Review Article

Community-associated Methicillin-resistant *Staphylococcus aureus*: Overview and Local Situation

Limin Wijaya,[1] *MBBS (Mel), MRCP (UK)*, Li-Yang Hsu,[1] *MBBS, MRCP (UK), DTMH (Gorgas)*, Asok Kurup,[1] *MBBS, MRCP (UK), FAMS*

Abstract

Introduction: Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has emerged worldwide. In contrast to healthcare-associated MRSA (HA-MRSA), CA-MRSA isolates are usually susceptible to multiple non-beta-lactam antibiotics and cause a distinct spectrum of infections in epidemiologically disparate populations – in particular, cutaneous abscesses, necrotising fasciitis and necrotising pneumonia. They arise from a broader genetic background, and possess differing virulence genes. We aim to describe the distribution of different molecular subtypes of CA-MRSA among various regions and discuss briefly the implications of CA-MRSA from a local perspective. Methods: Literature review of articles on CA-MRSA, focusing mainly on reports where the genetic background of isolates had been analysed using multi-locus sequence typing (MLST). Singapore data were obtained from the local CA-MRSA database. Results: MLST analysis demonstrated the presence of epidemic subtypes of CA-MRSA within most geographic areas. In parts of the United States, community MRSA infections currently exceed those caused by their methicillin-susceptible counterparts. In Singapore, CA-MRSA infections are increasing, predominantly as a result of the spread of ST30 clones. Conclusion: Available evidence suggests that the emergence of MRSA from the community is not going to be a transient phenomenon. Local guidelines for dealing with this phenomenon at both therapeutic and preventive levels are needed prior to the potential development of a situation mirroring that of meso-endemic HA-MRSA in local hospitals or CA-MRSA epidemics in parts of USA.

Ann Acad Med Singapore 2006;35:479-86

Key words: Bacterial typing, Epidemic, Epidemiology, Infection control

Introduction

The emergence and spread of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from the community that are distinct from their archetypal healthcare-associated counterparts (HA-MRSA) marked a critical evolutionary milestone for the organism. In less than 2 decades, particularly in the last 3 years, this initially sporadic phenomenon of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has become a global reality, leading to paradigm shifts in the perception and management of staphylococcal infections in countries where CA-MRSA has reached epidemic proportions.[1,2]

In the past year alone, more than 200 articles on CA-MRSA have been published in the medical literature, of which at least 10 have been review articles. Nevertheless, this is a fast evolving field and the incidence of CA-MRSA infections is increasing at an alarming rate locally. In this article, we aim to provide a brief review of the current state of knowledge regarding this organism, with an emphasis on the implications of this phenomenon in the local context.

The Fundamental Organism

S. aureus is a gram-positive coccoid bacterium named for its tendency to form clusters ("staphyle" is the Greek expression for "bunch of grapes") and its golden pigmentation ("aureus" is Latin for "golden") on culture plates. Its importance as a human pathogen has increased in the previous century as a result of the evolution of antimicrobial resistance – to penicillin and subsequently methicillin in particular – and the evolution of medical care resulting in ever-increasing and extensive use of intravascular devices.[3] The actual impact was recently estimated by Norskin and co-workers:[4] inpatients with

[1] Department of Internal Medicine
Singapore General Hospital, Singapore
Address for Reprints: Dr Hsu Li-Yang, Department of Internal Medicine, Singapore General Hospital, Outram Road, Singapore 169608.
Email: liyang_hsu@yahoo.com

S. aureus infections in US hospitals had approximately 3 times the length of hospital stay, 3 times the total charges (US$48,824 versus US$14,141) and 5 times the risk of in-hospital death when compared to inpatients without infection.

Methicillin Resistance

MRSA was first isolated at hospitals in Surrey, UK in 1961, one year after methicillin was introduced. But the mechanism of methicillin resistance was not elucidated until 1981, when Hartman and co-workers[5] discovered altered penicillin-binding proteins (PBP2') in MRSA that had markedly reduced affinity for all currently available beta-lactam antibiotics while maintaining effective cell wall-building activity. PBP2' is encoded by the *mecA* gene which is carried on a mobile DNA element, the staphylococcal cassette chromosome *mec* (SCC*mec*).[6] The other important component of SCC*mec*, the chromosome cassette recombinase (*ccr*) genes, encodes for proteins that enable precise integration into and excision from a specific site of the *S. aureus* chromosome (*attBscc*).[6]

Five *SCCmec* subtypes have officially been identified to date, varying in size from ~20 kilobase pairs (kb) to 68 kb.[6,7] SCC*mec* types I, IV and V possess no antimicrobial resistance determinants other than *mecA*, whereas SCC*mec* types II and III possess multiple other resistant determinants such as *Tn554* (encodes for macrolide resistance). This partially accounts for the larger sizes of the latter, which is thought to have limited their capability for horizontal transference.[6] It is currently postulated that methicillin resistance did not arise within *S. aureus*, but was transferred from other staphylococcal species.[8,9]

From Hospital to the Community, Worldwide

Since 1961, successive waves of epidemic MRSA have spread throughout hospitals and other chronic healthcare facilities worldwide to the extent that it is now the most commonly isolated antimicrobial-resistant pathogen in many countries.[10-12] Despite the apparent success of MRSA in the nosocomial setting, it was originally hardly ever isolated from the community. This observation was attributed to the much slower growth rates of earlier MRSA isolates (probably a result of the fitness costs of SCC*mec* I to III) compared to methicillin-susceptible *S. aureus* (MSSA) isolates, a factor which may be of crucial ecological importance in settings where antibiotic selection pressure is not the main evolutionary imperative.[13]

The initial description of sporadic cases of CA-MRSA infections in patients without the usual risk factors for nosocomial MRSA acquisition did not generate much attention.[14-16] However, the deaths of 4 children in North Dakota and Minnesota from severe CA-MRSA infections dramatically highlighted the potential threat of these organisms, and drummed in the concept that CA-MRSA had evolved separately from HA-MRSA, and possessed a distinct virulence profile.[17] These concerns have only escalated with the subsequent profusion of reports from all around the world.[6,18-40]

In a predictable but unpleasant development, the latest reports suggest that in parts of the USA, the problem of CA-MRSA has reached epidemic proportions. This occurred initially in situations where conditions of overcrowding and poor sanitation prevail, such as among the homeless in San Francisco,[40] within US correctional facilities,[41] or among players of contact sports.[27] Subsequent sobering analyses have demonstrated that CA-MRSA currently accounts for almost 50% of all outpatient staphylococcal infections in some states,[29] and are starting to replace HA-MRSA as a cause of nosocomial infections.[42,43] These developments have rendered prophetic the comment that the evolution of methicillin resistance in *S. aureus* may in time reprise that of penicillin resistance.[44]

The reason for the recent steep upsurge in CA-MRSA cases worldwide is not well understood. Experts have attributed this to the relatively new SCC*mec* subtypes – in particular SCC*mec* IV – which had probably been introduced into *S. aureus* only in the recent 2 or 3 decades, but which are now present in more clones of *S. aureus* than the earlier 3 SCC*mec* subtypes.[45,46] The small size of SCC*mec* IV (20-25 kb) probably allows for greater promiscuity in horizontal transference, and importantly, does not confer a replicative disadvantage compared to MSSA.[6] Circumstantial corroborative evidence is provided by the simultaneous expansion of new healthcare-associated MRSA (HA-MRSA) clones bearing SCC*mec* IV within various hospitals internationally.[47,48]

Differentiating CA-MRSA from HA-MRSA

A plethora of differing definitions of CA-MRSA in the early literature led to confusion and non-comparability of some of these reports. Most authors had initially used the isolation of MRSA from a clinical specimen obtained as outpatient or within 48 to 72 hours of hospitalisation as a yardstick.[14,45] However, it is known that patients may be colonised with HA-MRSA for years before developing infection.[49] Similarly, nosocomial outbreaks of CA-MRSA have been reported,[24,50] and the experience of a single institute in Atlanta, USA suggest that these lines are likely to be blurred further in the future.[42,43] For these reasons, any attempt at distinguishing CA-MRSA from HA-MRSA using purely epidemiologic criteria will neither be sensitive nor specific.

Investigators from Lyon had highlighted 2 potential molecular markers for CA-MRSA: the Panton-Valentine leukocidin (PVL) genes and SCC*mec* IV.[51] While PVL

genes are present in the majority of CA-MRSA isolates reported in the literature, their absence alone in any MRSA isolate is not proof against a community origin. This can be seen from the example of Australia, where the majority of CA-MRSA isolates lack PVL genes.[52] SCC*mec* IV has also been well described in HA-MRSA isolates.[47,48]

What is clear is that CA-MRSA isolates at present have different genetic backgrounds compared to HA-MRSA isolates. Using molecular methods such as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) – the latter being a PCR-based method for characterising organisms based on differences between the internal fragments of highly-conserved housekeeping genes – a picture of a globally diverse though regionally conserved distribution of CA-MRSA clones has emerged.[18-40,51] These facts debunk the misconception that CA-MRSA had initially originated from HA-MRSA that had adapted to survival in the community, and suggest that the horizontal transference of methicillin resistance had occurred separately and near-simultaneously in different parts of the world.

Therefore molecular typing, complemented by patient epidemiology, is the most sensitive and specific method for differentiating CA- from HA-MRSA. While this adds costs in terms of resources and manpower (not forgetting that HA-MRSA have to be typed as well), it allows for an accurate depiction of the problem of CA-MRSA, and for timely and directed infection control intervention if required.

A summary of current predominant CA-MRSA clones in various countries as typed via MLST is shown in Table 1. It is to be cautioned that this list is transient and subject to change over time. For example, ST1-MRSA-IV (USA400) was the initial CA-MRSA reported from the USA but this was quickly overtaken by the more stable and highly transmissible ST8-MRSA-IV (USA300) clone. Furthermore, with the ease and frequency of travel, CA-MRSA clones may be transplanted across the world, an event that has already been documented several times.[22,53]

Table 1. Summary of Predominant Community-associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) Clones According to Geographic Region as Classified by Multi Locus Sequence Typing (MLST), Staphylococcal Chromosome Cassette *mec* (SCC*mec*) Subtype and Presence of Panton-Valentine Leukocidin (PVL) Genes

Country*	Sequence Type†	SCC*mec*†	PVL†	Reference
USA	8	IV	Positive	27, 29, 36
Canada	1	IV	Positive	34
Brazil	30	IV	Positive	25
Uruguay	30	IV	Positive	39
Belgium	80	IV	Positive	31
Denmark	80	IV	Positive	26
France	80	IV	Positive	18, 51
Germany	80	IV	Positive	24
Greece	80	IV	Positive	32
Holland	80	IV	Positive	33
Latvia	30	IV	Positive	37
Norway	8	IV	Positive	36
Sweden	30	IV	Positive	37
Switzerland	80	IV	Positive	51
United Kingdom	80	IV	Positive	35
Taiwan	59	V	Positive	21
China - Hong Kong SAR	30	IV	Positive	30
Australia				
- Queensland	93	IV	Negative	51
- Western Australia	1	IV	Negative	38
New Zealand/West Samoa	30	IV	Positive	51

* Based on articles available on PubMed until January 2006. Countries that have reported CA-MRSA cases but without MLST being performed on the isolates (India, Nigeria) are not listed.

† Only one predominant CA-MRSA clone from each country is listed, unless different regions or states within a country have differing major clones. Note that most countries (in particular, Australia and Switzerland) have reported multiple unrelated CA-MRSA clones.

Risk Factors for CA-MRSA

CA-MRSA clusters have been described in various population groups such as participants of contact sports,[27] military personnel,[28] intravenous drug abusers (IVDA),[14,29,40] prison inmates,[41] aboriginal groups,[15,19,20] and people belonging to a lower socio-economic stratus.[18,40] In each of these settings, perhaps with the exception of IVDA, overcrowded facilities, close contact and lack of sanitation had contributed to the spread of MRSA. The majority of published cases have occurred, however, in patients who do not belong to these population groups, are young, immunocompetent, and lack traditional risk factors for MRSA infection such as recent hospitalisation, presence of indwelling medical devices or catheters, dialysis and residence in chronic healthcare facilities.[16,21,22,24]

In brief, excluding regional specific epidemiological risk factors, patients with CA-MRSA infections are little different from patients with community-associated MSSA infections.

Clinical Presentation

The archetypal presentation of CA-MRSA infection is a young, healthy outpatient with a cutaneous abscess.

In one of the earliest comparison studies between CA- and HA-MRSA, Naimi and co-workers[54] found that skin and soft tissue infections were far more common among patients with CA-MRSA (75%) than those with HA-MRSA (37%). Cutaneous infections, particularly in the form of furuncles or abscesses, have thus far consistently remained as the most commonly reported manifestation of CA-MRSA.[16,18-40] This is probably a consequence of the

association of PVL genes with CA-MRSA.

PVL is an extracellular bicomponent toxin that targets and induces leukocyte death with release of cytokines and intracellular proteases by creating pores in the cell membrane. Its production is practically deterministic of the ensuing infection – either cutaneous furuncles/abscesses or, more rarely, necrotising pneumonia.[55] The latter condition is rare perhaps because other virulence factors are required.[56]

As mentioned, more fulminant presentations such as septicaemia and necrotising pneumonia have been described,[17] and new, severe manifestations not commonly associated with staphylococcal infections such as Waterhouse-Friderichsen syndrome[57] and necrotising fasciitis have emerged. Miller and co-workers[58] reported 16 cases of the latter condition over a 2-year period in Los Angeles.

Curiously, nosocomial infections attributed to PVL-producing CA-MRSA appear to be less distinctive and are similar from those caused by HA-MRSA. These include wound infections,[24,50] septicemia,[24,43] and implant infections.[42] The reason for this is not clear at present, although it is possible that the portal of entry (open wounds and lines in nosocomial cases) may play a part in determining the type of infection subsequently arising.

PVL-negative CA-MRSA infections are less commonly reported. Infections attributed to these organisms are not distinct from other MSSA or even HA-MRSA infections, although they also occur in young patients without the usual risk factors for HA-MRSA infection.

Whether CA-MRSA is more virulent than HA-MRSA is not a question that is easily answered. On the whole, CA-MRSA isolates possess a greater array of known virulence factors than their healthcare-associated counterparts,[51,59] and USA300 has been associated with several spectacular and fulminant presentations.[57,58] However, the vast majority of cases have been cutaneous abscesses, resulting in

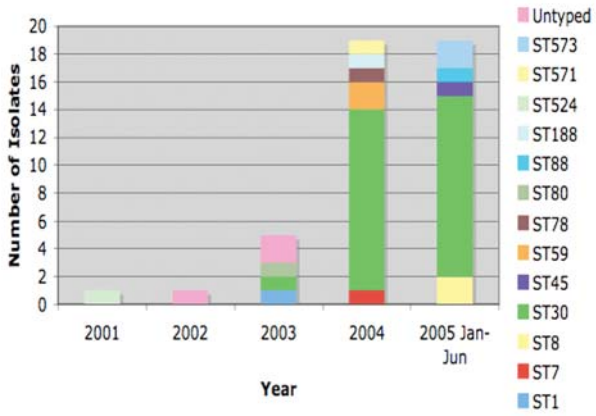

Fig. 1. Distribution of non-duplicate community-associated methicillin-resistant *Staphylococcus aureus* cases in Singapore by year and multilocus sequence type.

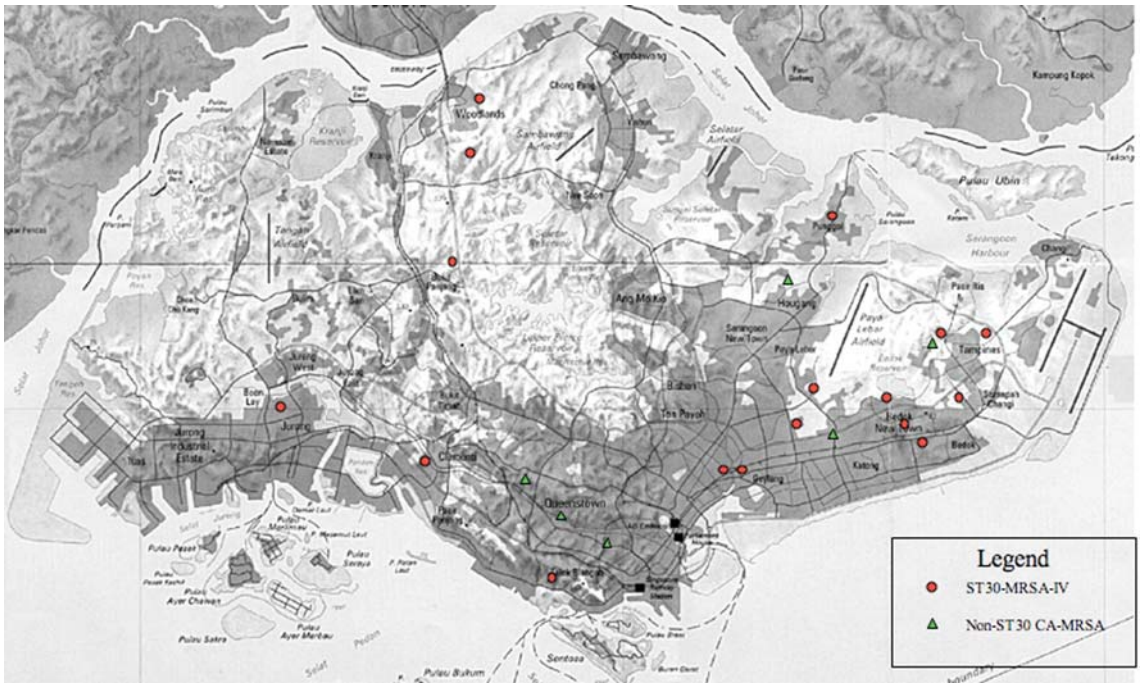

Fig. 2. Distribution of community-associated methicillin-resistant *Staphylococcus aureus* in Singapore by the residential addresses of 23 cases.

Table 2. Distribution of Local Community-associated Methicillin-Resistant *Staphylococcus aureus* According to Multi Locus Sequence Type, Patient Ethnicity and Types of Infection Caused

Sequence type	SCC*mec*	PVL	Patient ethnicity (No. of cases)	Type of infection (No. of cases)
1	V	Positive	Chinese (1)	Cutaneous abscess (1)
7	V	Negative	Chinese (1)	Exfoliative dermatitis (1)
8	V	Positive	Caucasian (1)	Cutaneous abscess (1)
			Chinese (1)	Wound infection (1)
30	IVa	Positive	Malay (1)	Cutaneous abscess (1)
	IVc	Positive	Caucasian (1)	Cutaneous abscess (1)
			Chinese (14)	Cutaneous abscess (13) Wound infection (1)
			Malay (6)	Bacteremia (1) Cutaneous abscess (5)
			Filipino (5)	Cutaneous abscess (5)
45	V	Positive	Chinese (1)	Cutaneous abscess (1)
59	V	Positive	Chinese (2)	Cutaneous abscess (2)
78	IVa	Negative	Chinese (1)	Bacteremia/Endocarditis (1)
80	IVa	Positive	Filipino (1)	Cutaneous abscess (1)
88	V	Positive	Malay (1)	Cutaneous abscess (1)
188	V	Negative	Chinese (1)	Cutaneous abscess (1)
524	V	Positive	Indian (1)	Bacteremia/Pneumonia (1)
571	IVa	Negative	Chinese (1)	Osteomyelitis (1)
573	V	Negative	Chinese (2)	Cutaneous abscess (2)

PVL: Panton-Valentine leukocidin; SCC*mec*: staphylococcal chromosome cassette *mec*

estimated attributable morbidity and mortality rates that are much lower than that quoted for HA-MRSA infections, although this has to be balanced against the fact that patients who develop HA-MRSA infections tend to be older and have more co-morbidities. More longitudinal data are required before the truth can be determined, but it is probably fair comment to state that at this point in time, CA-MRSA is not more virulent than HA-MRSA according to conventional measures.

Local Context

A search through the microbiology archives of the Singapore General Hospital (SGH) from January 2001 to April 2004 yielded 8 possible CA-MRSA cases, of which only 5 isolates remained available for typing. These were found to be completely unrelated to each other, and based on strain typing and patient epidemiologic data, at least 3 were likely to have been imported from other countries.[22]

Between May 2004 and June 2005, however, a further 37 CA-MRSA isolates were confirmed by molecular typing. Although a reporting bias is evident (many isolates had been contributed by hospitals other than SGH), this nevertheless marks a real and progressive increase in CA-MRSA cases locally (Fig. 1). 70.3% (26 of 37) of the new isolates had identical PFGE patterns and belonged to ST30 on MLST. This is highly suggestive that local transmission had occurred, and that ST30 clones have become the predominant CA-MRSA in Singapore.[60] ST30 clones currently form perhaps the most globally widespread clonal group among CA-MRSA, and it is interesting but worrying to note these isolates have probably evolved from the pandemic phage type 80/81 penicillin-resistant MSSA that had caused considerable morbidity and mortality in the 1950s, particularly during the global outbreak of Asian influenza.[61]

The distributions of CA-MRSA according to the sequence type, infections caused, and ethnicity of the patients is shown in Table 2, and the residential addresses of the patients (only 23 available) is depicted in Figure 2. A significant proportion had been isolated from Filipino expatriates working as maids or construction workers locally, but it is not clear whether they had imported their CA-MRSA isolates or had been infected while working in Singapore. Despite a slight congregation of cases in the eastern part of the island, it is apparent that CA-MRSA – including ST30 isolates – has spread island-wide. The mean age of patients was 34.9 years (range, 1 to 74), and cases were almost equally distributed between the sexes (male-to-female ratio = 1.1:1).

Cutaneous abscesses had accounted for the vast majority of local CA-MRSA infections (35 of 42 cases), which is consistent with global reports. Three cases with cutaneous abscesses had experienced at least 1 relapse. There have only been 2 cases with severe infections to date – a diabetic who had necrotising pneumonia in 2001, and an IVDA with tricuspid valve endocarditis and septic pulmonary embolism in 2004. Both survived. Two patients had wound infections post surgery where CA-MRSA (demonstrated by molecular typing) was isolated from wound cultures, reinforcing the lack of sensitivity of epidemiologic criteria alone in the identification of such organisms.

While the number of local cases appears to be relatively small, this figure is biased by the fact that only hospitalised patients and CA-MRSA isolates submitted to public hospital microbiology laboratories are likely to be detected. The actual prevalence and incidence of CA-MRSA infections locally is not known, but is probably much higher than reported.

Therapy and Control

The current approach towards a suspected *S. aureus* infection from the community involves the use of beta-lactam antibiotics along with removal of foci of infection, e.g., abscess drainage. Severe community-acquired infections such as pneumonia or septicaemia are treated with broad-spectrum intravenous beta-lactam antibiotics in the majority of cases. While uncomplicated cutaneous abscesses caused by CA-MRSA are often cured by incision and drainage alone,[62] the delay in initiating appropriate antibiotic therapy for severe MRSA infections might prove costly.

In regions where CA-MRSA incidence is high, empiric antimicrobial therapy guidelines have been changed to reflect this. Intravenous vancomycin is now given empirically for selected septicaemic patients in Northern Territory, Australia,[2] and oral clindamycin and trimethoprim/sulfamethoxazole are now first-line drugs for the treatment of cutaneous infections in parts of the USA.[63] However, although CA-MRSA infections – in contrast to the majority of HA-MRSA – are generally susceptible to most non-beta-lactam antibiotics,[51] resistance to various older non-beta-lactam antibiotics may be relatively easily acquired. Widespread use of clindamycin had resulted in a small but significant rise in resistance within 2 years in northeast USA.[64]

The recent and impending release of new antibiotics targeting gram-positive organisms such as linezolid, daptomycin, tigecycline and newer glycopeptides appears to blunt the impact of CA-MRSA. But it has to be kept in mind that the newer antibiotics come at an initially prohibitive price (the cost of a 1-day supply of linezolid is >100 times the cost of cloxacillin and >15 times the cost of vancomycin in the SGH formulary), particularly for outpatient therapy of mild-to-moderate infections.

Control of the spread of CA-MRSA is an issue for which no solution looks to be readily available. Time-honoured infection control measures that have proven important in preventing the spread of antibiotic-resistant organisms within institutions, such as isolation and contact precautions, can hardly be applied in the open community without creating a backlash of public outrage against the authorities or ostracism towards those who are isolated. And once a state is reached where both CA- and HA-MRSA rates are high – a situation that is analogous with that of penicillin resistance in *S. aureus* – it might no longer be cost-effective or even possible to control MRSA.

Active contact tracing and de-colonisation of carriers were successful in ending a CA-MRSA outbreak in Denmark.[26] This is an extension of part of the "search and destroy" policy practised by most Scandinavian, Dutch and Western Australian healthcare institutions. But whereas such a policy is feasible within the limited confines of hospitals, it would be daunting to reproduce and operate such a programme indefinitely in open democratic societies, and careful consideration of the costs and resources required to perpetuate such a program should be undertaken prior to implementation.

In Singapore, we are at the ascending phase of the CA-MRSA outbreak. The small number of cases to date does not justify any alteration in antibiotic prescription guidelines. However, a more rigorous surveillance programme, especially at the primary healthcare level, would be ideal in order to gauge the situation more accurately, and guidelines should be drawn up to prepare for the eventuality where community MRSA infections outnumber those caused by their methicillin-susceptible counterparts.[40,41]

Conclusion

The phenomenon of CA-MRSA has swept worldwide within a couple of decades. The crux of the matter is whether this is an immutable evolutionary process following on the steps of penicillin resistance, or if it can potentially be reversed or contained. Current evidence favours the former theory, although there may be a window period for collaborative human efforts to halt the process. To draw a parallel from the development of penicillin resistance in *S. aureus*, it may take years to decades before methicillin resistance becomes as prevalent as penicillin resistance worldwide.[44]

While there is a clear need locally to keep track of the CA-MRSA situation and to formulate guidelines for empirical therapy and for minimising spread of CA-MRSA should the situation worsen, it is equally important to focus on

antibiotic stewardship both in Singapore and elsewhere in order to reduce the evolutionary pressure for the generation of yet more resistant pathogenic organisms.

Acknowledgement

We would like to thank Dr Tan Thean Yen, Dr Prabha Krishnan, Dr Timothy Barkham, Dr Nancy Tee and Dr Raymond Lin – microbiologists from Changi General Hospital, Tan Tock Seng Hospital, Kandang Kerbau Hospital and National University Hospital respectively – for their continued support and collaboration in our projects on community-associated S. aureus *in Singapore.*

We would also like to thank the staff of the SGH Microbiology Laboratory, in particular Mdm Grace Wang and Ms Ong Lan Huay, for their invaluable assistance in the microbiological and molecular work-up of local isolates.

REFERENCES

1. Mongkolrattanothai K, Daum RS. Impact of community-associated, methicillin-resistant *Staphylococcus aureus* on management of the skin and soft tissue infections in children. Curr Infect Dis Rep 2005;7:381-9.
2. Murray RJ, Lim TT, Pearson JC, Grubb WB, Lum GD. Community-onset methicillin-resistant *Staphylococcus aureus* bacteremia in Northern Australia. Int J Infect Dis 2004;8:275-83.
3. Steinberg JP, Clark CC, Hackman BO. Nosocomial and community-acquired *Staphylococcus aureus* bacteremias from 1980 to 1993: impact of intravascular devices and methicillin resistance. Clin Infect Dis 1996;23:255-9.
4. Noskin GA, Rubin RJ, Schentag JJ, Kluytmans J, Hedblom EC, Smulders M, et al. The burden of *Staphylococcus aureus* infections on hospitals in the United States: an analysis of the 2000 and 2001 Nationwide Inpatient Sample Database. Arch Intern Med 2005;165:1756-61.
5. Hartman B, Tomasz A. Altered penicillin-binding proteins in methicillin-resistant strains of *Staphylococcus aureus*. Antimicrob Agents Chemother 1981;19:726-35.
6. Okuma K, Iwakawa K, Turnidge JD, Grubb WB, Bell JM, O'Brien FG, et al. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J Clin Microbiol 2002;40:4289-94.
7. Ito T, Ma XX, Takeuchi F, Okuma K, Yuzawa H, Hiramatsu K. Novel type V staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase, ccrC. Antimicrob Agents Chemother 2004;48:2637-51.
8. Wu S, Piscitelli C, de Lencastre H, Tomasz A. Tracking the evolutionary origin of the methicillin resistance gene: cloning and sequencing of a homologue of mecA from a methicillin susceptible strain of *Staphylococcus sciuri*. Microb Drug Resist 1996;2:435-41. Erratum in: Microb Drug Resist 1997;299. Microb Drug Resist 1997;3:212.
9. Katayama Y, Takeuchi F, Ito T, Ma XX, Ui-Mizutani Y, Kobayashi I, et al. Identification in methicillin-susceptible *Staphylococcus hominis* of an active primordial mobile genetic element for the staphylococcal cassette chromosome mec of methicillin-resistant *Staphylococcus aureus*. J Bacteriol 2003;185:2711-22.
10. Diekema DJ, BootsMiller DJ, Vaughn TE, Woolson RF, Yankey JW, Ernst EJ, et al. Antimicrobial resistance trends and outbreak frequency in United States hospitals. Clin Infect Dis 2004;38:78-85.
11. Lee K, Chang CL, Lee NY, Kim HS, Hong KS, Cho HC. Korean nationwide surveillance of antimicrobial resistance of bacteria in 1998. Yonsei Med J 2000;41:497-506.
12. Goossens H. European status of resistance in nosocomial infections. Chemotherapy 2005;51:177-81.
13. Ender M, McCallum N, Adhikari R, Berger-Bächi B. Fitness cost of SCCmec and methicillin resistance levels in *Staphylococcus aureus*. Antimicrob Agents Chemother 2004;48:2295-7.
14. Centers for Disease Control (CDC). Community-acquired methicillin-resistant *Staphylococcus aureus* infections — Michigan. MMWR Morb Mortal Wkly Rep 1981;30:185-7.
15. 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.
16. Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA 1998;279:593-8.
17. Centers for Disease Control and Prevention. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus* – Minnesota and North Dakota, 1997-1999. JAMA 1999;282:1123-5.
18. Dufour P, Gillet Y, Bes M, Lina G, Vandenesch F, Floret D, 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.
19. Adhikari RP, Cook GM, Lamont I, Lang S, Heffernan H, Smith JM. Phenotypic and molecular characterization of community occurring, Western Samoan phage pattern methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 2002;50:825-31.
20. Centres for Disease Control and Prevention (CDC). Community-associated methicillin-resistant *Staphylococcus aureus* in Pacific Islanders – Hawaii, 2001-2003. MMWR Morb Mortal Wkly Rep 2004;53:767-70.
21. 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.
22. Hsu LY, Tristan A, Koh TH, Bes M, Etienne J, Kurup A, et al. Community-associated methicillin-resistant *Staphylococcus aureus*, Singapore. Emerg Infect Dis 2005;11:341-2.
23. Harbarth S, Francois P, Shrenzel J, Fankhauser-Rodriguez C, Hugonnet S, Koessler T, et al. Community-associated methicillin-resistant *Staphylococcus aureus*, Switzerland. Emerg Infect Dis 2005;11:962-5.
24. Linde H, Wagenlehner F, Strommenger B, Drubel I, Tanzer J, Reischl U, et al. Healthcare-associated outbreaks and community-acquired infections due to MRSA carrying the Panton-Valentine leucocidin gene in southeastern Germany. Eur J Clin Microbiol Infect Dis 2005;24:419-22.
25. Ribeiro A, Dias C, Silva-Carvalho MC, Berguo L, Ferreira FA, Santos RN, et al. First report of infection with community-acquired methicillin-resistant *Staphylococcus aureus* in South America. J Clin Microbiol 2005;43:1985-8.
26. 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;26:144-9.
27. Kazakova SV, Hageman JC, Matava M, Srinivasan A, Phelan L, Garfinkel B, et al. A clone of methicillin-resistant *Staphylococcus aureus* among professional football players. N Engl J Med 2005;352:468-75.
28. Campbell KM, Vaughn AF, Russell KL, Smith B, Jimenez DL, Barrozo CP, et al. Risk factors for community-associated methicillin-resistant *Staphylococcus aureus* infections in an outbreak of disease among military trainees in San Diego, California, in 2002. J Clin Microbiol 2004;42:4050-3.
29. Clancy MJ, Graepler A, Breese PE, Price CS, Burman WJ. Widespread emergence of methicillin resistance in community-acquired *Staphylococcus aureus* infections in Denver. South Med J 2005;98: 1061-2.
30. Ho PL, Tse CW, Mak GC, Chow KH, Ng TK. Community-acquired methicillin-resistant *Staphylococcus aureus* arrives in Hong Kong. J Antimicrob Chemother 2004;845-6.
31. Denis O, Deplano A, De Beenhouwer H, Hallin M, Huysmans G, Garrino MG, et al. Polyclonal emergence and importation of community-acquired methicillin-resistant *Staphylococcus aureus* strains harbouring Panton-

Valentine leucocidin genes in Belgium. J Antimicrob Chemother 2005;56:1103-6.

32. Vourli S, Perimeni D, Makri A, Polemis M, Voyiatzi A, Vatopoulos A. Community acquired MRSA infections in a paediatric population in Greece. Euro Surveill 2005;10:78-9.
33. Wannet WK, Spalburg E, Heck ME, Pluister GN, Tiemersma E, Willems RJ, et al. Emergence of virulent methicillin-resistant *Staphylococcus aureus* strains carrying Panton-Valentine leucocidin genes in the Netherlands. J Clin Microbiol 2005;43:3341-5.
34. Mulvey MR, MacDougall L, Cholin B, Horsman G, Fidyk M, Woods S, et al. Community-associated methicillin-resistant *Staphylococcus aureus*, Canada. Emerg Infect Dis 2005;11:844-50.
35. Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. *Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. J Clin Microbiol 2005;43:2384-90.
36. Hanssen AM, Fossum A, Mikalsen J, Halvorsen DS, Bukholm G, Sollid JU. Dissemination of community-acquired methicillin-resistant *Staphylococcus aureus* clones in northern Norway: sequence types 8 and 80 predominate. J Clin Microbiol 2005;43:2118-24.
37. Miklasevics E, Haeggman S, Balode A, Sanchez B, Martinsons A, Olsson-Liljequist B, et al. Report on the first PVL-positive community acquired MRSA strain in Latvia. Euro Surveill 2004;9:29-30.
38. Dailey L, Coombs GW, O'Brien FG, Pearman JW, Christiansen K, Grubb WB, et al. Methicillin-resistant *Staphylococcus aureus*, Western Australia. Emerg Infect Dis 2005;11:1584-90.
39. Ma XX, Galiana A, Pedreira W, Mowszowicz M, Christophersen I, Machiavello S, et al. Community-acquired methicillin-resistant *Staphylococcus aureus*, Uruguay. Emerg Infect Dis 2005;11:973-6.
40. Young DM, Harris HW, Charlebois ED, Chambers H, Campbell A, Perdreau-Remington F, et al. An epidemic of methicillin-resistant *Staphylococcus aureus* soft tissue infections among medically underserved patients. Arch Surg 2004;139:947-51.
41. Centers for Disease Control and Prevention (CDC). Methicillin-resistant *Staphylococcus aureus* infections in correctional facilities – Georgia, California, and Texas, 2001-2003. MMWR Morb Mortal Wkly Rep 2003;52:992-6.
42. Kourbatova EV, Halvosa JS, King MD, Ray SM, White N, Blumberg HM. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA 300 clone as a cause of healthcare-associated infections among patients with prosthetic joint infections. Am J Infect Control 2005;33:385-91.
43. Seybold U, Kourbatova EV, Johnson HG, Halvosa JS, Wang YF, King MD, et al. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA 300 genotype as a major cause of healthcare-associated blood stream infections. Clin Infect Dis 2006; 42:647-56.
44. Chambers HF. The changing epidemiology of *Staphylococcus aureus*? Emerg Infect Dis 2001;7:178-82.
45. Daum RS, Ito T, Hiramatsu K, Hussain F, Mongokolrattanothai K, Jamklang M, et al. A novel methicillin-resistance cassette in community-acquired methicillin-resistant *Staphylococcus aureus* isolates of diverse genetic backgrounds. J Infect Dis 2002;186:1344-7.
46. Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2003;47:3926-34.
47. Donnio PY, Preney L, Gautier-Lerestif AL, Avril JL, Lafforgue N. Changes in staphylococcal cassette chromosome type and antibiotic resistance profile in methicillin-resistant *Staphylococcus aureus* isolates from a French hospital over an 11 year period. J Antimicrob Chemother 2004;53:808-13.
48. Hsu LY, Koh TH, Singh K, Kang ML, Kurup A, Tan BH. Dissemination of multisusceptible methicillin-resistant *Staphylococcus aureus* in Singapore. J Clin Microbiol 2005;43:2923-5.
49. Sanford MD, Widmer AF, Bale MJ, Jones RN, Wenzel RP. Efficient detection and long-term persistence of the carriage of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 1994;19:1123-8.
50. Saiman L, O'Keefe M, Graham PL 3rd, Wu F, Said-Salim B, Kreiswirth B, et al. Hospital transmission of community-acquired methicillin-resistant *Staphylococcus aureus* among post-partum women. Clin Infect Dis 2003;37:1313-9.
51. 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.
52. O'Brien FG, Lim TT, Chong FN, Coombs GW, Enright MC, Robinson DA, et al. Diversity among community isolates of methicillin-resistant *Staphylococcus aureus* in Australia. J Clin Microbiol 2004;42:3185-90.
53. Tietz A, Frei R, Widmer AF. Transatlantic spread of the USA300 clone of MRSA. N Engl J Med 2005;353:532-3.
54. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, et al. Comparison of community- and healthcare-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA 2003;290:2976-84.
55. 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.
56. de Bentzmann S, Tristan A, Etienne J, Brousse N, Vandenesch F, Lina G. *Staphylococcus aureus* isolates associated with necrotizing pneumonia bind to basement membrane type I and IV collagens and laminin. J Infect Dis 2004;190:1506-15.
57. Adem PV, Montgomery CP, Husain AN, Koogler TK, Arangelovich V, Humilier M, et al. *Staphylococcus aureus* sepsis and the Waterhouse-Friderichsen syndrome in children. N Engl J Med 2005;353:1245-51.
58. Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, et al. Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. N Engl J Med 2005;352:1445-53.
59. Baba T, Takeuchi F, Kuroda M, Yuzawa H, Aoki K, Oguchi A, et al. Genome and virulence determinants of high virulence community-acquired MRSA. Lancet 2002;359:1819-27.
60. Hsu LY, Koh YL, Loomba-Chlebicka N, Tan TY, Krishnan P, Lin RT, et al. Establishment of ST30 as the predominant clonal type among community-associated methicillin-resistant *Staphylococcus aureus* isolates in Singapore. J Clin Microbiol 2006;44:1090-3.
61. Robinson DA, Kearns AM, Holmes A, Morrison D, Grundmann H, Edwards G, et al. Re-emergence of early pandemic *Staphylococcus aureus* as a community-acquired methicillin-resistant clone. Lancet 2005;365:1256-8.
62. Lee MC, Rios AM, Aten MF, Mejias A, Cavuoti D, McCracken GH Jr, et al. Management and outcome of children with skin and soft tissue abscesses caused by community-acquired methicillin-resistant *Staphylococcus aureus*. Pediatr Infect Dis J 2004;23:123-7.
63. Buescher ES. Community-acquired methicillin-resistant *Staphylococcus aureus* in pediatrics. Curr Opin Pediatr 2005;17:67-70.
64. Braun L, Craft D, Williams R, Tuamokumo F, Ottolini M. Increasing clindamycin resistance among methicillin-resistant *Staphylococcus aureus* in 57 northeast United States military treatment facilities. Pediatr Infect Dis J 2005;24:622-6.