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Comparative molecular analysis of community-associated and healthcare-associated methicillin-resistant *Staphylococcus aureus* isolates from children in northern Taiwan

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ABSTRACT

From August 2004 to July 2005, 210 clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were collected prospectively from 173 children admitted to Chang Gung Children's Hospital in Taiwan. A comparative molecular analysis of the 111 community-associated (CA) isolates from 102 children and the 99 healthcare-associated (HA) isolates from 71 children was conducted. In comparison to the HA isolates (31%), the CA isolates (90%) were more likely to have been isolated from pus ($p < 5 \times 10^{-8}$). For each patient with MRSA infection, only the first isolate was selected for molecular analysis. The molecular characteristics differed significantly between the CA and the HA isolates ($p < 5 \times 10^{-8}$). The clone characterized as sequence type (ST)59/pulsotype D (similar to USA1000)/staphylococcal chromosomal cassette (SCC)*mec* V_T/Panton–Valentine leukocidin (PVL)-positive accounted for 69% of the CA isolates, and another clone, characterized as ST239/pulsotype A (Hungary clone)/SCC*mec* III/PVL-negative, accounted for 45% of the 71 HA isolates. The CA clone of ST59 also accounted for 20% of the HA isolates, including 47% of the 17 community-onset isolates. It was concluded that the molecular characteristics of clinical MRSA isolates from children differed significantly between the CA and the HA isolates in northern Taiwan. However, the CA clone of ST59 was also identified in a substantial proportion of HA isolates.

Keywords Community-associated, healthcare-associated, methicillin-resistant *Staphylococcus aureus*, molecular characteristics, Taiwan

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INTRODUCTION

Recent reports indicate that community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections are increasing and may now involve individuals without risk factors predisposing for acquisition of MRSA [1–11]. CA-MRSA strains have been recognized as a novel pathogen group that is genetically different from nosocomial MRSA [5,8,9,11]. They are usually characterized by limited antibiotic resistance (except to β-lactams). They possess different exotoxin gene profiles (e.g. Panton–Valentine leukocidin (PVL)) and carry the type IV staphylococcal cassette chromosome (SCC*mec* IV). The major clinical manifestations are usually cellulitis and abscesses. However, CA-MRSA clones vary among different continents, countries, and even areas. For example, clones of multilocus sequence type (ST)1 (USA400) and ST8 (USA300) are found mainly in the USA and Canada [9–14], clones of ST80 are found mainly in Europe [12,15], and clones of ST30 are found worldwide, including the USA, Europe, Oceania and Japan [12,16,17].

In Taiwan, several retrospective studies have shown that between 1997 and 2003, MRSA accounted for 9.8–36% of CA *S. aureus* infections in children without risk factors [18–21]. Most of these retrospectively collected CA clinical isolates

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were characterized by a specific pulsed-field gel electrophoresis (PFGE) pattern (similar to USA1000) and resistance to clindamycin and erythromycin. They contain a specific type of SCC*mec* (type V_T, recently tentatively designated as type VII by Takano *et al*. [17]) gene and possess PVL genes [22–24]. However, there has been no prospective study that has systemically collected and molecularly characterized the CA-MRSA clinical isolates in Taiwan. Therefore, this prospective study was conducted to elucidate the situation and compare the molecular characteristics of the CA and the healthcare-associated (HA) isolates.

MATERIALS AND METHODS

Chang Gung Children's Hospital is a university-affiliated hospital, situated in northern Taiwan (near Taipei city), that provides a range of care, from primary to tertiary, and is part of Chang Gung Memorial Hospital. Between August 2004 and July 2005, an investigator (C.-F. Ho) evaluated the medical records of hospitalized children (excluding those from neonatal units) when an MRSA isolate was identified microbiologically. The definitions of CA-MRSA and HA-MRSA infection used in the study were according to those proposed by Naimi *et al*. [5]. MRSA infection identified after 48 h of hospitalization or yielding a positive culture from a lesion absent at admission was categorized as hospital-onset (HO). If the infection was classified as community-onset (CO), which was defined as MRSA infection identified within 48 h of admission, the parents or the care provider of the patients were interviewed, using a questionnaire concerning hospitalization, surgery, dialysis, a permanent indwelling catheter or percutaneous medical device, a known MRSA-positive culture before this infection, and history of residence in a long-term-care facility within the previous 12 months. Information about household members working in healthcare facilities was also recorded. Patients with any of these conditions or with a hospital onset of MRSA infection were classified as having HA infections; otherwise, they were classified as having CA infections. Patients less than 1 year of age were classified as having CA infections if they did not have any above condition after birth, except birth in the hospital.

Identification of MRSA was confirmed according to CLSI guidelines [25]. PFGE with *Sma*I-digested DNA was performed according to the procedures described previously [19,26,27]. The genotypes were designated in alphabetical order. PFGE patterns with differences in fewer than four bands as compared with an existing genotype were defined as subtypes of that genotype [28].

The SCC*mec* typing was carried out using a multiplex PCR strategy described previously [29]. Control strains for SCC*mec* types I, II, III and IVa, kindly provided by K. Hiramatsu, were as follows: type I, NCTC10442; type II, N315; type III, 85/2082; and type IVa, JCSC4744. SCC*mec* typing of type V_T was carried out using a particular primer described elsewhere [24], and the strain TSGH-17, kindly provided by C.-C. Wang, was used as a control. However, the SCC*mec* typing method for type V_T yielded inconsistent results. Thus, an alternative method, described elsewhere, was used [30]. The presence of PVL genes was determined by a PCR strategy described previously [31]. Some isolates of representative PFGE patterns were subjected to multilocus sequence typing (MLST) as described elsewhere [32]. The allelic profiles were assigned by comparison of the sequences at each locus with those of the known alleles in the *S. aureus* MLST database, and were defined as STs accordingly. The results were analysed statistically by a chi-square test or Fisher's exact test if appropriate. A difference was considered significant if $p < 0.05$.

RESULTS

During the study period, 394 *S. aureus* isolates were identified from 357 children, and 210 of the isolates from 173 children were methicillin-resistant (Table 1). Among them, 130 MRSA isolates from 119 children were classified as CO, 111 isolates from 102 children were classified as CA, and 99 isolates from 71 children were classified as HA. Table 2 illustrates the distribution of specimens in which the 210 MRSA isolates were identified. Ninety per cent of the 111 CA-MRSA isolates were from pus, whereas this was the case in only 31% of the 99 HA-MRSA isolates. Isolates in the CA group were more likely to have been recovered from pus ($p < 5 \times 10^{-8}$), whereas those in the HA group were more likely to have been recovered from blood ($p < 0.05$), sputum ($p < 5 \times 10^{-8}$), and central venous catheters ($p < 0.001$). Within the HA group, CO infection-causing MRSA isolates were more likely to be obtained from pus ($p < 0.0005$), whereas in the case of the HO infections, MRSA isolates were more likely to be obtained from sputum ($p < 0.05$).

Of the 102 children with CA-MRSA infections, 47 (46%) were male and 55 (54%) female. Eighty-eight patients (86%) presented with simple skin and/or soft tissue infection. Six patients (5.9%) had soft tissue infection with a scarlatiniform rash. Septic arthritis was noted in two cases (both blood and joint fluid yielded MRSA), pneumonia in two cases (blood culture yielded MRSA in one case), necrotizing fasciitis with sepsis in one case (blood culture positive for MRSA), and varicella

Table 1. Source of *Staphylococcus aureus* infections in 357 children as related to methicillin resistance

Source	Methicillin-resistant[a]	Methicillin-sensitive	Total no.
Community-associated	102 (56)	81 (44)	183
Healthcare-associated	71 (41)	103 (59)	174

Values are given as *n* (%).
[a]The rate of methicillin resistance among community-associated isolates was significantly higher than that among healthcare-associated isolates ($p < 0.005$).

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Table 2. Distribution of 210 clinical methicillin-resistant *Staphylococcus aureus* isolates stratified according to specimen types

Origin	No. of isolates	Blood	Sputum	Pus	CVC	Urine	CSF	Others[a]
Community-associated[b]	111	4 (3.6)	5 (4.5)	100 (90)	0	1 (0.9)	0	1 (0.9)
Healthcare-associated[b]	99	13 (13)	37 (37)	31 (31)	9 (9)	4 (4)	3 (3)	2 (2)
Community-onset[c]	19	1 (5.3)	3 (16)	13 (68)	0	1 (5.3)	1 (5.3)	0
Hospital-onset[c]	80	12 (15)	34 (43)	18 (23)	9 (11)	3 (3.8)	2 (2.5)	2 (2.5)

Values are given as *n* (%).
CVC, central venous catheter; CSF, cerebrospinal fluid.
[a]Ascites and tissue.
[b]A significant difference was found between the community-associated and healthcare-associated isolates with respect to the specimens from blood ($p < 0.05$), CVC ($p < 0.001$), sputum and pus ($p < 5 \times 10^{-8}$).
[c]A significant difference was found between the community-onset and hospital-onset isolates with respect to the specimens from sputum and pus ($p < 0.05$).

Table 3. Antibiotic susceptibility of 173 clinical methicillin-resistant *Staphylococcus aureus* isolates stratified according to the origin of acquisition

Origin	No. of isolates	Penicillin	Erythromycin	Clindamycin	Trimethoprim/sulfamethoxazole[a]	Vancomycin	Teicoplanin
Community-associated	102	0	6 (5.9)	8 (7.8)	97 (95)	102 (100)	102 (100)
Healthcare-associated	71	0	2 (2.8)	6 (8.5)	34 (48)	71 (100)	71 (100)
Community-onset	17	0	0	2 (12)	11 (65)	17 (100)	17 (100)
Hospital-onset	54	0	2 (3.7)	4 (7.4)	23 (43)	54 (100)	54 (100)

Values are given as *n* (%).
[a]The susceptibility to trimethoprim/sulfamethoxazole was significantly different between the community-associated and healthcare-associated isolates ($p < 5 \times 10^{-8}$).

gangrenosum with septic shock due to MRSA in one case. Two patients had bacteraemia. None of the 102 patients died during the study period.

For microbiological characterization, only the first MRSA isolate from a single patient was included for analysis. The detailed antimicrobial susceptibility distribution of the isolates is shown in Table 3. All of the 173 MRSA isolates were susceptible to vancomycin and teicoplanin but resistant to penicillin. Most isolates were resistant to erythromycin and clindamycin. However, CA-MRSA isolates were significantly more susceptible to trimethoprim–sulphamethoxazole than were HA-MRSA isolates ($p < 5 \times 10^{-8}$).

Table 4 illustrates the detailed distribution of PFGE patterns, SCC*mec* types and the presence of PVL genes in these MRSA isolates. In total, 13 PFGE patterns were identified; ten patterns were found among the CA isolates, and nine among the HA isolates. PFGE pattern D, the most common pattern among the CA isolates, accounted for 73% of the CA isolates but only 21% of the HA isolates. In contrast, pattern A, the most common pattern among the HA isolates, accounted for 45% of the HA isolates but 4.9% of the CA isolates. The distribution of PFGE patterns was significantly different between the two groups ($p < 5 \times 10^{-8}$). Four SCC*mec* types were identified

Table 4. Comparison of molecular characteristics between community-associated and healthcare-associated methicillin-resistant *Staphylococcus aureus* isolates from 173 children

		PFGE pattern				SCC*mec* type				
Origin	No. of isolates	A	C	D	Other	II	III	IV	V_T	Presence of PVL genes
Community-associated[a]	102	5 (4.9)	12 (12)	74 (73)	11 (11)	3 (2.9)	7 (6.9)	21 (21)	71 (70)	81 (79)
Healthcare-associated[a]	71	32 (45)	17 (24)	15 (21)	7 (9.9)	1 (1.4)	35 (49)	20 (28)	15 (21)	14 (20)
Community-onset[b]	17	3 (18)	5 (29)	8 (47)	1 (5.8)	0	3 (18)	6 (35)	8 (47)	8 (47)
Hospital-onset[b]	54	29 (54)	12 (22)	7 (13)	6 (11)	1 (1.9)	32 (59)	14 (26)	7 (13)	6 (11)
Sequence type		239	59	59, 338, new[c]	5, 8, 22, 30, 59, 89, 239					

Values are given as *n* (%).
PFGE, pulsed-field gel electrophoresis; SCC*mec*, staphylococcal chromosomal cassette; PVL, Panton–Valentine leukocidin.
[a]A significant difference was found between the community-associated and healthcare-associated isolates with respect to PFGE type C ($p < 0.05$), types A and D, and SCC*mec* III and V_T, and the presence of PVL genes ($p < 5 \times 10^{-8}$).
[b]A significant difference was found between the community-onset and hospital-onset isolates with respect to PFGE types A and D, and SCC*mec* III and V_T, and the presence of PVL genes ($p < 0.01$).
[c]A single-locus variant of ST1 (single nucleotide difference).

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among the isolates. Type V_T was the predominant type among the CA isolates, whereas type III was the predominant type among the HA isolates. PVL genes were detected in 79% of the CA isolates but in only 20% of the HA isolates. The distribution of SCC*mec* types and the presence of PVL genes were significantly different between the two groups ($p < 5 \times 10^{-8}$). A similar picture was also found when the characteristics of CO isolates were compared with those of HO isolates ($p < 0.01$). The molecular characteristics of nearly half of the CO isolates were the same as those of CA isolates. However, the molecular characteristics were significantly different between the CA isolates and the CO isolates in terms of PFGE patterns ($p < 0.031$, using Fisher's exact test) and the presence of PVL genes ($p < 0.012$, using the same test).

MLST was performed for 56 isolates, and nine STs were identified (Table 4). ST59 accounted for 30 of the 34 PFGE type D isolates, seven of the seven PFGE type C isolates, and one each of the PFGE type AA and PFGE type AN isolates. ST338, a single-locus variant of ST59 (one nucleotide difference in the *gmk* locus), accounted for three other isolates of PFGE type D. A PFGE type D isolate was found to be of a new ST, a single-locus variant of ST1. ST239 accounted for all six isolates of PFGE type A subjected to MLST and one isolate of PFGE type AP. Altogether, three dominant clones were identified among the clinical MRSA isolates; they were characterized as ST59/PFGE type D/SCC*mec* V_T/PVL(+), ST59/PFGE type C/SCC*mec* IV/PVL(−) and ST239/PFGE type A/SCC*mec* III/PVL(−) (Table 5). The former clone was significantly more frequently found in the CA isolates than in the HA isolates ($p < 5 \times 10^{-8}$). In contrast, the latter clone was significantly more likely to be found in the HA isolates than in the CA isolates ($p < 5 \times 10^{-8}$).

DISCUSSION

The results from this study indicated that the microbiological characteristics of MRSA clinical isolates, including the source of specimens, the antibiotic susceptibility pattern, the PFGE patterns, the SCC*mec* types, and the presence of PVL genes, were significantly different between the CA isolates and the HA isolates in northern Taiwan. These results suggest that CA-MRSA isolates in Taiwan had not spread from the healthcare facilities but originated *de novo* in the community. Furthermore, the rate of methicillin resistance among the CA *S. aureus* isolates was even higher than that among the HA isolates (56% and 41%, respectively, $p < 0.005$), implying that a CA-MRSA clone had disseminated in the community in northern Taiwan.

The significant difference in the source of the specimens obtained between the CA and the HA isolates reflected the significantly different disease spectra of the patients. The majority (90%) of the CA isolates were cultured from pus and, indeed, 92% of the 102 children with CA-MRSA infection presented with skin and soft tissue infections, which is compatible with reports from the USA [5,6,11]. In contrast, the specimen sources of the HA isolates were relatively diverse. Pus was the source of only 31% of the 99 HA isolates, whereas pneumonia and catheter-related bacteraemia might account for more than half of the hospital-associated infections (the numbers may depend on the source of specimens obtained).

Table 5. Distribution of three major clones of methicillin-resistant *Staphylococcus aureus* among the clinical isolates in northern Taiwan

Origin	PFGE D/SCC*mec* V_T/PVL(+)	PFGE C/SCC*mec* IV/PVL(−)	PFGE A/SCC*mec* III/PVL(−)
Sequence type	59 (338)[a]	59	239
Community-associated[b] (n = 102)	70 (69)	9 (8.8)	4 (3.9)
Hospital-associated[b] (n = 71)	14 (20)	16 (23)	32 (45)
Community-onset[c] (n = 17)	8 (47)	5 (29)	3 (18)
Hospital-onset[c] (n = 54)	6 (11)	11 (20)	29 (54)

Values are given as n (%).
PFGE, pulsed-field gel electrophoresis; SCC*mec*, staphylococcal chromosomal cassette; PVL, Panton–Valentine leukocidin.
[a]ST338 is a single-locus variant of ST59 and accounted for three of 33 isolates of PFGE type D undergoing multilocus sequence typing.
[b]A significant difference was found between the community-associated and healthcare-associated isolates in terms of the second clone ($p < 0.05$), and first and third clones ($p < 5 \times 10^{-8}$).
[c]A significant difference was found between the community-onset and hospital-onset isolates in terms of the first and third clones ($p < 0.01$).

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In the current study, more than 90% of the clinical MRSA isolates, either CA or HA, were also resistant to clindamycin and erythromycin. This scenario is different from that in the USA [1–11], where most CA-MRSA isolates were reported to still be susceptible to erythromycin and clindamycin. Apparently, in Taiwan, for children with severe diseases possibly caused by *S. aureus* infection, clindamycin alone is no longer appropriate, and a glycopeptide (vancomycin or teicoplanin) or linezolid should be used empirically. However, more than 90% of the CA isolates remained susceptible to trimethoprim–sulphamethoxazole, indicating that oral trimethoprim–sulphamethoxazole may still be suitable for simple skin and soft tissue infections caused by CA-MRSA.

The molecular characteristics of the CA-MRSA isolates were significantly different from those of HA-MRSA isolates in the current study, a picture similar to that seen in the USA [5,8]. However, the molecular characteristics of the CA isolates from Taiwan are totally different from those of isolates identified in the USA. Consistent with previous reports from Taiwan [22–24], CA isolates in the current study shared a common pulsotype (type D), which was similar to PFT USA1000. It belonged to ST59 or its variant ST338, carried a newly identified subtype of SCC*mec* V, called SCC*mec* V_T (recently tentatively designated as type VII by Takano *et al*. [17]), and carried PVL genes. A clone characterized as ST59/pulsotype C/SCC*mec* IV/PVL(−), although accounting for fewer than 10% of the CA isolates in the current study, was the predominant clone among the colonizing isolates from the healthy children without risk factors in Taiwan [30,33,34]. The question of whether the PVL genes, which are reported to constitute a virulence factor associated with necrotizing pneumonia and abscesses [35], may be associated with the ability of the PVL-positive clone to cause infection requires further investigation.

Among the HA isolates, an epidemic clone, previously called the Hungarian or Brazilian clone and characterized as ST239/SCCmec III/PVL(−), was predominant and accounted for nearly half of the HA isolates in the current study. This clone of ST239 has prevailed in Taiwanese hospitals since 1994 [36], and accounted for more than 70% of the clinical MRSA isolates in Taiwan between 1997 and 2001 [26,37,38]. However, the CA clones of ST59 accounted for 45% of the HA isolates, and the rate reached 76% among the CO isolates, suggesting that the CA clones of ST59 had spread into hospitals and emerged as an HA clone that may be a major HA clone in the near future. A similar scenario has also been reported in some areas of the USA [39].

TRANSPARENCY DECLARATION

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