

# Increasing Resistance in Multiresistant Methicillin-Resistant *Staphylococcus aureus* Clones Isolated from a Chinese Hospital Over a 5-Year Period

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**Background:** The aim was to study the changes in the antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) clones over a 5-year period (from 2000 to 2005) at a representative hospital in Beijing, China. **Methods:** A total of 100 randomly selected MRSA strains were analyzed using antimicrobial susceptibility testing, pulsed-field gel electrophoresis, *spa* typing, multilocus sequence typing, *SCC<sub>mec</sub>* typing, and PCR for the Panton-Valentine leukocidin virulence factor. **Results:** Resistance to rifampin greatly increased from 32% (16/50) to 68% (34/50). High-level mupirocin-resistant isolates were found only in 2005, when four were identified. Intermediate susceptibility to quinupristin-dalfopristin increased from 22% (11/50) to 52% (26/50) between 2000 and 2005. The main antimicrobial resistance profiles changed from TC-GM-CI-EM-CM in 2000 to TC-GM-CI-EM-CM-RI in 2005. The main pulsed-field gel electrophoresis type changed from types C, L, and E in 2000 to types J, F, and N, respectively, in 2005. ST239-MRSA-III was the most predominant clone in 2000 and 2005, whereas ST5-MRSA-II was found only in 2005. **Conclusions:** There were increasing levels of antimicrobial resistance and epidemiological changes in the hospital-associated MRSA strains isolated in this facility between 2000 and 2005.

## Introduction

**S**TAPHYLOCOCCUS AUREUS HAS become a very important pathogen that causes several diseases, ranging from minor infections of the skin to wound infections, bacteremia, and necrotizing pneumonia. The epidemiological characteristics of *S. aureus*, especially methicillin-resistant *S. aureus* (MRSA), are changing rapidly. From the first MRSA strain reported until the present, various hospital-associated MRSA (HA-MRSA) clones have been disseminated worldwide.<sup>3,5,6,12,26</sup> The emergence of new strains of community-associated MRSA in many parts of the world is well documented<sup>15,21</sup> and causes an increasing proportion of healthcare-associated infections.<sup>13</sup>

In China, MRSA is highly endemic in hospitals with mean prevalence rates of >50% in 2005.<sup>33</sup> Two major epidemic MRSA clones with a unique geographic distribution across China, ST239-MRSA-III and ST5-MRSA-II, have been reported recently.<sup>22</sup> Multilocus sequence typing for a few isolates over 5 years was completed by us and provided an important clue.<sup>34</sup> To investigate the dynamics of MRSA over long time periods, we characterized MRSA isolates from a Chinese hospital collected between 2000 and 2005 to identify

changes in antimicrobial resistance, molecular typing, and virulence factors.

## Materials and Methods

### Isolates

A total of 100 MRSA strains were isolated from several clinical sources including the respiratory tract ( $n=76$ ), blood ( $n=3$ ), drainage ( $n=8$ ), pus ( $n=7$ ), wounds ( $n=3$ ), and other sources ( $n=3$ ) that were randomly selected from Beijing Union Medical Hospital, which has about 1,800 beds. Fifty strains were isolated in 2000, and 50 more were isolated in 2005. All strains were confirmed by PCR analysis of the *mecA* and *nuc* genes.<sup>4</sup>

### Antimicrobial susceptibility tests

A total of 14 antimicrobial agents were tested, including linezolid, chloramphenicol, tetracycline, gentamicin, vancomycin, ciprofloxacin, erythromycin, clindamycin, rifampin, quinupristin-dalfopristin, teicoplanin, mupirocin, fusidic acid, and trimethoprim. Vancomycin was tested by broth dilution according to Clinical Laboratory Standards Institutes, and

the other antimicrobial agents were tested by Etest (AB Biodisk). Clinical Laboratory Standards Institutes breakpoints were used for minimum inhibitory concentration (MIC) interpretation. The resistance breakpoints for mupirocin and fusidic acid were defined as previously described.<sup>9,23</sup>

### Molecular typing

All isolates were investigated by pulsed-field gel electrophoresis (PFGE), *SCCmec* typing, and *spa* typing. Multilocus sequence typing was performed on 29 strains that included representatives of each *spa* type. Genomic DNA and plasmids were extracted using the DNeasy blood and tissue kit and the QIAprep Spin Miniprep kit, respectively (Qiagen GmbH). Amplifications were performed in a Mycycler™ thermal cycler (Bio-Rad). PFGE was performed using the CHEF-DR III System (Bio-Rad).

PFGE using *Sma*I was set up according to the American CDC Standardized Protocol for Molecular Subtyping of *Listeria monocytogenes* by PFGE ([www.cdc.gov/PULSENET/protocols.htm](http://www.cdc.gov/PULSENET/protocols.htm)). The MRSA suspension incorporated into the agarose block was standardized to have an optical density (OD) of 5.5–6.5, and 2 µl lysostaphin (1 mg/ml) was added to 250 µl of bacterial suspension, which was immediately mixed with an equal volume of 1% low-melting-point agarose. Digital images were analyzed by BioNumerics software (v. 4.6; Bio-Rad) using the Dice coefficient and were represented by unweighted pair group method arithmetic averages (UPGMA) with 1.5% tolerance and 1% optimization settings. A similarity cutoff of 80% and the criterion of a difference of six bands as described by Tenover *et al.*<sup>30</sup> were used to define a cluster. Isolates showing identical or related PFGE patterns were considered to belong to the same clone. Clones were labeled with a capital letter (A, B, C, and so on), and related profiles were indicated by adding a number (A1, A2, B1, B2, and so on).

*spa* typing, multilocus sequence typing, and *SCCmec* typing were performed as previously described.<sup>7,8,14,27,35</sup>

### *pvl* and *mupA* genes detection

Panton-Valentine leukocidin (PVL) genes (*lukS-PV* and *lukF-PV*) were detected by PCR.<sup>21</sup> The high-level mupirocin-resistant isolates (based on the Etest method) were detected by PCR for the *mupA* gene as previously described.<sup>1</sup>

### Statistical analysis

Statistical analysis was performed with the SPSS software package using chi-square and Fisher's exact tests.

## Results

### Antimicrobial susceptibility testing

Isolates from the various time periods did not demonstrate resistance or increases in the MIC of vancomycin, teicoplanin, and linezolid. Resistance to rifampin greatly increased from 32% (16/50) to 68% (34/50,  $p < 0.001$ ). Four high-level mupirocin-resistant isolates were found only in samples from 2005. Intermediate susceptibility rates to quinupristin-dalfopristin increased from 22% (11/50) to 52% (26/50,  $p < 0.01$ ) from 2000 to 2005. Resistance to cipro-

floxacin was universal. Resistance to chloramphenicol, tetracycline, erythromycin, and clindamycin slightly increased from 4% to 6%, 94% to 98%, 98% to 100%, and 98% to 100%, respectively. On the other hand, gentamicin, quinupristin-dalfopristin, fusidic acid, and trimethoprim resistance decreased from 100% to 94%, 12% to 8%, 4% to 0%, and 4% to 0%, respectively.

### Antibiotic resistance profiles and molecular epidemiology

Overall, 15 PFGE strain types (A through O) and 4 clusters (1 through 4) were identified among the 100 MRSA isolates. Five PFGE types, type (C, J, N, F, and L) were the dominant types, constituting 82% of all isolates. Types C, L, and E were the dominant types from isolates collected in 2000, accounting for 88% (44/50), whereas types J, F, and N were the dominant types in 2005, accounting for 74% (37/50).

Eleven antimicrobial resistance profiles were identified in total. Two predominant profiles (TC-GM-CI-EM-CM-RI and TC-GM-CI-EM-CM) contained 47 and 40 isolates, respectively. The antimicrobial resistance profile TC-GM-CI-EM-CM-RI included 14 isolates in 2000, corresponding to PFGE types E, L, and M, and included 33 isolates in 2005, corresponding to PFGE types A, F, I, J, K, L, and N. The antimicrobial resistance profile TC-GM-CI-EM-CM included 29 isolates in 2000, corresponding to PFGE types C, D, and N, and included 11 isolates in 2005, corresponding to PFGE types C, F, G, and L (Table 1).

Typing all isolates yielded five *spa* types (Table 1). T037 was the predominant *spa* type in 2000, accounting for 74% (37/50); and the second most prominent was t030, accounting for 24% (12/50). T030 was the most predominant *spa* type in 2005, accounting for 66% (33/50); whereas the second was t002, accounting for 30% (15/50,  $p < 0.001$ ).

Twenty-nine strains that included representatives of each *spa* type yielded three STs belonging to two clonal complexes (Table 1). ST239 was the predominant ST, which belonged to clonal complex CC8 and constitutes 72.41% of isolates (21/29). The second ST was ST5, which belonged to clonal complex CC5 and constitutes 24.14% of isolates (7/29). ST5 was only found in 2005.

From all isolates, two *SCCmec* types were identified, namely II and III (Table 1). The most common *SCCmec* type was type III, which was found in 80 isolates (80/100 or 80%), including 45 isolates from 2000 and 35 isolates (35/50 or 70%) from 2005. The second was type II, which was only found in 15 isolates (15/100 or 15%) from 2005. A total of five isolates (5/100 or 5%) were nontypeable by the multiplex *SCCmec* typing method.

### Detection of *pvl* and *mupA*

No isolate was *pvl* positive. The four high-level mupirocin-resistant isolates were all positive for *mupA*.

## Discussion

MRSA strains associated with community acquisition were not found to have been introduced into this hospital, but there were epidemiological changes in the types of HA-MRSA strains in the facility over the 5 years included in our

TABLE 1. MULTILOCUS SEQUENCE TYPING, *spa*, *SCCmec*, AND PULSED-FIELD GEL ELECTROPHORESIS TYPES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES AND THEIR ANTIBIOTIC RESISTANCE PROFILES IN 2000 AND 2005

ST (CC)	<i>spa</i> type	<i>SCCmec</i>	PFGE type	Antibiotic resistance profile	% of isolates in 2000	% of isolates in 2005			
5 (5)	t002	II	F	TC-GM-CI-EM-CM	0	16			
			G	CL-TC-GM-CI-EM-CM-MU	0	4			
			H	TC-GM-CI-EM-CM	0	2			
				TC-CI-EM-CM-MU	0	2			
				CL-TC-GM-CI-EM-CM-MU	0	2			
72 (8)	t148	Unknown	L1	TC-CI-EM-CM	0	2			
			O	GM-CI-EM-CM	2	0			
239 (8)	t1152	III	A	TC-GM-CI-EM-CM-RI	0	2			
			B	CL-TC-GM-CI-EM-CM-RI-QDA-MU-FU-TR	2	0			
	t037	III	C1	TC-GM-CI-EM-CM	50	2			
				TC-GM-CI-EM-CM-FU-TR	2	0			
				GM-CI-EM-CM	2	0			
			C2	TC-GM-CI-EM-CM	4	0			
				GM-CI-EM-CM	2	0			
			D	TC-GM-CI-EM-CM	2	0			
			E	CL-TC-GM-CI-EM-CM	2	0			
			Unknown	E	Unknown		TC-GM-CI-EM-CM-RI	8	0
						F	TC-GM-CI-EM-CM-RI	0	2
			t030	III	III	I1	TC-GM-CI-EM-CM-RI	0	6
						I2	TC-GM-CI-EM-CM-RI	0	2
						J1	TC-GM-CI-EM-CM-RI	0	6
						J2	TC-GM-CI-EM-CM-RI	0	22
							TC-CI-EM-CM-RI	0	2
						K	TC-GM-CI-EM-CM-RI	0	2
						L1	TC-GM-CI-EM-CM-RI-QDA	2	0
							TC-GM-CI-EM-CM-RI	16	0
						L2	TC-GM-CI-EM-CM-RI	0	2
						M	TC-GM-CI-EM-CM-RI	4	0
			N1	TC-GM-CI-EM-CM-RI	0	4			
			N2	TC-GM-CI-EM-CM			TC-GM-CI-EM-CM	2	0
							TC-GM-CI-EM-CM-RI	0	18

CI, ciprofloxacin; CL, chloramphenicol; CM, clindamycin; EM, erythromycin; FU, fusidic acid; GM, gentamicin; MU, mupirocin; PFGE, pulsed-field gel electrophoresis; QDA, quinupristin/dalfopristin; RI, rifampin; TC, tetracycline; TR, trimethoprim.

study, with an associated change in antimicrobial resistance patterns.

The main antimicrobial resistance profile changed from TC-GM-CI-EM-CM in 2000 to TC-GM-CI-EM-CM-RI in 2005. Resistance to rifampin increased from 32% to 68%, and the MIC<sub>50</sub> for rifampin increased from 0.008 to 256 µg/ml. Combination therapy with rifampin has been used to treat *S. aureus* infections<sup>24,29</sup> and has been widely used in China in recent years to treat esophagitis, dysentery (difficult to cure or resistant), skin infections, hordeolum, purulent otitis media, and mycoplasma pneumonia. From our data, we found that an increasing number of resistant isolates are emerging with the wide spread use of rifampin.

The data in this study revealed that resistance to mupirocin increased from 2% to 8%. We found that four high-level-resistant (MupRH) isolates with *mupA* in 2005,<sup>11</sup> which were PFGE types F and H, belonged to the same *spa* type and STs (t002, ST5-MRSA-II) and belonged to two antimicrobial resistant profiles (CL-TC-GM-CI-EM-CM-MU and TC-CI-EM-CM-MU). One low-level-resistant (MupRL)<sup>2,10</sup> isolate from 2000 was PFGE type B, t037, ST239-MRSA-III, and resistant to 11 antimicrobial drugs (CL-TC-GM-CI-EM-CM-RI-QDA-MU-FU-TR). This result indicated the MupRL

isolates and the MupRH isolate were different clones. Beginning in December 2005, mupirocin could be purchased OTC in China. From the experience in other countries,<sup>31</sup> more attention to it should be paid to this trend. Caution should be taken whenever high-level resistance strains are detected; and control measures should be implemented, because transmissible plasmids have led to outbreaks.<sup>20</sup>

The other interesting finding was that 10% isolates were resistant to quinupristin-dalfopristin whereas 37% (37/100) of isolates displayed intermediate susceptibility to the drug. The rate of intermediate susceptibility to quinupristin-dalfopristin increased from 22% (11/50) to 52% (26/50) between 2000 and 2005. We are quite curious how this has occurred, because quinupristin-dalfopristin had not been marketed in China until the present. Virginiamycin, another streptogramin A/B combination, has long been used in animal feed as a growth promoter in many countries, including China. This type of use selects for virginiamycin-resistant strains of *Enterococcus faecium*, which are cross-resistant to quinupristin-dalfopristin<sup>18</sup> and that may pose a risk to public health. As far as we know, staphylococci are generally host-specific, although some exceptions have been noted, such as

MRSA from pigs that can colonize humans, as observed in the Netherlands.<sup>16</sup> Thus, whether these isolates resistant to quinupristin-dalfopristin originated from animals needs to be further investigated.

ST5-MRSA-II was a new type found in the hospital in 2005; this strain has also spread widely in European countries and is the predominant MRSA clone in Korea and Japan.<sup>19</sup> Some published data have shown that clonal evolution can occur within a single hospital. In a Mexico City hospital, a local clone (ST30-MRSA-IV) was predominant between 1997 and 2000, but it was completely replaced over a 2-year period by the New York/Japan clone (ST5-MRSA-II).<sup>32</sup> Similarly, a study in Spain showed that ST247-MRSA-I was replaced by ST36-MRSA-II between 1998 and 2002.<sup>28</sup> The ST5-MRSA-II clone emerged after 2000 and was not previously found in Hong Kong.<sup>17</sup> These results indicate that ST5-MRSA-II has become more common in this Beijing hospital in recent years. Recently, research on the ST5-MRSA clone has provided strong evidence that the geographical spread of MRSA over long distances and across cultural borders is a rare event compared with the frequency with which the staphylococcal cassette chromosome island has been imported.<sup>25</sup> Thus, continuous surveillance of MRSA and methicillin-sensitive staphylococcus aureus (MSSA) in hospitals and communities is of great importance for understanding the local epidemiology of MRSA.

## Conclusion

Our study showed that there were increasing levels of antimicrobial resistance and epidemiological changes in the HA-MRSA strains collected in the facility between 2000 and 2005.

## Acknowledgment

This work was supported by "major infectious diseases such as AIDS and viral hepatitis prevention and control" technology major projects (2008ZX10004-002) from the Ministry of Science and Technology. We thank Beijing Union Medical Hospital for the MRSA isolates supported.

## Disclosure Statement

No competing financial interests exist.

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