Evolving EMRSA-15 epidemic in Singapore hospitals

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The aim of this study was to determine the extent of EMRSA-15 spread in hospitals in Singapore. Molecular analysis of 197 non-duplicate meticillin-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 \( \mu \text{g ml}^{-1} \). 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 meticillin-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.

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). 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 \textit{ermA–C} was performed on all EMRSA-15 isolates (Sutcliffe et al., 1996).

All strains were subject to PFGE (Maslow et al., 1993), multilocus variable-number tandem-repeat analysis (MLVA) (Sabat et al., 2003), accessory gene regulator \textit{agr} allele profiling (Lina et al., 2003) and SCC\textit{mec} 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 \textit{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 \textit{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-\(\beta\)-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 \textit{ermA} and \textit{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 \(\mu\)g ml\(^{-1}\), although only two ST239 strains exceeded this MIC. The vancomycin MICs of the community-associated MRSA strains were lower (0.75 \(\mu\)g ml\(^{-1}\), range 0.5–1.5 \(\mu\)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 \textit{agr} alleles theoretically suggest co-existence rather than competition in shared ecological niches (Lina et al., 2003), this was not borne out \textit{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).
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, 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 $\geq 2\ \mu g\ ml^{-1}$. 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 hetero-glycopeptide-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.

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

<table>
<thead>
<tr>
<th>Sequence type</th>
<th>Antimicrobial resistance profile</th>
<th>No. of isolates for each vancomycin MIC ($\mu g\ ml^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\leq 0.5$</td>
</tr>
<tr>
<td>239</td>
<td>E, Cip, Cli, Gen, Tet, SXT</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>E, Cip, Cli</td>
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<tr>
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<tr>
<td>59</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>88</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

**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 clindamycin 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.
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