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Epidemiological Analysis of Methicillin-Resistant *Staphylococcus aureus* in a Community Hospital in Hiroshima

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen in hospitals, including community hospitals with relatively small numbers of beds (1). Molecular analysis of MRSA isolates in a hospital, using restriction fragment length polymorphisms of genomic DNA using pulsed-field gel electrophoresis (PFGE), is essential for assessment of hospital infection controls (2).

The present study was conducted in a hospital in Hiroshima with two wards and 100 beds. In September 2001, MRSA was isolated from two patients' sputa and one patient's urine in ward I and four patients' sputa, one patient's bile, and one patient's nasal swab in ward II. MRSA was isolated from the sputum of five patients and the pus of one in ward I, and the sputum of three patients in ward II in December 2000. The isolates were tested for chromosomal DNA typing by using a contour-clamped homogeneous electric field system (CHEF Mapper™: Bio-Rad Laboratories, Hercules, Calif., USA), enterotoxin serotyping (SET-RPLA: Denka Seiken Co., Tokyo), toxic shock syndrome toxin-1 (TSST-1) production (TST-RPLA: Denka Seiken), and coagulase serotyping (Denka Seiken).

From nine MRSA isolates, seven different PFGE patterns of *Sma*I DNA digests were detected (Fig. 1). Isolate No. 898 from ward I and No. 903 from ward II, and isolates Nos. 901 and 905 from ward II had the same PFGE pattern, respectively. All isolates except Nos. 899 and 900 produced enterotoxin type C, TSST-1, and coagulase type II (Table 1). Isolate No. 899 produced TSST-1 and coagulase type II, but not entero-

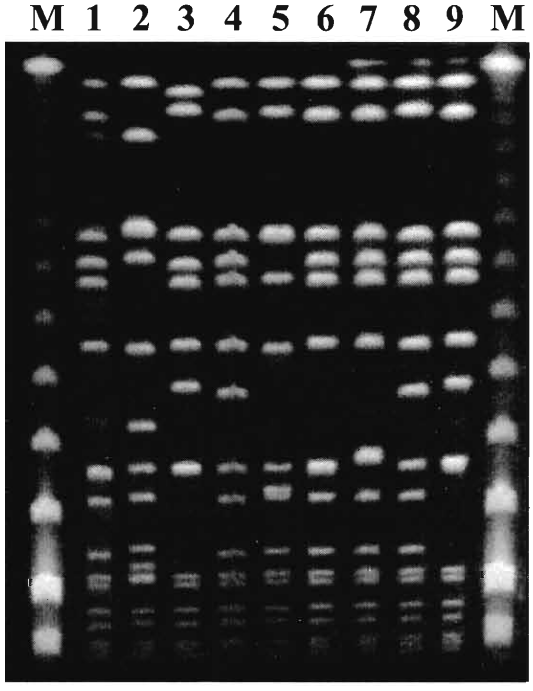

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. Lane 1: MRSA isolate No. 898, lane 2: No. 899, lane 3: No. 900, lane 4: No. 901, lane 5: No. 902, lane 6: No. 903, lane 7: No. 904, lane 8: No. 905, lane 9: No. 907. M: low range PFG Marker.

Table 1. Phenotypic and genotypic characterization of *S. aureus* isolates

Ward	Isolate No.	Specimen	PFGE pattern	Enterotoxin	TSST-1	Coagulase
I	898	sputum	A1	C	+	II
	899	urine	C3	–	+	II
	900	sputum	A6	A, C	+	II
II	901	bile	A2	C	+	II
	902	sputum	A12	C	+	II
	903	sputum	A1	C	+	II
	904	sputum	A3	C	+	II
	905	sputum	A2	C	+	II
	907	nasal cavity	A7	C	+	II

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toxin. Isolate No. 900 produced enterotoxin types A and C, TSST-1, and coagulase type II.

The PFGE patterns of these MRSA isolates were compared with those of MRSA isolates obtained in the same hospital in August and December 2000 (2). From a total of 31 isolates, 20 different PFGE patterns were detected (Fig. 2A). Band-based cluster analysis of these patterns (Molecular Analyst™: Bio-Rad) revealed six clusters A to F (Fig. 2B) (patterns sharing a similarity of 70% or higher were grouped into a cluster). The PFGE pattern and the isolation term of all MRSA isolates were summarized in Table 2. Six of nine MRSA isolates in August 2000, nine of 13 isolates in December 2000, and eight of nine isolates in September 2001 were of cluster type A. Three isolates from ward I in August, five from the same ward in December, and one from ward I (No. 898) and one from ward II (No. 903) in September were of the same PFGE pattern (A1). One isolate from ward II in August and two isolates from ward II (Nos. 901 and 905) in September were of the same PFGE pattern. Other MRSA isolates were of different PFGE patterns from those with patterns A1 and A2, and from each other (A3 to A12, B, C1 to C3, and E1 and E2, and F). Collectively, the above observation indicates that the

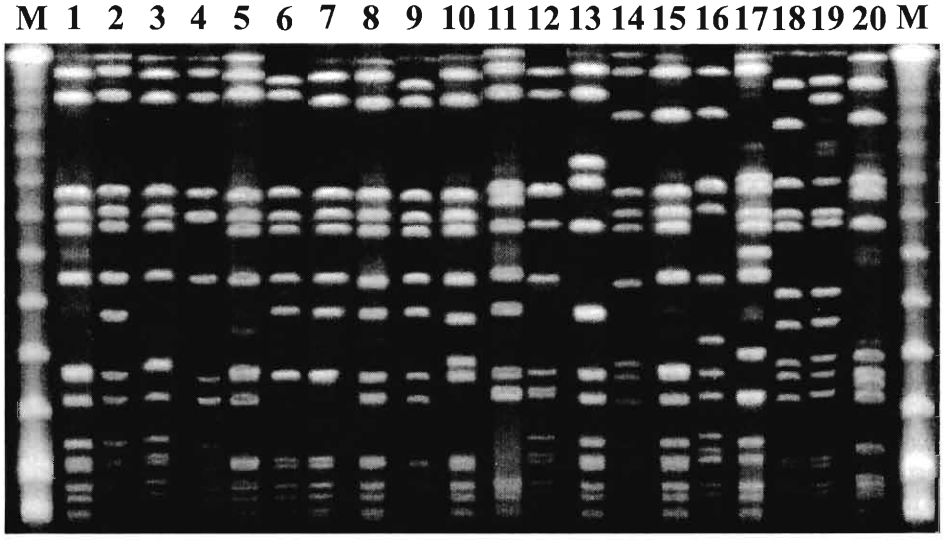

Fig. 2A. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. Lane 1: MRSA isolate No. 898 (pattern A1), lane 2: No. 901 (pattern A2), lane 3: No. 904 (pattern A3), lane 4: No. 401 (1) (pattern A4), lane 5: No. 635 (1) (pattern A5), lane 6: No. 900 (pattern A6), lane 7: No. 907 (pattern A7), lane 8: No. 636 (1) (pattern A8), lane 9: No. 396 (1) (pattern A9), lane 10: No. 634 (1) (pattern A10), lane 11: No. 629 (1) (pattern A11), lane 12: No. 902 (pattern A12), lane 13: No. 623 (1) (pattern B), lane 14: No. 399 (1) (pattern C1), lane 15: No. 627 (1) (pattern C2), lane 16: No. 899 (pattern C3), lane 17: No. 630 (1) (pattern D), lane 18: No. 398 (1) (pattern E1), lane 19: No. 404 (1) (pattern E2), lane 20: No. 628 (1) (pattern F). M: low range PFG Marker.

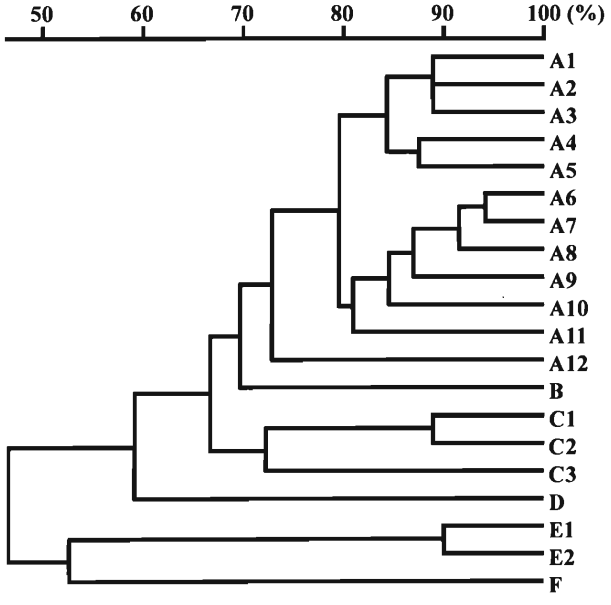

Fig. 2B. Cluster analysis of MRSA isolates based on PFGE patterns of *Sma*I-digested genomic DNA.

Table 2. PFGE pattern of MRSA isolates

PFGE pattern	Aug. 2000		Dec. 2000		Sep. 2001	
	I	II	I	II	I	II
A1	●●●		●●●●●		●	●
A2		●				●●
A3						●
A4	●					
A5			●			
A6					●	
A7						●
A8				●		
A9		●				
A10			●			
A11				●		
A12						●
B			●			
C1	●					
C2			●			
C3					●	
D				●		
E1		●				
E2		●				
F			●			

spread of a single MRSA strain (pattern A1) occurred in ward I in August and December 2000. The MRSA outbreak of a single strain was being stamped out in ward I in September 2001. Meanwhile, most other MRSA infections in this hospital appeared to occur sporadically, indicating that these MRSA were mainly come from other healthcare facilities in the same community.

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