Current status of oxacillin-susceptible mecA-positive Staphylococcus aureus infection in Shanghai, China: A multicenter study

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Abstract

The worldwide reported oxacillin-susceptible mecA -positive Staphylococcus aureus (OS-MRSA) represents a distinctly important challenge to detection and treatment of MRSA, but finite data on current status of OS-MRSA infection in Chinese hospitals are available. The present multicenter study carried out a battery of phenotypic susceptibility tests as well as diagnostic tests (PBP2a detection, mecA , and mecC PCR) for a collection of 956 clinical S. aureus isolates from 10 hospitals in Shanghai, molecular typing was performed for all identified OS-MRSA strains. OS-MRSA represented 1.8% (17/956) of total isolates and were commonly borderline oxacillin-susceptible (Oxacillin-MIC of 1 or 2 mg/L). 10 of 17 OS-MRSA were ST59 lineages, followed by ST965 (3/17). Unlike oxacillin-resistant MRSA that commonly exhibit a multidrug resistant (MDR) phenotype, OS-MRSA were less likely to be MDR and displayed MIC pattern remarkably differential from OR-MRSA. OS-MRSA showed oxacillin-inducible oxacillin resistance and the majority of them (15/17) were cefoxitin-resistant by cefoxitin disk diffusion. S. aureus with borderline susceptible oxacillin MICs (1 or 2 mg/L) should be confirmed by cefoxitin disk diffusion in clinical practice, whereas susceptible isolates reclassified by cefoxitin disk diffusion should still be subjected to PBP2a testing and (or) mecA (mecC) PCR. The presented study has characterized phenotypically and molecularly an atypical type of MRSA showing cryptic but inducible resistance to oxacillin, but its underlying mechanisms warrant further elucidation.

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA), which stands for the most serious issue of gram-positive bacteria [1], has acquired resistance to methicillin and other β-lactams by either the altered penicillin-binding protein, namely PBP2a, encoded by *mecA* or *mecC* (*mec*A_{LGA251}) [2], or other mechanisms [3]. Appropriate antimicrobial therapy against MRSA relies on accurate detection of methicillin (oxacillin) resistance. PCR amplification of *mecA* is a recognized “gold standard” for MRSA identification [4, 5], but clinical laboratories routinely employ phenotypic susceptibility tests to detect *mecA*-mediated resistance, including oxacillin minimum inhibitory concentration (MIC) and cefoxitin disk diffusion assay, as Clinical Laboratory Standards Institute (CLSI) has recommended [6] .

MRSA are typically resistant to oxacillin, but this is not always the case. Cryptic antibiotic-resistant organisms in *S. aureus*, such as oxacillin-susceptible MRSA (OS-MRSA), which carry *mecA* or *mecC* but show MIC of oxacillin in the susceptible range (≤2 mg/L), have been described worldwide over recent years [7-10]. However, the prevalence of OS-MRSA in Chinese hospitals was reported to be 1.6% (34 of 2068) only in a single study [10], limited study has investigated the characteristics of OS-MRSA currently prevalent within Chinese hospitals and offered insights into the management of this special group of MRSA for clinical laboratories.

Such phenotypic oxacillin susceptibility of OS-MRSA has contributed to their misidentification as methicillin-sensitive *S. aureus* (MSSA) by conventional phenotypic susceptibility tests, thus leading to potential therapeutic failure [8]. Moreover, OS-MRSA could rapidly generate high-level oxacillin-resistant subclones upon oxacillin exposure [11]. Therefore, it’s indispensable to provide more detailed characterization of OS-MRSA and optimize detection procedures for reducing their misreport. With these purposes, we performed a battery of phenotypic susceptibility assays as well as diagnostic tests (PBP2a detection, *mecA*, and *mecC* PCR) for a collection of 956 clinical *S. aureus* isolates from 10 hospitals in Shanghai. For isolates matching the criterion of OS-MRSA (OXA-MIC by broth microdilution ≤2 mg/L but *mecA*-positive), in addition to antimicrobial susceptibility tests, molecular typing including multi-locus sequence typing (MLST), *spa* typing, and SCC*mec* typing were carried out to understand their genotypic characteristics.

Methods

Bacterial isolates

A collection of clinical *S. aureus* isolates was recovered from 10 hospitals in Shanghai between Feb 2017 and Sep 2018. All of the collected isolates were subsequently validated as *S. aureus* by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS; Bruker Daltonics); then a total of 956 *S. aureus* isolates was included in this study. All isolates were kept at 80°C in tryptic soy broth (TSB) containing 30% glycerol and sub-cultured to ensure purity before testing.

Antimicrobial susceptibility testing by the Vitek2 system

Antimicrobial susceptibility testing (AST) by Vitek2 (BioMerieux) was conducted by inoculating a bacterial suspension equivalent to a 0.5 McFarland standard into Vitek2 AST card and then performing on the instrument according to the manufacturer's instructions. Notably, as the Vitek2 AST card incorporates cefoxitin screen as well as the determination of oxacillin MIC, MSSA was interpreted as both oxacillin sensitive and cefoxitin screen negative by the VITEK® 2 software (version 8.01)'s Advanced Expert System™ (AES) for on-line result validation [12].

Determination of MIC by broth microdilution

Broth microdilution assay was performed according to CLSI recommendations. Briefly, a bacterial suspension equivalent to a 0.5 McFarland standard was inoculated into cation-adjusted Mueller-Hinton broth (with the addition of 2% NaCl for testing oxacillin) and then incubated for 24h at 35 ℃. Oxacillin was tested at concentrations ranging on a log2 scale from 0.25 to 128 g/ml, for each isolate oxacillin-MIC determining was repeated 2 or 3 times to validate oxacillin susceptibility. *mecA*-positive isolates whose oxacillin-MICs determined by broth microdilution were ≤2 mg/L were identified as OS-MRSA. The reference strains *S. aureus* ATCC 29213 and ATCC 43300 were used as negative and positive quality control, respectively. Results of all antibiotics were interpreted by CLSI's latest interpretative criteria [6], except amikacin [13] .

Disk diffusion testing

The disk diffusion assay was also performed according to CLSI recommendations. In brief, Mueller-Hinton agar plates were inoculated by swabbing the surfaces with a 0.5 McFarland standard suspension for *S. aureus* isolates and incubated for 24h at 35°C after a cefoxitin disk was placed on the surface. Isolates were to be considered cefoxitin-resistant when the diameters of inhibition were less than or equal to 21 mm, in accordance with the CLSI breakpoint [6]. Each isolate was tested repeatedly 2 or 3 times to confirm its cefoxitin-susceptibility. *S. aureus* ATCC 25923 and ATCC 43300 were used as negative and positive quality control, respectively.

Amplification of the *mecA* and *mecC* gene, PBP2a detection

PCR for detecting the *mecA* and *mecC* genes was performed as previously described [14]. Standard phenol–chloroform extraction procedure was used to prepare DNA template. PCR products were visualized on 1.5% agarose gels. Positive results were indicated by the presence of specific amplified DNA fragment. For PBP2a detection, the Alere PBP2a SA culture colony test (Alere, San Diego, CA) was performed according to the manufacturer's instructions.

Molecular typing

Chromosomal DNA was extracted following culture on blood agar plates by a standard phenol–chloroform extraction procedure and used as template for PCR reaction. Multi-locus sequence typing (MLST) was carried out as previously described by sequencing an internal fragment of seven unlinked housekeeping genes to determine allelic profiles in the following: carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glp*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triosephosphate isomerase (*tpi*), and acetyl coenzyme A acetyltransferase (*yqiL*) [15]. The sequences of the PCR products were assigned allele numbers by comparing with the existing sequences available on the MLST website (http://www.pubmlst.net) for *S. aureus,* and the alleles of the seven genes define the *S. aureus* lineage, resulting in an allelic profile designated ST. *spa* typing was carried out by amplification and sequencing of polymorphic X region of *spa*, whereafter *spa* types were assigned by using the *spa* database website (https://www.spaserver.ridom.de). The SCC*mec* typing was performed as described previously [16].

Inducibility of oxacillin resistance

To determine whether oxacillin-resistance of OS-MRSA strains is oxacillin-inducible, we employed a method adapted from what Nicas et al. described [17]. Culture of OS-MRSA was grown overnight at 37℃ in TSB containing oxacillin of 0.5-fold MIC, the overnight culture was then subcultured by being diluted 1:100 into fresh TSB with 1 g/mL oxacillin, the obviously grown subculture (at an approximate OD_{600} of 2) was successively subcultured into fresh TSB with gradually increasing concentrations of oxacillin (Log_2 scale from 2 to 32 g/mL). The successfully grown culture with 32 g/mL oxacillin was plated onto MHA plates supplemented with oxacillin of 4 g/mL, colonies that formed on MHA were used for confirming oxacillin resistance by broth microdilution. Cultures that failed to grow with oxacillin within 5 days was considered oxacillin-noninducible.

Results

OS-MRSA are commonly borderline oxacillin susceptible

Vitek2 system is one of the automated susceptibility testing (AST) systems widely used in clinical microbiological laboratories, however, with *mecA* gene PCR as the gold standard, we noticed that Vitek2-Oxacillin miscategorized an especially high proportion (33%, 15/46) of *S. aureus* isolates for which the Vitek2-Oxacillin MIC were 1 or 2 mg/L, furthermore, CLSI-recommended reference broth microdilution (rBMD) confirmed the oxacillin-susceptibility of 13 out of the 15 miscategorized *mecA*-positive isolates (Table 1). Another 4 *mecA*-positive *S. aureus* isolates with Vitek2-OXA MIC of ≤0.25 mg/L and 0.5 mg/L were further validated as oxacillin susceptible by reference broth microdilution, thus a total of 17 OS-MRSA strains was identified (Table 2).

Oxacillin-MIC of more than half of these OS-MRSA strains (11/17) were determined to be 1-2 mg/L by rBMD (close to CLSI oxacillin-susceptible breakpoint), namely borderline oxacillin susceptible (Table 2). Additionally, OS-MRSA were more frequently found in *S. aureus* isolates with Vitek2-oxacillin MIC of 1 or 2 mg/L (13/46, 28.26%), whereas less frequently isolated among isolates with Vitek2-oxacillin MIC of ≤0.25 mg/L and at 0.5 mg/L (0.32% and 1.25%, respectively) (Figure 1).

The frequent occurrence of OS-MRSA in *S. aureus* isolates with Vitek2-oxacillin MIC of 1 or 2 mg/L) partly explain why false susceptible results by Vitek2 system were predominately found in these

borderline susceptible population, while with the exception of these borderline susceptible isolates (1-2 mg/L), categorical results of Vitek2-oxacillin highly agree with those of *mecA* PCR, cefoxitin disk and oxacillin MIC by rBMD (99.2%-100%) (Table 1).

Genotypic and phenotypic characteristics of OS-MRSA

ST59 clones represented the predominant clone for 58.8% (10/17) of OS-MRSA based on MLST, followed by ST965 (3/17, 17.6%) and ST630 (2/17, 11.8%) (Table 2, Figure 2A). In addition to MLST, *spa* typing revealed that 7 of 10 ST59 OS-MRSA isolates were t172, one of four remaining isolates was t163, and the other two isolates were t437. Of 17 OS-MRSA isolates, 11 carried SCC*mec* type IV, other 5 carried SCC*mec* type V (Table 2). Taking all typing methods into account, ST59-t172-IV was the major contributor of OS-MRSA (Table 2).

In addition, large parts of OS-MRSA were recovered from pediatric patients (11/17; 64.7%) (Figure 2B), yet only 23.3% (223/956) of total isolates were from pediatric patients.

Though susceptible to oxacillin, 15 out of 17 OS-MRSA were classified as cefoxitin-resistant by cefoxitin disk diffusion (Figure 2C). Further antimicrobial susceptibility tests revealed that unlike oxacillin-resistant MRSA (OR-MRSA) that commonly exhibited a multi-drug resistant (MDR) phenotype, OS-MRSA isolates displayed a significantly lower MDR rate and were less likely to be MDR ($p<0.05$), and so were MSSA (Table 3). Moreover, resistant rates of OS-MRSA were near to those of MSSA for all antibiotics tested ($p>0.05$) but were obviously lower relative to that of OR-MRSA for several kinds of antibiotics ($p<0.05$) (Table 3). What's more, the remarkably differential MIC pattern between OS-MRSA and OR-MRSA further suggested that OS-MRSA appeared to be less susceptible *in vitro* to a majority of representative antibiotics, including cefazolin, one of the first-generation cephalosporin (Figure 2D).

Notably, oxacillin resistances of all identified OS-MRSA strains were inducible, as prior incubation with oxacillin *in vitro* could convert all OS-MRSA isolates to highly oxacillin-resistant strains (MIC ≥32mg/L), in which the presence of *mecA* perhaps play a part (Table 2).

Optimized detection procedures for OS-MRSA

OS-MRSA is a particular type of MRSA and inherently oxacillin-susceptible. Its phenotypic trait makes OS-MRSA easier to be misinterpreted as MSSA by conventional phenotypic tests based on oxacillin susceptibility (Table 1 and Table 2).

Accordingly, special attention should be paid to borderline susceptible *S. aureus* population (OXA MICs of 1-2 mg/L) in routine tests, considering the frequent appearance of OS-MRSA in borderline oxacillin susceptible group (Table 1, Figure 1). Though the Vitek2 AST card incorporates oxacillin MIC as well as cefoxitin screening to provide simultaneous determination for *S. aureus,* 11 of 17 OS-MRSA strains still couldn't be adjusted to MRSA by the AES software on the basis of the cefoxitin result (Table 2, Figure 2C). By contrast, 15 of 17 OS-MRSA could be corrected to cefoxitin-resistant MRSA by disk diffusion test (Table 2, Figure 2C). Generally, the results of cefoxitin disk diffusion highly correlated with *mecA* gene PCR; it was found to be 100% specific and demonstrated higher sensitivity (98.4%) in detecting *mecA* positive isolates than the Vitek2 system (Table 4). However, the PBP2a test yielded better correlation with

mecA PCR (Table 4) as compared to the cefoxitin disk diffusion test, whereas *mecA* PCR, as well as PBP2a test, are costly and not accessible for all clinical microbiological laboratories.

For these reasons, it's recommended that oxacillin-susceptible *S. aureus* with borderline MIC (1-2 g/mL) and negative cefoxitin screen by Vitek2 should be retested in routine management by cefoxitin disk diffusion, which is accessible to nearly all microbiological laboratories. These supplementary tests are not laborious as borderline oxacillin-susceptible population only represent a small part of all (46/956, 5%). Notably, in case the results obtained by cefoxitin disk diffusion test remain negative, final reports should be based on detection of *mecA* or PBP2a.

Discussion

OS-MRSA has frequently been reported in geographically distinct regions [7, 9, 18, 19], yet limited studies have investigated the characteristics of OS-MRSA amongst Chinese mainland hospitals and provided management for reducing misreport of this cryptic MRSA in clinical practice. Moreover, the prevalence of OS-MRSA among *mecA*-positive *S. aureus* (17 of 377, 4.5%) in the present study and their inducible high-level oxacillin resistance both imply that such atypical MRSA deserve significant attention.

OS-MRSA was initially regarded as a subclass of community-associated MRSA (CA-MRSA) mainly due to its antimicrobial susceptibility, coagulase type and toxin genes, which were close to CA-MRSA but distinguishable from healthcare-associated MRSA (HA-MRSA) [7, 20]. Similarly, though clinical MRSA commonly behave as MDR [21], OS-MRSA strains in our study were less likely to be MDR, for which the frequent presence of SCC*mec* type IV element in OS-MRSA isolates may account. The SCC*mec* type IV element, which is closely associated with prevalent CA-MRSA strains, lacks genes conferring non-β-lactam antimicrobial resistance that type II and III elements commonly carry [22]. MLST characterized 59% of OS-MRSA in this study as ST59, the most widespread CA-MRSA clones in large parts of Asia [23]. Although initially considered as a lineage exclusively associated with CA-MRSA, ST59 clones have been observed generating nosocomial infection readily [24-26]. The growing prevalence of ST59-MRSA in Chinese hospitals has been previously described [21], suggesting its successful spread among healthcare settings. Moreover, the predominance of ST59 clones was more evident among Chinese children in both communities and hospitals [27, 28], which partly explains the frequent recovery of OS-MRSA in children rather than in adults as observed in this study. Furthermore, these oxacillin-susceptible ST59-MRSA strains are able to disseminate unnoticed in hospital environment due to improper antibiotic therapy, probably contributing to their rapid and successful prevalence.

Even if the OS-MRSA collected in our study represented a low proportion (1.8%) compared with OR-MRSA, it should be noted that as the dominant OS-MRSA-associated clones, the Asian epidemic CA-MRSA ST59 clones demonstrate more pronounced virulence over HA-MRSA ST5 and ST239 clones, to which the enhanced expression of several virulent determinants (e.g. α-toxin, PSMα) contributed [29]. Furthermore, oxacillin resistance of OS-MRSA is inducible, exposure to oxacillin convert OS-MRSA into highly resistant clones, possibly due to the instability of *mecA* gene carried by OS-MRSA, as disclosed by a recent study. Therefore, efficient and timely detection for OS-MRSA should be initiated to prevent patients from worse consequences, particularly for isolates from cases in which β-lactam therapy failed.

However, accurate detection of OS-MRSA is challenging, as conventional susceptibility tests probably generate false susceptible results because of their phenotypic oxacillin susceptibility. Additionally, a rare type of MRSA, *mecC*-positive MRSA, may be easily misreported as well because of its borderline resistant phenotype [30], although none was detected in this study. Despite the widely applied Vitek2 system's capability to produce results rapidly, its deficiency in detecting some of OS-MRSA has been noticed in the present trial, even if accuracy of Vitek2 obviously improved with the addition of a cefoxitin screen to the test panels, as suggested previously [4, 31]. Even more noteworthy is the particularly frequent recovery of OS-MRSA among borderline oxacillin susceptible group (1-2 mg/L), which perhaps warrant an intermediate category (1-2 mg/L) in CLSI guideline. Also, special care and supplementary confirmatory testing for those borderline oxacillin susceptible isolates should be taken in clinical practice. Cefoxitin has been considered a better predictor of the presence of *mecA* than oxacillin, as it has been observed that cefoxitin disk diffusion provided considerable sensitivity for detection of *S. aureus* expressing borderline oxacillin MICs [32, 33]. In our evaluation, the cefoxitin disk diffusion test correctly classified the majority of OS-MRSA (15/17) as compared to Vitek2 cefoxitin screening (6/17). Therefore, the cefoxitin disk diffusion test represents a reliable alternative to avoid misidentification as MSSA for *S. aureus* isolates with borderline OXA-MIC that may escape from routine susceptibility tests by automated AST systems, such as Vitek2.

The occurrence of OS-MRSA also raises concerns about optimal therapeutics to target infections. It is commonly believed that most OS-MRSA strains exhibit hetero-resistance to oxacillin, which results in treatment failure of β-lactam antibiotics [8]. Notably, mupirocin could lead to conversion of OS-MRSA from a heterogeneous into a highly homogeneous resistant phenotype [34]. Dicloxacillin, a derivative of oxacillin, demonstrated comparable efficacy with vancomycin against OS-MRSA [35]. Nevertheless, we found that all OS-MRSA could be induced to highly oxacillin-resistant clones following exposure to oxacillin *in vitro*. Therefore, this atypical type of MRSA should be treated with antibiotics active against MRSA (e.g., linezolid, vancomycin) instead of β-lactams or according to results of susceptibility testing, as some studies have suggested [8].

The underlying mechanisms responsible for OS-MRSA are still not entirely clear, the genetic background may play amajor role in regulation of β-lactam resistance in MRSA [36]. A previous study ascribed the reduced oxacillin resistance in OS-MRSA to the *bla* system with a truncated *blaR1* gene [37]; additionally, nucleotide polymorphisms within the *mecA* gene sequences of clinical ST59 strains appear to have a substantial influence on their oxacillin MICs [38]. Other factors outside *mecA*, such as lack of penicillin binding protein 4 (PBP4) [39] and Fem enzyme activity [40], may play a part in low oxacillin responsiveness.

The study as presented investigated phenotypical and genotypic characteristics of atypical MRSA showing cryptic but inducible resistance to oxacillin. Cefoxitin disk diffusion test and, if available, *mecA* PCR or PBP2a detection should be supplemented into the routine procedure and would reduce misreport when encountering *S. aureus* with borderline susceptible OXA-MICs. The genetic characteristics and potential factors that mediate the reduced oxacillin resistance among these OS-MRSA strains remain to be elucidated in our further study.

Conclusions

OS-MRSA is an increasingly important issue for both clinical laboratories and clinician. Its phenotypic oxacillin susceptibility make itself easier to be misreported by the widely used Vitek2 system. Most of OS-MRSA was found to be ST59 lineages, the Asian epidemic CA-MRSA clone. OS-MRSA were less likely to be MDR and displayed MIC pattern remarkably differential from OR-MRSA. Considering the borderline oxacillin susceptibility of MRSA and the considerable performance of cefoxitin disk diffusion, *S. aureus* with Vitek2-OXA MIC of 1-2 mg/L and negative cefoxitin screen should be subjected to supplementary tests by cefoxitin screen, and if available, *mecA* (*mec*

C) PCR and (or) PBP2a detection.

Abbreviations

MSSA: Methicillin-sensitive Staphylococcus aureus; MRSA: Methicillin-resistant Staphylococcus aureus; OS-MRSA: Oxacillin-susceptible *mecA*-positive *Staphylococcus aureus*; OR-MRSA: Oxacillin-resistant *mecA*-positive *Staphylococcus aureus*; PCR: Polymerase chain reaction; MIC: Minimum inhibitory concentration; MLST: Multi-locus sequence typing; *Spa*: *Staphylococcus aureus* protein A gene; SCC*mec*: Staphylococcal cassette chromosome *mec*; AST: Automated susceptibility testing; rBMD: Reference broth microdilution; TSB: Tryptic soy broth; MALDI-TOF-MS: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry; AES: Advanced expert system; ST: sequence type; MHA: Muller-Hinton agar; MDR: Multi drug resistant; CLSI: Clinical Laboratory Standards Institute.

Declarations

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Authors' contributions

LJ, LT, and ZN performed the experiments and wrote the manuscript. WX, JJ, ZW, TR, GY, LY, HJ, and HL participated in collection of isolates and data analysis. WW and TJ supported with experimental planning. LM conceived of and designed the experiments and extensively reviewed and critically revised the manuscript. All authors read and approved the final manuscript.

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analysis and interpretation of results in this study.

Ethics approval and consent to participate

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Institutional Review Board Ethics Committee of Renji Hospital, School of Medicine, Shanghai Jiaotong University. For this type of retrospective study, formal consent is not required.

Availability of data and materials

The datasets used and analyzed during the present study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

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Tables

Table 1 Categorical agreement between Vitek2-oxacillin MIC and indicated methods for a collection of 956 *S. aureus* isolates[a]

Vitek2-oxacillin MIC (mg/L)(No. of isolates,proportion)	VITEK2 Cefoxitin screen		Cefoxitin disk diffusion		Oxacillin MIC by broth microdilution		*mecA*		*mecC*	
	+	—	R	S	R	S	+	—	+	—
	No. of isolates (% agreement)									
≤0.25(n=312, 33%)	0(0%)	312(100%)	0(0%)	312(100%)	0(0%)	312(100%)	1(0.3%)	311(99.7%)	0	312
0.5(n=241, 25%)	1(0.4%)	240(99.6%)	2(0.8%)	239(99.2%)	2(0.8%)	239(99.2%)	5(2.1%)	236(97.9%)	0	241
1-2(n=46, 5%)	**7(15.2%)**	**39(84.8%)**	**15(32.6%)**	**31(67.4%)**	**2(4.3%)**	**44(95.7%)**	**15(32.6%)**	**31(67.4%)**	**0**	**46**
≥4(n=357, 37%)	356(99.7%)	1(0.3%)	354(99.2%)	3(0.8%)	356(99.7%)	1(0.3%)	356(99.7%)	1(0.3%)	0	357

[a]Results were interpreted according to the CLSI M100 recommendations: Cefoxitin disk diffusion (30mg): R:≤21mm, S:≥22mm; Oxacillin MIC: R≥4 (mg/L), S:≤2 (mg/L).

Table 2 Phenotypic and genotypic characteristics for 17 clinical oxacillin-susceptible *mecA*-positive *S. aureus*(OS-MRSA) isolates[a]

Strain	Vitek2 OXA-MIC(mg/L)	Vitek2 FOX-Screen	OXA-BMD(mg/L)	FOX-DD(mm)	*mecA*	PBP2a	Clonal lineage	SCC*mec* type	OXA-inducible OXA-resistance
1	≤0.25	—	0.5	22	+	+	ST398-t034	V	+
2	0.5	—	0.5	19	+	+	ST59-t437	IV	+
3	0.5	—	1	20	+	+	ST59-t437	IV	+
4	1	—	1	18	+	+	ST59-t163	IV	+
5	1	—	2	15	+	+	ST59-t172	IV	+
6	1	—	2	19	+	+	ST59-t172	IV	+
7	2	—	0.5	18	+	+	ST59-t172	V	+
8	2	—	2	12	+	+	ST59-t172	IV	+
9	2	—	2	18	+	+	ST59-t172	IV	+
10	2	—	0.5	15	+	+	ST121-t2086	IV	+
11	2	—	0.5	15	+	+	ST630-t4549	V	+
12	2	+	2	20	+	+	ST630-t4549	V	+
13	1	+	1	10	+	+	ST965-t062	IV	+
14	1	+	2	13	+	+	ST965-t062	IV	+
15	2	+	0.5	11	+	+	ST965-t062	IV	+
16	2	+	2	12	+	+	ST59-t437	V	+
17	0.5	+	1	30	+	+	ST59-t172	NT	+

[a]Abbreviations: FOX: Cefoxitin; BMD: Broth microdilution; DD: disk diffusion test; NT: not typeable.

Table 3 Resistance rates of MSSA, OS-MRSA, and OR-MRSA to 10 classes of antimicrobial agents[a]

Antimicrobial agents	MSSA(n=25)	OS-MRSA(n=17)	OR-MRSA(n=25)	*P* value[c]	
				OS-MRSA vs MSSA	OS-MRSA vs OR-MRSA
AMK	0%	5.9%	8%	0.4048	>0.9999
ERY	36%	52.9%	88%	0.3483	**0.0289**
TET	0%	5.9%	68%	0.4048	**<0.0001**
SXT	8%	5.9%	8%	>0.9999	>0.9999
RIF	0%	5.9%	4%	0.4048	>0.9999
LEV	12%	17.6%	76%	0.6720	**0.0004**
DA	28%	41.2%	72%	0.5076	0.0605
LZD	0%	0%	0%	NA	NA
VAN	0%	0%	0%	NA	NA
CZO(MIC_{50})[b]	0.25	1	256	NA	NA
MDR rates	16%	23.5%	76%	0.6939	**0.0014**

[a]Abbreviations: AMK, amikacin; ERY, erythromycin; CZO, cefazolin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; RIF, Rifampin; LEV, levofloxacin; DA, clindamycin; LZD, linezolid; VAN, vancomycin; MDR, multi-drug resistant (resistant to ≥ 3 classes of antibiotics except β-lactams). Antibiotic resistances were interpreted according to CLSI recommendations based on MICs obtained by broth dilution method.

[b]Results of susceptibility tests for cefazolin were shown as MIC_{50} (mg/L) due to the absence of breakpoints in CLSI recommendations.

[C]The differences between resistances (%) of indicated groups were tested by Fisher exact tests, $p<0.05$ was considered significantly different.

TABLE 4 Performance of different methods in detecting a collection of 956 clinical isolates of *S. aureus*[a]

Method	*mecA* positive	*mecA* negative	Performance, %(95% Confidence Interval)			
	No (%)	No (%)				
			Sensitivity	Specificity	PPV[a]	NPV[a]
Vitek2	377(39.4%)	579(60.6%)	96.6(94.0-98.1)	99.8(98.9-100)	99.7(98.2-100)	97.8(96.2-98.8)
Oxacillin by BMD[a]			95.5(92.7-97.3)	100(99.2-100)	100(98.7-100)	97.1(95.4-98.3)
Cefoxitin disk diffusion			98.4(96.4-99.4)	100(99.2-100)	100(98.7-100)	99.0(97.7-99.6)
PBP2a test			100(98.7-100)	100(99.2-100)	100(98.7-100)	100(99.2-100)

[a]Abbreviations: BMD, Broth microdilution; PPV, positive predictive value; NPV, negative predictive value

Figures

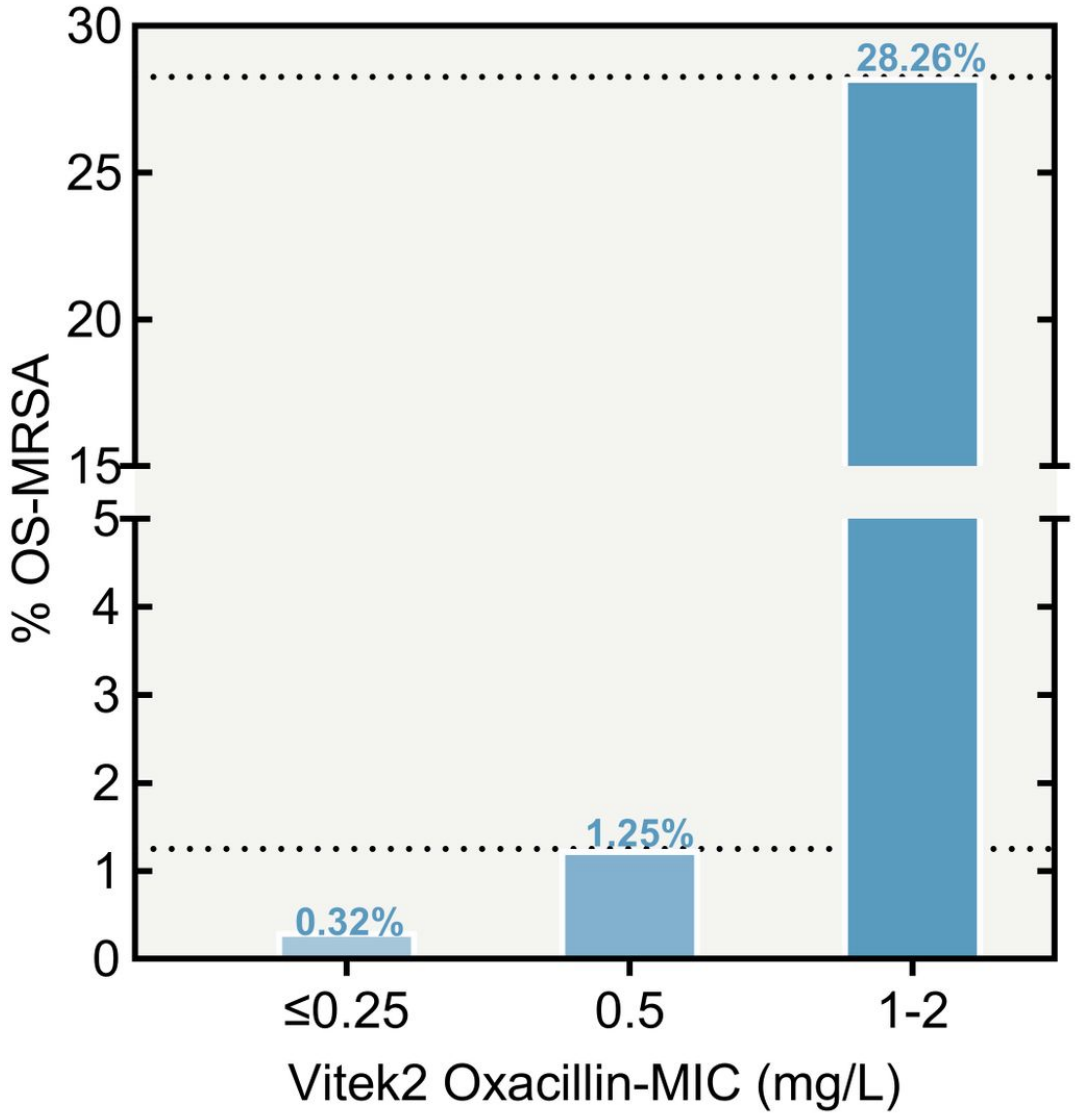

Figure 1

The prevalence of OS-MRSA among S. aureus with different Vitek2-determined oxacillin MICs. The percentages of OS-MRSA in indicated group are shown above the columns. The number of included isolates with Vitek2-OXA MIC of≤0.25 mg/L, 0.5 mg/L, and 1-2 mg/L are 312, 241, and 46 respectively.

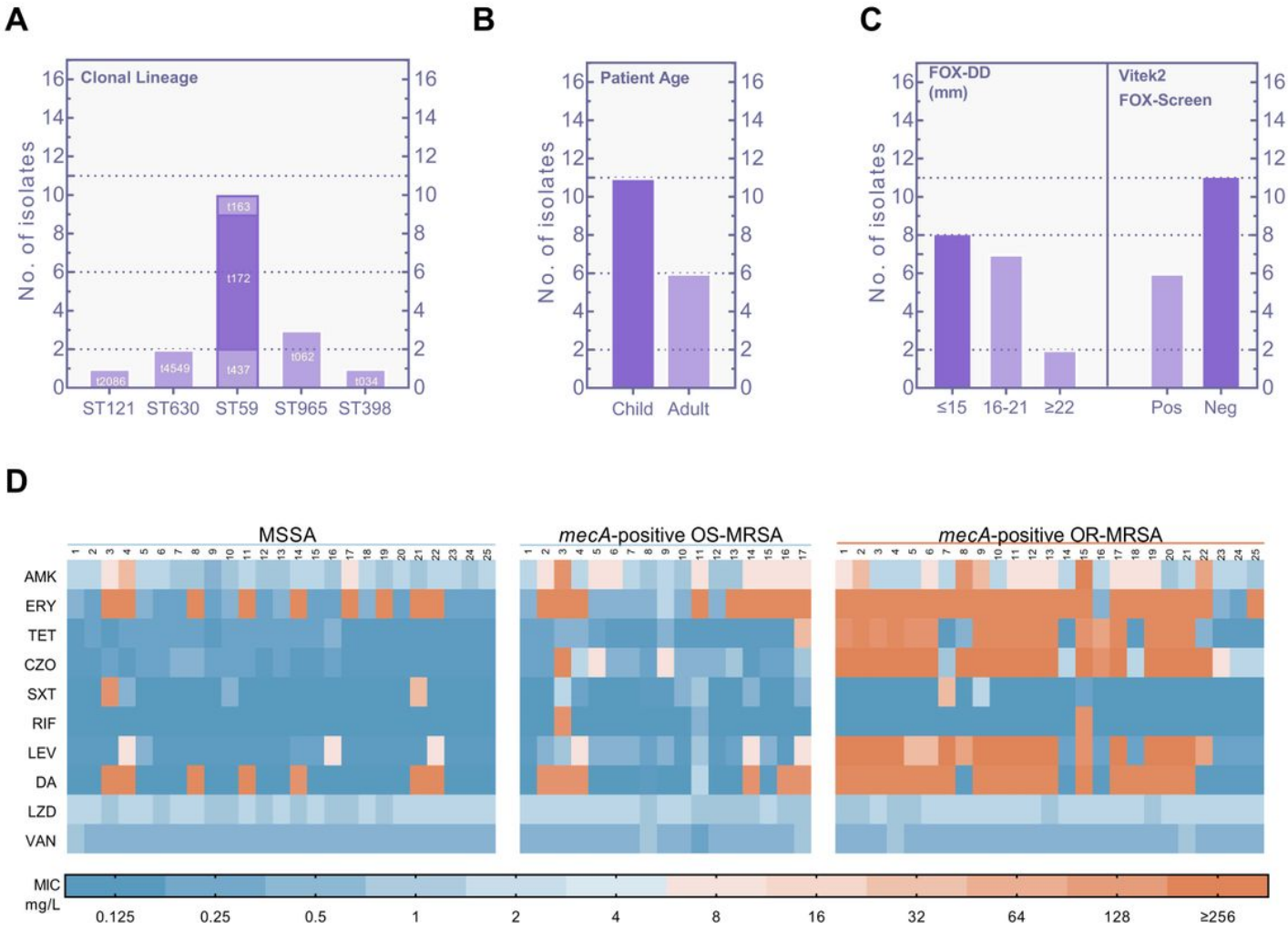

Figure 2

Characteristics of OS-MRSA strains identified in this study. (A) clonal lineages by MLST and spa typing. (B) Ages of patients from who OS-MRSA were isolated. (C) Inhibition zone diameters by cefoxitin disk diffusion test and results of Vitek2 Cefoxitin screen. Pos: positive; Neg: negative. (D) MIC pattern of OS-MRSA, MSSA and OR-MRSA. Each number represents one S. aureus isolate of indicated type. OS-MRSA(OXA-MIC by BMD ≤2mg/L and mecA negative, n=25) and OR-MRSA(OXA-MIC by BMD ≥4mg/L and mecA positive, n=25) were both randomly selected.