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

M.-Q. WEI and W. B. GRUBB*

School of Biomedical Sciences, Curtin University of Technology, GPO Box U1987, Perth 6102
Western Australia

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 *Sma*I 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 1962¹ and although, between 1964 and 1967, the incidence of MRSA amongst *S. aureus* isolates was reported to be 5.7%² they do not appear to have caused serious problems.³ However, the emergence of gentamicin-resistant MRSA⁴ appears to have been associated with a resurgence of MRSA and by the early 1980s the epidemic spread of MRSA was being reported,⁵ and, in some cases, up to 50 % of all *S. aureus* isolates were MRSA.⁵

The causes for this resurgence in resistant strains are not known. Possible reasons that have been advanced include antibiotic abuse, bad cross-infection control⁶ and the emergence of a new strain with a particular property.^{7,8} 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)⁸ are different from earlier isolates (classic MRSA).⁸ These results also indicated that, except for occasional differences in plasmid content, a particular strain had spread to the different Australian hospitals.^{8–11}

Despite many attempts, no successful methods for typing the EA MRSA have emerged. Because most of the isolates are untypable with the standard inter-

national phage typing set, phage typing has not been very successful even though supplementary phages have been useful.¹² 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^{13,14} 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.¹⁵ The development of pulsed field gel electrophoresis (PFGE) has enabled large fragments of DNA (> 9000 kb) to be separated.¹⁵ Variations of this technique are Contour-clamped Homogeneous Electric Field (CHEF)¹⁶ and Field Inversion Gel Electrophoresis (FIGE).¹⁷ 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.^{18,19} 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.²⁰ Examples of the applications of these methods are the use of the CHEF to size and map the chromosomes of *Escherichia coli*²¹ and *Haemophilus influenzae*,²² and of the FIGE to fingerprint *Pseudomonas aeruginosa*²³ and staphylococci.²⁴

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

Received 9 Sept. 1991; accepted 19 Nov. 1991.

* Correspondence should be sent to Professor W. B. Grubb.

Fig. 1. DNA bands and banding patterns of chromosomes of MRSA digested with *Sma*I. Isolates from (A) Melb-2: lane a, V1202; b, V1204; c, V1207; d, V1210; e, V1212; f, V1218; (B) Melb-1: g, V2801; h, V2802; i, V2803; j, V2804; k, V2805; l, V2828; (C) Syd-1: m, V4621; n, V4624; o, V4636; (D) Adel: p, V7718; q, V7713; r, V7714; (E) Syd-2: s, V9082; t, V9083; u, V9085; (F) Bris: v, V5701; w, V5702; x, V5711; y, V5719; z, V5735; z', size control, phage λ concatemers.

hospitals.²⁵ This paper reports the application of restriction endonuclease digestion in the typing of genomic DNA in clinical isolates.

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 $c. 6 \times 10^5$ cells/ml. The cells were then suspended in agarose 1% and lysed. Portions of the blocks were treated with *Sma*I 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

*Sma*I 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; there were 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

State	Hospital	Isolate V no.	RFLP Group	Fragment size (kb)				
				700	400	200	50	10
VIC	Melb-2	1202	S1					
		1204, 1207	S2					
		1210	S3					
		1212	S4					
		1218	S5					
VIC	Melb-1	2801	S6					
		2802, 2803	S7					
		2804	S8					
		2805	S9					
		2828	S10					
NSW	Syd-1	4621	S11					
		4624	S7					
		4636	S12					
QLD	Bris	5701	S13					
		5702, 5719	S2					
		5711	S14					
		5735	S7					
SA	Adel	7718	S7					
		7713	S13					
		7714	S15					
NSW	Syd-2	9082	S16					
		9083	S2					
		9085	S17					

Fig. 2. Stylised RFLP patterns of *Sma*I 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.^{13,14} 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 *Sma*I.

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.²⁷ These results indicate that the MRSA isolates in Australia at the time of this survey²⁵ were not all the same. Studies on

Table. Percentage similarity of isolates based on their RFLP patterns

Hospital	Isolate no.	RFLP pattern	RFLP pattern																									
			S1	S2	S2	S3	S4	S5	S6	S7	S7	S8	S9	S10	S11	S7	S12	S13	S2	S14	S2	S7	S13	S15	S7	S16	S2	
Syd-2	9085	S17	85	94	94	91	69	88	75	94	94	88	79	82	79	94	79	91	94	88	94	94	91	84	91	85	94	
	9083	S2	97	100	100	91	71	88	85	94	94	88	79	88	85	94	79	94	100	94	100	94	97	90	97	91		
	9082	S16	88	91	91	88	66	91	81	82	82	85	81	77	88	85	81	94	88	85	88	85	94	93	94			
Adel	7718	S7	94	97	97	94	65	85	81	100	100	91	81	85	88	100	81	88	97	91	97	100	91	93				
	7714	S15	94	90	90	80	62	84	80	84	84	71	80	77	87	84	87	93	84	84	84	84	93					
	7713	S13	94	97	97	94	65	85	81	91	91	91	81	85	88	91	81	100	97	91	97	91						
Bris	5735	S7	85	94	94	91	71	94	79	100	100	88	79	72	79	100	75	91	97	88	97							
	5719	S2	91	100	100	91	69	88	79	97	97	97	79	88	79	97	79	97	100	94								
	5711	S14	97	94	94	85	63	82	85	88	88	94	79	88	79	88	79	91	94									
	5702	S2	91	100	100	91	69	88	79	97	97	97	79	88	79	97	79	97										
	5701	S13	94	97	97	94	65	85	81	91	91	91	81	85	88	91	81											
Syd-1	4636	S12	75	85	85	81	71	73	75	73	73	73	69	73	81	73												
	4624	S7	85	94	94	91	71	94	79	100	100	88	79	72	79													
	4621	S11	81	85	85	81	77	79	75	79	79	79	81	73														
Melb-1	2828	S10	79	88	88	73	71	82	85	88	88	88	73															
	2805	S9	75	79	79	81	65	79	75	79	79	73																
	2804	S8	85	94	94	85	69	82	79	88	88																	
	2803	S7	85	94	94	91	71	94	79	100																		
	2802	S7	85	94	94	91	71	94	79																			
	2801	S6	81	79	79	82	65	73																				
Melb-2	1218	S5	79	88	88	80	69																					
	1212	S4	65	69	69	65																						
	1210	S3	82	86	86																							
	1207	S2	91	100																								
	1204	S2	91																									
	1202	S1																										

the EA MRSA⁸ 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.⁹⁻¹¹ 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 hospitals²⁵ so long after the epidemic of the gentamicin-resistant strain⁵ 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 study²⁸ but close to the sizes of 2860²⁹ and 2748³⁰ reported by others.

This work was funded in part by grants to WBG from Markalinga Management (Western Diagnostic Services) and the National Health and Medical Research Council of Australia. M-QW was the recipient of grants from the Australia-China Council, the Cohen Fellowship, Beechman Australia Pty Ltd and Essex Asia Limited. We thank Miss Alison Vickery and the NSSG for providing the isolates. The NSSG was funded by Eli Lilly Australia.

References

1. Lane WR. Methicillin resistance in staphylococci. *Med J Aust* 1962; **49**: 962-965.
2. Rountree PM, Beard MA. Hospital strains of *Staphylococcus aureus*, with particular reference to methicillin-resistant strains. *Med J Aust* 1968; **2**: 1163-1168.
3. Rountree PM. History of staphylococcal infection in Australia. *Med J Aust* 1978; **2**: 543-546.
4. Perceval A, McLean AJ, Wellington CV. Emergence of gentamicin resistance in *Staphylococcus aureus*. *Med J Aust* 1976; **2**: 74.
5. Pavillard R, Harvey K, Douglas D *et al.* Epidemic of hospital-acquired infection due to methicillin-resistant *Staphylococcus aureus*, in major Victorian hospitals. *Med J Aust* 1982; **1**: 451-454.
6. Lacey RW. Antibiotic resistance in *Staphylococcus aureus* and streptococci. *Br Med Bull* 1984; **40**: 77-83.
7. McDonald PJ. Methicillin-resistant staphylococci: a sign of the times? *Med J Aust* 1982; **1**: 445-446.
8. Townsend DE, Ashdown N, Pearman JW, Annear DI, Grubb WB. Genetics and epidemiology of methicillin-resistant *Staphylococcus aureus* isolated in a Western Australian hospital. *Med J Aust* 1985; **142**: 108-111.
9. Grubb WB, Townsend DE, Greed LC, Ashdown N, Momoh M. Characteristics of methicillin-resistant *Staphylococcus aureus* epidemic in Australian hospitals. In: Spitzzy KH, Karrer K (eds) Proceedings of the 13th international

- congress of chemotherapy. Vienna, Verlag H. Egermann. 1983; Part 83: 23–26.
10. Townsend DE, Grubb WB, Ashdown N. Genetics of drug resistance in methicillin-resistant *Staphylococcus aureus* from Australian hospitals. *J Hosp Infect* 1983; **4**: 331–337.
11. Lyon BR, Iuorio JL, May JW, Skurray RA. Molecular epidemiology of multiresistant *Staphylococcus aureus* in Australian hospitals. *J Med Microbiol* 1984; **17**: 79–89.
12. Beard-Pegler MA, Vickery AM. Lysogenicity of methicillin-resistant strains of *Staphylococcus aureus*. *J Med Microbiol* 1985; **20**: 147–155.
13. Jordens JZ, Hall LMC. Characterisation of methicillin-resistant *Staphylococcus aureus* isolates by restriction endonuclease digestion of chromosomal DNA. *J Med Microbiol* 1988; **27**: 117–123.
14. Burnie JP, Matthews RC, Lee W, Murdoch D. A comparison of immunoblot and DNA restriction patterns in characterising methicillin-resistant isolates of *Staphylococcus aureus*. *J Med Microbiol* 1989; **29**: 255–261.
15. Schwartz DC, Cantor CR. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* 1984; **37**: 67–75.
16. Vollrath D, Davis RW. Resolution of DNA molecules greater than 5 megabases by contour-clamped homogeneous electric fields. *Nucleic Acids Res* 1987; **15**: 7865–7876.
17. Carle GF, Frank M, Olson MV. Electrophoretic separations of large DNA molecules by periodic inversion of the electric field. *Science* 1986; **232**: 65–68.
18. Fan JB, Chikashige Y, Smith CL, Niwa O, Yanagida M, Cantor CR. Construction of a *NotI* restriction map of the fission yeast *Schizosaccharomyces pombe* genome. *Nucleic Acids Res* 1988; **17**: 2801–2818.
19. Orbach MJ, Vollrath D, Davis RW, Yanofsky C. An electrophoretic karyotype of *Neurospora crassa*. *Mol Cell Biol* 1988; **8**: 1469–1473.
20. Smith CL, Klco S, Cantor CR. Pulsed-field gel electrophoresis and the technology of large DNA molecules. In: Davies KE (ed) *Genome analysis: a practical approach*. Oxford, IRL Press. 1988: 41–72.
21. Smith CL, Econome JG, Schutt A, Klco S, Cantor CR. A physical map of the *Escherichia coli* K12 genome. *Science* 1987; **236**: 1448–1453.
22. Kauc L, Mitchell M, Goodgal SH. Size and physical map of the chromosome of *Haemophilus influenzae*. *J Bacteriol* 1989; **171**: 2474–2479.
23. Grothues D, Tümmler B. Genome analysis of *Pseudomonas aeruginosa* by field inversion gel electrophoresis. *FEMS Microbiol Lett* 1987; **48**: 419–422.
24. Goering RV, Duensing TD. Rapid field inversion gel electrophoresis in combination with an rRNA gene probe in the epidemiological evaluation of staphylococci. *J Clin Microbiol* 1990; **28**: 426–429.
25. Turnidge J, Lawson P, Munro R, Benn R. A national survey of antimicrobial resistance in *Staphylococcus aureus* in Australian teaching hospitals. *Med J Aust* 1989; **150**: 69–72.
26. Wei M-Q, Wang F, Grubb WB. Use of contour-clamped homogeneous electric field electrophoresis to type methicillin-resistant *Staphylococcus aureus*. *J Med Microbiol* 1992; **36**: 172–176.
27. Dice LR. Measures of the amount of ecological association between species. *Ecology* 1945; **26**: 297–302.
28. McClelland M, Jones R, Patel Y, Nelson M. Restriction endonucleases for pulsed field mapping of bacterial genomes. *Nucleic Acids Res* 1987; **15**: 5985–6005.
29. Weil MD, McClelland M. Enzymatic cleavage of a bacterial genome at a 10-base-pair recognition site. *Proc Natl Acad Sci USA* 1989; **86**: 51–55.
30. Patel AH, Foster TJ, Pattee PA. Physical and genetic mapping of the protein A gene in the chromosome of *Staphylococcus aureus* 8325-4. *J Gen Microbiol* 1989; **135**: 1799–1807.