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

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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 (SCCmec) 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

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) was first isolated in United Kingdom hospitals in 1961, two years after the introduction of methicillin(10). Methicillin resistance is conferred by the product of the mecA 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. This gene is localised within a mobile genetic element designated staphylococcal chromosome cassette mec (SCCmec). Five major structural types of SCCmec have been described to date, 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.

Epidemic clones from five major lineages of MRSA have spread worldwide and are responsible for practically all healthcare-associated MRSA infections globally. MRSA was introduced into Singapore in the early 1980s, 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). A small proportion (<5%) of MRSA infections locally are caused by a strain related to the New York/Japan clone (ST5-MRSA-II).

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. This phenomenon was subsequently noticed again in the late 1980s in West Australia and Chicago. The term “community-associated MRSA” (CA-MRSA) was coined for this phenomenon, which has since become global in scope, reaching epidemic proportions in parts of the USA where conditions of overcrowding and poor sanitation prevail.

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, SCCmec types, and genetic heritage. Of note is the fact that the majority of CA-MRSA isolates possessed geographically-related genetic backgrounds, SCCmec 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 \textit{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 \textit{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.

\textit{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\(^{27}\). 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:

\textit{luk-PV1}, 5’-ATCATTTAGTAAATGTCTGGACA TGATCCA-3’

\textit{luk-PV2}, 5’-GCATCAAGTGATTTGGATAGC AAA AGC-3’

For SCC\textit{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\(^{13,21}\).

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\(^{29}\). MLST and SCC\textit{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
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 SCCmec 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 SCCmec 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(22), but similar strains have since been found in Hong Kong(27), parts of Europe and USA(28), 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 transferece 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(23); 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(27).

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, SCCmec 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

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

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

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.

<table>
<thead>
<tr>
<th>MRSA profile</th>
<th>Antimicrobial susceptibility</th>
<th>Molecular typing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community</td>
<td>Oxa</td>
<td>Ery</td>
</tr>
<tr>
<td>Isolate 1</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Isolate 3</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Isolate 4</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Isolate 5</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Isolate 6</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Brazilian</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>EMRSA-15</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>NY/Japan</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

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;
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 (SCCmec):
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 SCCmec 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), SCCmec type and methicillin/glycopeptide susceptibility(39). 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

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