Emergence of community-associated methicillin-resistant *Staphylococcus aureus* in Singapore: a further six cases

Hsu L Y, Koh T H, Tan T Y, Ito T, Ma X X, Lin R T, Tan B H

ABSTRACT

Introduction: The clinical features and molecular epidemiology of further cases of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infection in Singapore are described.

Methods: Six cases of CA-MRSA infection that occurred between April and October 2004 are described. The bacterial isolates were tested for the presence of Panton-Valentine leukocidin (PVL) genes and typed via pulsed-field gel electrophoresis, staphylococcal chromosomal cassette *mec* (SCC*mec*) and multi-locus sequence typing. The results were compared with that of previously-reported local and international CA-MRSA isolates.

Results: There were four cases of cutaneous abscesses and one each of chronic osteomyelitis and endocarditis. CA-MRSA isolates from the last two cases tested negative for PVL genes. Three isolates were identical and related to the Oceanian clone, and one isolate to the predominant Taiwanese clone. The isolate causing osteomyelitis had a novel sequence type.

Conclusion: CA-MRSA, though uncommon, is being isolated with increasing frequency in Singapore. A predominant clone (ST30-MRSA-IV) seems to be emerging locally.

Keywords: community infection, Methicillin-resistant *Staphylococcus aureus*, Methicillin resistance, Panton-Valentine leukocidin, *Staphylococcus aureus*

Singapore Med J 2006; 47(1):20-26

Department of Internal Medicine
Singapore General Hospital
Outram Road
Singapore 169608

Hsu L Y, MBBS, MRCP, DTMH
Associate Consultant

Tan B H, MBBS, MRCP
Consultant and Head

Department of Pathology
Singapore General Hospital

Koh T H, MBChB, MRCPath, FAMS
Consultant

Department of Laboratory Medicine
Changi General Hospital
2 Simei Road Street 3
Singapore 523889

Tan T Y, MBBS, MRCPath
Consultant

Department of Bacteriology
School of Medicine
Juntendo University
2-1-1 Hongo
Bunkyo-ku
Tokyo
113-8421 Japan

Ito T, MD, PhD
Associate Professor

Ma X X, MD, PhD
Research Associate

Department of Pathology
KK Women's and Children's Hospital
100 Bukit Timah Road
Singapore 229899

Lin R T, MBBS, FRCPA, FAMS
Consultant and Head

Correspondence to:
Dr Hsu Li Yang
Tel: (65) 6321 4976
Fax: (65) 6225 3931
Email: liyang_hsu@yahoo.com

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first isolated in United Kingdom hospitals in 1961, two years after the introduction of methicillin[1]. Methicillin resistance is conferred by the product of the *mec*A gene, an altered penicillin-binding protein (PBP2a or PBP2') with reduced affinity for practically all beta-lactam antibiotics while maintaining effective cell wall building activity[2]. This gene is localised within a mobile genetic element designated staphylococcal chromosome cassette *mec* (SCC*mec*)[3]. Five major structural types of SCC*mec* have been described to date[4], and it is believed that these elements were horizontally transferred into methicillin-susceptible *Staphylococcus aureus* (MSSA) at different time points by distantly-related bacterial species[5].

Epidemic clones from five major lineages of MRSA have spread worldwide and are responsible for practically all healthcare-associated MRSA infections globally[6]. MRSA was introduced into Singapore in the early 1980s[7], and the current two major local healthcare-associated strains are related to the Brazilian clone (ST239-MRSA-III) and to UK-EMRSA-15 (ST22-MRSA-IV)[8]. A small proportion (<5%) of MRSA infections locally are caused by a strain related to the New York/Japan clone (ST5-MRSA-II)[8].

Initially thought to be confined to hospitals and chronic healthcare facilities, sporadic cases of MRSA infections in patients without the usual risk factors for nosocomial MRSA acquisition were first noted in Michigan, USA in 1981[9]. This phenomenon was subsequently noticed again in the late 1980s in West Australia[10] and Chicago[11]. The term "community-associated MRSA" (CA-MRSA) was coined for this phenomenon, which has since become global in scope[12-17], reaching epidemic proportions in parts of the USA where conditions of overcrowding and poor sanitation prevail[18,19].

The concept that CA-MRSA sprang from feral nosocomial isolates that had somehow managed to thrive in the community was dispelled by a series of comparison studies demonstrating differing clinical presentations and antimicrobial susceptibilities[20], SCC*mec* types[21,22], and genetic heritage[23]. Of note is the fact that the majority of CA-MRSA isolates possessed geographically-related genetic backgrounds, SCC*mec* IV and Panton-Valentine

leukocidin (PVL) genes[22], although this is not necessarily true of CA-MRSA described from all countries[16,23].

The predominant concern with CA-MRSA is neither the clinical diseases nor the epidemiological problems caused by these organisms; rather, it is the fact that this phenomenon probably represents yet another evolutionary step forward for *Staphylococcus aureus*[24]. It is conceivable that the development of methicillin resistance will mirror that of penicillin resistance, which had initially also been restricted to nosocomial *S. aureus* strains, but is now so widespread that few doctors would contemplate using penicillin for the empiric treatment of staphylococcal infections.

We had recently described a small series of local CA-MRSA cases identified between January 1, 1997 and April 15, 2004 at the Singapore General Hospital (SGH)[25]. No predominant clone existed in that series, and the majority of those isolates were likely to have been imported from overseas. However, given the experience of physicians in other parts of the world[12,15,16,18,19], it was clear that further monitoring of the situation was required. Since then, we have started a basic surveillance program aimed at identifying likely CA-MRSA infections at SGH. Microbiologists and infectious diseases physicians from other public hospitals in Singapore have also been invited to send us MRSA isolates with unusual antibiograms or clinical presentations for typing, and some of these isolates have met the epidemiological criteria for CA-MRSA.

METHODS

Multi-susceptible (defined by susceptibility to trimethoprim-sulfamethoxazole and gentamicin as determined by the Kirby-Bauer disk diffusion method following NCCLS guidelines[26]) MRSA strains isolated at SGH Microbiology Lab between April 16 and October 30, 2004 were screened for likelihood of community acquisition. There were also two MRSA isolates contributed by Changi General Hospital (CGH) and one MRSA isolate contributed by KK Women's and Children's Hospital (KKWCH) during this period.

S. aureus were identified by colony morphology, coagulation of citrated rabbit plasma with EDTA (BBL Becton Dickinson and company, Cockeysville, MD, USA), and production of clumping factor and protein A (BactiStaph, Remel, Lenexa, KS, USA). Methicillin resistance was determined by susceptibility testing using an oxacillin disk[26]. Strains were deemed to be community-associated if they had been isolated within 48 hours of hospitalisation in patients who had not been admitted to any healthcare facility for one year prior to the current hospitalisation.

Clinical and epidemiological data were obtained via a review of the patients' medical records. Travel and relevant contact history was obtained via telephone interview with the patients or their family.

Community-associated strains were tested for the presence of PVL genes as previously described[27]. The following primers were used:
luk-PV1, 5´-ATCATTAGGTAAAATGTCTGGACA TGATCCA-3´
luk-PV2, 5´-GCATCAAGTGTATTGGATAGCAAA AGC-3´

For SCC*mec* typing, the multiplex PCR method described by Oliveira was used initially[28]. Strains were sent to the Department of Bacteriology at Juntendo University, Japan, for verification of results using methods pioneered by their laboratory[3,21].

For pulsed-field gel electrophoresis (PFGE), restriction endonuclease SmaI was used for PFGE of all strains following an established protocol[29]. SmaI macrorestriction patterns were digitised and analysed by using Molecular Analyst v1.6 software to calculate Dice coefficients of correlation and to generate a dendrogram by the unweighted pair group method using arithmetic averages (UPGMA) clustering.

For multilocus sequence typing (MLST), PCR products were obtained using primers and protocols as established previously[30]. A commercial company performed DNA purification and sequencing, and the sequences were then submitted to an internet database (http://www.mlst.net) for the generation of an allelic profile and sequence type.

Molecular typing and results of current strains were compared with previous local strains[25]. MLST and SCC*mec* results were also compared with similar published results from other countries. The local ethics committee granted approval for the study.

RESULTS

There were six CA-MRSA strains (including the strains from CGH and KKWCH) isolated within the specified period. The demographical and clinical profiles of the patients are shown in Table I. Patient one had undergone an uncomplicated open reduction of a traumatic fracture of his right tibia in SGH in 1974. Although the presenting symptoms were acute, the radiological and subsequent histopathological findings were in keeping with chronic osteomyelitis of the former fracture site. It was impossible to deduce when the infection had started. Patient three had congenital lymphoedema of both lower limbs

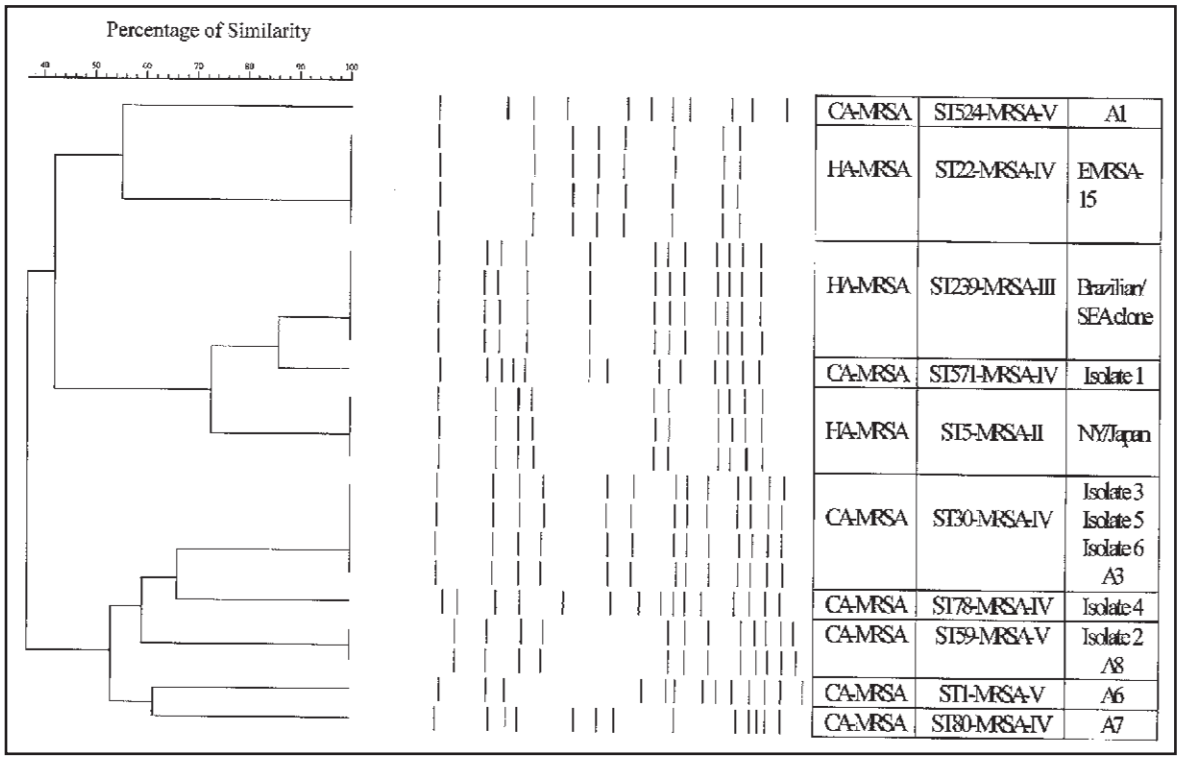

Fig. 1 Pulsed field gel electrophoresis (PFGE) pattern of nosocomial (HA) and community-associated (CA) MRSA from Singapore. *Sma*I macrorestriction patterns were digitised and analysed using Molecular Analyst v1.6 software to calculate Dice coefficients of correlation and to generate a dendrogram by the unweighted pair group method using arithmetic averages (UPGMA) clustering. A1, A3, A6-8 were CA-MRSA isolates from a previous study[25]. Isolates 1 to 6 are from the current study.

and was on regular outpatient follow-up at SGH. Her previous hospitalisation was just over a year ago. None of the other patients had co-morbid conditions or healthcare-associated contacts. Other than for patient one, none of the others had travelled in the six-month period prior to developing their infections. None of the patients were epidemiologically linked on questioning to each other or to previously-described cases.

The antimicrobial resistance and molecular typing results of community-associated and major nosocomial MRSA strains are shown in Table II. CA-MRSA strains tended to be susceptible to ciprofloxacin and some strains to erythromycin, whereas the vast majority of nosocomial strains were resistant to these antibiotics. Half of the strains belonged to multilocus sequence type (ST) 30, which was first ascribed to CA-MRSA isolates from Oceania[22]. One strain belonged to ST59, and possessed SCC*mec* V – this is identical to the predominant Taiwanese CA-MRSA strains[16]. The other two strains were PVL negative – Isolate 1 had a novel ST as confirmed by the curator of the MLST database (submitted to MLST database on 27th November 2004. ID number: 1255. ST571), and Isolate 4 had a sequence type described in an Australian CA-MRSA[31].

A phylogenetic dendrogram comprising selected nosocomial MRSA strains and all local CA-MRSA strains is shown in Fig. 1. This confirmed that CA-MRSA isolates as a whole were unrelated to nosocomial MRSA. All CA-MRSA belonging to ST30 were clonal, as were the two strains belonging to ST59.

DISCUSSION

We had previously described a diverse variety of CA-MRSA strains isolated at SGH that may have been directly imported from different parts of the world[25]. Our latest results suggest that this is no longer the case, and that CA-MRSA is in the process of establishing itself in Singapore. Five of the six patients had no history of antecedent travel prior to the onset of their infections. Although the date of onset of Patient one's infection could not be ascertained, the unique sequence type of his MRSA isolate suggested that this was probably a local MSSA strain that had somehow acquired SCC*mec* IV.

A clone of ST30-MRSA-IV was isolated from half of our current cases. This is also identical to Isolate 3 from our previous study[25], and may well become the predominant CA-MRSA clone in Singapore. PVL-positive ST30-MRSA-IV strains appear to be highly adaptable and transmissible

– these were first isolated from Pacific islanders from Oceania[32], but similar strains have since been found in Hong Kong[17], parts of Europe and USA[31], making it possibly the most widely-prevalent and successful CA-MRSA clonal type.

There were no clear epidemiological links between our patients with clonal CA-MRSA strains, suggesting the presence of intermediate asymptomatic carriers and on-going low-level transmission in the community. It would have been ideal to demonstrate this conclusively by means of contact tracing and screening; unfortunately we lacked the resources to accomplish this. Of course, persistent carriage following transference from remote overseas contacts or local acquisition from foreign MRSA carriers has not been ruled out, but the presence of both clonal (Isolates 3, 5 and 6) and unique (Isolate 1) MRSA strains argue against these alternatives.

There has possibly been an increase in the number of CA-MRSA cases locally, with seven cases identified in the first ten months of this year. This is equivalent to the sum total of cases identified from 2001 to 2003[25], and the trend is likely to be real despite the obvious confounding fact that the isolates have come from three hospitals instead of just SGH alone. Although these numbers appear very insignificant, it is to be cautioned that only the hospitalised cases are detected, whereas asymptomatic carriers and those with minor skin infections are missed.

The true prevalence of CA-MRSA can only be determined via widespread community-level screening, which we are unable to undertake at present. At this point in time, we feel that the prevalence is unlikely to be high and measures beyond continued surveillance may not be cost-effective in our local context. Should the number of cases increase significantly, however, community-level infection control programmes might be necessary in addition to changes in antibiotic prescription – an outbreak of CA-MRSA in Denmark was brought under control only after active contact tracing and de-colonisation of carriers[33]; intravenous vancomycin is now given empirically for selected septicaemic patients in Northern Territory, Australia[34]; and oral clindamycin and trimethoprim/sulfamethoxazole are now first-line drugs for the treatment of cutaneous abscesses in parts of USA[35].

A review of all our patients failed to reveal any clinical or epidemiological characteristic that could be used as a possible indicator of CA-MRSA infection prior to obtaining culture results. The majority of our patients had cutaneous abscesses, but this condition is commonly caused by MSSA as well[36]. The lack of risk factors is consistent with the experience at most areas where CA-MRSA is described with the exception of San Francisco, where CA-MRSA infections are common among the urban poor and intravenous drug abusers[37].

A comparison of antimicrobial susceptibility profiles appears to indicate that ciprofloxacin susceptibility with or without erythromycin susceptibility could be used locally to identify MRSA as being community-associated independently of molecular or epidemiological markers. However, we would urge caution in this respect as antimicrobial susceptibility profiles tend to change over time, and ciprofloxacin resistance is relatively easily acquired by *S. aureus*[38]. A combination of epidemiological screening and molecular profiling (PVL detection, SCC*mec* determination and typing via either PFGE or MLST) remains the best way to identify CA MRSA at present.

Two of our CA-MRSA isolates lacked PVL genes, which are found in the majority of CA-MRSA strains in countries other than Australia[22,23]. The presence of PVL is practically deterministic of the types of infections produced[27,36], hence the majority of CA-MRSA strains rarely cause infections other than cutaneous abscesses or necrotising pneumonia[20]. Determining whether a PVL-negative MRSA is from the community may be problematic unless the predominant nosocomial strains have been typed. In our series, the 2 PVL-negative isolates had markedly different genetic profiles compared to local nosocomial strains.

In conclusion, our data suggests that the problem of CA-MRSA is unlikely to disappear from Singapore, but will most likely escalate over time. More work is clearly required to define the epidemiology of this problem locally, and continued surveillance of the situation at a national (rather than just at the hospital) level seems advisable. Should this reach the epidemic proportions present in various US cities, empiric therapy for community cases of soft tissue infections and severe sepsis will have to include antibiotics with activity against CA-MRSA, although it is to be hoped that successful community-level infection control programs can be implemented prior to such an eventuality.

ACKNOWLEDGEMENTS

We would like to thank the staff of the SGH Microbiology Laboratory, especially Ms Grace Wang and Ms Ong Lan Huay, for their help in providing logistical support for this study. This work was funded by Grant number P026/04 from the Department of Clinical Research, Singapore General Hospital.

Table I. Demographical and clinical data of patients with community-acquired MRSA infections.

Patient/isolate	1	2	3	4	5	6
Hospital	SGH	KKWCH	SGH	CGH	SGH	CGH
Date of MRSA isolation	April 2004	April 2004	June 2004	September 2004	October 2004	October 2004
Ethnicity	Chinese	Chinese	Malay	Chinese	Eurasian	Filipino
Age	57	7	33	28	35	35
Sex	M	F	F	M	M	M
Co-morbid conditions	Diabetes mellitus	Nil	Milroy's disease	IVDU[1]	Nil	Nil
Travel history[2]	Yes	No	No	No	No	No
Infection type	Tibial Osteomyelitis	Buttock abscess	Thigh abscess	Endocarditis	Neck abscess	Chest wall abscess
Therapy[3]	Bone curettage, IV Vancomycin	I&D, oral cloxacillin	I&D, oral amoxicillin and cloxacillin	IV Vancomycin	I&D, oral amoxicillin/ clavulanate	I&D, oral cloxacillin
Appropriate antibiotic usage[4]	Yes	No	No	Yes	No	No
Outcome	No relapse at six months follow-up	Cured	Cured	Cured	Cured	Cured

[1] Intravenous drug abuse.

[2] Travel within six months prior to onset of infection. For patient one, exact time point of start of infection was not known.

[3] Therapy: IV = intravenous, I&D = incision and drainage of abscess.

[4] Use of an antibiotic with activity against MRSA.

Table II. Antimicrobial resistance profiles and molecular typing of community-acquired and selected nosocomial MRSA isolates.

MRSA profile	Antimicrobial susceptibility[1]									Molecular typing			
Community	Oxa	Ery	CC	SXT	CIP	TET	GEN	FUS	VAN	PVL	SCCmec	MLST[2]	ST[3]
Isolate 1	R	R	R	S	S	S	S	S	S	–	IV	7-3-1-1-4-4-10	571
Isolate 2	R	R	R	S	S	R	S	S	S	+	V	19-23-15-2-19-20-15	59
Isolate 3	R	S	S	S	S	S	S	S	S	+	IVc	2-2-2-2-6-3-2	30
Isolate 4	R	R	R	S	S	S	S	S	S	–	IVa	22-1-14-23-12-53-31	78
Isolate 5	R	S	S	S	S	S	S	S	S	+	IVc	2-2-2-2-6-3-2	30
Isolate 6	R	S	S	S	S	S	S	S	S	+	IVc	2-2-2-2-6-3-2	30
Nosocomial													
Brazilian	R	R	V	R	R	R	R	V	S	–	III	2-3-1-1-4-4-3	239
EMRSA-15	R	R	S	S	R	S	S	S	S	–	IV	7-6-1-5-8-8-6	22
NY/Japan	R	R	R	S	R	S	R	S	S	–	II	1-4-1-4-12-1-10	5

[1]OXA = oxacillin; ERY = erythromycin; CC = clindamycin; SXT = trimethoprim-sulfamethoxazole; CIP = ciprofloxacin; TET = tetracycline; GEN = gentamicin; FUS = fusidic acid; VAN = vancomycin; R = resistant; S = susceptible; V = variable susceptibility;

[2] Multi-locus sequence typing allelic profile;

[3] ST - sequence type based on multi-locus sequence typing

GLOSSARY

Panton-Valentine leukocidin (PVL):
An extracellular bicomponent toxin produced by *Staphylococcus aureus* that targets leukocytes by creating pores in their cell membranes. These disrupt the permeability barrier, resulting in the release of cytokines, activation of intracellular proteases, occasionally induction of apoptosis, and ultimately cell death.

Staphylococcal chromosomal cassette *mec* (SCC*mec*):
A mobile genetic element within which the methicillin resistance gene *mecA* is incorporated. This genetic element has been found in the chromosome of almost all beta-lactam resistant staphylococci, including MRSA. Five different structural types have been described to date. Types I to III are typically found in nosocomial MRSA – these are large genetic elements (especially SCC*mec* III) ranging between 34 to 66 kilobase pairs (kbp) in size, with many other antimicrobial resistance determinants incorporated within each cassette. Types IV and V are typically found in community-associated MRSA, although Type IV has also been identified with increasing frequency in multi-susceptible nosocomial MRSA. These latter two genetic elements are much smaller in size (20-25 kbp), with fewer antimicrobial resistance determinants, and are postulated to be more readily transferred in between strains.

Multilocus sequence typing (MLST):
A highly discriminatory but expensive method of characterising bacterial isolates based on differences between the internal (~450 base pair) fragments of seven conserved housekeeping genes. Different sequences for each gene fragment are assigned as different alleles, and each bacterial isolate is defined by the alleles at each of the seven housekeeping loci (sequence type [ST]). As an example, the Brazilian clone of MRSA has the allelic profile 2-3-1-1-4-4-3, and is ST239. MLST schemes are in place for multiple other bacteria including *Burkholderia pseudomallei, Streptococcus pneumoniae* and *Neisseria meningitides*. More details can be found at http://www.mlst.net/

***Staphylococcus aureus* nomenclature:**
A new nomenclature for *S. aureus* which has gained fairly widespread acceptance at this current time was proposed by Enright and co-workers based on sequence type (ST), SCC*mec* type and methicillin/glycopeptide susceptibility[5]. Hence the Brazilian clone of MRSA will be ST239-MRSA-III, and the Oceanian clone of community-associated MRSA will be ST30-MRSA-IV.

REFERENCES

1. Jevons MP, Coe AW, Parker MT. Methicillin resistance in staphylococci. Lancet 1963; 1:904-7.
2. Fuda C, Suvorov M, Vakulenko SB, Mobashery S. The basis for resistance to β-lactam antibiotics by penicillin-binding protein 2a (PBP2a) of methicillin-resistant *Staphylococcus aureus*. J Biol Chem 2004; 279:40802-6.
3. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcus cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 2000; 44:1549-55.
4. Ito T, Ma XX, Takeuchi F, Okuma K, et al. Novel type V staphylococcal chromosome cassette *mec* driven by a novel cassette chromosome recombinase, ccrC. Antimicrob Agents Chemother 2004; 48:2637-51.
5. Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin resistant *Staphylococcus aureus*. Trends Microbiol 2001;9:486-93.
6. Enright MC, Robinson DA, Randle G, et al. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc Natl Acad Sci USA. 2002; 99:7687-92.
7. Esuvaranathan K, Kuan YF, Kumarasinghe G, et al. A study of 245 infected wounds in Singapore. J Hosp Infect 1992; 21:231-40.
8. Hsu LY, Koh TH, Singh KS, et al. Dissemination of multi-susceptible methicillin-resistant *Staphylococcus aureus* in Singapore. J Clin Microbiol 2005; 43:2923-5.
9. CDC. Community-acquired methicillin-resistant Staphylococcus aureus infections-Michigan. MMWR Morb Mortal Wkly Rep 1981; 30:185-7.
10. Udo EE, Pearman, JW, Grubb WB. Genetic analysis of community isolates of methicillin-resistant *Staphylococcus aureus* in Western Australia. J Hosp Infect 1993; 25:97-108.
11. Herold BC, Immergluck LC, Maranan MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA 1998; 279:593-8.
12. Dufour P, Gillet Y, Bes M, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton-Valentine leukocidin. Clin Infect Dis 2002; 35:819-24.
13. Okuma K, Iwakawa K, Turnidge JD, et al. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J Clin Microbiol 2002; 40:4289-94.
14. Adhikari RP, Cook GM, Lamont I, et al. Phenotypic and molecular characterization of community occurring, Western Samoan phage pattern methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 2002; 50:825-31.
15. CDC. Community-associated methicillin-resistant *Staphylococcus aureus* in Pacific Islanders – Hawaii, 2001-2003. MMWR Morb Mortal Wkly Rep 2004; 53:767-70.
16. Wang CC, Lo WT, Chu ML, Siu LK. Epidemiological typing of community-acquired methicillin-resistant *Staphylococcus aureus* isolates from children in Taiwan. Clin Infect Dis 2004; 39:481-7.
17. Ho PL, Tse CW, Mak GC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* arrives in Hong Kong. J Antimicrob Chemother 2004; 54:845-6.
18. Young DM, Harris HW, Charlebois ED, et al. An epidemic of methicillin-resistant *Staphylococcus aureus* soft tissue infections among medically undeserved patients. Arch Surg 2004; 139:947-51.
19. CDC. Methicillin-resistant *Staphylococcus aureus* infections in correctional facilities – Georgia, California, and Texas, 2001-2003. MMWR Morb Mortal Wkly Rep 2003; 52:992-6.
20. Naimi TS, LeDell KH, Como-Sabetti K, et al. Comparison of community- and healthcare-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA 2003; 290:2976-84.
21. Ma XX, Ito T, Tiensasitorn C, et al. Novel type of staphylococcal chromosome cassette mec identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. Antimicrob Agents Chemother 2002; 46:1147-52.
22. Vandenesch F, Naimi T, Enright MC, et al. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 2003; 9:978-84.
23. O'Brien FG, Lim TT, Chong FN, et al. Diversity among community isolates of methicillin-resistant *Staphylococcus aureus* in Australia. J Clin Microbiol 2004; 42:3185-90.

24. Chambers HF. The changing epidemiology of *Staphylococcus aureus*? Emerg Infect Dis 2001; 7:178-82.
25. Hsu LY, Tristan A, Koh TH, et al. Community-associated methicillin-resistant *Staphylococcus aureus*, Singapore. Emerg Infect Dis 2005; 11:341-2.
26. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. Wayne, PA: The Committee; 2004. NCCLS document M100-S14, vol 24, no 1.
27. Lina G, Piemont Y, Godail-Gamot F, et al. Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis 1999; 29:1128-32.
28. 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.
29. Maslow J, Slutsky A, Arbeit R. The application of pulsed field gel electrophoresis to molecular epidemiology. In: Persing H, Smith T, Tenover F, White T, eds. Diagnostic Molecular Microbiology: Principles and Applications. Washington, DC: American Society for Microbiology, 1993: 563-72.
30. Enright MC, Day NP, Davies CE, et al. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-sensitive clones of *Staphylococcus aureus*. J Clin Microbiol 2000; 38:1008-15.
31. *Staphylococcus aureus* multilocus sequence typing database. Available at: www.saureus.mlst.net/ Accessed October 15, 2004.
32. Nimmo GR, Schooneveldt J, O'Kane G, et al. Community acquisition of gentamicin-susceptible methicillin-susceptible *Staphylococcus aureus* in Southeast Queensland, Australia. J Clin Microbiol 2000; 38:3926-31.
33. Urth T, Juul G, Skov R, Schonheyder HC. Spread of a methicillin-resistant *Staphylococcus aureus* ST80-IV clone in a Danish community. Infect Control Hosp Epidemiol 2005; 25:144-9.
34. Murray RJ, Lim TT, Pearson JC, et al. Community-onset methicillin-resistant *Staphylococcus aureus* bacteremia in Northern Australia. Int J Infect Dis 2004; 8:275-83.
35. Buescher ES. Community-acquired methicillin-resistant *Staphylococcus aureus* in pediatrics. Curr Opin Pediatr 2005; 17:67-70.
36. Hsu LY, Koh TH, Kurup A, et al. High incidence of Panton Valentine leukocidin-producing *Staphylococcus aureus* in a tertiary public hospital in Singapore. Clin Infect Dis 2005; 40:486-9.
37. Charlebois ED, Bangsberg DR, Moss NJ, et al. Population-based community prevalence of methicillin-resistant *Staphylococcus aureus* in the urban poor of San Francisco. Clin Infect Dis 2002; 34:425-33.
38. Tanaka M, Wang T, Onodera Y, et al. Mechanism of quinolone resistance in *Staphylococcus aureus*. J Infect Chemother 2000; 6:131-9.