First Report of Oxacillin Susceptible *mecA*-Positive *Staphylococcus aureus* in a Children's Hospital in Kunming, China

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Purpose: The present study investigated the prevalence characteristics of oxacillin susceptible *mecA*-positive *Staphylococcus aureus* (OS-MRSA) in a children's hospital in Kunming from January 2019 to December 2020.

Methods: A total of 499 *S. aureus* strains were included in the study and tested for oxacillin susceptibility using the VITEK 2 Compact automated antimicrobial susceptibility test system. All oxacillin-susceptible strains were detected *mecA* and *mecC* by polymerase chain reaction (PCR). E-test was used to compare the minimum inhibitory concentration (MIC) values of methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), and OS-MRSA for oxacillin, cefoxitin, penicillin, vancomycin, erythromycin, and clindamycin. Molecular typing of OS-MRSA was performed by MLST and SCC*mec* typing. Toxin genes were detected by PCR.

Results: Forty-five OS-MRSA strains were detected, for an overall rate of 9.02% (45/499). The MICs of MSSA, OS-MRSA, and MRSA against oxacillin were concentrated at 0.38, 0.38, and 12 µg/mL, respectively; the cefoxitin MICs of MSSA and MRSA were concentrated at 2 and 32 µg/mL respectively; and MICs of OS-MRSA were concentrated at 2 and 8 µg/mL; penicillin, vancomycin and erythromycin MICs against MSSA, OS-MRSA, and MRSA showed same centralized points and were 32, 1, and 256 µg/mL, respectively; the MICs of clindamycin against MSSA were 0.5 µg/mL, while that against OS-MRSA and MRSA were concentrated at 256 µg/mL. Molecular typing of OS-MRSA was dominated by ST59-SCC*mec* IV. The carrier rates of hemolysin genes (*hl-a*, *hl-d*) and fibrinogen-binding clumping factor genes (*clfA*, *clfB*) were 100% in OS-MRSA, followed by 40% (18/45) for enterotoxin genes (*sea*, *seb*).

Conclusion: OS-MRSA has a high detection rate in children, and main molecular typing is ST59-SCC*mec* IV in Kunming. The identification ability of automated antibacterial drug sensitivity test detection systems for OS-MRSA is very limited. A combination of phenotypic analysis and molecular detection should be used to improve OS-MRSA identification.

Keywords: *Staphylococcus aureus*, MRSA, OS-MRSA, children, oxacillin

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the common pathogens causing nosocomial and community-acquired infections, often leading to severe infections such as endocarditis, osteomyelitis, and sepsis. The mechanism of MRSA resistance to methicillin and other β-lactam antibiotics is mainly due to the carriage of *mecA* on the mobile genetic element staphylococcal chromosomal cassette *mec* (SCC*mec*), which encodes the synthetic low-affinity penicillin-binding

protein PBP2a. Recent studies have found that the *mecC* gene also mediates drug resistance development.¹

MRSA drug resistance has become a global issue of the high concern. The correct selection of antibiotics to treat MRSA infections in clinical practice depends on accurate laboratory identification of MRSA strains. Positive detection of the *mecA* or *mecC* gene is the “gold standard,” but its application in clinical laboratories is limited due to the complex, time-consuming, and expensive testing. At present, clinical laboratories generally use phenotypic analyses to identify MRSA. According to the recommendations of the Clinical and Laboratory Standards Institute (CLSI), they mainly include the minimum inhibitory concentration (MIC) method of oxacillin and cefoxitin. That is, oxacillin MIC ≤ 2 $\mu\text{g/mL}$ or cefoxitin MIC ≤ 4 $\mu\text{g/mL}$ is determined as methicillin-susceptible *S. aureus* (MSSA); oxacillin MIC ≥ 4 $\mu\text{g/mL}$ or cefoxitin MIC ≥ 8 $\mu\text{g/mL}$ is determined as MRSA. There is also the disk diffusion method with 30 μg cefoxitin; that is, an inhibition zone diameter ≥ 22 or ≤ 21 mm is determined as MSSA or MRSA, respectively.² However, phenotypic analyses do not allow for the interpretation for strains with MICs between susceptible and resistant strains. More importantly, increasing studies have found that some clinical strains carry the *mecA* gene, but phenotypic analysis shows they are oxacillin or cefoxitin susceptible, named of Oxacillin-susceptible *mecA* gene-positive *S. aureus* (OS-MRSA).^{3–5} Routine phenotypic testing in clinical laboratories often misinterprets such strains as MSSA. Once these bacteria are exposed to β -lactam antibiotics, they are prone to induce MRSA with high resistance, resulting in clinical treatment failure.^{6,7} Therefore, identifying differences between OS-MRSA phenotypes and genotypes is challenging in clinical laboratory testing, and there are also risks for clinical empirical treatment.

There is a growing body of research on OS-MRSA in various regions and countries. Kampf et al⁷ showed that the detection rate of OS-MRSA was 1.6% in hospital healthcare workers, with six OS-MRSA strains that became highly resistant under repeated induction of oxacillin and cefotaxime. Only three true MRSA strains were detected in the present study, suggesting that *S. aureus* carried by healthcare workers may be insidious. Failure to identify such strains could lead to resistance development during antibiotic treatment, and then transmission of these strains to patients is more dangerous than MRSA. Petinaki et al⁸ detected OS-MRSA in up to 11.1% of Greek inpatients, suggesting that such strains might

have been spreading but traditional detection methods failed to identify them. Hososaka et al⁹ found that OS-MRSA was detected in 1.25% of cases in Japan, and SCC*mec* typing showed that the main type was type III with molecular characteristics similar to community acquired MRSA. The detection rates were 3%, 10.9%, 1.17%, 1.2%, and 1.64–3.78% in Virginia (US), Taiwan (China), Iran, Wessex region (UK), and China, respectively.^{5,10–14} OS-MRSA strains are also detected in other animal species. Pu et al¹⁵ found a high detection rate (17.31–62.5%) in cow mastitis in many areas of China, suggesting that OS-MRSA strains may be closely related to this condition. Mistry and colleagues also confirmed the high detection rate of OS-MRSA in cow mastitis (48.71%) in India.¹³ OS-MRSA has also been detected in cheese and meat foods, which can cause food poisoning.^{16–18} Therefore, OS-MRSA is widely prevalent throughout the world, but the detection rate and molecular prevalence characteristics have significant geographical distribution differences.

Despite the growing number of reports on OS-MRSA in recent years, the relevant studies mainly focused on the prevalence and distribution of OS-MRSA in various regions and countries with case reports and molecular mechanism studies. Notably, several studies have shown that up to 60% of OS-MRSA strains are isolated from children, suggesting that the carrier rate may be higher in this population.^{4,11,19} However, no further research has assessed the specific prevalence of OS-MRSA in children, including molecular types, toxin genes carriage and resistance to commonly used antibiotics. Because children have an immature immune system and are more susceptible to pathogen infection than adults, it is of great clinical significance and value to understand the prevalence characteristics of OS-MRSA strains in children. The present study is the first investigation of the prevalence characteristics of OS-MRSA in children, including Multilocus sequence typing (MLST) and SCC*mec* typing, toxin genes carriage, and MIC distribution characteristics against commonly used antimicrobial agents.

Materials and Methods

Strain Collection and Identification

Duplicate samples obtained from the same site from the same patient were excluded, and 499 *S. aureus* strains were isolated from children visiting Kunming Children's Hospital

from January 2019 to December 2020 (229 in 2019 and 270 in 2020). Clinical sample types included sputum, lavage fluid, pus, various secretions, venous blood, and pleural effusion. Each strain was identified by colony morphology observation, Gram staining microscopy, and VITEK 2 Compact Automated Antimicrobial Susceptibility Test System card GP (bioMérieux, Marcy-l'Étoile, France).

VITEK 2 Compact Automated Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed according to the manufacturer instructions for the VITEK 2 Compact automated antimicrobial susceptibility test. Bacterial suspensions with a McFarland turbidity of 0.5 were prepared. The instrument automatically added suspension to the antimicrobial susceptibility test card GP-67. After incubation for 18–24h, the Advanced Expert System™ system could automatically read and analyze the susceptibility of various antibacterial drugs. The GP-67 card enabled cefoxitin screening and MIC test for oxacillin. *S. aureus* ATCC29213 was used as the quality control strain.

Polymerase Chain Reaction (PCR) Detection of *mecA* and *mecC* Genes in Oxacillin-Susceptible Strains

Based on the VITEK 2 Compact antimicrobial susceptibility test results, all oxacillin-susceptible strains were subjected to PCR for *mecA* and *mecC* gene detection. Bacterial genomic DNA was extracted using a bacterial genomic DNA extraction kit (TaKaRa Biotechnology, Shiga, Japan). The primer sequences of the *mecA* and *mecC* are listed in Table 1. PCR conditions were 94 °C for 15 min, 30 × (94 °C for 30 s, 59 °C for 1min, 72 °C for 1 min), and 72 °C for 10 min.²⁰

E-Test to Compare the MICs of MSSA, OS-MRSA, and MRSA Strains

We randomly selected 30 MRSA strains and 30 MSSA strains for testing; all OS-MRSA strains were tested. We detected the MIC values against cefoxitin, oxacillin, penicillin, vancomycin, clindamycin, and erythromycin using E-test antimicrobial strips and Mueller-Hinton agar plates (Autobio Diagnostics, Zhengzhou, China). *S. aureus* ATCC29213 was used as a quality control strain, and the MIC₅₀ and MIC₉₀

Table 1 The Main Primers Used for PCR Amplification in the Study

Gene	Primer Sequence (5'-3')	Amplicon Size (bp)	Tm (°C)
<i>mecA</i>	F: TCCAGATTACAACCTTCACCAGG R: CCACTTCATATCTTGTAACG	162	59
<i>mecC</i>	F: GAAAAAAGGCTTAGAACGCCTC R: GAAGATCTTTCCGTTTTCAGC	138	59
<i>sea</i>	F: TTATCAATGTGCGGGTGG R: TTTCCTGTAAATAACGTCTTGC	219	54
<i>seb</i>	F: ACTGAGCATAATGGAAACCAA R: GCATCATGTCATACCAAAAGC	250	54
<i>hla</i>	F: CTGATTACTATCCAAGAAATTCGATTG R: CTTTCCAGCCTACTTTTTTATCAGT	210	57
<i>hld</i>	F: TACGTGGCAAACCTGGTCAA R: AGCACTGAGTCCAAGGAACTA	561	57
<i>clfA</i>	F: GCATTAGTTTAGCGGCAGTAG R: TAATTGGTGGCACTTTAGCAG	255	57
<i>clfB</i>	F: CGAAGAAAGTAGCGGTAAAGTAAG R: GTTCTGGATCTGGCGTTGG	482	57
<i>pvl</i>	F: ATCATTAGGTAAAATGTCTGGACATGATCC R: CATCAACTGTATTGGACATGATCCA	430	54
<i>tst-I</i>	F: GCCCTTTGTTGCTTGCG R: GGCTGATGCTGCCATCTGTG	213	54

represented the antibacterial drug concentrations that could inhibit the growth of 50% and 90% of bacteria, respectively.

MLST and SCCmec Molecular Typing of OS-MRSA Strains

All OS-MRSA strains were subjected to molecular typing by Multilocus sequence typing (MLST) and SCCmec typing. Seven housekeeping genes of MLST (*aecC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) primer sequences and reaction conditions amplified by PCR were obtained from the MLST public database (<https://pubmlst.org/>). SCCmec typing performed as described previously.²¹

Toxin Genes Detection of OS-MRSA Strains

Toxin genes of OS-MRSA strains were detected by PCR, including hemolysin (*hl-a*, *hl-d*), fibrinogen-binding clumping factor (*clfA*, *clfB*), enterotoxin (*sea*, *seb*), Pantón–Valentine leukocidin (*pvl*), and toxic shock syndrome 1 (*tst-I*); the primer sequences of toxin genes are shown in Table 1.²² The PCR amplification reaction conditions of *pvl*, *tst-I*, *sea*, and *seb* genes were 94 °C for 5 min, 30 × (94 °C for 30 s, 54 °C for 35 s, 72 °C for 1 min), and 72 °C for 10 min. Those for *hl-a*, *hl-d*, *clfA*, and *clfB* genes were: 94 °C for 5 min, 32 × (94 °C for 30 s, 57 °C for 35 s, 72 °C for 50 s), and 72 °C for 6 min.

Results

Sample Types and Patient Characteristics

The distribution of clinical sample types of 499 *S. aureus* strains were 239 (47.90%) from sputum; 114 (22.85%) from secretion; 90 (18.04%) from pus,

and the remaining 56 (11.2%) from blood, bone marrow, Bronchoalveolar Lavage Fluid (BLAF) and pleural effusion. The patient group comprised 305 males and 194 females, and the mean age was 3.39 ± 4.28 years (range: 1 day to 17 years).

Identification of OS-MRSA Strains

The VITEK 2 compact detection results showed that among 499 *S. aureus* strains, 345 (166 strains in 2019, 179 strains in 2020) were susceptible to oxacillin, 45 (20 strains in 2019, 25 strains in 2020) were positive for the *mecA* gene by PCR. No strains were positive for *mecC* genes. Carriers of OS-MRSA strains were mainly children ≤ 1 year old, accounting for up 55.56% (25/45). The main types of clinical specimens were sputum accounting for 33.33% (15/45), secretions accounting for 31.11% (14/45), pus accounting for 24.44% (11/45), and a few isolated from blood and BLAF, accounting for 8.89% (4/45) and 2.22% (1/45), respectively. Most patients were from orthopedic surgery department accounting for 22.22% (10/45), followed by the intensive care unit (ICU) and burn (wound) plastic surgery department, both 17.78% (8/45), and neonatology 15.56% (7/45). The remaining strains were detected in other departments such as general surgery, respiratory medicine, and hematological oncology (Figure 1).

MIC Distribution Characteristics of MSSA, OS-MRSA, and MRSA Strains

The MIC values of 30 randomly selected MSSA strains, 30 MRSA strains, and 45 OS-MRSA strains against oxacillin, cefoxitin, penicillin, vancomycin, clindamycin, and erythromycin were assessed with E-tests. The MICs of the

Figure 1 The distribution of patient ages, sample types and clinic departments among 45 OS-MRSA strains. (A) ages; (B) sample types; (C) clinic departments.

experimental quality control strain *S. aureus* ATCC29213 against six antimicrobial agents were within the quality control ranges. The distributions of MIC values of MSSA, OS-MRSA, and MRSA strains against oxacillin mainly concentrated at 0.38, 0.38, and 12 $\mu\text{g/mL}$, respectively; the distributions of cefoxitin MICs of MSSA and MRSA were concentrated at 2 and 32 $\mu\text{g/mL}$ respectively, and MICs of OS-MRSA were concentrated at 2 and 8 $\mu\text{g/mL}$; the distributions of penicillin, vancomycin, and erythromycin MICs against MSSA, OS-MRSA, and MRSA strains were mainly concentrated at 32, 1, and 256 $\mu\text{g/mL}$, respectively; the MIC of clindamycin against MSSA was 0.5 $\mu\text{g/mL}$, while those against OS-

MRSA and MRSA were mainly concentrated at 256 $\mu\text{g/mL}$ (Figure 2). Further analyses of the MIC₅₀ and MIC₉₀ results showed that the values against oxacillin and cefoxitin showed an upward trend for MSSA, OS-MRSA, and MRSA strains, as did the MIC₅₀ values against penicillin and clindamycin (Table 2). No vancomycin-resistant strains were identified.

MLST and SCCmec Molecular Typing of OS-MRSA Strains

Except for three OS-MRSA strains for which the MLST database could not determine the ST type, the remaining 42 OS-MRSA strains were distributed in 14 different ST

Figure 2 The MICs distribution of three kinds of *S. aureus* strains against oxacillin, cefoxitin, penicillin, vancomycin, clindamycin, and erythromycin.

Table 2 MIC₅₀ and MIC₉₀ Values of Three Kinds of *S. aureus* Strains Against Oxacillin, Cefoxitin, Penicillin, Vancomycin, Clindamycin, and Erythromycin

Antibiotics	MIC Range of ATCC29213 (μg/mL)	MSSA (n=30)		OS-MRSA (n=45)		MRSA (n=30)	
		MIC ₅₀ (μg/mL)	MIC ₉₀ (μg/mL)	MIC ₅₀ (μg/mL)	MIC ₉₀ (μg/mL)	MIC ₅₀ (μg/mL)	MIC ₉₀ (μg/mL)
Oxacillin	0.25	0.38	0.75	1	4	12	32
Cefoxitin	2	2	2	4	16	32	32
Penicillin	0.25–0.5	1.5	32	2	32	32	32
Vancomycin	1	1	1	1	1	1	1
Erythromycin	0.75	256	256	256	256	256	256
Clindamycin	0.094–0.5	0.5	256	0.38	256	0.75	256

types, of which ST59 (11/45) was the most predominant ST type, followed by ST338 (8/45) and ST22 (5/45). SCCmec IV (44/45) was the most common SCCmec classification (Table 3). Therefore, ST59-SCCmec IV is the most common type of OS-MRSA strain from children in Kunming, followed by ST338-SCCmec IV.

Toxin Genes Detection of OS-MRSA Strains

The results of PCR for the detection of five common toxin genes of *S. aureus* showed that the hemolysin (*hl-a*, *hl-d*) and fibrinogen-binding clumping factor (*clfA* and *clfB*) genes were present in 100% (45/45) of the OS-MRSA strains, followed by enterotoxin gene in 40% (18/45), which was mainly *seb* (17/18). The carrier rates of toxic shock syndrome (*tsst-I*) and Panton–Valentine leukocidin (*pvl*) genes were low at 6.67% (3/45) and 4.44% (2/45), respectively (Table 3).

Discussion

The present investigation was designed to assess the prevalence characteristics of OS-MRSA in children. To our knowledge, this is the first study on this topic. Previous reports on OS-MRSA were mainly in adult patients, even though some studies have shown that up to 60% of isolated OS-MRSA strains were from children, suggesting that that may have a higher carrier rate for this pathogen class. In the present study, the overall detection rate of OS-MRSA in children in Kunming was 9.02% for 2 years. The detection rate was 8.73% in 2019 and 9.30% in 2020 respectively, showing an increasing trend. The most common molecular type was ST59-SCCmecIV. Several groups have investigated the prevalence of OS-MRSA in China. He et al¹⁴ reported a detection rate of 3.78% that increased year by year (1.9–4.8%). The molecular types

of the strains were mainly ST59/338-t437-SCCmecIV/V. Song et al³ showed that the detection rate of OS-MRSA was 1.64% with the same molecular types of strains (mainly ST338/59-t437-SCCmecIV/V). The detection rate was slightly lower than that reported by He and colleagues, which may be due to the different cities included in the two studies. In addition, Liu et al¹⁹ showed that the detection rate of OS-MRSA was 1.8% in Shanghai, China, and the most common molecular type was ST59-SCCmecIV. Notably, 11 (64.71%) of the 17 OS-MRSA strains in that study were isolated from children. The results of Conceicao et al also showed that 60% of OS-MRSA strains were isolated from children.⁴ The molecular types detected in the present study were same with those reported in the relevant studies published in China, but the detection rate of OS-MRSA was significantly higher than that in the existing study reports. Possible reasons are as follows. (i) Previous relevant reports covered patients of all ages. These investigations focally reflected the overall prevalence of OS-MRSA in the region. Since the carrier rate of OS-MRSA may be different by age group, it could not accurately reflect the prevalence rates of such strains in children. (ii) The relevant studies showed that *S. aureus* ST59 was the main epidemic type in Asia, and the prevalence of ST59 was higher in children than in adults. This could lead to a higher carrier rate of OS-MRSA in children to a certain extent.^{23–25} (iii) Relevant studies reported that the antimicrobial susceptibility tests mainly used the broth microdilution method, which reveals higher susceptibility to MRSA.^{11,26} However, due to the complexity of the experiments and time required, this method is rarely used in clinical laboratories, which typically use automated antimicrobial susceptibility test detection systems. MSSA screening in the present study was based on the VITEK 2 Compact, an

Table 3 Characteristics of 45 OS-MRSA Strains in the Study

Strains	Molecular Typing		VITEK 2 Compact		Toxins							
	MLST	SCC _{mec}	Oxacillin MIC (µg/mL)	Cefoxitin Screening	PVL	TSST-I	Enterotoxin		Haemolysin		Cif	
					<i>pvl</i>	<i>tst-I</i>	<i>sea</i>	<i>seb</i>	<i>hl-a</i>	<i>hl-d</i>	<i>CifA</i>	<i>CifB</i>
1	ST188	IV	0.5	—	—	—	—	+	+	+	+	+
2	ST338	IV [*]	1	—	—	—	—	+	+	+	+	+
3	ST5	IV	0.5	—	—	—	—	+	+	+	+	+
4	ST398	IV	0.5	—	—	—	—	—	+	+	+	+
5	ST896	IV	≤0.25	—	—	—	+	+	+	+	+	+
6	ST30	IV	≤0.25	—	—	+	+	—	+	+	+	+
7	ST22	V	0.5	—	—	—	—	—	+	+	+	+
8	ST338	IV	≤0.25	—	—	—	—	—	+	+	+	+
9	ST22	IV	≤0.25	—	—	—	—	—	+	+	+	+
10	ST15	IV	≤0.25	—	—	—	—	—	+	+	+	+
11	ST2290	IV	0.5	—	—	—	—	—	+	+	+	+
12	ST5	IV	≤0.25	—	—	—	—	—	+	+	+	+
13	ST59	IV	2	+	+	+	—	—	+	+	+	+
14	ST59	IV	2	+	—	—	—	+	+	+	+	+
15	ST398	IV	0.5	+	—	—	—	—	+	+	+	+
16	ST59	IV	1	+	—	—	—	+	+	+	+	+
17	ST59	IV	1	+	—	—	—	+	+	+	+	+
18	ST398	IV	≤0.25	+	—	—	—	—	+	+	+	+
19	ST338	IV	2	+	—	—	—	—	+	+	+	+
20	ST25	IV	≤0.25	—	—	—	—	+	+	+	+	+
21	ST96	IV	≤0.25	+	+	—	—	—	+	+	+	+
22	ST30	IV	0.5	—	—	—	—	—	+	+	+	+
23	ST6	IV	≤0.25	—	—	—	—	—	+	+	+	+
24	ST338	IV	2	+	—	—	—	—	+	+	+	+
25	ST338	IV	2	+	—	—	—	—	+	+	+	+
26	ST5	IV	0.5	—	—	—	—	—	+	+	+	+
27	ST338	IV	0.5	—	—	—	—	+	+	+	+	+
28	ST338	IV	1	+	—	—	—	+	+	+	+	+
29	ST59	IV	1	+	—	—	—	—	+	+	+	+
30	ST338	IV	1	+	—	—	—	—	+	+	+	+
31	ST59	IV	1	+	—	—	+	+	+	+	+	+
32	ST59	IV	2	+	—	—	—	+	+	+	+	+
33	ST30	IV	≤0.25	—	—	+	+	+	+	+	+	+
34	ST59	IV	2	+	—	—	+	+	+	+	+	+
35	N	IV	2	+	—	—	—	—	+	+	+	+
36	N	IV	≤0.25	—	—	—	—	—	+	+	+	+
37	ST 59	IV	≤0.25	—	—	—	—	+	+	+	+	+
38	ST22	IV	4	—	—	—	—	—	+	+	+	+
39	ST2167	IV	2	+	—	—	—	—	+	+	+	+
40	ST59	IV	2	+	—	—	—	+	+	+	+	+
41	ST22	IV	0.5	—	—	—	—	—	+	+	+	+
42	ST5	IV	≤0.25	—	—	—	—	—	+	+	+	+
43	N	IV	1	+	—	—	—	—	+	+	+	+
44	ST59	IV	2	+	—	—	+	+	+	+	+	+
45	ST22	IV	4	+	—	—	—	—	+	+	+	+

automated antimicrobial susceptibility test system widely used in clinical laboratories, so the OS-MRSA detection rate is more in line with the actual clinical situation. In addition, the CLSI recommended the use of cefoxitin screening instead of oxacillin for MRSA identification in 2008. However, we found that 23 of 45 OS-MRSA strains were still negative for cefoxitin screening, so the identification ability of the automated antibacterial drug sensitivity test detection system for OS-MRSA was very limited. A combination of phenotypic analysis and molecular detection is required for accurate OS-MRSA identification.

In the present study, most OS-MRSA strains were isolated from children aged ≤ 1 year, which may be related to their immature immune systems that make them more susceptible to pathogens. The main types of clinical specimens were sputum (33.33%), secretion (31.11%), and pus (24.44%), which were consistent with the findings of Liu et al.¹⁹ Patients were commonly seen in the orthopedic surgery department, followed by the ICU and burn (wound) plastic surgery. Since most of the secretion and pus samples came from the orthopedic surgery, ICU, and burn (wound) plastic surgery departments, the types of specimen were consistent with the distribution of departments. Taking into account the analysis of disease characteristics of patients, many patients treated in these departments undergo invasive procedures such as surgery, ventilation, gastric tube placement, tracheal intubation or incision that can damage the skin and respiratory mucosal barriers, facilitating *S. aureus* infection.

There are few reports on the distribution characteristics of OS-MRSA MICs against antimicrobial agents. In the present study, we analyzed and compared the MIC distribution characteristics of MSSA, OS-MRSA, and MRSA against commonly used antimicrobials (oxacillin, cefoxitin, penicillin, vancomycin, erythromycin, and clindamycin). The MIC distribution characteristics of oxacillin in the three kinds of *S. aureus* strains showed that all MSSA strains were in the susceptible range (MIC ≤ 2 $\mu\text{g/mL}$). Except for three strains of MRSA with MIC of 3 $\mu\text{g/mL}$, the MICs of the other strains were in the resistant range (MIC ≥ 4 $\mu\text{g/mL}$). The MICs of 33 strains of OS-MRSA were in the susceptible range, and 10 strains were in the resistant range, with corresponding MIC values mainly concentrated in 0.38 and 4 $\mu\text{g/mL}$, respectively. As expected, the MSSA and MRSA MICs against cefoxitin were in the susceptible (MIC ≤ 4 $\mu\text{g/mL}$) and resistant (MIC ≥ 8 $\mu\text{g/mL}$) ranges, respectively. However, the MIC values of OS-MRSA showed that half were susceptible

and half were resistant, and there were also two centralized points of MIC values at 2 and 8 $\mu\text{g/mL}$. Oxacillin and cefoxitin are the antibacterial drugs recommended by the CLSI for clinical identification of MRSA. However, we found that there were two centralized points in the MIC values of OS-MRSA against these two drugs: one located in the susceptible range and one located at the susceptible cut-off point. Several groups reported that strains located at the cut-off point were often misinterpreted as MSSA, which was confirmed in the present study.^{27,28} There was no significant difference in the MIC distribution characteristics of MSSA, OS-MRSA, and MRSA strains against penicillin, vancomycin, and erythromycin. With regard to clindamycin MICs, the values of MSSA strains were mainly distributed at 0.5 $\mu\text{g/mL}$, while those of OS-MRSA were the same as those of MRSA, mainly concentrated at 256 $\mu\text{g/mL}$. Further analysis of the MIC₅₀ and MIC₉₀ of three types of strains against oxacillin, cefoxitin, penicillin, and clindamycin showed that the values of MSSA, OS-MRSA and MRSA strains against oxacillin and cefoxitin showed an upward trend, as did the MIC₅₀ values against penicillin and clindamycin. This suggests that OS-MRSA may be a special type of *S. aureus* with a unique resistance profile, and the resistance range was between that of MSSA and MRSA. Controversy remains surrounding the treatment of OS-MRSA infection and whether β -lactam antibiotics can be used in clinical practice, and several studies have found that OS-MRSA strains can be induced to become highly resistant once exposed to β -lactam antibiotics, resulting in clinical treatment failure.^{6,7}

Previous studies have shown that virulence factors play an important role in severe *S. aureus* infection, and the types and numbers of *S. aureus* strains carrying toxin genes are closely related to their invasiveness and pathogenicity.^{29,30} At present, there are very limited reports on the study of OS-MRSA virulence factors. We detected five common toxin genes of *S. aureus* and found that the carrier rates of the hemolysin (*hla*, *hld*) and fibrinogen-binding clumping factor (*clfA*, *clfB*) genes in OS-MRSA strains were up to 100% (45/45). The hemolysin gene is one of the most common toxins of *S. aureus*, and the *hla* and *hld* genes are important toxins causing *S. aureus* pneumonia and sepsis in clinical practice as they encode synthetic toxins that can destroy a variety of host cells and promote the development of inflammation. Fibrinogen-binding clumping factors encoded by the *clfA* and *clfB* genes are important

clumping factors of *S. aureus* that can mediate binding to fibrin or fibrinogen of host cells to inhibit phagocytosis and promote bacterial invasion into the host, especially in the initial stage of infection. The Hososaka study also showed a high hemolysin gene carrier rate in OS-MRSA strains, but the distribution of fibrinogen-binding clumping factor genes in OS-MRSA strains has not been reported.⁹ In addition, we found that enterotoxin genes associated with food poisoning (*sea*, *seb*) had a high detection rate (40%) in OS-MRSA strains, mainly carrying *seb*. However, the six OS-MRSA strains studied by Hososaka et al⁹ were not found to carry enterotoxin genes. One study reported that 47% of MRSA strains in Chinese pediatric ICUs carried enterotoxin genes, similar to the results of the present study,³¹ while another group reported *S. aureus* in 68.5% of acquired bloodstream infections in Chinese children's hospitals.³² Enterotoxin gene distributions may vary in different countries and regions with various population distributions and infection types. The low carrier rates of the toxic shock syndrome (*tsst-I*) and Panton–Valentine leukocidin gene (*pvl*) genes are consistent with the findings of Hososaka and colleagues.

Conclusion

We observed a high carrier rate (9.02%) of OS-MRSA in children, and the molecular typing was mainly ST59-SCC *mecIV* in Kunming. The MICs of OS-MRSA to antimicrobial agents are somewhat different from those of MSSA and MRSA and have unique distribution characteristics. Unfortunately, automated antibacterial drug sensitivity test detection system has very limited OS-MRSA identification ability. For children with *S. aureus* infection, it is important to determine the infected strain is OS-MRSA. Clinical laboratories should use a combination of phenotypic analysis and molecular detection to ensure accurate identification of these strains. This is the first study to describe the prevalence characteristics of OS-MRSA in children, but the limitations include the short surveillance duration and small sample size. Future prospective, longer-term investigations are needed to confirm the conclusion of the present study.

Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki. The research protocol was approved by the Ethics Committee of Kunming Children's Hospital (reference number 2020-03-020-K01). The Ethics Committee of Kunming Children's

Hospital did not require written informed consent from patients because the data of the patients used in the study were anonymized or maintained with confidentiality.

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Disclosure

The authors declare that they have no conflicts of interest for this work.

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