Comparative Analysis of Multidrug-Resistant, Non-Multidrug-Resistant, and Archaic Methicillin-Resistant Staphylococcus aureus Isolates from Central Sydney, Australia

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In this study, the phenotypic and genotypic characteristics of 50 methicillin-resistant Staphylococcus aureus (MRSA) isolates (43 contemporary and 7 archaic strains from the mid-1960s) from four Sydney hospitals in the central Sydney area were compared. Phenotypic analysis based on antibiotic profiles and phage typing patterns categorized the MRSA isolates into three major groups: multidrug resistant (mMRSA), non-multidrug resistant (nmMRSA), and archaic. The nmMRSA isolates could be further subdivided into nmMRSA group 1, which was phage typeable and similar to the archaic group; nmMRSA group 2, which was non-phage typeable and only resistant to ciprofloxacin; and nmMRSA group 3, which was also nontypeable and generally resistant to other antibiotics. The characterization of all five phenotypic groups was then extended by genetic analysis. Restriction fragment length polymorphism (RFLP) analysis showed the 50 isolates could be sorted into 20 group-specific pulsortypes. mecA gene deletions and mutations at various percentages among the five MRSA groups were detected by sequencing. Several mec promoter mutations were also found. The overall findings indicated that nmMRSA strains may have independently acquired mecA DNA and are more likely to be newly emergent strains than nmMRSA variants.

Following its first isolation in 1961 (9), methicillin-resistant Staphylococcus aureus (MRSA) has spread around the world and become a major cause of hospital-acquired infection. The evolution of MRSA, or more specifically, the transfer of genes or staphylococcal cassette chromosome mec (SCCmec) between species of staphylococci, has been the subject of intensive investigation since the discovery that the mec genes were responsible for methicillin resistance (8, 15, 18, 29).

Explanations for this transfer are controversial, one being that mec transmission has occurred only once or twice (14) and another being that this transmission has occurred several times (20). More recent evidence has shown that common environmental coagulase-negative staphylococci such as S. sciuri carry mecA and may be the reservoir of SCCmec now found in S. aureus (4).

Our understanding of how MRSA has spread would be clarified if it could be shown that MRSA populations are clonal and that populations change via clonal displacement. Some of the earliest MRSA strains in Australia were isolated at Royal Prince Alfred Hospital (RPAH), Sydney, Australia (29), and then spread nationally. These early MRSA strains as well as subsequent MRSA strains have been preserved in the Staphylococcal Reference Collection at RPAH. Our phage typing data on strains from this collection suggested a population shift might have occurred in the late 1970s (33). We subsequently examined the collection genotypically and provided evidence to suggest that clonal displacement of the then- endemic strains by new strains of MRSA had occurred in this Australian hospital (6).

Infections due to MRSA in eastern Australia are predominantly due to multidrug-resistant MRSA (mMRSA) and are usually termed “hospital-acquired MRSA” (HAMRSA). However, in the last decade there has been an increase in the number of community-acquired infections that are predominantly caused by non-multidrug-resistant MRSA (nmMRSA) strains; that is, they are resistant to fewer antibiotics, other than β-lactams, than mMRSA strains (3). Our previous data have led us to hypothesize that new horizontal gene transfer events may explain the emergence of nmMRSA, because phage typing and antibiograms indicate that these strains have different characteristics from mMRSA strains.

In this study, 43 MRSA isolates from individual patients presenting at Central Sydney Area Hospitals (RPAH, Concord Repatriation General Hospital [CRGH], Canterbury Hospital, and Balmain Hospital) during 1999 to 2000 were studied (14 mMRSA isolates from RPAH and 29 nmMRSA isolates from persons attending all four hospitals), together with 7 archaic strains isolated at RPAH during 1966 to 1967.

Pulsed-field gel electrophoresis, the “gold standard” in genotyping MRSA, was carried out as previously described (6), and the results were analyzed based on guidelines published by Tenover et al. (32) and by phylogenetic analysis. Phage typing was carried out by the method of Blair and Williams (1). The 23 phages of the basic international set of typing phages were supplemented by three experimental phages (no. 187, 90, and 23) of the basic international set of typing phages.
Phenotypic characterization. The phenotypic characteristics of all MRSA isolates studied are shown in Table 1. Based on these results, the MRSA isolates could be divided into three distinct groups: mMRSA, archaic MRSA, and an nmMRSA group, which could further be divided into three subgroups.

Molecular characterization. Table 1 shows restriction fragment length polymorphism (RFLP) data of all 50 isolates supported the groupings based on antibiograms and phage typing analysis. The RFLP band patterns were divisible into 20 reproducible group-specific pulsotypes, with several subtypes designated by a number following the letter of the main pulsotype to which it is closest. The 10 nmMRSA group 1 isolates fell into four main pulsotypes (A, B, C, and D), the 14 mMRSA isolates were made up of 7 pulsotypes (F, G, H, I, J, K, and T), and the archaic MRSA isolates comprised 4 pulsotypes (M to P), while nmMRSA groups 2 and 3 had 4 pulsotypes (E, Q, R, and S) and shared similar pulsotypes between them.

Phylogenetic analysis. Figure 1 illustrates a rooted dendrogram produced by DRAWGRAM. Two main clusters are visible. The cluster originating from node A consists primarily of isolates from nmMRSA groups 2 and 3, whereas the isolates originating from node B are comprised mainly of mMRSA. Archaic MRSA isolates and isolates from nmMRSA group 1 are found grouped together on the lower half of the dendrogram, but are not clustered.

Overall, the mecI gene was deleted in 34 of the 50 (68%) isolates (all 7 archaic MRSA isolates; 11 of 11 nmMRSA group 2 isolates, 7 of 8 [88%] nmMRSA group 3 isolates, 8 of 10 [80%] mmRSA group 1 isolates, and 1 of 14 [7%] mmRSA isolates) as shown in Table 1. Sequence analysis of the 16 mecI-positive isolates revealed point mutations in 12 of 13 (93%) mmRSA isolates, 1 mmRSA group 3 isolate, and 2 mmRSA group 1 isolates.

Analysis of the mec promoter sequence data is shown in Table 1. In all, 4 mutations were identified: the G→T substitution in the mecA Shine-Dalgarno (SD) sequence was the most common change, being identified in 8 isolates (6 of the 10 [60%] mmRSA group 1 isolates, and 2 of 8 [25%] mmRSA group 3 isolates).

In this study, our findings are consistent with previous investigations of mmRSA and mRSA, showing increased antibiotic susceptibility to non-β-lactam antibiotics in the former and major differences in phage typing patterns (3, 24). Our data illustrated that mmRSA groups 2 and 3, which were not phage typeable with either the basic or experimental phage set and were largely resistant to ciprofloxacin, were similar to the EMRSA-15 isolates described by Richardson and Reith (27) and O’Neill et al. (25). This relationship warrants further analysis. Our study is novel in that we were able to revisit the first MRSA isolates isolated in Australia in the mid-1960s, and the data showed that the archaic MRSA isolates had phage profiles similar to those of isolates in the current mmRSA group 1.

Phylogenetic analysis categorizes our isolates into three major groups: mMRSA, nmMRSA groups 2 and 3, and archaic MRSA/mmRSA group 1. The dendrogram showed that most mMRSA and mmRSA groups 2 and 3 were clustered separately and the nmRSA group 1 isolates were grouped with the archaic MRSA isolates rather than with the other nmRSA groups. These results generally supported the phenotypic data, which separated mmRSA group 1 from mMRSA. Interestingly, although mmRSA group 1 isolates and archaic isolates did not cluster separately, they were mixed together. Group 2 and 3 nmRSA isolates were phenotypically similar and were also shown to be closely related on the dendrogram, although both groups appeared to be more related to the mmRSA isolates than the mmRSA group 1 isolates. RFLP analysis also illustrated the clear differences between mMRSA and mmRSA group 1, because there were no common or related pulsotypes. Isolates from mmRSA groups 2 and 3 made up distinctive pulsotypes compared to the other groups, but shared pulsotypes between them. This confirms the close relatedness observed on the dendrogram and their phenotypic profiles. Archaic MRSA isolates also showed unique pulsotypes by this method, in contrast to phylogenetic analysis, in which they appeared to group with mmRSA group 1. Nonetheless, because the RFLP/pulsotype method of analysis is considered inappropriate for studies of organisms collected over extended periods of more than 1 year (32), this may explain the observed pulsotype discrepancies between mmRSA group 1 and archaic MRSA if any relationship does exist.

Analysis of mecI in contemporary MRSA from other countries has shown deletion rates ranging from 16 to 73% (11, 13, 26, 31, 34). Our study of mec mutations has novel features: it provides the first such data from this part of the world and, unlike previous surveys, distinguishes multidrug-resistant from non-multidrug-resistant strains. The overall mecI deletion rate among the 43 contemporary MRSA isolates at 63% was within the range of published data, but there were marked differences between mmRSA, mmRSA group 1, and mmRSA groups 2 and 3 (7, 80, and 95%, respectively). The absence of mecI in all seven of our archaic MRSA isolates was of considerable interest, since this is the first report of mecI status from archaic Australian MRSA and, to our knowledge, is only the second
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Table 1. Phenotypic and genotypic characterization of the 50 MRSA isolates by different methods.

- **E, erythromycin; T, tetracycline; C, chloramphenicol; C, fusidic acid; R, rifampin; V, vancomycin; Tri, trimethoprim; G, gentamicin; Cip, ciprofloxacin; R, resistant; S, susceptible. All 50 strains were resistant to penicillin and methicillin and sensitive to rifampin and vancomycin.**

- **NT, nontypeable.**

- **/H11002, mecI deletion.**

- **1, 202C T; 2, 324T G; 3, 93A G; 4, 5-G insertion; 5, 208A T; 6, G T mecA SD; 7, A insertion upstream of mecA SD; 8, A deletion at mecA /H11002 35; 9, G insertion in mecA SD.**

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report globally. The lack of mecI in archaic MRSA is in agreement with a previous study by de Lencastre et al. (5).

The eight nmMRSA group 1 isolates with mecI deletions may share a common ancestor, but a detailed analysis of deletion junctions would be needed to verify this. Among the mutations in the two nmMRSA group 1 isolates with an intact mecI gene, a C→T substitution in J28 at position 202 has been reported in MRSA isolates from several countries around the world (7, 12, 31, 34). In marked contrast to findings in nmMRSA, 13 of the 14 mMRSA isolates retained the mecI gene. All shared a common mutation, an A→G substitution at position 93, which suggests that these isolates are related. This mutation has not been reported previously, suggesting that it is clonal rather than a “hotspot.” Three isolates possessed a second common mutation at nucleotide 5 indicating that they may be more closely related to each other than to the other

FIG. 1. Rooted dendrogram illustrating relevant genetic distances calculated from $\rho$ values.
mMRSA isolates. Several mec promoter mutations in our isolates have not been previously reported, and their biological significance remains unknown.

Some nmMRSA group 2 and group 3 isolates provided conflicting mutation data on their relationship between nmMRSA and the nmMRSA group 1 MRSA. For example, J33 shared the same pulotype as two other group 3 isolates, but retained mecI, unlike all other group 2 and 3 isolates and carried a mecI point mutation identical to that found in mMRSA. This is consistent with the notion that the SCCmec in mMRSA and nmMRSA groups 2 and 3 had a common origin, which is also implied by their relative positions on the dendrogram. A theory similar to this was suggested by Lemaitre et al. and others to explain the appearance of gentamicin-susceptible MRSA in France (2, 16, 17). Nonetheless, J37 and J39 had distinct pulotypes and mec promoter mutations identical to those of several group 1 isolates, which is in conflict with the evidence that groups 2 and 3 may be related to mMRSA.

The inclusion of seven archaic MRSA isolates meant that in an epidemiological niche with limited antimicrobial pressure.

In conclusion, we have demonstrated clear differences between the nmMRSA and mMRSA isolates, with evidence from antibiograms, phage typing, RFLPs, and mec regulatory region mutations suggesting that nmMRSA isolates are not nosocomial mMRSA strains that have lost their resistance to multiple antibiotics, but are new clones arising from independent mec transfer events.

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