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Original Article

Risk factors for and molecular characteristics of methicillin-resistant *Staphylococcus aureus* nasal colonization among healthy children in southern Taiwan, 2005–2010

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Abstract *Background/purpose:* Nasal colonization of *Staphylococcus aureus* is a well-defined risk factor for subsequent infection. This study investigated the prevalence of methicillin-resistant *S. aureus* (MRSA) in southern Taiwan and aimed to identify the host factors for *S. aureus* colonization and the virulence factor of Panton-Valentine Leukocidin (PVL) genes.
Methods: In a hospital-based study in Kaohsiung from Oct. 2005 to Dec. 2010, we performed nasal swab in the healthy children aged 2–60 months. We examined the relationship between the demographic characteristics and *S. aureus* nasal colonization. MRSA isolates were further analyzed for antimicrobial susceptibility and molecular characteristics.
Results: Among 3020 healthy children, 840 (27.8%) children had *S. aureus* nasal colonization. Of 840 isolates, 246 (29.3%) isolates were MRSA. MRSA colonization was significantly associated with age 2–6 months, day care attendance, and influenza vaccination. Breastfeeding was a protective factor against MRSA colonization. Most MRSA isolates were susceptible to trimethoprim-sulfamethoxazole and doxycycline. Ninety-four percent of MRSA isolates carried either type IV staphylococcal cassette chromosome *mec* (SCC*mec*) or SCC*mec* V_T and 87%

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belonged to the local community strains, namely clonal complex 59/SCC*mec* IV or V_T. MRSA isolates with PVL-negative was associated with children with passive smoking.
Conclusions: Between 2005 and 2010, 27.8% and 8.14% of healthy children in southern Taiwan had nasal carriage of *S. aureus* and MRSA, respectively. Most MRSA isolates were local community strains. Several demographic factors associated with nasal MRSA colonization were identified.

Introduction

Staphylococcus aureus is a major pathogen of human bacterial infections worldwide, and causes substantial morbidity and mortality.[1] Easy acquisition of resistance to antibiotics is characteristic of *S. aureus*.[2] Methicillin-resistant *S. aureus* (MRSA) was reported shortly after the methicillin was introduced.[3] Traditionally MRSA was associated with hospitals and other healthcare settings. Healthcare-associated MRSA (HA-MRSA) infection occurs in individuals with risk factors, such as hospitalization, surgery or dialysis.[4] By contrast, community-associated MRSA (CA-MRSA) infections can occur in healthy individuals without risk factors.[5] Additionally, CA-MRSA infections have become prevalent in some countries including Taiwan.[6] These features suggest CA-MRSA strains have greater virulence and transmission than do traditional HA-MRSA strains. However, the contributing factors responsible for CA-MRSA virulence and transmission have not been well-defined. Understanding these factors may provide useful strategies to prevent the transmission of CA-MRSA.

S. aureus can colonize many parts of human bodies including nostrils, throat, axilla, groin and perirectal area without any symptom. The nostrils are the major reservoir among these sites.[7] Nasal carriage of *S. aureus* is a well-known risk factor for subsequent infections.[8,9] In addition, *S. aureus* carriers are an important source of spread of infection.[10] Thus, nasal colonization of *S. aureus* may serve as a parameter of the burden of staphylococcal diseases. Continuous surveillance of *S. aureus* nasal colonization and infection is an important issue.

Previous studies have revealed that the nasal carriage rate of MRSA among children in southern Taiwan was lower than in northern Taiwan.[11,12] However, the nasal carriage rate of MRSA in southern Taiwan had increased from 2001 to 2006.[13] Our previous study revealed that MRSA nasal colonization in Taiwanese children significantly increased from 2005 to 2008.[12] To further investigate the local molecular epidemiology of MRSA in southern Taiwan in the long-term period, we conducted this study in southern Taiwan from 2005 to 2010. This study would also like to identify the host factors for *S. aureus* colonization and the virulence factor of Panton-Valentine Leukocidin (PVL) genes. Awareness of these factors may alleviate CA-MRSA transmission and subsequent infections.

Materials and methods

Study design

This prospective study was approved by the institutional review board of Chang Gung Memorial Hospital (CGMH). From October 2005 to December 2010, all children aged 2—60 months who presented to well-child clinics in Kaohsiung CGMH were invited to participate in this study. Children in nursery or kindergarten were also invited into this study. Informed consent from their parents or guardians was obtained. Children with chronic renal failure, thalassemia major, chronic cardiovascular diseases, chronic lung disease, nephrotic syndrome, liver cirrhosis, diabetes mellitus, immunodeficiency, human immunodeficiency virus infection, asplenia, and indwelling devices were excluded. Children with prematurity without complications were eligible in this study. Standardized questionnaires about demographic information, potential environmental factors for carriage, and health conditions were recorded. The day care attendance was defined as attendance at nursery or kindergarten. Influenza vaccination was defined as "ever received influenza vaccination before."

The nasal swabs of both anterior nares were performed, placed in transport medium, and then sent to the microbiologic laboratory for detection of *S. aureus* by culture. *S. aureus* was identified by catalase and coagulase testing, and oxacillin susceptibility was assessed by the disc diffusion method. The nasopharyngeal swabs were also collected for isolation of *Streptococcus pneumoniae*, which was confirmed by optochin sensitivity and bile solubility. All *S. aureus* and *S. pneumoniae* isolates were sent to Linko CGMH, Taoyuan for molecular characterization.

Molecular characterization of MRSA isolates

Identification of MRSA was confirmed according to Clinical and Laboratory Standards Institute 2010 guidelines.[14] MRSA isolates were further analyzed for antimicrobial susceptibility and molecular characteristics. Pulse-field gel electrophoresis (PFGE) with *Sma* I digestion was performed to fingerprint the MRSA isolates according to procedures described previously.[15] The genotypes were designated in alphabetical order, as in the previous studies.[15—18] Any

Table 1 Demographic data for methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA) nasal carriage by univariate analysis.

Factors	Non-carrier (n = 2180)	MSSA carrier (n = 594)	p_1 value[a]	MRSA carrier (n = 246)	p_2 value[b]
Age 2–6 months	229 (10.5%)	138 (23.2%)	<0.0001	50 (20.3%)	<0.0001
Male (%)	1197 (54.9%)	331 (55.7%)	NS	140 (56.9%)	NS
Breastfeeding	1719 (78.9%)	440 (74.1%)	0.0129	176 (71.5%)	0.0086
Period (mo)[c,e]	6.06 ± 5.93	5.30 ± 5.66	0.0152	4.94 ± 6.48	0.0178
No. of children in the family[e]	1.91 ± 0.89	1.91 ± 1.00	NS	1.91 ± 0.86	NS
House size (m^2)[e]	193.16 ± 120.75	193.10 ± 101.19	NS	185.66 ± 90.29	NS
No. of toilets[e]	2.63 ± 1.17	2.71 ± 1.18	NS	2.64 ± 1.06	NS
Sleeping with parents	1906 (87.4%)	498 (83.8%)	0.0224	209 (85.0%)	NS
Handwashing frequency (time/day)[e]	7.47 ± 3.81	7.00 ± 3.96	0.0091	7.08 ± 4.63	NS
Passive smoking	1082 (49.6%)	283 (47.6%)	NS	129 (52.4%)	NS
Day care attendance	334 (15.3%)	87 (14.6%)	NS	61 (24.8%)	0.0001
Duration of stay (h/wk)[d,e]	40.28 ± 5.48	39.93 ± 6.51	NS	40.82 ± 4.24	NS
No. of classmates[e]	17.27 ± 6.34	17.22 ± 6.36	NS	17.97 ± 5.66	NS
Pneumococcal vaccination	555 (25.5%)	151 (25.4%)	NS	53 (21.5%)	NS
Pneumococcal nasal colonization	324 (14.9%)	38 (6.4%)	<0.0001	39 (15.9%)	NS
Flu vaccination	951 (43.6%)	234 (39.4%)	NS	124 (50.4%)	0.0424
Prematurity	190 (8.7%)	39 (6.6%)	NS	26 (10.6%)	NS
History of AOM	237 (10.9%)	62 (10.4%)	NS	32 (13.0%)	NS
URI within 2 weeks	648 (29.7%)	118 (19.9%)	<0.0001	68 (27.6%)	NS
Received antibiotics within 2 weeks	202 (9.3%)	31 (5.2%)	0.0016	19 (7.7%)	NS

[a] Statistical test between MSSA carriers and non-carriers.
[b] Statistical test between MRSA carriers and non-carriers.
[c] Data derived from those with a history of breastfeeding.
[d] Data derived from those attending day care.
[e] Values are expressed as mean ± SD.
AOM = acute otitis media; URI = upper respiratory tract infection; NS = not significant.

identified new genotype was designated consecutively. PFGE patterns with three or fewer band differences from an existing genotype were defined as subtypes of that genotype.[19]

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was performed by two multiplex PCR strategies described previously.[20] The control strains for SCC*mec* types I, II, III, and IVa, kindly provided by Keiichi Hiramatsu, were as follows: type I, NCTC10442; type II, N315; type III, 85/2082; and type IVa, JCSC4744. SCC*mec* type V_T was determined by using a particular primer described elsewhere,[21] and strain TSGH-17, kindly provided by Chi-Chien Wang,[21] was used as a control.

The presence of PVL genes was determined by a PCR technique described elsewhere.[22] Some isolates with representative PFGE patterns were selected and underwent multi-locus sequence typing (MLST) (http://www.mlst.net) as described previously.[23] 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 sequence types accordingly.

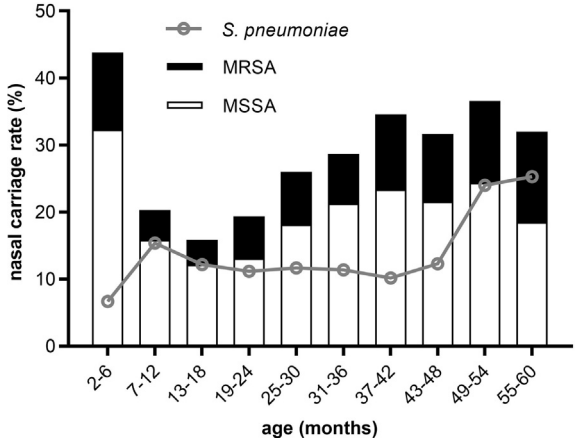

Figure 1. Age-specific distribution of methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), and *S. pneumoniae* carriage.

Statistical analysis

MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium; 2016) was used for statistics and GraphPad Prism (version 7 for Windows, GraphPad Software, La Jolla, California, USA) for figures plotting. Statistical significance of differences in categorical variables, such as gender, breastfeeding, was determined by the Chi-square test. Statistical significance of differences in continuous variables, such as age, breastfeeding period, was determined by a two-sample t test. If the variances of the two samples cannot be assumed to be equal and the t-test with a correction for unequal variances (Welch test) was adopted. Multiple logistic regression analysis was

adopted to examine factors associated with MSSA and MRSA colonization. A p value < 0.05 was considered statistically significant.

Results

Initially 3280 children aged 2—60 months were invited to participate into this study. About 50 children were recruited for study each month. After examining for eligibility, four children due to underlying diseases were excluded. These four children each had cirrhosis, nephrotic syndrome, chronic cardiovascular disease and indwelling device, respectively. Of 3276 eligible children, 3020 children completed this study, including 2957 from well-child clinics, 1 from nursery, 62 from kindergarten.

Among 3020 healthy children, 840 (27.8%) children had *S. aureus* nasal colonization. The carriage rate of *S. aureus* ranged from 16.4% in the second quarter (Q2) of 2007 to 34.6% in the fourth quarter (Q4) of 2009. The carriage rate of *S. aureus* was higher in Q3 and Q4 (28.2% in Q3, 31.4% in Q4) than in Q1 and Q2 (24.2% in Q1, 26.2% in Q2) ($p = 0.0087$). Of these 840 *S. aureus* isolates, 246 (29.3%) isolates were MRSA. The yearly carriage rates of MRSA were 6.0%, 9.1%, 10.1%, 8.4%, 6.7%, and 7.2% from 2005 to 2010, respectively. The carriage rate of MRSA increased non-significantly from 2005 to 2007 ($p = 0.0895$), and then decreased from 2007 to 2009 ($p = 0.067$). The overall carriage rate of MRSA was 8.1% during the study period. The yearly carriage rates of *S. pneumoniae* were 10.7%, 16.9%, 13.1%, 13.2%, 11.2%, and 10.6% from 2005 to 2010, respectively. The carriage rate of *S. pneumoniae* was higher in Q1 and Q2 (16.6% in Q1, 14.2% in Q2) than in Q3 and Q4 (11.4% in Q3, 11.4% in Q4) ($p < 0.01$).

In term of the age of colonization, the carriage rate of both Methicillin-sensitive *S. aureus* (MSSA) and MRSA was significantly higher among children aged 2—6 months old (32.3% for MSSA and 11.5% for MRSA) (Table 1 and Fig. 1). MSSA colonization was highest in age 2—6 months (32.3%) and lowest in age 13—18 months (12.2%). Children aged 49—54 and 55—60 months old had the highest and second highest carriage rate of MRSA (12.2% and 13.5%), respectively. MRSA colonization was lowest in children aged 13—18 months old (3.7%). The carriage rate of both MSSA and MRSA significantly increased from age 12 months—42 months ($p < 0.02$). *S. pneumoniae* colonization was lowest in age 2—6 months (6.7%) and highest in age 55—60 months (25.3%). The carriage rate of *S. pneumoniae* significantly increased from age 2 months—12 months ($p < 0.0001$), and increased from age 37 months—60 months ($p < 0.0001$).

The demographic data and characteristics of *S. aureus* colonization are shown in Table 1. Breastfeeding was a protective factor for MSSA and MRSA colonization, and non-carriers had a longer duration of breastfeeding than both MSSA and MRSA carriers ($p < 0.02$). By univariate analysis, sleeping with parents, more handwashing frequency, pneumococcal nasal colonization, upper respiratory tract infection (URI) within 2 weeks, and receiving antibiotics within 2 weeks were protective factors for MSSA colonization ($p < 0.03$). Day care attendance and influenza vaccination were associated with MRSA colonization ($p < 0.05$). By multivariate logistic regression, only age 2—6 months was significantly associated with MSSA colonization, while age 2—6 months, day care attendance, and influenza vaccination were significantly associated with MRSA colonization (Table 2). Breastfeeding, *S. pneumoniae* colonization, and URI within 2 weeks were protective factors against MSSA colonization, while only breastfeeding was a protective factor against MRSA colonization (Table 2). Among 252 subjects receiving antibiotics within 2 weeks,

Table 2 Risk factors for methicillin-resistant *S. aureus* (MRSA) carriage by multivariate logistic regression analyses.

Factors	MRSA carrier vs. non-carrier	
	Adjusted OR (95% CI)	p value
Age 2—6 months	3.5397 (2.3837—5.2565)	<0.0001
Breastfeeding	0.6896 (0.5086—0.9349)	0.0167
Day care attendance	1.9530 (1.4035—2.7176)	0.0001
Flu vaccination	1.7316 (1.2781—2.3460)	0.0004

OR = odds ratio; CI = confidence interval.

Table 3 Association of pulsed-field electrophoresis (PFGE) patterns with multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) types, and presence of Panton-Valentine Leukocidin (PVL) genes for 246 methicillin-resistant *S. aureus* (MRSA) isolates.

PFGE pattern	No. isolates (%)	No. subtypes	SCC*mec* type (*n*)	PVL-positive	Sequence type (*identified no./checked no.*)
A	2 (1)	2	III (1), III_A (1)	0	ST239 (1/1)
C	178 (72)	40	IV (175), V_T (3)	2	ST59 (8/8)
D	38 (15)	8	IV (6), V_T (31), V (1)	38	ST59 (3/4), ST338[a] (1/4)
F	2 (1)	1	II	0	ST5 (1/1)
AF	9 (4)	7	II	0	ST89 (2/2)
AG	1 (0.4)	1	IV	1	ST30
AI	1 (0.4)	1	IV	1	ST8
AK	12 (5)	5	IV	0	ST45 (1/2), ST508[b] (1/2)
AW	2 (1)	1	IV	0	ST78 (1/1)
U	1 (0.4)	1	IV	0	ST573

[a] A single locus variant of ST59.
[b] A single locus variant of ST45.

Table 4 Comparison between methicillin-resistant *S. aureus* (MRSA) isolates with and without Panton-Valentine Leukocidin (PVL) genes.

Factors	PVL-positive $n = 42$	PVL-negative $n = 204$	*p value*
Age 2–6 months	4 (9.5%)	46 (22.5%)	0.0566
Male	24 (57.1%)	116 (56.9%)	0.9734
Breastfeeding	26 (61.9%)	150 (73.5%)	0.1292
No. of children in the household[a]	2 (1–5)	2 (1–5)	0.3322
House size (m^2)[a]	198.3 (82.6–396.7)	165 (26.4–495)	0.2168
Toilet number[a]	3 (1–5)	2 (1–8)	0.7734
Sleeping with parents	35 (83.3%)	174 (85.3%)	0.7467
Hand washing < 8 times/day	16 (38.1%)	104 (51.0%)	0.1290
Passive smoking	16 (38.1%)	113 (55.4%)	0.0414
Daycare attendance	15 (35.7%)	46 (22.5%)	0.0726
Pneumococcal nasal colonization	8 (19.0%)	31 (15.2%)	0.5346
Flu vaccination	22 (52.4%)	102 (50.0%)	0.7791
AOM history	6 (14.3%)	26 (12.7%)	0.7874
URI within 2 weeks	13 (31.0%)	55 (27.0%)	0.5991
Received antibiotics within 2 weeks	3 (7.1%)	16 (7.8%)	0.8772

[a] Median (range).
AOM = acute otitis media; URI = upper respiratory infection.

113 (44.8%) subjects have documented antibiotics. Of the 113 documented antibiotics, 72 (63.7%) have activity against MSSA.

The distribution of PFGE patterns, SCC*mec* types, the presence of PVL genes, and sequence type among these 246 MRSA isolates are shown in Table 3. Totally 10 PFGE patterns were identified. Pattern C was the most common pattern and accounted for 72% of these isolates, followed by pattern D (15%). Six types of SCC*mec* gene were identified. SCC*mec* type IV (80%) and type V_T (14%) were the two most predominant types. PVL genes were present in 42 isolates (17%). PVL genes were present in all isolates with PFGE pattern D but absent in most isolates with PFGE pattern C. MLST was selectively done in twenty-two isolates, and eleven sequence types were identified. Sequence type 59 (ST59) was the most common sequence type and accounted for 8 of 8 PFGE type C isolates and 3 of 4 PFGE type D isolates. The other isolate of PFGE type D was ST338, which is a single-locus variant of ST59 (a single nucleotide difference in the *gmk* locus). The MRSA isolates characterized by ST59/PFGE type C/SCC*mec* IV/absence of PVL genes and ST59/PFGE type D/SCC*mec* V_T/presence of PVL genes were the two most common clones and accounted for 70% and 12% of the analyzed isolates, respectively. All of these 246 colonizing MRSA isolates were susceptible to linezolid, teicoplanin, fusidic acid and vancomycin. Most isolates were susceptible to trimethoprim-sulfamethoxazole (TMP-SMX) (98.8%) and doxycycline (99.2%). Fewer isolates were susceptible to clindamycin (12.6%), penicillin (2.8%), and erythromycin (8.9%).

Table 4 reveals the comparison between MRSA isolates with and without PVL genes. MRSA isolates with absence of the PVL genes were associated with children with passive smoking ($p = 0.0414$). Although children with age other than 2–6 months and day care attendance were more likely to have carriage of MRSA isolates with PVL genes, there was no statistically difference.

Discussion

The nasal MRSA colonization among healthy children in southern Taiwan was 8.1% during the period from October 2005 to December 2010. Although the nasal MRSA

Table 5 Selected publications for methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) colonization among healthy children in Taiwan.

Periods	Location in Taiwan	Subjects	MSSA identified no./case no. (%)	MRSA identified no./case no. (%)	References
2001	Southern	Children aged 2–18y	281/987 (28.5)	33/987 (3.3)	Lu et al.[13]
2001–2002	Northern	Children aged 3–12y	90/262 (34.4)	5/262 (1.9)	Huang et al.[17]
2004–2009	Northern	Children aged <14y	453/3200 (14.2)	371/3200 (11.6)	Lo et al.[23]
2005	Northern	Newborn within 24h	28/94 (29.8)	1/94 (1.1)	Huang et al.[24]
2005–2010	Northern	Children aged 2m–5y	589/3226 (18.3)	330/3226 (10.2)	Tsai MS et al.[25]
2005–2010	Southern	Children aged 2m–5y	594/3020 (19.7)	246/3020 (8.1)	This study
2010–2011	Northern	High school students	278/1232 (22.6)	45/1232 (3.7)	Chen et al.[26]
2012–2013	Northern	Infant aged 1–12m	61/555 (11.0)	208/555 (37.5)	Tsai MH et al.[27]

colonization rate increased from 2005 to 2007, and then decreased from 2007 to 2010, there was no significant trend. Compared with a previous study[13] conducted in southern Taiwan in 2001, though the study population was different, the nasal MRSA colonization among healthy children in southern Taiwan increased significantly, from 3.3% in 2001 to 8.1% during the period from 2005 to 2010 ($p < 0.0001$). By contrast, the nasal MSSA colonization among healthy children in southern Taiwan decreased significantly, from 28.5% in 2001 to 19.7% during the period from 2005 to 2010 ($p < 0.0001$). MRSA colonization rate among healthy children in Taiwan from 2001 to 2013 ranged from 1.1% to 37.5% (Table 5).[13,17,24–28] The high variation of colonization rate may be attributed to different subject age, study locations, and time periods.

Seasonal variation was found in the carriage of *S. pneumoniae* and carriage of *S. aureus*. *S. pneumoniae* colonization was higher in Q1/Q2 than in Q3/Q4. The temperature in southern Taiwan is a little cooler in Q1/Q2 than in Q3/Q4 (Monthly mean temperature: http://www.cwb.gov.tw/V7e/climate/monthlyMean/tx.htm). The association of cool climate and *S. pneumoniae* colonization were also found elsewhere.[29] In contrast, *S. aureus* colonization was higher in Q3/Q4 than in Q1/Q2. Although an association of warm climate with *S. aureus* skin and soft-tissue infections appears to exist, the seasonality of *S. aureus* colonization is little known.[30] Children with *S. pneumoniae* colonization had a significant lower risk for MSSA colonization. This phenomenon was consistent with previous epidemiological studies demonstrating an inverse correlation between carriage of MSSA and carriage of *S. pneumoniae*.[31–33] In addition, our previous study has observed the inverse correlation between carriage of MRSA and carriage of *S. pneumoniae*.[12] However, this inverse correlation was not found in the present study. Compared with MSSA, MRSA behaved to be resistant to *S. pneumoniae* interference. This characteristic may explain CA-MRSA strains are more transmissible than other strains of *S. aureus*.[34]

In this study, MRSA colonization rate was higher in age 2–6 months and the lowest in age 13–18 months. These results were similar to a longitudinal study conducted in children less than 2 years old in Taiwan, which showed MRSA colonization reached the peak at the age of 2 months and was down to the lowest level at the age of 18 months.[35] In contrast, *S. pneumoniae* colonization was lower in young infants in Taiwan. *S. pneumoniae* carriage significantly increased from 2 to 12 months, and also increased from 37 to 60 months. Children aged >49 months old had the highest carriage rate of both MRSA and *S. pneumoniae* among children aged 2–60 months. That may be associated with their day care center/kindergarten attendance.

The interplay between the host immune system and bacteria determines *S. aureus* nasal colonization.[36] The present study surveyed the possible associated factors for *S. aureus*. A preceding breastfeeding history associated with lower risk for subsequent *S. aureus* colonization implicated that breastfeeding influenced the host immunity in the long run. Longer duration of breastfeeding has more impact on the immunity against *S. aureus* colonization. Among the environmental factors, day care attendance was associated with increased risk for MRSA colonization, consistent with our previous study.[12] It means contact with MRSA carriers in contaminated environment may facilitate subjects to be colonized regardless of the number of classmates. Crowding in the family also did not influence the risk for colonization in the present study. This study result of crowding in the family was different from our previous study.[12] The mean house size was larger in southern Taiwan than northern Taiwan. Given the similar number of children in the family, the family population density was lower in southern Taiwan. That may contribute to the discrepancy of the results. In addition to pneumococcal nasal colonization, other factors with an inverse correlation with MSSA included breastfeeding and URI within 2 weeks. URI within 2 weeks may predispose subjects to receive antibiotics. More than half of documented antibiotic usage can cover MSSA. That may lead to URI within 2 weeks associated with a lower risk of MSSA colonization, but not MRSA. However, antibiotics usage within 2 weeks was a protective factor against MSSA significantly by univariate analysis, but not reached as a significant factor by multivariate analysis. Unexpectedly, influenza vaccination was significantly associated with MRSA colonization. To the best of our knowledge, no study has demonstrated any association between MRSA carriage and influenza vaccination yet. Further studies are needed to validate and investigate the underlying mechanism.

As previously reported, two major clones, characterized as ST59/PFGE type C/SCC*mec* IV/PVL-negative and ST59/PFGE type D/SCC*mec* V_T/PVL-positive, accounted for most of the MRSA isolates (82%) in the present study. The antimicrobial susceptibility test revealed that both clones were highly resistant to penicillin, erythromycin, and clindamycin, but susceptible to doxycycline and trimethoprim-sulfamethoxazole (TMP-SMX) in addition to glycopeptides, linezolid and daptomycin. Compared with other dominant global clones of CA-MRSA, the CA-MRSA clones in Taiwan are less susceptible to non-beta lactam antibiotics.[37] The antimicrobial susceptibility profile of MRSA colonizing isolates in the present study was similar to that of CA-MRSA clinical isolates in previous studies from Taiwan.[38–41] However, the clone of ST59/PFGE type C/SCC*mec* IV/PVL-negative accounted for most of colonizing isolates (70%) in the present study. By contrast, the clone of ST59/PFGE type D/SCC*mec* V_T/PVL-positive was responsible for most of clinical isolates.[42] The clone of ST59/PFGE type D/SCC*mec* V_T/PVL-positive have been demonstrated to have a greater virulence than the clone of ST59/PFGE type C/SCC*mec* IV/PVL-negative in both humans and an animal infection model while their adhesion to respiratory epithelial cells is indistinguishable.[43]

In this study, nasal colonization of MRSA isolates with absence of PVL genes was associated with children with passive smoking while previous antibiotic usage within 2 weeks was not associated with MRSA isolates with or without PVL genes. However, one study conducted in northern Taiwan revealed that antibiotic usage in the past 12 months was associated with nasal colonization of MRSA isolates with presence of PVL genes among healthy children.[44]

There were some limitations in the present study. First, only children aged 2–60 months were recruited in the present study. All children were recruited from one tertiary medical center in Kaohsiung, a city located in southern-

western Taiwan. Therefore, the prevalence of *S. aureus* colonization may not be generalized to the entire pediatric population in southern Taiwan. Second, some demographic characteristics which may be associated with *S. aureus* colonization might not have been collected and analyzed. For instance, information about household members' occupation and the colonization status of contacts were not collected. Living with health care workers and carriers was described to be associated with an increased risk for *S. aureus*, including MRSA, colonization.[45,46]

In conclusion, 8.1% of previously healthy children in southern Taiwan had nasal carriage of MRSA between 2005 and 2010. The carriage rate of MRSA non-significantly increased from 2005 to 2007, and decreased from 2007 to 2010. Age 2—6 months, day care attendance, and influenza vaccination were associated with MRSA colonization, while breastfeeding provided protection from MRSA colonization among healthy children. Passive smoking seems to be associated with the carriage of PVL-negative MRSA isolates. Two major CA-MRSA clones were identified as previously reported from Taiwan. Validation of the association of influenza vaccination and MRSA colonization, and investigation of the underlying mechanism are needed in future studies.

Conflicts of interest

All authors declare no conflicts of interest.

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