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Original Article

Prevalence of and risk factors for nasal methicillin-resistant *Staphylococcus aureus* colonization among children in central Taiwan

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Abstract *Background/purpose: Staphylococcus aureus (S. aureus)* causes diseases ranging from mild skin infections to invasive diseases. Carriage of *S. aureus*, including methicillin-resistant *S. aureus* (MRSA), is a significant risk factor for subsequent staphylococcal infection. Several studies discussed MRSA colonization in Taiwan, but mostly in northern Taiwan. This is the first study that estimates the prevalence of MRSA nasal colonization in healthy children and identifies the potential risk factors in central Taiwan.
Methods: A total of 3144 healthy children aged 2—60 months who visited Taichung Veterans General Hospital (TCVGH) were screened for nasal *S. aureus* carriage from July 2005 to December 2010. Questionnaires included demographic information and potential risk factors for carriage of *S. aureus* were completed by parents/guardians.

Abbreviations: CI, confidence interval; [aOR], adjusted odds ratio; *S. aureus*, *Staphylococcus aureus*; MRSA, Methicillin-resistant *S. aureus*; TCVGH, Taichung Veterans General Hospital; *S. pneumoniae*, *Streptococcus pneumoniae*; CA-MRSA, community-associated MRSA; PVL, Panton-Valentine leukocidin; SCCmec, staphylococcal cassette chromosome mec; PFGE, Pulsed-field gel electrophoresis; MLST, multilocus sequence typing; CD4, cluster of differentiation 4.

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Results: Prevalence of MSSA and MRSA were 12.09% and 5.25%, respectively. The youngest group aged 2—6 months had the highest *S. aureus* carriage rate, and the carriage rate revealed a peak in summer. The nasal colonization of *Streptococcus pneumoniae (S. pneumoniae)* was a protective factor against *S. aureus* colonization. 85% of the MRSA colonizing isolates belonged to clonal complex 59/staphylococcal cassette chromosome type IV or V_T, the local community clone in Taiwan.

Conclusion: An increasing trend of MRSA nasal carriage rate in Taiwan had been brought forward, however, it was not observed in central Taiwan during the period of 2005—2010. We found a summer peak on both MRSA and MSSA carriages.

Introduction

Staphylococcus aureus, a common potential pathogen both in healthcare and community settings, causes diseases ranging from mild skin infections to invasive diseases, such as pneumonia, osteomyelitis, and endocarditis.[1] MRSA was a common hospital-associated pathogen until the late 1990s.[2] The emergence of community-associated MRSA (CA-MRSA) in healthy individuals without underlying risk factors to get MRSA[3] was reported to be associated with rapidly fatal disease or serious complications, such as septic shock, necrotizing pneumonia, and necrotizing fasciitis.[4] CA-MRSA strains, which were genetically different from health care-associated MRSA (HA-MRSA), had relatively less antibiotic resistance pattern, mostly had the characteristic PVL genes and type IV or V staphylococcal cassette chromosome (SCCmec).[5] Nasal or skin colonization of *S. aureus*, including MRSA, is quite common among children, and a potential high risk factor for subsequent invasive staphylococcal infection.[6] The anterior nares are the most consistent sites of colonization.[7]

In Taiwan, previous report[8,9] found that MRSA isolates increased from 9.8% in 1997—2000 to 56% during 2004—05 among all CA *S. aureus* infection in children. The nasal MRSA carriage rate among well-child healthcare visits and/or in school children increased from 1.9% in 2001 to ~15.1% in 2007—2009.[10,11] It was found that MRSA colonization in children were associated with the more number of children in the family and day care attendance.[12] These data were mostly from the northern part of Taiwan. We conducted a 5-year survey to estimate the trend of MRSA nasal colonization in healthy children, identify the potential risk factors and present the evolving epidemiology in central Taiwan.

Methods

Study design and data collection

This prospective observational study was conducted from July 2005 to Dec 2010 at TCVGH, a 1500-bed tertiary teaching hospital in central Taiwan.

Eligible children were 2- to 60-months of age without underlying medical condition, including chronic cardiovascular diseases, chronic lung disease, liver cirrhosis, nephrotic syndrome, chronic renal failure, dialysis, indwelling devices, diabetes mellitus, thalassemia major, asplenia, congenital immunodeficiency, human immunodeficiency virus infection, malignancy or receiving immunosuppressant agents. However, infant of being prematurity without complication was not excluded. Study participants were recruited from general health checkup clinics. After obtaining written informed consent, parents/guardians completed questionnaires that included demographic information (age, gender, and history of breast feeding), and data regarding potential risk factors for carriage of *S. aureus*.[12] We divided the age into seven age groups, including those aged >2—6 months, >6—12 months, >12—18 months, >18—24 months, >2—3 years, >3—4 years, and >4—5 years. The potential risk factors included environmental factors and health condition. Season, number of children in the household, frequency of hand washing, passive smoking, the recognition of the main caregiver during the day and kindergarten or day care attendance were environmental factors. The health condition included vaccine history of *Streptococcus pneumoniae*, history of recent (<2 weeks) upper respiratory tract infection, and recent (<2 weeks) antibiotic use. The data in the questionnaires were digitalized in a computer core laboratory before carrying on statistical analysis. This study was approved by the Institutional Review Board of the Taichung Veterans General Hospital (IRB No. S05089).

Cultures, bacterial strains and antimicrobial susceptibility testing

We collected a swab (BBL CultureSwab Plus; Becton Dickinson and Company) of both anterior nares and another flexible swab (Venturi Transystem; Copan Innovation) from deep in the nasopharyngeal space from each subject. The sampling procedure was done by one well-trained nurse, and the specimens were brought to and processed in the microbiological laboratories within 4 h of the sampling. *S. aureus* and *S. pneumoniae* were identified with standard methods, and the oxacillin susceptibility was determined by the disc diffusion method according to Clinical and Laboratory Standards Institute 2006 guidelines.[13]

Molecular characterization of MRSA isolates

We sent all the *S. aureus* isolates to the Linko Medical Center of Chang Gung Memorial Hospital for further microbiological characterization. All the MRSA isolates were arranged for molecular characterization. Pulsed-field gel electrophoresis (PFGE) with SmaI digestion used to fingerprint the MRSA isolates was performed according to procedures described previously.[14] The genotypes were designated in alphabetical order, as in the previous studies.[11,14,15]; identified new genotype was designated consecutively. Subtypes of the existing genotype were defined as fewer than four band differences from that existing genotype.

Staphylococcal chromosomal cassette mec (SCC*mec*) typing of isolates was determined by a multiplex PCR strategy described previously.[16] The control strains for SCCmec types I, II, III, and IVa were as follow: type I, NCTC10442; type II, N315; type III, 85/2082; and type IVa, JCSC4744. SCC*mec* typing for type V_T was identified with a particular primer described elsewhere,[17] and the strain TSGH-17 was used as a control.

We identified the presence of Panton-Valentine leukocidin (PVL) genes with a PCR technique described by Lina et al.[18] Some isolates with representative PFGE patterns were selected for multilocus sequence typing (MLST) as described elsewhere.[19] The allelic profiles were determined through a comparison of the sequence at each locus with the sequences of the known alleles in the *S. aureus* MLST database and were defined as sequence types accordingly.

Statistics

As analyzing the carriage rate of MSSA and MRSA in each season, we used generalized additive models to evaluate smoothed predicted curves. We also tested the linear trend of MSSA and MRSA colonization. The comparison of categorical variables between study groups was performed with a chi-square test, while the differences between study groups in the continue variables were tested by the student *t* test. Multiple logistic regression analysis was conducted to evaluate the association between potential factors with MSSA or MRSA colonization. SAS software version 9.4 (SAS Institute Inc., Cary, NC) was applied to perform statistic, and *p* value of <0.05 was considering statistical significance.

Results

Characteristics of study subjects

A total of 3144 children with ages ranging from 2 to 60 months were enrolled in this study. More than a half (52.7%) subjects were male, and 6.6% of the participants had a birth history of prematurity.

Nasal carriage of *S. aureus* was detected in 545 (17.33%) subjects, which included 380 (12.09%) as MSSA and 165 (5.25%) as MRSA. 442 (14.06%) subjects were carriers of *S. pneumoniae*. The youngest group aged 2–6 months was with the highest *S. aureus* carriage rate and the lowest *S. pneumoniae* carriage rate (Fig. 1). MSSA carriage rate reached the lowest point around two years old and gradually increased between 2 and 5 years of age. MRSA carriage

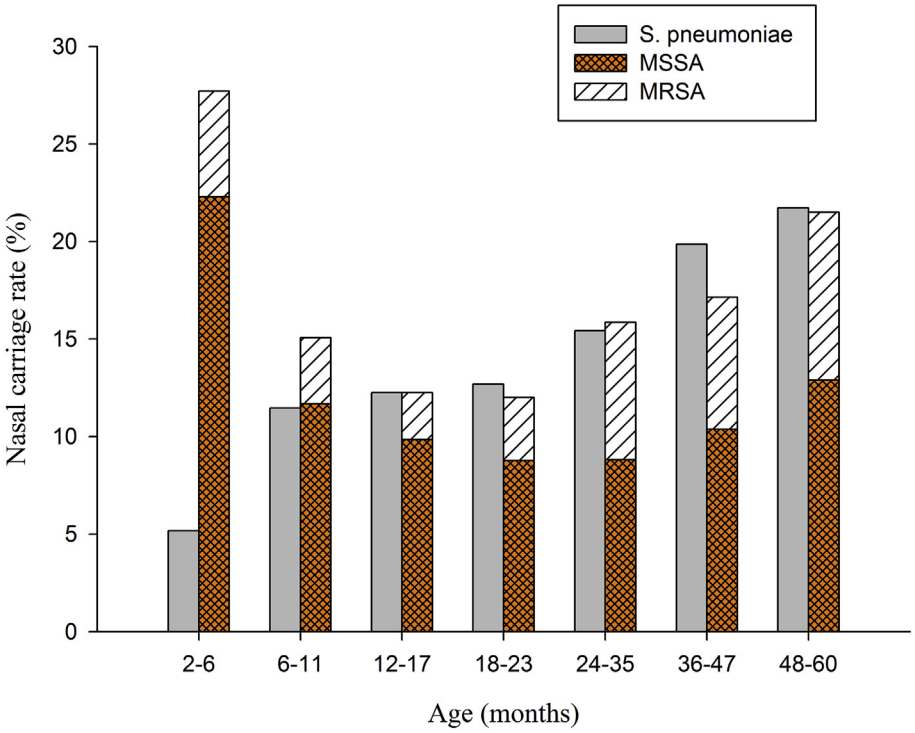

Figure 1. MRSA, MSSA, and *S. pneumoniae* carriage rates in different age groups in children younger than 6 years old. MRSA = Methicillin-resistant *Staphylococcus aureus*; MSSA = Methicillin-sensitive *Staphylococcus aureus*; *S. pneumoniae* = *Streptococcus pneumoniae*.

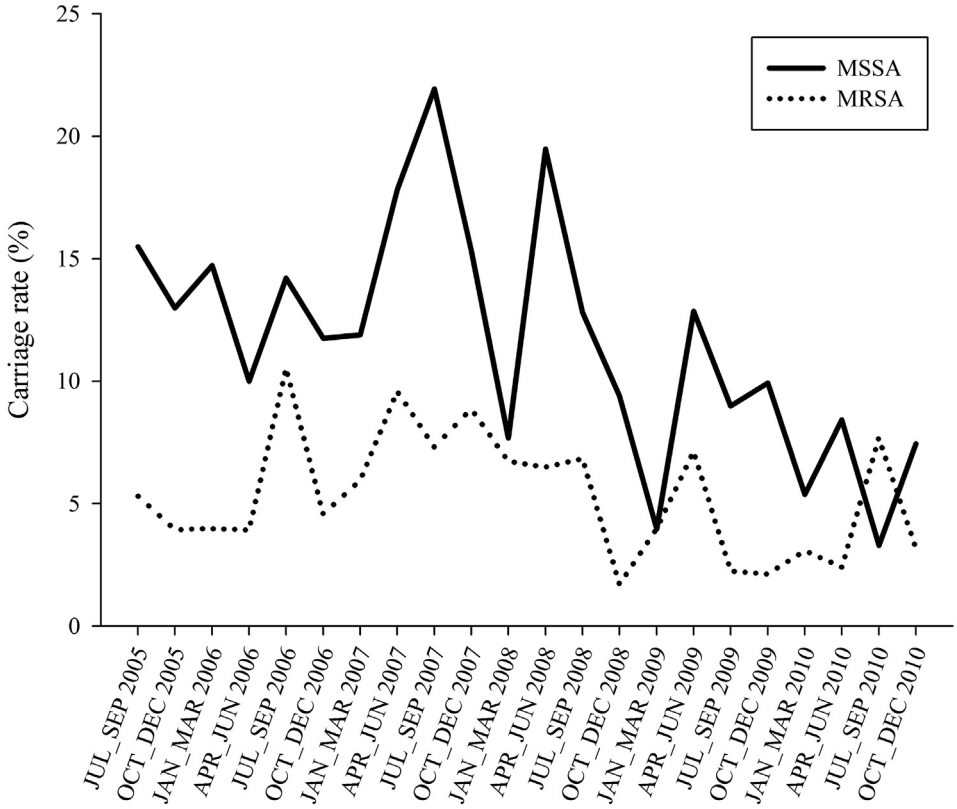

Figure 2. Time trend of MRSA and MSSA nasal colonization in children in central Taiwan from July 2005 to December 2010. MRSA = Methicillin-resistant *Staphylococcus aureus*; MSSA = Methicillin-sensitive *Staphylococcus aureus*.

rate touched bottom around one year old. *S pneumococcus* carriage rate increased with age.

During the period of study, the prevalence of MRSA did not change over time ($p = 0.3445$). However, there was a decreasing trend of MSSA carriage ($p = 0.0042$) (Fig. 2). The incidence of MRSA and MSSA carriage rate varied with season. We found significantly higher carriage rate in summer (MRSA, aOR, 1.731; 95% CI, 1.10—2.74; $p = 0.019$; MSSA, aOR, 1.450; 95% CI, 1.04—2.01; $p = 0.027$).

Association between *S. aureus* and *S. pneumoniae* colonization

There were 442 (14.06%) children colonized with *S. pneumoniae* in our study. Among them, 38 (8.6%) co-colonized with *S. aureus*. Subjects colonized with *S. aureus* had a rate of 6.97% for *S. pneumoniae* co-colonization, while subjects didn't colonize with *S. aureus* had a rate of 15.54% for *S. pneumoniae* colonization ($p < 0.001$). We found a negative correlation between *S. aureus* and *S. pneumoniae* colonization, which also revealed when we assessed *S. aureus* further to MSSA or MRSA (Table 1). There was no difference among MSSA carriers and MRSA carriers in *S. pneumoniae* co-colonization rate (P = 0.361).

Epidemiological factors associated with MRSA and MSSA carriage

Univariate analysis demonstrated the factors associated with MRSA and MSSA carriage (Table 2). Both MSSA and MRSA

Table 1 Association between nasal carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in children

Carriage of *S. pneumoniae*	Carriage of *S. aureus*			OR[a] (95% CI)	P[a]	OR[b] (95% CI)	P[b]	OR[c] (95% CI)	P[c]
	MSSA n = 380	MRSA n = 165	Non n = 2599						
No	356 (93.68%)	151 (91.52%)	2195 (84.46%)	—	—	—	—	—	—
Yes	24 (6.32%)	14 (8.48%)	404 (15.54%)	0.366 (0.24—0.56)	<0.001	0.504 (0.29—0.88)	0.016	0.407 (0.29—0.58)	<0.001

MRSA = methicillin-resistant *Staphylococcus aureus*; MSSA = methicillin-sensitive *Staphylococcus aureus*.
[a] Statistical test between MSSA carriers and noncarriers.
[b] Statistical test between MRSA carriers and noncarriers.
[c] Statistical test between MSSA carriers + MRSA carriers and noncarriers.

Table 2 Epidemiologic factors associated with MRSA and MSSA nasal carriage in children in central Taiwan from July 2005 to December 2010

Factor	Value			P[a]	P[b]
	MSSA (n = 380)	MRSA (n = 165)	No carrier (n = 2599)		
Demographic					
Age (months)				<0.001	<0.001
2–6 (n = 444)	99 (26.05%)	24 (14.55%)	321 (12.35%)		
6–11 (n = 471)	55 (14.47%)	16 (9.70%)	400 (15.39%)		
12–17 (n = 457)	45 (11.84%)	11 (6.67%)	401 (15.43%)		
18–23 (n = 433)	38 (10%)	14 (8.48%)	381 (14.66%)		
24–35 (n = 454)	40 (10.53%)	32 (19.39%)	382 (14.7%)		
36–47 (n = 443)	46 (12.11%)	30 (18.18%)	367 (14.12%)		
48–60 (n = 442)	57 (15%)	38 (23.03%)	347 (13.35%)		
Male (n = 1657)	198 (52.11%)	85 (51.52%)	1374 (52.87%)	0.781	0.736
Breast feeding (n = 2266)	275 (72.37%)	111 (67.27%)	1880 (72.34%)	0.989	0.160
Period (months, Mean ± SD)	5.35 ± 5.1	5.26 ± 4.9	6.42 ± 6.28	0.002	0.019
Environment					
Season				0.040	0.024
Spring (APR–JUN)	96 (25.26%)	40 (24.24%)	594 (22.85%)		
Summer (JUL–SEP)	115 (30.26%)	58 (35.15%)	663 (25.51%)		
Autumn (OCT–DEC)	102 (26.84%)	36 (21.82%)	745 (28.66%)		
Winter (JAN–MAR)	67 (17.63%)	31 (18.79%)	597 (22.97%)		
No. of children in the household (Mean ± SD)	1.79 ± 0.92	1.85 ± 0.86	1.86 ± 0.91	0.163	0.916
Hand washing frequency (no./day)				0.002	0.461
0–3 (n = 995)	148 (38.95%)	49 (29.70%)	798 (30.70%)		
4–7 (n = 1580)	182 (47.89%)	79 (47.88%)	1319 (50.75%)		
≥8 (n = 569)	50 (13.16%)	37 (22.42%)	482 (18.55%)		
Passive smoking (n = 1308)	168 (44.21%)	74 (44.85%)	1066 (41.02%)	0.238	0.332
Primary caregivers				0.161	0.089
Parents	178 (46.84%)	57 (34.55%)	1116 (42.94%)		
Grandparents	98 (25.79%)	39 (23.64%)	624 (24.01%)		
Day care centers	73 (19.21%)	50 (30.3%)	610 (23.47%)		
Babysitters	23 (6.05%)	18 (10.91%)	209 (8.04%)		
Others	8 (2.11%)	1 (0.61%)	40 (1.54%)		
Day-care or kindergarten attendance (n = 733)	73 (19.21%)	50 (30.3%)	610 (23.47%)	0.065	0.046
Duration (months)				0.016	0.523
<3 (n = 207)	28 (38.36%)	17 (34%)	162 (26.56%)		
3–8 (n = 214)	10 (13.7%)	16 (32%)	188 (30.82%)		
8–16 (n = 174)	19 (26.03%)	11 (22%)	144 (23.61%)		
≥16 (n = 138)	16 (21.92%)	6 (12%)	116 (19.02%)		
No. of classmates (Mean ± SD)	16.18 ± 6.72	16.22 ± 5.78	16.52 ± 7.05	0.697	0.772
Duration of stay (h/wk, Mean ± SD)	35.75 ± 5.59	36.4 ± 4.62	35.97 ± 5.9	0.767	0.537
Health conditions					
Pneumococcal vaccination	56 (14.74%)	31 (18.79%)	391 (15.04%)	0.876	0.195
URI within 2 weeks	86 (22.63%)	55 (33.33%)	669 (25.74%)	0.193	0.032
Received antibiotics within 2 weeks	11 (2.89%)	13 (7.88%)	102 (3.92%)	0.326	0.014

MRSA = Methicillin-resistant *Staphylococcus aureus*; MSSA = Methicillin-sensitive *Staphylococcus aureus*; *S. pneumoniae* = *Streptococcus pneumoniae*; URI = upper respiratory tract infection.

[a] Statistical test between MSSA carriers and noncarriers.

[b] Statistical test between MRSA carriers and noncarriers.

were correlated with demographic factors as age and breast feeding duration. When it came to health and environmental factors, MSSA colonization was influenced by season, lower frequency of hand washing and longer duration of day-care or kindergarten attendance; MRSA colonization was affected with season, day-care or kindergarten attendance, upper respiratory tract infection and use of antibiotics within the previous 2 weeks.

Adjusted for other risk factors, multiple logistic regression analysis showed that the independent variable

correlated with MRSA carriage was age (a non-linear but U-shaped relationship) and season (Table 3). On the other hand, independent factors for incidence of MSSA carriage were colonization by *S. pnuemoniae*, age, breast feeding period and season.

Molecular characteristics and antimicrobial susceptibilities of MRSA isolates

Of 165 MRSA isolates, 9 PFGE types with 61 subtypes were identified. Types C and D were the two most frequent types which accounted for 64% and 21% respectively. Four SCC*mec* types (type II, III, IV and V_T) were identified and type IV (72.1%) was the most common type, followed by type V_T (18.8%). PVL genes were found in 35 isolates (21%), which was mostly presented in isolate with PFGE type D (91%). MLST was performed for 26 isolates, and ten sequence types with two new types were identified. The most common sequence type was ST 59, which accounted for eight of nine pulsotype C isolates (a new sequence type of single locus variant of ST 59 for the other one) and four of six pulsotype D isolates (ST 338, also single locus variant of ST 59, for the other two). We sorted out the association of PGEF patterns with SCC*mec* types, sequences types and the presence of the PVL genes of these isolates in Table 4. Of the 165 isolates, nearly all were sensitive to vancomycin (100%) and teicoplanin (99.39%). Sensitive to trimethoprim-sulfamethoxazole (SXT) (96.36%) and doxycycline (95.15%) was high. On the other hand, most isolates exhibited high rates of resistance to penicillin (98.18%), erythromycin (92.73%) and clindamycin (89.09%).

Discussion

MRSA colonization in healthy children has increased significantly in the past decade. As colonization generally precedes infection, this increase may be a major factor in the emergence of community-associated MRSA infection. Prevalence rate of nasal carriage of MRSA or MSSA for

Table 3 Multiple logistic regression analysis of risk factors for MRSA and MSSA nasal carriage in children in central Taiwan from July 2005 to December 2010

Factors	Model 1[a] MRSA carrier v.s. noncarrier		Model 2[a] MSSA carrier v.s. noncarrier	
	Adjusted OR (95% C.I.)	P	Adjusted OR (95% C.I.)	P
Colonization by *S. pneumoniae*	0.436 (0.25—0.78)	0.005	0.396 (0.255—0.613)	<0.001
Demographic				
Age (ref: 2—6 months)				
6—11 months	0.570 (0.30—1.10)	0.094	0.474 (0.33—0.69)	<0.001
12—17 months	0.394 (0.19—0.84)	0.015	0.382 (0.26—0.57)	<0.001
18—23 months	0.546 (0.27—1.12)	0.097	0.354 (0.23—0.55)	<0.001
24—35 months	1.249 (0.67—2.34)	0.487	0.399 (0.26—0.62)	<0.001
36—47 months	1.374 (0.70—2.71)	0.360	0.560 (0.35—0.90)	0.015
48—60 months	2.060 (0.997—4.26)	0.051	0.846 (0.51—1.42)	0.526
Male (ref: female)	0.988 (0.72—1.36)	0.939	0.988 (0.79—1.23)	0.914
Breast feeding (ref: no)	0.848 (0.60—1.20)	0.356	0.990 (0.77—1.27)	0.939
Period (per add 1 quarter)	0.978 (0.95—1.01)	0.107	0.961 (0.94—0.98)	<0.001
Environment				
Season (ref: Winter (JAN—MAR))				
Spring (APR—JUN)	1.225 (0.75—2.00)	0.417	1.439 (1.03—2.02)	0.036
Summer (JUL—SEP)	1.731 (1.10—2.74)	0.019	1.450 (1.04—2.01)	0.027
Autumn (OCT—DEC)	0.923 (0.56—1.52)	0.752	1.212 (0.87—1.69)	0.258
No. of children in the household	0.961 (0.80—1.16)	0.675	0.964 (0.85—1.10)	0.573
Hand washing frequency (ref: 0—3)				
4—7	0.764 (0.49—1.18)	0.227	0.956 (0.72—1.26)	0.751
≥8	0.812 (0.47—1.39)	0.450	0.711 (0.48—1.07)	0.098
Passive smoking (ref: no)	1.158 (0.84—1.60)	0.376	1.151 (0.92—1.44)	0.221
Day-care or kindergarten attendance (ref: no)	0.775 (0.48—1.24)	0.291	0.76 (0.52—1.11)	0.159
Health conditions (ref: no)				
Pneumococcal vaccination	1.379 (0.89—2.14)	0.154	1.345 (0.97—1.87)	0.080
URI within 2 weeks	1.270 (0.88—1.84)	0.208	0.983 (0.74—1.30)	0.906
Received antibiotics within 2 weeks	1.783 (0.92—3.46)	0.088	0.864 (0.44—1.70)	0.671

MRSA = Methicillin-resistant *Staphylococcus aureus*; MSSA = Methicillin-sensitive *Staphylococcus aureus*; *S. pneumoniae* = *Streptococcus pneumoniae*; URI = upper respiratory tract infection.

[a] Adjusted for Colonization by *S. pneumoniae*, Age, Sex, Breast feeding, Timing of enrollment, No. of children in the family, Hand washing frequency, Passive smoking, Day-care or kindergarten attendance, Pneumococcal vaccination, URI within 2 weeks, Premature birth, Received antibiotics within 2 weeks.

Table 4 Distribution of PFGE patterns, SCCmec types, and presence of PVL genes among 165 MRSA isolates

PFGE pattern (N)	No. of subtypes	SCC*mec* type (N)	PVL genes-positive (N)	Sequence type
A (3)	3	III (3)	0	ST239
C (106)	32	IV(105), V_T(1)	3	ST59, new[a]
D (35)	13	IV(7), V_T(28)	32	ST59, ST338[a]
CP (2)	2	V_T(2)	0	ST9, new[c]
AF (11)	5	II(11)	0	ST89
AK (5)	2	IV(5)	0	ST508[b]
AQ (1)	1	IV(1)	0	ST508[b]
S (1)	1	IV(1)	0	ST5
BM (1)	1	V(1)	0	ST45

N = no. of isolates; PFGE = Pulsed-field gel electrophoresis; PVL = Panton-Valentine leukocidin; SCC*mec* = staphylococcal cassette chromosome mec.
[a] A single locus variant of ST 59.
[b] A single locus variant of ST 45.
[c] A single locus variant of ST 9.

previously healthy children in Taiwan during 2001–2010 was summarized in Table 5. The incidence of MRSA carriage in children living in northern Taiwan raised from 1.9% during 2001–2002, 9.5% during 2005–2008 to 15.1% during 2007–2009.[10–12] The MRSA carriage also increased in children who lived in southern Taiwan, from 3.3% in 2001 to 7.7% during 2005–2008.[12,20] However, the carriage rate in central Taiwan decreased from 6.2% during 2005–2008 to 5.25% during 2005–2010.[12] The temporal trend analysis of our study from 2005 to 2010 showed decreasing in MSSA

Table 5 Prevalence rate of nasal carriage of MRSA or MSSA for previously healthy children in Taiwan during 2001–2010

Study period	Setting	Age	Area	Sample size	No (%)	Reference
MRSA						
2001	Community	2–18 years	Southern	987	33 (3.3)	20
2001–2002	Community	3–12 years	Northern	262	5 (1.9)	11
2003	Community/Hospital	<12 years	Northern	640	34 (5.3)	17
2004–2006	Community/Hospital	<14 years	Northern	1615	131 (8.1)	10
2005–2006	Hospital	2 months to 5 years	Northern	1279	121 (9.5)	15
			Central	1011	49 (4.8)	15
			Southern	756	51 (6.7)	15
2005–2008	Hospital	2 months to 5 years	Northern	2017	192 (9.5)	12
			Central	2017	125 (6.2)	12
			Southern	2023	156 (7.7)	12
2007–2009	Community/Hospital	<14 years	Northern	1585	240 (15.1)	10
2005–2010	Hospital	2 months to 5 years	Central	3144	165 (5.25)	[a]
MSSA						
2001	Community	2–18 years	Southern	987	281 (28.5)	20
2001–2002	Community	3–12 years	Northern	262	90 (34.4)	11
2003	Community/Hospital	<12 years	Northern	640	109 (17.0)	17
2003–2008	Community/Hospital	1 months to 6 years	Northern	3305	495 (15)	21
2004–2006	Community/Hospital	<14 years	Northern	1615	323 (20)	10
2005–2006	Hospital	2 months to 5 years	Northern	1279	223 (17.4)	15
			Central	1011	131 (13.0)	15
			Southern	756	138 (18.3)	15
2005–2008	Hospital	2 months to 5 years	Northern	2017	361 (17.9)	12
			Central	2017	284 (14.1)	12
			Southern	2023	286 (14.1)	12
2007–2009	Community/Hospital	<14 years	Northern	1585	130 (8.2)	10
2005–2010	Hospital	2 months to 5 years	Central	3144	380 (12.1)	[a]

[a] Our study.
Community in setting means subjects from day-care centers or schools. Hospital in setting means subjects as children for healthcare visits.

carriage and no significant change in MRSA carriage. However, one study with nearly the same time period as 2004—2009 revealed decreased MSSA colonization and increased MRSA colonization.[10] Seasonal variation in the occurrence of *S. aureus* infection appeared to exist, particularly an association of warm-weather months with *S. aureus* skin and soft-tissue infections.[21] In our study, both MRSA and MSSA carriage rate varied with season, which was significantly higher in summer. Lewnard JA et al. also found that the late-summer coincided with a seasonal peak in *S. aureus* carriage.[22] However, Hassoun A et al. described that *S. aureus* and MRSA were frequently detected in both winter and summer.[23]

Our study found the colonization of *S. pneumoniae* decreased the colonization of *S. aureus*. *S. aureus* carrier state inversely associated with *S. pneumoniae* carrier state in children was previously described.[24] However, the carriage of *S. pneumoniae* did not affect that of *S. aureus* in older children (6—17 years) and children with chronic disease.[25,26] *S. aureus* carriage was affected with *S. pneumoniae* exposure under the suggestion that acquired immune responses resulting from earlier carriage may mediate inverse interspecies associations with *S. aureus* carriage.[22] Interleukin 17—expressing $CD4^+$ T cells triggered in response to *S. pneumoniae* and *Haemophilus influenzae* facilitate phagocytic clearance of *S. aureus*.[27]

Several studies discussed risk factors related to MRSA colonization in children,[10,12,28] but no conclusive statement was found. MRSA colonization was positively associated with the number of children in the family and day care attendance, but negatively associated with breast feeding and colonization with *S. pneumoniae*.[12] Male sex, larger family size, antibiotic use and admission during the previous three months, and parental smoking were independent risk factors for nasal colonization with MRSA.[28] However, except for colonization by *S. pneumoniae*, age and season, none of the discussed factors were independently associated with *S. aureus* colonization in our study.

This is the first study discussed the MRSA nasal colonization in children in central Taiwan. In Taiwan, MRSA ST59 (and its variants, such as ST338) was the most common sequence type[15] involving CA-MRSA infection[17,29,30] as well as colonization.[12] In the current study, most colonizing isolates shared common molecular characteristics, and more than 75% of the isolates belonged to one of two major clones, characterized by ST59/PFGE type C/SCCmec IV/PVL-negative or ST59/PFGE type D/SCCmec V_T/PVL-positive. In previous studies, ST59/PFGE type C/SCCmec IV/PVL-negative was dominant among the colonizing isolates,[15] while ST59/PFGE type D/SCCmec V_T/PVL-positive was prevalent among the clinical isolates.[5] In antibiotic susceptibility testing, most MRSA colonization isolates were resistant to erythromycin and clindamycin, but sensitive to SXT and doxycycline, which was similar to the antibiogram of CA-MRSA in Taiwan.[30]

Several limitations were considered in this study. First, subjects were come from one hospital, which might not represent the generalized pediatric population in central Taiwan. However, TCVGH was a medical center, where patient might come from entire central area. Second, *S. aureus* not only colonized in the nose and pharynx, but also on the rectum, groin and etc. The *S. aureus* colonization rate might be underestimated, while the nose was the site most frequently found to yield *S. aureus*. Third, recall bias might exist while parents or guardians tried to memorize past events and exposures asked in the questionnaire. Finally, a cross-sectional survey might miss some children who were intermittently colonized.

In conclusion, this prospective observational study found the incidence of MRSA nasal colonization not increased in central Taiwan in 2005—2010, and a decrease in the prevalence of MSSA nasal colonization. During this period, summer was a seasonal peak in both MRSA and MSSA carriages.

Conflicts of interest

All authors have no conflicts of interest to declare.

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