Typing of Australian methicillin-resistant *Staphylococcus aureus* strains by pulsed field gel electrophoresis

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Summary. Twenty-six clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) collected from six Australian hospitals by a National Staphylococcal Study Group were examined by analysis of restriction fragment length polymorphisms (RFLPs) of chromosomal DNA with pulsed field gel electrophoresis. Digestion with the restriction endonuclease *SmaI* produced 13–17 bands of 7–700 kb. The digestion patterns were easily distinguished and isolates could be classified into 17 groups based on their RFLPs. Isolates giving a pattern associated with one group were from four hospitals in four different states. In another group, the isolates responsible were from three hospitals in two states and in a further group, the isolates were derived from two hospitals in different states. The remaining groups comprised only one member each. The method has promise for typing and studying the epidemiology of MRSA.

Introduction

The isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) in Australia was reported in 1962 and although, between 1964 and 1967, the incidence of MRSA amongst *S. aureus* isolates was reported to be 5–7% they do not appear to have caused serious problems. However, the emergence of gentamicin-resistant MRSA appears to have been associated with a resurgence of MRSA and by the early 1980s the epidemic spread of MRSA was being reported, and, in some cases, up to 50% of all *S. aureus* isolates were MRSA. The causes for this resurgence in resistant strains are not known. Possible reasons that have been advanced include antibiotic abuse, bad cross-infection control and the emergence of a new strain with a particular property. Although it has not been possible to ascribe any particular property to the isolates which would account for their success, plasmid analysis and the location of resistance determinants have shown that the more recent strains from eastern Australian hospitals (EA MRSA) are different from earlier isolates (classic MRSA). These results also indicated that, except for occasional differences in plasmid content, a particular strain had spread to the different Australian hospitals.

Despite many attempts, no successful methods for typing the EA MRSA have emerged. Because most of the isolates are untypeable with the standard inter-national phage typing set, phage typing has not been very successful even though supplementary phages have been useful. New molecular techniques are now available for studying total genomic differences between isolates. Chromosomal DNA can be cut with restriction enzymes and the resulting restriction fragment length polymorphisms (RFLPs) analysed by gel electrophoresis. High frequency cutting restriction enzymes have been used to compare MRSA but the large numbers of bands produced are difficult to compare and the use of low frequency cutting restriction enzymes produces fragments which cannot be resolved satisfactorily by conventional gel electrophoresis. The development of pulsed field gel electrophoresis (PFGE) has enabled large fragments of DNA (> 9000 kb) to be separated. Variations of this technique are Contour-clamped Homogeneous Electric Field (CHEF) and Field Inversion Gel Electrophoresis (FIGE). These methods have been used primarily to analyse the genomes of eukaryotic organisms such as fungi and protozoa which are up to about 10 megabases in size. However, more recently, these methods have been used to study the chromosomes of bacteria. The cells have been lysed and cut with restriction enzymes in agarose blocks to prevent shearing of the DNA. Examples of the applications of these methods are the use of the CHEF to size and map the chromosomes of *Escherichia coli* and *Haemophilus influenzae*, and of the FIGE to fingerprint *Pseudomonas aeruginosa* and staphylococci.

The results of a National Staphylococcal Study Group (NSSG) in Australia have indicated that MRSA continue to be a problem in Australian
Materials and methods

Strains of MRSA

Twenty-six MRSA isolates collected from six hospitals around Australia by the NSSG25 were analysed. They were supplied by Ms Alison Vickery, Royal Prince Alfred Hospital Sydney, New South Wales (NSW). Isolates V4621, V4624 and V4636 were from one Sydney Hospital (Syd-1); V9082, V9083 and V9085 from another Sydney Hospital (Syd-2); V2801-5 and V2828 from a Melbourne, Victoria (VIC), Hospital (Melb-1); V1202, V1204, V1207, V1210, V1212 and V1218 from another Melbourne hospital (Melb-2); V5701, V5702, V5711, V5719 and V5735 from a Brisbane, Queensland (QLD), Hospital (Bris), and V7713, V7714 and V7718 from an Adelaide, South Australia (SA), hospital (Adel).

Preparation of chromosomal DNA and CHEF electrophoresis

These have been described previously.26 Briefly, MRSA were grown to late log phase, washed and resuspended in 50 mM EDTA buffer to a concentration of c. 6 x 10⁶ cells/ml. The cells were then suspended in agarose 1% and lysed. Portions of the blocks were treated with SmaI restriction enzyme and loaded on to an agarose 1% slab gel and electrophoresed at 120 V for 42 h at 9°C in Tris borate EDTA buffer in a CHEF-DR® II PFGE system (BioRad, USA) with mol. wt standards. The similarity of the RFLPs was determined by the Dice coefficient.27

Results

CHEF RFLP banding patterns

SmaI digestion of the DNA of the 26 MRSA produced from 13–17 bands ranging from 7 to 700 kb. The conditions required to obtain separation of the large bands (fig. 1) did not always enable the bands of < 20 kb to be visualised on the same gel. Electrophoresis conditions that enabled bands below 20 kb to be visualised showed that all isolates consistently had three bands of the same size which were < 20 kb (photographic data not shown). However, these bands are represented in fig. 2. Based on the summation of the bands, the average genome size for the isolates was c. 2800 kb.

The banding patterns of the isolates enabled them to be classified into 17 different groups (fig. 2). The most prevalent pattern was that of group S7. Isolates producing this pattern were found in four different hospitals in four states; one isolate from each of Adel, Bris and Syd-1, and two isolates from Melb-1. The next most prevalent group was S2. It was found in isolates from three hospitals in three states; two isolates from Melb-2, two from Bris and one from Syd-2. There were only two isolates that gave a pattern represented by group S13; one was from Adel and the other from Bris. Each of the other groups was represented by only one isolate.

It is interesting that there was no evidence that a particular type was prevalent in any one hospital. In some cases the groups were quite different and percentage similarity was as low as 62%, e.g., S4 with S15 (table). In other cases, there was only a single band difference between groups, e.g., S13 and S16 (table). These small differences appear to be reliable as the method appeared to be very reproducible. Three isolates were chosen for repeat tests and, when the same preparation for each isolate was electrophoresed on different gels, the same results were obtained each
<table>
<thead>
<tr>
<th>State</th>
<th>Hospital</th>
<th>Isolate V no.</th>
<th>RFLP Group</th>
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<td>Melb-2</td>
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<td>S1</td>
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<tr>
<td></td>
<td></td>
<td>1204,1207</td>
<td>S2</td>
<td>400</td>
</tr>
<tr>
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<td>S10</td>
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<td>Syd-1</td>
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<td>4636</td>
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<td>S13</td>
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<tr>
<td></td>
<td></td>
<td>9085</td>
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</table>

Fig. 2. Stylised RFLP patterns of Smal digested chromosomes of MRSA.

time. Additionally, when different inserts of different preparations were electrophoresed on the same gel the same result was again obtained for each isolate.

Discussion

Restriction endonuclease digestion analysis of DNA has been used to compare MRSA isolates. However, shearing of the DNA during preparation and the high frequency cutting enzymes used have produced numerous bands which have been difficult to interpret. Shearing has been overcome by lysing the cells and digesting the DNA in agarose blocks and the number of bands has been kept to an easily readable number by digesting with the low frequency cutting restriction endonuclease Smal.

CHEF electrophoresis has enabled fragments from c. 10 kb to 700 kb to be visualised on the same gel. The bands were easy to read (fig. 1) and the results were reproducible.

The isolates were readily placed into groups based on their banding patterns and their relatedness can be calculated by the Dice coefficient. These results indicate that the MRSA isolates in Australia at the time of this survey were not all the same. Studies on
Table. Percentage similarity of isolates based on their RFLP patterns

<table>
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<tr>
<th>Hospital</th>
<th>Isolate no.</th>
<th>RFLP pattern</th>
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<tr>
<td></td>
<td></td>
<td>S1  S2  S3  S4  S5  S6  S7  S8  S9  S10  S11  S12  S13  S14  S15  S16  S2</td>
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<tr>
<td>Syd-2</td>
<td>9085 S17</td>
<td>85  94  94  91  69  88  75  94  94  88  79  82  79  94  97  91  94  99  94  94  94</td>
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<tr>
<td></td>
<td>9083 S2</td>
<td>97 100 100 91 71 88 85 94 94 88 79 88 85 94 79 94 100 94 100 94 97 97 97 91</td>
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<td></td>
<td>9082 S16</td>
<td>88 91 91 88 66 91 81 82 82 85 81 77 88 85 81 94 88 85 88 85 94 93 94</td>
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<td>7718 S7</td>
<td>94 97 97 94 65 85 81 100 100 91 81 85 88 100 81 88 97 91 97 100 91 93</td>
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<td>7714 S15</td>
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<tr>
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<td>91</td>
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<tr>
<td></td>
<td>1202 S1</td>
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</table>

The EA MRSA\(^a\) of the early 1980s, based on the location of the resistance determinants and plasmid profiles, had indicated that the isolates were very similar and that a particular strain had spread around Australia.\(^8\)\(^-\)\(^31\) Although plasmid analysis has been very useful in typing MRSA, it does not reflect the overall genetic relatedness of isolates. Also, the relatedness of isolates can be obscured by the fact that plasmids can be lost or gained.

The advantage of the results reported here is that they reflect the overall relatedness of the complete bacterial genome. The results also indicate that the method could be useful in studying the epidemiology of MRSA within hospitals and between hospitals. It may enable the identification of strains which are more endemic or epidemic and should provide a means of studying the evolution of MRSA in Australia. It is clear that MRSA continues to be a problem in Australian hospitals\(^5\) so long after the epidemic of the gentamicin-resistant strain\(^8\) of the early 1980s. It will be interesting to use CHEF electrophoresis to compare the isolates studied here with the EA MRSA of the 1980s and with future isolates.

The genome size of c. 2800 kb calculated from these results is slightly larger than that reported in one study\(^28\) but close to the sizes of 2860\(^9\) and 2748\(^30\) reported by others.

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References


