Journal of Microbiology, Immunology and Infection (2018) 51, 756–762

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

Nasal methicillin-resistant *Staphylococcus aureus* colonization among otherwise healthy children aged between 2 months and 5 years in northern Taiwan, 2005–2010

Meng-Shan Tsai [a], Chih-Jung Chen [a,b], Tzou-Yien Lin [a,b], Yhu-Chering Huang [a,b,*]

[a] *Divisions of Pediatric Infectious Diseases, Department of Pediatrics, Chang Gung Memorial Hospital, Guishan, Taoyuan, Taiwan*
[b] *College of Medicine, Chang Gung University, Guishan, Taoyuan, Taiwan*

Received 5 April 2017; received in revised form 10 July 2017; accepted 27 July 2017
Available online 12 August 2017

KEYWORDS
Methicillin-resistant *Staphylococcus aureus*;
Community-associated;
Sequence type 59;
Taiwan;
Children;
Colonization

Abstract *Background:* Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections have been increasingly reported worldwide and are associated with nasal colonization. In Taiwan, available data disclosed a similar trend. We conducted a study for the updated childhood nasal MRSA carriage.
Methods: From July 2005 to December 2010, children aged between 2 months and 5 years who presented for a well-child health care visit to a medical center or from kindergarten/daycare center were invited and a nasal swab specimen was obtained for the detection of MRSA. All MRSA isolates were characterized.
Results: A total of 3226 children were included and the rate of nasal MRSA carriage was 10.2%. Children aged 2–6 months and >3 years were significantly associated with MRSA carriage, while pneumococcus colonization ($p = 0.033$) and breastfeeding ($p = 0.025$) were negatively associated with MRSA carriage. Of the 330 MRSA isolates, a total of 13 pulsotypes with two major patterns (type C, 47.0% and D, 29%) were identified. Most MRSA isolates belonged to two major clones, characterized as sequence type 59 (ST59)/pulsotype C/staphylococcal cassette chromosome (SCC*mec*) IV/Panton-Valentine leukocidin (PVL)-negative (45.8%) and ST59/pulsotype D/SCC*mec* V_T/PVL-positive (22.7%). Two new clones as ST 508/SCC*mec* IV (9.7%) and ST573/SCC*mec* IV (7.3%) emerged and increased markedly since 2007.
Conclusion: Between 2005 and 2010, 10.2% of healthy children in northern Taiwan carried MRSA in anterior nares, with the highest carriage rate for infants aged 2–6 months. Two

* Corresponding author. Division of Pediatric Infectious diseases, Department of Pediatrics, Chang Gung Memorial Hospital, No. 5, Fuxing St., Guishan Dist., Taoyuan 333, Taiwan. Fax: +886 3 3288957.
E-mail address: ychuang@adm.cgmh.org.tw (Y.-C. Huang).

http://dx.doi.org/10.1016/j.jmii.2017.07.014

emerging clones, ST 508 and ST 573, were identified and the clinical significance needs further surveillance.

Introduction

Not a long time after methicillin has been introduced in 1959, methicillin-resistant *Staphylococcus aureus* (MRSA) first identified from UK[1] has become famous around the world.[2] Plenty of scientists investigated its evolution during the past decades and found that increasing community-acquired MRSA infections in patients without significant risk factors, instead of hospital-acquired, were reported worldwide since the mid-1990s.[3] The disease burden from MRSA usually correlates to the rate of healthy carriers, as proved in a population-based study.[4] An updated local epidemiology of MRSA carriage among healthy populations is useful for clinicians, including those in Taiwan.

Taiwan is not an exception of increasing community-associated MRSA infections as well as the nasal MRSA carriage rate based on previous reports.[5–10] In early 2000s the nasal MRSA carriage rate ranged from 1.9% to 3.3% for school children, 5.3% for healthy children presented for health care visits, 10.8% for health care workers and 13.6% for contacts of CA-MRSA infection. Regarding those truly with diseases due to community-acquired *S. aureus* infection, MRSA accounted for up to 36%.[7] However, these studies were not comparable to each other due to the different study designs, and study populations. Thus, to renew the prevalence of childhood nasal MRSA carriage and the related risk factors, we conducted this prospective study in northern Taiwan. Further molecular analysis was also done for all MRSA isolates.

Material and methods

Subject collection

This study was approved by the Institutional Review Board of the Chang Gung Memorial Hospital. During July 2005 to December 2010, we invited all children aged between 2 and 60 months who presented for a well-child health care visit of Chang Gung Memorial Hospital at Linkou, a tertiary medical center in northern Taiwan, to join this study. The enrolled cases were distributed into seven separated age groups as 2–6 months, >6–12 months, >12–18 months, >18–24 months, >2–3 years, >3–4 years and >4–5 years. Each participant would complete a questionnaire after the parents or legal guardians signed an informed consent form. The questionnaire was designed for potential risk factors of *S. aureus* or MRSA colonization. Other than demographic data, the questionnaire included the environmental factors, such as size of living area, number of bathrooms and siblings, day-care center attendance, bed-sharing with others and as a second smoker; hygiene habits as hand washing frequency and the relationship of other pathogens like flu vaccination status, *Streptococcus pneumoniae* colonization and antibiotics usage within 2 weeks. Breast milk feeding and its duration were also recorded.

A swab from anterior nares of each subject was obtained for detection of MRSA. The swabs were obtained with a cotton swab, placed in the transport medium (Venturi Transystem; Copan Innovation Ltd., Limerick, Ireland), and then brought to and processed within four hours of samplings. All *S. aureus* were further studied for their microbiological characteristics. Some preliminary results of this study were published previously.[10,11]

Antimicrobial susceptibility tests

Nine antibiotics were tested for antimicrobial susceptibility of MRSA in this study including penicillin, clindamycin, erythromycin, doxycycline, trimethoprim/sulfamethoxazole (SXT), vancomycin, teicoplanin, linezolid and fusidic acid, using the disk-diffusion method in accordance with the 2011 Guideline of Clinical and Laboratory Standard Institutes.[12]

Molecular characterizations

Pulsed-field gel electrophoresis (PFGE) with *Sma*I digestion was performed according to the procedure published previously.[6] The genotypes were designated in alphabetical order, as in our previous studies; any new genotype, if identified, was designated consecutively. PFGE patterns with fewer than 4-band differences from an existing genotype were defined as subtypes of that genotype. Staphylococcal cassette chromosome (SCC*mec*) typing was done using a multiplex PCR strategy[13,14] as previously described; type V_T was done by a modified method.[10] Control strains for SCC*mec* types were NCTC10442, N315, 85/2082, JCSC4744, and TSGH-17 accordingly for type I, II, III, IVa and V_T. The presence of Panton-Valentine leukocidin (PVL) genes was determined by a PCR strategy described previously.[14] Some isolates of representative PFGE patterns were selected and underwent multilocus sequence typing (MLST) as described elsewhere.[14]

Statistics

Prevalence and risk factors of *S. aureus* and MRSA colonization were showed via Chi-square and ANOVA for univariates; logistic regression for multivariate (only significant risk factors in univariate analysis were included), using SPSS version 20. Fisher's exact test was used under small sample size. A difference was considered significant if $p < 0.05$.

Results

A total of 3282 children were recruited in this study. After excluding one inappropriate age (less than 2-month-old) and 55 subjects with some missing data, a total of 3226 children were included. Overall, 919 (28.5%) subjects were colonized with *S. aureus*. Three hundred and thirty MRSA accounted for 10.2 percent of the whole studied population (Fig. 1). The yearly nasal MRSA carriage rate ranged from 8.7% in 2006 to 12.5% in 2010. The MRSA carriage rate between 2005 and 2007 was not significantly different from that between 2008 and 2010 ($p = 0.245$) (Fig. 1).

Risk factors of *S. aureus*/MRSA colonization

By univariate and multivariate logistic regression analysis (Tables 1 and 2), we identified several risk factors associated with *S. aureus* or MRSA nasal colonization among the children. Children within the two ends of the seven age groups (two to six-month-old and >four to five-year old) were significantly associated with colonization of both *S. aureus* and MRSA more than those in other age groups while pneumococcus colonization was negatively associated with both *S. aureus* and MRSA colonization. Breast milk feeding significantly reduced MRSA colonization while antibiotic usage within two weeks was negatively associated with *S. aureus* colonization. However, we did not find a relationship between the duration of breast milk feeding and MRSA colonization.

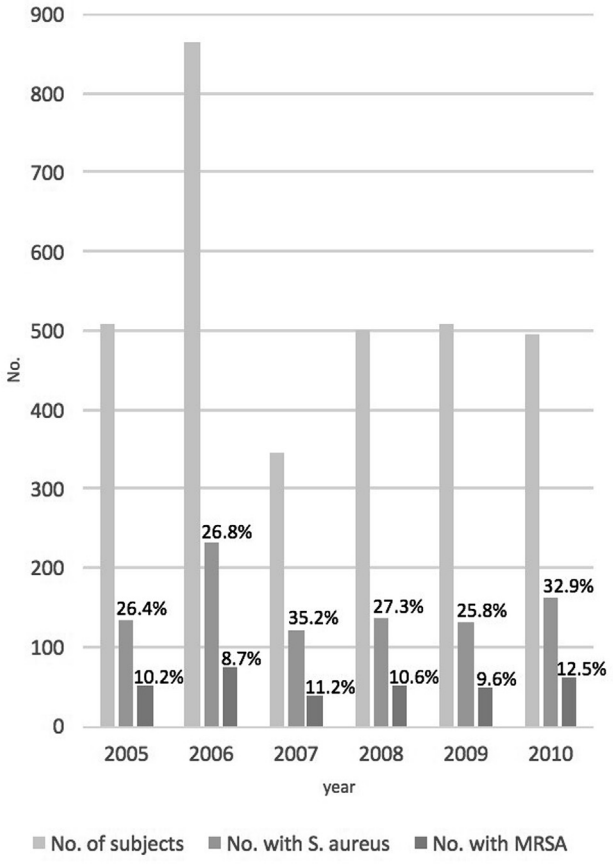

Figure 1. Nasal carriage rate of *S. aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) among children aged 2–60 months in northern Taiwan, 2005–2010. Percentages of nasal *S. aureus* and MRSA carriage are showed in the top of each bar.

Antibiotic susceptibility testing

All 330 MRSA isolates were susceptible to vancomycin, teicoplanin, linezolid and fusidic acid (Table 3) and more than 90% of the isolates susceptible to doxycycline and trimethoprim/sulfamethoxazole (SXT). The susceptibility rates of the MRSA isolates to clindamycin, erythromycin and penicillin were quite low and all less than 25%. The susceptibility rate of clindamycin significantly increased from 13.3% during 2005–2007 to 35.4% during 2008–2010 ($p < 0.001$). Also, an increasing trend was noted for erythromycin.

Molecular characteristics

The detailed molecular characteristics of all MRSA isolates are shown in Table 4. A total of 13 PFGE patterns were identified and patterns C and D were the most frequent ones. Five types (types II, IIIA, IV, V and V_T) of SCC*mec* were identified among the isolates, with type IV being the predominant type, followed by type V_T. All but one isolates with PVL genes were identified from the isolates with PFGE pattern D. Two major clones were identified as ST59/PFGE C/SCC*mec* IV/PVL-negative (45.8%) and ST59/PFGE D/SCC*mec* V_T/PVL-positive (22.7%), which were reported to be the most common CA-MRSA clones in Taiwan. However, two emerging clones characterized as ST 508/PFGE AK/SCC*mec* IV/PVL-negative (9.7%) and ST 573/PFGE U/SCC*mec* IV/PVL-negative (7.3%) were also found and became more prominent during the later period (2008–2010) in this study ($p < 0.001$).

Two new sequence types, including ST3331 and ST3336, were identified and both were a single locus variant of ST 9, the major clone of livestock-associated MRSA in Taiwan. Besides, ST 966, a single locus variant of ST59, was also newly found in this study.

Discussion

Previous studies regarding nasal carriage of MRSA among children presenting for a well-child healthcare visit and/or in school children in Taiwan revealed that he carriage rate increased significantly from 1.9% in 2001 to 15.1% during 2007–2009 for northern Taiwan, from 4.8% in 2005–2006 to 7.1% in 2007–2008 and from 3.3% in 2001 to 9.2% in 2007–2008 for southern Taiwan.[9,15,16] In a survey conducted during 2004–2009, Lo et al.[16] recruited 3200 children from birth to 14 years of age presenting for health maintenance visits or attending 1 of 57 kindergartens in Taipei city (northern Taiwan) and found that the prevalence of nasal MRSA carriage was 11.6%, with an increase from 8.1% during 2004–2006 to 15.1% during 2007–2009. Results from the present study indicated that nasal MRSA colonization rate among otherwise healthy children less than five years of age in northern Taiwan was steady during

Table 1 Univariate analysis of epidemiologic factors associated with methicillin-resistant *Staphylococcus aureus* (MRSA) and *S. aureus* carriage in children in northern Taiwan.

Factor	No. (%) of subjects					
	MRSA carriers (n = 330)	MRSA non-carriers (n = 2896)	p	*S. aureus* carriers (n = 919)	*S. aureus* non-carriers (n = 2307)	p
Age group			<0.001	–	–	<0.001
2–6 months (n = 458)	73 (22.1)	385 (13.3)		209 (22.7)	249 (10.8)	
>6–12 months (n = 464)	40 (12.1)	424 (14.6)		112 (12.2)	352 (15.3)	
>2–18 months (n = 470)	25 (7.6)	445 (15.4)		91 (9.9)	469 (20.3)	
>18–24 months (n = 454)	24 (7.3)	430 (14.8)		68 (7.4)	386 (16.7)	
>2–3 years (n = 477)	43 (13.0)	434 (15.0)		135 (14.7)	342 (14.8)	
>3–4 years (n = 455)	55 (16.7)	400 (13.8)		131 (14.3)	324 (14.0)	
>4–5 years (n = 448)	70 (21.2)	378 (13.1)		173 (18.8)	275 (11.9)	
Female gender	137 (41.5)	283 (48)	0.033	420 (45.7)	1026 (44.5)	0.276
Size of living area (m^2), mean ± SD	152.4 ± 83.6	155.4 ± 106.1	0.600	150.1 ± 88.9	156.4 ± 109.4	0.364
Number of bathrooms at home, mean ± SD	2.2 ± 1.0	2.2 ± 1.0	0.121	2.2 ± 1.0	2.2 ± 1.0	0.228
Number of siblings, mean ± SD	2.1 ± 1.0	2.0 ± 1.0	0.369	2.0 ± 1.0	2.1 ± 1.0	0.785
Number of hand washing per day	5.0 ± 3.5	5.1 ± 3.0	0.005	4.9 ± 3.3	5.0 ± 3.5	<0.001
DCC attendance	76 (23.0)	440 (15.2)	<0.001	165 (18.0)	351 (15.2)	0.032
Bed-sharing	292 (88.5)	2528 (87.3)	0.302	790 (86.0)	2039 (88.0)	0.066
Second smoker	173 (52.4)	1418 (49.0)	0.129	460 (50.1)	1131 (49.0)	0.312
Pneumococcal colonization	28 (8.5)	338 (11.7)	0.047	63 (6.9)	303 (13.1)	<0.001
Recent antibiotic use	16 (4.8)	159 (5.5)	0.370	34 (3.7)	141 (6.1)	0.006
Flu vaccination	156 (47.3)	1229 (42.4)	0.100	374 (40.7)	1011 (43.8)	0.057
Breast milk feeding	204 (61.8)	1970 (68.0)	0.026	612 (66.6)	1562 (67.7)	0.285
Breast milk feeding duration (month), mean ± SD	3.6 ± 5.4	4.3 ± 5.7	0.924	4.0 ± 5.8	4.3 ± 5.6	0.110

Abbreviations: SD, standard deviation; DCC, day care center.

Table 2 Multivariate analysis of factors associated with MRSA and *S. aureus* carriage in children in northern Taiwan.

Factors	*S. aureus*			MRSA		
	Adjusted OR	95% CI	p	Adjusted OR	95% CI	p
Age group						
2–6 months	Referent			Referent		
>6–12 months	0.389	0.294–0.516	<0.001	0.508	0.336–0.769	<0.001
>12–18 months	0.296	0.220–0.397	<0.001	0.307	0.188–0.499	<0.001
>18–24 months	0.219	0.16–0.301	<0.001	0.308	0.187–0.506	<0.001
>2–3 years	0.488	0.371–0.64	<0.001	0.537	0.350–0.826	0.005
>3–4 years	0.519	0.394–0.684	<0.001	0.731	0.478–1.116	0.146
>4–5 years	0.853	0.651–1.117	0.248	0.871	0.583–1.410	0.575
Female gender	–	–	–	0.878	0.695–1.110	0.277
DCC attendance	1.089	0.823–1.44	0.551	0.763	0.522–1.114	0.162
No. of hand washing	0.987	0.958–1.016	0.373	0.984	0.943–1.028	0.475
Pneumococcus colonization			<0.001			
Positive	Referent			Referent		
Negative	2.067	1.542–2.771		1.571	1.037–2.381	0.033
Recent antibiotics use			0.012			
Yes	Referent			–	–	–
No	1.685	1.116–2.464		–	–	–
Breast milk feeding						0.025
Yes	–	–	–	Referent		
No	–	–	–	1.314	1.034–1.671	–

Abbreviations: OR, odds ratio; CI, confidence interval.

Table 3 Antibiotic susceptibility rates of 330 colonizing methicillin-resistant *Staphylococcus aureus* isolates from children in northern Taiwan, 2005–2010.

Year	Isolates number	Susceptibility, No. (%)				
		Penicillin	Clindamycin	Erythromycin	Doxycycline	SXT
2005	52	0 (0)	5 (9.6)	3 (5.8)	52 (100)	52 (100)
2006	75	2 (2.7)	8 (10.7)	7 (9.3)	73 (97.3)	74 (98.7)
2007	39	0 (0)	9 (23.1)	3 (7.7)	39 (100)	39 (100)
2008	53	0 (0)	27 (50.9)	7 (13.2)	40 (75.5)	52 (98.1)
2009	49	0 (0)	15 (30.6)	11 (22.4)	48 (98.0)	49 (100)
2010	62	3 (4.8)	16 (25.8)	6 (9.7)	60 (96.8)	61 (98.4)
Total	330	5 (1.5)	80 (24.2)	37 (11.2)	312 (94.5)	327 (99.0)

Abbreviation: SXT, trimethoprim-sulfamethoxazole.
All isolates were 100% susceptible to vancomycin, teicoplanin, linezolid and fusidic acid.
The susceptibility rate to clindamycin was significantly higher in 2008–2010 than in 2005–2007 ($p < 0.001$).

Table 4 Molecular characteristics of 330 colonizing methicillin-resistant *Staphylococcus aureus* isolates stratified by pulsed-field electrophoresis (PFGE) patterns.

PFGE pattern(n)	No. of subtypes	SCC*mec* type(n)	PVL-positive(n)	Sequence type
A (1)	1	IIIA (1)	0	ST239
AF (9)	4	II (9)	0	ST89
AI (1)	1	Non-typeable	0	ST8
AK (32)	7	IV (32)	0	ST508
AN (3)	2	IV (3)	0	ST59
AQ (4)	3	IV (4)	0	ST508
AR (1)	1	IV (1)	0	ST15
BA (1)	1	IV (1)	0	ST97
C (155)	37	IV (152), V_T (3)	1	ST59, ST966[a]
CP (2)	1	V (1), V_T (1)	0	ST3331, ST3336
D (96)	20	IV (13), V (3), V_T (80)	88	ST59
F (1)	1	II (1)	0	ST5
U (24)	2	IV (24)	0	ST573

SCC*mec*, staphylococcal cassette chromosome; PVL, Panton-Valentine leukocidin.
[a] ST966, a single locus variant of ST59; ST 3331 & 3336, a single locus variant of ST9.

2005 and 2010, from 8.7% in 2006 to 12.5% in 2010, with an average of 10.2%. The prevalence of nasal MRSA carriage among children in Taiwan, though increased significantly during 2000s, seemed to reach plateau around 2010. With the increase of nasal MRSA carriage rate, the rate of MRSA amongst childhood CA *S. aureus* infections, since first reported in 2002, also increased significantly from 9.8% (17/173) in 1999–2000 to 56% (102/183) in 2004–2005 in Taiwan.[7,9,15] Continued surveillance is needed. As regards global trend, it is hard to be clarified due to lack of the updated colonization rate in each country. However, instead of carriage rate, MRSA infection rate also reached a steady state in USA since 2005.[17] The possible explanations might be improved hygiene habits and wound care etc.

The more we know about the risk factors of MRSA colonization, the better is the control of spreading in the community, especially the information specified to the local area. Age, sex, hygiene habits, living in urban area, day care attendance and seasonality were reported as risk factors of nasal *S. aureus*/MRSA carriage in developed or developing countries previously.[18–20] Most of these factors were also identified as risk factors of MRSA carriage in the present study by univariate analysis. However, by multivariate analysis, only age (2–6 months and >3 years) was significantly associated with MRSA carriage, while pneumococcus colonization and breastfeeding were negatively associated with MRSA carriage. Different study populations in different areas may be associated with different risk factors for MRSA colonization, some in common while some different. Human alpha-lactalbumin made lethal to tumor cells (HALMET)[21] purified from the human milk acts as an antimicrobial adjuvant that can increase the activity of a broad spectrum of antibiotics including methicillin, against multi-drug resistant *S. aureus*. HALMET works both in antimicrobial assays against planktonic and biofilm bacteria and in an in-vivo model of nasopharyngeal colonization.[22] *S. pneumoniae* carriage was reported to be negatively associated with *S. aureus* carriage in children,[23] which is again demonstrated in the present study. Pneumococcal conjugate vaccine may reduce vaccine-serotype pneumococcal carriage among recipients. Previous studies also mentioned that MRSA probably arose due to antibiotic selective pressure.[24] In this study, the only impact of recent antibiotics use was reduction of *S. aureus* carriage but nothing to do with MRSA. Different kinds of antibiotics used might affect the results, which needs further studies.

Younger age between 2 and 6 months was also significantly associated with MRSA colonization in the present study, which is consistent with previous studies.[11,25]

The two major clones identified in the present study are the same as those reported previously from Taiwan and are ST59/PFGE C/SCC*mec* IV/PVL-negative, known as the Asia-Pacific clone, and ST59/PFGE D/SCC*mec* V_T/PVL-positive, known as Taiwan clone. They are characterized with relatively low drug resistance but usually resistant to clindamycin and erythromycin, which is different from that of USA300 strain. For CA-MRSA isolates, instead of USA300 prevailing in the United States, the clones in Asian countries are heterogeneous,[9,15] like Europe.[26] Additionally, two new clones accounting for more than 20 isolates were identified in the present study and included ST 508/pulsotype AK/SCC*mec* IV/PVL-negative and ST 573/pulsotype U/SCC*mec* IV/PVL-negative. The former clone was first identified in 2006 and increased markedly since 2007, while the latter clone was first identified in 2007 and increased since 2008. Most isolates of both clones were sensitive to clindamycin, which resulted in significant increase of clindamycin susceptibility rate during the later period from 2008 and 2010 in this study. The clone of ST 573 was reported to be a dominant clone in our previous study[25] regarding a longitudinal survey of MRSA colonization among 304 infants conducted during 2009 and 2011. The clinical significance and impact of the emergence of this clone in Taiwan needs further studies and observation.

There are several limitations in this study. First, this is a cross-sectional study and we did not follow up the patients to assess the correlation of colonization with subsequent infection. Second, only one site (nares) was swabbed for samples, which may underestimate the prevalence of MRSA colonization. The more sampling sites were obtained, the more MRSA colonization could be detected.[27] Lastly, given distinct strain types/lineages present in different countries, these findings may not be generalizable outside of Taiwan.

Conclusions

Nasal MRSA colonization rate among otherwise healthy children less than five years of age in northern Taiwan was 10.2% during 2005 and 2010, and the rate was relatively steady during the study period. Young infants aged 2—6 months had a highest colonization rate. Pneumococcal colonization and breastfeeding are negatively associated with MRSA colonization. Though the clone of ST 59 was still the major clone in Taiwan, two new clones of ST 573 and ST 508 were identified and the clinical significance needs further surveillance.

Acknowledgment

This study was supported by the grants from Chang Gung Memorial Hospital (CMRPG3C1582 and CMRPG3C1883).

References

1. Jevons MP. "Celbenin"-resistant staphylococci. *BMJ* 1961;**1**:124—5.
2. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *PNAS* 2002;**99**:7687—92.
3. David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev* 2010;**23**:616—87.
4. Liu C, Graber CJ, Karr M, Diep BA, Basuino L, Schwartz BS, et al. A population-based study of the incidence and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* disease in San Francisco, 2004—2005. *Clin Infect Dis* 2008;**46**:1637—46.
5. Boyle-Vavra S, Ereshefsky B, Wang CC, Daum RS. Successful multiresistant community-associated methicillin-resistant *Staphylococcus aureus* lineage from Taipei, Taiwan, that carries either the novel staphylococcal chromosome cassette mec (SCCmec) type VT or SCCmec type IV. *J Clin Microbiol* 2005;**43**:4719—30.
6. Chen CJ, Huang YC, Chiu CH, Su LH, Lin TY. Clinical features and genotyping analysis of community-acquired methicillin-resistant *Staphylococcus aureus* infections in Taiwanese children. *Pediatr Infect Dis J* 2005;**24**:40—5.
7. Huang YC, Chen CJ. Community-associated meticillin-resistant *Staphylococcus aureus* in children in Taiwan, 2000s. *Int J Antimicrob Agents* 2011;**38**:2—8.
8. Huang YC, Su LH, Chen CJ, Lin TY. Nasal carriage of methicillin-resistant *Staphylococcus aureus* in school children without identifiable risk factors in northern Taiwan. *Pediatr Infect Dis J* 2005;**24**:276—8.
9. Chen CJ, Huang YC. New epidemiology of *Staphylococcus aureus* infection in Asia. *Clin Microbiol Infect* 2014;**20**:605—23.
10. Huang YC, Hwang KP, Chen PY, Chen CJ, Lin TY. Prevalence of methicillin-resistant *Staphylococcus aureus* nasal colonization among Taiwanese children in 2005 and 2006. *J Clin Microbiol* 2007;**45**:3992—5.
11. Chen CJ, Hsu KH, Lin TY, Hwang KP, Chen PY, Huang YC. Factors associated with nasal colonization of methicillin-resistant *Staphylococcus aureus* among healthy children in Taiwan. *J Clin Microbiol* 2011;**49**:131—7.
12. Clinical and Laboratories Standards Institute. *Perform stand antimicrob susceptibility test; 21st informational supplement*. 2011. M100-s21.
13. Oliveira DC, de Lencastre H, Multiplex PCR. Strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2002;**46**:2155—61.
14. 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.
15. Chuang YY, Huang YC. Molecular epidemiology of community-associated meticillin-resistant *Staphylococcus aureus* in Asia. *Lancet Infect Dis* 2013;**13**:698—708.
16. Lo WT, Wang CC, Lin WJ, Wang SR, Teng CS, Huang CF, et al. Changes in the nasal colonization with methicillin-resistant *Staphylococcus aureus* in children: 2004—2009. *PLoS One* 2010;**5**:e15791.
17. Johnson NB, Hayes LD, Hoo EC, Ethier KA. CDC National Health Report: leading causes of morbidity and mortality and associated risk and protective factors — United States, 2005—2013. *MMWR Suppl* 2014;**63**:3—27.
18. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying panton-valentine leukocidin genes worldwide emergence. *Emerg Infect Dis* 2003;**9**:978—84.
19. Eibach D, Nagel M, Hogan B, Azuure C, Krumkamp R, Dekker D, et al. Nasal carriage of *Staphylococcus aureus*

among children in the Ashanti region of Ghana. *PLoS One* 2017;**12**:e0170320.
20. Sdougkos G, Chini V, Papanastasiou DA. Community-associated *Staphylococcus aureus* infections and nasal carriage among children- molecular microbial data and clinical characteristics. *Clin Microbiol Infect* 2008;**14**:995—1001.
21. Marks LR, Clementi EA, Hakansson AP. Sensitization of *Staphylococcus aureus* to methicillin and other antibiotics in vitro and in vivo in the presence of HAMLET. *PLoS One* 2013;**8**:e63158.
22. Marks LR, Clementi EA, Hakansson AP. The human milk protein-lipid complex HAMLET sensitizes bacterial pathogens to traditional antimicrobial agents. *PLoS One* 2012;**7**:e43514.
23. Regev-Yochay G, Dagan R, Raz M, Carmeli Y, Shainberg B, Derazne E, et al. Association between carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in children. *JAMA* 2004;**292**:716—20.
24. Shorr AF. Epidemiology of staphylococcal resistance. *Clin Infect Dis* 2007;**45**(Suppl. 3):S171—6.
25. Huang YC, Chen CJ. Nasal carriage of methicillin-resistant *Staphylococcus aureus* during the first 2 years of life in children in northern Taiwan. *Pediatr Infect Dis J* 2015;**34**:131—5.
26. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated methicillin-resistant *Staphylococcus aureus*. *Lancet* 2010;**375**:1557—68.
27. Senn L, Basset P, Nahimana I, Zanetti G, Blanc DS. Which anatomical sites should be sampled for screening of methicillin-resistant *Staphylococcus aureus* carriage by culture or by rapid PCR test? *Clin Microbiol Infect* 2012;**18**:E31—3.