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Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in a Tokyo Hospital in 2003

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important nosocomial pathogens in healthcare facilities. Epidemiological analysis is therefore indispensable for assessing infection control measures (1-3).

In October 2003, 241 MRSA isolates were obtained from 72 inpatients in a hospital with 24 wards and 925 beds in Tokyo. Among the samples, 65 were derived from a single patient and were analyzed in terms of the following: chromosomal DNA typing with a contour-clamped homogeneous electric field system (CHEF Mapper™: Bio-Rad Laboratories, Hercules, Calif., USA), antibiotic resistance (WalkAway™: Dade Behring, Greefield, Ill., 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). Isolates showing the same pulsed-field gel electrophoresis (PFGE) patterns were probably of the same origin.

Thirty-eight different PFGE patterns of *Sma*I DNA digests were detected (Fig. 1). A band-based cluster analysis (Molecular Analyst™: Bio-Rad), in which PFGE-band similarity exceeding 70% was used as the criterion for cluster formation, revealed the following 15 clusters: A, AT, Y, AU, AV, AB, AW, AE, AX, J, AY, AZ, BA, BB, and BC (Fig. 2A). The frequency distribution of these different PFGE-pattern isolates of MRSA is shown in Fig. 2B. Cluster A was the cluster type of 50% of the total isolates, and the most frequent pattern was A1, which represented 17% of the isolates. The distribution of MRSA isolates in this study is shown in Table 1. Isolates belonging to cluster A were found in 14 of 24 wards; more specifically, PFGE pattern A1 was identified in 10 wards, pattern A3 in four wards, and patterns A4 and A29 in two wards, respectively. Pattern Y4 was found in two wards.

The sensitivity to antibiotics is shown in Table 2. Fifteen different patterns were identified. The isolates were found to be resistant to 8 - 13 of 18 tested drugs. None of the isolates were resistant to vancomycin, teicoplanin, nor sulfamethoxazole/trimethoprim. All of the 11 isolates with pattern A1 had an antibiotic pattern of j, k, or ab. No correlation was found between the antibiotic patterns and PFGE patterns.

Among 65 isolates, 61 produced coagulase type II, three isolates produced coagulase type IV, and one produced coagulase type III. Forty-four isolates produced enterotoxin type C, nine isolates enterotoxin type B, four isolates enterotoxin types B and C, and one isolate enterotoxin type A, while the remaining seven isolates produced no enterotoxins. Fifty isolates produced TSST-1, but 15 did not. Collectively, among 65 MRSA isolates, 44 produced coagulase type II, enterotoxin type C, and TSST-1.

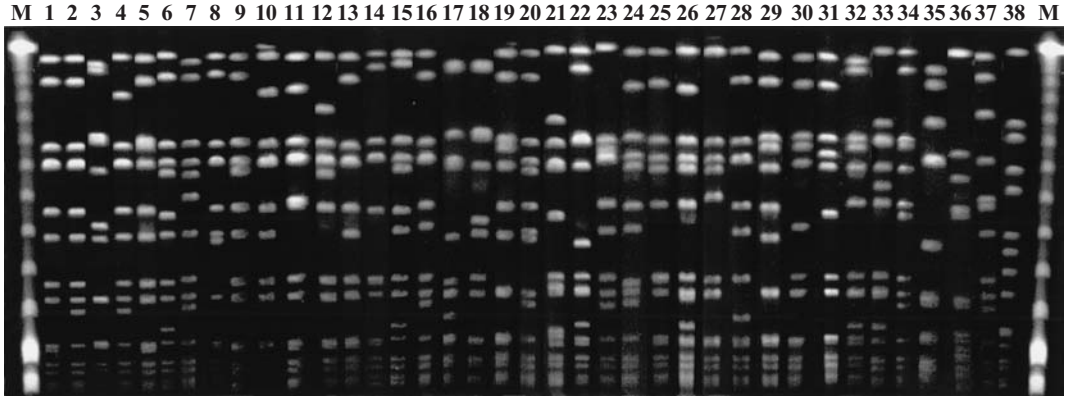

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. M: low range PFG Marker. Lanes 1 to 38: MRSA isolates with different PFGE patterns A1 to BC shown in Fig. 2.

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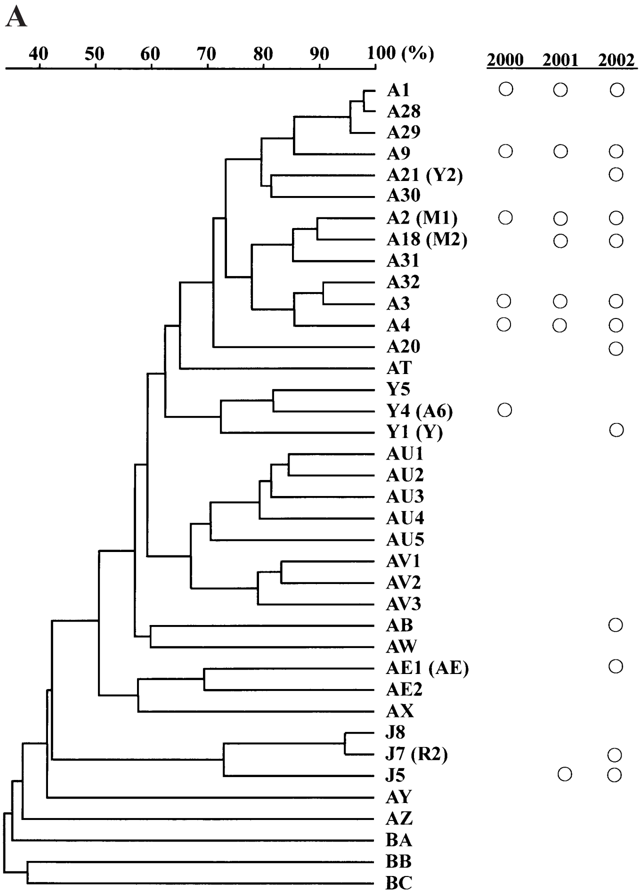

PFGE-based MRSA surveillance was conducted in the same hospital in December 2000 (1), October 2001 (2), and October 2002 (3). Four PFGE patterns, A1, A2(M1), A3, A4, and A9, were detected in all of those surveillance studies, as well as in present study. Patterns A18(M2) and J5, detected in the present study, were detected in the October 2001 and October 2002 studies, but they were not found in the December 2000 study. PFGE patterns A20, A21(Y2), Y1(Y), AB, AE1(AE), and J7(R2) were detected in the present study, and were also detected in the October 2002 study, but not in the December 2000 and October 2001 studies. Patterns A28 to 32, J8, Y5, AE2, AT, AU1 to 5, AV1 to 3, AW,

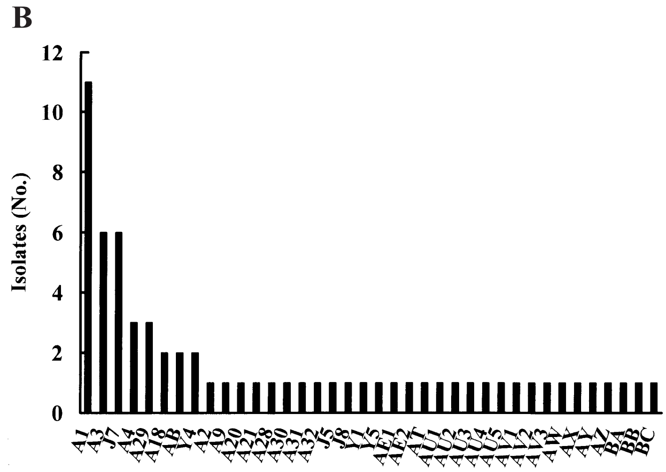

Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns. MRSA isolates indicated by circles were also detected in previous surveillance (1-3).

Table 1. Distribution of MRSA in a hospital

Ward	A													J			Y			AB	AE		AT	AU					AV			AW	AX	AY	AZ	BA	BB	BC
	1	2	3	4	9	18	20	21	28	29	30	31	32	5	7	8	1	4	5		1	2		1	2	3	4	5	1	2	3							
4N																																						
4S				2#														1																			1	1
5N																				2		1								1								
5S																																						
6N																																						
6S																																						
7N	2		1			2								1			1									1												
7S										1																												
8N	1																		1				1											1		1		
8S	1								1																													
9N			3																																			
9S	1																															1			1			
10N																																						
10S	1																																					
11N	1																	1																				
11S	1		1	1							1																											
12N	1	1								2																												
12S	1						1	1				1	1												1								1					
13N																																						
13S															6	1								1														
14																					1								1									
15	1				1																							1			1							
16			1																																			
ICU																																						

#: Number of patients with MRSA.

Table 2. Antibiotic pattern classified by antibiotic pattern of 18 antibiotics against MRSA

Antibiotic pattern	Antibiotics									
	EM	LVFX	CLDM	FOM	GM	ABK	MINO	ST	TEIC	VCM
c	R	R	R	R	R	S	I	S	S	S
d	R	R	R	R	R	S	S	S	S	S
e	R	I	R	R	R	S	I	S	S	S
f	R	R	R	I	R	S	S	S	S	S
i	R	R	S	R	R	S	S	S	S	S
j	R	R	R	R	S	S	I	S	S	S
k	R	R	R	R	S	S	S	S	S	S
o	R	R	R	S	S	S	S	S	S	S
p	R	R	R	I	S	S	S	S	S	S
q	R	S	R	S	R	S	S	S	S	S
ab	R	R	R	I	S	S	I	S	S	S
ad	R	S	S	S	R	S	S	S	S	S
aq	R	R	S	I	R	S	S	S	S	S
ar	R	S	S	S	S	S	S	S	S	S
as	S	S	S	S	S	S	S	S	S	S

All the isolates were resistant to PCG, MPIPC, ABPC, CEZ, CTM, CFDN, FMOX, IPM.
PCG: benzyl-penicillin, MPIPC: oxacillin, ABPC: ampicillin, CEZ: cefazolin, CTM: cefotiam, CFDN: cefdinir, FMOX: flomoxef, IPM: imipenem/cilastatin, EM: erythromycin, LVFX: levofloxacin, CLDM: clindamycin, FOM: fosfomycin, GM: gentamicin, ABK: arbekacin, MINO: minocyclin, ST: sulfamethoxazole/trimethoprim, TEIC: teicoplanin, VCM: vancomycin, R: resistant, S: susceptible, I: intermediate.

AX, AY, AZ, BA, BB, and BC were detected only in the present study, i.e., new patterns emerged as of this study. Among these patterns, A28 and A29 were identical to pattern A1, with only a single band difference. This study suggested the presence of two types of MRSA in this hospital setteing, i.e., those that persist for a long duration, and those appearing for only a short time. The MRSAs that persist long-term appear to have undergone constant evolution within the hospital.

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