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Clonal and drug resistance dynamics of methicillin-resistant *Staphylococcus aureus* in pediatric populations in China

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ABSTRACT

Importance: Regional clonal replacements of methicillin-resistant *Staphylococcus aureus* (MRSA) are common. It is necessary to understand the clonal and drug resistance changes in specific areas.

Objective: To evaluate the clonal and drug resistance dynamics of MRSA in Chinese children from 2010 to 2017.

Methods: MRSA was isolated from patients in Beijing Children's Hospital from 2010 to 2013 and from 2016 to 2017. The molecular characteristics and antibiotic resistance were determined.

Results: In total, 211 MRSA isolates were collected, and 104 isolates were classified as community-associated MRSA (CA-MRSA). ST59-SCC*mec* IV was the most prevalent type in both CA-MRSA (65.4%) and healthcare-associated-MRSA (HA-MRSA) (46.7%). ST239-SCC*mec* III accounted for 21.5% of all HA-MRSA, which were not detected in 2016, and only three isolates were detected in 2017. The *pvl* gene carrying rate of CA-MRSA was significantly higher than that of HA-MRSA (42.3% vs. 29.0%, $P = 0.0456$). Among CA-MRSA, resistance rate to all tested antibiotics excluding chloramphenicol remained stable over the periods of 2010–2013 and 2016–2017. HA-MRSA displayed an overall trend of decreased resistance to oxacillin, gentamicin, tetracycline, ciprofloxacin, and rifampin, and increased resistance to chloramphenicol, consistent with the difference of antibiotic resistance patterns between ST59-SCC*mec* IV and ST239-SCC*mec* III isolates. Vancomycin minimal inhibitory concentration (MIC) creep was found in the study period in all MRSA and ST59-SCC*mec* IV isolates.

Interpretation: ST59-SCC*mec* IV has spread to hospitals and replaced the traditional ST239-SCC*mec* III clone, accompanied by changes in drug resistance. Furthermore, vancomycin MIC creep indicated that the rational use of antibiotics should be seriously considered.

KEYWORDS
Methicillin-resistant *Staphylococcus aureus* (MRSA), Clonal lineage, Drug resistance, Pediatric, China

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a major pathogenic cause of both community- and healthcare-associated infections, which can cause diseases ranging from minor to potentially life-threatening infections, such as skin and soft tissue infections, bacteremia, pneumonia, osteomyelitis, and endocarditis.[1] The mortality rate of MRSA bloodstream infection can reach 30% or higher.[2,3]

MRSA was initially recognized as a nosocomial pathogen causing infections only in patients with healthcare-associated risk factors.[4] However, in the 1990s, a novel MRSA strain causing infections in previously healthy individuals without any predisposing risk factors emerged, and was defined as community-associated MRSA (CA-MRSA).[5] Subsequently, a variety of CA-MRSA lineages emerged and rapidly spread around the world.[6] Previous studies stressed the differences between CA- and hospital-acquired MRSA (HA-MRSA). CA-MRSA usually carries staphylococcal cassette chromosome *mec* (SCC*mec*) types IV or V, and exhibits susceptibility to most non-β-lactam antibacterial agents; conversely, HA-MRSA strains usually carry SCC*mec* types I-III, and multidrug-resistance (MDR) is common.[7] However, recent studies found that CA-MRSA has invaded hospitals, and the traditional perceptions of CA-MRSA and HA-MRSA may no longer be applicable.

CA-MRSA strains have caused outbreaks in hospital settings in the US, Canada, Brazil, Uruguay, Taiwan, France, Germany, Switzerland, the UK, Israel, and Greece, often affecting pediatric and obstetric populations.[5,8] Furthermore, a deterministic mathematical model suggested that CA-MRSA may eventually replace the traditional HA-MRSA lineages and become the dominant MRSA strains in hospitals.[9] In addition, clonal replacements of both epidemic CA-MRSA and HA-MRSA in a specific area have been extremely common during the last two decades. For example, ST239-IIIA-t037, the most common in Portugal in the late 1990s has been progressively replaced by ST22-IV-t022 and ST5-II-t067 clones.[10] A Germany study covering 2000-2010 illustrated that CC5/ST228-MRSA-I and CC45-MRSA-IV were replaced by CC5-MRSA-II and CC22-MRSA-IV,[11] and CC22-MRSA-IV is currently the major clone.[12]

Along with the shifts in predominant clonal lineages, the drug resistance patterns of MRSA will inevitably change. Therefore, it is necessary to understand clonal and drug resistance changes in specific areas. Recent research about the changes of MRSA clones in China was predominantly conducted in adult populations,[13-15] whereas the epidemiology of MRSA infections among children is less studied, although it appears to be extremely different from that in adults. Thus, the purpose of this study was to investigate the clonal and drug resistance dynamics of CA-MRSA and HA-MRSA in a pediatric tertiary care university teaching hospital in Beijing, China from 2010 to 2017.

METHODS

Bacterial isolates

The study was performed at Beijing Children's Hospital in China, a tertiary care university teaching hospital. This study was reviewed and approved by the Ethics Committee of Beijing Children's Hospital, Capital Medical University. No ethical problems existed in this study.

A total of 211 nonduplicate MRSA isolates were recovered between 2010 and 2017 (32 from 2010, 45 from 2012, 31 from 2013, 59 from 2016, and 44 from 2017). These isolates were confirmed to be MRSA based on their colony morphological characteristics, coagulase test results[16], and detection of the *nuc* gene.[17] Methicillin resistance was determined using cefoxitin discs (30 mg, Oxoid) and confirmed by detecting the presence of the *mecA* gene via polymerase chain reaction (PCR).[18] All strains were stored at −80°C until use.

The 211 MRSA strains originated from 88 females and 123 males. The median patient age was 12.7 months (range, 1 day to 15.9 years). These strains were isolated from several clinical sources, including the respiratory tract (2 from throat swab, 59 from sputum and 27 from bronchial alveolar lavage fluid), skin and soft tissue (34 from pus, 17 from secretions of omphalitis, 8 from skin secretions, 14 from wound surface, 5 from eye secretions, and 2 from ear secretions), sterile sites (31 from blood, 4 from pleural effusion, 3 from bone marrow, 2 from joint effusion), and midstream urine (1 isolate). MRSA infections were categorized as HA-MRSA or CA-MRSA according to previously established epidemiological definitions.[19]

Molecular typing and detection of the *pvl* gene

Multilocus sequence typing (MLST) was conducted as described previously.[20] The PCR products of the seven housekeeping genes (*arcC, aroE, glpF, gmk, pta, tpi*, and *yqiL*) were compared with the known sequences from the MLST database (http://saureus.mlst.net/), and the allelic profiles (allele numbers) and sequence types (STs) were determined using the database. Multiplex PCR was performed for SCC*mec* typing according to the methods described by Milheiriço et al.[21] The presence of the *pvl* gene was confirmed using previously described primers and conditions.[22]

Antimicrobial susceptibility testing

Susceptibility to penicillin G, gentamicin, rifampin, ciprofloxacin, clindamycin, erythromycin, chloramphenicol, tetracycline, linezolid, and vancomycin (National Institutes for Food and Drug Control, China)

were tested by agar dilution method as described by Wiegand et al.[23] In addition, the E-test method was used to determine the MICs of all isolates for sulfamethoxazole/trimethoprim (SXT) (bioMérieux, France). Mueller-Hinton agar (MHA) plates were inoculated by streaking the standardized inocula (0.5 McFarland, approximately 1.5×10^8 CFUs/mL) using a sterile swab. The SXT E-test strips (bioMérieux, France) were placed on the plates, followed by incubation at 35°C for 16–20 h. The minimal inhibitory concentration (MIC) reading for both the E-test and agar dilution methods was conducted independently by a senior experimenter, with the result confirmed by a second reader. The MIC results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints for *Staphylococcus* spp.[24] *Staphylococcus aureus* ATCC29213 was used as a quality control. MDR was defined as isolates resistant to ≥3 classes of non-β-lactam antimicrobials.

Statistical analysis

SAS JMP Statistical Discovery v11.0 was used for statistical analysis. Categorical variables were analyzed using the chi-squared (χ^2) test or Fisher's exact test. $P < 0.05$ was considered statistically significant.

RESULTS

Genetic characterization

All 211 clinical isolated strains were confirmed to be MRSA, including 104 CA-MRSA and 107 HA-MRSA isolates. The genotypic characteristics of the CA-MRSA and HA-MRSA isolates are shown in Tables 1 and 2, respectively. Among the CA-MRSA isolates, 19 STs were identified. ST59 (70.2%, 73/104) was the most prevalent, followed by ST88 (4.8%, 5/104) and ST1 (4.8%, 5/104). The frequencies of the remaining STs were extremely low, ranging from 1.0% (1/104) to 3.8% (4/104). SCC*mec* typing illustrated that 78.8% (82/104) of the CA-MRSA isolates harbored SCC*mec* type IV, followed by SCC*mec* type V (17.3%, 18/104), and III (1.9%, 2/84). The SCC*mec* type of two isolates could not be determined. Combined analysis revealed that ST59-SCC*mec* type IV was the most prevalent genotype (65.4%, 68/104). The dominant clone of CA-MRSA remained stable from 2010 to 2017, with ST59-SCC*mec* type IV accounting for 63.2% (12/19), 70.0% (14/20), 58.8% (10/17), 75% (24/32) and 50% (8/16) of isolates in 2010, 2012, 2013, 2016 and 2017, respectively (Figure 1A).

TABLE 1 Genotypic characteristics of CA-MRSA recovered from 2010 to 2017

Genotype	Number of isolates (%)	*pvl* (+)	Years				
			2010 (*n*=19)	2012 (*n*=20)	2013 (*n*=17)	2016 (*n*=32)	2017 (*n*=16)
ST1-SCC*mec* IV	5 (4.8)	1			1	1	3
ST5-SCC*mec* IV	1 (1.0)	0				1	
ST6-SCC*mec* IV	1 (1.0)	0					1
ST22-SCC*mec* V	2 (1.9)	2				1	1
ST30-SCC*mec* IV	1 (1.0)	1				1	
ST59-SCC*mec* IV	68 (65.4)	27	12	14	10	24	8
ST59-SCC*mec* V	5 (4.8)	5	1	1	3		
ST72-SCC*mec* IV	1 (1.0)	0	1				
ST88-SCC*mec* III	2 (1.9)	1	2				
ST88-SCC*mec* IV	1 (1.0)	1		1			
ST88-SCC*mec* NT	2 (1.9)	2	1			1	
ST97-SCC*mec* V	1 (1.0)	0				1	
ST120-SCC*mec* V	1 (1.0)	0		1			
ST121-SCC*mec* V	1 (1.0)	0				1	
ST338-SCC*mec* V	4 (3.8)	4		2			2
ST375-SCC*mec* IV	1 (1.0)	0	1				
ST398-SCC*mec* V	2 (1.9)	0		1			1
ST630-SCC*mec* V	1 (1.0)	0			1		
ST950-SCC*mec* IV	1 (1.0)	0			1		
ST965-SCC*mec* IV	1 (1.0)	0	1				
ST1224-SCC*mec* IV	1 (1.0)	0				1	
ST1777-SCC*mec* V	1 (1.0)	0			1		

NT, nontypable.

TABLE 2 Genotypic characteristics of HA-MRSA recovered from 2010 to 2017

Genotype	Number of isolates (%)	*pvl* (+)	Years				
			2010 (*n* = 13)	2012 (*n* = 25)	2013 (*n* = 14)	2016 (*n* = 27)	2017 (*n* = 28)
ST1-SCC*mec* IV	3 (2.8)	1				1	2
ST5-SCC*mec* IV	1 (0.9)	0		1			
ST9-SCC*mec* IV	1 (0.9)	0	1				
ST22-SCC*mec* V	5 (4.7)	5				1	4
ST59-SCC*mec* IV	50 (46.7)	17	4	7	6	21	12
ST59-SCC*mec* V	4 (3.7)	0		3			1
ST59-SCC*mec* NT	1 (0.9)	0				1	
ST72-SCC*mec* IV	1 (0.9)	0			1		
ST88-SCC*mec* IV	1 (0.9)	0					1
ST88-SCC*mec* V	1 (0.9)	0					1
ST88-SCC*mec* NT	1 (0.9)	1		1			
ST239-SCC*mec* I	1 (0.9)	0					1
ST239-SCC*mec* III	23 (21.5)	1	6	8	6		3
ST239-SCC*mec* IV	1 (0.9)	1		1			
ST239-SCC*mec* NT	2 (1.9)	0		1	1		
ST338-SCC*mec* V	2 (1.9)	2				1	1
ST398-SCC*mec* IV	2 (1.9)	1	1	1			
ST509 -SCC*mec* IV	1 (0.9)	0					1
ST585-SCC*mec* III	1 (0.9)	0		1			
ST896-SCC*mec* V	1 (0.9)	1				1	
ST965-SCC*mec* IV	1 (0.9)	1	1				
ST1295-SCC*mec* IV	1 (0.9)	0					1
ST1296-SCC*mec* V	1 (0.9)	0		1			
ST1821-SCC*mec* NT	1 (0.9)	0				1	

NT, nontypable.

Among the HA-MRSA isolates, 17 STs were identified. ST59 (51.4%, 55/107) was the most common type, followed by ST239 (25.2%, 27/107) and ST22 (4.7%, 5/107). The frequencies of the remaining STs ranged from 0.9% (1/107) to 2.8% (3/107). The most frequent detected SCC*mec* type was also SCC*mec* type IV (58.9%, 63/107), followed by SCC*mec* type III (22.4%, 24/107), V (13.1%, 14/107), I (0.9%, 1/107). Combined analysis illustrated that ST59-SCC*mec* IV was the most prevalent clone (46.7%, 50/107), followed by ST239-SCC*mec* III (21.5%, 23/107). The SCC*mec* type of five isolates could not be determined. However, the predominant clone of HA-MRSA changed between 2010 and 2017. ST59-SCC*mec* type IV (46.7%, 50/107) accounted for 30.8% (4/13), 28% (7/25), 42.9% (6/14), 77.8% (21/27) and 42.9% (12/28) of HA-MRSA strains recovered in 2010, 2012, 2013, 2016, and 2017, respectively. ST239-SCC*mec* type III (21.5%, 23/107) accounted for 46.2% (6/13), 32.0% (8/25), 42.9% (6/14), 0, and 10.7% of strains in 2010, 2012, 2013, 2016, and 2017, respectively (Figure 1B).

The prevalence rate of the *pvl* gene was significantly higher in CA-MRSA (42.3%, 44/104) than in HA-MRSA (29.0%, 31/107) ($P = 0.0456$).

Trends of drug-resistance rates between 2010–2013 and 2016–2017

Antimicrobial susceptibility test results are shown in Figure 2. All values obtained using ATCC29213 were within the quality control ranges for all antimicrobial agents tested. Among the CA-MRSA isolates, the drug resistance rate for chloramphenicol was significantly higher in 2016–2017 (68.8%, 33/48) than in 2010–2013 (46.4%, 26/56) ($P = 0.0291$), and there was no significant difference in the rate for the other tested antibiotics between the two time periods (all $P > 0.05$) (Figure 2A). However, the rates of HA-MRSA resistance to oxacillin (94.2% vs. 80.0%, $P = 0.0430$) , tetracycline (75% vs. 40.0%, $P = 0.0004$), gentamicin (50.0% vs. 10.9%, $P < 0.0001$), ciprofloxacin (57.7% vs. 30.9%, $P = 0.0066$) and rifampin (34.6% vs. 5.5%, $P = 0.0002$) tended to decrease between 2010–2013 and 2016–2017, whereas resistance to chloramphenicol increased (44.2% in 2010–2013 vs.

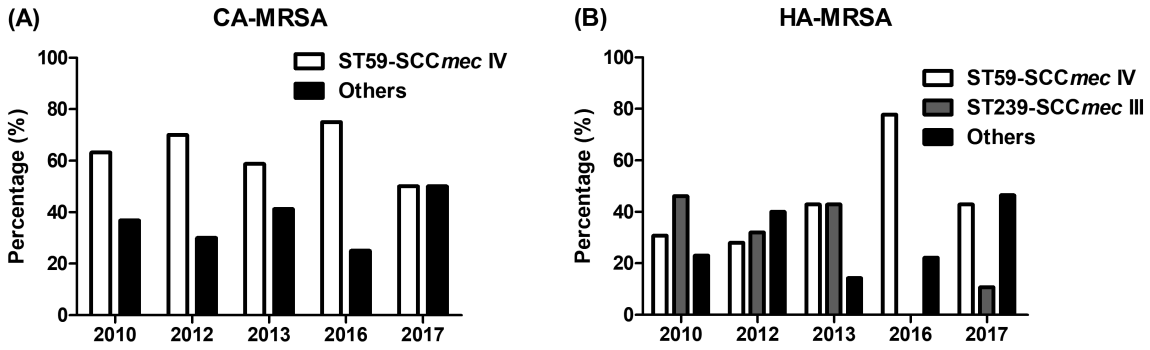

FIGURE 1 Dynamics of epidemic MRSA in pediatric populations in China. (A) Dynamics of dominant clones among CA-MRSA isolates. (B) Dynamics of dominant clones among HA-MRSA isolates. MRSA, methicillin-resistant *Staphylococcus aureus*; CA-MRSA, community-associated MRSA; HA-MRSA, hospital-acquired MSRA.

70.9% in 2016–2017, $P = 0.0064$) (Figure 2B).

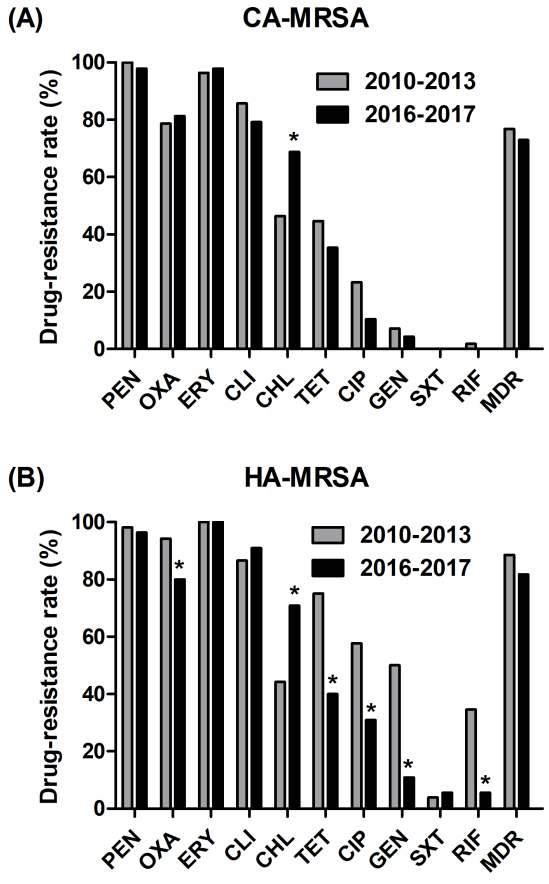

FIGURE 2 Drug resistance rates of CA-MRSA (A) and HA-MRSA (B) in pediatric populations in China from 2010 to 2017. All isolates were susceptible to vancomycin and linezolid, which are not listed in the figure. MRSA, methicillin-resistant *Staphylococcus aureus*; CA-MRSA, community-associated MRSA; HA-MRSA, hospital-acquired MSRA; PEN, penicillin; OXA, oxacillin; ERY, erythromycin; CLI, clindamycin; TET, tetracycline; GEN, gentamicin; CHL, chloramphenicol; CIP, ciprofloxacin; RIF, rifampin; SXT, trimethoprim-sulfamethoxazole; MDR, multidrug resistance, resistant to ≥ 3 classes of non-β-lactam antimicrobials. *$P < 0.05$.

Drug-resistance rates among different genotypes

The drug-resistance rates of MRSA stratified by genotype are shown in Figure 3. ST239-SCC*mec* type III strains exhibited significantly higher rates of resistance to oxacillin, tetracycline, gentamicin, ciprofloxacin, SXT, and rifampin than ST59-SCC*mec* type IV and other clones (all $P < 0.05$), whereas the ST59-SCC*mec* type Ⅳ strains had a significantly higher rate of resistance to chloramphenicol (67.0%, 79/118) than ST239-SCC*mec* type III (34.8%, 8/23) and other clones (48.6%, 34/70) (Figure 3A).

The rate of resistance of ST59-SCC*mec* IV strains to all tested antibiotics excluding chloramphenicol remained stable between 2010–2013 and 2016–2017 (Figure 3B). Furthermore, there were no significant differences in drug resistance rate between CA- and HA-MRSA isolates belonging to ST59-SCC*mec* IV (Figure 3C).

Trends in the MIC distribution of vancomycin

The percentage of isolates with MIC = 1 mg/L increased from 48.1% (52/108) in 2010–2013 to 83.5% (86/103) in 2016–2017. In addition, the percentage of isolates with MIC = 2 mg/L increased from 0.9% (1/108) to 5.8% (6/103) over this period, whereas the percentage of isolates with MIC = 0.5 mg/L decreased from 50.9% (55/108) to 10.7% (11/103). The difference in the MIC distribution of MRSA isolates between the two time periods was statistically significant ($P < 0.0001$) (Figure 4A).

There were similar trends in the MIC distribution of vancomycin among isolates belonging to ST59-SCC*mec* type IV (Figure 4B). The percentage of isolates with MIC = 1 mg/L increased from 47.2% (25/53) in 2010–2013 to 81.5% (53/65) in 2016–2017. The percentage of isolates with MIC = 2 mg/L increased from 1.9% (1/53) to 6.2% (4/65) over this period, whereas the percentage of isolates with MIC = 0.5 mg/L decreased from 50.9% (27/53) to 12.3% (8/65). The MIC distribution of ST59-SCC*mec* IV isolates between the two periods was also statistically significant different ($P < 0.0001$).

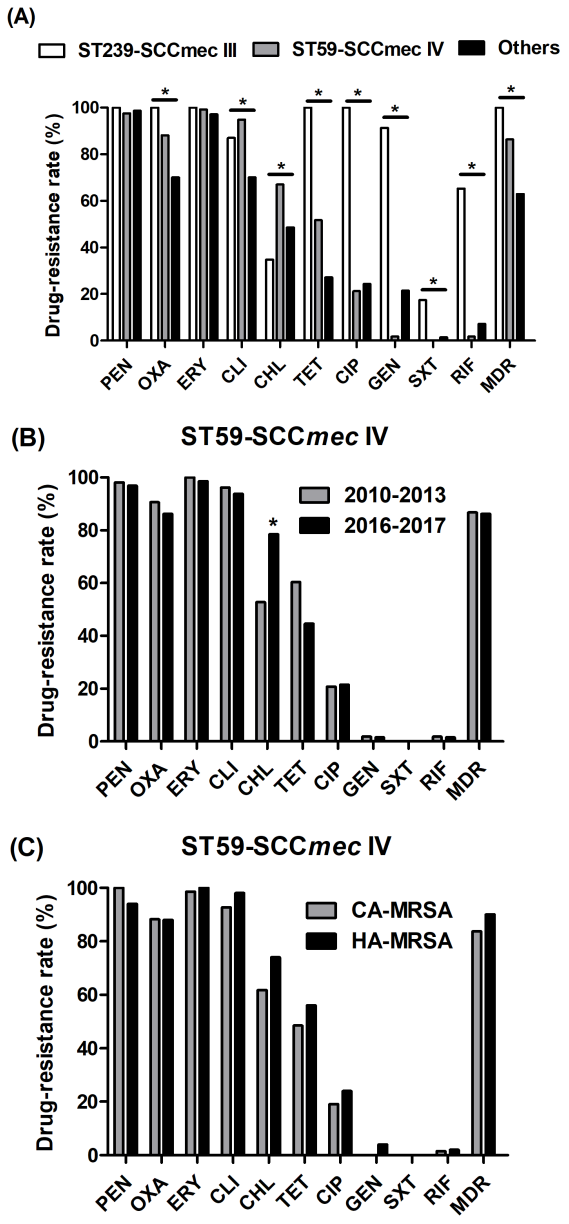

FIGURE 3 Drug resistance rates of MRSA in pediatric populations stratified by genotype. (A) Drug-resistance rates for ST239-SCC*mec* type III, ST59-SCC*mec* IV and other genotypes. (B) Drug resistance rates for ST59-SCC*mec* IV between 2010–2013 and 2016–2017. (C) Drug resistance rate for CA-MRSA and HA-MRSA among ST59-SCC*mec* IV isolates. All isolates were susceptible to vancomycin and linezolid, which are not listed in the figure. MRSA, methicillin-resistant *Staphylococcus aureus*; CA-MRSA, community-associated MRSA; HA-MRSA, hospital-acquired MRSA; PEN, penicillin; OXA, oxacillin; ERY, erythromycin; CLI, clindamycin; TET, tetracycline; GEN, gentamicin; CHL, chloramphenicol; CIP, ciprofloxacin; RIF, rifampin; SXT, trimethoprim-sulfamethoxazole; MDR, multidrug resistance, resistant to ≥ 3 classes of non-β-lactam antimicrobials. *$P < 0.05$.

DISCUSSION

The molecular epidemiology and drug resistance of MRSA are changing rapidly. Understanding the epidemiological dynamics of MRSA in a specific area can promote the rational use of antibiotics, which may eventually reduce the incidence of infection. In the present study, we described the molecular characteristics and drug resistance dynamics of MRSA in a pediatric population in China from 2010 to 2017.

Our research revealed that ST59-SCC*mec* type IV, which was usually considered a community-associated clone, had spread to hospitals and replaced the traditional hospital-associated ST239-SCC*mec* type Ⅲ clone. Similar to our findings, an investigation of MRSA infections in Shenzhen, China from January to December 2014 illustrated that CC59-t437-SCC*mec* IV/V-*agr* group I (46.4%) was the most prevalent clone, and CC239-t030/t037-SCC*mec* III-*agr* I accounted for only 4.4% of 183 MRSA isolates.[25] In addition, many studies also demonstrated that CA-MRSA strains are beginning to replace HA-MRSA strains as the predominant causes of healthcare infections in several countries, such as the US, Greece, Denmark, and Korea.[5]

The potential reason for the replacement of traditional HA-MRSA strain by CA-MRSA strains remains unclear, but it may be attributable to the following factors. First, CA-MRSA clones carry a much smaller version of SCC*mec* (usually type IV and V) and fewer antimicrobial resistance genes than HA-MRSA clones (usually types I, II and III). Thus, the fitness cost of antibiotic resistance may be minimized through the carriage of smaller or fewer genes.[9] Second, CA-MRSA clones were more likely to carry the *pvl* gene, suggesting the involvement of the gene. However, not all CA-MRSA isolates harbored the gene, and CA-MRSA clones, which do not harbor the gene can also cause outbreaks in healthcare settings.[26] Finally, CA-MRSA strains can express much higher levels of RNAIII [the mainly effector of the accessory gene regulator (*agr*) system, which can regulate the expression of multiple toxins] than HA-MRSA strains and maintain virulence in the absence of antibiotic exposure, but it can trade *agr* activity for methicillin resistance once exposed to antibiotics.[27] Contrarily, for HA-MRSA, the expression level of virulence factors is relative low; thus, it may become difficult for this clone to infect healthy people. Further studies are needed to explore these questions.

During the study period, HA-MRSA exhibited an overall trend of decreased resistant to tetracycline, gentamicin, ciprofloxacin, and rifampin and increase resistance to chloramphenicol. The results were consistent with the difference in the antibiotic resistance patterns between ST59-SCC*mec* type IV and ST239-SCC*mec* type III isolates. Thus, the decreasing trend of antibiotic resistance among HA-MRSA clones may be related to the decreasing detection of highly drug-resistant ST239-SCC*mec* type III clone. The association between clonal replacement and changes in antibiotic resistance are supported by several other studies. Olearo et al[28] found a general upward trend of non-MDR MRSA in Switzerland between 2004

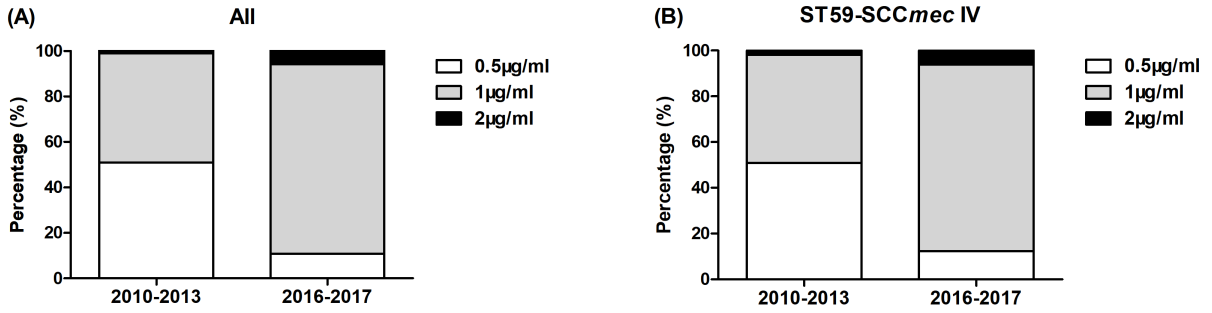

FIGURE 4 Trends in the vancomycin susceptibility of MRSA strains in pediatric populations in China in 2010–2017. (A) and (B) present the distribution of MICs for vancomycin among all isolates and isolates belonging to ST59-SCC*mec* IV, respectively. MRSA, methicillin-resistant *Staphylococcus aureus*; MIC, minimal inhibitory concentration.

and 2014, in line with the decreased frequency of MDR HA-MRSA isolates and the rapid spread of CA-MRSA. Amorim et al[29] also found that the increasing isolation of non-MDR MRSA strains was associated with the decline and massive replacement of the MDR ST239-IIIA clone (from 69% in 1996–2000 to 12% in 2003–2005) by the epidemic ST22-IV clone (80%), in which resistance to non-β-lactam antibiotics is uncommon. Furthermore, the resistance of the dominant ST59-SCC*mec* type IV clone to all tested antibiotics remained stable from 2010 to 2017. This result could explain why the drug resistance rate of CA-MRSA (ST59-SCC*mec* type IV as the major clone) did not change over time. Although ST59-SCC*mec* type IV isolates had spread to hospitals, they did not become more resistant to antibiotics under the high selective pressure of antibiotic exposure in healthcare settings during the study period. These studies including ours demonstrated the importance of monitoring genetic backgrounds and drug resistance simultaneously.

Vancomycin is active against MRSA, including MDR strains. Although the isolates tested in this study were all sensitive to vancomycin, a statistically significant increase of the vancomycin MIC was found between 2010 and 2017. This result was similar to previous findings by Zhuo et al[30] in China, who reported that the percentages of MRSA isolates with a vancomycin MIC exceeding 1 mg/L were 26.0%, 23.5%, 21.6%, 27.8%, 30.6% and 42.8% in 2006, 2007, 2008, 2009, 2010, and 2011, respectively, with significant increases noted over time ($P < 0.05$). A global analysis (including 56 countries) based on the Tigecycline Evaluation and Surveillance Trial also demonstrated that the percentage of MRSA isolates with MICs of at least 2mg/L increased from 5.6% in 2004 to 11.1% in 2009 ($P < 0.001$).[31] However, a systematic review and meta-analysis failed to find evidence of the MIC creep phenomenon.[32] Such differences may be explained by the fact that the MIC creep phenomenon may be influenced by differences in the susceptibility testing methods used (including storage of isolates) and differences in epidemiological and clinical factors among study sites.[33,34] Therefore, dynamic monitoring of vancomycin MICs in a specific area is highly warranted. Many studied revealed that increased and prolonged therapy and suboptimal exposure to vancomycin might contribute to MIC creep and promote the emergence of drug-resistant bacteria,[35,36] indicating that the appropriate and accurate use of antibiotics should be seriously considered.

The limitations of our study are worth noting. First, because bacteria isolated from vaginal secretions and fecal samples were not included and only pathogenic bacteria were selected for further study, the sample size was relatively small. Second, *Staphylococcus aureus* collection was not conducted in 2014 and 2015, and thus, the time period was not continuous. Third, all isolates were collected from a single hospital. However, our hospital provides both primary and tertiary care for more than 3 000 000 children in Beijing and the surrounding areas, making the results representative to some extent.

In conclusion, our study demonstrated that ST59-SCC*mec* type IV clone, which was usually considered as a community-associated clone, had spread to hospitals and replaced the traditional hospital-associated ST239-SCC*mec* type III clone, and ST59-SCC*mec* type IV clone was dominant among both CA-MRSA and HA-MRSA in a pediatric population in China. Replacement of clonal lineages were accompanied by changes in drug resistance in our study. In addition, vancomycin MIC creep indicated that the rational use of antibiotics should be seriously considered. Considering the rapid changes of MRSA epidemiology, continuous surveillance is necessary to keep monitor clonal and drug resistance changes in a specific area.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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