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Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in a Tokyo Hospital in 2002

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a prevalent nosocomial pathogen in healthcare facilities. Epidemiological analysis of MRSA by means of restriction fragment length polymorphisms of genomic DNA using pulsed-field gel electrophoresis (PFGE) is essential for assessment of hospital infection controls (1-4).

Two hundred eighty-six MRSA isolates were obtained from 97 inpatients in October 2002 in a hospital with 24 wards and 925 beds in Tokyo. Sixty-eight of the isolates, each derived from a single patient, were analyzed for chromosomal DNA typing by using a contour-clamped homogeneous electric field system (CHEF Mapper™, Bio-Rad Laboratories, Hercules, Calif., USA), and for antibiotic resistance (WalkAway™, Dade Behring, Deerfield, 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 PFGE patterns were probably of the same origin.

Thirty-two different PFGE patterns of *Sma*I DNA digests were detected (Fig. 1). A band-based cluster analysis of these patterns (Molecular Analyst™, Bio-Rad), in which PFGE-band similarity exceeding 70% was used as the criterion of cluster formation, revealed that there existed 15 clusters: patterns A, X, Y, Z, G, M, AA, AB, AC, AD, AE, AF, J, R, AG (Fig. 2A). The frequency distribution of MRSA of these different PFGE patterns isolates is shown in Fig. 2B. The most frequent pattern (A1) represented 26%. The most frequent clusters of patterns (A1 to A4, A9, A15, A16, A20 to A24) made up 54% of total isolates. Distribution of MRSA isolates in the wards is shown in Table 1. The isolates belonging to PFGE pattern A were found in 17 of 24 wards; more specifically, PFGE pattern A1 was in 11 wards, patterns A4 and A16 were in three wards, respectively, and patterns A3 and A22 were in two wards, respectively. Pattern X was in three wards, and G3 and Z were in two wards, respectively.

Sensitivity to antibiotics is shown in Table 2. The MRSA isolates had a wide spectrum of drug-resistance with 21 different patterns. Those isolates were resistant to 9-14 of 17 tested drugs. No isolate was resistant to either vancomycin or sulfamethoxazole/trimethoprim. All of the 18 isolates having PFGE pattern A1 had antibiotic patterns j, k, m, or am. No correlation was found between other antibiotic patterns and

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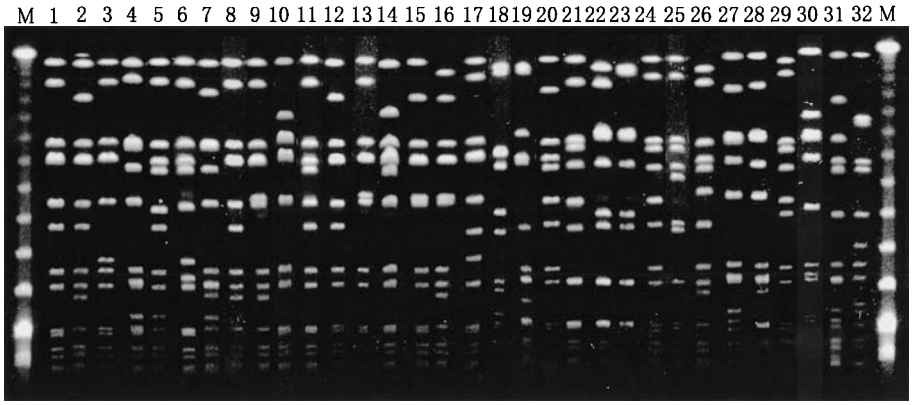

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. M: low range PFG Marker. Lanes 1 to 32: MRSA isolates with different PFGE patterns A1 to AG shown in Fig. 2.

PFGE patterns (data not shown).

Among 68 MRSA isolates, 65 isolates produced coagulase type II, and the remaining three produced coagulase type III. Fifty-seven isolates produced enterotoxin type C, four isolates enterotoxin type B, three isolates enterotoxin types B and C, while the other isolates produced no enterotoxins. Forty-eight isolates produced TSST-1, and the other 10 did not. Collectively, among 68 MRSA isolates, 55 isolates produced coagulase type II, enterotoxin type C, and TSST-1.

PFGE-based MRSA surveillance was conducted in the same hospital in December 2000 (1) and October 2001 (2). PFGE patterns A1, A2(M1), A3, A4, A9, and G3, were detected in the present study and in that conducted in December 2000 and October 2001. Patterns A15, A16, M2(A18), J3, and R1(R) were detected in the present study and in that conducted in October 2001 but not in December 2000. PFGE patterns A23(B) were detected in the present study and in that conducted in December 2000 but not in October 2001. PFGE patterns A20, A21, A22, A24, X, Y, Z, M7, AA1, AA2, AB, AC1 to 3, AD, AE, AF, J5, R2, and AG were newly detected in the present study. The study suggested the persistence of some MRSA strains as well as the rapid turnover of MRSA in the hospital.

MRSA with PFGE pattern A1 expanding in the hospital was detected in other Japanese hospitals in Kumamoto (3) and in Hiroshima (4), indicating that some MRSA strains persist in healthcare facilities in Japan.

Table 1. Distribution of MRSA in a hospital

Ward	PFGE pattern																																
	A												G	J		M		R		X	Y	Z	AA		AB	AC			AD	AE	AF	AG	
	1	2	3	4	9	15	16	20	21	22	23	24	3	3	5	2	7	1	2				1	2		1	2	3					
4N																																	
4S				1[#]							1																				1		
5N																				1													
5S																																	
6N	1																																
6S	1						1																										
7N	2													1		4					1												
7S				2			1				1		3																				
8N	1								1																								
8S	2												1																			1	
9N																							1										
9S							2			1																		3		1			
10N	1	1																						1									
10S	1																																
11N	4																										1						
11S																																	
12N	1		1			1																1			1		1						
12S	3		1		1															1											1		
13N																		1	1														
13S										1																							
14																																	
15	1													1		1						1											
16							1													1													
ICU				1																													

[#] Number of patients with MRSA.

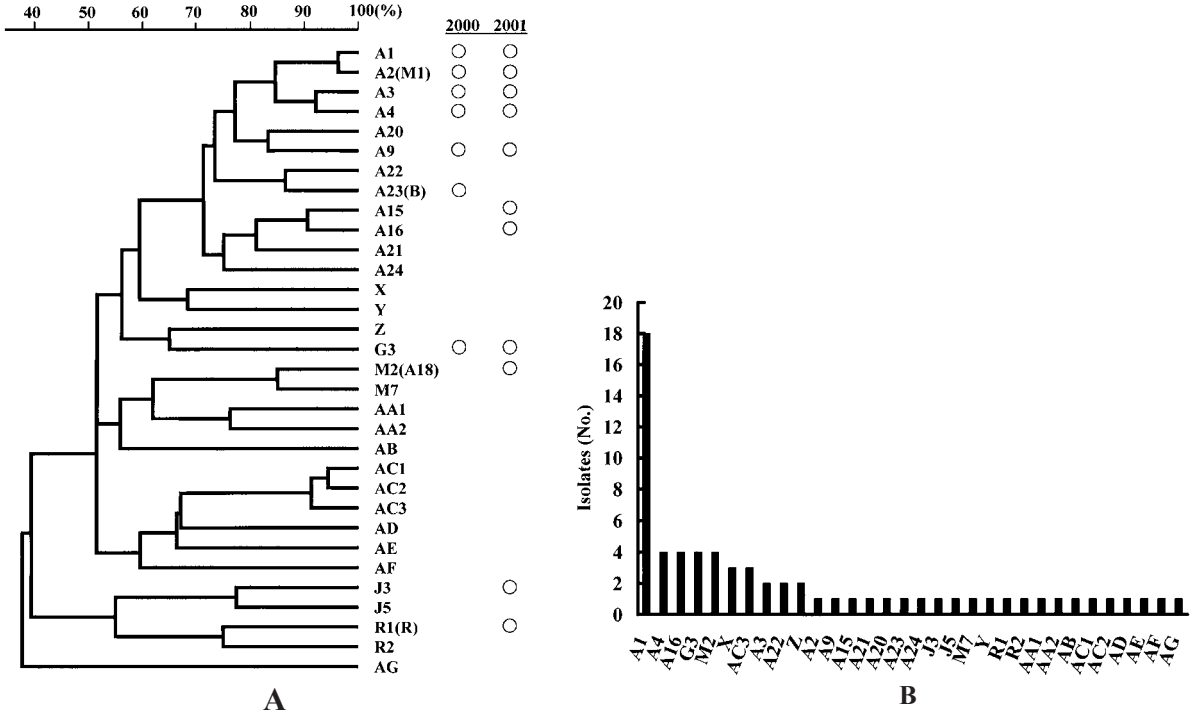

Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns. MRSA isolates indicated by circles were also detected in the previous surveillance (1,2).

Table 2. Antibiotic pattern classfied by antibiotic pattern of 17 antibiotics against MRSA

Antibiotic pattern	Antibiotics								
	EM	LVFX	CLDM	FOM	GM	ABK	MINO	ST	VCM
c	R	R	R	R	R	S	I	S	S
d	R	R	R	R	R	S	S	S	S
e	R	I	R	R	R	S	I	S	S
f	R	R	R	I	R	S	S	S	S
i	R	R	S	R	R	S	S	S	S
j	R	R	R	R	S	S	I	S	S
k	R	R	R	R	S	S	S	S	S
l	R	I	R	R	S	S	I	S	S
m	R	I	R	R	S	S	S	S	S
o	R	R	R	S	S	S	S	S	S
p	R	R	R	I	S	S	S	S	S
q	R	R	S	S	R	S	S	S	S
s	S	R	S	S	R	S	S	S	S
u	R	R	S	I	S	S	S	S	S
ai	R	R	R	R	R	R	S	S	S
ak	R	R	R	I	R	S	I	S	S
al	R	R	S	S	R	S	I	S	S
am	S	I	R	R	S	S	S	S	S
an	R	I	R	S	R	S	S	S	S
ao	R	I	R	I	S	S	S	S	S
ap	S	S	S	S	R	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, VCM: vancomycin, ST: sulfamethoxazole/trimethoprim, R: resistant, S: susceptible, I: intermediate.

REFERENCES

1. Fujino, T., Mori, N., Kawana, A., Kawabata, H., Kuratsuji, T., Kudo, K., Kobori, O., Yazaki, Y. and Kirikae, T. (2001): Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a Tokyo hospital in 2000. Jpn. J. Infect. Dis., 54, 91-93.
2. Fujino, T., Mori, N., Kawana, A., Naiki, Y., Kawahata, H., Kuratsuji, T., Kudo, K., Kobori, O., Yazaki, Y. and Kirikae, T. (2001): Molecular epidemiology of methicillin-

resistant *Staphylococcus aureus* in a Tokyo hospital in 2001. Jpn. J. Infect. Dis., 54, 240-242.
3. Kawano, F., Miyazaki, H., Takami, J., Fujino, T., Saruta, K. and Kirikae T. (2002): Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a Kumamoto hospital in 2001. Jpn. J. Infect. Dis., 55, 29-30.
4. Sasaki, R., Fujino, T., Saruta, K., Kawasaki, J., Shigeto, N. and Kirikae, T. (2002): Molecular epidemiological surveillance of methicillin-resistant *Staphylococcus aureus* in a Hiroshima community hospital in 2002. Jpn. J. Infect. Dis., 55, 93-95.