



Antimicrobial Resistance and Molecular Characteristics of Nasal *Staphylococcus aureus* Isolates From Newly Admitted Inpatients

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Staphylococcus aureus, or methicillin-resistant *S. aureus* (MRSA), is a significant pathogen in both nosocomial and community infections. Community-associated MRSA (CA-MRSA) strains tend to be multi-drug resistant and to invade hospital settings. This study aimed to assess the antimicrobial resistance and molecular characteristics of nasal *S. aureus* among newly admitted inpatients. In the present study, 66 *S. aureus* isolates, including 10 healthcare-associated MRSA (HA-MRSA), 8 CA-MRSA, and 48 methicillin-sensitive *S. aureus* (MSSA) strains, were found in the nasal cavities of 62 patients by screening 292 newly admitted patients. Antimicrobial resistance and molecular characteristics of these isolates, including spa-type, sequence type (ST) and SCCmec type, were investigated. All isolates were sensitive to linezolid, teicoplanin, and quinupristin/dalfopristin, but high levels of resistance to penicillin and erythromycin were detected. According to D-test and *erm* gene detection results, the cMLS_B and iMLS_B phenotypes were detected in 24 and 16 isolates, respectively. All 10 HA-MRSA strains displayed the cMLS_B phenotype mediated by *ermA* or *ermA/ermC*, while the cMLS_B CA-MRSA and MSSA strains carried the *ermB* gene. Molecular characterization revealed all 10 HA-MRSA strains were derived from the ST239-SCCmec III clone, and four out of eight CA-MRSA strains were t437-ST59-SCCmec V. The results suggest that patients play an indispensable role in transmitting epidemic CA-MRSA and HA-MRSA strains.

Key Words: *Staphylococcus aureus*, CA-MRSA, Nasal carriage, Antimicrobial resistance, Molecular characteristics

Received: November 5, 2015

Revision received: January 21, 2016

Accepted: January 29, 2016

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Staphylococcus aureus is a significant pathogen in both nosocomial and community infections. This bacterium is capable of colonizing various bodily sites, especially the nose [1]. Nasal carriage of *S. aureus* or methicillin-resistant *S. aureus* (MRSA) is considered a risk factor for further endogenous infections such as sepsis [2]. Various carriage rates of nasal *S. aureus*

have been reported, and recent data suggest a carriage rate of 20-30%. Moreover, scientific interest has focused on nasal community-associated MRSA (CA-MRSA), with respect to nasal prevalence, antibiotic resistance, and molecular characterization [3, 4]. MRSA strains first emerged in hospitals in the early 1960s, and several clones have become prevalent in healthcare

settings worldwide. However, several community infection-associated clones, referred to as CA-MRSA, emerged in the 1990s and changed the concept of MRSA. Worse still, CA-MRSA tend to be multi-drug resistant and to invade hospital settings. Nasal carriers of CA-MRSA strains may play a role in infection transmission between community and hospital settings. Therefore, it is of great importance to detect nasal carriage of *S. aureus* among inpatients at hospital admission.

The healthcare-associated MRSA (HA-MRSA) sequence type (ST) 239 clone is prevalent in China, whereas methicillin-sensitive *S. aureus* (MSSA) strains show great diversity in molecular characteristics [5]. Several CA-MRSA clones have been reported from China, and ST59, or its single locus variant ST338, is the most common clone [5, 6]. Nevertheless, molecular epidemiology of nasal *S. aureus* among healthy individuals and community populations from China indicate molecular heterogeneity. Limited data on nasal *S. aureus* among inpatients was reported. In the present study, we analyzed nasal carriage rates of *S. aureus* among newly admitted inpatients by microbiologic examination, antimicrobial susceptibility testing, and molecular characterization.

A total of 66 nasal *S. aureus* isolates were collected from 292 patients within 48 hr after admission to a tertiary hospital in Shanghai, China. Bacterial identification was performed by a combination of phenotypic tests, such as microscopic examination and production of coagulase and DNase [7, 8]. Molecular identification was verified by amplification of the *nuc* gene with specific primers [8]. Susceptibility testing was carried out by using the disk diffusion method according to the CLSI guidelines [9]. Tested antimicrobials included penicillin (10 units), cefoxitin (30 µg), teicoplanin (30 µg), gentamicin (10 µg), kanamycin (30 µg), tobramycin (10 µg), erythromycin (15 µg), clindamycin (2 µg), quinupristin/dalfopristin (15 µg), tetracycline (30 µg), minocycline (30 µg), ciprofloxacin (5 µg), sulfamethoxazole/trimethoprim (25 µg), chloramphenicol (30 µg), rifampicin (5 µg), linezolid (30 µg), and fusidic acid (10 µg). Susceptibility to penicillin was also observed by the penicillin zone-edge test. Inducible resistance to clindamycin was tested by using the D-test, and high-level mupirocin resistance (MUP^{Hi}) was screened by using a mupirocin disk (200 µg). *S. aureus* ATCC 25923 was used as the control strain for susceptibility testing.

MRSA strains were screened by cefoxitin disk and confirmed by detection of the *mecA* gene. The *erm* genes (*ermA*, *ermB*, and *ermC*) and *ileS-2* gene were detected by PCR as previously described [10, 11]. Multi-locus sequence typing (MLST) and *spa* typing, with additional *SCCmec* typing for MRSA strains,

were performed [10-12]. Analysis of these molecular characteristics was conducted by grouping isolates with eBURST based on ST. Moreover, a series of clinically significant toxin genes encoding staphylococcal enterotoxins (*sea-see* and *seg-sej*), exfoliative toxin (*eta* and *etb*), toxic shock syndrome toxin 1 (*tst*), and Panton-Valentine leukocidin (*pvl*) were detected [13].

By cefoxitin screening and *mecA* gene detection, 18 out of 66 (27.3%) nasal *S. aureus* isolates were *mecA*-positive MRSA. The results of antimicrobial susceptibility testing revealed that all isolates were sensitive to linezolid, teicoplanin, and quinupristin/dalfopristin. Two isolates, one MRSA strain and one MSSA strain isolated from the same patient, exhibited the MUP^{Hi} phenotype and harbored the *ileS-2* gene. High resistance rates for penicillin (92.4%), erythromycin (60.6%), kanamycin (45.5%), tobramycin (40.9%), tetracycline (37.9%), clindamycin (36.4%), and gentamicin (28.8%) were detected. Resistance rates for ciprofloxacin, chloramphenicol, sulfamethoxazole/trimethoprim, rifampicin, minocycline, and fusidic acid were 21.2%, 13.6%, 7.6%, 7.6%, 1.5%, and 1.5%, respectively. The antimicrobial resistance patterns (ARP) of the nasal *S. aureus* isolates, including 18 MRSA and 48 MSSA are shown in Table 1.

By molecular typing, 10 out of 18 (55.6%) MRSA isolates were HA-MRSA clones, all of which were ST239-SCC*mec* III with different *spa*-types. The remaining eight MRSA isolates were genotypic CA-MRSA strains, a half of which were t437-ST59-SCC*mec* V. On the other hand, MSSA strains displayed diverse molecular characteristics with some prominent clones, including t091-ST7 (six isolates), t616-ST182 (five isolates), t189-ST188 (four isolates), and t002-ST5 (four isolates). Toxin gene profiling revealed that most isolates (35/66, 53%) did not harbor any toxin genes. HA-MRSA strains tended to carry the *sea* gene, while CA-MRSA strains usually carried the *seb* gene. Moreover, all four CA-MRSA isolates, which were t437-ST59-SCC*mec* V, were positive for the *pvl* gene. Different staphylococcal enterotoxin genes were found in some MSSA isolates, especially ST5 and ST182 clones. Detailed molecular characterization and toxin gene profiles are shown in Table 1 and the Supplemental Data Table S1.

According to our D-test results, the phenotypes cMLS_B, characterized by resistance to both erythromycin and clindamycin, and iMLS_B (positive D-test) were detected in 24 and 16 isolates, respectively. However, no isolates exhibited the MS_B phenotype, characterized by susceptibility to clindamycin and erythromycin resistance with a negative D-test.

The *erm* genes were detected, and their distribution among different strains, including CA-MRSA, HA-MRSA, and MSSA,

Table 1. Antimicrobial resistance patterns and molecular characteristics of nasal *S. aureus* isolates

Antimicrobial resistance patterns (ARP)	MSSA	MRSA	Molecular characteristics*
None	2		t616-ST182, t12280-ST398
E	2		t616-ST182, t12281-ST new1 [†]
TE	1		t286-ST1
P	18		t616-ST182 (3), t548-ST5 (2), t084-ST15, t127-ST1, others [‡]
P-CN-K-TOB-E-CC	3		t002-ST5 (2), t571-ST398 (1)
P-CN-K-TOB-E-CC-CL	1		t002-ST5
P-CN-K-TOB-E-CIP-CC	2		t189-ST188 (2)
P-CN-K-TOB-E-TE-CC	1		t002-ST5
P-CN-K-TOB-E-TE-CC-CL	1		t437-ST59
P-E	4		t164-ST20, t436-ST28, t189-ST188, t8296-ST2148
P-E-FD	1		t12277-ST630
P-E-MH	1		NT-ST1
P-E-TE	2		t853-ST15, t895-ST5
P-K-E-CC-CL	1		t441-ST59
P-K-TOB-E-CL	1		t091-ST7
P-K-TOB-E-TE	2		t091-ST7, t091-ST789
P-K-TOB-TE-CL	1		t091-ST7
P-TE	1		NT-ST1667
P-TOB	1		t091-ST7
P-TOB-E-TE	1		t091-ST7
P-TOB-TE-MUP ^{hi}	1		t091-ST7
P-CX-CN-K-TOB-E-TE-CIP-CC-RA		4	t632-ST239-SCCmec III (4)
P-CX-CN-K-TOB-E-TE-CIP-CC-RA-MUP ^{hi}		1	t030-ST239-SCCmec III
P-CX-CN-K-TOB-E-TE-CIP-CC-SXT		5	t037-ST239-SCCmec III (5)
P-CX-CN-K-TOB-E-TE-CIP-CL		1	t008-ST8-SCCmec IV
P-CX-E		1	t034-ST804-SCCmec V
P-CX-K-E-CC-CL		2	t437-ST59-SCCmec V (2)
P-CX-K-E-TE-CC		2	t437-ST59-SCCmec V, t437-ST59-SCCmec IV
P-CX-K-E-TE-CC-CL		1	t437-ST59-SCCmec V
P-CX-K-TOB-CIP		1	t127-ST1-SCCmec IV
Total	48	18	

*Molecular characteristics shown as spa-type, sequence type (ST), (and SCCmec type for MRSA strains); No. of isolates with the same molecular characteristics indicated in the bracket when necessary; [†]ST new1, allelic profile 1-1-1-1-238-40; [‡]Others including t163-ST59, t164-ST20, t186-ST88, t189-ST188, t693-ST1, t701-ST6, t1341-ST5, t6378-ST new2 (allelic profile 1-1-1-1-58-1-1), t12276-ST45, t12280-ST398, NT-ST96.

Abbreviations: MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; P, penicillin; CX, cefoxitin; CN, gentamicin; K, kanamycin; TOB, tobramycin; E, erythromycin; CC, clindamycin; TE, tetracycline; MH, minocycline; CIP, ciprofloxacin; SXT, sulfamethoxazole/trimethoprim; CL, chloramphenicol; RA, rifampicin; FD, fusidic acid; MUP^{hi}, high-level mupirocin resistance; None, sensitive to all tested antimicrobial drugs; NT, not typable.

was explored. As shown in Table 2, all 10 HA-MRSA strains displayed the cMLS_B phenotype, which was mediated by the *ermA* or *ermA/ermC* genes. CA-MRSA and MSSA strains exhibiting the cMLS_B phenotype carried the *ermB* gene. All D-test positive strains, both MSSA and CA-MRSA, were only positive for the

ermC gene. Unexpectedly, four patients were found to be colonized with two nasal *S. aureus* strains with different molecular characteristics. Brief information on these four patients and the strains is listed in Table 3.

This study investigated the status of nasal *S. aureus* from new-

Table 2. Distribution of *erm* genes among different strains

Phenotype	Strain	N of isolates carrying					Total
		<i>ermA</i>	<i>ermB</i>	<i>ermC</i>	<i>ermA+ermC</i>	<i>ermB+ermC</i>	
cMLS _B (24)	CA-MRSA		5				5
	HA-MRSA	4			6		10
	MSSA		8			1	9
iMLS _B (16)	CA-MRSA			2			2
	MSSA			14			14
Total		4	13	16	6	1	40

Abbreviations: CA-MRSA, community-associated methicillin-resistant *Staphylococcus aureus*; HA-MRSA, healthcare-associated methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*.

Table 3. Brief information on four patients who were co-colonized with two nasal *S. aureus* strains

No.	Sex	Age	Strain One	Strain Two	Note
Patient 1	m	40	t091-ST7	t030-ST239-SCC <i>mec</i> III	Both strains were MUP ^{Hi}
Patient 2	m	39	t037-ST239-SCC <i>mec</i> III	t632-ST239-SCC <i>mec</i> III	Both strains were multi-drug resistant (MDR)
Patient 3	m	21	NT-ST96	t437-ST59-SCC <i>mec</i> V	Strain Two carried <i>seb</i> and <i>pvl</i>
Patient 4	m	20	t163-ST59	t616-ST182	Strain One carried <i>seb</i> ; Strain Two carried <i>sei</i>

Abbreviations: m, male; ST, sequence type; MUP^{Hi}, high-level mupirocin resistance.

lyadmitted inpatients, and the results revealed that 62 out of 292 (20.5%) inpatients carried *S. aureus* in the nose. Among the 62 inpatients, 66 *S. aureus* isolates were detected, as four patients were co-colonized with two nasal *S. aureus* strains with different molecular characteristics. Of these 66 *S. aureus* isolates, 18 were MRSA strains including 10 HA-MRSA and 8 CA-MRSA strains. High resistance rates for penicillin and erythromycin were observed for both MSSA and MRSA strains.

Nasal colonization of *S. aureus* can be seen as a risk factor for further endogenous infection [2]. In this study, 20.5% of newlyadmitted inpatients carried *S. aureus* in the nose, suggesting that it is essential to detect and eliminatenasal *S. aureus* in inpatients at hospital admission, especially those of high-risk departments, such as orthopedics or the burn unit [14, 15]. The antibiotic erythromycin was commonly used for combating *S. aureus* in China, yet high resistance to erythromycin was observed in these nasal isolates. In Table 2, we show that different kinds of strains displayed diverse resistance phenotypes mediated by various resistance genes. It is difficult to eliminate or treat nasal *S. aureus* efficiently; mupirocin is a good option despite the fact that some mupirocin-resistant *S. aureus* strains were detected in the present study.

Molecular characterization of the nasal *S. aureus* isolates revealed that all 10 HA-MRSA strains were ST239-SCC*mec* III, a major clone causing nosocomial infection, and that four out of

eight CA-MRSA strains were t437-ST59-SCC*mec* V, reported to be a prevalent clone in community settings. As reported, HA-MRSA and CA-MRSA strains may co-exist with the interaction and dynamic of hospital and community populations [16]. In the present study, molecular data typically provided microbiological and epidemiological information on the role of patients in exchanging epidemic CA-MRSA and HA-MRSA strains. Therefore, the nasal carrier patients can be regarded as an important risk factor for the introduction of CA-MRSA into hospitals. It would also be helpful to prevent and monitor hospital infections if some measures were taken to detect the nasal carriage status of inpatients in China.

Unexpectedly, it was found that four patients were co-colonized by two nasal *S. aureus* strains. It was suspected when distinct bacterial colonies with different morphology and hemolysis were observed on blood agar plates, and was confirmed by molecular typing. In one patient, both strains (one MSSA t091-ST7 and one MRSA t030-ST239-SCC*mec* III) exhibited the MUP^{Hi} phenotype and harbored the *ileS-2* gene, indicating potential horizontal resistance determinant transfer *in vivo* in the nasal cavity.

In this study, antimicrobial resistance and molecular characteristics of nasal *S. aureus* isolates from newlyadmitted inpatients were investigated. All isolates were sensitive to linezolid, teicoplanin, and quinupristin/dalfopristin, but high resistance

against erythromycin was detected. Molecular evidence suggests that patients play an indispensable role in transmitting epidemic CA-MRSA and HA-MRSA strains in different environments, thus making it essential to detect the nasal carriage status of inpatients to control emerging genotypic CA-MRSA strains in hospitals.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Acknowledgments

This study was supported in part by the Research Project of Science and Technology Commission of Shanghai (15YF1407300). We acknowledge the use of the *S. aureus* MLST database (Imperial College London, London, UK; funded by the Wellcome Trust). The authors would also thank Prof. Yuxing Ni and Dr. Lizhong Han (Department of Clinical Microbiology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine) for their kind help.

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