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Clinical Characteristics, Antibiotic Resistance and Molecular Typing of Methicillin-Resistant *Staphylococcus aureus* in a Teaching Hospital at South Taiwan

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Authors' contributions

This work was carried out in collaboration with all authors. Authors MHL, HLY, YFH, LLH, WLS and YPL designed the study, wrote the protocol and managed the analyses of the study. Authors HHH and HLY wrote the manuscript, performed the statistical analysis and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has become an issue of public health worldwide. The clinical features, molecular epidemiology and antibiotic resistance of MRSA isolates from South Taiwan from January to December 2009 were collected and analyzed.

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Methods: A total of 439 patients were invited to participate in this investigation. Antibiotic resistance was assessed by broth microdilution and pulsed-field gel electrophoresis (PFGE) was performed to identify the molecular genotypes.
Results: Among 439 culture-proven *S. aureus* isolates, MRSA accounted for 47.8% (210/439). The remaining 52.2% (229/439) isolates were methicillin-susceptible *Staphylococcus aureus* (MSSA). MRSA isolated from intensive care unit (ICU) were significantly more than MSSA and the percentage of MRSA strains isolated from the respiratory tract was significantly higher than that of MSSA. The resistant rates of MRSA to penicillin, ampicillin/sulbactam, clindamycin and erythromycin were over 80% as no MRSA isolates were resistant to vancomycin, linezolid and teicoplanin. Antimicrobial data of MRSA strains were categorized into 10 patterns, of which 5 main patterns accounted for 95.7% (n=201). PFGE characterization of MRSA was grouped into 20 genotypes (A through T). Among 20 major pulsotypes, clusters A, E, J, and N of MRSA isolates were associated with clinical and antimicrobial importance.
Conclusion: This study revealed crucial information of MRSA typing and essential connections among clinical characteristics, antimicrobial patterns and MRSA pulsotypes. The PFGE pulsotype may be coupled to distinct antibiotic-resistant patterns, special specimen sources and specific hospital department where MRSA isolated. The results can be regionally used in infection control and antibiotic stewardship of MRSA.

Keywords: Antibiotic resistance; molecular epidemiology; MRSA; MSSA; South Taiwan.

1. INTRODUCTION

Staphylococcus aureus (*S. aureus*) is one of the most crucial and commonly isolated gram-positive bacteria. MRSA has become a hygienic issue worldwide and the control of MRSA remains an important part of the infection control strategies in hospitals and community. MRSA isolated from ocular infections in north Taiwan has been shown to have greater resistance than MSSA (Methicillin-susceptible *Staphylococcus aureus*) to clindamycin, erythromycin and sulfamethoxazole / trimethoprim [1]. MRSA are important pathogens in community- and hospital/healthcare-associated infections [2,3] and hence MRSA are categorized into two groups: community-associated MRSA (CA-MRSA) and healthcare-associated MRSA (HA-MRSA) [4]. The transmission of MRSA has been found to emerge between hospitals in the US and Taiwan and even between countries [5-7]. The first MRSA hospital outbreak in the US was reported in 1968 as from 1999-2000 the number of MRSA infection was diagnosed over 120000 in the US [8,9]. Types of MRSA infection or syndrome included bacteremia, pneumonia, endocarditis, cellulitis and osteomyelitis [10,11]. The percentages of MRSA in the isolated staphylococci have been shown to increase in different areas in the world. In the US, the proportion of MRSA clearly raised from 22% in 1995 to 57% in 2001 [10] and it was showed that approximately 40% *S. aureus* infections are MRSA [12]. Over the past decades, high or increasing incidence rate of MRSA among *S. aureus* isolates have been reported in Europe and Taiwan [13-17]. In many European countries, including the UK, Germany, Belgium and Republic of Ireland, a rise of MRSA prevalence between 1999 and 2002 was reported [13]. From 1980s to 1990s, increasing incidence of MRSA infections in north Taiwan has been reported [14] as between 1990 and 2000, prevalence of MRSA were also markedly increased [15]. MRSA has been shown to occupy 53-83% of total *S. aureus* isolates in most major hospitals in Taiwan [15-17].

The antimicrobial resistance of MRSA has been clinically and hygienically concerned over the past decades. It has been shown that CA-MRSA are usually sensitive to clindamycin in the US, gentamicin in Australia, and ciprofloxacin in England whereas increasing non-â-lactam antimicrobial resistance among CA-MRSA, particularly to clindamycin has been reported and noticed in the community in the US [18]. In north Taiwan between 1999 and 2008, MRSA isolated from ocular infections has been shown to have greater resistance than MSSA (Methicillin-susceptible Staphylococcus aureus) to clindamycin, erythromycin and sulfamethoxazole / trimethoprim [1]. On the other hand, PFGE typing has been widely applied to molecular studies of MRSA strains in Taiwan as well as in other countries [4,6,19-21]. Importantly, the associations of PFGE genotypes with characteristics of patients and antimicrobial resistance patterns have been investigated in Europe to help surveillance and infection control

of MRSA [20,21]. A retrospective study was conducted here to investigate the clinical features, antimicrobial resistance and PFGE typing of MRSA while combined analyses of clinical, antimicrobial and PFGE data of MRSA were also carried out to uncover their essential associations with each other in Taiwan.

2. MATERIALS AND METHODS

2.1 Hospital and Setting

This study was conducted in a teaching hospital with more than 600 beds in south Taiwan from January to December 2009. All 439 patients were eligible for and invited to participate in this investigation and the specimens were collected from pus, respiratory tract, blood, urine and other sources (Table 3). This study was approved by the institutional review board of Pingtung Christian Hospital and a written informed consent was obtained from each subject.

2.2 Bacterial Identification

Identification of *S. aureus* was carried out by morphological examination, gram stain, catalase test and coagulase test. For the detection of clumping factor and protein A associated with *S. aureus,* rapid latex agglutination test was performed to further identify *S. aureus* using Staphaurex Plus (Remel, Lenexa, KS, USA) according to the manufacturer's instructions. *S. aureus* ATCC 25923 was used as positive control and *Streptococcus pyogenes* ATCC 19615 was used as negative control.

2.3 Antimicrobial Susceptibility Test

The susceptibility test was accomplished using broth microdilution on VITEK 2 system (Biomerieux, Durham, NC, USA) to examine the resistance of *S. aureus* isolates to antimicrobials penicillin (P), ampicillin/sulbactam (SAM), clindamycin (CC), erythromycin (E), oxacillin, rifampicin (RA), trimethoprim-sulfamethoxazole (SXT), fusidic acid (FA), teicoplanin, linezolid, and vancomycin. Minimum inhibitory concentration (MIC) was determined according to the guideline 2009 of Clinical and Laboratory Standards Institute (CLSI). The criteria for antibiotic resistance/susceptibility are shown in supplementary 5.

2.4 Molecular Characterization

All the MRSA isolates were molecularly characterized by pulsed-field gel electrophoresis (PFGE) with SmaI digestion [22]. After bacterial identification, MRSA colonies grown overnight on blood agar were suspended in 100 mM Tris HCl-100 mM EDTA (pH 8) and cast into gel plugs at 55°C. The plugs were incubated with lysis buffer (6 mM Tris HCl, 1M NaCl, 100 mM EDTA, 0.5% Brij-58, 0.2% sodium deoxycholate, 0.5% sodium lauroylsarcosine) at 37°C overnight. Approximately 2 mm slices of DNA plugs were cut and incubated overnight with 200 μl of restriction buffer containing 20 U SmaI at 25°C. The fragments were separated on a contour-clamped homogeneous electric field (CHEF-DRIII; BioRad) at 14°C. Electrophoresis was conducted under the conditions as below: initial switch time 5 sec; final switch time 40 sec; run time 21 h; voltage gradient 6 V/cm. Images were analyzed by BioNumerics software (Applied. Math, Sint-Martens-Latem, Belgium).

2.5 Statistical Analysis

Patients with MSSA or MSSA isolates were statistically the control group and Patients with MRSA or MRSA isolates were the study group. Categorical variables in each analytic group were presented as numbers and percentages. Continuous variables were presented as means and standard deviations. Two-proportional t test or chi-square test was performed to compare categorical data, as appropriate. Continuous data were statistically examined by two-sample t test. All analyses are two-tailed and P value ≤ 0.05 indicated statistical significance. The statistical analyses were carried out using STATA software.

3. RESULTS

3.1 The Rate of MRSA in *S. aureus* Isolates

A total of 439 *S. aureus* were examined in the present study. The rate of MRSA among *S. aureus* strains isolated from January to December 2009 in southern Taiwan was 47.8% (210/439). Of these, 229 were MSSA (229/439, 52.2%).

3.2 Profiles of Clinical Data (Tables 1-4)

The inpatient/outpatient ratio of MRSA isolates had no significant difference compared with that of MSSA isolates (P=0.1680, Table 1). The average age of patients with MRSA was 58.59±26.32 years. The age of the 229 patients

with MSSA ranged from 0 to 94 years. The average age of patients with MSSA was 55.50±24.36 years. Proportions of MRSA patients 0~14 and >64 years old were significantly higher than those of MSSA patients (*P*=0.0440 and *P*=0.0102, respectively). Patients with MSSA accounted for higher percentages than MRSA patients in the group of 15-64 years old (*P*=0.0001, Table 2). Data on gender of patients with MRSA and MSSA showed ratio of sex is significantly different between MSSA and MRSA patients (*P*=0.0340, Table 1). The percentage of MRSA isolates from respiratory tract was significantly higher than that of MSSA (*P*=0.0006). Table 4 represents that MRSA isolates from ICU were significantly more than MSSA (*P*=0.0004) as MSSA isolates from department of surgery were more than MRSA isolates (*P*=0.0178). In a total of 210 MRSA isolates, the patient group aged >64 years old accounted for 111 isolates (52.9%), which was more than the other younger groups (Table 2) and MRSA was isolated more from male patients than from the female counterparts (Table 1). The highest number/proportion of specimen sources of MRSA isolates was pus (83/39.5%), followed by respiratory tract (75/35.7%), blood (32/15.2%), urine (12/5.7%) and others (8/3.8%) (Table 3) while the highest number/proportion of departments from which MRSA isolated was internal medicine and ICU (74/35.2%), followed by surgery (45/21.4%), pediatrics (16/7.6%) and gynecology (1/0.5%) (Table 4).

Table 1. Comparison of characteristics between MRSA patients and MRSA counterparts

Characteristics of patients	MRSA (n=210)	MRSA (n=229)	*P* value
Average age(Mean±SD years)[a]	58.59±26.32	55.50 ±24.36	0.2024
Gender(F/M)[b]	98/112	84/145	0.0340
Service(Inpatient/Outpatient)[b]	180/30	185/44	0.1680

[a]Two sample t test, [b]Chi-square test

Table 2. Comparison of age distribution between MRSA and MSSA patients

Age	Numbers of isolates(n=439)	Proportion(%) of all strains	No.(%) of strains		*P* value ł
			MSSA (n=229)	MRSA (n=210)	
0.14	47	10.7	18 (7.9)	29 (13.8)	0.44
15.64	188	42.8	118 (51.5)	70 (33.3)	0.0001
>64	204	46.5	93 (40.6)	111 (52.9)	0.0102

ł Two-proportional t test

Table 3. Comparison of specimen sources between MRSA and MSSA isolates

Specimen source	Numbers of solates(n=439)	Proportion(%)of all strains	No.(%) of strains		*P* value ł
			MSSA (n=229)	MRSA (n=210)	
Pus	191	43.5	108 (47.2)	83 (39.5)	0.1069
Respiratory tract	123	28	48 (21.0)	75 (35.7)	0.0006
Blood	80	18.2	48 (21.0)	32 (15.2)	0.1208
Urine	32	7.3	20 (8.7)	12 (5.7)	0.2241
Others	13	3	5 (2.1)	8 (3.8)	0.3154

ł Two-proportional t test

Table 4. Comparison of hospital departments of MRSA patients with MSSA counterparts

Departments	Numbers of solates(n=439)	Proportion(%)of all strains	No.(%) of strains		*P* value ł
			MSSA (n=229)	MRSA (n=210)	
Internal medicine	165	37.6	91 (39.7)	74 (35.2)	0.3309
Surgery	117	26.7	72 (31.4)	45 (21.4)	0.0178
Pediatrics	35	8	19 (8.3)	16 (7.6)	0.7934
Gynecology	2	0.4	1 (0.4)	1 (0.5)	0.951
ICU[a]	120	27.3	46 (20.1)	74 (35.2)	0.0004

ICU[a],intensive care unit, ł Two-proportional t test

3.3 Antibiotics Resistance of MRSA and MSSA (Table 5)

MRSA isolates accounted for 100% resistance rate to penicillin and ampicillin/sulbactam, which significantly higher than MSSA isolates (*P*<0.0001). Over 80% MRSA isolates were resistant to clindamycin (86.2%, n=181) and erythromycin (87.1%, n=183) as the resistance rates of MRSA to rifampicin and trimethoprim / sulfamethoxazole were 34.3% (n=72) and 28.1% (n=59), respectively. All *S. aureus* (MRSA and MSSA) isolates in this study were not resistant to teicoplanin, linezolid and vancomycin. MRSA isolates showed higher resistance than MSSA to clindamycin (*P*<0.0001), erythromycin (*P*<0.0001), rifampicin (*P*<0.0001) and trimethoprim / sulfamethoxazole (*P*<0.0001).

3.4 Antibiotics Resistance Patterns of MRSA and MSSA (Table 6)

The antimicrobial data of MRSA isolates were categorized into 10 patterns. The 5 major patterns were “SAM, P, CC, E”, “SAM, P, CC, E, RA”, “SAM, P, CC, E, RA, SXT”, “SAM, P” and “SAM, P, CC, E, SXT” (Table 6A). In contrast, the antibiotics resistance features of MSSA were grouped into 8 patterns as the 2 main patterns were “P” and “P, CC, E” (Table 6B).

Table 5. Comparison of antimicrobial resistance between MRSA and MSSA isolates

Antimicrobials	No.(%) of resistant strains		*P* value ł
	MRSA (n=210)	MSSA (n=229)	
Oxacillin	210 (100)	0 (0)	<0.0001
Penicillin	210 (100)	210 (91.7)	<0.0001
Ampicillin/Sulbactam	210 (100)	0 (0)	<0.0001
Erythromycin	183 (87.1)	35 (15.3)	<0.0001
Clindamycin	181 (86.2)	32 (14)	<0.0001
Rifampicin	72 (34.3)	3 (1.3)	<0.0001
Trimethoprim/Sulf[a]	59 (28.1)	3 (1.3)	<0.0001
Fusidic Acid	3 (1.4)	3 (1.3)	<0.9149
Teicoplanin	0 (0)	0 (0)	-
Linezolid	0 (0)	0 (0)	-
Vancomycin	0 (0)	0 (0)	-

[a]*Trimethoprim/Sulf: Trimethoprim / Sulfamethoxazole, ł Two proportional test*

Table 6A. Antimicrobial resistance patterns of MRSA isolates

Antimicrobial resistance	Numbers of resistant strains	(%)
SAM, P, CC, E, RA, SXT	32	15.2
SAM, P, CC, E, RA	37	17.6
SAM, P, CC, E, SXT	21	10.0
SAM, P, CC, E	88	41.9
SAM, P	23	11.0
SAM, P,RA	2	1.0
SAM, P,E	2	1.0
SAM, P,SXT	1	0.5
SAM,P,CC, E, SXT	1	0.5
E, SXT,CC, E, SXT, FA	3	1.4

[A]*Antimicrobial resistance: SAM, Ampicillin/Sulbactam; P, Penicillin; CC, Clindamycin; E, Erythromycin; RA, Rifampicin; SXT Trimethoprim/Sulfamethoxazole; FA, Fusidic Acid*

Table 6B. Antimicrobial resistance patterns of MSSA isolates

Antimicrobial resistance	Numbers of resistant strains	(%)
P	188	73.4
P,CC, E	30	13.1
P,SXT	3	1.3
P, E	3	1.3
P,RA	2	0.9
P,CC,RA	1	0.4
CC, E	1	0.4
P, FA	3	1.3

3.5 PFGE typing of MRSA Isolates (Table 7)

Molecular typing of MRSA isolates was defined and separated using a cutoff of 80% [22,23]. A total of 148 PFGE patterns were obtained and 20 major genotypes of MRSA were determined after cutoffs of > 80% relatedness, designated clusters A through T. Twenty major clusters of PFGE and the phylogenetic tree of MRSA isolates are represented in Fig. 1. Among the 20 genotypes, the proportion of clusters A, E, G, J, N, O, P and T accounted for 76.2% as 64.1% of all MRSA isolates, which clustered in pulsotypes A, E, J and N (Supplementary 1~4).

3.6 Combined Analysis of Clinical, Antimicrobial and Molecular Typing Data (Tables 8 and 9)

The MRSA antimicrobial resistance pattern "SAM, P, CC, E" was the highest occupied (41.9%). This MRSA antimicrobial resistance pattern mainly belonged to the age 15~64 as well as > 64 and the isolations from internal medicine. The PFGE genotypes A and E, of 83.33% and 77.87% antibiotic-resistance pattern "SAM, P, CC, E", respectively, were both mainly isolated from pus specimens. The second highest antimicrobial resistance pattern "SAM, P, CC, E, RA" accounted for 17.6% of total MRSA isolates and was mainly matched by PFGE cluster N. Men aged over 64 and respiratory tract specimens were essentially belonged to this genotype, suggesting that older MRSA positive male patients with respiratory infection may be associated with pulsotype N. The antimicrobial resistance pattern "SAM, P, CC, E, RA" of MRSA strains was distributed in genotype N and these MRSA primarily isolated from department of internal medicine as well as ICU (intensive care units). MRSA strains with pulsotype J were found the most in the antimicrobial resistance pattern "SAM, P, CC,E, RA, SXT" and this MRSA pattern was mainly isolated from ICU and the male patient group of age over 64. Blood and respiratory tract specimens for MRSA strains clustered the most in pulsotype J, implicating that this genotype may be relavent with bacteremia, septicemia and respiratory infection in MRSA positive patients.

Table 7. Twenty major PFGE types of MRSA isolates

PFGE types	Numbers of strains	Proportion(%) of strains
N	38	18.0
E	37	17.6
J	36	17.1
A	24	11.4
G	8	3.8
T	7	3.3
O	5	2.4
P	5	2.4
H	4	1.9
L	4	1.9
C	3	1.4
D	3	1.4
I	3	1.4
B	2	1.0
F	2	1.0
K	2	1.0
Q	2	1.0
R	2	1.0
S	2	1.0
M	1	0.5
Total	190	90.4

4. DISCUSSION

The current work investigated the epidemiological and clinical data, antimicrobial resistance patterns and PFGE typing of MRSA in Taiwan. The crucial associations between the three data groups of MRSA were further analyzed. MRSA occurrence was higher than MSSA in the groups below 14 as well as above 64 years old while MSSA showed higher incidence than MRSA in 15~64 years old patients. These results suggested that MRSA

Table 8. Associations of 10 main antimicrobial resistance patterns of MRSA isolates with characteristics of patients and PFGE plusotypes

		SAM, P, CC, E, RA, SXT (n=32)	SAM, P, CC, E, RA (n=37)	SAM, P, CC, E, SXT (n=21)	SAM, P, CC, E (n=88)	SAM, P (n=23)
Age						
0.14		1	1	1	20	5
15-64		8	12	5	34	9
>64		23	24	15	34	9
Gender						
Male		21	27	11	43	8
Female		11	10	10	45	15
Departmaent						
Internal medicine		11	17	7	32	7
Surgery		3	7	7	19	7
Pediatris					13	3
Gynecology					1	
ICU[b]		18			23	6
Specimen						
Respiratory		14	23	6	25	4
Pus		4	27	11	25	10
Blood		8	4	3	10	5
Urine		2	3		2	3
Others		4		1	1	1
PFGE types (Number of strains)	E(2)	J(16), N(2)	A(1),E(2), J(2)	E(2), J(9), I(2)	A(20), E(28), J(6)	A(2), E(2), T(4)
	K(1)	L(3), S(1)	N(28), G(1), T(2)	F(1), N(1), R(2)	N(7),G(7), P(4)	C(1), D(2),H(1)
					B(2), C(2),D(1) F(1),H(3),1(1) K(1),O(1),Q(2)	L(1), O(3),P(2) S(1)

infection may be more risky for the elderly and children. Isolations of total *S. aureus* strains were more among males (58.5%) than females (41.5%) while identification of MRSA isolates was also higher in males (53.3%) than females (46.7%). Also, gender of MRSA patients was significantly different from that of MSSA (*P*=0.0340), suggesting gender could be a factor for MRSA/MSSA infections. The similar gender differences in MRSA prevalence have also been shown in European countries from 1999 to 2002 by Tiemersma et al. [13], implicating the presence of gender differences in MRSA occurrence in different countries or areas.

In the current study, most *S. aureus* isolates (over 90%) came from departments of internal medicine, surgery and ICU while MRSA (210 isolates) were found the most from departments of internal medicine (74/35.2%) and ICU (74/35.2%). MRSA isolated form the rest departments (surgery, pediatrics and gynecology) accounted for less than 30%. Interestingly, in ICU the proportion of MRSA strains was clearly higher than MSSA (*P*=0.0004) while in surgery wards MSSA were isolated more than MRSA (*P*=0.0178). Similarly, in 1992 proportion of MRSA reached to 57% among ICU acquired *S. aureus* infections documented in the European Prevalence of Infection in Intensive Care (EPIC) study while data from January 1999 to December 2002 in Europe revealed that the highest MRSA occurrence (35%) among patients admitted to ICU [13]. Staying in ICU has been indicated as a risk factor for MRSA infection. This may be that ICU patients are usually with chronic illness and the frequent use of invasive indwelling devices in ICU [12]. On the other hand, focusing on the specimen sources of MRSA, isolates from pus (83/39.5%) and respiratory tract (75/35.7%) were

Table 9. Association of 8 major PFGE types of MRSA isolates with characteristics of patients and antimicrobial resistance patterns

characteristics of patients	N (n=38)	E (n=37)	J (n=36)	A (n=24)	G (n=8)	T (n=7)	O (n=5)	P (n=5)
Age								
0-14	1	12	3	4	2		2	
15-64	11	14	7	9	3		1	4
>64	26	11	26	11	3	7	2	1
Gender								
Male	27	16	22	12	3	3	2	3
Female	11	21	14	12	5	4	3	2
Department								
Internal medicine	15	12	13	11		2		
Surgery	7	10	6	3	3	2	2	4
Pediatrics		8	1	3	1		1	
Gynecology					1		1	
ICU[b]	16	7	16	7	3	3	1	1
Specimen								
Respiratory tract	24	5	14	7	3	4	1	2
Pus	8	29	7	12	5	2	3	1
Blood	3	2	10	3		1	1	2
Urine	3		3	1				
Other		1	2	1				
Antimicrobial resistance								
SAM, P,CC ,E, RA, SXT	2	2	16					
SAM, P,CC,E,RA	28	2	2	1	1	2		
SAM, P, CC, E, SXT	1	2	9					
SAM, P, CC, E	7	28	6	20	20	4	2	3
SAM, P		2		2	2		3	2

predominately more than those from blood (32/15.2%), urine (12/5.7%) and others (8/3.8%), implicating the possible association between MRSA isolates and respiratory system. MRSA was isolated more than MSSA from the respiratory tract (*P*=0.0006). This should be highlighted since pulmonary disease has been shown as a risk factor for CA-MRSA infections [24] and detection of MRSA has been indicated to be associated with lower lung function and worse survival in a common lethal autosomal recessive disorder, cystic fibrosis [25,26].

PFGE is a valuable technique with high discriminatory power for investigation of *S. aureus* infections [22,27]. It has been considered as the "gold standard" in molecular typing of MRSA isolates [28,29]. The work by Senna et al. [30] indicated that PFGE is still more reliable than PCR and PCR-based methods exhibited insufficient discriminatory power and limited reproducibility compared with PFGE [28]. It is noteworthy that all MRSA isolates were resistant to at least 2 antibiotics. Moreover, nearly 90% MRSA isolates possessed resistance to 3~6 antibiotics (Table 6A), suggesting that MRSA are indeed multi-antibiotic resistant bacterial strains. The antimicrobial pattern MRSA "SAM, P, CC, E" clustered the most among the main 10 patterns, which accounted for 41.9% of MRSA isolates and mainly belonged to PFGE genotypes E (28/88) and A (20/88). Cluster N was most dominant in the antimicrobial pattern "SAM, P, CC, E, RA" (28/37) as genotype J appeared the most in both the patterns "SAM[a], P, CC,E, RA, SXT" (16/32) and "SAM, P, CC,E, SXT" (9/21). These data demonstrate that different antimicrobial patterns are correlated with PFGE

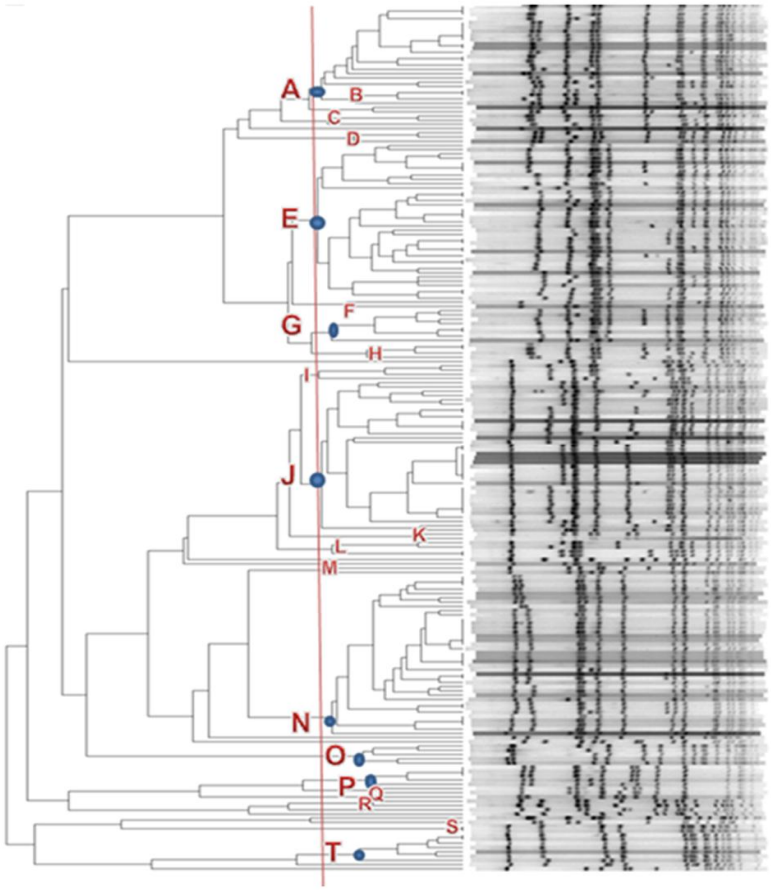

Fig. 1. Twenty PFGE major pulsotypes and phylogenetic tree of MRSA isolates. The isolates with ≧80% similarity were grouped into an identical type. Twenty major pulsotypes were designated as A through T

typing clusters for MRSA isolates and a designated genotype of MRSA may possess activity against specific antibiotic patterns. These connections show that PFGE is an applicatible approach for MRSA studies.

We found that *S. aureas* with oxacillin/methicillin resistance were coupled with higher resistance to other 6 antibiotics than MSSA (P<0.0001, Table 5), demonstrating resistance to methicillin is clinically and hygienically a critical factor for selection of antibiotics against *S. aureas*. The antimicrobial results showed that vancomycin, teicoplanin and linezolid are the most effective agents against MRSA isolates. This is in accordance with the antibiotic susceptibility data in Taiwan [31] as well as the results in north Taiwan[1]. Similarly, in the current study MRSA isolates collected from south Taiwan show resistance rate of 28.1% to trimethoprim / sulfamethoxazole (SXT) compared with MRSA isolated from ocular infection in north Taiwan (susceptible rate 73.1% for SXT). MRSA isolates with the SXT resistance involved patterns here are mostly resistant to penicillin, ampicillin / sulbactam, clindamycin and erythromycin. It has also been shown that MRSA isolates in Taiwan with higher SXT resistance seem more resistant to clindamycin and erythromycin and their MLST (multilocus sequence typing) type (ST239) is different from those with lower resistance to SXT (ST59) [31]. In the current study, some common typing characteristics can also be found in the antimicrobial patterns containing SXT resistance. PFGE pattern J is occupied over 50% by the two main antimicrobial patterns with SXT resistance, “SAM, P, CC, E, SXT” and “SAM, P, CC, E, RA, SXT”, which were not found in other antimicrobial patterns without SXT resistance. These typing data of MRSA may partially explain why MRSA

strains in Taiwan have various resistance to SXT. On the other hand Egyptian report in 2007 showed that over 80% MRSA/ORSA isolates in Cairo are susceptible to SAM [32]. Investigation in Japan showed that SAM may be effective for MRSA isolates [33]. However, the resistance to SAM of 210 MRSA isolates in the current study constitutively reached 100%, implicating the existence of regional differences in SAM resistance for MRSA. One of the reasons should be the differences in antibiotics of choice among countries and areas.

Moreover, our study found that both erythromycin and clindamycin have quite low activity against MRSA, which is consistent with the previous data collected from north Taiwan or throughout Taiwan [1,31]. The report has shown that in a healthcare center in Boston the resistance of MRSA to erythromycin and clindamycin is 96% and 57%, respectively [34] while data focused on CA-MRSA SSTIs among MSM (men who have sex with men) patients in New York revealed a similar clindamycin susceptibility of 37% [35]. Compared with these data, the earlier report in the US showed that higher susceptibilities of MRSA. CA-MRSA and HCA-MRSA (health care-associated MRSA) isolates, respectively, are 48% and 29% for erythromycin, and 74% and 53% for clindamycin [36]. It seems that in the US and Taiwan MRSA have conferred increasing antibiotic resistance to both erythromycin and clindamycin in addition to oxacillin and penicillin and hence these antimicrobial agents may not be active against MRSA. Since most MRSA isolates in the current study are multi-antibiotic resistant, the antimicrobial data uncovered in our investigation are essential. Compared with the listed antibiotics against multi-antibiotic resistant MRSA in 2005, except lower activity of rifampicin in the current study, the active antimicrobial agents against MRSA are mostly in agreement with the antibiotics selected by Rayner and Munckhof [37] ENREF 39. In addition, our antimicrobial data showed that 73.4% MSSA isolates have resistance merely to penicillin and 7.9% MSSA displayed no resistance to 12 antimicrobial agents (Table 6B), demonstrating that in comparison with MRSA, most MSSA isolates are not multi-antibiotic resistant bacterial strains (Table 5). These antimicrobial data of MSSA isolates are coherent with the antibiotics of choice for MSSA in the previous report [37] ENREF 39 ENREF 39 ENREF 39.

5. CONCLUSION

Our study provides important data on the antimicrobial resistance and PFGE clusters of MRSA as well as on comparative analysis between MRSA and MSSA isolates in Taiwan. PFGE successfully differentiated the MRSA isolates into 20 main clusters and types A, E, G, and J were essentially relevant with clinical and antibiotics resistance data. The statistical results showed that *S. aureas* isolated from ICU and the respiratory tract of patients account for more MRSA than MSSA, suggesting that ICU and the respiratory tract are potentially risky factors for MRSA. Vancomycin, teicoplanin and linezolid were the most active agents against MRSA isolates. More than 25% MRSA isolates were resistant to sulfamethoxazole / trimethoprim while resistance to ampicillin / sulbactam, clindamycin and erythromycin for MRSA isolates dominantly reached 86.2~100%. In contrast, most MSSA isolates are not multi-antibiotic resistant in our study. Combined analysis of characteristics of patients, antibiotic-resistant patterns and PFGE typing profiles further reveal the crucial associations and clues among the three microbiological parameters of MRSA and demonstrate that PFGE clusters of MRSA isolates can be linked with their distinct antibiotic-resistant patterns, special specimen sources or clinical implications as well as specific hospital departments where MRSA isolated. This study offers essential information of MRSA typing to aid understanding molecular epidemiology and selection of antimicrobial agents of MRSA and can be utilized in infection control and antimicrobial stewardship in Taiwan.

COMPETING INTERESTS

All authors have no conflict of interest.

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