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ORIGINAL ARTICLE

# Evaluation of double locus (*clfB* and *spa*) sequence typing for studying molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Taiwan

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**Abstract** *Background:* Pulsed-field gel electrophoresis (PFGE) is the "gold standard" for epidemiological investigation of methicillin-resistant *Staphylococcus aureus* (MRSA), but several DNA sequence-based methods have been developed in MRSA typing because of the unambiguous results.
*Methods:* Ninety-one MRSA isolates were collected from the blood cultures of different patients from July 2008 to December 2008 in central Taiwan. The molecular characteristics of each isolate, including double locus sequence typing (DLST; *spa* and *clfB* typing), *Staphylococcus* cassette chromosome *mec* (SCCmec), and PFGE were determined for comparison.
*Results:* Five major *clfB* types (types A–E), 18 *spa* types, 33 DLST genotypes, five SCCmec types, 17 pulsotypes have been observed. Three major DLST genotypes (A1-t002, C0-t037, and B1-t437) and two major pulsotypes (6 and 8) were identified. Most *clfB* type A isolates (97.1%) were SCCmec type II and all *clfB* type C isolates (100%) were SCCmec type III. Most *clfB*

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type B isolates (88.9%) were SCCmec type IV (59.3%) and $V_T$ (29.6%). All (100%) *clfB* subtypes A1, A2, and C isolates and 70.4% of *clfB* type B isolates belonged to healthcare-associated-MRSA. The average congruence was 57.7% between DLST and PFGE, and 96.6% between *clfB* and SCCmec type. The index of discrimination of SCCmec, *clfB*, *spa*, PFGE, and DLST was 0.72, 0.79, 0.80, 0.81, and 0.87, respectively.
*Conclusion: ClfB* type has high congruence with SCCmec type. The DLST method in this study yielded a higher discriminatory power than PFGE in local investigation of molecular epidemiology of MRSA and a promising alternative to PFGE.
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## Introduction

*Staphylococcus aureus* usually causes infections of the skin, wound, cardiac valve, central nervous system, bone, and blood.[1] Methicillin-resistant *Staphylococcus aureus* (MRSA) can cause a pandemic outbreak in hospitals. Several genotyping methods[2–4] have been developed for the epidemiological investigation of MRSA. Although pulsed-field gel electrophoresis (PFGE) is currently the "gold standard" in MRSA typing, a DNA sequence-based typing method has been promoted recently because of the unambiguous results.[5] Multilocus sequence typing (MLST) is suitable for the determination of macro-variations or long-term revolution in large populations.[2] Staphylococcal cassette chromosome *mec* (SCCmec) typing is the most reliable method to distinguish between health care-associated MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA).[6–8]

A double locus sequence typing (DLST) method was developed recently by combining the protein A gene (*spa*) and clumping factor B gene (*clfB*) sequence. *Spa* typing, based on the highly variable repeat region (X region) of the *spa* gene, is useful in local investigations due to its great discriminatory power.[9–11] The *clfB* gene contains a highly variable serine-aspartate (SD) repeat region,[12] and forms a microbial surface components recognizing adhesive matrix molecules[13] protein to bind to fibrinogen[14] and keratin, which make *S. aureus* colonize the nasal mucosa of humans[15,16]. The combination of the two methods increased discriminatory power, and was indeed comparable to the performance of PFGE, with expenditure of less time, cost, and labor, in both local epidemiologic and international outbreak investigations.[17–21]

However, there has been no study of DLST of MRSA in Taiwan. For this study, we collected 91 MRSA isolates from blood cultures of different patients in central Taiwan and compared the DLST (*spa* and *clfB*) with SCCmec, MLST, and PFGE.

## Methods

### MRSA isolates

Ninety-one MRSA isolates were collected from blood cultures of different patients from July 2008 to December 2008 in the bacteriological laboratory of China Medical University Hospital in central Taiwan.[2] The samples were then streaked on both Trypticase soy agar containing 5% sheep blood and Levine eosin methylene blue agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and incubated at 37°C for appropriate time periods. MRSA identification was performed with the BD Phoenix Automated Microbiology System (Becton Dickinson), and stocked in a −70°C freezer before use.

### Clinical definition of MRSA isolates

Definition of community-associated MRSA patients: (1) those patients without dialysis, surgery, prior admission to an acute care facility, residence in a long-term care facility, or surgery during the prior year; (2) presence of an indwelling catheter or percutaneous medical device; or (3) history of MRSA infection or hospitalization $>$ 48 hours prior to the index culture.

Definition of health care-associated MRSA patients: those patients other than community-acquired MRSA patients.[22,23]

The trial was approved by the Institutional Ethics Committee and Review Board at China Medical University Hospital (No. DMR101-IRB-125).

### DNA extraction

The DNA of all MRSA isolates was extracted using the Genomic DNA Mini Kit (Geneaid, Taiwan). The method was modified for *S. aureus* by the inclusion of lysostaphin (Sigma–Aldrich) at a final concentration of 30 mg/mL at the cell lysis step.

### SCCmec typing

Detection and identification of SCCmec were performed using multiplex polymerase chain reaction (PCR) as described previously.[24] The size of the PCR products of SCCmec type V was 325 bp, and that of SCCmec $V_T$ was 600 bp.

### MLST typing

Seven housekeeping genes (*arcC*, *aroE*, *glp*, *gmk*, *pta*, *tpi*, and *yqiL*) of *S. aureus* were used for the MLST typing. Twenty-one of 91 isolates were selected for MLST typing. Amplification of a portion of each gene was performed as described previously. The amplified products were

sequenced, and the sequences thus obtained were analyzed using the software at http://saureus.mlst.net/sql/multiplelocus.asp.

### *spa* typing

A PCR capable of identifying *spa* types was performed as described previously.

### *clfB* detection

A PCR capable of identifying *clfB* types was performed as described previously.[12]

### DLST

The DLST type was constituted by combining both *spa* and *clfB* types. DLST phylogenetic relationships were analyzed as shown in Figure 1 by using bionumerics software (Applied Meths, Belgium).

### PFGE

Bacterial isolates were typed by PFGE[25] following digestion of genomic DNA with *Sma*I. DNA fragments were separated on 1% SeaKem Gold agarose gel (Lonza Rockland, USA) in 0.5× tris/borate/ethylenediaminetetraacetic acid buffer with CHEF DRIII (BioRad, Hercules, CA, USA) with 6 V/cm, pulse time from 2.2 seconds to 54.2 seconds for 19 hours at 14°C. The PFGE results of MRSA isolates were interpreted with phylogenetic tree analysis (BioNumerics program, Applied Meths). Comparison of the patterns was performed by the unweighted pair group method with arithmatic mean clustering method. A similarity coefficient of 80% was selected to define the pulsotype clusters.

### Index of discrimination

Discriminatory power was evaluated by the number of types and the index of discrimination (ID).[26] The ID was calculated from the distribution of types with the Discriminatory Power Calculator (http://biophp.org/stats/discriminatory_power/demo.php). An ID value of 1 indicated that each isolate could be distinguished from all others by the typing method. Conversely, an index of 0 indicated that all isolates were the identical type.

## Results

All 91 MRSA isolates were typeable by *Sma*I macrorestriction, *spa*, *clfB*, DLST, SCCmec. The size of the PCR product of the *spa* gene after amplification was 200—400 bp, and that of the *clfB* gene was 500—1000 bp.

### *clfB* analysis

Five types (A—E), including 16 *clfB* subtypes compared with those published by Lamers et al[20] were identified. Every *clfB* subtype has its variation of nucleotide sequences of SD repeats and repeat profiles. The three major *clfB* types were A, B, and C (Table 1).

The most common *clfB* type was A (35, 38.5%), including 33 isolates (A1) with deletion of four repeat numbers (29-11-23-32), 1 isolate (A2) with deletion of four repeat numbers (29-11-23-32), and one substituted repeat number

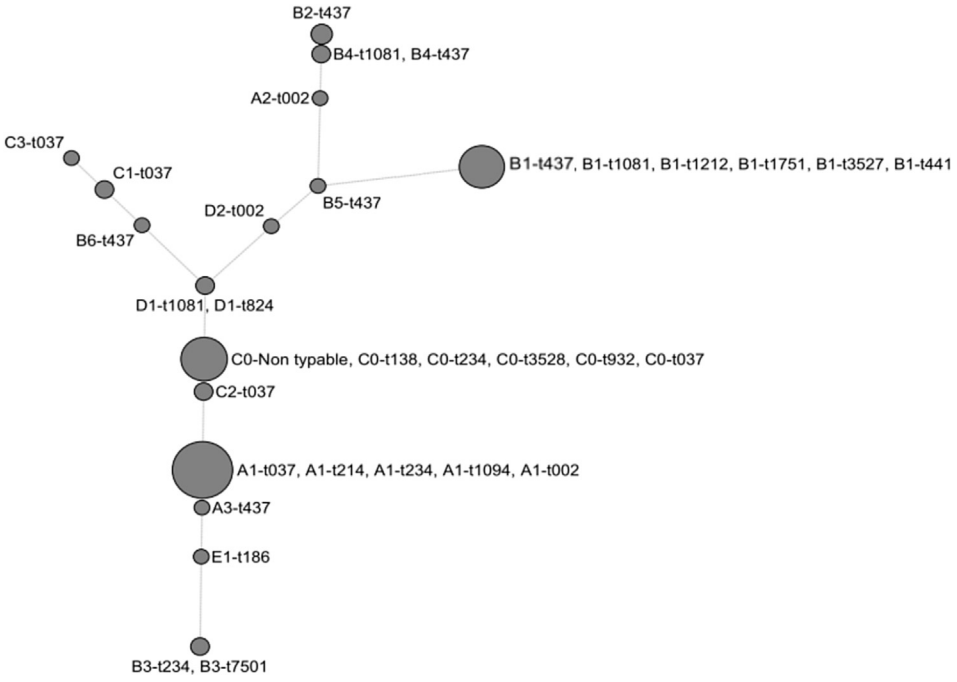


**Figure 1.** Ninety-one MRSA isolates were grouped by BioNumerics with double locus sequence typing phylogenetic relationship. Each circle represents DLST-types and the diameter of the circles reflects the number of isolates.

**Table 1** Five *clfB* types, 16 *clfB* subtypes, and repeat profiles in 91 methicillin-resistant *Staphylococcus aureus*.

| *clfB* type (No., %) | Repeat profiles | Reference types[b] |
|---|---|---|
| Type A (35, 38.5) | 21-2-34-35-36-11-35-37-14-14-35-36-11-37-26-27-28-25-25-29-11-23-32-23-14-23-23-25-23-14-38-11-39-11-40-45 | 2—10 |
| A1 (33, 36.3) | 21-2-34-35-36-11-35-37-14-14-35-36-11-37-26-27-28-25-25-x-x-x-x-23-14-23-23-25-23-14-38-11-39-11-40-45 | |
| A2 (1, 1.1) | 21-2-34-35-36-11-35-37-14-14-35-36-11-37-26-27-28-25-25-x-x-x-x-23-14-23-23-25-23-14-**29**-11-39-11-40-45 | |
| A3 (1, 1.1) | 21-2-34-35-36-11-35-37-14-14-14-36-11-37-26-27-28-25-25-29-11-11-32-23-14-23-23-32-14-23-14-38-11-39-14-36-11-40-29-32-40-41-6 | 2—3 |
| Type B (27, 29.7) | 1-2-3-4-5-5-6-6-7-8-4-5-6-7-8-7-9-10-6-11-11-10-12-7-13-14-15-12-13-16-17-18-7-15-10-19-20-14-17 | 1—1 |
| B1 (18, 19.8) | 1-2-3-4-5-5-6-6-**6**-7-8-4-5-6-7-8-**50-14-8**-7-9-10-6-11-11-10-12-7-13-14-15-12-13-16-17-18-7-15-10-19-20-14-17 | |
| B2 (3, 3.3) | 1-2-3-4-5-5-6-6-**6**-7-8-4-5-6-**7**[a]-8-**50-14-8**-7-9-10-6-11-11-10-12-7-13-14-15-12-13-16-17-18-7-15-10-19-20-14-17 | |
| B3 (2, 2.2) | 1-2-3-4-5-5-6-6-**6**-7-8-4-5-6-7-8-**50-14-8**-7-9-10-6-11-11-10-12-7-13-14-15-12-13-16-17-18-**68**-15-10-19-20-14-17 | |
| B4 (2, 2.2) | 1-2-3-4-5-5-6-6-**6**-7-8-x-x-x-x-x-**50-14-8**-7-9-10-6-11-11-10-12-7-13-14-15-12-13-16-17-18-7-15-10-19-20-14-17 | |
| B5 (1, 1.1) | 1-2-**27**-4-5-5-6-6-**6**-7-8-4-5-6-7-8-**50-14-8**-7-9-10-6-11-11-10-12-7-13-14-15-12-13-16-17-18-7-15-10-19-20-14-17 | |
| B6 (1, 1.1) | 1-2-3-4-5-5-6-6-7-8-4-5-6-7-8-**50-14-8**-7-9-10-6-11-11-10-12-7-13-14-15-12-13-16-17-18-7-15-10-19-20-14-17 | |
| Type C (25, 27.5) | 45-2-24-45-6-46-27-47-14-7-48-30-49-50-47-47-46-27-17-44-21-56-7-45-45-47-7-52-7-53-21-41-20-50-16-42-14-33-33-7-29-13-55-7-17 | 3—50 |
| C0 (20, 22) | 45-2-24-45-6-46-27-47-14-7-48-30-49-50-47-47-46-27-17-44-21-56-7-45-45-47-7-52-7-53-21-41-20-50-16-42-14-33-33-7-29-13-55-7-17 | |
| C1 (2, 2.2) | 45-2-24-45-6-46-27-47-14-7-48-30-49-50-47-47-46-27-17-44-21-56-7-45-45-47-**56-7**-7-53-21-41-20-50-16-42-14-33-33-7-29-13-55-7-17 | |
| C2 (2, 2.2) | 45-2-24-45-6-46-27-47-14-7-48-30-49-50-47-47-46-27-17-44-21-56-7-45-45-47-7-52-x-x-x-x-x-x-x-x-42-14-33-33-7-29-13-55-7-17 | |
| C3(1, 1.1) | 45-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-**92**-29-13-55-7-17 | |
| Type D (3, 3.3) | 7-63-64-7-21-14-44-20-20-15-14-48-7-9-68-13-14-48-70-7-20-15-16-52-48-7-20-15-16-41-13-7-20-15-16-52-48-7-20-15-16-41-109-7-32 | 5—59 |
| D1 (2, 2.2) | 7-63-64-7-**63**[a]-**64-7**-21-14-44-20-20-x-x-x-x-x-x-13-14-48-70-7-20-15-16-52-48-7-20-15-16-41-13-7-20-15-16-52-48-7-20-15-16-41-109-7-32 | |
| | 7-63-64-7-63-64-7-21-16-17-65-69-14-20-20-15-14-48-7-9-68-13-14-48-70-7-20-15-14-9-68-17 | 5—32 |
| D2 (1, 1.1) | 7-63-64-7-63-64-7-21-16-17-65[a]-69-14-20-20-15-14-48-7-9-68-13-14-48-70-7-20-15-14-**20-15-14**-9-68-17 | |
| Type E (1, 1.1) | 27-2-24-52-103-32-104-21-22-53-15-14-37-43-23-41-35-36-37-13-14-37-43-14-29-7-29-23-35-35-11-13-14-37-43-27-17-23-25-31-14-29-25-19-31-25-30-52-47-32-23-25-17 | 8—40 |
| E1 (1, 1.1) | 27-2-24-52-**37-103**-103-32-104-21-22-53-15-14-**32**-43-23-41-53-13-7-23-37-23-14-29-30-67[a]-31-25-19-31-25-30-52-47 | |

[a] Single nucleotide mutation.
[b] Based on lineage-type reference by Lamers et al.[20]
Bold characters = insertion of repeat number; underlined bold characters = substituted repeat number; x = deletion of a sequence repeat.

(38→29), and one isolate (A3) with a sequence identical to lineage 2—3. The second most common *clfB* type was B (27, 29.7%). It included 18 isolates (B1), three isolates (B2) with one new repeat number, 7, with C to T change in the nucleotide sequence (Table 2), two isolates (B3), two isolates (B4), one isolate (B5), and one isolate (B6) compared with lineage 1—1. The third most common *clfB* type was C (25, 27.5%), which included 20 isolates (C0; identical to 3—50), two isolates (C1), two isolates (C2), and one isolate (C3) compared with lineage 3—50.

The other *clfB* type included two isolates of type D1 with one new repeat number, 63, with C to T change in nucleotide sequence, one isolate of type D2 with one new repeat number, 65, with A to C change in nucleotide sequence

**Table 2** Single nucleotide mutation in nucleotide sequences of serine-aspartate repeat number in *clfB* subtypes B2, D1, D2, E1.

| Repeat No. | Nucleotide sequences |
|---|---|
| 7 | TCA GAT TCA GAC AG**C** GAT[b] |
| 7[a] in B2 | TCA GAT TCA GAC AG**T** GAT |
| 63 | TCA GAC T**C**A GGT AGC GAT[b] |
| 63[a] in D1 | TCA GAC **TT**A GGT AGC GAT |
| 65 | T**C**A GAT TCC GAT AGT GAC[b] |
| 65[a] in D2 | T**C**C GAT TCC GAT AGT GAC |
| 67 | T**C**C GAC AGC GAT[b] |
| 67[a] in E1 | T**C**A GAC AGC GAT |

[a] Repeat number with single nucleotide mutation (in bold type).
[b] Reference nucleotide sequences by Lamers et al.[20]

(Table 2), and one isolate of type E1 with one new repeat number, 67, with C to A change in nucleotide sequence (Table 2) compared with lineage 8—40. *clfB* types B2, D1, D2, and E1 displayed a single nucleotide mutation in nucleotide sequences (Table 2).

## DLST (*spa* and *clfB*) analysis

In the analysis of DLST (*clfB* and *spa*), 33 DLST genotypes were observed in 91 MRSA isolates (Table 3). The majority of isolates (60.9%) belonged to the three predominant DLST genotypes A1-t002 (29.7%, 27/91), C0-t037 (16.5%, 15/91), and B1-t437 (14.3%, 13/91). Using BioNumerics, all 91 isolates with a DLST genotype were grouped into three major clusters (A1, B1, C0), six minor clusters (B2, B3, B4, C1, C2, D1), and seven singletons (A2, A3, B5, B6, C3, D2, E1) in DLST phylogenetic relationships (Figure 1).

## Typing results by PFGE, *clfB*, *spa*, DLST, and SCCmec

The PFGE of 91 MRSA isolates produced 17 different pulsotypes. The most common pulsotype was pulsotype 6 (35.2%, 32/91) followed by pulsotype 8 (23.1%, 21/91), pulsotype 10 (11.0%, 10/91), and pulsotype 15 (8.8%, 8/91) (Table 3).

The most common types were A1-t002, SCCmec II, pulsotype 6 (21 isolates), followed by C0-t037, SCCmec III, pulsotype 8 (12 isolates), B1-t437, SCCmec IV, pulsotype 10 (4 isolates), and B1-t437, SCCmec $V_T$, and pulsotype 15 (4 isolates; Table 3). All 34 *clfB* subtypes A1 and A2 isolates were found to have SCCmec type II (100%), but subtype A3 was SCCmec type IV. Most *clfB* type A isolates (80%, 28/35) were pulsotype 6. The most frequent genotype of *clfB* type A was A1-t002 (77.1%, 27/35), 21 isolates (77.8%, 21/27) of which were pulsotype 6. The most frequent SCCmec type in the *clfB* type B isolates was type IV (59.3%, 16/27), followed by type $V_T$ (29.6%, 8/27), type II (3.7%, 1/27), type III (3.7%, 1/27), and type V (3.7%, 1/27). The most frequent genotype of *clfB* type B (48%, 13/27) was B1-t437. Eight isolates (61.5%, 8/13) of B1-t437 were SCCmec IV, four of which were pulsotype 10. The other five isolates (38.5%, 5/13) were SCCmec $V_T$, and four of them were pulsotype 15. All 25 *clfB* type C (lineage 3—50) isolates were SCCmec type III, and most (80%, 20/25) were pulsotype 8. The most frequent genotype of *clfB* type C (60%, 15/25) was C0-t037, and 12 of 15 isolates were pulsotype 8 (Table 3).

Two isolates of *clfB* subtype D1 (lineage 5—59) were SCCmec type V, but the isolate of *clfB* subtype D2 (lineage 5—32) was SCCmec type II. The isolate of *clfB* subtype E1 (lineage 8—40) was SCCmec IV (Table 3).

## Typing results by MLST

Ten of 35 *clfB* type A isolates were randomly selected for MLST typing and belonged to ST5 (Table 3). Five of 27 *clfB* type B isolates and six of 25 *clfB* type C isolates randomly selected for MLST typing were ST59 and ST239, respectively.

## Congruence between DLST and PFGE

The congruence between the two methods (DLST and PFGE) was evaluated for each pulsotype by calculating the percentage of isolates belonging to the corresponding genotype. We found that 21 isolates (65.6%) of A1-t002 were pulsotype 6, and 12 isolates (57.1%) of C-t037 were pulsotype 8. The congruence was 40% between B1-t437 and pulsotype 10 and 50% between B1-t437 and pulsotype 15, respectively. This analysis showed an average congruence of 57.7% between the two methods (Table 4).

## Congruence between *clfB* and SCCmec type

The analysis showed a congruence of 97.1% between *clfB* type A and SCCmec II, 100% between *clfB* type C and SCCmec III, and 92.6% between *clfB* type B and SCCmec IV, V, and Vt, respectively. The average congruence was 96.6% between the two methods (Table 5).

## Correlation of CA/HA-MRSA and SCCmec, *clfB*, spa type, and DLST genotype

Using the clinical definition of MRSA, 10 patients (11%) had CA-MRSA, and 81 patients (89%) had HA-MRSA (Table 3). All 10 CA-MRSA patients were SCCmec IV or $V_T$ and eight of them were *clfB* type B. Sixty-one (75.3%) of the 81 HA-MRSA patients carried SCCmec II (35 patients, 43.2%) or III (26 patients, 32.1%), and 59 (96.7%) of 61 patients had *clfB* type A (34 patients, 55.7%) or C (25 patients, 41.0%). The remaining 20 (24.7%) of the 81 HA-MRSA patients carried SCCmec IV (12 patients, 14.9%) or V (four patients, 4.9%) or $V_T$ (four patients, 4.9%), and 17 (85%) of 20 patients had *clfB* type B.

All isolates (100%) of *clfB* subtype A1, A2, and type C belonged to HA-MRSA. *clfB* type B isolates, 29.6% (8/27), led to CA-MRSA infection and 70.4% (19/27) of *clfB* type B isolates caused HA-MRSA infection (Table 3). The HA-MRSA infection rate was higher in *clfB* types A and C than B.

In the spa type analysis, eight (80%) of 10 CA-MRSA patients were t437, and the other two patients carried t1751 and t186. In DLST analysis, genotypes A1-t002, B1-t437, and C0-t037 were found in 27 (33.3%), 9 (11.1%), and 15 (18.5%)

**Table 3** Summarized *clfB* types correlated with spa types, double locus sequence typing, Staphylococcal cassette chromosome *mec* type, pulsotype, and multilocus sequence typing.

| *clfB* type (*n*) | *clfB* subtype (*n*) | *spa* type (*n*) | DLST (*n*) | SCCmec type (*n*) | Pulsotype (*n*)[a] | MLST[e] (*n*) |
|---|---|---|---|---|---|---|
| Type A (35) | A1 (33) | t002 (27) | A1-t002 (27) | II (27) | 4 (1) | |
| | | | | | 6 (21) | ST5 (9) |
| | | | | | 7 (2) | |
| | | | | | 8 (1) | |
| | | | | | 9 (1) | |
| | | | | | 15 (1) | |
| | | t1094(3) | A1-t1094 (3) | II (3) | 6 (3) | |
| | | t037 (1) | A1-t037 (1) | II (1) | 6 (1) | |
| | | t214 (1) | A1-t214 (1) | II (1) | 6 (1) | |
| | | t234 (1) | A1-t234 (1) | II (1) | 6 (1) | |
| | A2 (1) | t002 (1) | A2-t002 (1) | II (1) | 6 (1) | ST5 (1) |
| | A3 (1) | t437 (1) | A3-t437 (1) | IV (1) | 14 (1)[b] | |
| Type B (27) | B1 (18) | t437 (13) | B1-t437 (13) | IV (8) | 10 (4)[c] | ST59 (1) |
| | | | | | 11 (1) | |
| | | | | | 12 (2) | |
| | | | | | 16 (1) | ST59 (1) |
| | | | | $V_T$ (5) | 13 (1) | |
| | | | | | 15 (4)[c] | ST59 (2) |
| | | t3527 (1) | B1-t3527 (1) | II (1) | 10 (1) | |
| | | t1081 (1) | B1-t1081 (1) | IV (1) | 6 (1) | |
| | | t1212 (1) | B1-t1212 (1) | $V_T$ (1) | 15 (1) | |
| | | t1751 (1) | B1-t1751 (1) | $V_T$ (1) | 15 (1)[b] | |
| | | t441 (1) | B1-t441 (1) | IV (1) | 17 (1) | |
| | B2 (3) | t437 (3) | B2-t437 (3) | IV (3) | 10 (3)[d] | ST59 (1) |
| | B3 (2) | t7501 (1) | B3-t7501 (1) | IV (1) | 10 (1) | |
| | | t234 (1) | B3-t234 (1) | III (1) | 5 (1) | |
| | B4 (2) | t437 (1) | B4-t437 (1) | IV (1) | 10 (1)[b] | |
| | | t1081 (1) | B4-t1081 (1) | V (1) | 1 (1) | |
| | B5 (1) | t437 (1) | B5-t437 (1) | $V_T$ (1) | 6 (1)[b] | |
| | B6 (1) | t437 (1) | B6-t437 (1) | IV (1) | 4 (1) | |
| Type C (25) | C0 (20) | t037 (15) | C0-t037 (15) | III (15) | 6 (1) | |
| | | | | | 8 (12) | ST239 (3) |
| | | | | | 9 (1) | ST239 (1) |
| | | | | | 15 (1) | |
| | | Non-typeable (1) | C0-Non-typeable | III (1) | 9 (1) | |
| | | t138 (1) | C0-t138 (1) | III (1) | 8 (1) | |
| | | t234 (1) | C0-t234 (1) | III (1) | 8 (1) | |
| | | t3528 (1) | C0-t3528 (1) | III (1) | 8 (1) | |
| | | t932 (1) | C0-t932 (1) | III (1) | 8 (1) | |
| | C1 (2) | t037 (2) | C1-t037 (2) | III (1) | 8 (2) | ST239 (1) |
| | C2 (2) | t037 (2) | C2-t037 (2) | III (2) | 8 (1) | |
| | | | | | 6 (1) | ST239 (1) |
| | C3 (1) | t037 (1) | C3-t037 (1) | III (1) | 8 (1) | |
| Type D (3) | D1 (2) | t824 (1) | D1-t824 (1) | V (1) | 1 (1) | |
| | | t1081 (1) | D1-t1081 (1) | V (1) | 1 (1) | |
| | D2 (1) | t002 (1) | D2-t002 (1) | II (1) | 2 (1) | |
| Type E (1) | E1 (1) | t186 (1) | E1-t186 (1) | IV (1) | 3 (1)[b] | |

[a] Clinical definition for community-acquired-methicillin-resistant *Staphylococcus aureus* or health care-associated methicillin-resistant *Staphylococcus aureus*.

[b] The isolate belonged to community-acquired-methicillin-resistant *Staphylococcus aureus*.

[c] Two of the four isolates belonged to community-acquired-methicillin-resistant *Staphylococcus aureus*; no marker belonged to health care-associated methicillin-resistant *Staphylococcus aureus*.

[d] One of the three isolates belonged to community-acquired-methicillin-resistant *Staphylococcus aureus*.

[e] Only parts of isolates were performed.

DLST = double locus sequence typing; MLST = multilocus sequence typing; SCCmec = Staphylococcal cassette chromosome *mec*.

**Table 4** Congruence between isolates belonging to pulsed-field gel electrophoresis pulsotypes 6, 8, 10, and 15, and corresponding double locus sequence typing genotypes A1-t002, B1-t437, and C0-t037.

| Pulsotype/DLST genotype | No. of isolates belonging to the pulsotype | No. of isolates belonging to the corresponding DLST genotype | Congruence (%) |
|---|---|---|---|
| 6/A1-t002 | 32 | 21 | 65.6% |
| 8/C0-t037 | 21 | 12 | 57.1% |
| 10/B1-t437 | 10 | 4 | 40% |
| 15/B1-t437 | 8 | 4 | 50% |
| Total | 71 | 41 | 57.7% |

DLST = double locus sequence typing.

**Table 5** Congruence between isolates belonging to *clfB* types A, B, C, and corresponding Staphylococcal cassette chromosome *mec* II, III, and IV/V/Vt.

| *clfB* type/SCCmec | No. of isolates belonging to the *clfB* type | No. of isolates belonging to the corresponding SCCmec | Congruence (%) |
|---|---|---|---|
| A/II | 35 | 34 | 97.1 % |
| C/III | 25 | 25 | 100 % |
| B/IV, V, Vt | 27 | 25 | 92.6 % |
| Total | 87 | 84 | 96.6 % |

SCCmec = Staphylococcal cassette chromosome *mec*.

of 81 HA-MRSA patients, respectively. However, genotypes A1-t002 and C0-t037 were not detected in 10 CA-MRSA patients, but B1-t437 was found in four (40%) of 10 CA-MRSA patients.

### ID of PFGE, *clfB*, *spa*, DLST, and SCCmec typing

The discriminatory power of PFGE, *clfB*, *spa*, DLST, and SCCmec typings was determined by calculating the ID. Seventeen pulsotypes, 16 *clfB* types, 18 *spa* types, 33 DLST genotypes, and five SCCmec types were identified. ID of SCCmec, *clfB*, spa, PFGE, and DLST was 0.72, 0.79, 0.80, 0.81, and 0.87, respectively.

## Discussion

Several genotyping methods are being used for the epidemiological investigation of *S. aureus*. PFGE has been the state-of-the-art method for MRSA typing during the past few decades. However, poor interlaboratory standardization, potentially ambiguous results, and labor-intensiveness were the main disadvantages of PFGE. In recent years, DNA sequence-based methods such as MLST,[5] spa,[10] *clf*B,[12] DLST, and SCCmec typing,[7,27] have been developed to achieve the goal of matching the resolution of PFGE.

The *clfB* gene has a unique repeat region encoding three SD dipeptides containing 18 bp in each repeat. The overall larger repeat region in the *clfB* gene and its individual repeat with 6 bp shorter than
that of the *spa* gene contributed to genetic microvariation during replication and recombination.[12] More subtypes in *clfB* type B than type A and C suggest that *clfB* type B has more genetic diversity (Tables 1 and 2). Our results demonstrated that the *clfB* gene is a highly variable marker for typing MRSA. Koreen et al[12] also suggested that the *clfB* repeat region was under three types of evolutionary pressure in the codon, amino acid level, and macrolevel, which appears to be recombinogenic and contributes to the typing ability of resolving differences within clonal groups. In the *clfB*-typing development study,[12] *clfB* type 3/lineage 3B (spa t002) was the most common type in the USA. Although this type was similar to *clfB* type B in our study according to the repeat profile, most *clfB* type B isolates were spa t437. However, *clfB* type A, mostly spa t002, was the most common type in our study (Table 1). Markers of the molecular typing system will accumulate mutations over time and thus are subject to change in different geographical areas.

In DLST analysis, a Swiss study[19] identified three major clones, DLST 2-2 (SCCmec II/ST105), 3-3 (SCCmec IV/ST8), and 4-4 (SCCmec I/ST228) in western Switzerland. The most common type, DLST 2-2 (SCCmec II/ST105), showed the same DLST and SCCmec types, but different sequence types (STs) as genotype A1-t002 (SCCmec II/ST5) in our study. Genotype A1-t002 had the same ST and SCCmec type as the Japan clone recovered in 1982 and 1996[19] and closed to DLST 2-2.[19] Genotype C0-t037 (SCCmec III/ST239) had the same ST and SCCmec type as the Poland clone recovered in 1992 and belonged to DLST 369-30.[19] Genotype B1-t437 (ST59/SCCmec IV/$V_T$), a regional clone in Taiwan was not found in Europe. The unambiguous DLST types combined with other sequence-based methods were suitable for international epidemiological comparison in different areas in the world.

From the point of view of genetic change in the *clfB* repeat profile (Table 1) and correlation with HA/CA-MRSA isolates (Table 3), most CA-MRSA isolates had insertion of duplication due to belonging to *clfB* type B, whereas diversity of genetic change such as insertion of duplication or

deletion or no change were found in most HA-MRSA isolates. The genetic change of the HA-MRSA isolates contributed to evolutionary change.

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.[27] In our study, 10 isolates of t002 in *clfB* type A belonged to ST5, five isolates of t437 in *clfB* type B belonged to ST59, and six isolates of t037 in *clfB* type C belonged to ST239. In a European MRSA molecular epidemiological study[28] including 26 countries, spa t032/ST22 was the most frequent type in Europe, followed by spa t008/ST-8 and t041/ST-111, whereas spa t002/ST5 and spa t037/ST239 were the fifth and eight most frequent types, respectively, in Europe. However, the spa t437 (t437/ST59) type was not found in this European study.

In this study, 75.3% (61/81) of HA-MRSA isolates belonged to SCCmec II and SCCmec III, and 100% (10/10) of CA-MRSA patients had SCCmec IV or $V_T$. Using molecular epidemiological definitions in SCCmec typing, some previous articles[29–33] clearly concluded that the major clone in HA-MRSA infections in Taiwan was SCCmec III/spa t037/ST239, followed by SCCmec II/spa t002/ST5. However, the major CA-MRSA strains in Taiwan were SCCmec IV or V($V_T$)/spa t437/ST59, which were similar to CA-MRSA strains reported in the USA (USA300: SCCmec IV/ST8/t008) and Europe (SCCmec IV/ST80/t044),[34] except the different ST and spa type. Our results also support this finding (Table 3), except more isolates of SCCmec II/ST5 than SCCmec III/ST239. However, 24.7% (20/81) of HA-MRSA isolates belonged to SCCmec IV/V/Vt. This higher proportion in contrast to previous Taiwan studies[29–33] demonstrated the geographical variation of SCCmec type distribution in different areas of Taiwan.

The higher congruence rate (96.6%) between *clfB* and SCCmec types suggested that *clfB* typing was comparable and highly associated with SCCmec typing (Tables 3 and 5). DLST clusters belonging to *clfB* type B had more heterogeneous evolutional change than *clfB* types A and C (Figure 1). This finding may be related to the highly association between *clfB* and SCCmec typing and the more homogenous SCCmec type in *clfB* types A and C.

Isolates in *clfB* type A belonging to the same pulsotype 6 could be divided into more different types by DLST, as with isolates in *clfB* types B and C (Table 3). However, the congruence in genotype B1-t437 between DLST and PFGE (Table 4) was relatively lower than that in genotypes A1-t002 and C0-t037. These results imply that genotype B1-t437 strains exhibit more clonal diversity than genotypes A1-t002 and C0-t037 strains. The average congruence rate (57.7%) between the two methods was lower than that (88%) reported by Basset et al.[19] This may be due to the small sample sizes in our study and the different definition of a PFGE clone for the PFGE pattern. A similarity of 80% is usually selected to define a PFGE clone in epidemiological studies.[35] Our study selected this definition of a PFGE clone but Basset et al[19] defined a PFGE clone by less than seven band differences in their PFGE patterns.

Either *spa* or *clfB* typing alone has discriminatory power comparable to PFGE, but DLST demonstrates more discriminatory power (ID: 86.76%) than PFGE (ID: 80.76%). Koreen et al[12] and Kuhn et al[17] both concluded that DLST had higher discriminatory power (94–99.5%) than either *spa* or *clfB* typing alone, and had discriminatory power comparable to that of PFGE. In our study, the relatively lower discriminatory power (ID: 86.76%) of DLST may be related to the homogenous genetic sequence within these 91 isolates in one hospital. Further study collecting more MRSA isolates from different areas in Taiwan should be carried out.

In conclusion, DLST method has two highly variable markers for typing MRSA, higher discriminatory power than PFGE, and provides unambiguous results allowing for interlaboratory comparisons and reproducibility. *clfB* typing was highly associated with SCCmec typing. DLST is more useful for local epidemiological investigation of MRSA than PFGE. In this, the first study to compare DLST with SCCmec, MLST, and PFGE in Taiwan, we found that DLST is a promising alternative to PFGE.

## Conflicts of interest

The authors have nothing to disclose.

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