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ORIGINAL ARTICLE

Fine typing of methicillin-resistant *Staphylococcus aureus* isolates using direct repeat unit and staphylococcal interspersed repeat unit typing methods

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Background/Purpose: Methicillin-resistant *Staphylococcus aureus* (MRSA) typing is an important epidemiologic tool for monitoring trends and preventing outbreaks. However, the efficiency of various MRSA typing methods for each SCC*mec* MRSA isolate is rarely evaluated.
Materials and methods: A total of 157 MRSA isolates from four different regions in Taiwan were typed with five different molecular methods, including SCC*mec* typing, multilocus sequence typing (MLST), *spa* typing, *mec*-associated direct repeat unit (*dru*) copy number determination, and staphylococcal interspersed repeat unit (SIRU) profiling.
Results: There were four SCC*mec* types, eight MLST types, 15 *spa* types, 11 *dru* types, and 31 SIRU profiles. The most common type determined by each molecular typing method was SCC*mec* III (115 isolates, 73.2%), ST239 (99 isolates, 63.1%), t037 (107 isolates, 68.2%), 14

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dru copies (76 isolates, 48.4%), and SIRU profile 3013722 (102 isolates, 65%), respectively. When using the combination of MLST, *spa* typing, and *dru* copy number, ST5-t002-4 ($n = 8$), ST239-t037-14 ($n = 68$), ST59-t437-9 ($n = 9$), and ST59-t437-11 ($n = 6$) were found to be the most common types of SCC*mec* types II ($n = 9$), III ($n = 115$), IV ($n = 21$), and V_T ($n = 11$) isolates, respectively. SCC*mec* type III isolates were further classified into 11 *dru* types. Of the 21 SCC*mec* type IV isolates, 14 SIRU profiles were found. Seven SIRU patterns were observed in the 11 SCC*mec* type V_T isolates.

Conclusion: Different typing methods showed a similar Hunter—Gaston discrimination index among the 157 MRSA isolates. However, *dru* and SIRU typing methods had a better discriminatory power for SCC*mec* type III and SCC*mec* types IV and V_T isolates, respectively, suggesting that *dru* and SIRU can be used to further type these isolates.

Introduction

Staphylococcus aureus is one of the most common pathogens. It can cause diseases such as cellulitis, myositis, food poisoning, septicemia, and toxic shock syndrome.[1] *S. aureus* infections are usually treated with methicillin. Unfortunately, methicillin-resistant *S. aureus* (MRSA) has emerged, and the incidence of infection caused by MRSA is increasing.[2,3] MRSA isolates harbor the *mecA* gene, which encodes the penicillin binding protein 2a (PBP 2a), rendering them resistant to some beta-lactamase antibiotics such as penicillin and methicillin.[4] The mortality rate of MRSA bacteremia has been shown to be as high as 39%.[5] MRSA can be divided into hospital-acquired (HA-MRSA) and community-acquired MRSA (CA-MRSA).[2] HA-MRSA was first isolated in 1961, shortly after the introduction of methicillin,[6] and CA-MRSA was first found in the United States in the 1990s.[2]

Strain typing is an important epidemiologic tool for monitoring trends and preventing outbreaks of microbial infections. Because of their high discriminatory power and good reproducibility, molecular typing methods are increasingly used for epidemiologic studies.[7] For MRSA, several molecular typing methods including staphylococcal chromosomal cassette *mec* (SCC*mec*) typing, multilocus sequence typing (MLST), determination of direct repeat unit (*dru*), and pulse field gel electrophoresis have been developed.[2] MLST requires sequencing technologies that may not be available in every laboratory. Pulse field gel electrophoresis is labor intensive, and results from different laboratories are difficult to compare because of the lack of a universal nomenclature system.[7] The *dru* locus is located in the hypervariable region of the *mec*A gene, between *tnp* and *orf*145 genes.[8] Different MRSA isolates may have different copies of *dru*. Determination of the staphylococcal interspersed repeat unit (SIRU) pattern is another method for MRSA typing. This method accesses the variable number of tandem repeat of the whole genome of MRSA.[9,10] In this study, we compared the efficiency of various MRSA typing methods and determined if the discrimination powers of these methods were different among each SCC*mec* MRSA isolate.

Materials and methods

Bacterial isolates

A total of 157 MRSA isolates from blood cultures were used in this study. These isolates were collected by the SMART (Surveillance of Multicenter Antimicrobial Resistance in Taiwan) program from March to August 2003 from nine medical centers in Taiwan.[11,12] The contributing hospitals of these isolates are listed in Table 1.

DNA extraction

MRSA isolates were grown on BAP agar plates (BBL Microbiology Systems, Becton Dickinson). Three to five colonies of each isolate were suspended in 600 μL of TE buffer (10 mM Tris, 1 mM EDTA, pH8.0). The cells were then pelleted by centrifugation. DNA was extracted from the bacterial pellet using the Genomic DNA Mini Kit (Geneaid, Taiwan) as described previously.[11]

SCC*mec* typing

Identification of various SCC*mec* types were performed by multiplex polymerase chain reaction (PCR) using the genomic DNA from each MRSA isolate as the template as described previously.[13] Types V and V_T were distinguished with the following primers[14]: F: 5′-GAACATTGTTA CTTAAATGAGCG-3′ and R: 5′-TGAAAGTTGTACCCTTGACACC-3′. The amplification was carried out with a 1-minute heating step at 94°C, followed by 30 cycles of 30 seconds at 94°C for denaturation, 60 seconds at 55°C for primer annealing, and 60 seconds at 72°C for extension, and then 5 minutes at 72°C for final extension. The PCR product of SCC*mec* type V was 325 bp, and that of SCC*mec* V_T was 1600 bp.

MLST typing

Seven housekeeping genes (*arc*, *aroE*, *glp*, *gmk*, *pta*, *tpi*, *yqiL*) of *S. aureus* were used for typing. The amplification of a portion of each gene was performed as described

Table 1 SCC*mec* types of the 157 MRSA isolates collected from nine medical centers in four different regions in Taiwan

SCC*mec* type (no.)	Northern			Central			Southern		Eastern
	N1	N2	N3	M1	M2	M3	S1	S2	E1
II (9)	2	1	—	1	—	3	1	—	1
III (115)	48	3	14	2	1	11	21	1	14
IV (21)	13	—	2	—	—	2	1	—	3
V (12)									
V_T (11)	7	—	1	—	—	1	1	—	1
Non-V_T (1)	—	—	—	—	—	1	—	—	—
Total (157)	70	4	17	3	1	18	24	1	19

E1 = Buddhist Tzu-Chi General Hospital, Hualien; M1 = Chung Shan Medical University Affiliated Hospital; M2 = China Medical University Hospital; M3 = Taichung Veterans General Hospital; N1 = National Taiwan University Hospital; N2 = Taipei Veterans General Hospital; N3 = Tri-Service General Hospital; S1 = National Cheng-Kung University Hospital, S2 = Kaohsiung Medical University Chung-Ho Memorial Hospital.

previously.[15] The amplified products were sequenced, and the sequences thus obtained were analyzed using the software on http://saureus.mlst.net/sql/multiplelocus.asp.

spa typing

The X region of the *spa* gene contains 21- to 27-bp variable number of repeats. The size of the most common repeat is 24 bp. The X region of each MRSA isolate was amplified by PCR with primers 1095F: 5′-AGACGATCCTTCGGT GAGC-3′ and 1517R: 5′-GCTTTTGCAATGTCATTTACTG-3′ as described previously.[16] The amplified products were sequenced, and the sequences obtained were analyzed using the Ridom StaphType software (version 1.4; Ridom, GmbH, Wurzburg, Germany; http://spa.ridom.de/index.shtml) to determine the repeat profile and the *spa* type of each isolate.[17]

SIRU typing

SIRU typing was performed as previously described.[9,10] Strain N315 was used as the reference (SIRU pattern: 1313A31). The number of repeats of each locus was determined by combining the size of the repeat unit and its flanking regions: (1) SIRU01, 239 bp = 55 bp (repeat unit) + 184 bp (flanking region); (2) SIRU13, 212 bp = 64 bp + 148 bp; (3) SIRU15, 343 bp = 131 bp + 212 bp; (4) SIRU16, 321 bp = 159 bp + 162 bp; (5) SIRU21, 120 bp = 24 bp + 96 bp; (6) SIRU05, 216 bp = 60 bp + 156 bp; (7) SIRU07, 247 bp = 56 bp + 191 bp. Each SIRU pattern was represented with a seven-digit number such as 3013722, where each digit indicates the number of repeats of seven different loci in the following order: SIRU 01, 13, 15, 16, 21, 05, and 07.[10] For a locus with more than 10 repeats, the following designations were used: A for 10, B for 11, and C for 12 repeats.

mec-associated dru copy numbers

The copy number of *dru* was determined as described previously.[18] Each repeat was 40 bp. The size of the flanking region was 517 bp, and the primer sequences for amplification of the *dru* locus were 5′-GTTAGCATATTACCTCTCCTTGC-3′ and 5′-GCCGATTGTGCTTGATGAG-3′.

Discriminatory power

The discriminatory power of each typing method was calculated using the Hunter–Gaston discriminatory index (HGDI)[19] as follows:

$$\mathrm{HGDI} = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} n_j(n_j - 1),$$

where N is the total number of isolates examined, s is the total number of types identified, and n_j is the total number of isolates belonging to the jth type.

Results

SCC*mec* typing by multiplex PCR

The results of SCC*mec* typing of the 157 MRSA isolates are shown in Table 1. SCC*mec* type III (n = 115) was the most common, followed by types IV (n = 21), V (n = 12), and II (n = 9). Most SCC*mec* type V isolates were SCC*mec* type V_T (11/12 = 91.7%). There was no SCC*mec* type I isolate.

MLST typing

ST239 was the most common type (99 isolates, 63.1%), followed by ST59 (27 isolates, 17.2%), ST241 (12 isolates, 7.6%), and ST5 (9 isolates, 5.7%) (Table 2). All nine SCC*mec* type II isolates belonged to ST5. Most SCC*mec* type III isolates were ST239 (99 isolates, 86.1%), whereas most SCC*mec* types IV and V_T isolates were ST59 [17 isolates (80.9%) and 10 isolates (90.9%), respectively]. The only SCC*mec* type V isolate had a new ST type.

spa typing

Fifteen *spa* types were observed. The most common one was t037 (n = 107), followed by t437 (n = 20), t002 (n = 8), and t421 (n = 7). The other 11 *spa* types were found in the remaining 15 MRSA isolates. Most t037 isolates were SCC*mec* type III (105/107, 98.1%) and ST 239 (92/107, 86%). All 20 t437 isolates were ST59 and belonged to SCC*mec* type IV (n = 11) or V_T (n = 9). All eight t002

Table 2 Molecular typing of the 157 MRSA isolates

SCC*mec* type	MLST type	*spa* type	No. of *dru*	SIRU profile
II ($n = 9$)	5 ($n = 9$)	t002 ($n = 8$)	4 ($n = 8$)	2313A31 ($n = 8$)
		t242 ($n = 1$)	4 ($n = 1$)	2313A31 ($n = 1$)
III ($n = 115$)	239 ($n = 99$)	t037 ($n = 92$)	14 ($n = 68$)	3013722 ($n = 63$)
				3012722 ($n = 2$)
				1013722 ($n = 1$)
				2013722 ($n = 1$)
				3013822 ($n = 1$)
			12 ($n = 9$)	3013722 ($n = 8$)
				3013721 ($n = 1$)
			10 ($n = 6$)	3013722 ($n = 6$)
			13 ($n = 4$)	3013722 ($n = 3$)
				4013722 ($n = 1$)
			11 ($n = 2$)	3013722 ($n = 2$)
			9 ($n = 2$)	3013722 ($n = 1$)
				2142522 ($n = 1$)
			5 ($n = 1$)	3013722 ($n = 1$)
		t421 ($n = 4$)	14 ($n = 3$)	3013722 ($n = 3$)
			13 ($n = 1$)	3013722 ($n = 1$)
		t138 ($n = 1$)	11 ($n = 1$)	3013622 ($n = 1$)
		t388 ($n = 1$)	12 ($n = 1$)	3013721 ($n = 1$)
		t3519 ($n = 1$)	14 ($n = 1$)	3013822 ($n = 1$)
	241 ($n = 12$)	t037 ($n = 9$)	6 ($n = 5$)	3013722 ($n = 5$)
			1 ($n = 2$)	3013722 ($n = 2$)
			3 ($n = 1$)	3013722 ($n = 1$)
			4 ($n = 1$)	3013722 ($n = 1$)
		t421 ($n = 3$)	6 ($n = 3$)	3013621 ($n = 3$)
	900 ($n = 2$)	t037 ($n = 2$)	14 ($n = 2$)	3013722 ($n = 2$)
	New ($n = 2$)	t037 ($n = 2$)	14 ($n = 2$)	3013722 ($n = 2$)
IV ($n = 21$)	59 ($n = 17$)	t437 ($n = 11$)	9 ($n = 9$)	21427B4 ($n = 3$)
				21327B4 ($n = 1$)
				21527B2 ($n = 1$)
				21427B2 ($n = 1$)
				21427A4 ($n = 1$)
				2142724 ($n = 1$)
				2132722 ($n = 1$)
			5 ($n = 2$)	21427B4 ($n = 1$)
				2152724 ($n = 1$)
		t3592 ($n = 3$)	9 ($n = 3$)	2142724 ($n = 1$)
				21327B4 ($n = 1$)
				21427B4 ($n = 1$)
		t1751 ($n = 2$)	9 ($n = 2$)	21427C4 ($n = 1$)
				21527C4 ($n = 1$)
		t084 ($n = 1$)	9 ($n = 1$)	4112772 ($n = 1$)
	537 ($n = 4$)	t3406 ($n = 2$)	9 ($n = 2$)	3242BA3 ($n = 2$)
		t037 ($n = 1$)	9 ($n = 1$)	3242B53 ($n = 1$)
		t3525 ($n = 1$)	9 ($n = 1$)	3252A93 ($n = 1$)
VT ($n = 11$)	59 ($n = 10$)	t437 ($n = 9$)	11 ($n = 6$)	2142784 ($n = 2$)
				2142722 ($n = 1$)
				2112724 ($n = 1$)
				2142793 ($n = 1$)
				2142794 ($n = 1$)
			9 ($n = 2$)	21427B4 ($n = 2$)
			12 ($n = 1$)	2142784 ($n = 1$)
		t3529 ($n = 1$)	11 ($n = 1$)	21427B4 ($n = 1$)
	338 ($n = 1$)	New ($n = 1$)	11 ($n = 1$)	21425B4 ($n = 1$)
V(non-VT) ($n = 1$)	New	t037	10	3013722 ($n = 1$)

SIRU profiles: A = 10, B = 11, C = 12.

isolates were SCC*mec* type II and ST 5. All seven t421 isolates were SCC*mec* type III and belonged to ST 239 ($n = 4$) or ST241 ($n = 3$).

mec-associated dru copy numbers

A total of 11 different *dru* copy numbers (from 1 to 14 except 2, 7 and 8) were observed (Table 2). Isolates with 14 copies of *dru* were the most common (76/157, 48.4%), followed by those with nine copies (23/157, 14.6%), 11 copies (11/157, 7%), 12 copies (11/157, 7%), four copies (10/157, 6.4%), six copies (8/157, 5.1%), 10 copies (7/157, 4.5%), 13 copies (5/157, 3.2%), five copies (3/157, 1.9%), one copy (2/157, 1.3%), and three copies (1/157, 0.6%). Seventy-six (66.1%) of 115 SCC*mec* type III isolates had 14 copies. All nine SCC*mec* type II isolates had four copies. Two *dru* types (5 and 9 copies) were found in the 21 SCC*mec* type IV isolates, and three *dru* types (9, 11, and 12 copies) were observed in the 11 SCC*mec* V_T isolates. Most SCC*mec* types IV isolates had nine (19/21, 90.5%) *dru* copies, and most SCC*mec* type V_T isolates had 11 (8/11, 72.7%) *dru* copies.

SIRU profiles

Thirty-one SIRU profiles were found (Table 2). Profile 3013722 was the most common (102/157, 65%), followed by profiles 2313A31 (9/157, 5.7%) and 21427B4 (8/157, 5.1%). As shown in Table 2, all SCC*mec* type II isolates belonged to SIRU pattern 2313A31. Most SCC*mec* III isolates belonged to pattern 3013722 (101/115, 87.8%). The SIRU patterns of SCC*mec* type IV and V_T isolates were more heterogeneous. Of the 21 SCC*mec* type IV isolates, 14 SIRU profiles were found. Seven SIRU profiles were observed in the 11 SCC*mec* type V_T isolates.

Combined with SCC*mec*, MLST typing, *spa* typing, *dru* copy numbers, and SIRU typing results, all nine SCC*mec* type II isolates were found to be ST5 and t002 with four copies of *dru* and SIRU profile 231313A, except one isolate, which was *spa* type t242. For SCC*mec* III-ST239 isolates, most of them were t037 (92/99, 93%) with SIRU profile 3220137 (88/99, 88.9%). For SCC*mec* III-ST241 isolates, most of them were t037 (9/12, 75%) with SIRU profile 3013722 (9/9, 100%). In ST239 isolates, *dru* copy numbers were 5, 9, 10, 11, 12, 13, or 14, but those of ST241 isolates were 1, 3, 4, or 6. Most SCC*mec* IV and V_T isolates were ST59 and t437 [11/21 (52.4%) and 9/11 (81.8%), respectively], but their *dru* types and SIRU patterns varied widely.

Discriminatory power

The HGDI values of different typing methods are listed in Table 3. For all isolates, different typing methods had similar discrimination powers. However, SIRU profiling showed a better discriminatory power with an HGDI of 0.9315 for SCC*mec* type IV and V_T isolates than SCC*mec* type III isolates (HGDI = 0.2287). The *dru* typing method displayed a better discriminatory power for SCC*mec* type III (HGDI = 0.5495) than for type IV isolates (HGDI = 0.5181).

Discussion

In this study, we typed 157 MRSA isolates from nine medical centers in Taiwan with several different methods and found that most of the predominant strains belonged to the same MLST and *spa* types. The most common MLST-*spa* types of SCC*mec* type II, III, IV, and V_T isolates were ST5-t002, ST239-t037, ST59-t437, and ST59-t437, respectively. This result is similar to that reported by two previous single-center studies in Taiwan.[20,21] The predominant SCC*mec* III strain found in our study was ST239-t037, which was the same type as the Brazilian/Hungarian strain that was determined to originate from the transfer of a 557-kb fragment from the chromosome of an ST30 isolate into an ST8 lineage by homologous recombination.[22] The ST5-t002 strain (USA100, New York/Japan strain) of SCC*mec* type II was derived from the acquisition of the type II SCC*mec* in ST5 methicillin-sensitive *S. aureus* (MSSA).[23] The origin of SCC*mec* IV or V MRSA is still unknown. The acquisition of SCC*mec* IV by PVL positive ST30 MSSA or ST398 MRSA has been speculated, because there are more SCC*mec* type IV strains than SCC*mec* type II or III strains.[24,25]

Although there was a predominant *dru* type in each of the SCC*mec* types (4, 14, 9, and 11 *dru* copies for SCC*mec* II, III, IV, and V_T, respectively), there were a total of 11 *dru* types. Nine different SIRU profiles were found in SCC*mec* type III isolates. All SCC*mec* type II isolates belonged to profile 2313A31, and most SCC*mec* type III isolates were 3013722 (101/115, 87.8%). Although isolates with SIRU patterns similar to 3013722 have been found in Turkey (3013622, SCC*mec* III-ST239-t030), Greece (4013722, SCC*mec* III-ST239-t361), and India (3113722, SCC*mec* III-ST239), isolates with pattern 3013722 have been found only in Taiwan to date.[10,26] All the isolates with similar SIRU profiles found in various countries belonged to SCC*mec* type III and ST239, but were different in *spa* types (t037 in Taiwan, t030 in Turkey, t361 in Greece). There were 15 and eight different SIRU profiles in SCC*mec* type IV and V_T isolates, respectively, and no predominant SIRU type was found in these two SCC*mec* types.

The high number of SIRU profiles in SCC*mec* type IV and V_T isolates provides a means to further type these isolates (HGDI = 0.9315). Similarly, the high number (11 total) of *dru* types in SCC*mec* type III isolates (11 different *dru* copy numbers) also allows fine typing of these isolates, although it has a lower HGDI (0.549). This possibility was supported by another study showing that *dru* typing was an effective method for discrimination of closely related SCC*mec* type III, ST239-t037 MRSA isolates.[27]

Table 3 Hunter–Gaston discriminatory index of different typing methods

	MLST typing	*spa* typing	DRU copy number	SIRU typing
All isolates ($n = 157$)	0.5661	0.517	0.7288	0.5732
SCC*mec* III (=115)	0.2496	0.1638	0.5495	0.2287
SCC*mec* IV and V_T ($n = 32$)	0.2802	0.6069	0.5181	0.9315

The low discrimination power for various molecular typing methods in both SCC*mec* type II and III isolates was probably attributable to clonal spreading.[20] This speculation was based on the fact that SCC*mec* type II and III isolates are always hospital-acquired and are under similar environmental selective pressure. Therefore, it is conceivable that these isolates are more homogenous than SCC*mec* types IV and V_T isolates that could be hospital or community acquired.[20]

In conclusion, MRSA is an important pathogen in Taiwan. Different SCC*mec* MRSA isolates had different MLST, *spa*, *dru*, and SIRU patterns. The SIRU profiles can be used for further discrimination of SCC*mec* type IV or V_T isolates. SIRU profile 3013722 may be unique to Taiwan as it has not been found elsewhere.

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