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ARTICLE

Clonal spreading of methicillin-resistant SCC*mec* *Staphylococcus aureus* with specific *spa* and *dru* types in central Taiwan

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Abstract The goal of this study was to delineate the molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) in Taiwan. Ninety-six MRSA isolates were collected from the blood cultures of different patients during the period July to December of 2008. The *spa* typing, staphylococcal chromosomal cassette (SCC*mec*) typing, *mec*-associated direct repeat unit (*dru*) copy numbers, and toxin genes (*sea*, *seb*, *sec*, *tst*, *lukS/F*) of each isolate were determined. Thirty-eight, 28, 18, and 12 MRSA isolates were SCC*mec* type II, SCC*mec* type III, SCC*mec* type IV, and SCC*mec* type V, respectively. Most (31/38, 81.6%) of the SCC*mec* type II isolates were of *spa* t002 with four *dru* repeats. Some of them also carried the *sec* or *tst* toxin gene (67.7 and 80.6%, respectively). Of the 28 SCC*mec* type III MRSA isolates, 15 (53.6%) were of t037 with 14 *dru* repeats, and all also carried the *sea* gene. Of the 18 SCC*mec* type IV MRSA isolates, 13 (72.2%) were of t437 with nine *dru* repeats, and ten of them also had the *seb* gene. Among the SCC*mec* type V MRSA isolates, nine were type V_T. Five (55.6%) of them were of t437 with 11 *dru* repeats, and all contained the *lukS/F* gene. The clonal spreading of SCC*mec* MRSA strains with specific *spa* and *dru* types was found. Further longitudinal, multiple-site surveillance is required in order to define the MRSA evolution in Taiwan.

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Introduction

Staphylococcus aureus is an important causative agent of a wide variety of diseases, including local skin and soft tissue infections, deep-seated abscesses, osteomyelitis, pneumonia, life-threatening septicemia, and endocarditis [1]. The clinical importance of *S. aureus* is attributed to its high virulence and rapid development of drug resistance. *S. aureus* virulent factors include surface proteins, toxins, and enzymes [2]. The antibiotics penicillin and methicillin that are commonly used to treat *S. aureus* infections almost always lead to the emergence of resistant strains within 1 to 2 years [3]. Although vancomycin does not always induce resistance, vancomycin-resistant *S. aureus* has emerged since 1996 [4]. Since the spreading of specific lineages of methicillin-resistant *Staphylococcus aureus* (MRSA) in the community or hospital varies in different areas [4, 5], we analyzed predominant lineages of MRSA isolates in our hospital using various methods, including multilocus sequence typing (MLST), staphylococcal chromosomal cassette (SCC*mec*) typing, accessory gene regulator (*agr*) and staphylococcal protein A (*spa*) typing, and *mec*-associated direct repeat unit (*dru*) locus typing.

Materials and methods

Ninety-six MRSA isolates were collected from the blood cultures of different patients from July to December of 2008 in the bacteriological laboratory of China Medical University Hospital located in central Taiwan. The BACTEC 9000 blood culture system (Becton Dickinson, Sparks, MD, USA) was used to grow and screen bacteria. Bacterial isolates were identified as *S. aureus* and the antibiotic susceptibility to various antimicrobial agents was determined using the BD Phoenix™ Automated Microbiology System (Becton Dickinson). The minimum inhibitory concentration (MIC) interpretive standard for various antibiotics' susceptibility was those illustrated by the Clinical Laboratory Standards Institute (CLSI) [6]. The basic and clinical information of each patient was collected by reviewing medical records. The community-acquired MRSA (CA-MRSA) infection were defined as a patient without histories of surgery, hospitalization, long-term care facility residence, dialysis, indwelling device, or catheter within the most recent year, or hospitalization>48 h before MRSA culture [7]. Hospital-acquired MRSA (HA-MRSA) were those other than CA-MRSA.

The DNA of all MRSA isolates was extracted by the Genomic DNA Mini Kit (Geneaid, Taiwan). Each typing method, including SCC*mec*, *agr*, *spa*, MLST, and *mec*-associated direct repeat unit (*dru*) copy numbers, were performed as described previously [8–13]. The types V and V_T SCC*mec* were distinguished as described previously [14]: the size of the polymerase chain reaction (PCR) products of SCC*mec* type V was 325 bp, and that of SCC*mec* V_T was 600 bp. The detection of the toxin genes, including enterotoxin A (*sea*), enterotoxin B (*seb*), enterotoxin C (*sec*), toxic shock toxin-1 (*tst*), and Panton–Valentine leukocidin (*lukS/F*), was performed as previously reported [15].

Results

Table 1 shows the clinical characteristics, diagnosis, and MIC distributions of various antibiotics of these 96 MRSA isolates. All strains were isolated from 91 adults (mean age: 65.9 years) and five children (mean age: 7 years). All isolates of SCC*mec* type II or III isolates were hospital-acquired. The percentage of hospital-acquired strains SCC*mec* type IV and V isolates were both 66.7%. The rate of hospital-acquired strains decreased to 33.3% for both SCC*mec* type IV and V isolates if the culture time (48 h) was the only consideration for the discrimination of hospital or community infections. The most common clinical diagnosis responsible for these MRSA septicemia were primary bacteremia (n=43), followed by central vascular catheter-related infections (n=30), soft tissue, joint, or bone infections (n=12), infective endocarditis (n=7), and pneumonia (n=4). All 96 isolates are sensitive to vancomycin, teicoplanin and linezolid. More than 90% isolate are resistant to erythromycin and clindamycin. There are 14 isolates with MIC values located in the susceptible range of clindamycin, except for five (5/14=35.7%), which are amended to resistance because of a positive D-zone test.

The results of the molecular typing and identification of virulence genes of the 96 MRSA isolates are shown in Table 2. There were 38 SCC*mec* type II, 28 SCC*mec* type III, 18 SCC*mec* type IV, and 12 SCC*mec* type V isolates. For *agr*, the most common was type I (55/96=57.3%), followed by type II (36/96=37.5%) and type IV (3/96=3.1%). There were only one type III *agr* and one non-typable isolates. Most (35/36, 97.2%) *agr* type II isolates belonged to SCC*mec* type II. For the 38 SCC*mec* type II isolates, *spa* type t002 with four *dur* repeat units was predominant (31/38=81.6%). All ten isolates selected for MLST typing were determined to be ST5 and *agr* type II. Twenty-one (67.7%) of 31 *agr* type II isolates also carried the *sec* gene, and 25 (80.6%) of these also harbored the *tst* gene. Twenty-seven of the 28 SCC*mec* III isolates were *agr* type I. Fifteen (53.6%) of these 28 SCC*mec* III isolates were *spa* t037 with 14 *dru* repeated units, also harbored the *sea* toxin gene, and belonged to MLST type 239. Sixteen of the 18 SCC*mec* type IV isolates belonged to *agr* type I. Most (13/18, 72.2%) of them harbored the *seb* toxin gene and were *spa* type t437 with nine *dru* repeat units and ST59. Among the 12 SCC*mec* type V isolates, nine (75%) were type V_T. Five (55.6%) of these nine V_T isolates were *spa* type t437 with 11 *dru* repeat units; these five isolates also carried the Panton–Valentine leukocidin (PVL or *lukS/F*) gene and were ST59.

Discussion

Several genotyping methods are being used for the epidemiological studies of *S. aureus*. MLST is suitable for the determination of macro-variations or long-term revolution in large populations, but PFGE is used for investigating micro-variations or short-term revolution in smaller populations [4]. The *spa* typing, based on a variable number of tandem repeats in the gene of protein A (*spa*), has a discrimination power between PFGE and MLST [16, 17].

Although MLST typing was not performed on all isolates in this study, the MLST types of the isolates can be inferred from their *spa* types because the isolates with the same *spa* type always belonged to the same MLST type but not vice versa [17]. The results of our random selection of isolates for MLST typing confirmed this observation. Combined with *agr* and SCC*mec* typing, we found some

Table 1 Clinical characteristics, diagnosis, and various antibiotics' minimum inhibitory concentration (MIC) distribution of 96 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates

	SCC*mec* type (no.)				
	II (*n*=38)	III (*n*=28)	IV (*n*=18)	V (*n*=12)	
				V_T (*n*=9)	Non-V_T (*n*=3)
Characteristic, no. (%)					
Adult (≧18 years)	38 (100%)	26 (92.9%)	17 (94.4%)	7 (77.8%)	3 (100%)
Child (< 18 years)	–	2 (7.1%)	1 (5.6%)	2 (22.2%)	–
Male	21 (55.3%)	18 (64.3%)	13 (76.5%)	5 (55.5%)	2 (66.7%)
Female	17 (44.7%)	10 (35.7%)	5 (27.8%)	4 (44.4%)	1 (33.3%)
Hospital-acquired	38 (100%)	28 (100%)	12 (66.7%)	5 (55.6%)	3 (100%)
Community-acquired	–	–	6 (33.3%)	4 (44.4%)	–
MRSA detection >48 h after admission	28 (73.7%)	19 (67.9%)	6 (33.3%)	2 (22.2%)	2 (66.6%)
Clinical diagnosis, no. (%)					
Primary bacteremia	23 (60.5%)	11 (39.3%)	7 (38.9%)	2 (22.2%)	1 (33.3%)
Central vascular catheter-related	11 (28.9%)	11 (39.3%)	4 (22.2%)	2 (22.2%)	2 (66.7%)
Soft tissue, joint, or bone infections	3 (7.9%)	3 (10.7%)	3 (16.7%)	3 (33.3%)	–
Infective endocarditis	–	2 (7.1%)	2 (11.1%)	2 (22.2%)	–
Pneumonia	1 (2.6%)	1 (3.6%)	2 (11.1%)	–	–
MIC (μg/ml) distribution of various antibiotics, no. (%)					
Erythromycin					
≦0.5 (S)	–	1 (3.6%)	4 (22.2%)	–	3 (100%)
≧8 (R)	38 (100%)	27 (96.4%)	14 (77.8%)	9 (100%)	–
Tetracycline					
≦4 (S)	34 (89.5%)	3 (10.7%)	9 (50%)	3 (33.3%)	–
8 (I)	1 (2.6%)	2 (7.1%)	8 (44.4%)	5 (55.6%)	–
≧16 (R)	3 (7.9%)	23 (82.1%)	1 (5.6%)	1 (11.1%)	3 (100%)
Clindamycin					
≦0.25 (S)	–	2 (7.1%)	4 (22.2%)	–	3 (100%)
≧4 (R)	37 (97.4%)	25 (89.3%)	11 (61.1%)	9 (100%)	–
D*(+) (R)	1 (2.6%)	1 (3.6%)	3 (16.7%)	–	–
Levofloxacin					
≦1 (S)	3 (7.9%)	2 (7.1%)	17 (94.4%)	8 (88.9%)	–
2 (I)	–	1 (3.6%)	–	–	–
≧4 (R)	35 (92.1%)	25 (89.3%)	1 (5.6%)	1 (11.1%)	3 (100%)
SXT					
≦0.5/9.5 (S)	25 (65.8%)	3 (10.7%)	18 (100%)	9 (100%)	3 (100%)
≧4/76 (R)	13 (34.2%)	25 (89.3%)	–	–	–
Vancomycin					
≦0.5 (S)	3 (7.9%)	2 (7.1%)	–	–	–
1 (S)	34 (89.5%)	21 (75%)	17 (94.4%)	9 (100%)	3 (100%)
2 (S)	1 (2.6%)	5 (17.9%)	1 (5.6%)	–	–
Teicoplanin					
≦0.5 (S)	5 (13.2%)	5 (17.9%)	16 (88.9%)	8 (88.9%)	3 (100%)
1 (S)	17 (44.7%)	19 (67.9%)	1 (5.6%)	–	–
2 (S)	13 (34.2%)	1 (3.6%)	1 (5.6%)	1 (11.1%)	–
4 (S)	3 (7.9%)	1 (3.6%)	–	–	–
8 (S)	–	2 (7.1%)	–	–	–
Linezolid					
1 (S)	23 (60.5%)	4 (14.3%)	3 (16.7%)	–	2 (22.2%)
2 (S)	14 (36.8%)	24 (85.7%)	15 (83.3%)	7 (77.8%)	3 (100%)
4 (S)	1 (2.6%)	–	–	–	–

S: susceptible, I: intermediate, R: resistant, SXT: trimethoprim–sulfamethoxazole

*D-zone: inducible clindamycin resistance

Table 2 Molecular typing and virulent genes of 96 MRSA isolates

SCC*mec* type (no.)	*spa* type: no. of DRUs[g] (no.)	MLST type[h]	*agr* type[g] (no.)	Virulent genes no. (%)				
				sea	*seb*	*sec*	*tst*	*lukS/F*
II (*n*=38)	t002: 4 (*n*=31)	5 (10/10)	II (*n*=31)	1 (3.2%)	3 (9.6%)	21(67.7%)	25(80.6%)	–
	Others[a] (*n*=7)		II (*n*=4)	–	1 (14.3%)	6 (85.7%)	6 (85.7%)	–
			I (*n*=3)					
III (*n*=28)	t037: 14 (*n*=15)	239 (5/5)	I (*n*=15)	15 (100%)	–	–	–	–
	t037: X[b] (*n*=7)	239 (2/2)	I (*n*=7)	5 (71.4%)	1 (14.3%)	–	1 (14.3%)	–
	Others[c] (*n*=6)		I (*n*=5)	3 (50%)	–	–	–	–
			N* (*n*=1)					
IV (*n*=18)	t437: 9 (*n*=13)	59 (3/3)	I (*n*=13)	–	10 (76.9%)	–	–	2 (15.4%)
	t437: 4 (*n*=1)		I (*n*=1)	–	–	–	–	–
	Others[d] (*n*=4)		I (*n*=2)	1 (25%)	3 (75%)	1 (25%)	-	1 (25%)
			II, III (*n*=1)					
V (*n*=12)	V_T (*n*=9)							
	t437: 11 (*n*=5)	59 (2/2)	I (*n*=5)	–	4 (80%)	–	–	5 (100%)
	t437: Y[e] (*n*=2)		I (*n*=2)	–	2 (100%)	–	–	1 (50%)
	Others[f] (*n*=2)		I (*n*=2)	–	–	–	–	1 (50%)
	Non-V_T (*n*=3)							
	t1081: 9 (*n*=1)		IV	–	–	–	–	–
	t1081: 10 (*n*=1)		IV	–	–	–	–	–
	t824: 9 (*n*=1)		IV	–	–	–	–	–

[a] t037: 14; t214: 4; t234: 13; t1094: 4 (*n*=3); t3527: 9

[b] X=6, 10, 12, 13 (*n*=2), 15; N non-typable

[c] t138: 14; t234: 10; t234: 13; t932: 14; t3528; 10, N*

[d] t186: 5; t411: 9; t1081: 9; new *spa* type: 9

[e] Y = 4, 12

[f] t1212: 4; t1751: 4

[g] Numbers in parentheses represent the no. of isolates with a certain *spa* no., DRUs, or *agr* type

[h] Numbers in parentheses represent the no. of isolates that underwent MLST analysis

*Non-typable

predominant strains with specific *spa* types in each SCC*mec* group. For type II and III SCC*mec* isolates, the predominant MLST *spa* types were ST5-t002 (USA100, New York/Japan clone) and ST239-t037 (Brazilian/Hungarian clone), respectively. These results were similar to those of the previous report on the analyses of isolates from northern Taiwan with slightly lower percentage rates (81.6/78.6% vs. 97/93%) [18]. High rates of ST5-t002 isolates containing both *sec* and *tst* toxin genes and ST239-t037 isolates containing the *sea* gene were also found. For type IV and V SCC*mec* isolates, most of them belonged to the ST59-t437 lineage; this is different from the predominant stains, such as ST8-t008 (USA 300), ST1-t127 (USA 400), ST80-t044 (European), and ST30-t012 (Southwest Pacific, USA1100) found in other countries [5, 19–21]. In type IV SCC*mec* isolates, the PVL-positive rate was significantly lower than those of other countries [22, 23]. In Taiwan, most PVL-positive MRSA isolates are SCC*mec* type V [24, 25]. However, most studies did not distinguish type V_T from type V, except the ones by Takano et al. and Boyle-Vavra et al. [26, 27]. In these two studies, most of the MRSA isolates were from colonization or infection of the skin and soft tissue. In our study, all MRSA isolates were from patients with bacteremia, and 70% of PVL-positive MRSA isolates were type V_T SCC*mec*.

In this study, we found some isolates with the same *spa* type and *dru* copy number in each of the four SCC*mec* groups. Interestingly, the SCC*mec* type, *spa* type, and *dru* copy numbers of the two vancomycin-intermediate *S. aureus* (VISA) strains in our previous study were identical to those of the major clones of SCC*mec* type III-t037-14 and SCC*mec* type IV-t437-9 found in this study, respectively [28]. This finding is consistent with the result of a previous study, which suggested the clonal dissemination of VISA in a hospital in Taiwan [29]. The phenomenon of the clonal spreading of VISA in Taiwan may be attributed to

the existence of these MRSA major clones, which could eventually lead to heteroresistant VISA (hVISA) and VISA after prolong vancomycin exposure.

For SCC*mec* type IV and V isolates, more than half belonged to hospital-acquired infections. This blurring distinction between hospital- and community-acquired MRSA had been mentioned by other studies [30]. The sensitivity results of various non-β-lactam antibiotics in our study result were different in some aspects compared to previous reports [18]. The rate of positive inducible resistance to clindamycin was 5.2%, which is between other studies' reported rates [31, 32]. In our study, there were no glycopeptides non-susceptible MRSA according to the CLSI criteria, but a higher MIC was found in minority isolates: 7.3% with vancomycin MIC 2 μg/ml and 22.9% with teicoplanin MIC 2–8 μg/ml. If the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint for teicoplanin was adopted (teicoplanin resistance was defined as MIC ≧4 μg/ml), there were six isolates (6.3%) in the non-susceptible range. Because of the low prevalence of VISA or hVISA in Taiwan [33] and inconsistencies between commercial and reference methods [34], further glycopeptide MIC testing by standard broth dilution or agar dilution methods for these higher MIC isolates would be suggested.

In summary, this study demonstrates that there are major MRSA clones sharing similar molecular characteristics, including *dru* copy number in the SCC*mec* region, the house keeping genes, X region of the *spa* gene, and various virulence genes among each SCC*mec* group in Taiwan. Further longitudinal, multiple-site surveillance for molecular characteristics and drug resistances of clinical MRSA isolates is essential in order to define the evolution history of MRSA in Taiwan.

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