International Journal of Infectious Diseases 79 (2019) 162–168

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Comparison of molecular epidemiology of bloodstream methicillin-resistant *Staphylococcus aureus* isolates between a new and an old hospital in central Taiwan

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ARTICLE INFO

Article history:
Received 4 October 2018
Received in revised form 3 December 2018
Accepted 4 December 2018
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Bloodstream infection
Methicillin-resistant *Staphylococcus aureus*
spa type
Sequence type
Taiwan

ABSTRACT

Objective: To compare the molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates between an old, urban hospital and a new, rural hospital over the same time period.
Methods: The molecular characteristics of 398 MRSA bloodstream isolates collected between 2007 and 2013 from two hospitals in Taiwan were analyzed retrospectively; 202 isolates were from the old hospital and 196 from the new hospital (opened in 2007).
Results: The rate of resistance to multiple antibiotics was significantly higher in the old hospital (93%) than in the new hospital (81%) ($p < 0.001$). Genetic community-associated MRSA carrying staphylococcal cassette chromosome (SCC) type IV or V accounted for 58% of all MRSA isolates in the new hospital, significantly higher than the rate in the old hospital ($p = 0.018$). The rate of *spa* t037-SCC*mec* III MRSA was significantly lower in the new hospital than in the old hospital ($p = 0.02$). A significant decreasing trend in *spa* t002-SCC*mec* II MRSA isolates was observed in the old hospital ($p = 0.006$), while the proportion of *spa* t037-SCC*mec* III MRSA decreased significantly in the new hospital (41.7% to 26.1%, $p = 0.022$).
Conclusions: The rate of multiple antibiotic resistance and the molecular characteristics of MRSA differed significantly between the old and new hospitals and changed over time.

Introduction

Since the first identification of methicillin-resistant *Staphylococcus aureus* (MRSA) in the United Kingdom in 1961, these organisms have become distributed worldwide and represent major pathogens of nosocomial infection. Through molecular methods, including pulsed-field gel electrophoresis (PFGE), staphylococcal cassette chromosome *mec* (SCC*mec*), multilocus sequence typing (MLST), and staphylococcal protein A (*spa*) typing, it has been shown that the molecular epidemiology of MRSA varies in different hospital settings and geographical regions and has evolved over time.

In Taiwan, MRSA accounted for 53–83% of all *S. aureus* isolates in most hospitals in the early 2000s (Chen and Huang, 2014). The majority of these MRSA belonged to a certain pulsotype, which was subsequently characterized as sequence type (ST) 239 with SCC*mec* type III and resistance to multiple non-β-lactam antibiotics (Chen et al., 2009; Chen et al., 2014; Huang et al., 2004). With the emergence of community-associated MRSA (CA-MRSA), a significant increase in the proportion of CA-MRSA infections has been recognized, including skin and soft tissue infections, deep-site infections, and bacteremia. Genotyping studies have shown that CA-MRSA strains in Taiwan are characterized as ST59-SCC*mec* type V or V_T, harboring Panton–Valentine leukocidin (PVL) genes (Chen

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https://doi.org/10.1016/j.ijid.2018.12.002

et al., 2009; Chen et al., 2007). There are significant differences between hospital-associated MRSA (HA-MRSA) and CA-MRSA isolates in terms of molecular characteristics, antibiograms, and clinical disease entities (Chen et al., 2007; Huang et al., 2008; Wang et al., 2010).

Previous studies have shown that temporal shifts in predominant MRSA clones have occurred over time worldwide, including in Taiwan (Chen and Huang, 2014; Chen et al., 2009; Chen et al., 2014). However, studies comparing the molecular epidemiology of MRSA isolates between old, urban hospitals and new, rural hospitals are scarce. The aim of this study was to elucidate this issue by comparing the molecular epidemiology of MRSA bloodstream isolates between two regional hospitals in central Taiwan, one of which is a new hospital that opened in the era of CA-MRSA epidemics.

Materials and methods

Study design and hospital setting

The molecular characteristics of MRSA bloodstream isolates that were collected prospectively between 2007 and 2013 from two regional hospitals in Changhua County in central Taiwan were analyzed retrospectively. The old hospital is Changhua Show Chwan Memorial Hospital, an 861-bed, 40-year-old hospital in the urban area of Changhua County. The new hospital is Chang Bing Show Chwan Memorial Hospital, a 768-bed new hospital (opened in September 2007) in the rural area of Changhua County. Both of these hospitals are general hospitals that provide a variety of clinical services; there are no differences in the medical specialties between them. Despite the old hospital being in an urban region and the new one in a rural region, these two hospitals are situated merely 17 km apart. In addition, comparisons were made of patient sex, age, disease entities, length of intensive care unit stay, 30-day mortality rate, etc. In general, there was no significant difference in these factors between the two study hospitals.

This study was approved by the Institutional Review Board of Show Chwan Memorial Hospital (approval No. 1040605), and the need for informed consent was waived owing to the retrospective nature of this study.

Clinical MRSA isolates

A total of 196 non-duplicate bloodstream MRSA isolates were collected throughout the study period at the new hospital, which opened in September 2007. At the old hospital, a total of 533 bloodstream MRSA isolates were collected during the study period. The difference in size of the patient populations may be the main contributor to this difference in number of bloodstream infections, with a larger population at the old hospital. After excluding duplicate isolates, only those isolates with a laboratory code ending with an even number were selected for further analysis in order to have a comparable number of isolates; furthermore, the number of selected isolates from the old hospital was matched to that from the new hospital in each study year. Finally a total of 202 MRSA isolates from the old hospital were included and characterized (nearly half of all non-duplicate MRSA isolates from the old hospital). Identification of *S. aureus* was confirmed by coagulase testing, and cefoxitin susceptibility was assessed by disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines of 2014.

Antimicrobial susceptibility tests

For each MRSA isolate, antimicrobial susceptibility to 11 antibiotics, including oxacillin, penicillin, trimethoprim–sulfamethoxazole (TMP–SXT), levofloxacin, clindamycin, erythromycin, gentamicin, fusidic acid, teicoplanin, and linezolid, was tested by disk diffusion and microdilution methods in accordance with the Clinical and Laboratory Standards Institute guidelines of 2014, as appropriate. Vancomycin susceptibility was determined by minimal inhibitory concentration.

Molecular characterization

SCC*mec* typing was determined using a multiplex PCR strategy, as described previously (Kondo et al., 2007); if this method was not successful in identifying a type, another multiplex PCR strategy was applied (Zhang et al., 2005). Control strains for SCC*mec* types I, II, III, and IVa were as follows: type I, NCTC10442; type II, N315; type III, 85/2082; and type IVa, JCSC4744. SCC*mec* typing for types V and V_T was determined using specific primers described elsewhere (Huang et al., 2008) and the strain TSGH-17, which was used as a control. MRSA isolates without the anticipated amplification product were regarded as untypeable (UT).

The presence of PVL genes was determined using a previously described PCR strategy (Chen et al., 2007). Screening for the presence of the *sas*X gene was conducted by PCR amplification of a 522-bp fragment using the primers and thermal cycles described previously (Li et al., 2012). Protein A gene (*spa*) typing was conducted for all MRSA isolates. The polymorphic X region of *spa* was amplified by PCR using the primers 1095F (5′-AGACGATCCTTCGGTGAGC-3′) and 1517R (5′-GCTTTTGCAATGTCATTTACTG-3′). PCR products were sequenced, and *spa* types were assigned by analysis of the nucleotide sequences using BioNumerics version 6.5 (Applied Maths NV). MLST was performed on a fixed proportion of isolates for each representative *spa* type and all singletons. Seven housekeeping genes of *S. aureus* were used for MLST typing (*arc*, *aroE*, *glp*, *gmk*, *pta*, *tpi*, and *yqiL*). Amplification of a portion of each gene was performed as described previously (Huang et al., 2008). The amplified products were sequenced, and the sequences thus obtained were analyzed using software available at http://saureus.mlst.net/sql/multiplelocus.asp

Definition of HA- and CA-MRSA by genetic features

Since the risk factors for MRSA acquisition could not be identified definitely through the retrospective chart review in this study, and growing evidence has shown that CA-MRSA carrying SCC*mec* IV, V, or V_T accounts for an increased proportion of HA-MRSA infections in Taiwan, the genetic features by SCC*mec* type were used to define CA-MRSA and HA-MRSA in this study (Chen et al., 2013; Otter and French, 2012). MRSA carrying SCC*mec* II or III was considered as genetic HA-MRSA, and MRSA carrying SCC*mec* IV, V, or V_T was considered as genetic CA-MRSA.

Statistical analysis

All statistical analyses were performed using SPSS software (PAWS Statistics 18.0.0; SPSS Inc., Chicago, IL, USA). Categorical variables were compared by Fisher's exact test or Pearson Chi-square test, as appropriate. All comparisons were two-tailed, and *p*-values of less than 0.05 were considered statistically significant.

Results

Antimicrobial non-susceptibility rate

During the study period 2007–2013, the annual rate of MRSA among *S. aureus* ranged from 54.3% to 75.7% in the old hospital and from 51% to 69% in the new hospital. Genetic CA-MRSA accounted for 58.1% of all MRSA isolates in the new hospital and this rate was significantly higher than that in the old hospital ($p = 0.018$). In contrast, HA-MRSA was still predominant in the old hospital (54.0%). The annual MRSA isolation rate, the proportions of genetic CA-MRSA and genetic HA-MRSA, the annual average number of patient admissions, and the annual daily defined dose of vancomycin and fluoroquinolones during each study year in each hospital are shown in Figure 1.

Of the 398 MRSA isolates, all were sensitive to vancomycin, teicoplanin, and linezolid. The detailed antibiograms of all isolates to the other six non-β-lactam antibiotics stratified by hospital and SCC*mec* type are shown in Table 1. The rate of resistance to each antibiotic varied from 21.6% for fusidic acid to 94.7% for erythromycin. The antimicrobial resistance rate to each antibiotic was significantly higher in the old hospital than in the new hospital, with the exception of fusidic acid ($p < 0.05$). Notably, up to 86.9% of isolates were resistant to more than three different classes of antimicrobial. The lowest multidrug resistance rate was observed for SCC*mec* IV MRSA (58.1%, $p < 0.05$).

*Distribution of SCC*mec *types,* spa *types, and STs*

Five SCC*mec* types were identified in this study, including types III ($n = 163$, 40.9%), IV ($n = 86$, 21.6%), II ($n = 77$, 19.3%), V_T ($n = 45$,

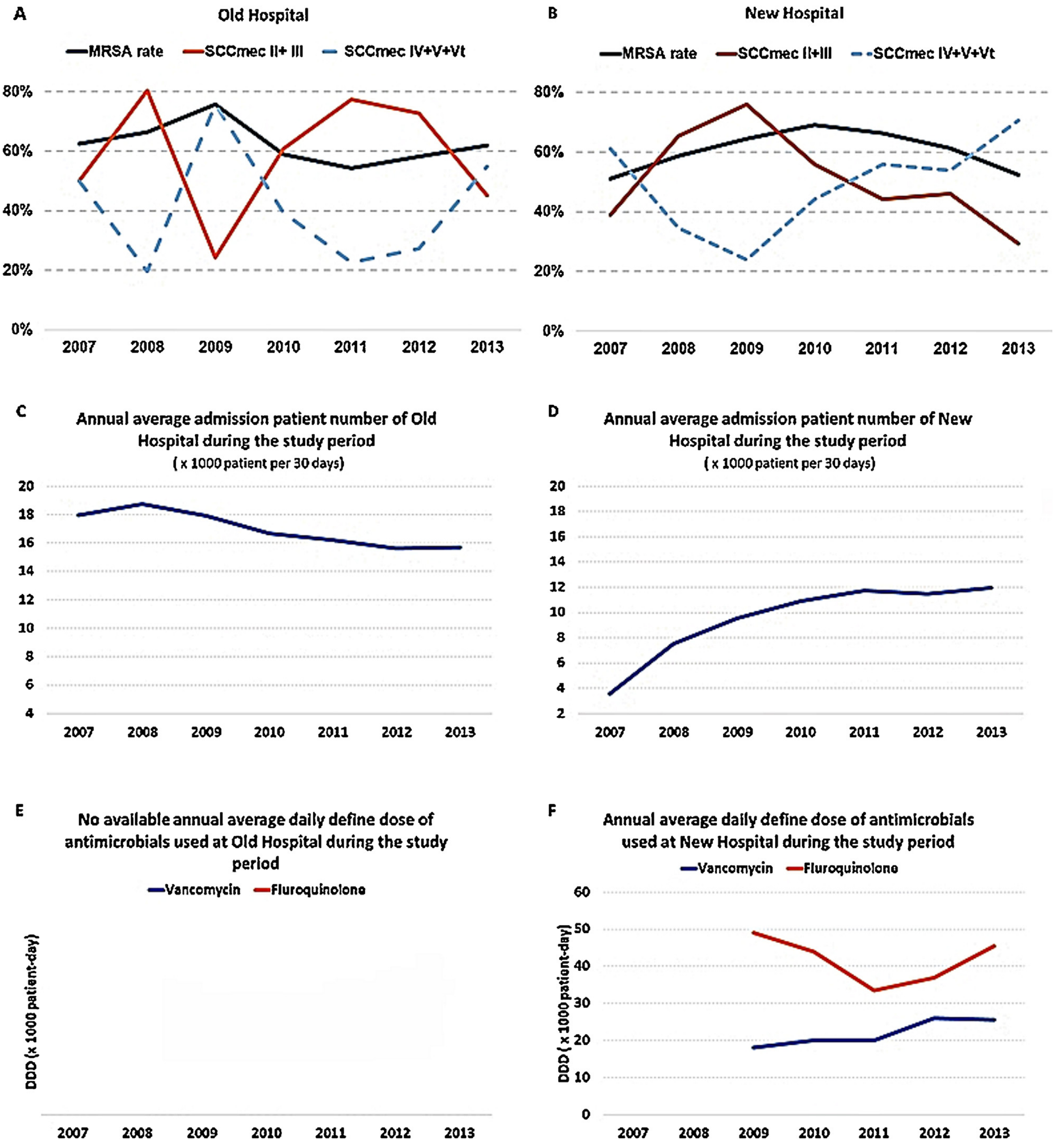

Figure 1. Annual prevalence of MRSA, inpatient numbers, and defined daily doses of antimicrobials in the old and new hospitals over the study period. (A) and (B): annual prevalence rates of MRSA and proportions of hospital-associated and community-associated MRSA. (C) and (D): annual average inpatient numbers depicted as 1000-patient–30 days over the study period. (E) and (F): defined daily doses of vancomycin and fluoroquinolones were only available for the new hospital from 2009.

Table 1
Antimicrobial non-susceptibility rates of 398 clinical MRSA bloodstream isolates, stratified by hospital and SCC*mec* type.

Antibiotics	Number of positive isolates (%)								
	Old hospital ($n = 202$)	New hospital ($n = 196$)	p-Value	SCC*mec* II ($n = 77$)	SCC*mec* III ($n = 163$)	SCC*mec* IV ($n = 86$)	SCC*mec* V ($n = 26$)	SCC*mec* V_T ($n = 45$)	SCC*mec* UT ($n = 1$)
CC	190 (94.1)	169 (86.2)	0.0139*	76 (98.7)	162 (99.4)	60 (69.8)	18 (69.2)	42 (93.3)	1 (100)
EM	198 (98.0)	179 (91.3)	0.0057*	77 (100)	163 (100)	72 (83.7)	20 (76.9)	44 (97.8)	1 (100)
SXT	141 (69.8)	99 (50.5)	0.0001*	37 (48.1)	156 (95.7)	27 (31.4)	7 (26.9)	13 (28.9)	0 (0)
LVX	168 (83.2)	130 (66.3)	0.0002*	76 (98.7)	154 (94.5)	35 (40.7)	19 (73.1)	14 (31.1)	0 (0)
GM	188 (93.1)	167 (85.2)	0.018*	75 (97.4)	159 (97.5)	61 (70.9)	20 (76.9)	39 (86.7)	1 (100)
FA	45 (22.3)	41 (20.9)	0.8356	12 (15.6)	61 (37.4)	2 (2.3)	8 (30.8)	3 (6.7)	0 (0)
≥3 drugs	188 (93.1)	158 (80.6)	0.0004*	76 (98.7)	160 (98.2)	50 (58.1)**	20 (76.9)	39 (86.7)	1 (100)

CC, clindamycin; EM, erythromycin; SXT, trimethoprim–sulfamethoxazole; LVX, levofloxacin; GM, gentamicin; FA, fusidic acid; UT, untypeable.

* $p < 0.05$, significant (Chi-square test, compared with old hospital).

** $p < 0.05$, significant (Chi-square test, compared with other SCC*mec* types).

11.3%), and V ($n = 26$, 6.5%); one isolate was untypeable. *spa* typing was conducted for all 398 isolates and revealed 33 different types. The distributions of SCC*mec* types and corresponding *spa* types are shown in Table 2. The five most common SCC*mec spa* types were SCC*mec* III-t037 ($n = 139$), SCC*mec* II-t002 ($n = 73$), SCC*mec* IV-t437 ($n = 38$), SCC*mec* VT-t437 ($n = 34$), and SCC*mec* V-t1081 ($n = 20$).

MLST was performed for 120 isolates, and 16 STs were identified. The detailed distributions of STs, *spa* types, and SCC*mec* types are shown in the **Supplementary Material** (Table S1). The leading five STs, SCC*mec* types, and *spa* types in combination were ST239-SCC*mec* III-t037 ($n = 139$, 34.9%), ST5-SCC*mec* II-t002 ($n = 73$, 18.3%), ST59-SCC*mec*IV-t437 ($n = 38$, 9.5%), ST59-SCC*mec* VT-t437 ($n = 34$, 8.5%), and ST45-SCC*mec* IV-t1081 ($n = 20$, 5.0%). Four major MRSA clones were identified, which were CC5-t002, CC45-t1081, CC239-t037, and CC 59-t437 (Figure 2). One ST9-SCC*mec* XII-t2922 MRSA strain, typically known to be associated with livestock, was identified in this study. This strain was isolated from a 71-year-old man with a right knee wound infection, proven MRSA septic arthritis, and bacteremia. He was discharged after 2 weeks of intravenous teicoplanin therapy and recovered uneventfully.

The presence of PVL and *sas*X genes and the distributions of each SCC*mec* type and ST are shown in Table 2. The presence of PVL genes was most commonly observed in SCC*mec* V_T MRSA (81.4%, $p < 0.001$) and ST59 MRSA (51.7%). In contrast, the *sas*X gene was most frequently identified in SCC*mec* III MRSA (91.6%, $p < 0.001$) and ST239 MRSA (100%).

Changes in the molecular epidemiology in each hospital between 2007–2009 and 2010–2013

Figure 3 shows the epidemiological changes in four major *spa* types and each SCC*mec* type between the periods 2007–2009 and 2010–2013. In general, there were no significant changes in trends for each *spa* type, with the exception of t002 MRSA, which exhibited a significantly decreasing trend (57.3% to 42.7%, $p = 0.002$). With respect to each *spa* type change in each hospital, the proportion of t002 MRSA decreased over time in both hospitals, but a significant decreasing trend for t002 MRSA isolates was only observed in the old hospital (Figure 3B, $p = 0.006$), and not in the new hospital (Figure 3C). Although there was no significant change in trend for the t037 type during the overall study period, the proportion of t037 MRSA decreased significantly during the period 2010–2013 in the new hospital (41.7% to 26.1%, $p = 0.022$). The proportion of *spa* t037-SCC*mec*III MRSA was also significantly lower in the new hospital than in the old one (Table 3, $p = 0.02$).

Discussion

In this study, four major clones were identified in two regional hospitals in central Taiwan during the 7-year period from 2007 to 2013, namely ST239-SCC*mec* III-t037, ST 59-SCC*mec* IV/V-t437, ST 5-SCC*mec* II-t002, and ST45-SCC*mec* IV-t1081; this is consistent with previous reports from Taiwan (Chen et al., 2014; Wang et al., 2010; Ho et al., 2016). These findings provide important insights into the distribution and evolution of CA- and HA-MRSA strains in the hospital setting in central Taiwan.

Interestingly, the results of this study showed that each hospital had its own unique MRSA epidemiology pattern (Chen et al., 2014; Chen et al., 2013). Genetic HA-MRSA still predominated in the old hospital, and the majority of isolates were ST239-SCC*mec*-III-t037. In contrast, genetic CA-MRSA, mostly ST59-SCC*mec* IV/V-t437, supplanted traditionally genetic HA-MRSA as the predominant clone in the new hospital from 2010. The new hospital opened during the CA-MRSA epidemic era in Taiwan, and CA-MRSA

Table 2
Molecular characteristics of 398 clinical MRSA bloodstream isolates stratified by *spa* typing.

spa clonal complex (n)	*spa* type (n)	SCC*mec* type (n)	MLST	PVL-positive, n (%)	*sasX*-positive, n (%)
t002 (79)	t002 (74)	II (73), III (1)	ST5 (16/16)	0	0
	t010 (1), t14715 (1), t668 (3)	II (4), UT (1)	ST5 (4/4)	0	0
t037 (147)	t037 (141)	III (139), IV (1), V (1)	ST239 (31/31)	0	139 (98.6)
	t074 (4), t275 (1), t5319 (1)	III (6)	ST239 (6/6)	0	6
t437 (88)	t437 (73)	IV (38) V (1) V_T (34)	ST59 (17/17)	34 (46.6)	0
	t441 (9), t1751 (2), t3485 (2), t3517 (1), t5795 (1)	IV (8), V_T (7)	ST59 (12/12)	7	0
t1081 (30)	t1081 (27)	IV (7) V (20)	ST45 (7/7)	0	0
	t12697 (3)	V (3)	ST45 (2/2)	0	0
t008 (8)	t008 (8)	IV (8)	ST8 (2/2)	7 (87.5)	0
Others (46)		III (17), IV (24), V (1), V_T (4)	ST188 (1/13), ST573 (4/13) ST508 (2/13), ST30 (2/13) ST2495 (1/13), ST338 (1/13) ST770 (1/13), ST9 (1/13)	8 (17.4)	15 (32.6)

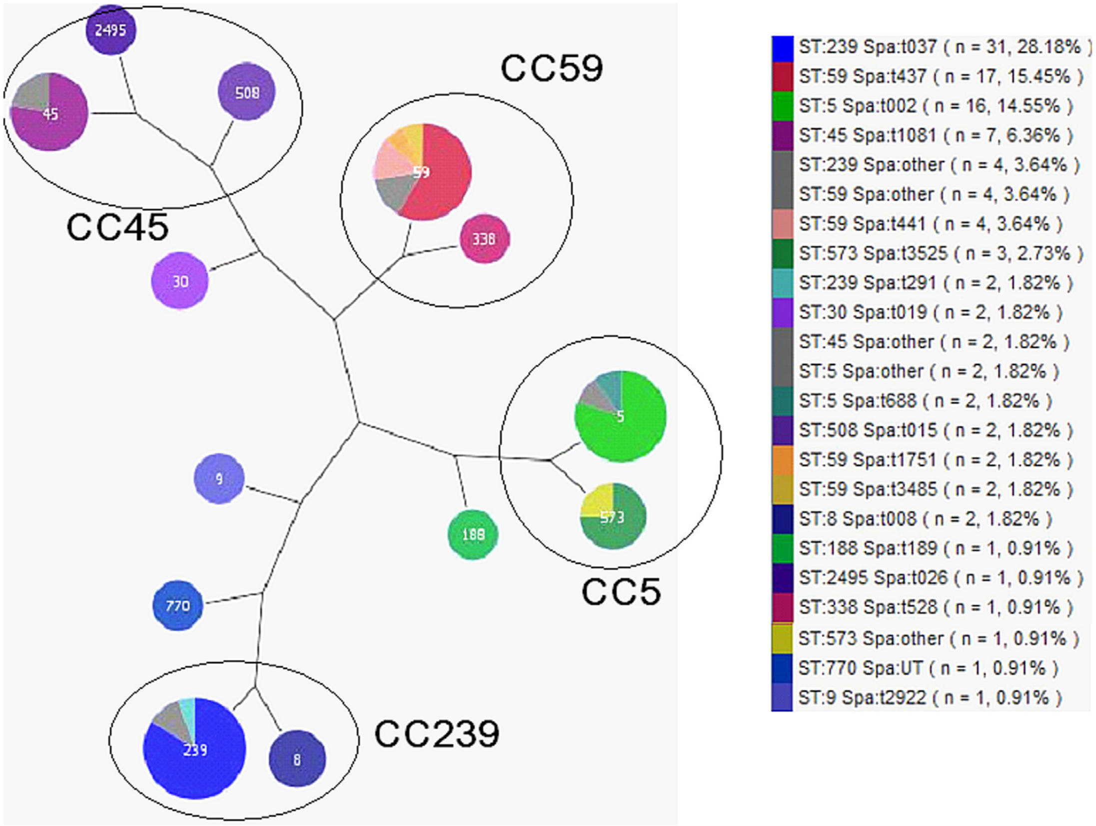

Figure 2. Major MRSA clones and phylogenetic analysis in this study. The phylogenetic tree was constructed using the software Phyloviz version 2 and the neighbor-joining method, and was based on the ST and *spa* type.

replaced the traditionally genetic HA-MRSA successfully in this hospital within 3 years.

To delineate any confounding factors for the change in genetic MRSA clones in the two hospitals, an analysis of the medical records related to these 398 MRSA bloodstream infections was performed. However, only 60% of the records were accessible. There were no statistically significant differences in terms of age, sex, laboratory findings, and rate of 30-day mortality. However, there were two statistically significant differences identified between these two study hospitals: (1) 53% of MRSA isolates

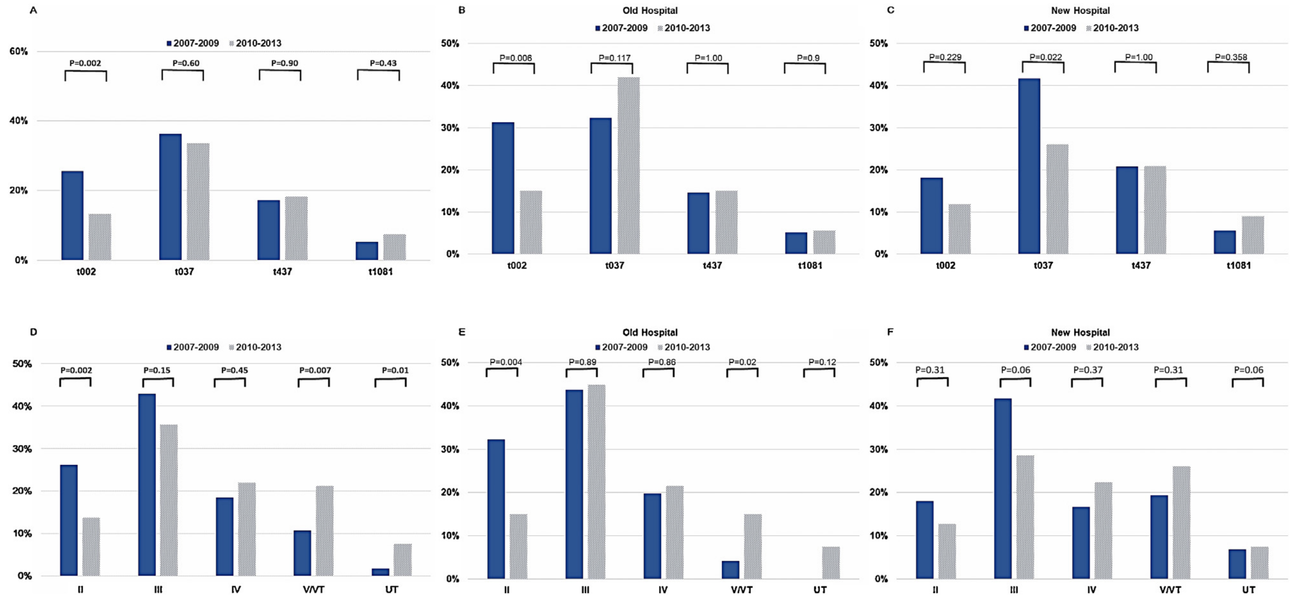

Figure 3. Changes in the proportions of four major *spa* types and SCC*mec* types of MRSA between 2007 and 2009 and between 2010 and 2013. (A)–(C): changes in four major *spa* types between the two study periods and decreasing trend of t002 in the old hospital. (D)–(F): changes in representative SCC*mec* types between the two study periods, the decreasing trend of SCC*mec* II, and the increasing trends in SCC*mec* V/V_T and UT in the old hospital.

Table 3
Comparison of major clones between old and new hospitals during two time periods.

Periods	Genetic HA-MRSA, No. (%)		Genetic CA-MRSA, No. (%)	
	spa t002-II	spa t037-III	spa t437-IV	spa t437-V_T
2007–2009				
Old hospital (N = 95)	29 (30.5)	31 (32.6)	13 (13.7)	1 (1.1)
New hospital (N = 71)	13 (18.3)	29 (40.8)	8 (11.3)	7 (9.9)
p-Value	0.10	0.35	0.81	0.02*
2010–2013				
Old hospital (N = 107)	15 (14.0)	45 (42.1)	8 (7.5)	8 (7.5)
New hospital (N = 125)	16 (12.8)	34 (27.2)	9 (7.2)	18 (14.4)
p-Value	0.85	0.02*	1.0	0.14
2007–2013				
Old hospital (N = 202)	44 (21.8)	76 (37.6)	21 (10.4)	9 (4.5)
New hospital (N = 196)	29 (14.8)	63 (32.1)	17 (8.7)	25 (12.8)
p-Value	0.092	0.252	0.611	0.004*

HA, hospital-associated; CA, community-associated; MRSA, methicillin-resistant *Staphylococcus aureus*.
* $p < 0.05$, significant (Chi-square test, compared with old hospital).

from the old hospital belonged to epidemiologically defined HA-MRSA, while only 36.8% of isolates from the new hospital were HA-MRSA ($p = 0.011$). (2) In terms of disease entities, there were more bacteremic skin and soft tissue infections identified in the new hospital than in the old one (26.3% vs. 13.9%, $p = 0.02$). In contrast, catheter-related MRSA bloodstream infections were more common in the old hospital (30.7% vs. 15.0%, $p = 0.004$). These findings explain why CA-MRSA predominated in the new hospital over the study period and highlight the change in molecular clones of MRSA in the community. Regarding the persistently high rate of genetic HA-MRSA in the old hospital, no matter what definition was used, the proportion of HA-MRSA was quite similar in the old hospital. Most of these MRSA isolates were characterized as SCC*mec* III-ST239. Although ST239 remains the predominant HA-MRSA clone in Taiwan, the possibility that there might have been HA-MRSA strains continuously colonizing the old hospital and contributing to a reservoir of MRSA infection cannot be excluded. However, MRSA screening of the patients admitted to the two hospitals was not conducted during the study period.

Previous studies have shown that CA-MRSA can adapt well to the hospital environment and increase the proportion of MRSA isolates over time (Chen et al., 2009; Chen et al., 2014; Huang et al., 2006; Pan et al., 2013). Moreover, in some countries, CA-MRSA has replaced traditional HA-MRSA successfully as the predominant MRSA strain (Nimmo et al., 2013; Sonnevend et al., 2012). However, the HA-MRSA clones continuously circulated in both hospitals and remained a significant challenge for infection control. This finding emphasizes the importance of molecular surveillance for MRSA and suggests that different infection control measures may be needed in different hospital settings.

This study identified a significant decreasing trend in ST5-t002 MRSA strains in both hospitals throughout the study period. ST5-t002 strains, previously defined as the New York/Japan clone, have been observed in Taiwan since the late 1990s, and they gradually became important hospital MRSA strains during the 2000s (Chen et al., 2009; Ho et al., 2016; Wang et al., 2012); these strains still accounted for 19% of bloodstream MRSA isolates from six major hospitals in Taiwan in 2010 (Chen et al., 2014). In the present study, the overall percentage of the ST5-t002 strain was 18.3%; however, there was a significant decrease in prevalence from 25.6% in 2007–2009 to 13.3% in 2010–2013, which was particularly noticeable in the old hospital. Whether this clone will continue to decline requires further surveillance. In contrast, there were no significant changes in the other three major clones.

Some specific MRSA ST types found in this study deserve more discussion. MRSA ST45 strain was first identified in 2006 in Taiwan in a respiratory care ward, colonizing a proportion of infected patients and healthcare workers (Lee et al., 2011). Furthermore, it was found to be the predominant colonizing clone among residents and healthcare workers in 14 nursing homes in a survey conducted in 2012 (Tsao et al., 2015). In this study the ST45-t1081 strain accounted for 6.6% of all MRSA bloodstream isolates, consistent with a previous report from Taiwan (Chen et al., 2014). Two ST8 isolates (USA300) were identified in this study. MRSA USA300 was first identified in Taiwan in 2005 and has been identified increasingly since 2010 (Huang et al., 2018). Notably, one ST9 isolate, which is a livestock-associated MRSA, was identified in this study. Unlike the widespread ST398 livestock-associated MRSA strains in European countries and North America, ST9 is predominant in most Asian countries, including Taiwan (Chen et al., 2018; Chuang and Huang, 2015; Fang et al., 2014). Whether these strains will continue to spread in Taiwan needs further observation.

In this study, the presence of two genetic markers of MRSA were evaluated, namely PVL genes and the *sas*X gene. The majority of PVL-positive MRSA in Taiwan belonged to the clone of ST59-t437-SCC*mec* V_T (Chen et al., 2014; Chen et al., 2007; Ho et al., 2016). In contrast, all the ST239-MRSA-SCC*mec*III-t037 isolates carried the *sas*X gene, but this gene was rarely detected in strains of other STs, consistent with previous reports (Chen et al., 2014; Li et al., 2014). Since ST239 MRSA is highly prevalent in China (Chen et al., 2013; Li et al., 2014), it is reasonable to assume that the *sas*X gene may be a key factor affecting the success of spread and transmission of ST239 MRSA; however, supportive evidence from other countries is limited. In contrast to China, the proportion of ST239-*sas*X-positive among MRSA bloodstream isolates in Taiwan declined significantly from 78% in 2000 to 38% in 2010 [Chen et al., 2014a], raising the question regarding the role of the *sas*X gene in the MRSA epidemic.

Resistance to multiple non-β-lactam antibiotics is not infrequently observed in both HA-MRSA and CA-MRSA isolates in Taiwan. In this study, the antimicrobial resistance rate to each antibiotic was significantly higher in the old hospital than in the new hospital, with the exception of fusidic acid. Notably, although all isolates were susceptible to vancomycin, teicoplanin, and linezolid, up to 86.9% of isolates were resistant to more than three different classes of antimicrobial. The rates of multidrug resistance ranged from 62.2% to 100% and correlated with SCC*mec* type; the rate was lowest for the isolates with SCC*mec* IV. These findings are consistent with those of previous reports from Taiwan (Chen and Huang, 2014; Chen et al., 2014).

In conclusion, the methicillin resistance rates of bloodstream *S. aureus* isolates in both the old and new hospital were comparable to the local epidemiological data in Taiwan, and unrelated to the timing of the hospital opening. However, the rate of multiple antibiotic resistance (which was correlated with the SCC*mec* type) and the proportion of genetic CA-MRSA among the MRSA isolates differed significantly between the new hospital and the old hospital. The molecular epidemiology of MRSA isolates changed over time in each hospital. Continued surveillance of MRSA molecular epidemiology is warranted.

Acknowledgements

This study was supported by a grant provided by the Chang Bing Show Chwan Memorial Hospital (RA14017).

Ethics statement

This study was approved by the Institutional Review Board of Show Chwan Memorial Hospital (approval No. 1040605), and the need for informed consent was waived owing to the retrospective nature of this study.

Conflict of interest

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2018.12.002.

References

Chen CJ, Su LH, Chiu CH, et al. Clinical features and molecular characteristics of invasive community-acquired methicillin-resistant *Staphylococcus aureus* infections in Taiwanese children. Diagn Microbiol Infect Dis 2007;59:287–93.

Chen CJ, Hsueh PR, Su LH, Chiu CH, Lin TY, Huang YC. Change in the molecular epidemiology of methicillin-resistant *Staphylococcus aureus* bloodstream infections in Taiwan. Diagn Microbiol Infect Dis 2009;65:199–201.

Chen CJ, Huang YC, Su LH, Wu TL, Huang SH, Chien CC, et al. Molecular epidemiology and antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* bloodstream isolates in Taiwan, 2010. PLoS One 2014;9:e101184.

Chen CJ, Huang YC. New epidemiology of *Staphylococcus aureus* infection in Asia. Clin Microbiol Infect 2014;20:605–23.

Chen CJ, Lauderdale TY, Lu CT, et al. Clinical and molecular features of MDR livestock-associated MRSA ST9 with staphylococcal cassette chromosome mecXII in humans. J Antimicrob Chemother 2018;73:33–40.

Chen X, Wang WK, Han LZ, et al. Epidemiological and genetic diversity of *Staphylococcus aureus* causing bloodstream infection in Shanghai, 2009-2011. PLoS One 2013;8:e72811.

Chuang YY, Huang YC. Livestock-associated meticillin-resistant *Staphylococcus aureus* in Asia: an emerging issue. Int J Antimicrob Agents 2015;45:334–40.

Fang HW, Chiang PH, Huang YC. Livestock-associated methicillin-resistant *Staphylococcus aureus* ST9 in pigs and related personnel in Taiwan. PLoS One 2014;9:e88826.

Ho CM, Lin CY, Ho MW, Lin HC, Peng CT, Lu JJ. Concomitant genotyping revealed diverse spreading between methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* in central Taiwan. J Microbiol Immunol Infect 2016;49:363–70.

Huang YC, Su LH, Wu TL, et al. Molecular epidemiology of clinical isolates of methicillin-resistant *Staphylococcus aureus* in Taiwan. J Clin Microbiol 2004;42:307–10.

Huang YC, Su LH, Wu TL, Lin TY. Changing molecular epidemiology of methicillin-resistant *Staphylococcus aureus* bloodstream isolates from a teaching hospital in Northern Taiwan. J Clin Microbiol 2006;44:2268–70.

Huang YC, Ho CF, Chen CJ, Su LH, Lin TY. Comparative molecular analysis of community-associated and healthcare-associated methicillin-resistant *Staphylococcus aureus* isolates from children in northern Taiwan. Clin Microbiol Infect 2008;14:1167–72.

Huang YC, Chen CJ, Kuo CC, Lu MC. Emergence, transmission and phylogeny of methicillin-resistant *Staphylococcus aureus* sequence type 8 (USA300) in Taiwan. J Hosp Infect 2018;, doi:http://dx.doi.org/10.1016/j.jhin.2018.02.014.

Kondo Y, Ito T, Ma XX, et al. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. Antimicrob Agents Chemother 2007;51:264–74.

Lee YT, Lin DB, Wang WY, et al. First identification of methicillin-resistant *Staphylococcus aureus* MLST types ST5 and ST45 and SCCmec types IV and Vt by multiplex PCR during an outbreak in a respiratory care ward in central Taiwan. Diagn Microbiol Infect Dis 2011;70:175–82.

Li M, Du X, Villaruz AE, et al. MRSA epidemic linked to a quickly spreading colonization and virulence determinant. Nat Med 2012;18:816–9.

Li S, Sun J, Zhang J, et al. Comparative analysis of the virulence characteristics of epidemic methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from Chinese children: ST59 MRSA highly expresses core gene-encoded toxin. APMIS 2014;122:101–14.

Nimmo GR, Bergh H, Nakos J, et al. Replacement of healthcare-associated MRSA by community-associated MRSA in Queensland: confirmation by genotyping. J Infect 2013;67:439–47.

Otter JA, French GL. Community-associated meticillin-resistant *Staphylococcus aureus*: the case for a genotypic definition. J Hosp Infect 2012;81:143–8.

Pan SC, Chen SY, Wang JT, et al. Changing epidemiology of community-onset *Staphylococcus aureus* bacteremia over nine years in an emergency department in Taiwan. J Infect 2013;66:187–9.

Sonnevend A, Blair I, Alkaabi M, et al. Change in meticillin-resistant *Staphylococcus aureus* clones at a tertiary care hospital in the United Arab Emirates over a 5-year period. J Clin Pathol 2012;65:178–82.

Tsao FY, Kou HW, Huang YC. Dissemination of methicillin-resistant *Staphylococcus aureus* sequence type 45 among nursing home residents and staff in Taiwan. Clin Microbiol Infect 2015;21:451–8.

Wang JL, Wang JT, Chen SY, Chen YC, Chang SC. Distribution of Staphylococcal cassette chromosome mec Types and correlation with comorbidity and infection type in patients with MRSA bacteremia. PLoS One 2010;5:e9489.

Wang WY, Chiueh TS, Sun JR, Tsao SM, Lu JJ. Molecular typing and phenotype characterization of methicillin-resistant *Staphylococcus aureus* isolates from blood in Taiwan. PLoS One 2012;7:e30394.

Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2005;43:5026–33.