Table 1. Clinical characteristics of patients with MRSA, *P. aeruginosa* and *S. marcescens*, and PFGE patterns of these isolates

Isolate date	Patient No.	Disease	Tracheotomy	Respirator	Specimen	PFGE pattern		
						MRSA	*P. aeruginosa*	*S. marcescens*
Dec. 2002	P1	Hypoxic encephalopathy	+	+	Sputum	B1	P.D	S.A1
	P2	Mental retardation	+	–	Sputum	AY3(AA1)	–	–
	P3	Hypoxic encephalopathy	+	+	Sputum	A3	P.G1	–
	P4	Cerebral palsy	+	–	Sputum	A3	P.C1	S.A1
	P5	Anoxic encephalopathy	+	+	Abscess	A16/BM	–	–
	P5	Anoxic encephalopathy	+	+	Sputum	–	P.B	–
	P6	Cerebral palsy	+	–	Sputum	A3	P.I1	S.A2
	P7	Cerebral palsy	+	–	Sputum	BM	P.A1	–
	P7	Cerebral palsy	+	–	Eye mucus	BM	–	–
	P8	Developmental disability	+	+	Sputum	A44	–	–
	P9	MELAS[1)]	+	+	Sputum	BK	–	–
	P10	Hypoxic encephalopathy	+	+	Sputum	–	P.E1	–
	P11	Sequelac of encephalitis	+	+	Sputum	–	P.G2	–
	P12	Cerebral palsy	+	–	Sputum	–	P.E2	–
	P13	Hypoxic encephalopathy	+	+	Sputum	–	–	S.B
	P14	Cerebral palsy	+	–	Sputum	–	–	S.A1
Aug. 2003	P1	Hypoxie encephalopathy	+	+	Sputum	–	P.F	S.A1
	P2	Mental retardation	+	–	Sputum	BJ	–	–
	P3	Hypoxic encephalopathy	+	+	Sputum	–	P.H	-
	P4	Cerebral palsy	+	–	Sputum	A3	–	S.A1
	P7	Cerebral palsy	+	–	Sputum	–	P.A1	–
	P8	Cerebral palsy	+	+	Sputum	–	P.K	–
	P11	Sequelae of encephalitis	+	+	Sputum	–	P.G2	S.A1
	P13	Hypoxic encephalopathy	+	+	Sputum	BN	–	–
	P14	Cerebral palsy	+	–	Sputum	–	P.A2	–
	P15	Hypoxic encephalopathy	+	–	Sputum	A1	P.J2	–
	P16	Developmental disability	–	–	Urine	AX2	–	–
	P17	Viral encephalitis[2)]	–	–	Urine	BL	–	–
	P18	Herpatic encephalitis	+	–	Sputum	–	P.G3	–
	P19	Hypoxic encephalopathy	+	+	Sputum	–	P.J1	–
	P20	Cerebral palsy	+	+	Sputum	–	P.I2	–
	P21	Cerebral palsy	+	–	Sputum	–	P.C2	–
	P22	Cerebral palsy	+	–	Sputum	–	P.J1	–
	P23	Herpatic encephalitis	+	+	Sputum	–	P.A3	–

[1)]: MELAS, nutochondrial myopathy and lactic acidosis.
[2)]: caused by measles virus.

sharing a similarity of 70% or higher were grouped into a cluster). No other clustering was observed.

Among 11 MRSA isolates found in December 2002, there were two clusters, one consisting of three isolates of PFGE pattern A3 and the other of three isolates of pattern BM. In contrast, in six isolates found in August 2003, clustering was not detected (Table 1). The PFGE patterns obtained from this study were compared with those identified in previous studies conducted in 2000-2003 in Tokyo (1-4), in 2002-2003 in Kumamoto (5-7), and in 2003 in Sendai (8). Among the patterns detected in the present study, pattern A1 was detected in 2000-2003 both in Tokyo and Kumamoto; pattern A3 in 2000-2003 in Tokyo and in 2003 in Sendai; and pattern A16 in 2001 and 2002 in Tokyo. The other nine patterns we identified were not detected in the previous studies.

The PFGE patterns of *P. aeruginosa* isolates are shown in Fig. 2A. From a total of 22 isolates, 19 different PFGE patterns were detected. Band-based cluster analysis of these patterns revealed six clusters, A, C, E, G, I, and J (Fig. 2B). The isolates from patients P19 and P22 in August 2003 were of the same pattern, P.J1. The isolates in December 2002 and August 2003 from patient P7 were of the same pattern P.A1, and those from patients P11 in the two surveys were also of the same pattern P.G2.

A total eight *S. marcescens* isolates were obtained. These represented three different PFGE patterns (Fig. 3A), two of which were similar to each other (Fig. 3A, 3B). Three of five isolates found in December and all of the three isolates found in August were of pattern S.A1.

Comparison of the August 2003 data with December 2002 data clearly shows reduction of MRSA carriers and disappearance of genetically related MRSA clusters in the second survey. Probably interventions taken after the first survey reduced MRSA transmission among the inpatients. The interventions taken were i) an educational program for the ward staff that dealt with infection control practice, ii) promotion of compliance with hand washing, and iii) replacement of the multi-use catheter with the sterile single-use catheter for suction of respiratory tract secretions. The data also suggested that the above interventions were not as successful for control of *P. aeruginosa* and *S. marcescens* that were present in the environment of the facility.

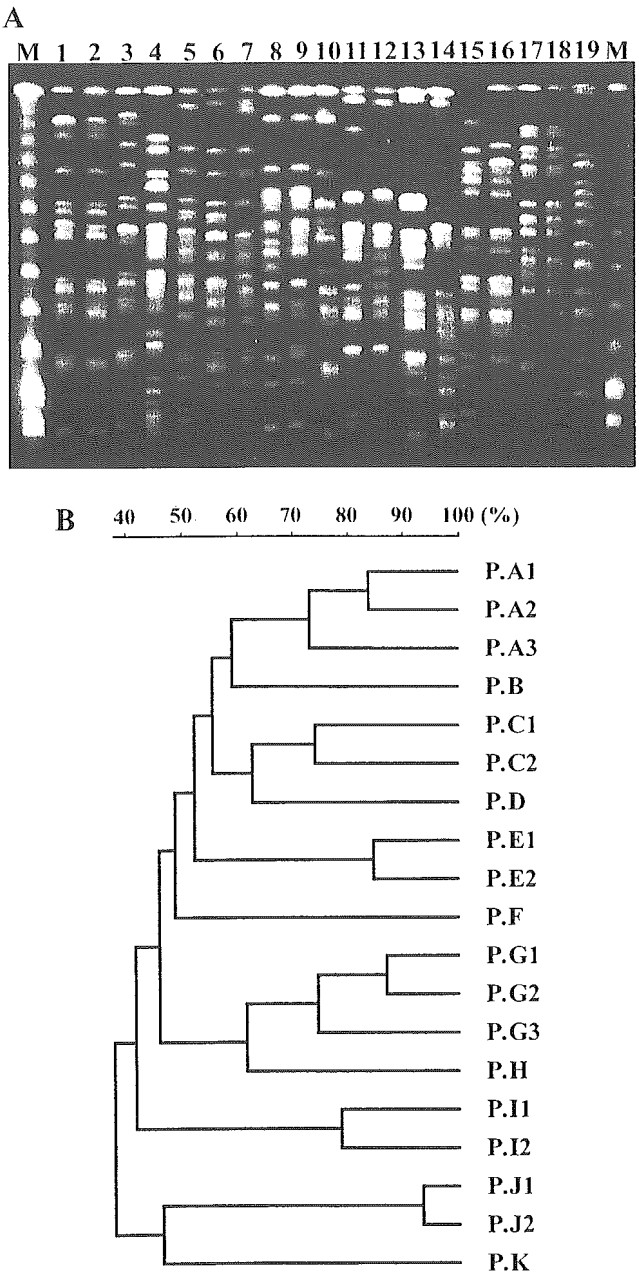

Fig. 2. Molecular analysis of *P. aeruginosa* isolate. A: pulsed-field gel electrophoresis of *Spe*I-digested genomic DNA from *P. aeruginosa* isolates. M: low range PFG Marker. Lanes 1 to 19 corresponding to the following PFGE pattern; 1: P.A1, 2: P.A2, 3: P.A3, 4: P.B, 5: P.C1, 6: P.C2, 7: P.D, 8: P.E1, 9: P.E2, 10: P.F, 11: P.G1, 12: P.G2, 13: P.G3, 14: P.H, 15: P.I1, 16: P.I2, 17: P.J1, 18: P.J2, 19: P.K. B: Cluster analysis of *P. aeruginosa* isolates based on PFGE patterns of *Spe*I-digested genomic DNA.

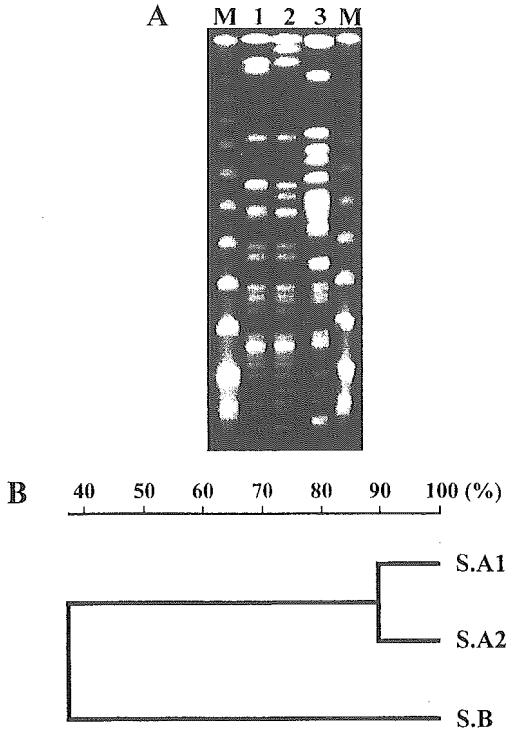

Fig. 3. Molecular analysis of *S. marcescens* isolate. A: pulsed-field gel electrophoresis of *Spe*I-digested genomic DNA from *S. marcescens* isolates. M: low range PFG Marker. Lanes 1 to 3 corresponding to the following PFGE pattern; 1: S.A1, 2: S.A2, 3: S.B. B: cluster analysis of *S. marcescens* isolates based on PFGE patterns of *Spe*I-digested genomic DNA.

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Jpn. J. Infect. Dis., 57, 2004

Laboratory and Epidemiology Communications

Detection of *Mycobacterium bovis* Bacillus Calmette-Guerin Using Quantum Dot Immuno-Conjugates

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Communicated by Masahiko Makino

(Accepted July 12, 2004)

Luminescent quantum dots (QDs) are a novel and promising class of fluorophores for cellular imaging (1,2). The benefits of QDs include their photostability, high brightness, multi-target labeling with several colors, and single-source excitation for QDs of all colors. We have developed procedures for using QDs to detect mycobacteria in a species-specific manner.

Mycobacterium bovis BCG strain 172 was obtained from Japan BCG Laboratory, Tokyo, Japan. A green fluorescent protein (GFP) expressing *M. bovis* BCG, containing plasmid pGFM-11, was supplied by C. Locht, Institut Pasteur de Lille, France. The BCG strains were grown in liquid Middlebrook 7H9 medium (Difco Laboratories, Detroit, Mich., USA) supplemented with 10% oleic acid-albumin-dextrose-catalase enrichment (OADC, Difco) and incubated at 37°C. Ten microliters of liquid medium was mounted on a glass coverslip beneath a hole in a plastic petri dish bottom (Matsunami Glass Industry., Ltd., Tokyo, Japan; code. D110100) and were subsequently air dried. Two percent glutaraldehyde in PBS was applied for 1 h at room temperature. After several rinses with PBS, the 1% bovine serum albumin (BSA) in PBS (BSA/PBS) was applied for 20 min at room temperature to block nonspecific binding. Antiserum obtained from rabbits immunized with heat-killed BCG was applied at a dilution of 1:4000 with BSA/PBS, and the dishes were incubated for 1 h at room temperature. After several rinses with 0.02% Tween 20 in PBS (PBS/Tween 20), Qdot™ 655 goat $F(ab')_2$ anti-rabbit IgG conjugate (H+L) highly cross-absorbed (antibodies QD-conjugate: Quantum Dot Corp., Hayward, Calf., USA) was applied at a dilution of 1:1000 with 1% BSA for 1 h at room temperature. The dishes were then rinsed three times with PBS/Tween 20, and microscopic examinations were conducted with a confocal laser scanning microscope (LSM 510, Carl Zeiss, Oberkochen, Germany) equipped with a × 100/1.40 oil immersion objective, an HBO 50 illuminator, and an FITC/Rhodamine dual-band filter set.

The results of immunofluorescent staining (A, B), conventional mycobacterial staining (C, D), and Ziehl-Neelsen staining (E, F) are shown in Fig. 1. BCG strains were labeled in red when treated with anti-BCG antibodies (Fig. 1A), whereas *Mycobacterium smegmatis* (Fig. 1B) was not labeled when treated with anti-BCG antibodies, indicating that these antibodies was specific to *M. bovis* BCG.

As shown by the confocal image in Fig. 2A, the surface of

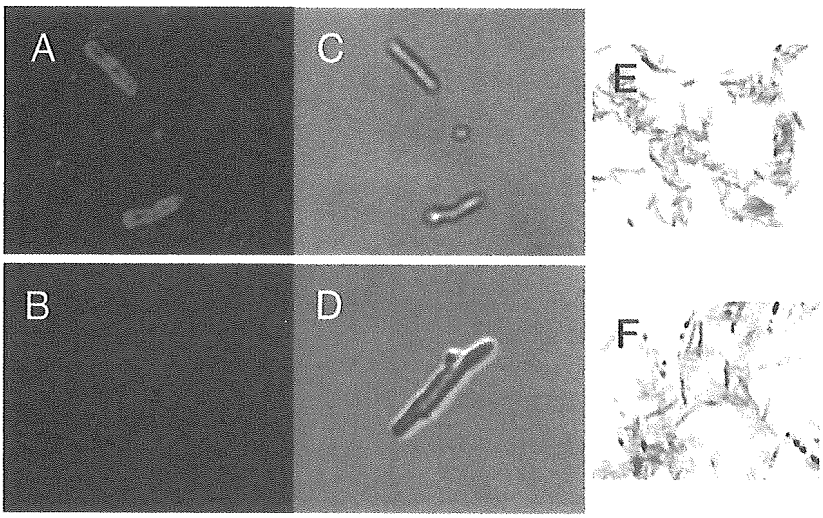

Fig. 1. Immunofluorescence staining of BCG (A, C, E) and *M. smegmatis* (B, D, F) strains (×1000).

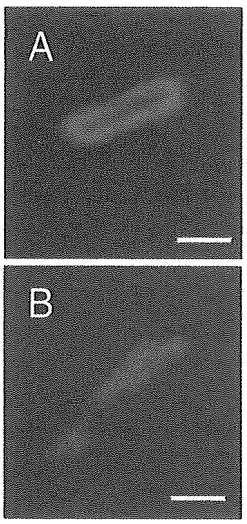

Fig. 2. Labeling of BCG (A) and GFP-expressed BCG (B) with anti-BCG antiserum and QD-conjugated anti-rabbit IgG. Scale bar, 1 μm.

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BCG strain 172 was labeled with red-colored QD-conjugated anti-rabbit IgG when treated with antiserum against BCG. The size of the labeled BCG was 3.5 (SD: 0.4) × 0.5 (SD: 0.1) μm ($n = 4$). The microorganisms were not labeled when treated with pre-immune serum. GFP-expressing BCG was stained using the same procedure (Fig. 2B). GFP was detected in the bacteria's intracellular region and was labeled only negligibly by QD-conjugate. The anti-BCG antibodies in combination with the QD-conjugated anti-IgG antibodies labeled the surface of BCG in a specific manner.

Acid-fast staining, such as Ziehl-Neelsen stain and auramine-rhodamine stain, are well-established procedures for detecting *Mycobacterium tuberculosis* and other mycobacterial spp. The immunostaining using QD-conjugates may be useful for identification of mycobacterial-specific antigen.

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Jpn. J. Infect. Dis., 57, 2004

Laboratory and Epidemiology Communications

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

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Communicated by Hiroshi Yoshikura

(Accepted March 25, 2004)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major hospital-acquired pathogen. Molecular epidemiological is essential for assessing hospital infection control measures (1-7).

Forty-five MRSA isolates were obtained from 24 inpatients during October 2003 in a hospital with 15 wards and 650 beds in Sendai city. Of these, 22 isolates, each derived from a single patient, 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 (disk diffusion tests by the National Committee for Clinical Laboratory standards method), 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 pulsed-field gel electrophoresis (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 of cluster formation, revealed the three following clusters, A, AV/AU, and BH (Fig. 2A). The frequency distribution of the PFGE patterns is shown in Fig. 2B. Pattern AV5 was shared by six isolates, pattern A3 by four, and pattern A4 by two. The other 10 PFGE patterns appeared in only a single isolate.

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As shown in Table 1, pattern AV5 was spread over five wards (e6, w2, w3, w4, and w6). Three isolates from the neonatal intensive care unit (NICU) were of pattern A3, and two from wards e6 and s6 were of pattern A4. Other patterns were unique to each ward.

The isolates showed a wide spectrum of drug resistance, with a total of 12 different patterns (Table 2). The isolates were resistant to 7-12 of 15 tested drugs. All of the isolates

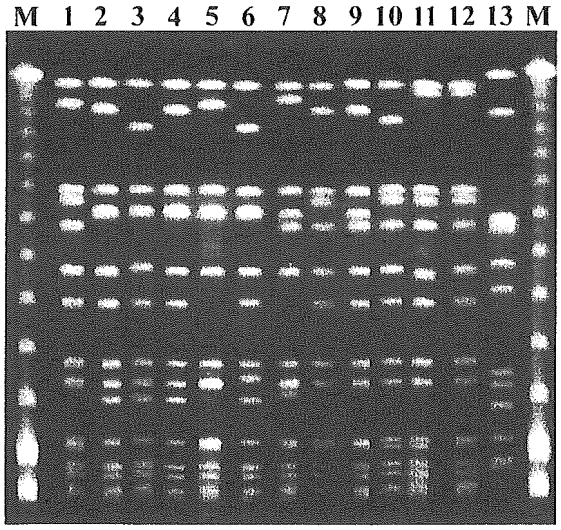

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. M: low range PFG Marker. Lanes 1 to 13: MRSA isolates with different PFGE patterns A3 to BH shown in Fig. 2.

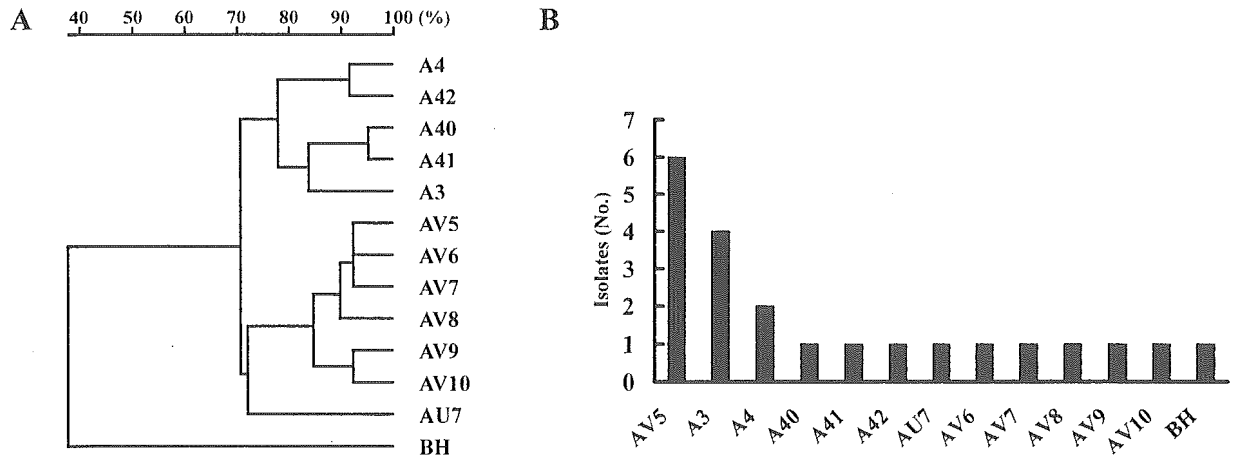

Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns of *Sma*I-digested genomic DNA.

Table 1. Distribution of MRSA in a hospital

PFGE pattern	Ward												Critical care center
	e					w					s		pediatrics	NICU	
	2	3	4	5	6	2	3	4	5	6	5	6			
A2													1		
A3		1#												3	
A4				1							1		1		
A40								1							
A41											1				
A42		1													
AV5				1		1	1	2		1					
AV6				1											
AV7								1							
AV8							1								
AV9							1								
BH							1								

#: Number of patients with MRSA.

Table 2. Antibiotic pattern classified by antibiotic pattern of 15 antibiotics against MRSA

Antibiotic pattern	Antibiotics							
	LVFX	GM	TC	MINO	AMK	ABK	ST	VCM
a	R	R	R	R	R	S	S	S
b	R	R	R	I	R	S	S	S
c	R	R	S	R	R	S	S	S
d	R	I	R	R	R	S	S	S
e	R	R	R	R	I	S	S	S
f	R	R	R	I	I	S	S	S
g	R	I	R	I	R	S	S	S
h	R	I	R	R	I	S	S	S
i	R	I	R	I	I	S	S	S
j	R	I	I	S	R	S	S	S
k	R	I	S	S	R	S	S	S
l	S	R	S	S	R	I	S	S

All the isolates were resistnat to MPIPC, ABPC, CEZ, FMOX, IPM, EM, CLDM.
MPIPC: oxacillin, ABPC: ampicillin, CEZ: cefazolin, FMOX: flomoxef,
IPM: imipenem/cilastatin, EM: erythromycin, CLDM: clindamycin,
LVFX: levofloxacin, GM: gentamicin, TC: tetracycline, MINO: minocyclin,
AMK: amikacin, ABK: arbekacin, ST: sulfametoxazole/trimethoprim,
VCM: vancomycin, R: resistant, S: susceptible, I: intermediate.

were sensitive to sulfametoxazole/trimethoprim and vancomycin. No antibiotic resistance pattern was found to be specific to a particular ward. No correlation was found between antibiotic patterns and PFGE patterns (data not shown).

Among 22 MRSA isolates, 21 produced coagulase type II, and the remaining isolate produced coagulase type I. Eleven isolates produced enterotoxin type C, nine isolates entero-

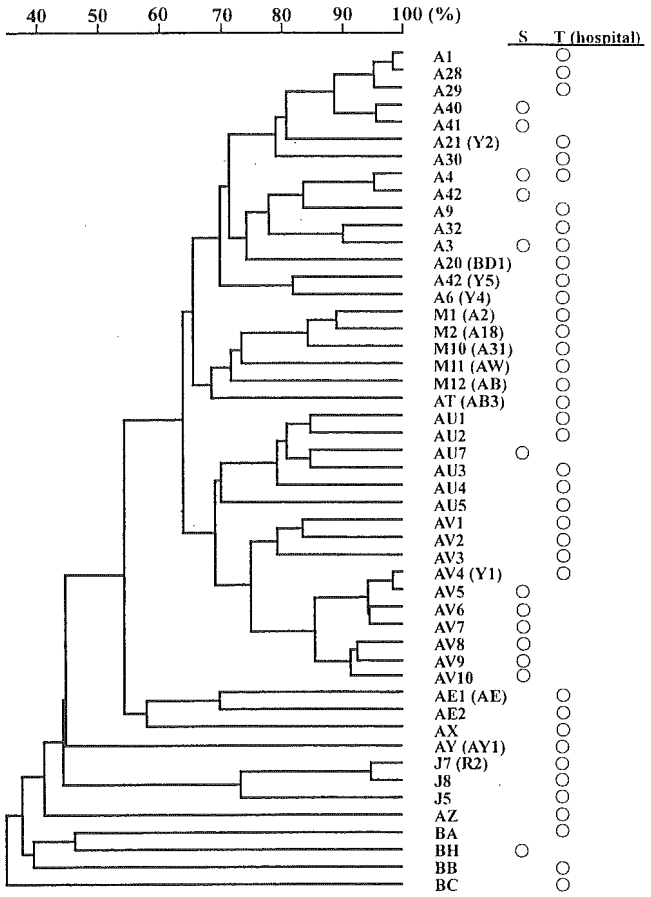

Fig. 3. Cluster analysis of MRSA isolates based on PFGE patterns. S: Sendai hospital, T: Tokyo hospital.

toxin type B, one isolate enterotoxin type D, and one isolate produced no enterotoxins. Twelve isolates produced TSST-1. Eleven isolates produced coagulase type II, enterotoxin type C, and TSST-1; their PFGE patterns were A3, A4, A40, A42, AU7, AV6, or AV7. Nine isolates produced coagulase type II, enterotoxin type B, but not TSST-1; their PFGE patterns were AV5, AV8, AV9, or AV10.

The PFGE patterns obtained in this study were compared with those identified in Tokyo in October 2003 (Fig. 3) (4). Patterns A3 and A4 were detected in both instances. Eleven patterns were unique to Sendai, and 36 were unique to Tokyo. Previous studies conducted in 2000-2003 in Tokyo (1-4) and in 2001-2003 in Kumamoto (5-7) indicated a clone propagation of the pattern A1 MRSA in both hospitals. In a Sendai hospital, a different clone with a PFGE pattern of AV5 was found to have spread (Table 1). Thus, it was determined that nosocomial infections in a hospital in Tokyo shared common clones with those in Sendai and those in Kumamoto. However, infections in hospitals in Sendai and in Kumamoto, which are geographically separated from each other, did not share clones. Considering the fact that patients, as well as doctors, nurses, and other health care workers are frequently sent to or visit large medical facilities in Tokyo, this data may help account for how MRSA spreads nationwide in Japan.

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Laboratory and Epidemiology Communications

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

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Communicated by Hiroshi Yoshikura

(Accepted March 25, 2004)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a prevalent nosocomial pathogen in healthcare facilities. Epidemiological analysis of MRSA isolates assisted by analysis of restriction fragment length polymorphisms of genomic DNA using pulsed-field gel electrophoresis (PFGE) is essential for achieving hospital infection control (1-3).

Sixty-seven MRSA isolates were obtained from 35 inpatients during October 2003 in a hospital with 11 wards and 550 beds in Kumamoto Prefecture. Of these, 34 isolates, each derived from a single patient, were analyzed for chromosomal DNA typing by using the following: a contour-clamped homogeneous electric field system (CHEF Mapper™: Bio-Rad Laboratories, Hercules, Calif., USA), 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).

Nineteen different PFGE patterns of *Sma*I DNA digests were detected (Fig. 1). A band-based cluster analysis (Molecular Analyst™: Bio-Rad) revealed 10 clusters, A/Y, AI/A, AY, BF, AB/AU, BD, AW, BG, AI, and BE (patterns with more than 70% similarity were considered to form a cluster) (Fig. 2A). The frequency distribution of the PFGE patterns of MRSA is shown in Fig. 2B. The most frequent pattern (A1) represented 26% of the total isolates. Pattern A35 was detected in four isolates, patterns A36 and AI2 in

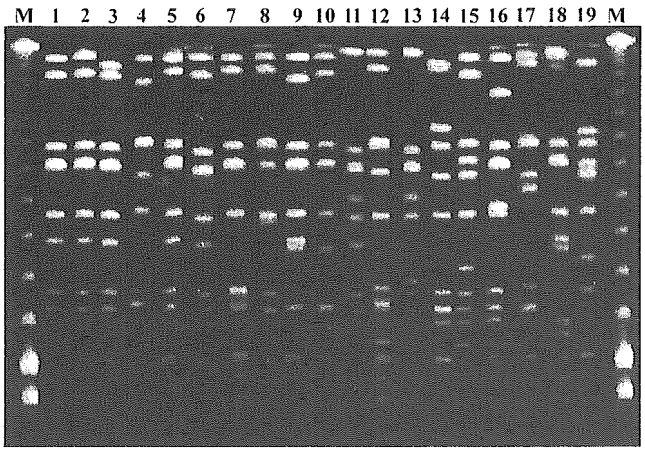

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. M: low range PFG Marker. Lanes 1-19 correspond to the following PFGE patterns; 1: A1, 2: A33, 3: A35, 4: A34, 5: A37(AH2), 6: Y6, 7: Y7, 8: A36, 9: AI1(G3), 10: A39, 11: AY2, 12: BF, 13: AB2, 14: AU6, 15: BD2, 16: AW2, 17: BG, 18: AI2(O5), 19: BE.

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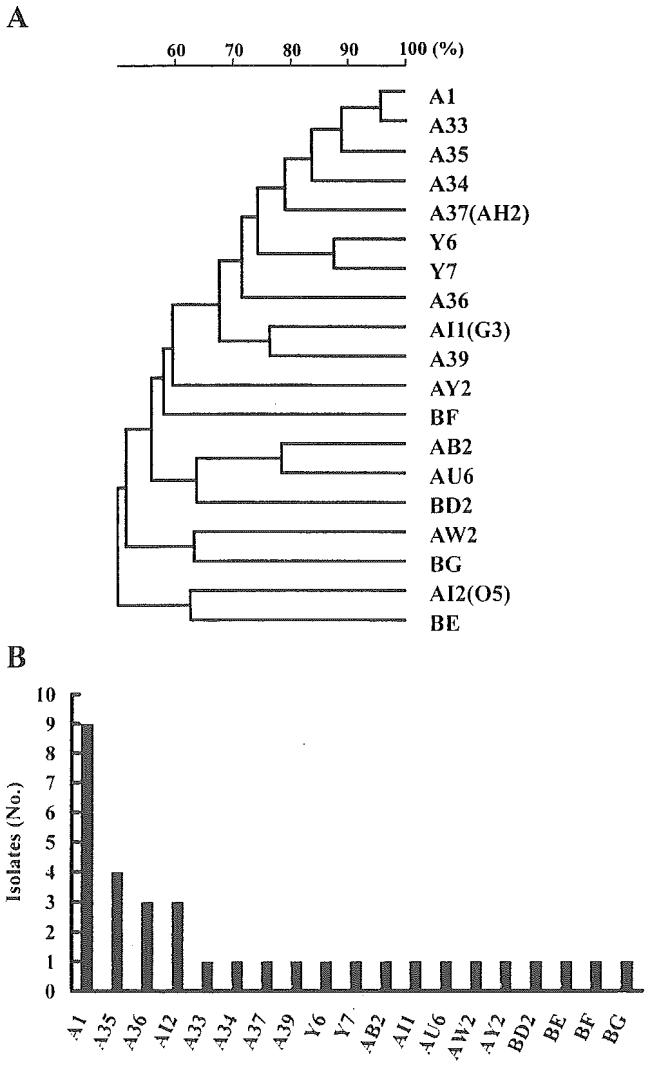

Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns of *Sma*I-digested genomic DNA.

Table 1. Distribution of MRSA in a hospital

PFGE pattern	Ward										ICU	Critical care center
	e			w			a					
	1	2	3	1	2	3	1	2	3	5	6	
A1	2#						1	2		3		1
A33		1										
A34					1							
A35		1					1				1	1
A36										1		2
A37								1				
A39				1								
Y6								1				
Y7							1					
AB2												1
AI1									1			
AI2									2			1
AU6												1
AW2												1
AY2								1				
BD2										1		
BE									1			
BF			1									
BG				1								

#: Number of patients with MRSA.

Table 2. Antibiotic pattern classified by antibiotic pattern of 15 antibiotics against MRSA

Antibiotic pattern	Antibiotics					
	GM	TC	MINO	ABK	VCM	TEIC
a	R	R	R	S	S	S
c	R	R	S	S	S	S
d	R	R	I	S	S	S
e	S	R	R	S	S	S
g	S	R	S	S	S	S
i	S	R	I	S	S	S
j	R	I	S	S	S	S
k	S	S	S	S	S	S

All the isolates were resistant to MPIPC, PCG, ABPC, PIPC, CEZ, CMZ, IPM, SBT/ABPC, EM.
MPIPC: oxacillin, PCG: benzyl-penicillin, ABPC: ampicillin, PIPC: piperacillin, 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.

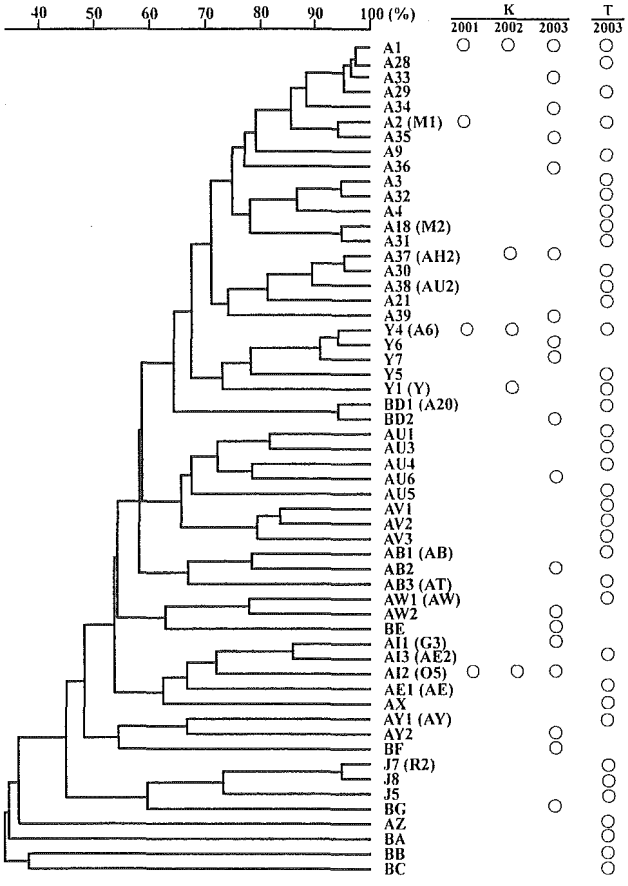

Fig. 3. Cluster analysis of MRSA isolates based on PFGE patterns.
K: Kumamoto hospital, T: Tokyo hospital.

three isolates. The remaining 15 patterns were identified in one isolate each.

MRSA with the pattern A1 was isolated from different wards as follows: one isolate was in the critical care center, two in ward e1, one in ward a1, two in ward a3, and three in ward a6. MRSA isolates with patterns A35, A36, and AI2 were detected in two or more wards. Four MRSAs with the pattern A35 were isolated from ward e2, ward a1, the intensive care unit, and the critical care center; three isolates with the pattern A36 were from ward a6 and the critical care center, and three isolates with the pattern A12(O5) were from ward a5 and the critical care center (Table 1). These results appear to suggest the multi-focal clonal expansion of MRSA in this hospital.

Sensitivity to antibiotics is shown in Table 2. The MRSA isolates were resistant to 9-12 of 15 drugs tested. All of the isolates were sensitive to arbekacin, vancomycin, and teicoplanin. Nine isolates having the PFGE pattern A1 had antibiotic pattern a or c. No correlation was found between antibiotic resistance and PFGE pattern.

All of the 34 MRSA isolates produced coagulase type II and TSST-1. Thirty-one isolates produced enterotoxin type C, one isolate produced enterotoxin types A and C, and one isolate produced enterotoxin types C and D. Collectively, among 34 MRSA isolates, 31 produced coagulase type II, enterotoxin type C, and TSST-1; i.e., most of the isolates shared common characteristics in terms of these parameters.

PFGE-based MRSA surveillance was conducted in the same hospital in October 2001 (1), October 2002 (2), and in a hospital in Tokyo in October 2003 (3). In these surveillance studies, a total of 56 PFGE patterns were detected (Fig. 3). PFGE patterns A1 and AI2(O5) were detected in Kumamoto in all of these surveillance studies conducted in 2001, 2002, and 2003. PFGE pattern A37(AH2) was detected in Kumamoto in 2002 and 2003. PFGE pattern Y4(A6) was detected in Kumamoto in 2001 and 2002. The other patterns were unique to each year (Fig. 3) (1,2). Pattern A33 was a newly detected pattern in the present study and was nearly identical to pattern A1, except the top band showed slightly slower migration. The present study indicates the co-existence of persistent and rapid turnover of MRSA in the hospital setting. Pattern A1 was detected in a hospital in Kumamoto in 2001, 2002, and 2003, and this pattern was also detected in a hospital in Tokyo (3). Among the patterns identified here, pattern A1 was most frequently detected in both hospitals (Fig. 2B) (3). The present data indicate the clonal expansion

of MRSA, not only within hospitals, but also nationwide.

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Jpn. J. Infect. Dis., 57, 2004

Laboratory and Epidemiology Communications

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

Tomoko Fujino, Jun-ichiro Sekiguchi, Akihiko Kawana, Hisami Konosaki, Haruo Nishimura, Katsutoshi Saruta, Koichiro Kudo, Tatsuya Kondo, Yoshio Yazaki, Tadatoshi Kuratsuji and Teruo Kirikae*

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Communicated by Hiroshi Yoshikura

(Accepted March 25, 2004)

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.

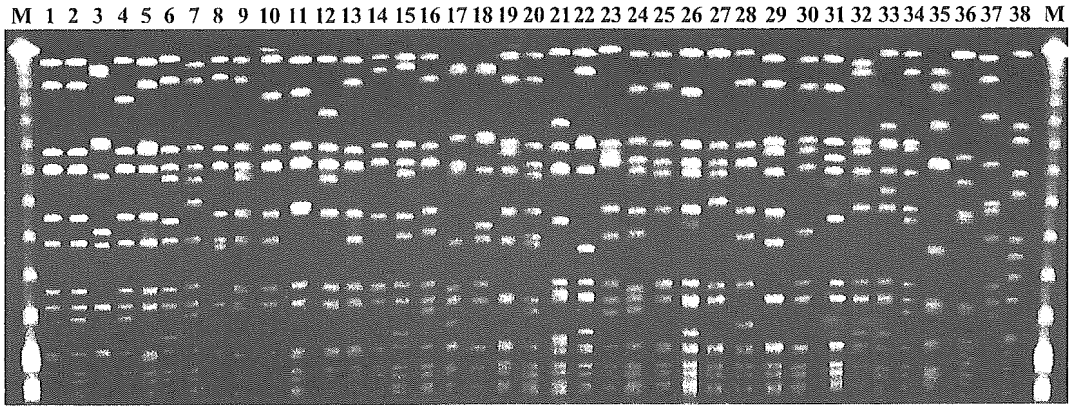

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. M: low range PFG Marker. Lanes 1 to 38: MRSA isolates with different PFGE patterns A1 to BC shown in Fig. 2.

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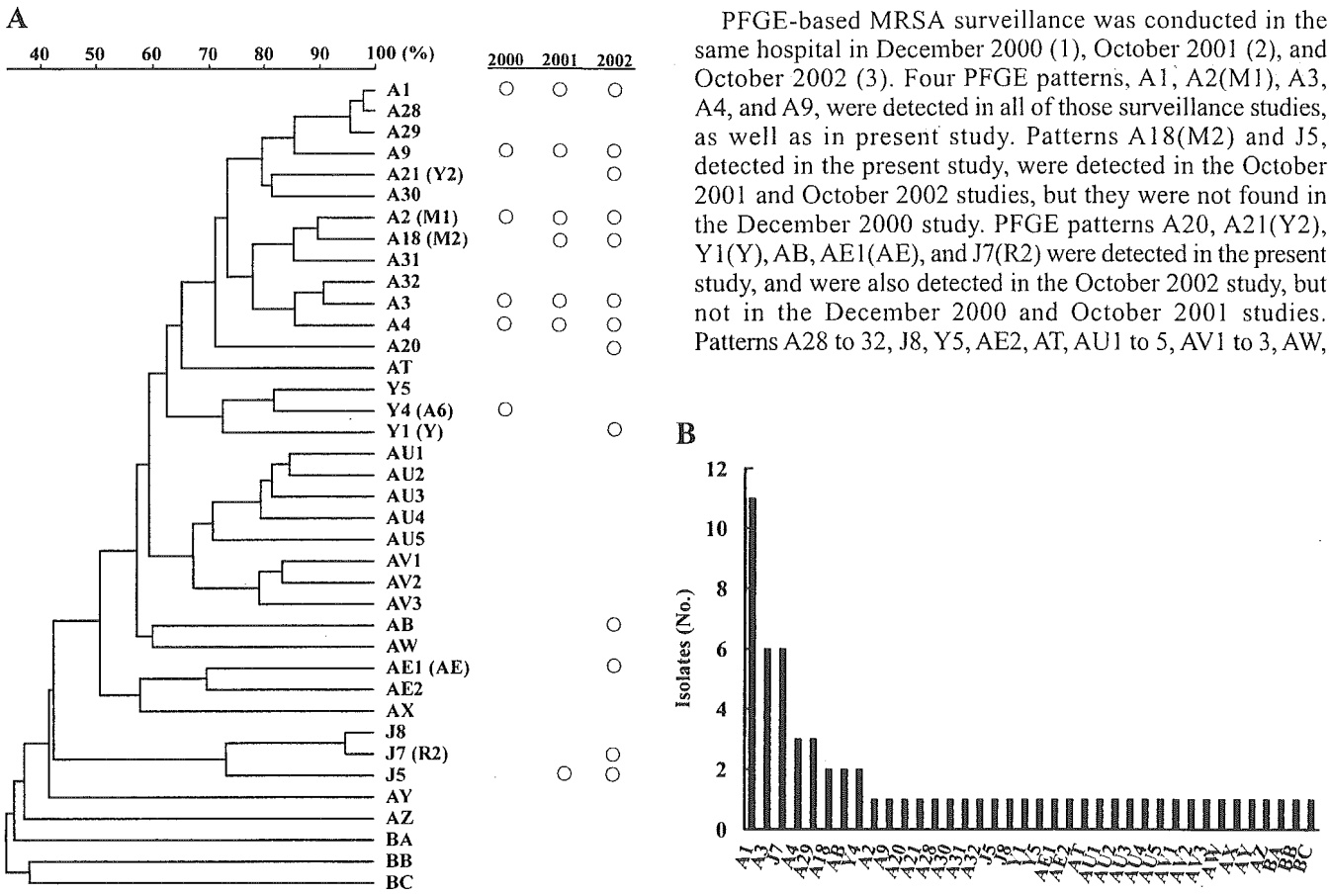

Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns. MRSA isolates indicated by circles were also detected in previous surveillance (1-3).

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,

Table 1. Distribution of MRSA in a hospital

Ward	PFGE pattern																																		
	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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Laboratory and Epidemiology Communications

Molecular Epidemiology of *Serratia marcescens* in a Hospital

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Communicated by Hiroshi Yoshikura

(Accepted March 12, 2004)

Serratia marcescens is an important nosocomial pathogen, particularly regarding catheter-related bacteremia, urinary tract infections, and respiratory infections. Pulsed-field gel electrophoresis (PFGE) is useful in determining the molecular epidemiology of various pathogens including *S. marcescens* (1).

In May 2003, two inpatients (P1 and P2) successively developed sepsis in a surgical ward of a hospital with 925 beds. Blood cultures of the two patients revealed the presence of *S. marcescens*. Both patients P1 and P2 had been inserted with vascular catheters for 12 days and 4 days, respectively, before developing sepsis. The two isolates from the respective patients had identical PFGE patterns. Epidemiological investigation conducted by the infection control team in the hospital, however, was unable to identify the source of the infection. PFGE-based surveillance of *S. marcescens* was then conducted to assess the possible risk of an outbreak of *S. marcescens* infections.

A total of 23 clinical isolates of *S. marcescens*, including the above two isolates and 21 isolates obtained from 21 inpatients during August and September 2003, were analyzed for chromosomal DNA typing by using a counter-clamped homogeneous electric field system (CHEF Mapper™: Bio-Rad Laboratories, Hercules, Calif., USA), and for antibiotic resistance (WalkAway™: Dade Behring, Deerfield, Ill., USA).

Twenty different PFGE patterns of the *Spe*I DNA digests of the isolates were detected (Figs. 1A and 1B). PFGE patterns A, J, and K (Fig. 1A) were shared respectively by

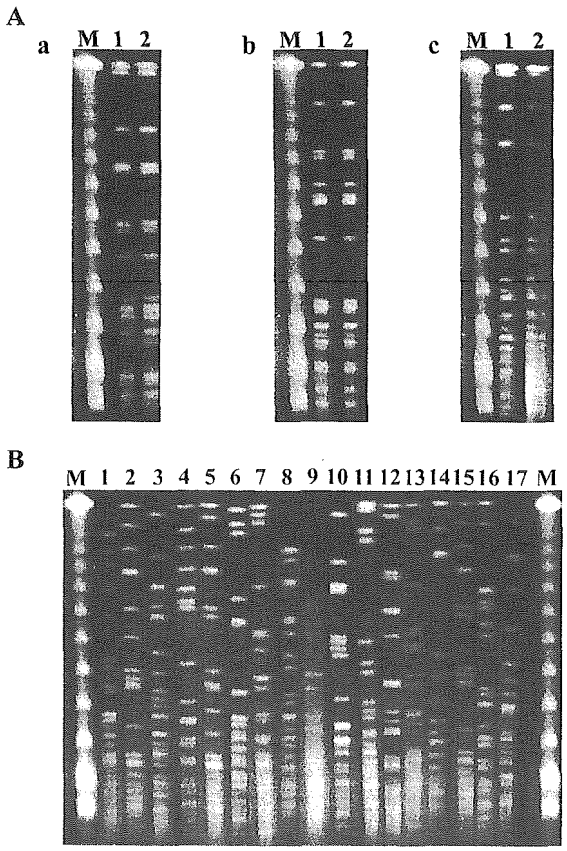

Fig. 1. Pulsed-field gel electrophoresis of *Spe*I-digested genomic DNA from *S. marcescens* isolates.
A: a) PFGE pattern K (isolates No. S6 and S7), b) PFGE pattern J (isolates No. S15 and S18), c) PFGE pattern A (isolates No. S21 and S38), M: low range PFG Marker.
B: lane 1: isolate No. S14, lane 2: No. S16, lane 3: No. S19, lane 4: No. S23, lane 5: No. S24, lane 6: No. S25, lane 7: No. S27, lane 8: No. S28, lane 9: No. S29, lane 10: No. S31, lane 11: No. S32, lane 12: No. S33, lane 13: No. S34, lane 14: No. S36, lane 15: No. S37, lane 16: No. S39, lane 17: No. S40.

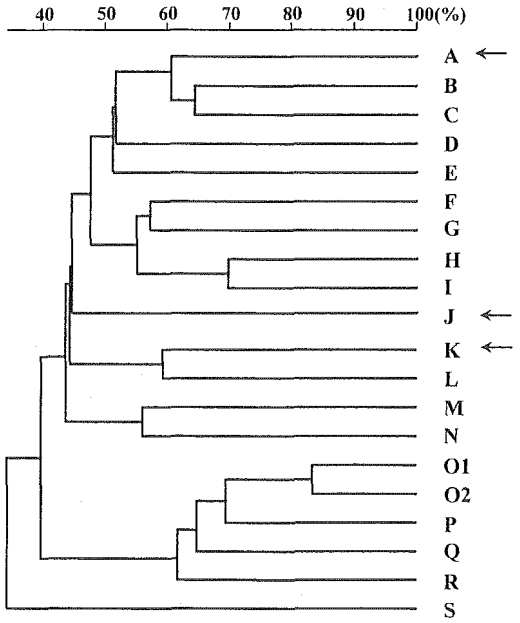

Fig. 2. Band-based cluster analysis of PFGE patterns of *S. marcescens* isolates.

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Table 1. Antibiotic pattern classified by antibiotic pattern of 18 antibiotics against *S. marcescens*

Antibiotic pattern	Antibiotics										
	ABPC	PIPC	CTM	CMZ	CTX	CAZ	FMOX	AZT	S/C	AMK	MINO
a	R	R	R	R	R	S	R	S	R	R	S
b	R	S	R	S	R	S	R	S	S	S	S
c	R	I	R	R	S	S	R	S	S	S	S
d	R	I	R	R	R	I	I	I	S	S	S
e	R	R	R	S	S	R	S	R	S	S	S
f	R	R	R	S	S	S	S	S	S	I	S
g	R	I	R	S	S	S	S	S	S	S	S
h	R	S	R	S	S	S	S	S	S	S	S
i	R	S	R	S	I	S	I	S	S	S	S
j	I	S	R	S	S	S	S	S	S	S	S
k	I	S	R	S	S	S	I	S	S	S	S
l	R	S	I	S	S	S	S	S	S	S	S
m	S	S	R	S	I	S	S	S	S	S	R
n	S	S	S	S	S	S	S	S	S	S	S

All isolates were resistant to CCL and CEZ, but sensitive to CPR, GM, IPM, LVFX, and ST.
ABPC: ampicillin, PIPC: piperacillin, CTM: cefotiam, CMZ: cefmetazole, CTX: cefotaxime, CAZ: ceftazidime, CCL: cefaclor, CEZ: cefazolin, CPR: cefpirome, FMOX: flomoxef, AZT: aztreonam, S/C: sulbactam/cefoperazone, AMK: amikacin, MINO: minocycline, GM: gentamicin, IPM: imipenem/cilastatin, LVFX: levofloxacin, ST: sulfametazole/trimethoprim, R: resistant, S: sensitive, I: intermediate.

Table 2. Phenotypic and genotypic characterization of *S. marcescens* isolates

Patient no.	Isolates no.	Specimen	Date	Ward	PFGE pattern	Antibiotic pattern
P1	S6	Venous blood	12-May	8N	K	k
P2	S7	Venous blood	19-May	8N	K	i
P3	S14	Venous blood	12-Aug	16	H	c
P4	S15	Venous blood	15-Aug	12S	J	d
P5	S16	Sputum	20-Aug	8N	O1	h
P6	S18	Urine	22-Aug	12S	J	j
P7	S19	Sputum	25-Aug	12N	F	o
P8	S21	Urine	27-Aug	7N	A	i
P9	S23	Sputum	28-Aug	7N	L	h
P10	S24	Sputum	29-Aug	11N	P	h
P11	S25	Sputum	1-Sep	ICU	I	k
P12	S27	Urine	8-Sep	6N	B	a
P13	S28	Sputum	8-Sep	7N	G	i
P14	S29	Sputum	8-Sep	9S	R	e
P15	S31	Urine	10-Sep	7N	S	l
P16	S32	Sputum	9-Sep	5S	C	i
P17	S33	Sputum	16-Sep	10N	Q	i
P18	S34	Urine	16-Sep	9S	D	f
P19	S36	Urine	18-Sep	7S	M	i
P20	S37	Venous blood	22-Sep	7N	O2	m
P21	S38	Urine	22-Sep	7N	A	b
P22	S39	Pleural cavity drain	24-Sep	12N	N	k
P23	S40	Urine	26-Sep	9S	E	n

isolates from different pairs of the patients (see below). The other 17 PFGE patterns were unique to each isolate (Fig. 1B). Band-based cluster analysis of these patterns (Molecular Analysis™: Bio-Rad) revealed a low level of similarity among the isolates except for patterns O1 and O2 that formed a cluster (a cluster was defined as a group of patterns sharing more than 70% similarity) (Fig. 2).

The majority of the *S. marcescens* isolates were resistant to ABPC, CCL, and CEZ, but sensitive to CAZ, IMP, and LVFX. They were resistant to 2 - 10 of 18 tested drugs (Table 1). Fifteen different drug resistance patterns were observed. No correlation was found between the antibiotic patterns and PFGE patterns (data not shown).

Three pairs of isolates having identical PFGE patterns were obtained from different patients in the same ward on similar dates. The strains with pattern K (isolate Nos. S6 and S7) were isolated from patients P1 and P2 in ward 8N in May. Those with pattern J (Nos. S15 and S18) were from patients P4 and P6 in ward 12S in August. Those with pattern A (Nos. S21 and S38) were from patients P8 and P21 in ward 7N in August and September. It was noteworthy that all these pairs of patients had undergone catheterization concurrently. The patients may have been infected with the pathogen from the same source related to cathters.

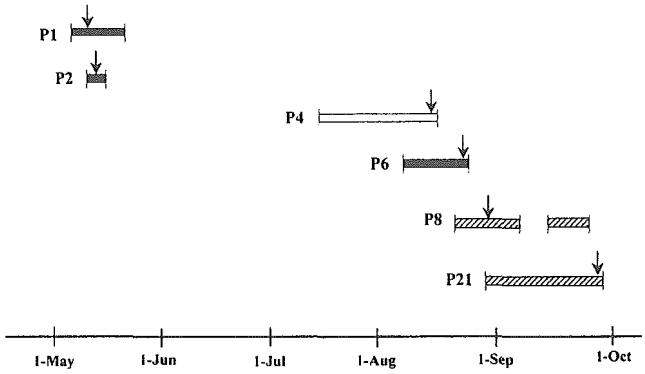

Fig. 3. Duration of catheterization. Intravenous, central venous, and urinary tract catheterization are represented by filled, open, and hatched boxes, respectively. Vertical arrows indicate the date of isolation of *S. marcescens*.

Catheterization was thus found to have a high risk of *S. marcescens* infection. In the hospital, its application including its duration was revised and a single use of heparin solution for the heparin lock technique was implemented. None of the patients involved in the above outbreak suffered serious consequences.

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Jpn. J. Infect. Dis., 57, 2004

Laboratory and Epidemiology Communications

Prevalence of Erythromycin-, Tetracycline-, and Aminoglycoside-Resistance Genes in Methicillin-Resistant *Staphylococcus aureus* in Hospitals in Tokyo and Kumamoto

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Communicated by Hiroshi Yoshikura

(Accepted March 12, 2004)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infections that are becoming increasingly difficult to combat because of their emerging resistance to all current antibiotic classes. Investigating the spread of the drug-resistance genes in MRSA is important for the control of its dissemination (1).

In our previous papers (2-4), a total of 179 MRSA clinical isolates obtained in December 2000, October 2001, and October 2002 from a hospital with 24 wards and 925 beds in Tokyo were assessed using restriction fragment length polymorphism (RFLP) of genomic DNA using pulsed-field gel electrophoresis (PFGE). A band-based cluster analysis of the PFGE patterns of these isolates revealed that 111 of the 179 MRSA isolates formed a cluster of PFGE patterns, called cluster A.

Chromosomal DNA was typed by using a contour-clamped homogeneous electric field system (CHEF Mapper™, Bio-Rad Laboratories, Hercules, Calif., USA). Plasmid DNA was typed by using agarose gel electrophoresis. The antibiotic resistance of MRSA to tetracycline (TC) was analyzed using WalkAway™ (Dade Behring, Deerfield, Ill., USA) and E-test™ (AB BIODISK, Dalvagen, Sweden). PCR was used to detect gentamicin (GM)-resistance genes [*aac6′-aph2″* and *aph(3′)-III*], erythromycin (EM)-resistance genes (*ermA*, *ermB*, and *ermC*), and TC-resistance genes (*tetK* and *tetM*), while Southern blot used to detect *aac6′-aph2″*, *ermA*, and *tetM*. Some of the PCR products were sequenced for confirmation. Based on these analyses, the isolates were classified into 33 types (Table 1).

Among the 111 MRSA isolates tested, all were resistant to EM, 13 were resistant to GM, and 102 were resistant to TC. The majority of the isolates (97 of 111) were resistant to EM and TC, but sensitive to GM. No isolates were sensitive to all three antibiotics (Table 1). PFGE of *Sma*I digests (Fig. 1A) revealed 23 different patterns. The most frequent pattern was A1, representing 31.5% of the total isolates (Table 1). The profiles of plasmid typing are shown in Fig. 2A. Plasmids of 27 different sizes, ranging from 2.4 kb to 300 kb, were detected. The isolates were classified into 26 plasmid patterns (Table 2). One-hundred-eight of 111 isolates had one or more different-sized plasmids. Three other isolates had no plasmids. Seventy-three isolates accounting for 68% of the total had plasmid pattern I, II, or III, and these isolates had both 50 kb and 35 kb plasmids (Table 2). Among the isolates with PFGE pattern A1, 34 had 50 kb plasmid. Among them, eight had plasmid pattern I, seven had plasmid pattern II, 10 had plasmid pattern III, one had plasmid pattern X, and eight had plasmid pattern XXIV.

The results of PCR analysis are summarized in Table 1. Among the 111 MRSA isolates, 13 were PCR-positive for *aac6′-aph2″*, all isolates were positive for *ermA*, and 103 were positive for *tetM*. No isolate was positive for *aph(3′)-III*, *ermB*, *ermC*, or *tetK*. The majority of the isolates (103 of 111) were positive for the genes *ermA* and *tetM*, but negative for the others. Twelve isolates with type Nos. 10-12, 24, 28, and 33 were positive for *aac6′-aph2″*, *ermA*, and *tetM*. One isolate with No. 4 was positive for *ermA* and *aac6′-aph2″*, and three with Nos. 3, 7, and 17 were positive for *ermA*.

Southern blotting detected *aac6′-aph2″* on 30 kb, 38 kb, 190 kb, or 200 kb plasmids carried by six isolates and on the chromosomes of 12 isolates. On the chromosomes, it was present in 110 kb and 220 kb *Sma*I fragments (two isolates), in a 220 kb *Sma*I fragment (one isolate), and in a 500 kb *Sma*I fragment (five isolates) (Fig. 1 and Table 1). The *ermA* was found on the chromosomes of all the isolates, mostly on 220 kb and 580 kb *Sma*I fragments. The *tetM* was found on the chromosomes of 104 isolates, mostly in the 290 kb *Sma*I fragment.

The PCR analysis gave data consistent with resistance pattern of the bacteria in all the cases except three, which were types Nos. 2, 5, and 14. An isolate of type No. 2 was sensitive to GM, resistant to EM, and intermediately resistant to TC, while negative for *aac6′-aph2″*, but positive for *ermA* and *tetM* in PCR. An isolate of type No. 5 was resistant to GM and EM, but sensitive to TC, while being PCR-negative for *aac6′-aph2″* and *tetM*, but positive for *ermA*. An isolate of type No. 14 was sensitive to GM and TC, but resistant to EM, while PCR-negative for *aac6′-aph2″*, but positive for *ermA* and *tetM*. This discordance may probably be brought about by mutations in the coding or promoter region of the PCR-detected genes.

Among 111 MRSA isolates obtained from a hospital in Tokyo and whose PFGE patterns showed A clusters, 34 isolates showing PFGE pattern A1 were sensitive to GM, and

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Table 1. PFGE pattarns of MRSA isolates; MICs of GM, EM and TC from these isolates; and distribution of GM-, EM-, and TC-resistance genes among these isolates

Typing no.[1]	PFGE pattern[2]	No. of isolates in			MIC(μg/ml) of			PCR product							Southern blot		
		2000	2001	2002	GM	EM	TC	A[3]	B	C	D	E	F	G	*aac6′-aph2″*	*ermA*	*tetM*
															plasmid/chromosome (kb)		
1	A1	7	10	17	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
2		0	0	1	<1(S)	>4(R)	5(I)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
3	A2(M1)	2	0	0	<1(S)	>4(R)	≦4(S)	−	−	+	−	−	−	−	—/—	—/220, 580	—/—
4		1	0	0	>8(R)	>4(R)	≦4(S)	+	−	+	−	−	−	−	38, 200/110, 220	—/220, 580	—/—
5		0	1	0	>8(R)	>4(R)	≦4(S)	−	−	+	−	−	−	−	—/500	—/220, 580	—/—
6		1	0	0	>8(R)	>4(R)	≦4(S)	+	−	+	−	−	−	−	—/500	—/220, 580	—/—
7		1	0	0	<1(S)	>4(R)	≦4(S)	−	−	+	−	−	−	−	—/—	—/220, 580	—/—
8		0	0	1	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 580	—/580
9	A3	3	8	0	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
10		0	1	0	>8(R)	>4(R)	≧16(R)	+	−	+	−	−	−	+	30, 38/—	—/220, 580	—/290
11		1	0	0	>8(R)	>4(R)	≧16(R)	+	−	+	−	−	−	+	—/220	—/220, 580	—/290
12		0	0	1	>8(R)	>4(R)	≧16(R)	+	−	+	−	−	−	+	—/500	—/220, 580	—/290
13	A4	2	7	4	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
14		0	1	0	<1(S)	>4(R)	≦4(S)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
15	A5	2	1	0	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 550	—/290
16	A6	1	0	0	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
17	A7	1	0	0	<1(S)	>4(R)	≦4(S)	−	−	+	−	−	−	−	—/—	—/220, 580	—/—
18	A8	1	0	0	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	−	—/—	—/220, 580	—/680
19	A9	1	3	1	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/100, 220, 580	—/290
20	A10	1	0	0	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 630	—/290
21	A11	1	1	0	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
22	A12	1	0	0	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
23	A13	0	1	0	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
24	A14	0	1	0	>8(R)	>4(R)	≧16(R)	+	−	+	−	−	−	+	—/500	—/220, 580	—/290
25	A15	0	2	1	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
26	A16	0	1	4	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
27	A17	0	1	0	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
28	A18(M2)	0	1	4	>8(R)	>4(R)	≧16(R)	+	−	+	−	−	−	+	—/500	—/210, 590	—/70, 590
29	A20	0	0	1	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
30	A21	0	0	1	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 230, 580	—/290
31	A22	0	0	2	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 580	—/290
32	A23	4	0	1	<1(S)	>4(R)	≧16(R)	−	−	+	−	−	−	+	—/—	—/220, 550	—/290
33	A24	0	0	1	>8(R)	>4(R)	≧16(R)	+	−	+	−	−	−	+	38, 190/110, 220	—/220, 580	—/290

[1]: Typing no. is corresponding to the lane No. shown in Fig. 1.
[2]: The PFGE patterns was reported in ref 2, 3, and 4.
[3]: A: *aac6′-aph2″*, B: *aph(3′)-III*, C: *ermA*, D: *ermB*, E: *ermC*, F: *tetK*, G: *tetM*.

resistant to EM and TC. They had *ermA* in 220 kb and 580 kb *Sma*I chromosomal digests and *tetM* in a 290 kb *Sma*I chromosomal digest, but they did not have plasmids harboring *ermA*, *tetM*, or any other of the drug-resistance genes tested. Previous studies (5,6) showed that MRSA isolates having the PFGE pattern A1 were wide spread in hospitals in Tokyo and in Kumamoto. Both the Kumamoto and the Tokyo isolates had *ermA* in 220 kb and 580 kb *Sma*I chromosomal fragments and *tetM* in 290 kb *Sma*I fragments. However, their antibiotic resistance patterns were different (1). Most Kumamoto isolates were resistant to GM, EM, and TC; they had a multidrug resistant 40 kb plasmid harboring *aac6′-aph2″*, *ermA*, and *tetM*, and 200 kb plasmid harboring *aac6′-aph2″*. They also had *aac6′-aph2″* in a 110 kb *Sma*I chromosome fragment. The Tokyo isolates, meanwhile, were found to be GM-sensitive, and had no 40 kb or 200 kb plasmids and no *aac6′-aph2″* in their chromosomes. In summary, there appears to have been a clonal expansion of closely related MRSAs in hospitals in Tokyo and in Kumamoto, but the MRSA in Kumamoto appeared to have recently acquired the GM-resistance gene, *aac6′-aph2″*, which was not found in the Tokyo isolates.

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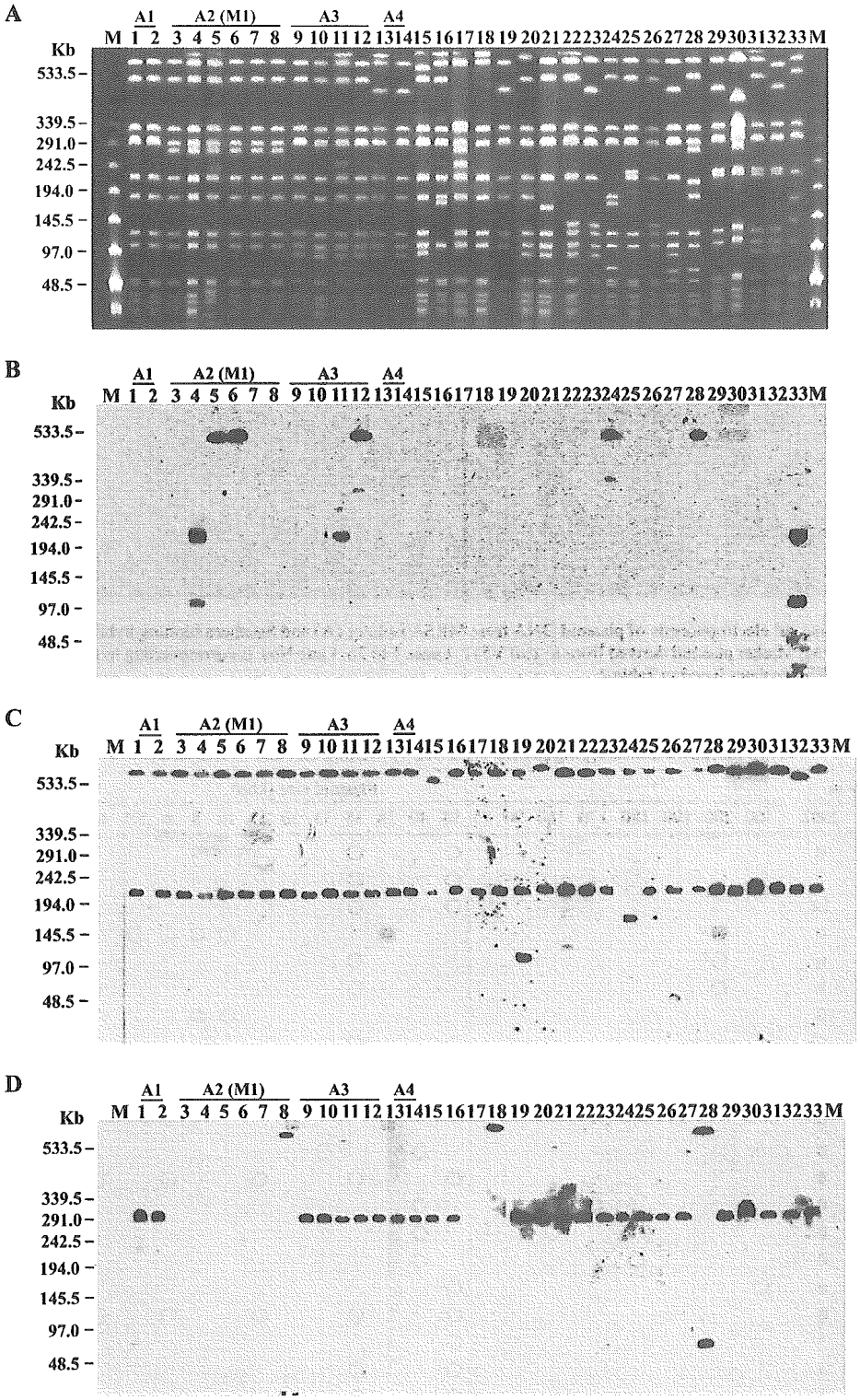

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates (A) and Southern blotting hybridized with *aac6'-aph2"* (B), *ermA* (C), and *tetM* (D). M: low range PFG Marker. Lanes 1 to 33: Lane Nos. is corresponding to the typing Nos. of MRSA isolates listed in Table 1.

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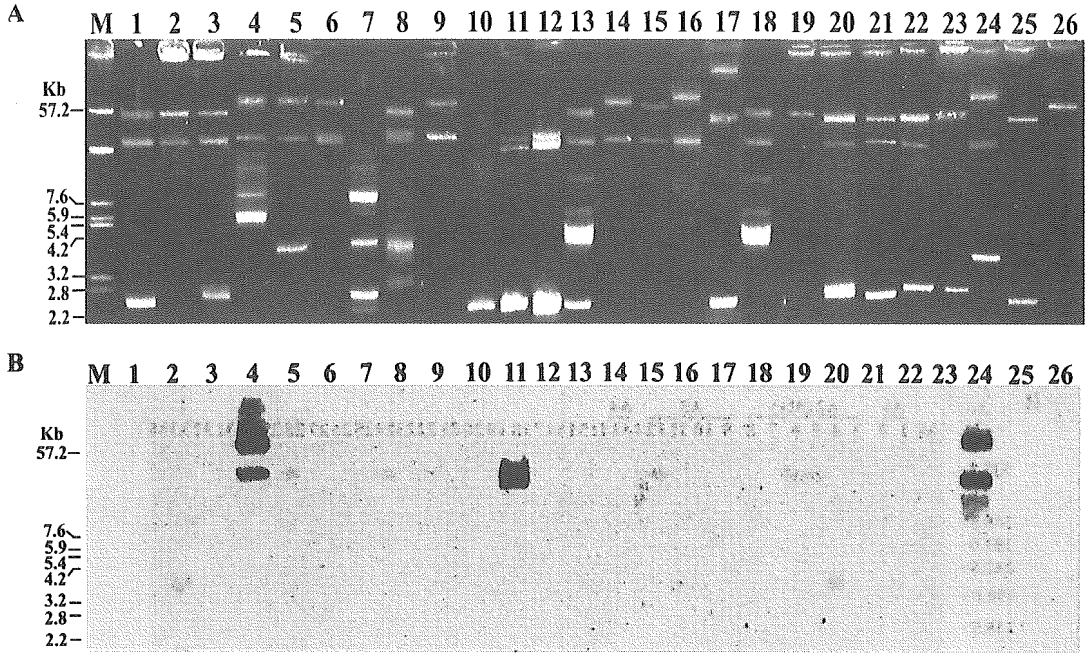

Fig. 2. Agarose gel electrophoresis of plasmid DNA from MRSA isolates (A) and Southern blotting hybridized with *aac6′-aph2″* (B). M: Marker plasmid derived from *E. coli* V517. Lanes 1 to 26: Lane Nos. is corresponding to the plasmid typing Nos. of MRSA isolates listed in Table 2.

Table 2. Plasmid typing pattern classified by plasmid size and its frequency

Plasmid pattern	Frequency in			Plasmid size (kbp)																								
	2000	2001	2002	300	200	190	180	170	160	90	85	50	40	38	35	33	30	11	10	8	6	5.5	4.5	4	3.5	2.9	2.8	2.7	2.6	2.4
I	10	26	0									○			○														○
II	6	3	7									○			○														
III	7	0	14									○			○														○	
IV	1	0	0		●[1]									●					○	○	○	○								
V	1	0	0		○[2]										○										○					
VI	1	0	0		○										○															
VII	1	0	0																○	○				○					○	○
VIII	1	0	0									○			○										○	○				
IX	1	0	0							○			○																	
X	0	3	0																										○	
XI	0	1	0											●			●												○	
XII	0	1	0										○			○													○	
XIII	0	1	0									○			○			○			○		○	○					○	
XIV	0	1	0					○					○																	
XV	0	1	0						○				○																	
XVI	0	1	0				○						○																	
XVII	0	1	0	○								○																○		
XVIII	0	1	0									○			○			○			○		○	○						
XIX	0	0	1									○																		
XX	0	0	3									○			○											○	○			
XXI	0	0	1									○			○												○			
XXII	0	0	1									○			○											○				
XXIII	0	0	1									○														○				
XXIV	0	0	1			●								●											○					
XXV	0	0	9									○																	○	
XXVI	0	0	1								○																			

[1]: Plasmid harboring *aac6′-aph2″*.
[2]: Plasmid not harboring any of the drug-resistant genes tested.