance to rifampicin in an emerging hospital-associated methicillin-resistant *Staphylococcus aureus* clone ST228, Spain. *BMC Microbiol*. 2010;10:68. doi:10.1186/1471-2180-10-68
- Gajdacs M. The continuing threat of methicillin-resistant *Staphylococcus aureus*. *Antibiotics (Basel)*. 2019;8. doi:10.3390/antibiotics8020052.
- Koreen L, Ramaswamy SV, Graviss EA, et al. spa typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. *J Clin Microbiol*. 2004;42:792–799. doi:10.1128/JCM.42.2.792-799.2004
- Walsh C. Deconstructing vancomycin. *Science*. 1999;284:442–443. doi:10.1126/science.284.5413.442
- Graziani AL, Lawson LA, Gibson GA, Steinberg MA, MacGregor RR. Vancomycin concentrations in infected and noninfected human bone. *Antimicrob Agents Chemother*. 1988;32:1320–1322. doi:10.1128/AAC.32.9.1320
- Howden BP, Davies JK, Johnson PD, Stinear TP, Grayson ML. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. *Clin Microbiol Rev*. 2010;23:99–139. doi:10.1128/CMR.00042-09
- Watanabe Y, Cui L, Katayama Y, Kozue K, Hiramatsu K. Impact of *rpoB* mutations on reduced vancomycin susceptibility in *Staphylococcus aureus*. *J Clin Microbiol*. 2011;49:2680–2684. doi:10.1128/JCM.02144-10
- Cui L, Isii T, Fukuda M, et al. An *rpoB* mutation confers dual heteroresistance to daptomycin and vancomycin in *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2010;54:5222–5233. doi:10.1128/AAC.00437-10
- Aiba Y, Katayama Y, Hishinuma T, et al. Mutation of RNA polymerase beta-subunit gene promotes heterogeneous-to-homogeneous conversion of beta-lactam resistance in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2013;57:4861–4871. doi:10.1128/AAC.00720-13

17. Matsuo M, Hishinuma T, Katayama Y, et al. Mutation of RNA polymerase β subunit (rpoB) promotes hVISA-to-VISA phenotypic conversion of strain Mu3. *Antimicrob Agents Chemother.* 2011;55:4188–4195. doi:10.1128/AAC.00398-11
18. Guerillot R, Gonçalves da Silva A, Monk I, et al. Convergent evolution driven by rifampin exacerbates the global burden of drug-resistant *Staphylococcus aureus*. *mSphere.* 2018;3. doi:10.1128/mSphere.00550-17
19. Aboshkiwa M, Rowland G, Coleman G. Nucleotide sequence of the *Staphylococcus aureus* RNA polymerase rpoB gene and comparison of its predicted amino acid sequence with those of other bacteria. *Biochim Biophys Acta.* 1995;1262:73–78. doi:10.1016/0167-4781(95)00054-k
20. Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. *JAMA.* 1998;279:1537–1541. doi:10.1001/jama.279.19.1537
21. Osmon DR, Berbari EF, Berendt AR, et al. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis.* 2013;56:e1–e25. doi:10.1093/cid/cis803
22. Senobar Tahaei SA, Stájer A, Barrak I, et al. Correlation between biofilm-formation and the antibiotic resistant phenotype in *Staphylococcus aureus* isolates: a laboratory-based study in Hungary and a review of the literature. *Infect Drug Resist.* 2021;14:1155–1168. doi:10.2147/IDR.S303992
23. Ju O, Woolley M, Gordon D. Emergence and spread of rifampicin-resistant, methicillin-resistant *Staphylococcus aureus* during vancomycin-rifampicin combination therapy in an intensive care unit. *Eur J Clin Microbiol Infect Dis.* 2006;25:61–62. doi:10.1007/s10096-005-0063-1
24. Aubry-Damon H, Soussy CJ, Courvalin P. Characterization of mutations in the rpoB gene that confer rifampin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother.* 1998;42:2590–2594. doi:10.1128/AAC.42.10.2590
25. Wichelhaus TA, Schafer V, Brade V, Boddingtonhaus B. Molecular characterization of rpoB mutations conferring cross-resistance to rifamycins on methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother.* 1999;43:2813–2816. doi:10.1128/AAC.43.11.2813
26. Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol.* 2012;19:455–477. doi:10.1089/cmb.2012.0021
27. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics.* 2014;30:2068–2069. doi:10.1093/bioinformatics/btu153
28. Gajdacs M. The concept of an ideal antibiotic: implications for drug design. *Molecules.* 2019;24:892. doi:10.3390/molecules24050892
29. Song JH, Hsueh PR, Chung DR, et al. Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: an ANSORP study. *J Antimicrob Chemother.* 2011;66:1061–1069. doi:10.1093/jac/dkr024
30. Cheng H, Yuan W, Zeng F, et al. Molecular and phenotypic evidence for the spread of three major methicillin-resistant *Staphylococcus aureus* clones associated with two characteristic antimicrobial resistance profiles in China. *J Antimicrob Chemother.* 2013;68:2453–2457. doi:10.1093/jac/dkt213
31. Chen H, Liu Y, Jiang X, Chen M, Wang H. Rapid change of methicillin-resistant *Staphylococcus aureus* clones in a Chinese tertiary care hospital over a 15-year period. *Antimicrob Agents Chemother.* 2010;54:1842–1847. doi:10.1128/AAC.01563-09
32. Beukers AG, Newton P, Hudson B, et al. A multicentre outbreak of ST45 MRSA containing deletions in the spa gene in New South Wales, Australia. *J Antimicrob Chemother.* 2020;75:1112–1116. doi:10.1093/jac/dkz560
33. Gao W, Cameron DR, Davies JK, et al. The rpoB H(4)(8)(1)Y rifampicin resistance mutation and an active stringent response reduce virulence and increase resistance to innate immune responses in *Staphylococcus aureus*. *J Infect Dis.* 2013;207:929–939. doi:10.1093/infdis/jis772

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