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Genetic Analysis of Methicillin-Resistant *Staphylococcus aureus* from Singapore Hospitals

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After some initial outbreaks, methicillin-resistant *Staphylococcus aureus* (MRSA) became quiescent (1). However, recently there has been a resurgence of outbreaks with reports coming from several countries (2). Genetic analyses have shown that the MRSA isolated prior to 1973 in the UK (3) and Australia (4) were similar and probably had the same clonal origin. Similar studies on isolates from recent outbreaks of MRSA have shown that they are genetically different from the clonal or classic MRSA (4–7). Although the isolates from some countries are different the epidemic MRSA of eastern Australia and London are similar indicating that a particular strain has recently spread internationally (7). These findings prompted an analysis of MRSA from Singapore hospitals. There is a large tourist trade between Australia and Singapore and it appeared likely that a similar spread could have occurred between the two countries.

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The isolation rates for MRSA in Singapore hospitals have been steadily increasing. In 1982 the Pathology Department, which does the bacteriological investigations for all hospitals in Singapore, recorded that 12% of all *Staphylococcus aureus* isolates were MRSA. In 1983 the figure was 16% and in 1984 and 1985 the figures increased to 21% and 27% respectively. During the first four months of 1986, 94 MRSA were isolated from patients with infections in the burns ward of a large general hospital. The majority (88) of the isolates were from infected wounds, three were from the respiratory tract, one from the urinary tract and two from blood cultures. Thirty-three of these isolates and 67 MRSA isolated in the latter period of 1985 were chosen for detailed genetic study. In order to see if there had been genetic changes in the MRSA with time, 15 isolates which had been lyopholized since 1982 were also examined. All the isolates examined were single colony isolates from different patients. The 1982 and 1985 isolates were from either wounds or sputa. All the isolates were phage typed by the Western Australian State Health Laboratory Services and were non-typable with the international bacteriophage typing set at routine test dilution (RTD). They were all non-typable at 100 RTD except for a few which were sensitive to phage 85.

Isolates were tested for penicillinase production and sensitivity to a range of antimicrobials (8, 9). In addition to being resistant to methicillin, all produced penicillinase. Their resistance to a range of antimicrobial agents is summarised in Table 1. Overall, the isolates were very similar in their spectrum of resistance to the agents tested. Although all the isolates were resistant to streptomycin, some had low-level resistance and others high-level (8). All the 1985 isolates and all the burns isolates had inducible erythromycin resistance (8). The 1982 isolates also had inducible erythromycin resistance except for a few which had constitutive erythromycin resistance (8). Except for a few of the 1985 isolates, all were resistant to propamidine isethionate and had nucleic acid-binding (NAB) resistance of the class 1 type (9). The only isolates resistant to trimethoprim were a few isolates from the burns ward. This may reflect the wide-spread use of co-trimoxazole in the burns ward to treat MRSA infections.

The isolates were examined for plasmids by agarose-gel electrophoresis (10). The types of plasmids found in the isolates are listed in Table 2. Except for a few 1985 isolates which were propamidine sensitive, all the isolates contained a plasmid of either 27, 30 or 33 kilobase pair (kbp). The isolates from the burns ward all had the same plasmid content and contained a 27, a 2.4 and 2.8 kbp plasmid. The other isolates varied in their plasmid content. The plasmid profile of a few of the isolates is shown in Figure 1. These were selected in order to show the range of plasmid sizes seen in the isolates.

Table 1: Percentage of isolates resistant to given antimicrobial agents.

Isolation	Gentamicin	Kanamycin	Neomycin	Streptomycin	Erythromycin	Lincomycin	Chloramphenicol	Tetracycline	Trimethoprim	Sulphonamides	Fusidic acid	Rifampicin	Novobiocin	Cadmium	Phenyl mercuric acetate	Arsenate	Propamidine isethionate
1982	100	100	36	100	100	21	29	93	0	100	0	0	0	100	100	0	100
1985	100	100	78	100	100	0	84	100	0	100	0	0	0	92	92	0	92
1986[a]	100	100	100	100	100	0	0	100	13	100	0	0	0	100	100	0	100

[a]Burns ward.

Table 2: Size (kbp) and phenotype[a] of plasmids in isolates.

Isolation	$Pi^RCd^RPma^R$	$Pi^RCd^RPma^RPase^+$	Tc^R	Cryptic
1982	27	30, 33	4.2	2.2, 2.4, 2.6, 2.8
1985		30, 33	4.2	2.2, 2.4, 2.6, 2.8
1986[b]	27			2.4, 2.8

[a]Cd = cadmium; Pma = phenyl mercuric acetate; Tc = tetracycline; $Pase^+$ = penicillinase produced; superscript R indicates resistance.
[b]Burns ward.

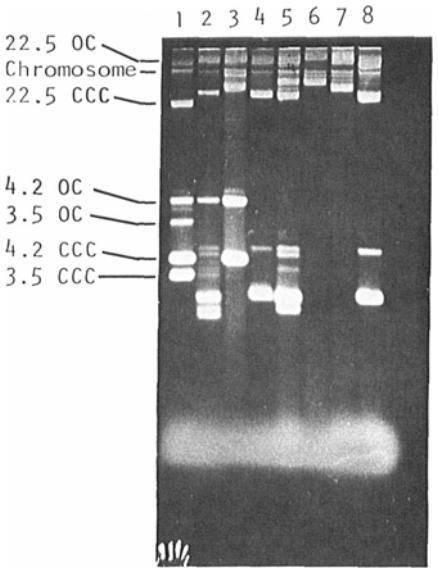

Figure 1: Track 1, molecular weight standard WBG4483 (11). Track 2, WBG1985 containing plasmids of 27, 4.2, 2.8, 2.6, 2.4 and 2.2 kbp. Track 3, WBG1986 containing plasmids of 30 and 4.2 kbp. Track 4, WBG6119 containing plasmids of 27 and 2.8 kbp. Track 5, WBG4997 containing plasmids of 33, 27, 2.8, 2.6 and 2.4 kbp. Track 6, Transcipient containing a 33 kbp plasmid. Track 7, Transcipient containing a 30 kbp plasmid. Track 8, WBG3164 containing a 27 and a 2.8 kbp plasmid.

The location of the resistance determinants was ascertained by transfer experiments (11) and curing of plasmids (8). Transfer was attempted by phage-mediated mixed culture and conjugation (11). Selection was made for the antimicrobial agents to which the isolates were resistant. Only phage-mediated transfer was obtained and WBG1876 and WBG3358 were found to be the best recipients (7). The results indicated that all the isolates studied had chromosomal resistance to gentamicin, kanamycin, streptomycin, erythromycin and sulphonamides. When the isolates were resistant to neomycin and lincomycin, resistance was also chromosomal. Tetracycline resistance was chromosomal in the isolates from the burns ward, but was either chromosomal, or borne on a 4.2 kbp plasmid in the other isolates.

The plasmids of 27, 30 and 33 kbp all encoded resistance to propamidine, cadmium, phenyl mercuric acetate and mercury. The two larger plasmids also encoded for penicillinase. When the penicillinase determinants were not plasmid-borne they were found to be chromosomal. This may indicate that the determinants are located on a transposon similar to that already reported (10). EcoRI endonuclease analysis of the three propamidine-resistance plasmids (Figure 2) indicated that they were closely related and had probably originated by recombination be-

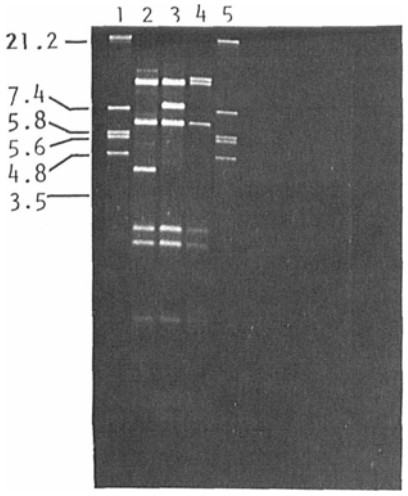

Figure 2: EcoRI restriction cuts. Tracks 1 and 5, λ DNA. Track 2, a 27 kbp plasmid. Track 3, a 30 kbp plasmid. Track 4, a 33 kbp plasmid.

tween a propamidine (NAB)-resistance plasmid and a penicillinase plasmid (12).

The isolates from Singapore are therefore quite different from the classic or clonal MRSA (4–7) and the eastern Australian and epidemic London MRSA (7). They are also different from the Irish (7), Japanese (6) and USA isolates (6). However, like the eastern Australian MRSA, most of them are resistant to propamidine and have class I NAB resistance (9). It has been shown that penicillinase plasmids and NAB-resistance plasmids both belong to incompatibility group 1 (12). The eastern Australian MRSA have acquired the NAB-resistance plasmids by locating the penicillinase plasmid determinants on the chromosome (12). The Singapore isolates appear to have taken a different course and the NAB-resistance plasmids have recombined with the penicillinase plasmids. NAB resistance also confers resistance to some disinfectants (9). However, the resistance conferred is low-level and it is arguable whether this level of resistance is effective in protecting the organisms against these disinfectants.

The strains of MRSA which are epidemic in eastern Australia and London hospitals appear to be able to spread in the hospital environment (5, 7). Although all the isolates from the burns ward were the same in their antimicrobial resistance and plasmid content, there is insufficient evidence at this stage to indicate whether this strain is more likely to spread than the other MRSA isolated from the hospitals in Singapore.

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