Evolving EMRSA-15 epidemic in Singapore hospitals

Li-Yang Hsu,¹ Nidhi Loomba-Chlebicka,² Yin-Ling Koh,² Thean-Yen Tan,³ Prabha Krishnan,⁴ Raymond Tzer-Pin Lin,⁵ Nancy Wen-Sin Tee,⁶ Dale Andrew Fisher¹ and Tse-Hsien Koh²

Correspondence

Li-Yang Hsu

liyang_hsu@yahoo.com

¹Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, 5 Lower Kent Ridge Road, Singapore 119074, Singapore

²Singapore General Hospital, Singapore

³Changi General Hospital, Singapore

⁴Tan Tock Seng Hospital, Singapore

⁵National University Hospital, Singapore

⁶Kandang Kerbau Women's and Children's Hospital, Singapore

Received 16 September 2006

Accepted 30 October 2006

The aim of this study was to determine the extent of EMRSA-15 spread in hospitals in Singapore. Molecular analysis of 197 non-duplicate methicillin-resistant *Staphylococcus aureus* (MRSA) isolates collected from five acute care public hospitals in Singapore in May 2005 revealed that 66 (33.5 %) were EMRSA-15 while 121 (61.4 %) belonged to the endemic multidrug-resistant ST239 clone. Median and mode vancomycin MIC for both major clones of health-care-associated MRSA were relatively high at 2.0 µg ml⁻¹. Subsequent laboratory surveillance data collected from the first half of 2006 confirmed increasing numbers of the EMRSA-15 clone – ranging from 25.0 to 66.1 % of all MRSA isolated in local hospitals – replacing the ST239 clone island-wide.

INTRODUCTION

EMRSA-15 emerged in 1991 in the UK and is at present one of two predominant health-care-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) clones in the country, having replaced most of the other HA-MRSA clones in the first decade of its emergence (O'Neill *et al.*, 2001). It has since spread worldwide, and is currently one of the most global HA-MRSA clones (Pearman *et al.*, 2001; Melter *et al.*, 2004; Hsu *et al.*, 2005; Smith & Cook, 2005; Udo *et al.*, 2006).

Clonal EMRSA-15 (ST22) strains were first noticed in a Singapore Public Hospital in 2003. Over the course of 2 years, it had displaced a progressively increasing proportion of the endemic ST239 clone in that hospital (Hsu *et al.*, 2005). Because Singapore is a very small country, with frequent transfer of patients between its six public acute care hospitals, it is likely that this clone has disseminated island-wide. We sought to determine the degree of penetration of EMRSA-15 in local hospitals.

Abbreviations: HA-MRSA, health-care-associated methicillin-resistant *Staphylococcus aureus*; MLST, multilocus sequence typing; MLVA, multilocus variable-number tandem-repeat analysis; MRSA, methicillin-resistant *Staphylococcus aureus*; SGH, Singapore General Hospital.

METHODS

Microbiology laboratories from five public hospitals (two tertiary hospitals, two secondary hospitals, and one hospital dedicated to obstetrics, gynaecology and paediatrics) contributed MRSA strains isolated from clinical specimens during May 2005, with duplicates removed via cross-referencing with patient identification numbers. Species identification was via colony morphology, coagulation of citrated rabbit plasma with EDTA (BBL Becton Dickinson), and production of clumping factor and protein A (BactiStaph). Antibiotic susceptibility results were obtained using the Kirby-Bauer disk diffusion method following Clinical & Laboratory Standards Institute (CLSI) guidelines (Clinical & Laboratory Standards Institute, 2005). Additionally, the MIC of vancomycin for each strain was determined via the use of Etest (AB Biodisk).

All strains were subject to PFGE (Maslow *et al.*, 1993), multilocus variable-number tandem-repeat analysis (MLVA) (Sabat *et al.*, 2003), accessory gene regulator *agr* allele profiling (Lina *et al.*, 2003) and SCCmec typing (Okuma *et al.*, 2002). Strains with novel PFGE results after comparison to our local database were further typed using multilocus sequence typing (MLST) (Enright *et al.*, 2000). In addition, PCR testing for macrolide resistance genes *ermA–C* was performed on all EMRSA-15 isolates (Sutcliffe *et al.*, 1996).

A surveillance program was also established to monitor the trends of MRSA in Singapore. The WHONET software program [World Health Organization. <http://www.who.int/drugresistance/whonetsoftware/en/> (accessed 8 September 2006)] was installed and used by all six public

hospital microbiology laboratories to extract and transmit antimicrobial susceptibility data for all non-duplicate MRSA strains for analysis every quarter.

RESULTS AND DISCUSSION

A total of 197 MRSA strains were typed, comprising 121 ST239-MRSA-III strains (61.4 %), 66 EMRSA-15 (ST22-MRSA-IV) strains (33.5 %), and 10 other strains deemed to be community-associated MRSA based on further epidemiological tracing (Fig. 1). These latter 10 strains have been described elsewhere (Hsu *et al.*, 2006). MLVA clusters correlated well with PFGE clusters and MLST sequence types in general, although MLVA appeared to be less discriminatory than PFGE for EMRSA-15 strains, and there was no exact matching of MLVA and PFGE profiles. All HA-MRSA isolates had *agr* allele 1. The percentage of EMRSA-15 to all MRSA ranged from 23.6 to 41.0 % in the different hospitals, with 28.3 % at the Singapore General Hospital (SGH) representing an increment of 9.9 % over the result of 18.4 % in 2004 (Hsu *et al.*, 2005).

The antibiotic resistance profiles of the strains were distinct (Table 1) and discriminated between the two HA-MRSA clones reliably. Ten EMRSA-15 strains were susceptible to all non- β -lactam antibiotics with the exception of ciprofloxacin – a novel finding compared to our survey a year ago (Hsu *et al.*, 2005). These strains could not be distinguished from other EMRSA-15 strains with PFGE, although the *ermA* and *ermC* genes present on all other strains could not be detected in these. Mode and median vancomycin MIC for both HA-MRSA strains were 2.0 $\mu\text{g ml}^{-1}$, although only

two ST239 strains exceeded this MIC. The vancomycin MICs of the community-associated MRSA strains were lower (0.75 $\mu\text{g ml}^{-1}$, range 0.5–1.5 $\mu\text{g ml}^{-1}$).

The results of the laboratory surveillance programme, using WHONET software, for January to June 2006 demonstrated unequivocally the significant presence of EMRSA-15 in all local hospitals (Fig. 2); 31.6 % of SGH MRSA had antibiotic susceptibility profiles that were identical to EMRSA-15. Although the time frames were not comparable, and none of the 2006 strains was typed, this nevertheless suggested a continued displacement of the ST239 clone by EMRSA-15. There was no overall increase in the numbers or rates of MRSA at SGH over previous years (data not shown).

Overall, our results indicate that EMRSA-15 has penetrated all local hospitals, with evidence pointing to a displacement (of the endemic clone) rather than an additive effect. Because of the frequent inter-transfer of patients and reactive focus of local infection control programs (active surveillance for MRSA is not practised), the spread of this new epidemic MRSA clone is unsurprising. Although the similar *agr* alleles theoretically suggest co-existence rather than competition in shared ecological niches (Lina *et al.*, 2003), this was not borne out *in vivo*, and the speed at which EMRSA-15 is displacing the endemic clone is noteworthy. This phenomenon has also been observed in the country of its origin and elsewhere (O'Neill *et al.*, 2001; Pearman *et al.*, 2001). However, the reasons for this are not completely clear, although hyper-transmissibility, partially attributed to prolonged environmental survival, may play a role (Hardy *et al.*, 2006).

Fig. 1. Comparison of 197 health-care- and community-associated MRSA isolates from five hospitals, using PFGE, MLVA, SCCmec typing and MLST. *Sma*I restriction patterns and MLVA gel images were digitized 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 means (UPGMA) clustering. Isolates were considered to be related according to PFGE if gel patterns demonstrated 80 % or greater similarity upon analysis.

Table 1. Antimicrobial resistance profiles, including vancomycin MIC, of 197 MRSA isolates stratified according to sequence type

Antimicrobials tested include erythromycin (E), ciprofloxacin (Cip), clindamycin (Cli), gentamicin (Gen), trimethoprim-sulfamethoxazole (SXT), tetracycline (Tet), fusidic acid and rifampicin. All isolates had <5% resistance to rifampicin and fusidic acid. ST239 strains demonstrated either inducible or constitutive resistance to clindamycin. ST22 (EMRSA-15) strains that were resistant to clindamycin manifested only inducible resistance. A small proportion (7.1%) of ST22 isolates resistant to both erythromycin and clindamycin were also resistant to tetracycline.

Sequence type	Antimicrobial resistance profile	No. of isolates for each vancomycin MIC ($\mu\text{g ml}^{-1}$)					
		≤ 0.5	1.0	1.5	2.0	3.0	4.0
239	E, Cip, Cli, Gen, Tet, SXT	0	12	46	61	1	1
22	E, Cip, Cli	0	5	18	33	0	0
	Cip	0	1	3	6	0	0
30	–	3	5	0	0	0	0
59	Tet	0	0	1	0	0	0
88	–	1	0	0	0	0	0

MLVA has proven to be an adequate typing tool for local surveillance. There was only one MLVA profile for EMRSA-15 strains in Singapore in contrast to three PFGE profiles,

Fig. 2. Distribution of different clones of MRSA (duplicates removed) in local hospitals from January to June 2006. ST239 strains (white bars) were identified based on resistance to ciprofloxacin, erythromycin, clindamycin, gentamicin and tetracycline. EMRSA-15 (ST22) strains (grey bars) were identified based on two antimicrobial resistance profiles: (a) resistance to ciprofloxacin, erythromycin, and inducible resistance to clindamycin, this comprised 92.3% of all presumptive EMRSA-15 strains; (b) resistance to ciprofloxacin alone, this comprised 7.7% of all presumptive EMRSA-15 strains. MRSA with differing antimicrobial resistance profiles were classed under 'others' (black bars). The proportion of EMRSA-15 is slightly overestimated because it is possible that other MRSA clones (i.e. community-associated MRSA) may possess resistance profiles identical to (b). However, we have not detected this locally as yet. *Hospital 5 MRSA strains were not included in May 2005 for typing/work-up; **hospital 6 is dedicated to obstetrics, gynaecology and paediatrics only.

implying lesser discriminatory power. An alternative explanation could be that there was insufficient time (approx. 4 years) for the various VNTR (variable number tandem repeat) loci in local EMRSA-15 to have acquired significant size alterations. ST239 has been in Singapore for more than 20 years, with sufficient time to generate a diverse range of related MLVA profiles.

A new EMRSA-15 subclone without *erm* genes has appeared locally, which is susceptible to all non- β -lactam antibiotics tested with the exception of ciprofloxacin. Although both MLVA and PFGE results imply that it might have been derived from local EMRSA-15 strains, the losses of presumably both plasmid *ermC* and chromosomal *ermA* are significant events. We were, however, not able to detect any intervening strains with only one type of *erm* gene in our admittedly small population of EMRSA-15 strains. It is impossible to determine the likelihood of this subclone being imported based on existing limited epidemiological data. Increasing diversity within the EMRSA-15 complex, and a lack of multi-drug resistance in a significant number of isolates has also been noted elsewhere (Smith & Cook, 2005).

Vancomycin MIC results are worrying, with almost 50% of HA-MRSA having MICs of $\geq 2 \mu\text{g ml}^{-1}$. This has potential implications for the success rates of therapy for local MRSA infections using vancomycin, although further study is required to ascertain this. We were not able to perform population analysis to determine the proportions of heteroglycopeptide-intermediate *S. aureus* in our strains.

In conclusion, our study highlights once again the remarkable success and transmissibility of EMRSA-15. Further surveillance is clearly indicated, and it is evident that infection control programmes that were barely able to contain more archaic endemic MRSA clones will not be able to cope with this and other newer epidemic HA-MRSA clones.

ACKNOWLEDGEMENTS

The authors would like to thank Ms Grace Wang, Ms Lan-Huay Ong and Mrs Mee-Lee Tan for their help in providing logistical support for this study. This study was funded by a grant (number NMRC/0903/2004) from the National Medical Research Council, Singapore. The authors do not have any conflicts of interest.

REFERENCES

- Clinical and Laboratory Standards Institute (2005).** *Performance Standards for Antimicrobial Susceptibility Testing*, approved standard M100-S16. Wayne, PA: Clinical and Laboratory Standards Institute.
- Enright, M. C., Day, N. P., Davies, C. E., Peacock, S. J. & Spratt, B. G. (2000).** Multilocus sequence typing for characterization of methicillin-resistant and methicillin-sensitive clones of *Staphylococcus aureus*. *J Clin Microbiol* **38**, 1008–1015.
- Hardy, K. J., Oppenheim, B. A., Gossain, S., Gao, F. & Hawkey, P. M. (2006).** A study of the relationship between environmental contamination with methicillin-resistant *Staphylococcus aureus* (MRSA) and patients' acquisition of MRSA. *Infect Control Hosp Epidemiol* **27**, 127–132.
- Hsu, L. Y., Koh, T. H., Singh, K., Kang, M. L., Kurup, A. & Tan, B. H. (2005).** Dissemination of multisusceptible methicillin-resistant *Staphylococcus aureus* in Singapore. *J Clin Microbiol* **43**, 2923–2925.
- Hsu, L. Y., Koh, Y. L., Chlebicka, N. L., Tan, T. Y., Krishnan, P., Lin, R. T., Tee, N., Barkham, T. & Koh, T. H. (2006).** Establishment of ST30 as the predominant clonal type among community-associated methicillin-resistant *Staphylococcus aureus* isolates in Singapore. *J Clin Microbiol* **44**, 1090–1093.
- Lina, G., Boutite, F., Tristan, A., Bes, M., Etienne, J. & Vandenesch, F. (2003).** Bacterial competition for human nasal cavity colonization: role of staphylococcal *agr* alleles. *Appl Environ Microbiol* **69**, 18–23.
- Maslow, J., Slutsky, A. & Arbeit, R. (1993).** The application of pulsed field gel electrophoresis to molecular epidemiology. In *Diagnostic Molecular Microbiology: Principles and Applications*, pp. 563–572. Edited by H. Persing, T. Smith, F. Tenover & T. White. Washington, DC: American Society for Microbiology.
- Melter, O., Aires de Sousa, M., Laskafeldova, K., Urbaskova, P., Wunschova, M. & de Lencastre, H. (2004).** Delineation of the endemic and sporadic methicillin-resistant *Staphylococcus aureus* clones in a Czech hospital. *Microb Drug Resist* **10**, 218–223.
- Okuma, K., Iwakawa, K., Turnidge, J. D., Grubb, W. B., Bell, J. M., O'Brien, F. G., Coombs, G. W., Pearman, J. W., Tenover, F. C. & other authors (2002).** Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. *J Clin Microbiol* **40**, 4289–4294.
- O'Neill, G. L., Murchan, S., Gil-Setas, A. & Aucken, H. M. (2001).** Identification and characterization of phage variants of a strain of epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA-15). *J Clin Microbiol* **39**, 1540–1548.
- Pearman, J. W., Coombs, G. W., Grubb, W. B. & O'Brien, F. (2001).** A British epidemic strain of methicillin-resistant *Staphylococcus aureus* (UK EMRSA-15) in Western Australia. *Med J Aust* **174**, 662.
- Sabat, A., Krzyszton-Russjan, J., Strzalka, W., Filipek, R., Kosowska, K., Hryniewicz, W., Travis, J. & Potempa, J. (2003).** New method for typing *Staphylococcus aureus* strains: multiple-locus variable-number tandem repeat analysis of polymorphism and genetic relationships of clinical isolates. *J Clin Microbiol* **41**, 1801–1804.
- Smith, J. M. & Cook, G. M. (2005).** A decade of community MRSA in New Zealand. *Epidemiol Infect* **133**, 899–904.
- Sutcliffe, J., Grebe, T., Tait-Kamradt, A. & Wondrack, L. (1996).** Detection of erythromycin-resistant determinants by PCR. *Antimicrob Agents Chemother* **40**, 2562–2566.
- Udo, E. E., Al-Sweih, N. & Noronha, B. (2006).** Characterisation of non-multiresistant methicillin-resistant *Staphylococcus aureus* (including EMRSA-15) in Kuwait Hospitals. *Clin Microbiol Infect* **12**, 262–269.