TABLE 3—*Continued*

Strain and position (bp)	No. of repeats	Gene no.	Product	Domain	Translation
1214721	5	Mb1116	PE_PGRS family protein	ORF	Poly(Gly)
1627966	5	Mb1485c	PE_PGRS family protein	ORF	Poly(Gly)
2339003	5	Mb2125c	Conserved hypothetical protein PE_PGRS family protein	ORF	Poly(Gly)
2339109	5	Mb2125c	Conserved hypothetical protein PE_PGRS family protein	ORF	Poly(Gly)
2367710	5	Mb2150c	Conserved hypothetical protein PE_PGRS family protein	ORF	Poly(Gly)
2604931	5	Mb2376c	PPE family protein	ORF	Poly(Ala)
2607401	5	Mb2377c	PPE family protein	ORF	Poly(Ala)
2607513	5	Mb2377c	PPE family protein	ORF	Poly(Ala)
2769065	5	Mb2517c	PE_PGRS family protein	ORF	Poly(Gly)
3706526	5	Mb3380c	PPE family protein	ORF	Poly(Ala)
3720437	5	Mb3385c	PPE family protein	ORF	Poly(Ala)
3755777	5	Mb3420	PE_PGRS family protein	ORF	Poly(Gly)
3912639	5	Mb3562	PPE family protein	ORF	Poly(Ala)
3915460	5	Mb3563c	PPE family protein	ORF	Poly(Ala)
3972250	5	Mb3618c	Probable conserved membrane protein	ORF	Poly(Pro)

containing $(CGG)_7$, PonA, encoded a penicillin-binding protein (Table 3). In strain CDC1551, the genes containing $(CGG)_5$ encoded the PPE, PE_PGRS, and PE families of proteins. A gene containing $(CGG)_6$ encoded a penicillin-binding protein (Table 3). In *M. bovis*, all genes containing $(CGG)_5$ encoded PPE and PE_PGRS family proteins, with the exception of two genes that encoded probable conserved membrane proteins (Table 3). In all three strains, the $(CGG)_5$ in the PPE genes translated to poly(Ala), and the $(CGG)_5$ and $(CGG)_6$ in the PE_PGRS and PE genes translated to poly(Gly). In both *M. tuberculosis* strains, the $(CGG)_6$ and $(CGG)_7$ in genes encoding penicillin-binding proteins translated to poly(Pro) (Table 3). In *M. bovis*, the two $(CGG)_5$ repeats in genes encoding probable conserved membrane proteins translated to poly(Ala) and poly(Pro) (Table 3). Most of the $(CGG)_5$ repeats within the PPE genes were located in the N-terminal PPE domain of the genes (data not shown). All $(CGG)_5$ and $(CGG)_6$ repeats within the PE_PGRS genes consisting of PE and PGRS domains were located in the PGRS domain (data not shown). Two $(CGG)_5$ repeats within the PE family-related gene (MT2159) in strain CDC1551 were located in the C-terminal domain of the genes (data not shown).

Genomic stability. To examine whether $(CGG)_5$ repeats in the genome are stable, two *M. tuberculosis* strains (H37Rv and IMCJ 541) were analyzed for $(CGG)_5$- and IS*6110*-probed fingerprints. The fingerprint patterns among culture periods were identical for strain H37Rv (Fig. 1A). These findings were confirmed with strain IMCJ 541 (Fig. 1B). The data indicate that $(CGG)_5$ repeats are stable in the genome for at least a few months. In the IS*6110*-probed fingerprints, the patterns did not change during the 9 weeks of culture of strain H37Rv or strain IMCJ 541 (data not shown), indicating that IS*6110* inserts are also stable over a few months.

Comparison of fingerprints between *M. tuberculosis* strains H37Rv and H37Ra. The virulent *M. tuberculosis* strain H37Rv and its avirulent derivative strain H37Ra were originally derived from the same strain, H37 (22, 23). It was reported that there are distinct differences between these strains with respect to IS*6110*-probed fingerprint patterns (3, 11). We investigated whether differences exist between these strains with respect to $(CGG)_5$-probed fingerprint patterns. DNA derived from the H37Rv and H37Ra strains were digested with 16 restriction enzymes as described in Materials and Methods. Unexpectedly, the patterns of $(CGG)_5$-based hybridization showed no differences between the H37Rv and H37Ra strains (Fig. 2A). For example, the $(CGG)_5$-based RFLP patterns of PvuII-digested fragments of H37Rv were identical to those of H37Ra (Fig. 2A, PvuII). However, the IS*6110*-based RFLP patterns of H37Rv were markedly different from those of H37Ra, which were analyzed with the use of the same blot of PvuII-digested fragments used in the $(CGG)_5$-based RFLP analysis (Fig. 2B). In the IS*6110*-based RFLP patterns, H37Rv showed 9 bands, and H37Ra showed 11 bands. Strain H37Rv but not H37Ra showed one band of 5.1 kb. Strain H37Ra but not H37Rv showed three bands of 1.1, 2.3, and 3.0 kb.

IS*6110*- and $(CGG)_5$-probed DNA fingerprinting of *M. tuberculosis* clinical isolates. To assess the potential usefulness of $(CGG)_5$ as an epidemiologic marker for *M. tuberculosis*, 109 clinical isolates obtained from Tokyo (76 isolates) and Warsaw (33 isolates) and the H37Rv and H37Ra strains were analyzed by the IS*6110*- and $(CGG)_5$-probed fingerprint methods. For IS*6110*-probed hybridization, DNA of these isolates was digested with PvuII according to a standardized protocol (26). For $(CGG)_5$-probed hybridization, DNA of the isolates was digested with AluI. When DNA of the H37Rv and H37Ra strains was digested with AatII, EcoRI, MluI, NruI, NsbI, PstI, PvuII, SacI, SalI, or XhoI, relatively higher-molecular-weight DNA fragments were visualized by the probe with a minimum size of 1 to 3.5 kb and a maximum size of more than 10 kb (Fig. 2A). When digested with AfaI, AluI, HinfI, Sau3AI, SmaI, or XspI, DNA fragments of sizes of 0.5 to 8 kb were visualized. When DNA of five clinical isolates selected at random were digested with AluI, clear $(CGG)_5$ fingerprint patterns with 10 to 14 copies of DNA fragments of 0.75 to 8 kb were detected (data not shown). Although we used AluI for this fingerprinting method, other enzymes may also be used.

IS*6110* fingerprint patterns obtained from clinical isolates and the corresponding dendrogram are shown in Fig. 3A. IS*6110* copies were detected in 110 of 111 isolates. One isolate from Japan had no copy. As indicated in Fig. 3A, 10 of 111 isolates (9.0% of tested isolates), including 8 isolates from Japan and 2 from Poland, possessed fewer than 6 copies of IS*6110*, which was insufficient to distinguish polymorphisms. Except for these 10 isolates with fewer than 6 copies of IS*6110*, the IS*6110* fingerprint patterns of 101 isolates showed ≥28% similarity; 98 patterns were found (Fig. 3A). Five clusters with

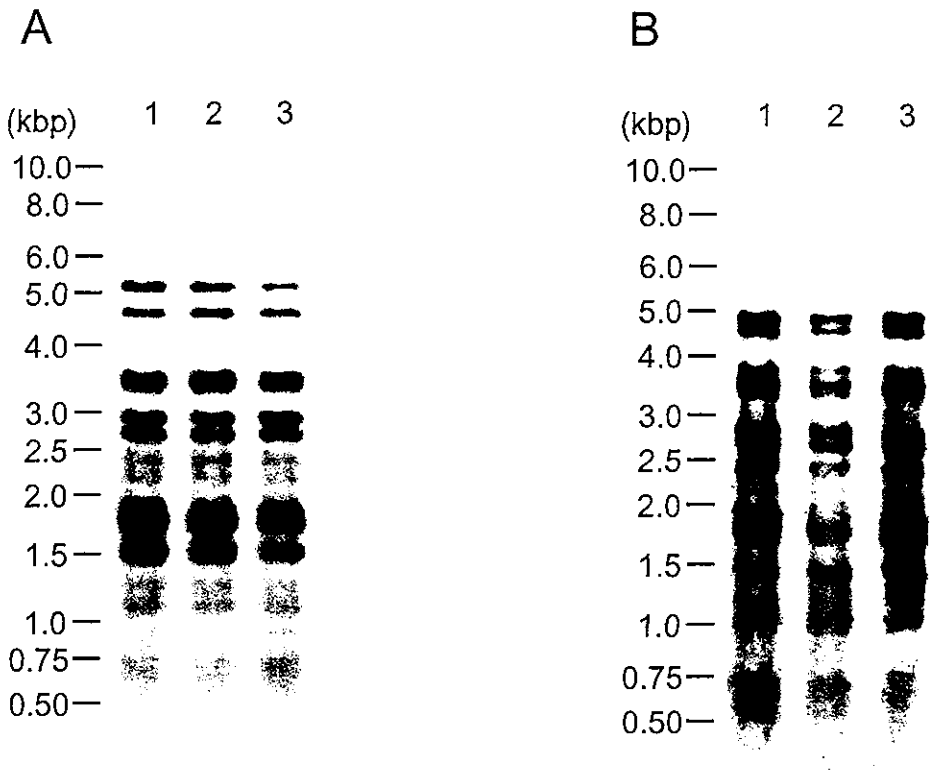

FIG. 1. (CGG)$_5$ fingerprinting of *M. tuberculosis* H37Rv (A) and clinical isolate IMCJ 541 (B), which were cultured and serially passaged weekly. The bacteria were harvested at 0 (lane 1), 3 (lane 2), and 9 (lane 3) weeks after culture.

≥44% similarity, including clusters Ia, IIa, IIIa, IVa, and Va, were detected (Fig. 3A). Cluster Ia was composed of seven Poland-derived isolates. Cluster IIa was composed of two H37 variants and 11 Japan- and 6 Poland-derived isolates. Cluster IIIa was composed of three Japan- and seven Poland-derived isolates. Cluster IVa was composed of four Japan- and five Poland-derived isolates. Cluster Va was composed predominantly of Japan-derived isolates (46 isolates from Japan and 2 from Poland). The majority of Japan-derived isolates (61%) and Poland-derived isolates (76%) belonged to cluster Va and to clusters Ia to IVa, respectively.

(CGG)$_5$ fingerprint patterns and the corresponding dendrogram are shown in Fig. 3B. (CGG)$_5$ copies were detected in all clinical isolates tested. The copy number ranged from 8 to 16, with a mean of 13.0 ± 1.5 per isolate. The number of (CGG)$_5$ copies of Japan- and Poland-derived isolates ranged from 8 to 16, with a mean of 12.9 ± 1.5 per isolate and from 11 to 15, with a mean of 13.2 ± 1.3 per isolate, respectively. A total of

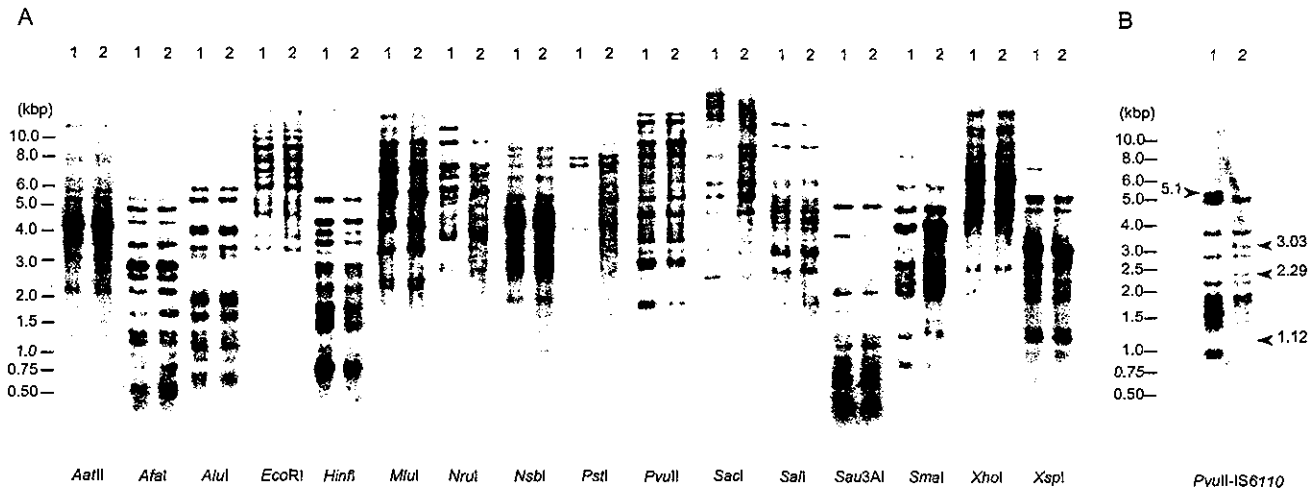

FIG. 2. (CGG)$_5$ (A) and IS*6110* (B) fingerprinting of *M. tuberculosis* strains H37Rv (lane 1) and H37Ra (lane 2). Genomic DNA was digested with 16 restriction enzymes. The digested fragments were separated by electrophoresis.

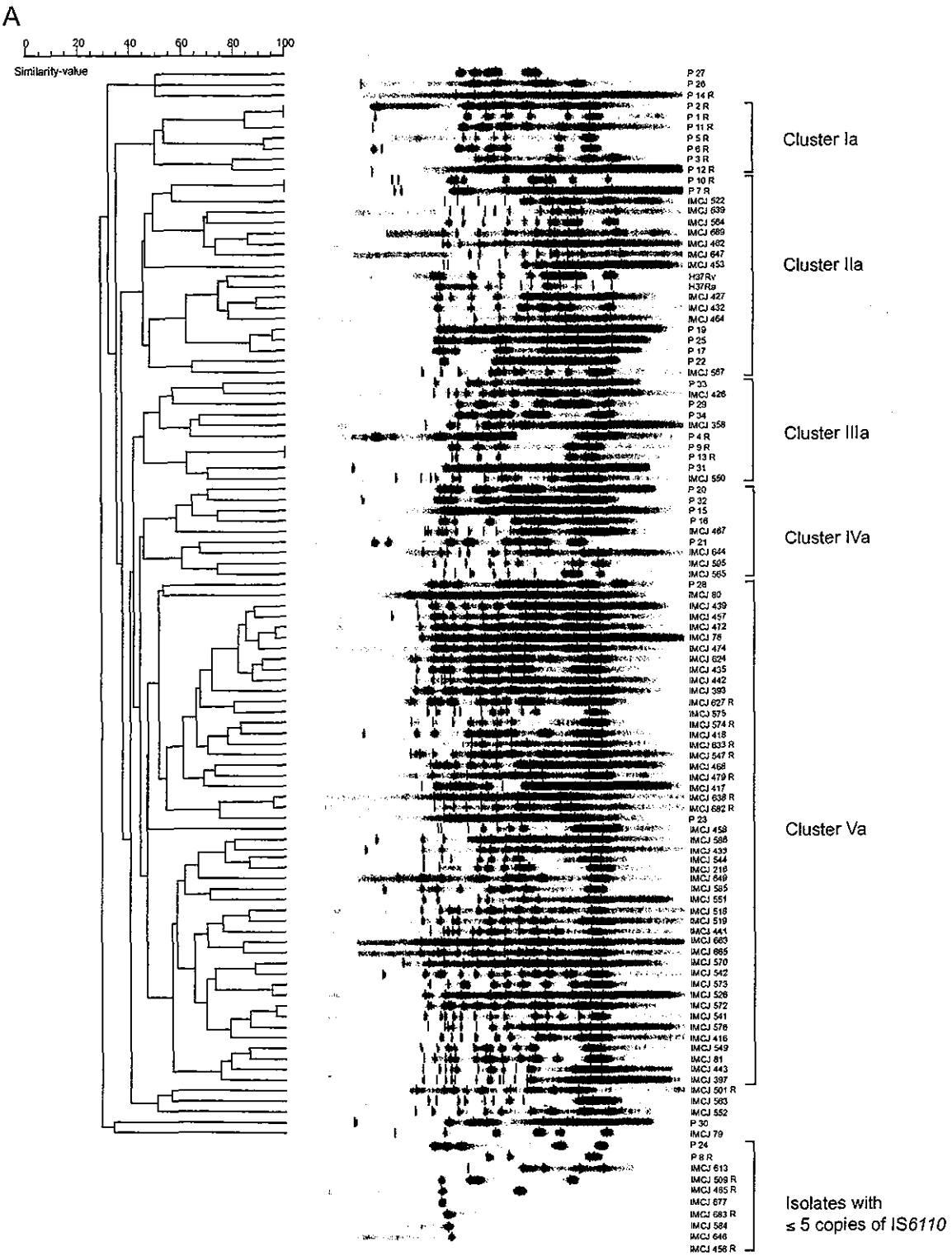

FIG. 3. IS*6110*- and $(CGG)_5$-probed DNA fingerprinting patterns of *M. tuberculosis* clinical isolates from Japan and Poland and the respective corresponding dendrograms. The fingerprint patterns are ordered by similarity. The corresponding dendrograms are to the left of the patterns. The position of each IS*6110* (A) or $(CGG)_5$ (B) band is normalized so that the patterns for all strains are comparable. The scale depicts the similarity of patterns calculated as described in Materials and Methods. In IS*6110*-probed DNA fingerprint patterns, five clusters showing a similarity of more than 44% were designated clusters Ia, IIa, IIIa, IVa, and Va. Isolates with five or less than five copies are indicated in panel A. In $(CGG)_5$-probed DNA fingerprint patterns, four clusters showing a similarity of more than 70% were designated clusters Ib, IIb, IIIb, and IVb. The isolates are named according to their origin as IMCJ (Japan) or P (Poland); the suffix R indicates drug resistance For example, IMCJ 627 R is a Japan-derived drug-resistant isolate.

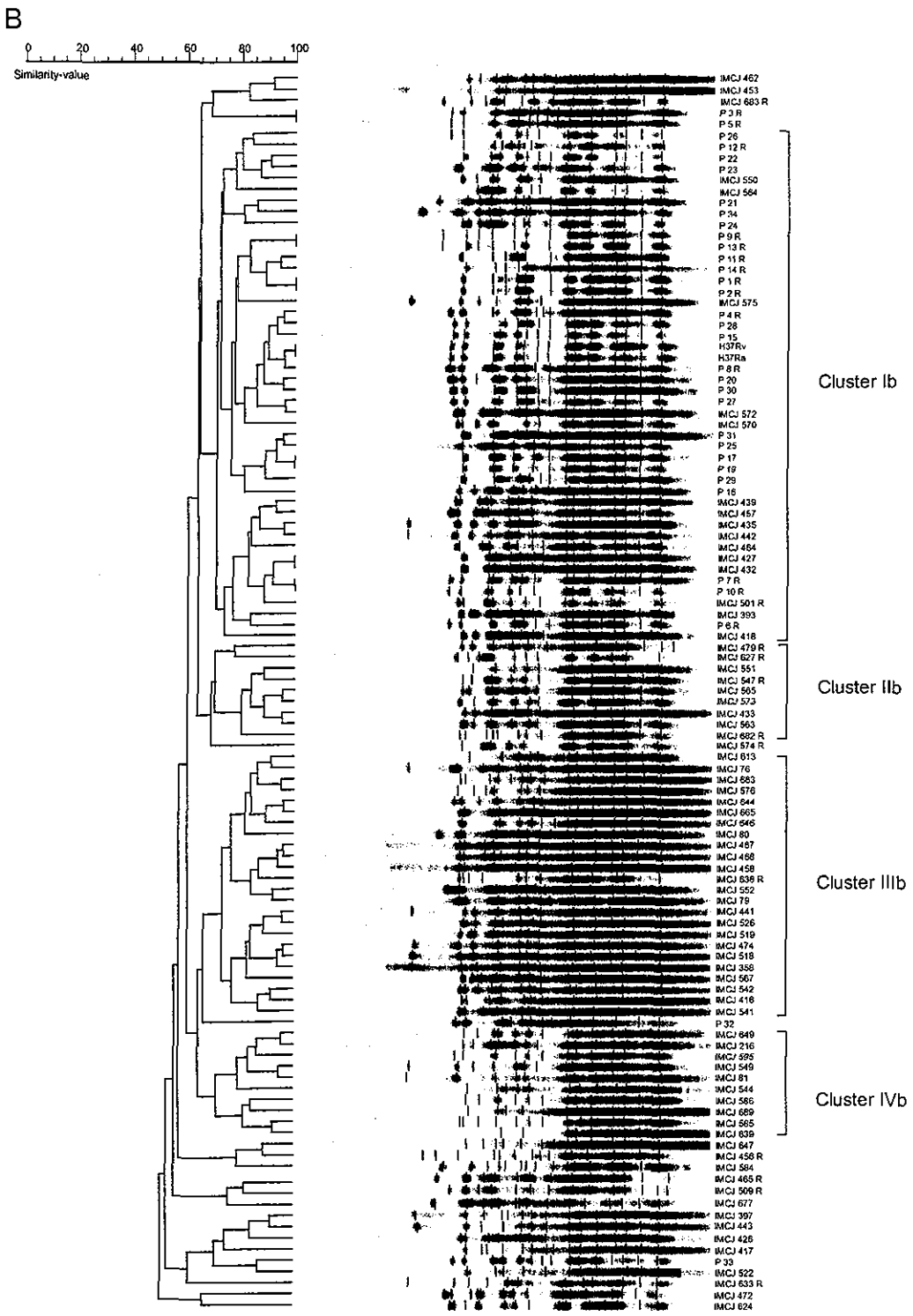

FIG. 3—*Continued.*

104 (CGG)$_5$ fingerprint patterns were found with ≥50% similarity (Fig. 3B). Four clusters with ≥70% similarity, including clusters Ib to IVb, were detected (Fig. 3B). Cluster Ib was composed of two H37 variants and 15 Japan- and 29 Poland-derived isolates. Clusters IIb, IIIb, and IVb were composed of 9, 24, and 10 Japan-derived isolates, respectively. Over half of the Japan-derived isolates (57%) and the majority of the Poland-derived isolates (88%) belonged to clusters IIb, to IVb and to cluster Ib, respectively (Fig. 3B).

Both the IS*6110* and (CGG)$_5$ fingerprint analyses showed an association between fingerprint pattern and geographic origin, indicating a correlation between them. Ten isolates that were

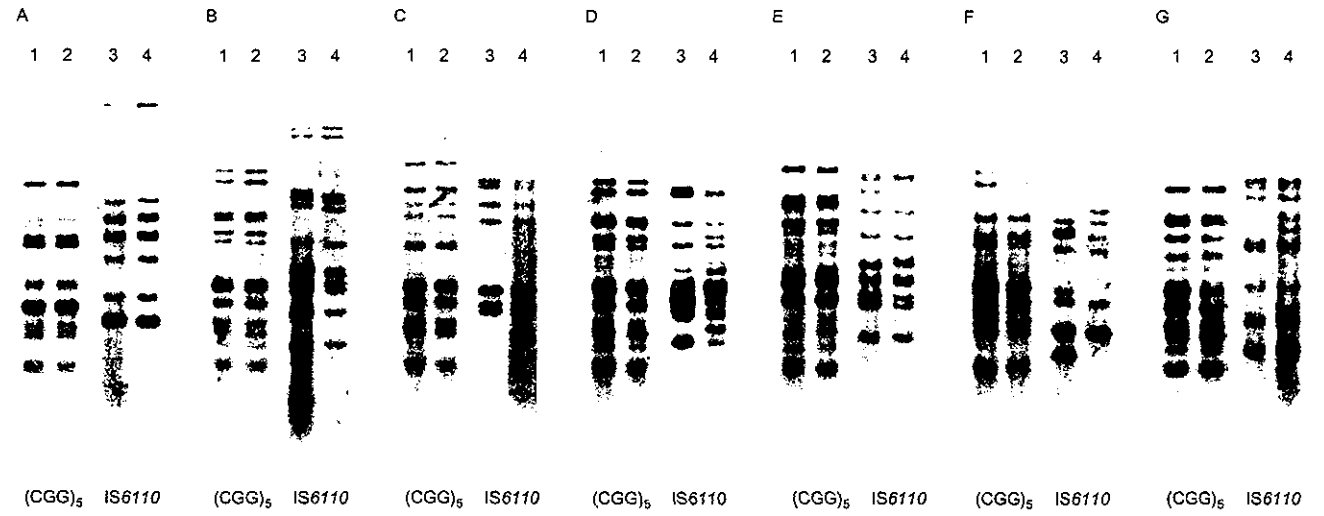

FIG. 4. $(CGG)_5$- and IS*6110*-probed DNA fingerprinting patterns of *M. tuberculosis* isolates that shared identical $(CGG)_5$ fingerprinting. (A) Lanes 1 and 3, P1; lanes 2 and 4, P2. (B) Lanes 1 and 3, P 7; lanes 2 and 4, P 10. (C) Lanes 1 and 3, P 9; lanes 2 and 4, P 13. (D) Lanes 1 and 3, H37Rv; lanes 2 and 4, H37Ra. (E) Lanes 1 and 3, IMCJ 427; lanes 2 and 4, IMCJ 432. (F) Lanes 1 and 3, P 3; lanes 2 and 4, P 5. (G) Lanes 1 and 3, P 17; lanes 2 and 4, P 19.

indistinguishable by IS*6110* RFLP because of the presence of few copies of the marker could be analyzed by $(CGG)_5$ marker. Three and seven pairs of isolates were identical to each other in the IS*6110* and $(CGG)_5$ fingerprint patterns, respectively (Fig. 4). The three pairs P 1 and P 2, P 7 and P 10, and P 9 and P 13 were identical to each other in the IS*6110* and $(CGG)_5$ fingerprint patterns (Fig. 4A to C, respectively). The four pairs H37Rv and H37Ra, IMCJ 427 and IMCJ 432, P 3 and P 5, and P 17 and P 19 were identical to each other in the $(CGG)_5$ fingerprint pattern but different in the IS*6110* fingerprint pattern (Fig. 4D, E, F, and G, respectively). The data suggest that the $(CGG)_5$ fingerprint patterns are more stable than the IS*6110* patterns.

Occurrence of $(CGG)_5$ among various mycobacterial strains. We investigated the presence of $(CGG)_5$ repeat sequences in mycobacterial species. $(CGG)_5$ hybridization patterns from various mycobacterial species are shown in Fig. 5. Bands ranging from 0 to 20 in number were seen. *Mycobacterium szulgai* possessed 20 bands. *M. bovis* BCG, *Mycobacterium marinum*, and *Mycobacterium kansasii* possessed 16 bands. *Mycobacterium nonchromogenicum*, *Mycobacterium terrae*, *Mycobacterium gastri*, *Mycobacterium simiae*, *Mycobacterium smegmatis*, and *Mycobacterium intracellulare* possessed 14, 12, 8, 5, 5, and 3 bands, respectively. *Mycobacterium peregrinum* possessed two bands. *Mycobacterium fortuitum* and *Mycobacterium chelonae* possessed one band. *Mycobacterium scrofulaceum*, *Mycobacterium avium*, *Mycobacterium xenopi*, and *Mycobacterium abscessus* showed no bands.

DISCUSSION

In this study, we found that various bacterial strains contain TRS in their genomes. In humans, TRS are associated with hereditary neurologic and neuromuscular disorders, including myotonic dystrophy, Huntington's disease, Fragile X syndrome, and Friedreich's ataxia (27). These diseases result from TRS expansion such as $(CTG)_n$, $(CGG)_n$, and $(GAA)_n$ (27). The TRS sizes associated with these diseases are usually quite large. For example, 80 to 3,000 repeats of CTG have been found in myotonic dystrophy, 230 to 2,000 repeats of CGG have been found in Fragile X syndrome, and 200 to 900 repeats of GAA have been found in Friedreich's ataxia (21). These expanded TRS can form hairpin structures or intramolecular triplex structures that result in genetic instability (21). The TRS sizes found in bacteria were relatively small. The largest size TRS identified was 21 repeats of GAA in *M. leprae*. The most frequently identified TRS was five repeats of CGG in *M. tuberculosis* and *M. bovis*. TRS found in bacteria are not likely to be linked to genetic instability because of the lower repeat number.

The $(CGG)_5$ TRS found in two strains of *M. tuberculosis* (H37Rv and CDC1551) and in one strain of *M. bovis* existed in genes encoding PE protein families, including a PE_PGRS subfamily and PPE protein families comprising 88 to 101 and 61 to 69 kinds of proteins, respectively, which occupy approximately 8% of the genome (4, 7, 8). The functional properties of $(CGG)_5$ in these genes are unknown, but $(CGG)_5$ should not play an important role in the development of the variations among different strains. $(CGG)_5$ in the PPE genes was located in the conserved N-terminal domain PPE but not in the C-terminal variable domain containing the major polymorphic tandem repeats with the consensus sequence of GCCGGT GTTG (10, 18). $(CGG)_5$ in the PE_PGRS genes was within the C-terminal variable domain containing the PGRS with the consensus sequence of CGGCGGCAA (18, 19). $(CGG)_5$ in the PE_PGRS genes did not comprise part of the consensus sequence of PGRS. $(CGG)_5$ was contained in 13 and 12 PE_PGRS genes in H37Rv and CDC1551, respectively. Among these genes, deletion or insertion was detected at one site of Rv1068c, two sites of Rv1087, and two sites of Rv1450c compared with their orthologs, MT1097, MT1118.1, and MT1497.1, respectively (data not shown). However, $(CGG)_5$ was not near these sites, indicating that it did not directly affect the deletion and insertion of PE_PGRS genes. $(CGG)_5$ in

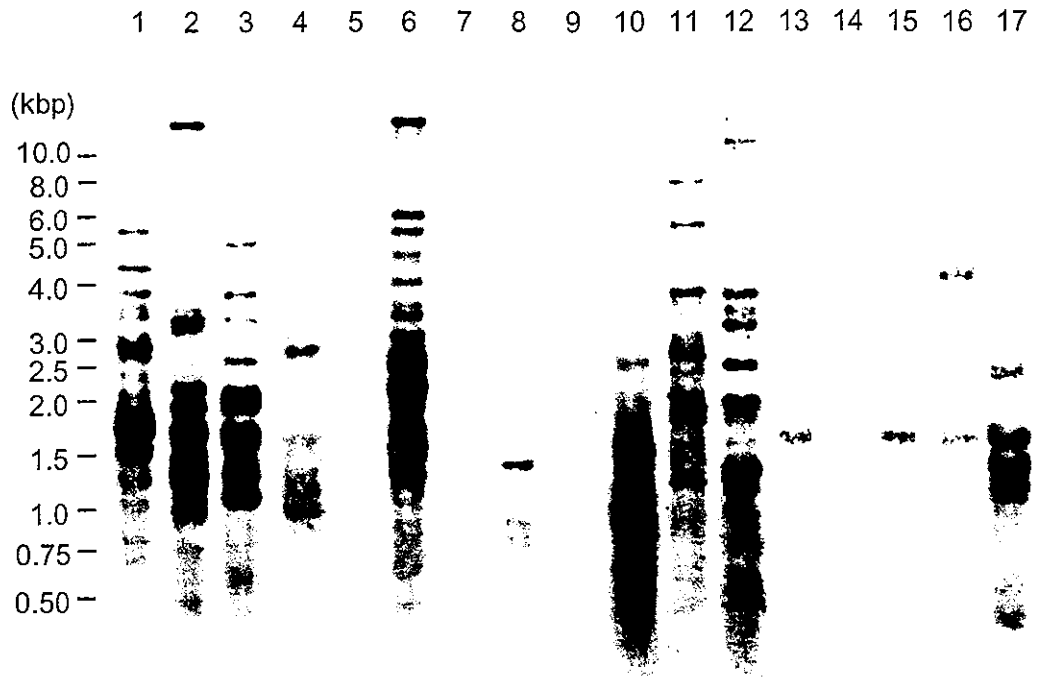

FIG. 5. (CGG)$_5$-probed fingerprinting of AluI-digested DNA from various mycobacterial species. Lane 1, *M. bovis* BCG; lane 2, *M. marinum*; lane 3, *M. kansasii*; lane 4, *M. simiae*; lane 5, *M. scrofulaceum*; lane 6, *M. szulgai*; lane 7, *M. avium*; lane 8, *M. intracellulare*; lane 9, *M. xenopi*; lane 10, *M. gastri*; lane 11, *M. terrae*; lane 12, *M. nonchromogenicum*; lane 13, *M. fortuitum*; lane 14, *M. abscessus*; lane 15, *M. chelonae*; lane 16, *M. peregrinum*; lane 17, *M. smegmatis*.

PPE, PE, and PE_PGRS genes translated to neutral-charged amino acids of poly(Ala) and poly(Gly), respectively, with no special substitution, indicating that these regions do not participate in the formation of unique structures within these proteins. Thus, the (CGG)$_5$ sequences in these genes will likely not have characteristic properties regarding function.

It is unclear whether TRS in bacteria, particularly (CGG)$_5$ in *M. tuberculosis* and *M. bovis*, participate in their pathogenesis. There was no difference between virulent strain H37Rv and the derived avirulent strain H37Ra in (CGG)$_5$-probed fingerprinting (Fig. 2). No correlation was found between the virulency of mycobacterial species and the numbers of bands in (CGG)$_5$-probed fingerprinting or copies of (CGG)$_5$ (Table 2 and Fig. 5). For example, *M. leprae* had no (CGG)$_5$ repeats (Table 2). Some rare etiologic agents of nontuberculous mycobacteria, such as *M. smegmatis* and *M. szulgai* (20), did possess several copies of (CGG)$_5$ in their genomes (Fig. 5), whereas some common etiologic agents, such as *M. avium*, *M. xenopi*, and *M. abscessus* (20), possessed no (CGG)$_5$ repeats (Fig. 5). These results indicate that (CGG)$_5$ repeats do not participate directly in the virulency of mycobacterial species.

Whereas fingerprinting analysis showed that both (CGG)$_5$ and IS*6110* were sufficiently stable epidemiologic markers, (CGG)$_5$ appeared to be more stable than IS*6110* (Fig. 1). We were unable to find any differences between strains H37Rv and H37Ra in (CGG)$_5$-probed fingerprinting by extensive studies with various restriction enzymes. However, four different bands were detected between these strains with PvuII-IS*6110* fingerprinting (Fig. 2B). Lari et al. (11) compared H37Rv and H37Ra strains maintained at their institution by IS*6110* fingerprinting with EcoNI, PstI, and PvuII and found different patterns between these strains. Bifani et al. (3) compared the PvuII-IS*6110* fingerprints of 15 and 3 different catalogued variants of H37Rv and H37Ra, respectively. Ten distinct fingerprint patterns, making up nine H37Rv variants and one H37Ra variant, were identified. A discrepancy between IS*6110*- and (CGG)$_5$-probed fingerprints of laboratory strains was observed in three pairs of clinical isolates (Fig. 4). In these cases, each isolate was identical in (CGG)$_5$ fingerprinting pattern but differed in its IS*6110* fingerprinting pattern. Our recent epidemiological case report of intrafamilial tuberculosis transmission showed that two clinical isolates from a father and son were identical in (CGG)$_5$-probed fingerprinting patterns, whereas one different band was detected between them by IS*6110*-probed fingerprinting (25). Collectively, IS*6110*-probed fingerprint patterns changed more rapidly than did (CGG)$_5$-probed patterns, suggesting that there are different mechanisms by which these patterns change. In other terms, although (CGG)$_5$-probed fingerprinting will hardly detect a few mutations in a clone of *M. tuberculosis*, it will easily detect an origin among the clones. The (CGG)$_5$-probed fingerprinting combined with IS*6110*-probed fingerprinting will provide more powerful information about tuberculosis epidemiology.

We collected and analyzed the isolates in this study in Japan and Poland. If isolates could be collected worldwide, it would provide more exact epidemiological data. In conclusion, the (CGG)$_5$ repeat is a useful probe for DNA fingerprinting of *M. tuberculosis*, because all strains tested here possessed more than eight copies. In addition, (CGG)$_5$-probed fingerprinting will be a useful tool for the investigation of *M. bovis*, *M. marinum*, *M. kansasii*, and *M. szulgai*.

ACKNOWLEDGMENTS

We thank M. Nakano (Jichi Medical School, Japan) for comments on the manuscript and A. S. Swierzko (Centre for Microbiology and Virology, Polish Academy of Sciences, Poland) for coordinating the international collaborative study.

The study was supported by the Health Sciences Research grants from the Ministry of Health, Labour and Welfare and by the Research on Health Sciences focusing on Drug Innovation (KH11008) from the Japan Health Sciences Foundation.

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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 patterns 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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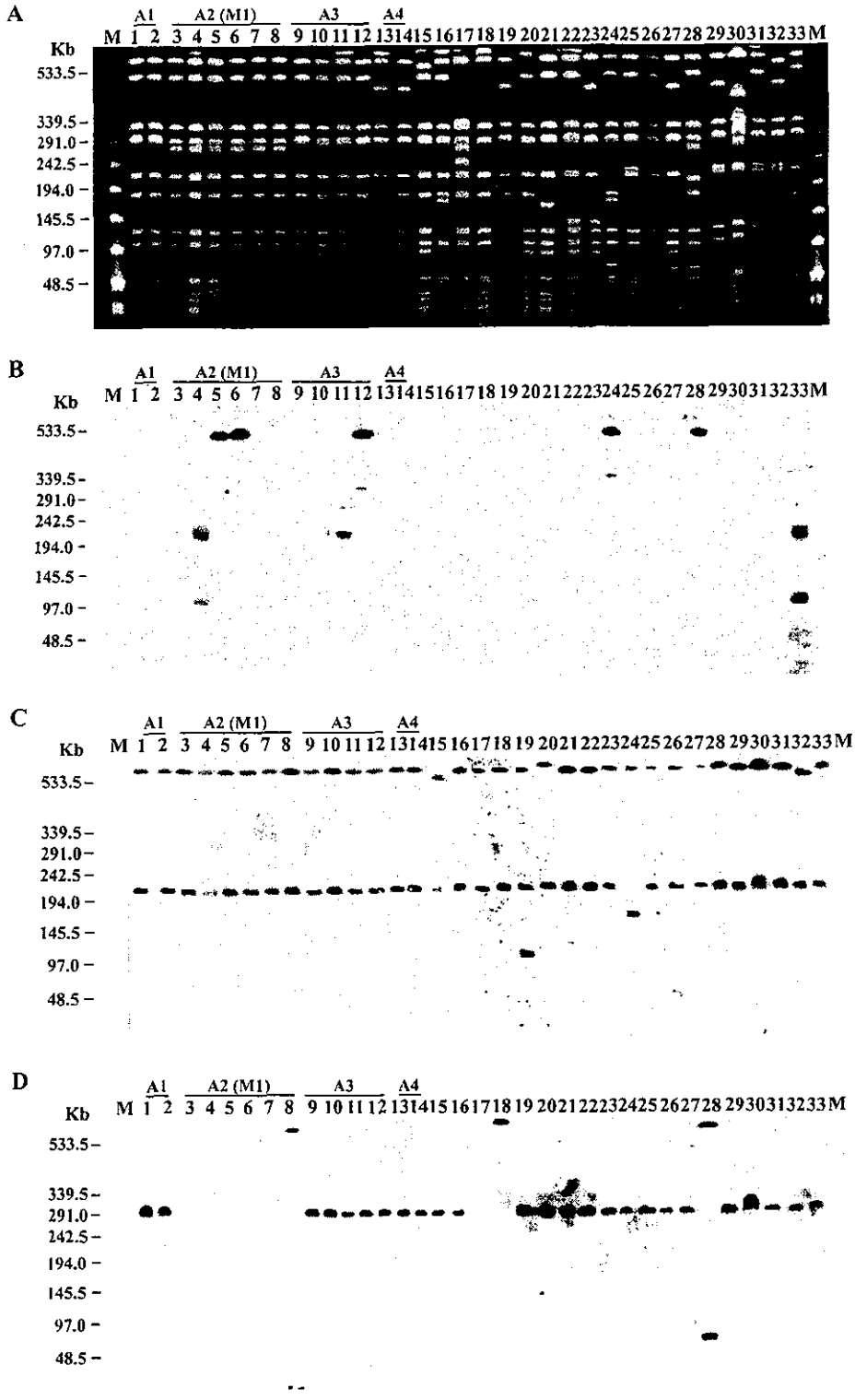

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates (A) and Southern blotting hybridized with *aac6'-aph2"* (B), *ermA* (C), and *tetM* (D). M: low range PFG Marker. Lanes 1 to 33: Lane Nos. is corresponding to the typing Nos. of MRSA isolates listed in Table 1.

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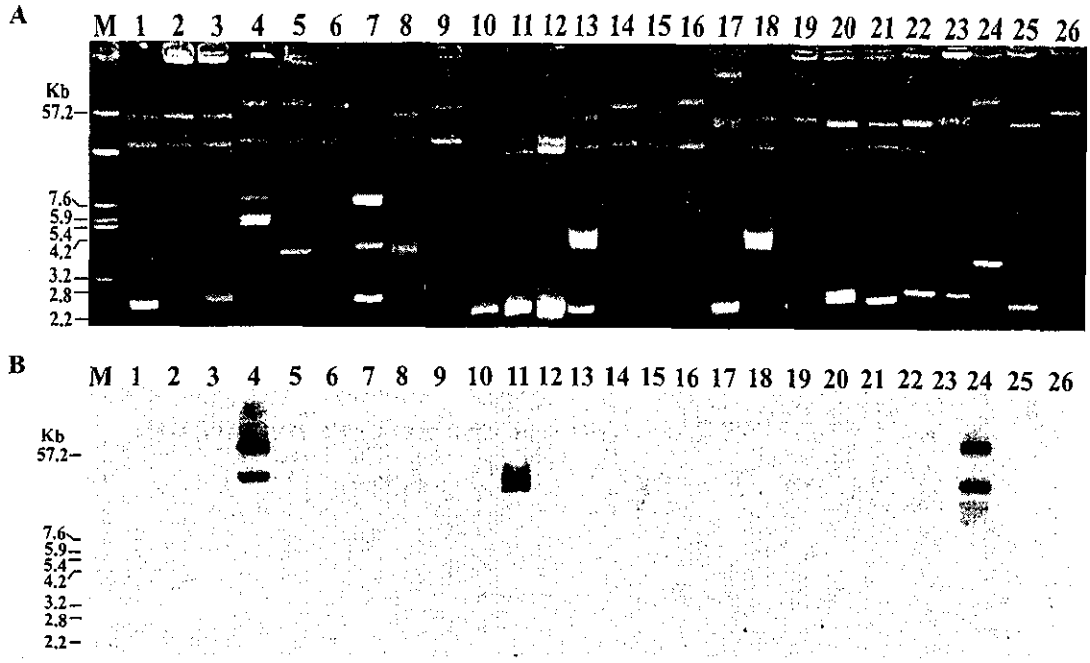

Fig. 2. Agarose gel electrophoresis of plasmid DNA from MRSA isolates (A) and Southern blotting hybridized with *aac6'-aph2"* (B). M: Marker plasmid derived from *E. coli* V517. Lanes 1 to 26: Lane Nos. is corresponding to the plasmid typing Nos. of MRSA isolates listed in Table 2.

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.

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

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

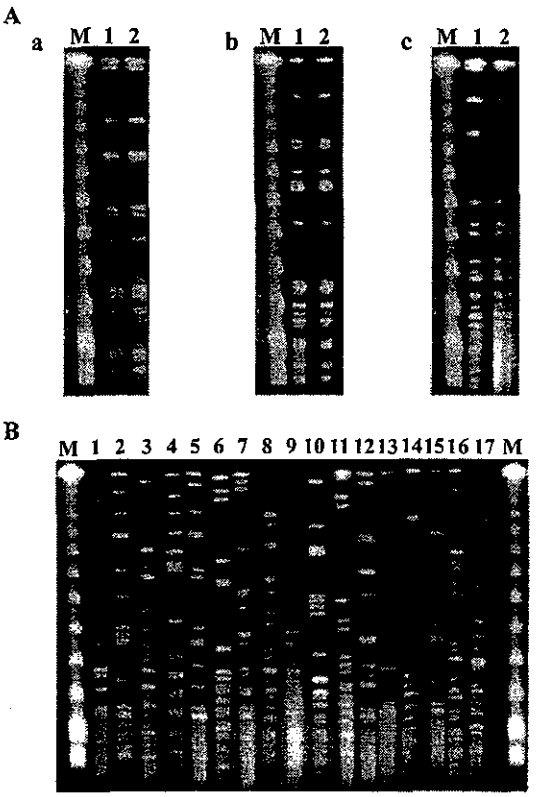

Fig.1. Pulsed-field gel electrophoresis of *Spe*I-digested genomic DNA from *S. marcescens* isolates.
A: a) PFGE pattern K (isolates No. S6 and S7), b) PFGE pattern J (isolates No. S15 and S18), c) PFGE pattern A (isolates No. S21 and S38), M: low range PFG Marker.
B: lane 1: isolate No. S14, lane 2: 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.

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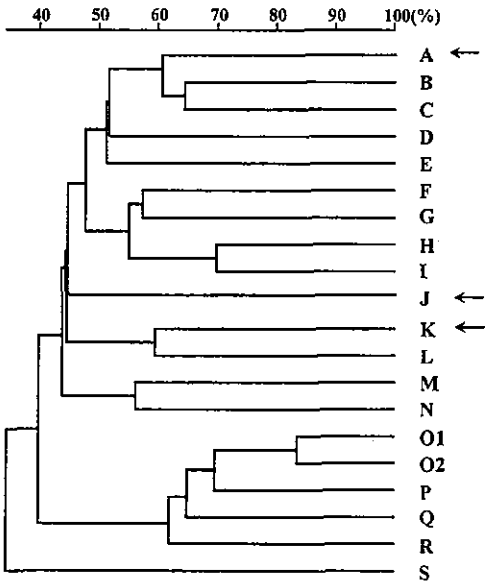

Fig. 2. Band-based cluster analysis of PFGE patterns of *S. marcescens* isolates.

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.

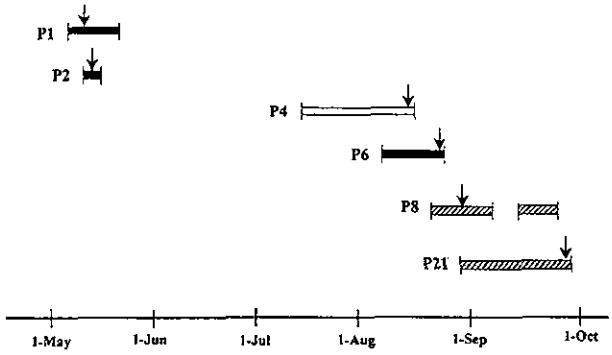

Fig. 3. Duration of catheterization. Intravenous, central venous, and urinary tract catheterization are represented by filled, open, and hatched boxes, respectively. Vertical arrows indicate the date of isolation of *S. marcescens*.

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

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

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Communicated by Hiroshi Yoshikura

(Aceepted 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.

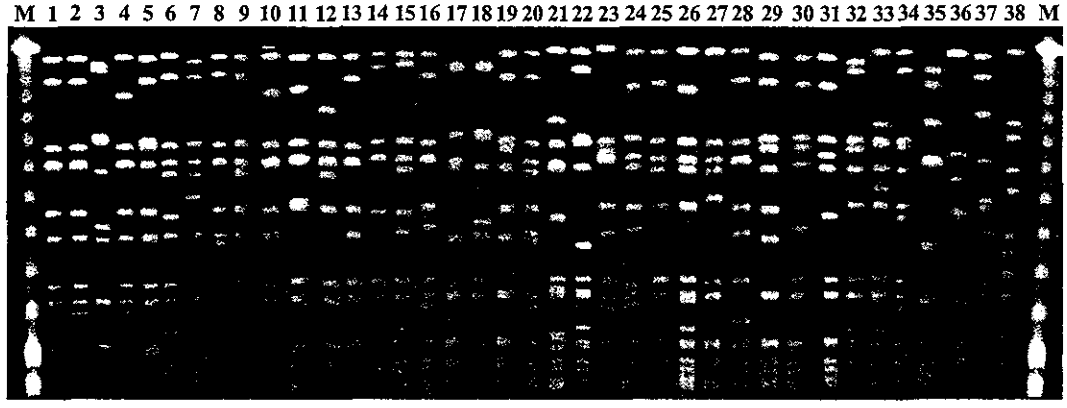

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. M: low range PFG Marker. Lanes 1 to 38: MRSA isolates with different PFGE patterns A1 to BC shown in Fig. 2.

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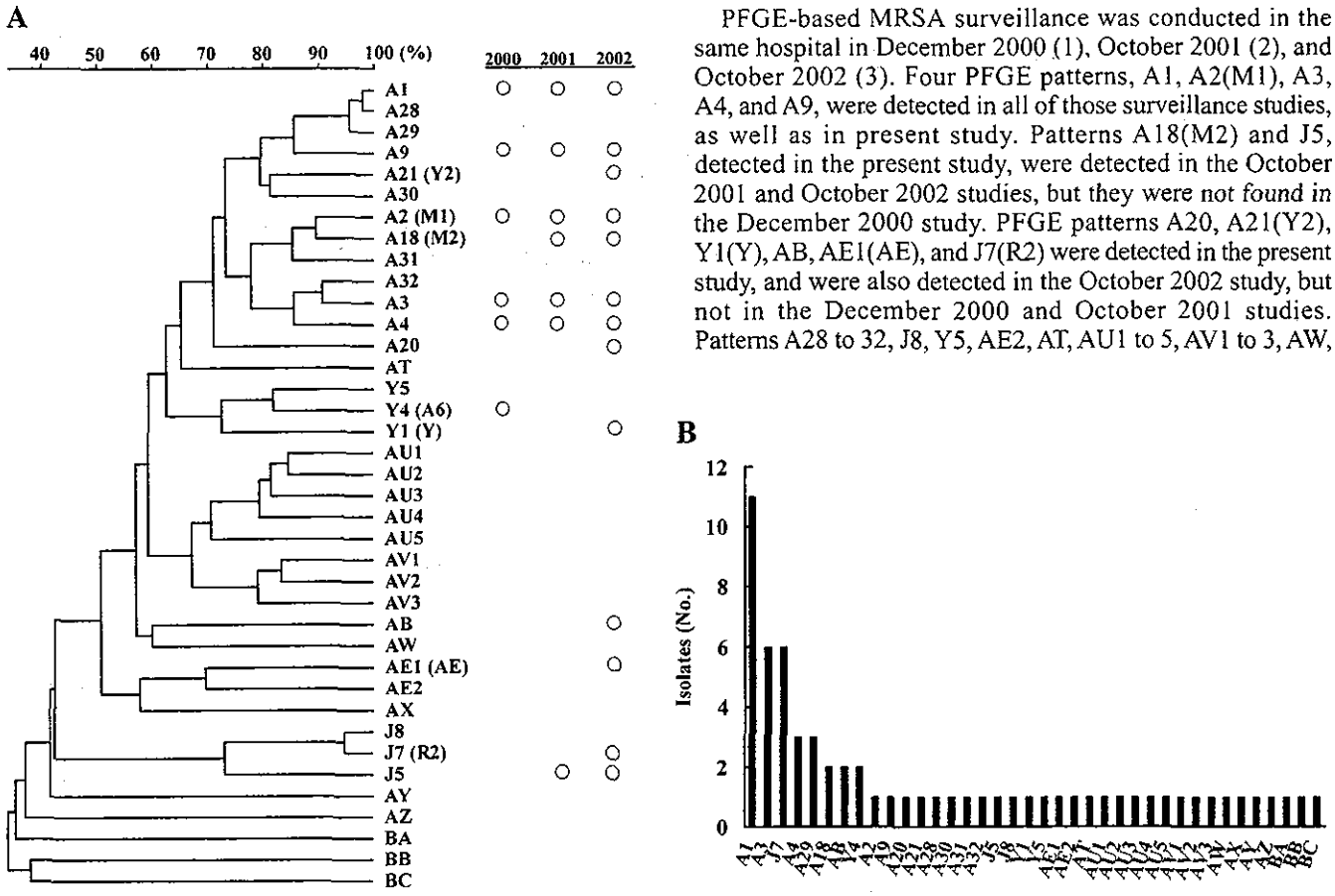

Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns. MRSA isolates indicated by circles were also detected in previous surveillance (1-3).

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

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

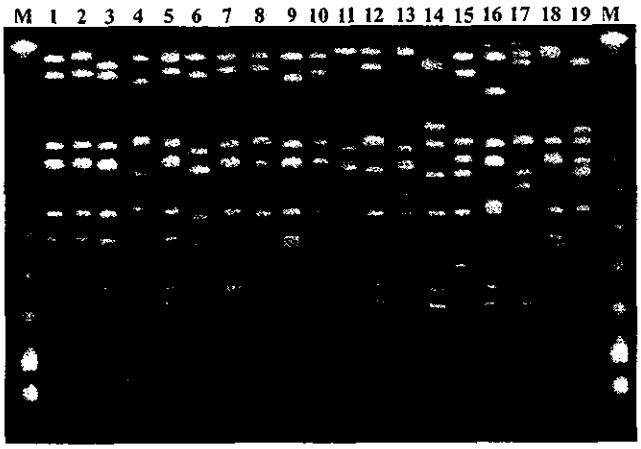

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. M: low range PFG Marker. Lanes 1-19 correspond to the following PFGE patterns; 1: A1, 2: A33, 3: A35, 4: A34, 5: A37(AH2), 6: Y6, 7: Y7, 8: A36, 9: AI1(G3), 10: A39, 11: AY2, 12: BF, 13: AB2, 14: AU6, 15: BD2,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

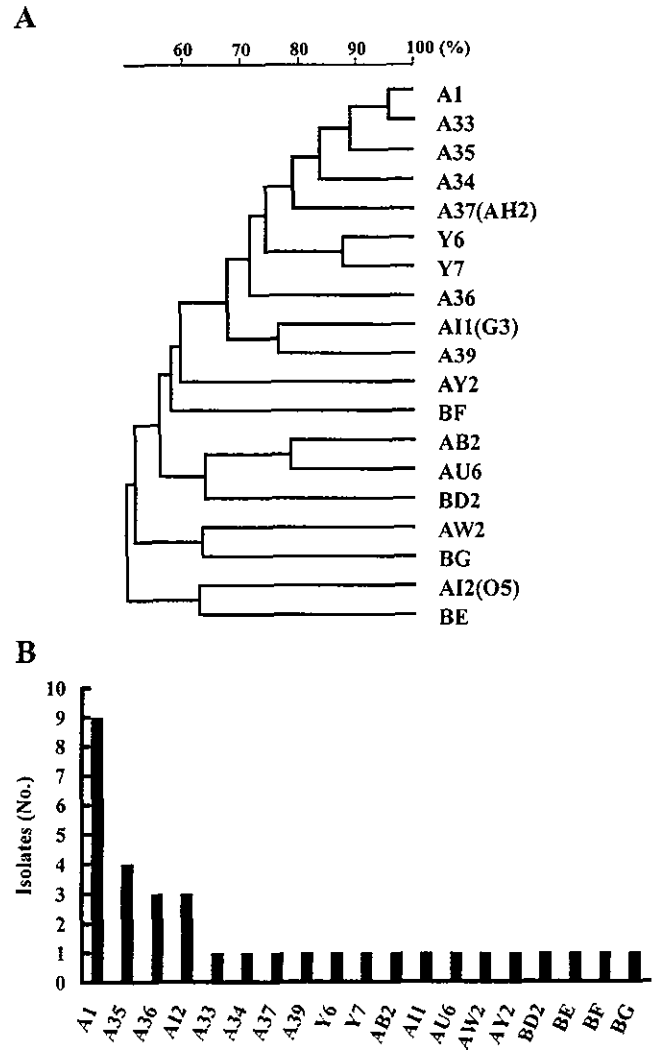

Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns of *Sma*I-digested genomic DNA.

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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.

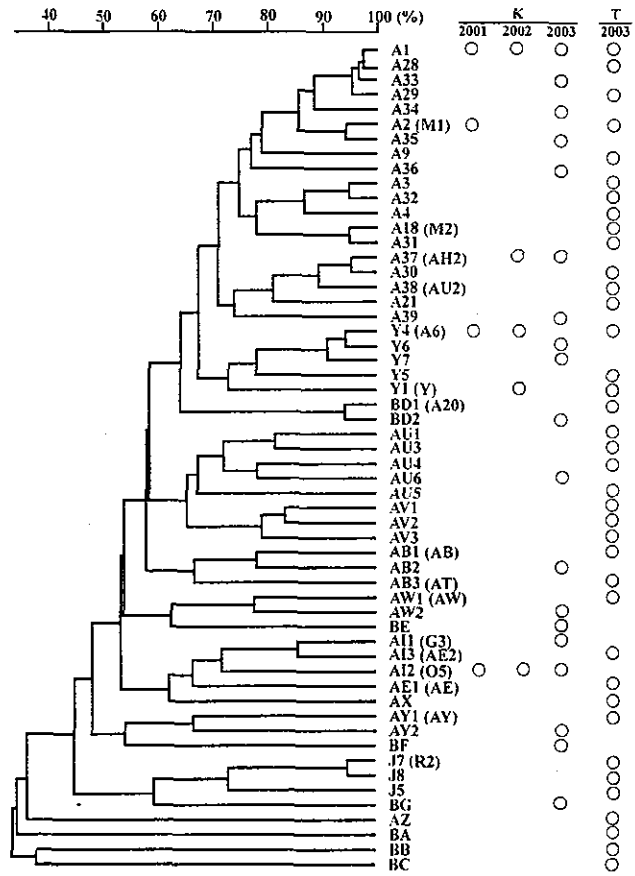

Fig. 3. Cluster analysis of MRSA isolates based on PFGE patterns.
K: Kumamoto hospital, T: Tokyo hospital.

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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