Molecular Epidemiologic Analysis of *Staphylococcus aureus* isolated from Clinical Specimens

Nosocomial infections caused by *Staphylococcus aureus* are clinically serious and control of such infections requires strain typing to identify the source of contamination. Recently, pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA (RAPD) assay have been introduced and have provided a high level of strain discrimination of *S. aureus* isolated from clinical specimens. This study was performed to classify 82 strains of *S. aureus* isolated from 4 hospitals in the Kwangju-Chonnam area by PFGE and RAPD assay. Methicillin-resistant *S. aureus* (MRSA) was identified by disk diffusion method using the oxacillin disk and polymerase chain reaction of *mecA* gene was done in 69 strains. Eighty-three strains including *S. aureus* ATCC 25923 were classified into 10 groups by RAPD assay, and into 8 groups by PFGE. Classified groups were not related to area or hospital. Classification was not characteristic between MRSA and methicillin-susceptible strains. Nosocomial infections due to outbreak were suggested because some strains disclosed identical band patterns by PFGE. These results indicate that medical personnels and instruments are routes of nosocomial infections caused by MRSA. PFGE and RAPD assay are powerful tools for the epidemiological study of *S. aureus*, but PFGE is more effective than RAPD assay. RAPD assay needs optimal combination of primers. *(JKMS 1997;12:190~8)*

Key Words : *Staphylococcus aureus, MRSA, PFGE, RAPD assay*

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INTRODUCTION

Nosocomial infections caused by *Staphylococcus aureus* continue to be a serious problem in many countries. Infection of methicillin-resistant *Staphylococcus aureus* (MRSA) has increased since MRSA was first reported in 1961 (1).

Epidemiologic typing such as bacteriophage typing, ribotyping, antibiogram, capsular typing, and DNA fragment length polymorphism have been performed to identify the source of nosocomial infection by *S. aureus* (2~7). The Center for Disease Control and Prevention (CDC) has used bacteriophage typing for 30 years to discriminate the source of contamination (8). Recently, pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA (RAPD) assay have been introduced, because bacteriophage typing has several weaknesses such as poor reproducibility, relatively low typing ability, and requirement of a large number of phage stocks (8~13). PFGE has been used to separate macrorestriction fragments of *S. aureus* generated by low-frequency-cleaving endonucleases that cut the chromosome at fewer than 30 sites. RAPD assay uses short

(<10-base) oligonucleotide primers less than 10 bases that anneal at a low stringency with multiple target sequences and that produce diverse DNA products that distinguish the isolates. These molecular analyses are more useful for epidemiologic study and clinical application than bacteriophage typing, ribotyping, etc. Therefore these methods are used for epidemiology and classification of several microorganisms as well as *S. aureus*.

Because the epidemiologic studies of *S. aureus*, which is a major source of nosocomial infection, are rare in Korea, the authors made a plan to classify the subgroups of *S. aureus* by genetic relatedness utilizing the PFGE and RAPD analyses and to approach the contamination routes of nosocomial infections caused by *S. aureus* and to evaluate the usefulness of PFGE and RAPD analyses as DNA-based *S. aureus* strain-typing techniques.

MATERIALS AND METHODS

Bacterial strains

A total of 83 strains of *S. aureus* were selected for

inclusion in this study. Seventy-six strains were isolated from patients of 4 general hospitals between February and March of 1995. Fifty of 76 strains (isolates No. 1~50) were isolated from clinical specimens of Chonnam University Hospital (CUH) and 11 strains (isolates No. V1~V11) were from Kwangju Veterans Hospital (KVH). Seven strains (isolates No. N1~N7) were from Namkwang Hospital (NH) and 8 strains (isolates No. C1~C8) were from St. Colomban's Hospital (SCH). On the other hand, 6 strains were isolated from the medical appliances and the hands of medical personnels, and one strain was *S. aureus* ATCC 25923. Isolates No. 39 and No. 40 from CUH were isolated from the blood and urine of one patient, and isolates No. 44 and No. 45 from CUH were isolated from the pus and urine of another patient.

Identification of methicillin-resistance

MRSA was identified by disk diffusion method using the oxacillin disk and *mecA* gene PCR method (14~17). The *mecA* gene PCR was performed in 6 strains isolated from the medical personnels and instruments, and 63 strains isolated from clinical specimens among 83 strains of *S. aureus*.

For the *mecA* gene PCR, bacterial colonies were suspended at a concentration of McFarland No. 4 in 150 μl of sterilized distilled water with 3 μl of lysostaphin (1mg/ml). The bacterial suspension was incubated at 100 ℃ for 15 min after 1 hour of incubation at 37℃. The lysate was centrifuged at 10,000 rpm for 10 min. The DNA concentration of the supernatant was measured by spectrophotometry at 260 nm and adjusted to 50~70 ng/μl. One microliter of template DNA solution was added to 24 μl of reaction solution consisting of 10 mM

Tris HCl (pH 9.0), 50 mM KCl, 2.5 mM MgCl$_2$, 0.01% gelatin, 0.1% Triton X-100, 0.2 mM dNTPs, and 0.5 μl of DynaZyme DNA polymerase (Finzymes Oy, Finland). Korea Biotech synthesized the primers for PCR according to the sequence of PBP-2' gene reported by Song and his colleagues (18). The concentrations of these primers, which consisted of sense primers (5'-AAAAT CGATGGTAAAGGTTGGC-3') and antisense primers, (5'-AGTTCTGCAGTACCGGATTTGC-3') were adjusted to 250 nM. DNA amplification was carried out for 40 cycles in 25 μl of reaction solution as follows : the first denaturation at 95℃ for 5 min, denaturation at 9 5℃ for 30 s, annealing at 50℃ for 30 s, and extension at 72℃ for 1 min with a final extension at 72℃ for 10 min. Ten-microliter samples of the PCR products were analyzed by 1.2% agarose gel electrophoresis (Fig. 1).

RAPD analysis

DNA extraction of the 83 strains was performed by the method described previously at the *mecA* gene PCR and DNA concentration was adjusted to 50~70 ng/μl. Reaction mixture for amplification was the same solution as those of *mecA* gene PCR. Each 10-base oligonucleotide primer (OPA 03, 5'-AGTCAGCCAC-3' ; OPA 04, 5'-AATCGGGCTG-3' ; OPA 05, 5'-AGGGGTCTTG-3' ; OPA 06, 5'-GGTCCCTGAC-3') of Operon Kit A (Operon Technologies, Inc., Atlanta, USA) was added to 24 μl of reaction mixture with 1 μl of template DNA solution. Low-stringency amplification was performed by using the PCR conditions of 40 cycles of consecutive denaturation (1 min, 95℃ ; 5 min, 95℃ at first), annealing (1 min, 36℃), and DNA chain extension (1 min, 72 ℃) with a final extension at 72℃ for 10 min. Ten
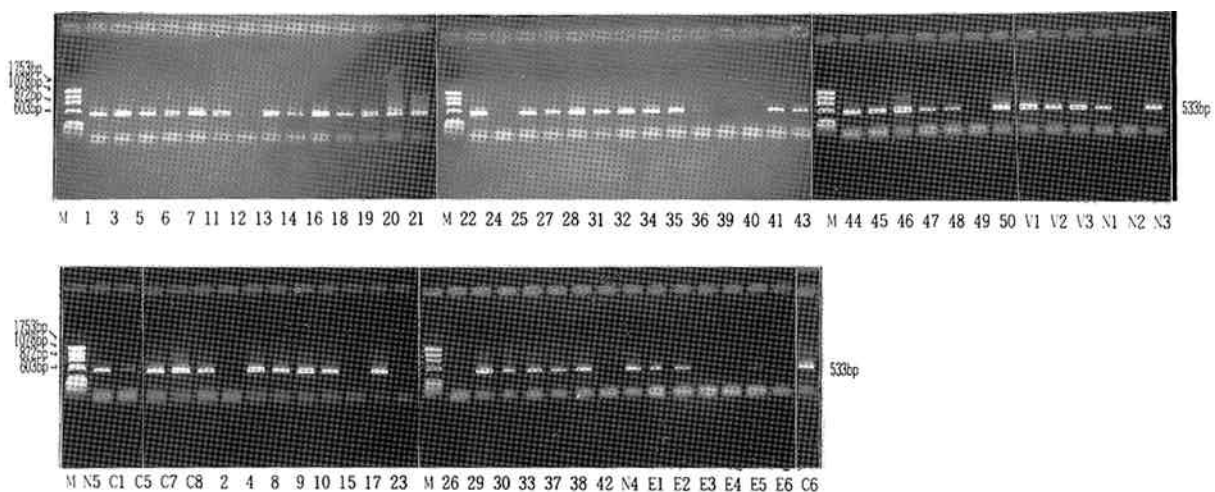

Fig. 1. Agarose gel electrophoresis of amplified 533-bp DNA fragment. The PCR for *mecA* gene was performed with lysates of 69 methicillin-resistant or -susceptible *S. aureus* strains. Molecular size markers were ϕX 174 RF DNA/*Hae* III fragments (lanes M).

microliters of the PCR product was analyzed by 1.2% agarose gel electrophoresis for 1 hour at 90 V and was visualized after staining with ethidium bromide. Molecular size marker (ΦX 174 RF DNA/Hae III ; Gibco BRL, USA) was used for reference purposes.

PFGE analysis

Eighty-three isolates of S. aureus were incubated overnight on blood agar plates, and bacterial colonies were suspended at a concentration of McFarland No. 2 in 100 μl of autoclaved TEN buffer (0.1 M Tris HCl, 0.15 M NaCl, 0.1 M EDTA). After centrifugation at 7,000 rpm for 5 min, the washed cells were resuspended in 100 μl of autoclaved EC buffer (6 mM Tris HCl, 1 M NaCl, 0.1 M EDTA, 0.5% Brij 58, 0.2% deoxycholate, 0.5% Sarkosyl), and the cell suspensions were briefly vortexed with 100 μl of 1.8% InCert agar (FMC BioProducts, USA) dissolved in EC buffer. The mixtures were quickly pipetted into a plug mold and the plugs were allowed to solidify at -20℃ for 10 min. The sliced plug was placed in a microtube (1.5 ml) containing 200 μl of EC buffer and 2 μl of 1 mg/ml lysostaphin solution (Sigma, USA) dissolved in 20 mM sodium acetate, and the cells in the plug were lysed overnight at 37℃ without shaking. After the lysing step, the EC buffer was removed and replaced with 500 μl of autoclaved TE buffer (10 mM Tris HCl, 5 mM EDTA) and the tube was incubated overnight at 55℃ without shaking. The plug was then transferred to 1 ml of fresh TE buffer for storage at 4℃ until electrophoresis.

The plug was cut into small slices (2 by 5 mm) and placed in a 125-μl restriction enzyme mixture containing 20 U of SmaI (Boehringer Mannheim, Germany). After a 4-hour incubation at 25℃ with shaking at 140 rpm, the trimmed slices of the plug were loaded into wells of a 1% SeaKem agarose running gel. After the wells containing the plug slices were sealed with 1% SeaKem agarose, chromosomal restriction fragments were separated by PFGE at an angle of 120 degrees by using a CHEF-DR II electrophoresis cell. The running parameters were as follows : initial pulse, 3 s; final pulse, 30 s ; voltage, 200 V ; time, 23 hours; and temperature, 12 to 14℃. The molecular size marker included the Saccharomyces cervisiae chromosome. The gels were stained with 1 μg/ml ethidium bromide for 10 min and photographed.

Cluster analysis of RAPD assay and PFGE results

Banding patterns of RAPD assay and PFGE were interpreted and transformed as follows : positive data, 1 and negative data, 0. After data matrix was established

for NTSYS-pc (numerical taxonomy system and multivariate analysis system) program by using transformed data, cluster analysis was performed by unweighted pair-group arithmetic average analysis (UPGMA). Cluster analysis was used to produce dendrograms showing estimates of the distance values and to anlyze the genetic relatedness among 83 strains of S. aureus. Twenty-five bands of the summed results for the four primers were used for cluster analysis in RAPD assay and 17 bands were used in PFGE.

RESULTS

Susceptibility pattern of antimicrobial agents

The mecA gene PCR was applied to 6 strains isolated from the medical personnels and instruments, and 63 strains isolated from clinical specimens (Fig. 1). Fifty-five strains were mecA-positive and 14 strains were mecA-negative (Table 1, 2). Fifty-five mecA-positive strains were methicillin-resistant, but 2 strains of 14 mecA-negative strains were classified as methicillin-resistant by disk diffusion method using oxacillin disk (Table 2).

RAPD analysis

RAPD assay of genomic DNAs of 83 strains was performed with the four primers. The number of distinct bands was 6 at primer OPA 03 (Fig. 2), 4 at primer OPA 04, 9 at primer OPA 05, and 6 at primer OPA 06. A total of 25 distinct bands were used for cluster analysis and 83 strains were classified into 10 groups on the basis of distance value 0.3 (Fig. 3). But the dendrogram using one primer revealed low discrimination power among strains. The analysis using primer OPA 03 displayed 4 groups and the analysis using OPA 04 displayed 8 groups. The dendrogram constructed by combination of primers OPA 03 and OPA 04 showed 6 groups, and the combination of primers OPA 05 and OPA 06 showed 10 groups.

When the cluster analysis was performed using the summed results for the four primers, group II which included 50 strains of S. aureus was bigger than other groups (Fig. 3). Although 6 strains of SCH (C2, C3, C4; C1, C6, C8) showed molecular similarity and 5 strains isolated from the medical environment (E1, E2, E3, E4, E5) belonged to group I, the classification was not related with hospitals or regional groups generally. Eight strains of 11 S. aureus strains isolated from KVH belonged to group II. Strains C2, C3 and C4 of SCH were clustered, and strains C1, C6 and C8 were also clustered in the dendrogram (Fig. 3). Strain ATCC 25923 was not similar

Table 1. Susceptibility pattern of antimicrobial agents in 14 *mecA* gene negative *S. aureus* isolated from clinical specimens and the medical personnels and instruments

No. of isolates	*mecA* gene by PCR	Methicillin susceptibility by oxacillin disk	E	P	CL	G	A	T	CM	CF	V	CT	Cl
2	Negative	Susceptible	S	R	S	S	S	S	S	S	S	S	S
12	Negative	Susceptible	S	R	S	S	S	S	S	S	S	S	S
15	Negative	Resistant	S	R	S	S	S	S	S	S**	S	S**	S
23	Negative	Susceptible	S	R	S	S	S	S	S	S	S	S	S
24	Negative	Susceptible	S	R	S	R	S	S	S	S	S	S	S
26	Negative	Susceptible	S	R	S	S	S	R	S	S	S	S	S
39	Negative	Resistant	S	R	S	S	S	S	S	S**	S	S**	S
40	Negative	Susceptible	S	R	S	S	S	R	S	S	S	S	S
42	Negative	Susceptible	R	R	S	S	S	S	S	S	S	S	S
49	Negative	Susceptible	S	R	S	S	S	I	S	S	S	S	S
E3	Negative	Susceptible	S	R	S	S	S	S	S	S	S	S	S
E4	Negative	Susceptible	S	R	S	S	S	S	S	S	S	S	S
E6	Negative	Susceptible	S	R	S	S	S	S	S	S	S	S	S
N2	Negative	Susceptible	S	R	S	S	S	S	S	S	S	S	S

* E, erythromycin ; P, penicillin ; CL, clindamycin ; G, gentamicin ; A, ampicillin ; T, tetracycline ; CM, chloramphenicol ; CF, cephalothir ; V, vancomycin ; CT, cefotaxime ; Cl, ciprofloxacin ; R, resistant ; S, susceptible.
** Non-applicable for clinical therapy.

Table 2. Correlation of *mecA* gene PCR results and methicillin resistance by oxacillin disk in 69 *S. aureus* isolates

	Methicillin-resistant	Methicillin-susceptible	Total
mecA gene positive	55	0	55
mecA gene negative	2	12	14
Total	57	12	69

to other strains by RAPD assay. On the other hand, the distribution of 14 *mecA*-negative strains was not specific.

The band patterns of strains 39 and 40 isolated from different specimens of one person were not identical,

though the two strains belonged to the same group (group III). But strains 44 and 45 isolated from different specimens of another person showed an identical band pattern (Fig. 3).
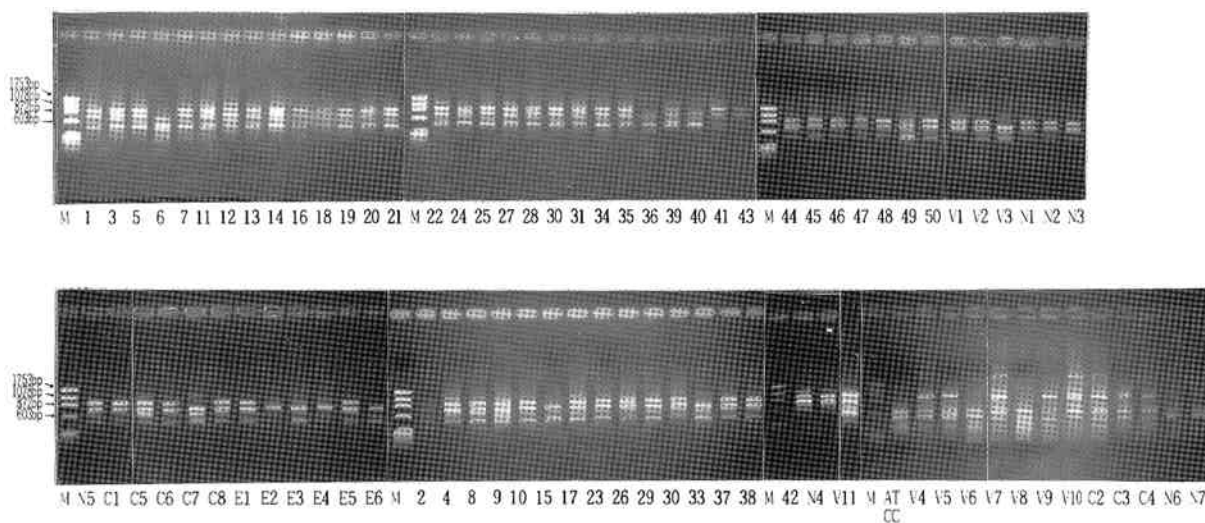

Fig. 2. Results of RAPD assay with Operon primer A3 on 83 *S. aureus* strains. Molecular size markers were φX 174 RF DNA/*Hae* III fragments (lanes M).
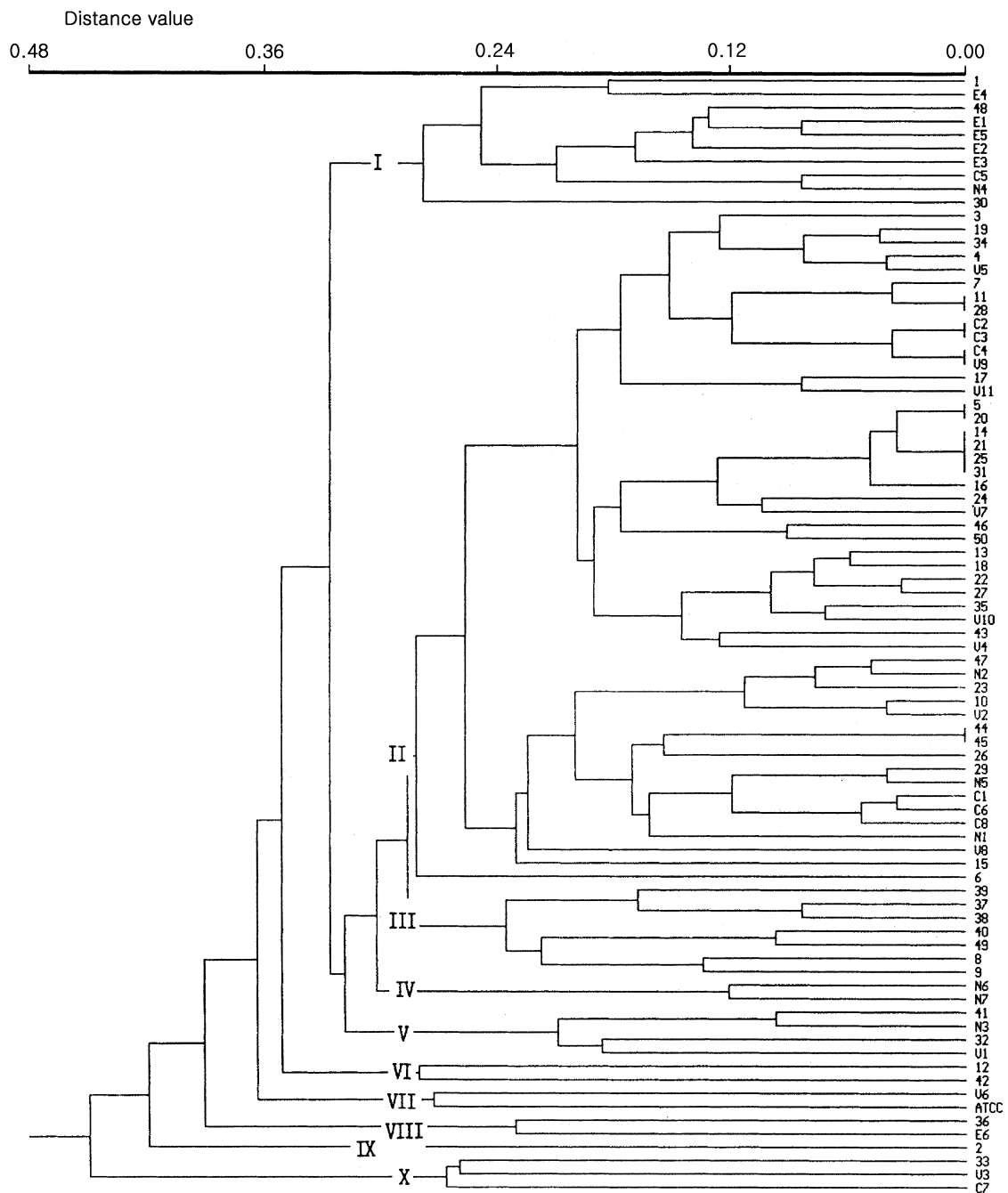
Fig. 3. Dendrogram showing estimates of the distance values using the results of RAPD assays with 4 primers among 83 *S. aureus* strains.

PFGE analysis

Seventeen distinct bands (>100 kb) utilized in cluster analysis were obtained by PFGE of *Sma*I restriction fragments on 83 *S. aureus* strains (Fig. 4). Eighty-three strains were clustered into 8 groups on the basis of distance value 0.3 (Fig. 5).

Group I contained 34 strains including some identical

band patterns (1, 7, 8, 9; 11, 18, 22, 27, 36, 43, 48, E1, E2; 34, E5; 32, 41; 44, 45; 19, 37; V4, V5). Strains E1 and E2 isolated from the medical personnels were MRSA and showed identical bands with 7 strains isolated from clinical specimens of the same hospital. Six strains except strain 36 were MRSA among the above 7 strains. Strains E5 and 34 that showed identical band pattern, were also MRSA. Two strains (V4, V5) among
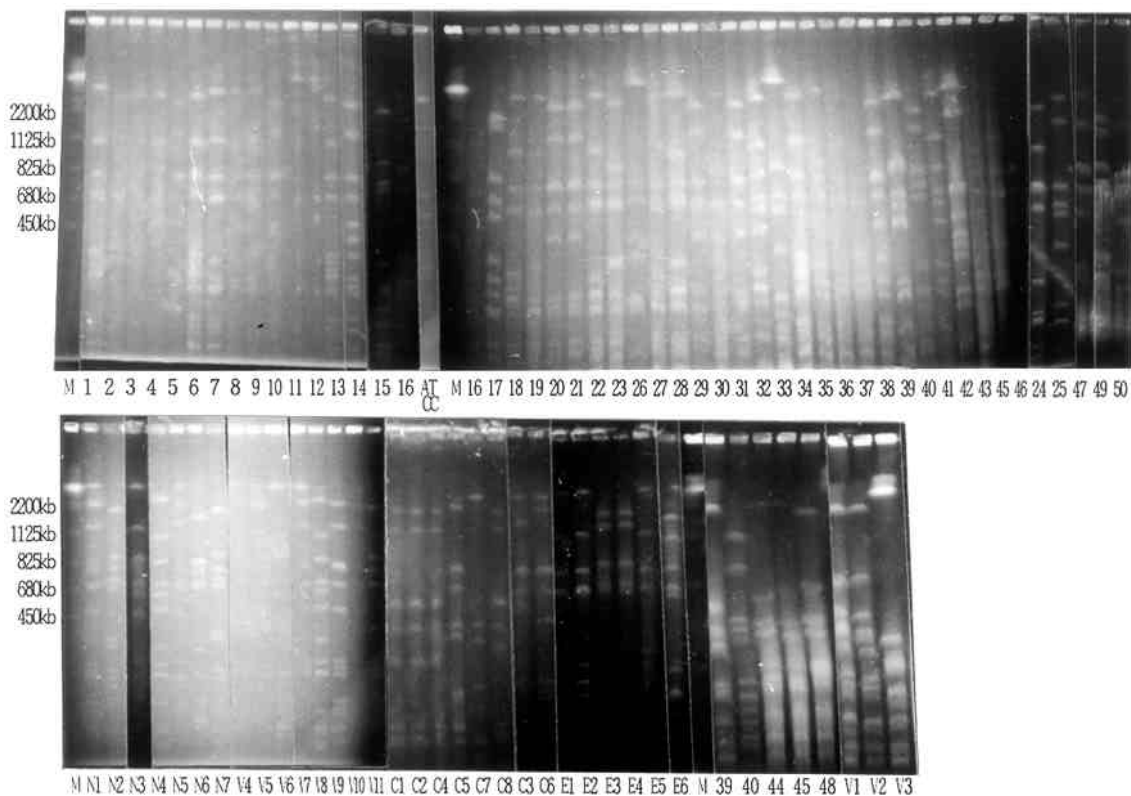
Fig. 4. PFGE patterns of 83 *S. aureus* isolates. Chromosomal DNAs were digested with *Sma*I restriction endonuclease, and the fragments were separated by CHEF-DR II PFGE system. Molecular size markers were obtained from *Saccharomyces cerevisiae* DNA concatemers (lanes M).

3 strains of KVH had identical bands.

Group IV contained 25 strains including 6 strains (C1, C2, C3, C4, C6, C8) that were isolated from SCH and showed similar band patterns by RAPD assay. The above 6 strains also showed similar band patterns by PFGE. Representatives of group V, VI, VII, and VIII were not specific. Six strains of SCH showed similar distribution on classification by PFGE, whereas distribution of isolates from the other 3 hospitals was not remarkable. Strains E3 and E4 among 14 *mecA*-negative *S. aureus* strains had identical band patterns.

Strains 39 and 40 isolated from different specimens of one person produced different band patterns by PFGE and were included in group I and III, respectively. On the other hand, strains 44 and 45 isolated from different specimens of another person produced identical band patterns.

DISCUSSION

S. aureus is major causative agent of nosocomial infection. In addition, because MRSA is resistant to several general antibiotics, the choice of antibiotics is difficult in the cases of nosocomial infection caused by MRSA. In this study *mecA* gene PCR was performed on 6 strains isolated from the medical personnels and instruments, and 63 strains isolated from clinical specimens. Two strains were *mecA*-negative by *mecA* gene PCR, but methicillin-resistant by disk diffusion method using oxacillin disk. Murakami et al. reported the *mecA*-positive and methicillin-susceptible strains and the *mecA*-negative and methicillin-resistant strains (14). By their studies, the *mecA*-positive and methicillin-susceptible strains had *mecA* gene not to express the low-affinity penicillin-binding protein (PBP-2'), and the *mecA*-negative and methicillin-resistant strains to oxacillin disk were methicillin-susceptible to methicillin disk (14). For this reason it is considered that minimal inhibitory concentration (MIC) of methicillin should be performed with the disk diffusion test using methicillin disks on isolates with conflicting results between the *mecA* gene PCR and the methicillin susceptibility test. On the other hand, Mulligan et al. reported that MRSA containing *mecA* gene showed methicillin-resistance due to intrinsic resistance and that methicillin-resistant strain due to a very high level production of β-lactamase was called BORSA for
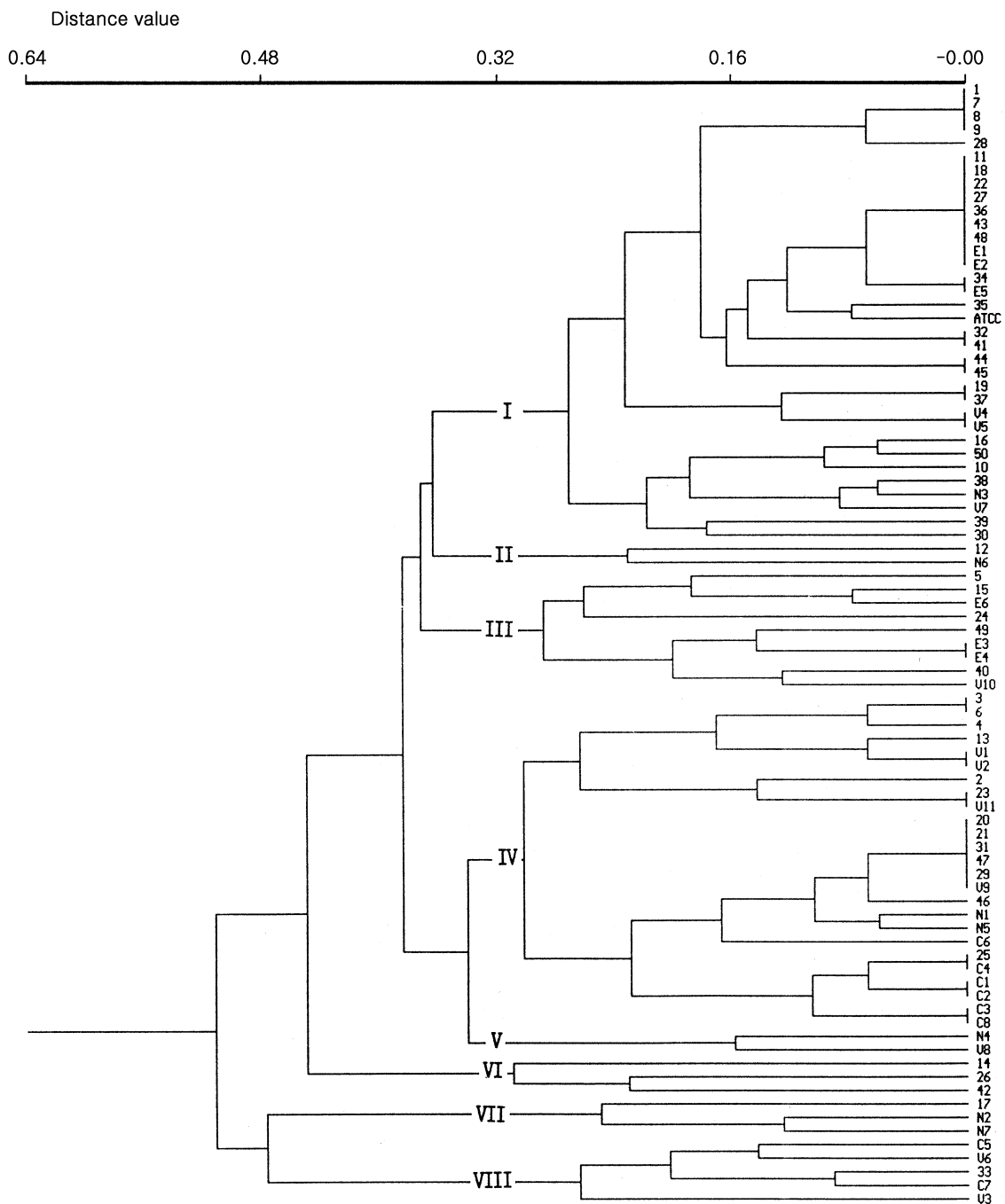
Fig. 5. Dendrogram showing estimates of the distance values using the results of the PFGE among 83 S. aureus strains.

"borderline oxacillin-resistant S. aureus" (19).

Conventional methods such as bacteriophage typing that have been performed for classification and epidemiologic study of microorganism have several weaknesses ; technical difficulty, unsuitableness to small laboratories, etc. Therefore RAPD assay and PFGE are used as the molecular typing techniques for classification and epidemiologic study of bacteria. Schlichting et al.

reported that PFGE was more effective for typing of S. aureus than bacteriophage typing (5). Prévost et al. reported that PFGE was more effective than ribotyping in distinguishing between MRSA isolates (9). On the other hand, van Belkum et al. reported that PCR fingerprinting by combination of PCR primers correlated well with established phage typing (12). Bannerman et al. reported that PFGE had advantages over bacterio-

phage typing in epidemiologic investigation of *S. aureus* isolates (8).

Struelens et al. reported that both RAPD assay and PFGE allowed useful epidemiologic typing of MRSA (11). However, Saulnier et al. reported that PFGE of *Sma*I restriction fragments was the best method of typing MRSA strains (10). The reasons were that the average percentage of similarity between strains was higher by the RAPD assay than by PFGE and that RAPD assay became more cumbersome with an increased number of primers.

In the present study the RAPD assay using the summed results for 4 primers produced 10 groups (Fig. 3), whereas PFGE of *Sma*I restriction fragments produced 8 groups (Fig. 5). The average distance values of RAPD assay and PFGE were similar, but cluster analysis of the results by RAPD assay showed low similarity and poor epidemiologic relatedness among the members of the group. On the other hand, it was not certain whether the RAPD assay using one primer represented true molecular similarity between the strains having identical bands, because there were too many isolates showing identical band patterns. But optimal combination of primers allowed useful classification for investigation of distribution and epidemiology of *S. aureus* strains. The dendrogram using the summed results of primers 03 and 04 showed 6 groups, whereas the dendrogram using the summed results of primers 05 and 06 showed 10 groups. Clustering by combination of 2 primers was more useful for epidemiological classification than by combination of 4 primers, but analysis using the results of 4 primers was more discriminant among the strains than by using the results of 2 primers. The epidemiologic tracking of *S. aureus* outbreaks needs a combination of a relatively large number of primers for discrimination of strains, while the investigation of distribution tendancy of many *S. aureus* strains needs a combination of only some primers. It is considered that the selection and combination of primers is important in RAPD assay and an optimal combination of primers for investigation is needed.

These results of RAPD assay were similar to Struelens' report that epidemiologic investigation was affected by alterations of band patterns according to combination and selection of primers. Although PFGE showing some identical band patterns may be difficult to distinguish among the causative agents of an outbreak, the major advantages of the PFGE technique are the good reproducibility, optimal number of restriction fragments, etc. In consequence PFGE analysis is better for investigation of regional distribution and epidemiologic relatedness of *S. aureus* than RAPD assay.

Because strains 39 and 40 isolated from different specimens of one person were included in different groups

by RAPD assay and PFGE, we concluded the case was a coinfected person by 2 different *S. aureus* strains. On the other hand, strains 44 and 45 isolated from different specimens of another person were the same strains, because the two isolates showed identical band patterns by RAPD assay and PFGE.

Fourteen *mecA*-negative strains were not clustered. This indicates that the DNA structure of MRSA is not so different from that of the methicillin-susceptible strain except for the fact that *mecA* gene exists or not.

In RAPD assay, isolates of the medical personnels and instruments of CUH showed low similarity of DNA polymorphism with isolates of clinical specimens of CUH. Six strains of SCH were included in the same group, but other strains did not show characteristic clustering.

In PFGE analysis 2 strains (E1, E2) isolated from the medical personnels showed identical band pattern with 7 strains (11, 18, 22, 27, 36, 43, 48) isolated from clinical specimens. This indicates that nosocomial infection can originate from the medical personnels and instruments. Nosocomial infections due to outbreak were also suggested, because some strains showed identical band patterns by PFGE.

In conclusion, the results of the present study indicate that medical personnels and instruments are routes of nosocomial infections caused by MRSA. PFGE and RAPD assay are powerful tools for the epidemiological study of *S. aureus,* but PFGE is more effective than RAPD assay. RAPD assay needs optimal combination of primers.

REFERENCES

1. Barber M. *Methicillin-resistant Staphlyococci. J Clin Pathol* 1961 ; 14 : 385-93.
2. Blanc DS, Lugeon C, Wenger A, Siegrist HH, Francioli P. *Quantitative antibiogram typing using inhibition zone diameters compared with ribotyping for epidemiological typing of methicillin-resistant Staphylococcus aureus. J Clin Microbiol* 1994 ; 32 : 2505-9.
3. Costas M, Cookson BD, Talsania HG, Owen RJ. *Numerical analysis of electrophoretic protein patterns of methicillin-resistant strains of Staphylococcus aureus. J Clin Microbiol* 1989 ; 27 : 2574-81.
4. Poddar SK, McClelland M. *Nonspecific primer and PCR generated hybridization probes for physical ordering large restriction fragments in complex genome of S. aureus. Biochem Biophys Res Commun* 1992 ; 185 : 1055-61.
5. Schlichting C, Branger C, Fournier JM, Witte W, Boutonnier A, Wolz C, Goullet P, Doring G. *Typing of Staphylococcus aureus by pulsed-field gel electrophoresis, zymotyping, capsular typing, and phage typing : Resolution of clonal relationships. J Clin Microbiol* 1993 ; 31 : 227-32.

6. Ryu PY, Kim YI, Rhee JH, Chung SS, Ahn TH, Shin JH, Ryang DW, Kim YH. *Antimicrobial resistance and plasmid profile of methicillin-resistant Staphylococcus aureus. Korean J Infect Dis 1995 ; 27 : 15-29.*

7. Blumberg HM, Rimland D, Kiehlbauch JA, Terry PM, Wachsmuth IK. *Epidemiologic typing of Staphylococcus aureus by DNA restriction fragment length polymorphisms of rRNA genes : Elucidation of the clonal nature of a group of bacteriophage-nontypeable, ciprofloxacin-resistant, methicillin-susceptible S. aureus isolates. J Clin Microbiol 1992 ; 30 : 362-9.*

8. Bannerman TL, Hancock GA, Tenover FC, Michael Miller J. *Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of Staphylococcus aureus. J Clin Microbiol 1995 ; 33 : 551-5.*

9. Prévost G, Jaulhac B, Piemont Y. *DNA fingerprinting by pulsed-field gel electrophoresis is more effective than ribotyping in distinguishing among methicillin-resistant Staphylococcus aureus isolates. J Clin Microbiol 1992 ; 30 : 967-73.*

10. Saulnier P, Bourneix C, Prévost G, Andremont A. *Random amplified polymorphic DNA assay is less discriminant than pulsed-field gel electrophoresis for typing strains of methicillin-resistant Staphylococcus aureus. J Clin Microbiol 1993 ; 31 : 982-5.*

11. Struelens MJ, Bax R, Deplano A, Quint WGV, van Belkum A. *Concordant clonal delineation of methicillin-resistant Staphylococcus aureus by macrorestriction analysis and polymerase chain reaction genome fingerprinting. J Clin Microbiol 1993 ; 31 : 1964-70.*

12. Van Belkum A, Bax R, Peerbooms P, Goessens WHF, van Leeuwen N, Quint WGV. *Comparison of phage typing and DNA fingerprinting by polymerase chain reaction for dis-crimination of methicillin-resistant Staphylococcus aureus strains. J Clin Microbiol 1993 ; 31 : 798-803.*

13. Yoon CS. *Examination of parameters affecting polymerase chain reaction in studying RAPD. Korean Mycol 1992 ; 20 : 315-23.*

14. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. *Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J Clin Microbiol 1991 ; 29 : 2240-4.*

15. Ubukata K, Nakagami S, Nitta A, Yamane A, Kawakami S, Sugiura M, Konno M. *Rapid detection of the mecA gene in methicillin-resistant staphylococci by enzymatic detection of polymerase chain reaction products. J Clin Microbiol 1992 ; 30 : 1728-33.*

16. Unal S, Werner K, DeGirolami P, Barsanti F, Eliopoulos G. *Comparison to tests for detection of methicillin-resistant Staphylococcus aureus in a clinical microbiology laboratory. Antimicrob Agents Chemother 1994 ; 38 : 345-7.*

17. Kim JM, Kubo N, Sakurabayashi I. *Clinical evaluation of PCR for rapid detection of methicillin-resistant Staphylococcus aureus. Korean J Clin Pathol 1993 ; 13 : 387-93.*

18. Song MD, Wachi M, Doi M, Ishino F, Matsuhashi M. *Evolution of an inducible penicillin-target protein in methicillin-resistant Staphylococcus aureus by gene fusion. FEBS Lett 1987 ; 221 : 167-71.*

19. Mulligan ME, Murray-Leisure KA, Ribner BS, Standiford HC, John JF, Korvick JA, Kauffman CA, Yu VL. *Methicillin-resistant Staphylococcus aureus: A consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management. Am J Med 1993 ; 94 : 313-28.*