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Original Article

Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* among nasal carriage strains isolated from emergency department patients and healthcare workers in central Taiwan

Tsung-Hua Wu [a,1], Chun-Yi Lee [b,1], Hui-Ju Yang [c], Yu-Ping Fang [b], Yu-Fen Chang [d], Shu-Ling Tzeng [e], Min-Chi Lu [f,g,*]

[a] *Department of Pediatrics, Chang Hua Show Chwan Memorial Hospital, Changhua, Taiwan*
[b] *Department of Pediatrics, Chang Bing Show Chwan Memorial Hospital, Changhua, Taiwan*
[c] *Department Surgical Intensive Care Unit, Chang Hua Show Chwan Memorial Hospital, Changhua, Taiwan*
[d] *Department Laboratory, Chang Bing Show Chwan Memorial Hospital, Changhua, Taiwan*
[e] *Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan*
[f] *Division of Infectious Diseases, Department of Internal Medicine, China Medical University Hospital, Taichung, Taiwan*
[g] *Department of Microbiology and Immunology, School of Medicine, China Medical University, Taichung, Taiwan*

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Nasal carriage;
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Abstract *Background and objective:* Screening and identification of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage are helpful for controlling MRSA dissemination in hospitals. The aim of our study was to determine the prevalence of nasal carriages and diversity of MRSA among patients and healthcare workers (HCWs) at two regional hospitals in Taiwan.
Methods: Nasal swabs were obtained prospectively from 204 patients visiting the emergency department (ED) and 326 HCWs in two regional hospitals in Changhua, Taiwan, between February 2015 and June 2015. All the MRSA isolates were further molecularly characterized.
Results: Of the 204 participating patients, the nasal carriage rates of *S. aureus* and MRSA were 22.1% and 7.8%, respectively. For HCWs, the *S. aureus* and MRSA carriage rates were 26.1% and 6.1%, respectively. There was no statistically significant difference in MRSA carriage rate

* Corresponding author. Department of Microbiology and Immunology, School of Medicine, China Medical University, Taichung, Taiwan.
E-mail address: luminchi@hotmail.com (M.-C. Lu).
[1] These authors contributed equally to this work.

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between patients and HCWs ($P = 0.447$). Patients receiving hemodialysis were significantly associated with MRSA colonization ($P = 0.012$). The leading three sequence types (ST) were ST59 (16, 44.4%), ST45 (11, 30.6%), and ST239 (3, 8.3%) for all 36 MRSA isolates. ST59/SCC*mec* IV/t437/PVL-negative and ST45/SCC*mec* V/t1081/PVL-negative were the predominant clones among HCWs (30%) and participating patients (19%), respectively.
Conclusion: Overall, a substantial proportion of patients visiting the ED and HCWs harbored CA-MRSA, mostly ST59 strains, in their nares. It is noteworthy that MRSA ST45 strains supplanted ST239 as the second leading nasal MRSA colonization strain in our study.

Introduction

Staphylococcus aureus, an important pathogen both in healthcare and community settings, causes a broad spectrum of diseases ranging from skin and soft-tissue infections to invasive diseases such as pneumonia, septicemia, osteomyelitis, and endocarditis.[1] The prevalence of methicillin-resistant *S. aureus* (MRSA) strains is increasing. MRSA, considered a hospital pathogen until the late 1990s, is classified as community-associated MRSA (CA-MRSA) and healthcare-associated MRSA (HA-MRSA) according to epidemiological or molecular characteristics.[2]

CA-MRSA strains have no healthcare-associated risk factors, including recent hospital admission or surgery, long-term accommodation in health care facilities, use of indwelling catheter, or hemodialysis.[3] The differences in the molecular characteristics of CA-MRSA and HA-MRSA include the relatively low antibiotic resistance and the presence of Panton-Valentine leukocidin (PVL) and type IV or V staphylococcal cassette chromosome (SCC*mec*) in CA-MRSA strains.[4] However, different types of MRSA clones have spread between communities and hospitals, particularly increasing the number of endemic CA-MRSA strains in many hospitals in the USA and Taiwan.[4,5]

Nasal colonization by *S. aureus*, including MRSA, is known to be a high risk factor for subsequent infection.[6] In Taiwan, previous studies reported that nasal MRSA colonization was common among children, with increase in carriage rate from 1.9% in 2001 to 11.6% in 2009 among well-child healthcare visits or school children.[7–9] However, the nasal MRSA carriage rate in pediatricians (6.8%) and healthcare workers (HCWs) (5.0–7.8%) were higher than that in the general adult population (3.8%).[4,10–12]

Strategies for controlling MRSA transmission in hospitals require baseline information on the prevalence and characteristics of circulating MRSA strains. Patients and HCWs are at the interface between hospitals and communities; particularly, patients visiting the emergency department (ED) may come from different settings. Screening and identification of MRSA carriage is helpful for controlling MRSA dissemination in hospitals. We conducted this study to determine the prevalence of nasal carriages and diversity of MRSA among patients visiting the ED and HCWs and to identify the risk factors associated with MRSA colonization.

Materials and methods

Study design

This study was approved by the Institutional Review Board of Show Chwan Memorial Hospital (IRB No. 1030906). A written informed consent was obtained from each subject before each survey for nasal carriage of MRSA. This study was conducted between February and June 2015 at two regional hospitals (Chang Hua Show Chwan Memorial Hospital and Chang Bing Show Chwan Memorial Hospital) situated in central Taiwan. Patients above 18 years of age visiting the ED of the two hospitals were invited and surveyed for nasal carriage of MRSA. HCWs working in the ED and intensive care unit (ICU) of the two hospitals were also invited and surveyed for nasal carriage of MRSA. A questionnaire regarding the risk factors for MRSA acquisition was also obtained. Demographic information included participants' age, gender, and working department. Information on any chronic underlying disease, including hypertension, diabetes mellitus, cardiovascular disease, hepatobiliary disease, dialysis, cancer, and respiratory disease, was also collected.

Laboratory methods

Nasal swab samples were collected using sterile swabs, from both anterior nares of each subject. Each swab was rubbed against the anterior 1 cm of the nasal vestibular wall of both nares. Then the swabs were sent to the microbiological laboratory for culture. There, swabs were streaked on 5% blood agar plates (BD Diagnostics, Sparks, MD, USA) and incubated at 37 °C overnight. *S. aureus* that were positive for the *mecA* gene and non-susceptible to cefoxitin by the disk diffusion method were identified as MRSA isolates according to the recommendations of the Clinical and Laboratory Standards Institute.[13]

Antimicrobial susceptibility testing

The susceptibilities of MRSA isolates were determined by BD Phoenix™ Automated Microbiology System PMIC/ID-62 containing 14 antibiotics, including clindamycin, cefazolin, daptomycin, erythromycin, cefoxitin, gentamicin, fusidic acid, linezolid, oxacillin, penicillin, trimethoprim/

sulfamethoxazole, tetracycline, teicoplanin, and vancomycin. Another antibiotic levofloxacin (5 μg bd) was determined by the disk-diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute.

Molecular typing

Chromosomal DNA was extracted from each MRSA isolate for molecular characterization. According to the measures described previously, all of them were characterized by SCC*mec* typing,[14,15] *spa* gene typing,[16] detection of the presence of PVL genes and *sas-X* genes, and multilocus sequence typing (MLST).[17–19]

Statistics

Continuous data obtained are expressed as the mean ± standard deviation (SD), and categorical data was examined using the chi-square test in Microsoft Excel version 2010. Fisher's exact test was performed when any expected count was less than five by statistical analysis. P values < 0.05 indicated statistically significant differences.

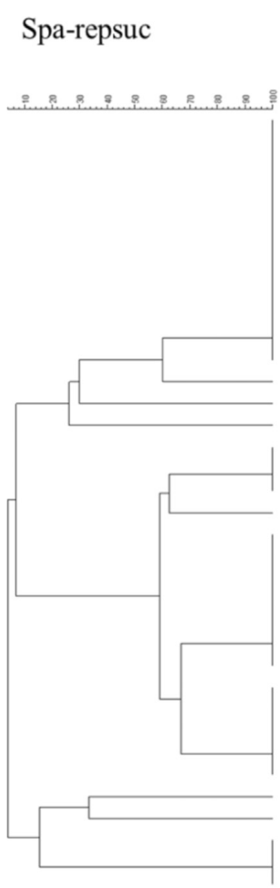
Results

In total, 530 adult eligible subjects were enrolled in this study, including 204 patients visiting the ED and 326 HCWs. The age of patients ranged from 19 to 91 years, with a mean age of 49.84 years, and 116 patients (56.9%) were male. Age of HCWs ranged from 21 to 67 years, with a mean age of 33.46 years, and 73 HCWs (22.4%) were male.

The overall prevalence of *S. aureus* and MRSA nasal carriage was 24.5% and 6.8%, respectively. The *S. aureus* and MRSA carriage rates were 22.1% and 7.8%, respectively, for patients visiting the ED, and 26.1% and 6.1%, respectively, for the HCWs. There was no statistically significant difference in the nasal carriage rate of MRSA between patients visiting the ED and HCWs ($P = 0.447$).

In terms of potential risk factors for MRSA acquisition, including age, gender, and underlying diseases, there was no statistically significant difference between participants (ED patients and HCWs) who did or did not harbor MRSA. However, the ED patients receiving hemodialysis ($P = 0.012$) were found to be significantly associated with nasal MRSA colonization.

The molecular characterization of all 36 MRSA isolates from 16 patients visiting the ED and 20 HCWs is shown in Fig. 1. Most MRSA isolates belonged to three major lineages



Spa-repsuc

rs_001	rs_002	rs_003	rs_004	rs_005	rs_006	rs_007	rs_008	rs_009	rs_010	Key	Spa Type	SCCmec type	PVL	sas-X	MLST	Staff	Hospital	Medical unit
5	21	18	21	18	26	34				045	t437	VT			59		A	
5	21	18	21	18	26	34				043	t437	VT	(+)		59		A	
5	21	18	21	18	26	34				039	t437	VT			59		A	
5	21	18	21	18	26	34				033	t437	IV			59	V	A	NCU
5	21	18	21	18	26	34				030	t437	IV			59	V	A	ED
5	21	18	21	18	26	34				040	t437	IV			59	V	A	MICU
5	21	18	21	18	26	34				024	t437	IV			59		A	
5	21	18	21	18	26	34				034	t437	IV			59	V	B	NICU
5	21	18	21	18	26					052	t3592	IV			59		B	
5	21	18	21	18	26	34				061	t437	IV			59		B	
5	21	18	21	18	26	34				059	t437	IV			59	V	B	ED
5	21	18	21	18	26	34				057	t437	IV			59	V	B	ED
5	21	18	26	34						032	t441	VT	(+)		59	V	A	ED
5	21	21	18	21	18	26	34			053	t4350	IV			59	V	B	ED
27	24	18	34	18	21	18	13	18	17	051	t002	III			5	V	B	ED
9	17	3	17	34	14	18	34	17	34	041	t015	IV			508		A	
9	17	3	17	34	14	18	34	17	34	028	t015	IV			508	V	A	ED
9	17	3	17	34	14	18	34	17	34	042	t015	IV			508	V	A	MICU
9	17	3	17	3	26	18	25			056	t019	IV			30		B	
9	17	3	42	34	18	34				038	t1081	V			45	V	A	SICU
9	17	3	42	34	18	34				020	t1081	IV			45	V	A	ED
9	17	3	42	34	18	34				064	t1081	V			45	V	B	ED
9	17	3	42	34	18	34				062	t1081	IV			45	V	B	ED
9	17	3	42	34	18	34				060	t1081	V			45		B	
9	17	3	42	34	18	34				058	t1081	V			45		B	
9	17	3	42	34	18	34				054	t1081	V			45		B	
9	17	34								047	t026	IV			45		A	
9	17	34								037	t026	IV			59	V	A	ED
9	17	34								048	t026	IV			45	V	A	MICU
9	17	34								055	t026	IV			45	V	B	ED
9	17	34								049	t026	IV			45	V	B	SICU
12	18	21	18	26	34					026	t4145	VT	(+)		59		A	
12	20	13	22	18	34	25	34	23	26	018	t008	IV	(+)		8		A	
16	13	17	3	26	18	25				035	t037	III		(+)	239	V	A	SICU
16	13	17	3	26	18	25				022	t037	III		(+)	239		A	
16	13	17	3	26	18	25				063	t037	IIIA		(+)	239		B	

Figure 1. Molecular characteristics of 36 methicillin-resistant *S. aureus* (MRSA) isolates. Most MRSA isolates belonged to sequence type (ST) 59 and ST45. Abbreviations: SCC*mec*, staphylococcal cassette chromosome; PVL, Panton-Valentine leukocidin genes; MLST, multilocus sequence type. Hospital A, Chang Hua Show Chwan Memorial Hospital; Hospital B, Chang Bing Show Chwan Memorial Hospital. NCU, Neurologic Intensive Care Unit; ED, Emergency Department; MICU, Medical Intensive Care Unit; NICU, Neonatal Intensive Care Unit; SICU, Surgical Intensive Care Unit.

as sequence type (ST) 59 (16 isolates, 44.4%), ST45 (11 isolates, 30.6%), and ST239 (3 isolates, 8.3%), respectively. The ST59 lineage was further classified into two clones, namely, SCC*mec* type IV (11 isolates) and SCC*mec* type V_T (5 isolates). The ST45 lineage was also further classified into two clones harboring the SCC*mec* type IV (6 isolates) and type V (5 isolates). Most *spa* types were t437 (all 11 isolates belonged to ST59) and t1081 (all 7 isolates belonged to ST45). PVL genes were detected in four isolates carrying ST59/SCC*mec* V_T/*spa* t437, ST59/SCC*mec* V_T/*spa* t441, ST59/SCC*mec* V_T/*spa* t4145, and ST8/SCC*mec* IV/*spa* t008. The *sas-X* genes were detected in three isolates harboring ST239/SCC*mec* III or IIIA/*spa* t037. ST59/SCC*mec* IV/t437/PVL-negative and ST45/SCC*mec* V/t1081/PVL-negative were the predominant clones among HCWs (30%) and participating patients (19%), respectively. In addition, the molecular characteristics of the 20 MRSA isolates from HCWs and their medical units were also appeared in Fig. 1. The rate of MRSA carriage was higher in HCWs of the ED (Chang Hua Show Chwan Memorial Hospital, 6.7%; Chang Bing Show Chwan Memorial Hospital, 9.6%). Moreover, the MRSA strains of HCWs were diverse and did not show any cluster infection.

Table 1 shows the antimicrobial susceptibilities of all 36 MRSA isolates to different antibiotics, stratified by MLST type. All these isolates were susceptible to vancomycin, teicoplanin, daptomycin, and linezolid, but were resistant to penicillin, cefazolin, cefoxitin, and oxacillin. Susceptibility to clindamycin, erythromycin, fusidic acid, gentamicin, trimethoprim/sulfamethoxazole (TMP-SMX), tetracycline, and levofloxacin was detected in 31%, 33%, 89%, 58%, 86%, 69%, and 72% isolates, respectively. Among MRSA isolates, the ST59 and ST45 lineages showed >80% susceptibility rates for fusidic acid and TMP-SMX. Five isolates belonged to ST45 with SCC*mec* V were resistant to tetracycline and levofloxacin. However, ST239 with SCC*mec* III showed multiresistance to clindamycin, erythromycin, gentamicin, TMP-SMX, and tetracycline.

Discussion

In this study, the MRSA nasal carriage rate in adult patients who visited the ED at two regional hospitals in central Taiwan was found to be 6.8%. This is higher than a previously reported MRSA nasal carriage rate of 3.8% in adult patients who visited the ED at hospitals in northern Taiwan in 2009,[20] and the MRSA nasal carriage rate of 1.5% determined from the nasal sample data of healthy college students in northern Taiwan collected in 2013.[21] However, this MRSA nasal carriage rate is significantly lower than the rates of 14.9—32% obtained from research data of patients in adult intensive care units in Taiwan hospitals.[22—24] In addition, large differences exist in the MRSA nasal carriage rate among various studies on patients who visited the ED in overseas hospitals. For instance, the rate in Iran was 3.2% in a 2014 report,[25] the rate in the USA was 13.5% in 2008—2009,[26] and the rate reported in Japan was 31.4% in 2009—2011.[27] A subset of patients who visit the adult ED may shuttle between hospitals and long-term care facilities frequently, and therefore, the MRSA nasal carriage rate in such patients is expected to be higher than that in typical community residents. Similarly, in this study, the MRSA nasal carriage rate in 326 HCWs from the ED and various intensive care units was 6.1%. During the 5-month study, clusters of MRSA infection did not occur within the health care units where the HCWs participating in this study worked. In comparison, the MRSA nasal carriage rates obtained in other studies on HCWs in Taiwan ranged from 5.0 to 7.8%,[4] and a rate of 7.6% was reported in another study on hospital workers in Serbia,[28] which are similar to the MRSA nasal carriage rate determined in this study.

An analysis of the potential risk factors associated with nasal MRSA colonization was attempted in this study. Among patients who visited the ED, only those receiving hemodialysis had a significant association with nasal MRSA colonization ($P = 0.012$). For HCWs, no statistically significant associations between risk factors and nasal MRSA colonization were found. However, in other studies conducted on domestic or foreign patients, the most commonly observed risk factor associated with nasal MRSA colonization was the presence of an indwelling catheter in the body.[20,29] We observed that for patients receiving hemodialysis, the prolonged presence of the dialysis catheters increased the risk of MRSA infection or colonization associated with the use of these catheters. In a study on the hemodialysis population in Taiwan, nasal swab samples were obtained before and after a certain period of hemodialysis, and it was observed that the MRSA carriage rate increased from 3.8% to 6.9%. However, in this study, the

Table 1 Distribution of antimicrobial susceptibility rates of 36 methicillin-resistant *S. aureus* (MRSA) isolates stratified by multilocus sequence typing (MLST).

MLST type	No.	CC, No. (%)	E, No.(%)	FA, No. (%)	GM, No. (%)	SXT, No. (%)	TE, No. (%)	LEV, No. (%)
Total	36	11 (31)	12 (33)	32 (89)	21 (58)	31 (86)	25 (69)	26 (72)
59	16	2 (13)	2 (13)	16 (100)	8 (50)	16 (100)	13 (81)	16 (100)
45	11	5 (45)	5 (45)	9 (82)	9 (82)	11 (100)	6 (55)	6 (55)
239	3	0 (0)	0 (0)	2 (67)	0 (0)	0 (0)	0 (0)	0 (0)
508	3	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)
5	1	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
8	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)
30	1	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)

CC, clindamycin; E, erythromycin; FA, fusidic acid; GM, gentamicin; SXT, trimethoprim/sulfamethoxazole; TE, tetracycline; LEV, levofloxacin.

strains identified in the nares of the subjects were CA-MRSA strains, and other risk factors associated with nasal MRSA colonization in hemodialysis patients were not found.[30] Several studies have reported a higher proportion of nasal MRSA colonization in males than in females for HCWs[29,31]; however, this was not observed in the present study.

In this study, MLST assigned 44.4% MRSA strains to ST59, and in particular, ST59/SCC*mec* IV/PVL-negative and ST59/SCC*mec* V_T/PVL-positive were the predominant clones, which are consistent with the current endemic CA-MRSA strains in Taiwan.[4,32] The ST59/SCC*mec* IV/PVL-negative clone is also known as the Asian-Pacific clone, and is the major nasal MRSA colonizing strain among healthy population in communities. The ST59/SCC*mec* V_T/PVL-positive clone, also known as the Taiwan clone, possesses higher toxicity compared to the Asian-Pacific clone, and is commonly isolated from patients with serious MRSA infections.[32] Epidemiological data on MRSA in Asian countries showed that heterogeneity exists in the distribution of CA-MRSA among different countries and regions. In addition to Taiwan, ST59 strains are endemic in China, Vietnam, Singapore, and Hong Kong.[7]

Another noteworthy point is that ST45 strains accounted for 30.6% of all MRSA isolates in this study, and thus occupied second position after ST59. Therefore, ST45 has supplanted ST239, which belongs to the HA-MRSA strains, and was reported as the second leading sequence type in previous Taiwanese studies.[4,33] ST45 strains mainly carry SCC*mec* type IV or V, and were first observed in Berlin hospitals in 1993; therefore, the strains are also known as the Berlin epidemic MRSA.[33] In recent years, ST45 strains have also been reported in different regions of Asia. Furthermore, studies conducted in Germany, Singapore, and Hong Kong showed that ST45 strains of MRSA are gradually posing a new threat in nursing homes or long-term care facilities.[34–36] In Taiwan, MRSA was the most common pathogen isolated from patients and environmental sources in long-term care facilities.[37] A study involving 523 subjects, who consisted of residents and employees of 14 nursing homes in Taiwan, was conducted in 2012. Results of the study indicated that ST45 accounted for half of the strains responsible for the nasal carriage rate of 20.1% among the subjects.[38] However, although ST45 strains are endemic in nursing homes or long-term care facilities, no study other than ours reported proportions of ST45 strains up to 30% in MRSA nasal colonization in Taiwanese hospitals. Further investigation is required to determine whether the increase in the proportion of ST45 strains is due to the dissemination of this strain via frequent shuttling of residents between nursing homes or long-term care facilities and hospitals for health care needs.

It is well-known that the antibiotic resistance gene *mec*A harbored by SCC*mec* is responsible for the antibiotic resistance of MRSA. However, in recent years, oxacillin-susceptible MRSA (OS-MRSA) strains have been identified in Taiwan and foreign countries.[39,40] OS-MRSA strains are characterized by the following characteristics: minimum inhibitory concentration (MIC) of oxacillin <2 μg/ml, *mec*A positivity, and preponderance of clones belonging to CA-MRSA SCC*mec* type V or V_T corresponding to ST59/t437 and ST45/t1081.[41] There is a need for careful evaluation of the performance of these methods for the detection of OS-MRSA to prevent their further spread in community settings.

This study is not without limitations. First, too few participants, regardless of whether they were patients visiting the ED or HCWs, were enrolled in the study. In addition, the proportions of HCWs from different health care units within the hospitals participating in this study varied, and therefore, the results obtained are not completely representative of the nasal carriage rates of the respective hospital units. Second, in addition to age, gender, and diseases of the patients, other important risk factors associated with MRSA acquisition were not recorded in this study, e.g., records of recent hospitalizations, antibiotic use, habits such as smoking or betel nut chewing, and place of residence of patients visiting the ED (nursing homes or long-term care facilities). Third, due to the limitation of resources in the microbiological laboratory, the MIC of vancomycin for MRSA strains was not tested. In addition, as the inhibition zone size determined using the vancomycin disk test correlated poorly with susceptibility test results obtained using the recommended standard dilution method, VSSA (vancomycin susceptible *S. aureus*, MIC < 2 μg/ml) and VISA (vancomycin intermediate *S. aureus*, MIC 4–8 μg/ml) could not be accurately distinguished. Therefore, at present, the vancomycin disk diffusion method is no longer recommended. Lastly, pulsed-field gel electrophoresis (PFGE) was not included in the molecular biology tests of MRSA strains; therefore, evidence regarding genetic relationships and similarities between MRSA strains is slightly inadequate.

In this study, nasal MRSA colonization was observed in 7.8% patients visiting the ED and 6.8% HCWs. Statistical analysis of data showed that a history of hemodialysis in patients visiting the ED was a risk factor associated with MRSA colonization. Characterization of the nasal MRSA isolates revealed that three-quarters of isolates belonged to CA-MRSA strains, and the leading sequence type was ST59, which is consistent with the current endemic CA-MRSA strains in Taiwan. However, a significant increase in the prevalence of ST45 strains was also observed in this study, with ST45 supplanting ST239 as the second leading sequence type. Therefore, a gradual dissemination of ST45 may occur within communities and hospitals.

References

1. Lowy FD. *Staphylococcus aureus* infections. *N Engl J Med* 1998; **339**:520–32.
2. 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.
3. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, et al. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA* 2003;**290**:2976–84.
4. Huang YC, Chen CJ. Community-associated meticillin-resistant *Staphylococcus aureus* in children in Taiwan, 2000s. *Int J Antimicrob Agents* 2011;**38**:2–8.
5. Bratu S, Eramo A, Kopec R, Coughlin E, Ghitan M, Yost R, et al. Community-associated methicillin-resistant *Staphylococcus aureus* in hospital nursery and maternity units. *Emerg Infect Dis* 2005;**11**:808–13.

6. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. *N Engl J Med* 2001;**344**:11–6.
7. Chen CJ, Huang YC. New epidemiology of *Staphylococcus aureus* infection in Asia. *Clin Microbiol Infect* 2014;**20**:605–23.
8. 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.
9. 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.
10. 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.
11. Wang JT, Liao CH, Fang CT, Chie WC, Lai MS, Lauderdale TL, et al. Prevalence of and risk factors for colonization by methicillin-resistant *Staphylococcus aureus* among adults in community settings in Taiwan. *J Clin Microbiol* 2009;**47**:2957–63.
12. Huang YC, Su LH, Lin TY. Nasal carriage of methicillin-resistant *Staphylococcus aureus* among pediatricians in Taiwan. *PLoS One* 2013;**8**:e82472.
13. Clinical and Laboratory Standard Institute (CLSI). *Performance standards for antimicrobial susceptibility testing; 20th informational supplement*. CLSI document M100-S19. Wayne:PA: CLSI; 2009.
14. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, et al. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. *Antimicrob Agents Chemother* 2007;**51**:264–74.
15. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2005;**43**:5026–33.
16. Harmsen D, Claus H, Witte W, Rothganger J, Turnwald D, Vogel U. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol* 2003;**41**:5442–8.
17. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, et al. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* 1999;**29**:1128–32.
18. Li M, Du X, Villaruz AE, Diep BA, Wang D, Song Y, et al. MRSA epidemic linked to a quickly spreading colonization and virulence determinant. *Nat Med* 2012;**18**:816–9.
19. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000;**38**:1008–15.
20. Lu SY, Chang FY, Cheng CC, Lee KD, Huang YC. Methicillin-resistant *Staphylococcus aureus* nasal colonization among adult patients visiting emergency department in a medical center in Taiwan. *PLoS One* 2011;**6**:e18620.
21. Chang CJ, Chen NC, Lao CK, Huang YC. Nasal *Staphylococcus aureus* and Methicillin-Resistant S. aureus Carriage among Janitors Working in Hospitals in Northern Taiwan. *PLoS One* 2015;**10**, e0138971.
22. Kao KC, Chen CB, Hu HC, Chang HC, Huang CC, Huang YC. Risk factors of methicillin-resistant *Staphylococcus aureus* infection and correlation with nasal colonization based on molecular genotyping in medical intensive care units: a prospective observational study. *Medicine (Baltimore)* 2015;**94**:e1100.
23. Chen CB, Chang HC, Huang YC. Nasal meticillin-resistant *Staphylococcus aureus* carriage among intensive care unit hospitalised adult patients in a Taiwanese medical centre: one time-point prevalence, molecular characteristics and risk factors for carriage. *J Hosp Infect* 2010;**74**:238–44.
24. Wang JT, Liao CH, Fang CT, Chie WC, Lai MS, Lauderdale TL, et al. Incidence of and risk factors for community-associated methicillin-resistant *Staphylococcus aureus* acquired infection or colonization in intensive-care-unit patients. *J Clin Microbiol* 2010;**48**:4439–44.
25. Rezaei M, Moniri R, Mousavi SG. Molecular analysis and susceptibility pattern of meticillin-resistant *Staphylococcus aureus* strains in emergency department patients and related risk factors in Iran. *J Hosp Infect* 2015;**89**:186–91.
26. Kecojevic A, Ranken R, Ecker DJ, Massire C, Sampath R, Blyn LB, et al. Rapid PCR/ESI-MS-based molecular genotyping of *Staphylococcus aureus* from nasal swabs of emergency department patients. *BMC Infect Dis* 2014;**14**:16.
27. Wakatake H, Fujitani S, Kodama T, Kawamoto E, Yamada H, Yanai M, et al. Positive clinical risk factors predict a high rate of methicillin-resistant *Staphylococcus aureus* colonization in emergency department patients. *Am J Infect Contr* 2012;**40**:988–91.
28. Cirkovic I, Stepanovic S, Skov R, Trajkovic J, Grgurevic A, Larsen AR. Carriage and Genetic Diversity of Methicillin-Resistant *Staphylococcus aureus* among Patients and Healthcare Workers in a Serbian University Hospital. *PLoS One* 2015;**10**, e0127347.
29. Peters C, Dulon M, Kleinmuller O, Nienhaus A, Schablon A. MRSA Prevalence and Risk Factors among Health Personnel and Residents in Nursing Homes in Hamburg, Germany - a Cross-Sectional Study. *PLoS One* 2017;**12**, e0169425.
30. Kang YC, Tai WC, Yu CC, Kang JH, Huang YC. Methicillin-resistant *Staphylococcus aureus* nasal carriage among patients receiving hemodialysis in Taiwan: prevalence rate, molecular characterization and de-colonization. *BMC Infect Dis* 2012;**12**:284.
31. Al-Humaidan OS, El-Kersh TA, Al-Akeel RA. Risk factors of nasal carriage of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* among health care staff in a teaching hospital in central Saudi Arabia. *Saudi Med J* 2015;**36**:1084–90.
32. Chen CJ, Unger C, Hoffmann W, Lindsay JA, Huang YC, Gotz F. Characterization and comparison of 2 distinct epidemic community-associated methicillin-resistant i clones of ST59 lineage. *PLoS One* 2013;**8**:e63210.
33. Chen CJ, Huang YC, Su LH, Wu TL, Huang SH, Chien CC, et al. Molecular epidemiology and antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* bloodstream isolates in Taiwan, 2010. *PLoS One* 2014;**9**, e101184.
34. Ghebremedhin B, Konig W, Konig B. Heterogeneity of methicillin-resistant *Staphylococcus aureus* strains at a German university hospital during a 1-year period. *Eur J Clin Microbiol Infect Dis* 2005;**24**:388–98.
35. Ho PL, Chow KH, Lo PY, Lee KF, Lai EL. Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* associated with spread of the ST45 lineage in Hong Kong. *Diagn Microbiol Infect Dis* 2009;**64**:131–7.
36. Chow A, Lim VW, Khan A, Pettigrew K, Lye DCB, Kanagasabai K, et al. MRSA transmission dynamics among interconnected acute, intermediate-term, and long-term healthcare facilities in Singapore. *Clin Infect Dis* 2017;**64**:S76–81.
37. Lee CM, Lai CC, Chiang HT, Lu MC, Wang LF, Tsai TL, et al. Presence of multidrug-resistant organisms in the residents and

environments of long-term care facilities in Taiwan. *J Microbiol Immunol Infect* 2017;**50**:133—44.

38. Tsao FY, Kou HW, Huang YC. Dissemination of methicillin-resistant *Staphylococcus aureus* sequence type 45 among nursing home residents and staff in Taiwan. *Clin Microbiol Infect* 2015;**21**:451—8.
39. Chen FJ, Hiramatsu K, Huang IW, Wang CH, Lauderdale TL. Panton-Valentine leukocidin (PVL)-positive methicillin-susceptible and resistant *Staphylococcus aureus* in Taiwan: identification of oxacillin-susceptible mecA-positive methicillin-resistant *S. aureus*. *Diagn Microbiol Infect Dis* 2009;**65**:351—7.
40. Chen FJ, Huang IW, Wang CH, Chen PC, Wang HY, Lai JF, et al. mecA-positive *Staphylococcus aureus* with low-level oxacillin MIC in Taiwan. *J Clin Microbiol* 2012;**50**:1679—83.
41. Ho CM, Lin CY, Ho MW, Lin HC, Chen CJ, Lin LC, et al. Methicillin-resistant *Staphylococcus aureus* isolates with SCCmec type V and spa types t437 or t1081 associated to discordant susceptibility results between oxacillin and cefoxitin, Central Taiwan. *Diagn Microbiol Infect Dis* 2016;**86**:405—11.