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

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a prevalent nosocomial pathogen in healthcare facilities. Epidemiological analysis of MRSA isolates present in a hospital, based, for example, on the restriction fragment length polymorphisms of genomic DNA determined using pulsed-field gel electrophoresis (PFGE), is essential for assessment of hospital infection control (1,2).

Fifty-six MRSA isolates were obtained from 24 inpatients during October 2002 in a hospital with 11 wards and 550 beds in Kumamoto Prefecture. Of these isolates, 24 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), plasmid DNA typing by use of agarose gel electrophoresis, antibiotic resistance (VITEK™: bioMerieux, Marcy-l'Etoile, France), 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).

Thirteen different PFGE patterns of *Sma*I DNA digests were detected (Fig. 1). A band-based cluster analysis of these patterns (Molecular Analyst™: Bio-Rad) revealed nine clusters (clusters A, AH, AI, AE, AM, AL, AN, AJ, and AK) (a cluster was defined as a group of patterns with more than 70% similarity (Fig. 2A). The frequency distribution of MRSA isolates based on PFGE patterns is shown in Fig. 2B. The most frequent pattern (A1) represented 45.8% of the total

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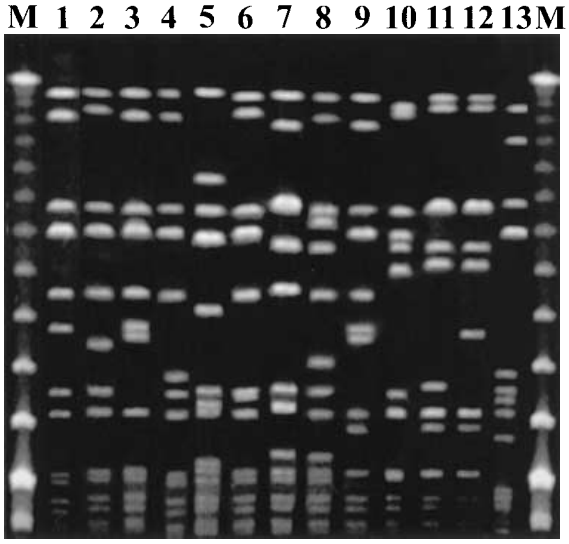

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. M: low range PFG Marker. Lanes 1 to 30: MRSA isolates with different PFGE patterns A1 to AK, respectively, shown in Fig. 2.

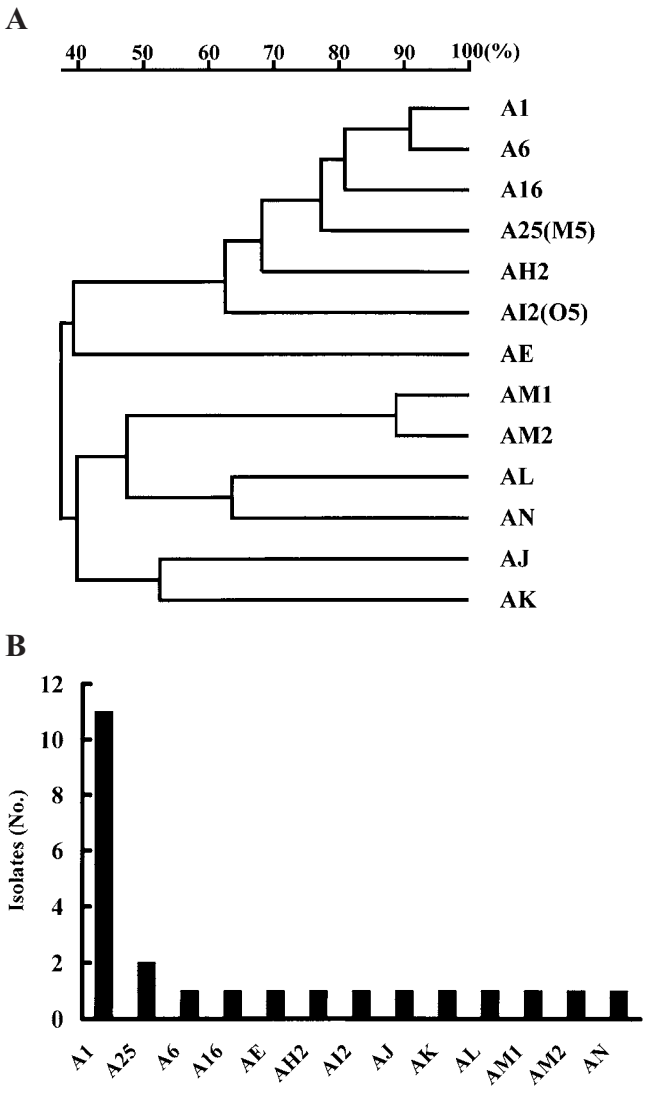

Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns of *Sma*I-digested genomic DNA.

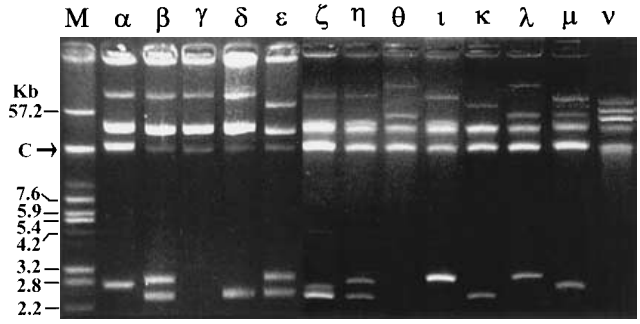

Fig. 3. Agarose gel electrophoresis of plasmid DNA from MRSA isolates. M: marker plasmids derived from *E. coli* V517. C: DNA fragments derived from genomes.

isolates. PFGE pattern A25 was detected in two isolates. The other 11 PFGE patterns were detected in only one isolate.

The profiles of plasmid typing are shown in Fig. 3. All MRSA isolates had plasmids. Twelve different sizes of plasmids, ranging from 2.4 kb to 280 kb, were detected. Each isolate had two to five different sized plasmids. Thirteen plasmids different in size were detected (Table 1). The frequency distribution of MRSA isolates based on plasmid patterns is shown in Table 1. All isolates had the 40 kb plasmid. Isolates with patterns α, β, and γ accounted for 50% of the total, and these isolates had both 200 kb and 40 kb plasmids.

The geographic distribution of MRSA isolates in the hospital is shown in Table 2. The two, two, two, one, three, and one isolates from intensive care unit and wards e2, w3, a2, a5, and a6, respectively, belonged to the same cluster, A1, suggesting a clonal spread of MRSA in the hospital. Among these isolates, one from ward a2 and two from ward a5 belonged to the same plasmid pattern α; and one from ward e2, one from ward a5 and one from ward a6 belonged to the pattern β, indicating that transmission of a clone of MRSA with PFGE pattern A1 and plasmid pattern α occurred in the ward a5. Two isolates belonging to PFGE pattern A25, but having different respective plasmid patterns (ζ and η) were isolated from wards a3 and a5, respectively. Isolates with other PFGE and plasmid patterns appeared to be limited to wards w3, e2, w2, a3, a5, e1, a3, w1, e1, a3, and w2.

Sensitivity to antibiotics is shown in Table 3. The MRSA

Table 1. Plasmid typing pattern classified by plasmid size and its frequency

Plasmid pattern	Frequency	Plasmid size (kb)										
		280	200	150	57	55	50	45	40	3.0	2.6	2.5	2.4
α	5		○						○		○		
β	4		○						○	○			○
γ	3		○						○				
σ	1		○						○				○
ε	1			○					○	○			○
ζ	1		○						○			○	○
η	1		○					○	○	○			○
θ	2	○					○		○				
ι	1		○					○	○	○			
κ	1				○				○				○
λ	1	○					○		○	○			
μ	2	○					○		○				○
ν	1			○		○	○		○				

Table 2. Distribution of MRSA in a hospital

PFGE pattern	Plasmid pattern	Ward										
		e			w			a					ICU
		1	2	3	1	2	3	1	2	3	5	6	
A1	α								1		2		1
	β		1[#]								1	1	
	γ						1						1
	δ						1						
	ε		1										
(A1[##]	α - ε		2				2		2		3	1	2)
A6	α						1						
A16	β		1										
A25	ζ									1			
	η										1		
AE	θ					1							
AH2	η									1			
AI2	ι										1		
AJ	θ	1											
AK	κ									1			
AL	λ				1								
AM1	μ	1											
AM2	μ									1			
AN	ν					1							

[#] Number of patient with MRSA.
[##] Total numbers of patients with MRSA having PFGE pattern A1.

Table 3. Antibiotic pattern classfied by antibiotic pattern of antibiotics against MRSA

Antibiotic pattern	Antibiotics						
	EM	GM	TC	MINO	ABK	VCM	TEIC
a	R	R	R	R	S	S	S
b	R	R	R	S	R	S	S
c	R	R	R	S	S	S	S
d	R	R	R	I	S	S	S
e	R	S	R	R	S	S	S
f	R	R	S	S	S	S	S
g	R	S	R	S	S	S	S
h	S	R	S	S	S	S	S

All the isolates were resistant to MPIPC, PCG, ABPC, PIPC, CEZ, CMZ, IPM, SBT/ABPC.
MPIPC: oxacillin, PCG: benzyl-penicillin, ABPC: ampicillin, PIPC: piperacllin, CEZ: cefazolin, CMZ: cefmetazole, IPM: imipenem/cilastatin, SBT/ABPC: sulbactam/ampicillin, EM: erythromycin, GM: gentamicin, TC: tetracycline, MINO: minocyclin, ABK: arbekacin, VCM: vancomycin, TEIC: teicoplanin.
R: resistant, S: susceptible, I: intermediate.

isolates were resistant to 9-12 of 15 tested drugs. Those isolates had a spectrum of drug-resistance showing eight different patterns. The spectra of drug-resistance were similar to each other; e.g., a difference in susceptibility was found against only one drug when isolates with pattern c were compared with those with pattern a, b, d, e, f, or g. One isolate was resistant to arbekacin (pattern b). Those isolates were resistant to 9-12 of 15 tested drugs. (One isolate with PFGE pattern AH2.) All the isolates were sensitive to vancomycin or teicoplanin. No correlation was found between the antibiotic resistance patterns and PFGE patterns (Table 4).

Among 24 MRSA isolates, 23 isolates produced coagulase type II, and the remaining isolate produced coagulase type III (Table 4). Twenty-two isolates produced enterotoxin type C; the twenty-third produced isolate enterotoxin types

Table 4. Genotypic and phenotypic characterization of MRSA

No.	PFGE pattern	Plasmid pattern	Antibiotic pattern	Enterotoxin	TSST-1	Coagulase
1245	A1	α	c	C	+	II
1247	A1	α	f	C	+	II
1250	A1	β	c	C	+	II
1251	A1	β	d	C	+	II
1252	A1	β	c	C	+	II
1255	A1	α	c	C	+	II
1256	A1	γ	c	C	+	II
1258	A1	α	c	C	+	II
1259	A1	δ	c	C	+	II
1261	A1	ε	g	C	+	II
1264	A1	γ	d	C	+	II
1246	A16	β	c	C	+	II
1263	A25(M5)	ζ	c	C	+	II
1257	A25(M5)	η	c	C	+	II
1248	A6	α	c	C	+	II
1268	AE	θ	h	C	+	III
1249	AH2	η	b	C	+	II
1253	AI2(O5)	ι	c	B,C	+	II
1267	AJ	θ	c	C	+	II
1262	AK	κ	e	C	+	II
1254	AL	λ	f	C	+	II
1265	AM1	μ	a	C	+	II
1260	AM2	μ	a	C	+	II
1266	AN	ν	f	–	–	II

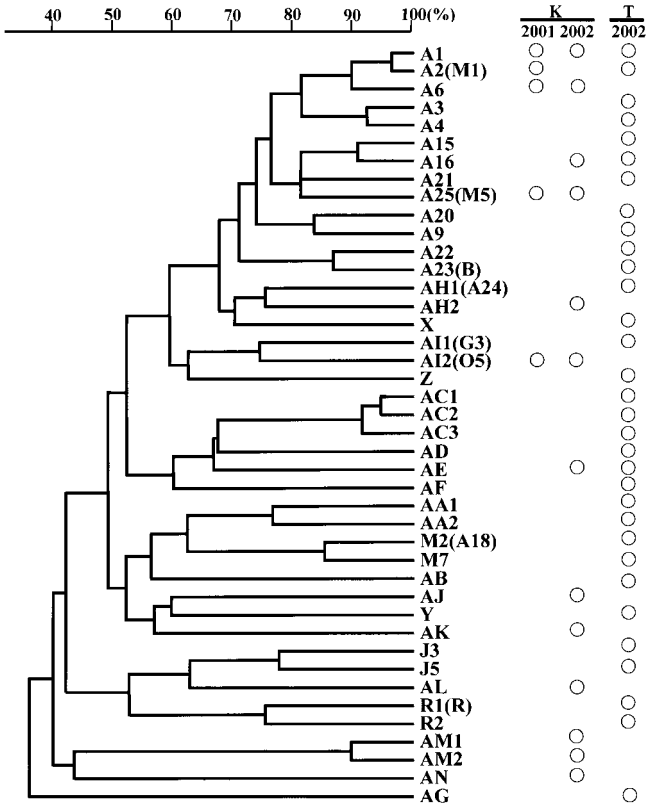

Fig. 4. Cluster analysis of MRSA isolates based on PFGE patterns.
K: Kumamoto hospital, T: Tokyo hospital.

B and C; and the last isolated produced neither enterotoxin types A, B, C, or D. Twenty-three isolates produced TSST-1; the twenty-fourth did not. Collectively, among 24 MRSA

isolates, 21 isolates produced coagulase type II, enterotoxin type C, and TSST-1; i.e., most of the isolates shared common characteristics regarding these parameters.

PFGE-based MRSA surveillance was conducted in the same hospital in October 2001 (1) and in a hospital in Tokyo in October 2002 (2). In these surveillance studies, a total of 42 PFGE patterns were detected (Fig. 4). PFGE patterns A1, A6, A25(M5), and AI2(O5) were common in Kumamoto in 2001 and 2002, whereas the other patterns were unique to each year (Fig. 4)(1), indicating the co-existence of persistence and rapid turnover of MRSA in a hospital. Patterns A1, A2(M1), A16, AE, detected in the hospital in Kumamoto in either 2001 or 2002, were also detected in a hospital in Tokyo in 2002 (Fig. 4)(2). Among these patterns, pattern A1 was most frequently detected in both hospitals (Fig. 2B)(2). The data indicate the clonal expansion of MRSA not only within hospitals but also nationwide.

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