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Comparison of Different PCR-Based Genotyping Techniques for MRSA Discrimination Among Methicillin-Resistant *Staphylococcus aureus* Isolates

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Abstract The usefulness of three PCR methods was evaluated for the epidemiological typing of *Staphylococcus aureus*: an enterobacterial repetitive intergenic consensus sequence PCR (ERIC-PCR), repetitive extragenic palindromic element PCR (REP-PCR), and 16S-23S intergenic spacer PCR (ITS-PCR). The analysis was performed using a collection of *S. aureus* strains comprised of 6 references and 79 isolates from patients with various diseases. Among the 85 *S. aureus* strains tested, 6 references and 6 isolates were found to be susceptible to methicillin, whereas the remaining 73 isolates were resistant to it. PCR methods are of special concern, as conventional phenotypic methods are unable to clearly distinguish among methicillin-resistant *S. aureus* (MRSA) strains. The ability of the techniques to detect different unrelated types was found to be as follows: ERIC-PCR, 19 types; REP-PCR, 36 types; and ITS-PCR, 14 types. On the basis of combining the ERIC, REP, and ITS fingerprints, the 85 *S. aureus* strains were grouped into 56 genetic types (designated G1 to G56). The diversities for the 85 *S. aureus* strains, calculated according to Simpson's index, were 0.88 for an ERIC-PCR, 0.93 for a REP-PCR, and 0.48 for an ITS-PCR, and the diversity increased up to 0.97 when an ERIC-PCR and REP-PCR were combined. The above discrimination indices imply that the genetic heterogeneity of *S. aureus* strains is high. Accordingly, this study demonstrates that DNA sequences from highly conserved repeats of a genome, particularly a combination of ERIC sequences and REP elements, are a convenient and accurate tool for the subspecies-specific discrimination and epidemiologic tracking of *S. aureus*.

Key words: ERIC-PCR, REP-PCR, ITS-PCR, *Staphylococcus aureus*, discrimination index, genetic heterogeneity

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a frequent cause of nosocomial (hospital-acquired) or community-acquired infections. MRSA can cause diseases in some individuals or be carried asymptomatically in others. Disease outbreaks caused by MRSA have become a major clinical problem in many parts of the world [28]. Outbreaks in hospitals or communities with a low incidence of MRSA are often initiated by the migration of patients or carriers from areas with a high prevalence of MRSA.

Specific and rapid epidemiologic typing is necessary for tracking the interhospital or intercommunity spread and evolution of MRSA strains. The high degree of genetic relatedness between MRSA strains has proven to be an obstacle to using routine bacteriological methods for epidemiologic analysis. Thus, recent studies have been based essentially on phenotypic traits, such as biochemical typing, serotyping, phage typing, and slime production [2, 3, 32, 33]. However, none of these methods has been found to be truly satisfactory because of insufficient discrimination, poor reproducibility, or lack of availability of specific reagents [26, 36]. The shortcomings of phenotypically-based typing methods have led to the development of typing methods based on the microbial genotype or DNA sequence, which minimize the problems associated with discrimination, typeability, and reproducibility, and enable the establishment of large databases of characterized organisms in some cases.

DNA-based typing of bacterial strains is based on the principle that epidemiologically related bacterial isolates have genetic features that distinguish them from other epidemiologically unrelated strains [23]. Yet, techniques used to type MRSA must be particularly discriminatory, as MRSA strains probably originate from a single clone or at least a few strain types [19], as the genetic differences between MRSA isolates are minor compared to differences between MRSA and methicillin-sensitive *S. aureus* (MSSA) strains. Therefore, the ideal system for typing *S. aureus* strains should combine a good discriminatory potential, typeability of all (or most) isolates, reproducibility, easily interpretable results, and practicality. Furthermore, it should

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be suitable for widespread use, so that the genotyping results obtained in different laboratories or countries can be compared. In Korea, different laboratories currently use different typing systems. To identify possible epidemic strains that are spread by the migration of patients or carriers, it is very important that the same suitable typing system be used. A PCR-based typing system would appear to be most appropriate because of its ease and speed of performance.

Repetitive extragenic palindromes (REP) constitute the best-characterized family of repetitive bacterial sequences [9]. REP sequences are 35 to 40 bp long and include an inverted repeat. A second family of repetitive elements, called intergenic repeat units or enterobacterial repetitive intergenic consensus (ERIC) sequences, are larger elements of 124 to 127 bp and contain a highly conserved central inverted repeat [13]. REP and ERIC sequences have been used as primer binding sites to amplify the genomes of a variety of bacteria including *S. aureus* for the REP-PCR and ERIC-PCR techniques, respectively [4, 5, 8, 16, 22, 34]. As primer binding sites for PCR reactions, the 16S rRNA gene itself was recently used to identify *Lactobacillus* or *Campylobacter* species [17, 20, 29]. But the sequence of the intergenic spacer region between the 16S and 23S rRNA genes is more variable than the sequence of the genes themselves. As such, the 16S-23S rDNA intergenic spacer PCR (ITS-PCR) method has also been proposed for the identification and typing of many other bacteria [1, 11, 30, 31], and already used to type *S. aureus* isolates [7, 24].

The objective of this study was to evaluate the usefulness of three different PCR-based molecular methods for typing MRSA isolates. The three different typing techniques of ERIC-PCR, REP-PCR, and ITS-PCR were also established to investigate the genetic relatedness of 73 MRSA isolates and 6 MSSA isolates, collected from patients with various infections in Korea.

MATERIALS AND METHODS

Isolation and Identification of *Staphylococcus aureus* Strains

Seventy-nine isolates of *S. aureus* were collected from patients suffering from inflammatory diseases in a hospital accommodating 1,000 beds in Korea, between September and November 1999. These 79 clinical isolates were identified by employing classical biochemical properties, such as Gram staining, production of coagulase, and growth characteristics on mannitol salt agar.

The reference type strains of enterotoxigenic *S. aureus* used in this study were from the American Type Culture Collection (ATCC) 19095 (enterotoxin type C) and ATCC 27664 (enterotoxin type E). The other reference enterotoxigenic strains of *S. aureus*, kindly supplied by Dr. Karsten Becker (Institute of Medical Microbiology, University of Munster, Germany), were KN556 (enterotoxin type A), H288a (enterotoxin type B), 1634/93 (enterotoxin type D), and H390 (enterotoxin types A and D).

Tests for Methicillin Resistance of *S. aureus*

The methicillin resistance of *S. aureus* was examined using a Cefinase® disk (BBL) for the production of β-lactamase, followed by an oxacillin susceptibility test using the agar dilution method.

Bacterial DNA Isolation

The template DNA used for the PCR amplifications was isolated by a method described previously [6] with modifications. The bacterial strains were cultured overnight at 37°C in 2 ml of a Luria-Bertani broth. The cells were centrifuged at 14,000 ×g for 5 min, rinsed twice with 1 M NaCl, and resuspended in 500 µl of 1× TE (10 mM Tris-HCl-1 mM EDTA, pH 8.0) buffer. Ten microliters of 1 mg/ml lysostaphin (Sigma Chemical Co., St. Louis, U.S.A.) solution and 20 µl of 10 mg/ml RNase A (Qiagen Inc., U.S.A.) solution were added to the suspension and incubated at 37°C for 40 min. Fifty microliters of 10% sodium dodecyl sulfate and 3 µl of proteinase K (20 mg/ml; Sigma Chemical Co., St. Louis, U.S.A.) were added and then incubated at 37°C for 1 h in order to lyse the bacterial cells. The lysate was extracted twice with equal volumes of phenol and then once with chloroform. The DNA was precipitated from the aqueous phase with 0.5 parts of 1 M ammonium acetate and 2.5 parts of absolute ethanol, and then resuspended in 50 µl of 1× TE (10 mM Tris-HCl-1 mM EDTA, pH 8.0) buffer.

ERIC-PCR and Amplification Conditions

The primers ERIC1R (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') used in this study were described previously by Versalovic *et al.* [35]. The PCRs were carried out with 1 µl of template DNA per reaction. The amplification reactions were performed in 50 µl of a solution containing 2 µM concentration of each of the two opposing primers (Bioneer, Seoul, Korea), 0.2 mM deoxyribonucleoside-5'-triphosphate (dNTP) (Bioneer, Seoul, Korea), 2 mM $MgCl_2$, 5 µl of 10× amplification buffer (100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol, 0.5% Tween20, 0.5% nonidet-P40, and 20 mM Tris-HCl, pH 8.0), and 1 U of *Taq* DNA polymerase (Bioneer, Seoul, Korea). The mixture was overlaid with 30 µl of light mineral oil, and amplifications were performed using an automated DNA thermocycler (PTC-100, MJ Research Inc., Massachusetts, U.S.A.) with initial denaturation (94°C, 4 min), followed by 40 cycles of denaturation (94°C, 1 min), annealing (25°C, 1 min), extension (74°C, 2 min), and single final extension (74°C, 10 min). The same low annealing temperature

has been employed for ERIC-PCR to identify *S. aureus* strains by van Belkum *et al.* [33] and Kluytmans *et al.* [18]. The amplified DNA products (10 µl) were electrophoretically separated in 1% agarose gel in 1× TAE buffer, stained with ethidium bromide, visualized on a UV transilluminator, and photographed with Polaroid 665 film.

REP-PCR and Amplification Conditions

The primer RW3A (5'-TCGCTCAAAACAACGACACC-3') used has been described previously by Del Vecchio *et al.* [6], who used this single REP-PCR primer successfully to identify *S. aureus* strains along with Hu *et al.* [12] and van der Zee *et al.* [34]. The PCRs were carried out with 1 µl of template DNA per reaction. The amplification reactions were performed in 50 µl of a solution containing 2 µM primer (Bioneer, Seoul, Korea), 0.2 mM dNTP, 2 mM $MgCl_2$, 5 µl of a 10× amplification buffer (100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol, 0.5% Tween20, 0.5% nonidet-P40, and 20 mM Tris-HCl, pH 8.0), and 1 U of *Taq* DNA polymerase (Bioneer, Seoul, Korea). Amplifications involved initial denaturation (94°C, 3 min), followed by 30 cycles of denaturation (94°C, 1 min), annealing (54°C, 1 min), extension (72°C, 2 min), and a single final extension (72°C, 5 min).

16S-23S rDNA Intergenic Spacer PCR (ITS-PCR) and Amplification Conditions

The primers 16S-2 (5'-TTGTACACACCGCCCGTC-3') and 23S-7 (5'-GGTACTTAGATGTTTCAGTTC-3') used have been described previously by Gurtler *et al.* [10]. The PCRs were carried out with 1 µl of template DNA per reaction. The amplification reactions were performed in 50 µl of a solution containing 2 µM of each of the two opposing primers (Bioneer, Seoul, Korea), 0.2 mM dNTP, 2 mM $MgCl_2$, 5 µl of a 10× amplification buffer (100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol, 0.5% Tween20, 0.5% nonidet-P40, and 20 mM Tris-HCl, pH 8.0), and 1 U of *Taq* DNA polymerase (Bioneer, Seoul, Korea). The amplifications involved initial denaturation (94°C, 4 min), followed by 30 cycles of denaturation (94°C, 1 min), annealing (50°C, 1 min), extension (72°C, 2 min), and a single final extension (72°C, 7 min).

Computer-Assisted Analysis of DNA Fingerprints

The gel images were normalized, the bands identified, and the data statistically analyzed using Bio-Gene software (version 96, Vilber Lourmat, Marne la Vallee, France). The positions of the DNA fragments on each gel were normalized using the 1-kb ladder from 201 to 4,072 bp as an external reference standard. Normalization using the same set of external standards facilitated the comparison of multiple gels. Three or four bands common to most of the isolates on each gel were also used as internal reference standards. The external and internal standards were corrected for smiling and other irregularities during electrophoresis. DNA fragments less than 200-bp long were not used in the analyses because they tended to be indistinct. Fingerprint images were added to a database and compared by performing a statistical analysis. Two fingerprints were considered identical if the same number of bands at the same positions were observed. Variations in intensity were not considered. The percentages of similarity between two profiles were calculated using the Jaccard coefficient [27]. The percentage of similarity ranged from 0% (complete dissimilarity) to 100% (identity). The unweighted pair group method with arithmetic linkages (UPGMA) was used to cluster the strains on the basis of the PCR fingerprinting patterns [21]. Strains with a percentage of similarity above 90% were considered as only potentially related.

Subsequently, Simpson's index (*D*) of diversity, based on the probability that two unrelated strain samples from the test population are placed into different typing groups, was calculated as described previously [5]. Simpson's index of diversity ranges from 0.0 to 1.0, where 1.0 indicates that a typing method is able to distinguish each member of a strain population from all other members of that population and, conversely, 0.0 indicates that all members

Table 1. Phenotypic characteristics of *S. aureus* strains used in this study.

Type	Strains	Characteristics		
		Coagulase	Mannitol salt agar	Methicillin resistance
MSSA	Reference strains of KN556, H288a, ATCC 19095, 1634/93, ATCC 27664, H390	+	+	–
	WCH 29, 31, 45, 66, 104, 137	+	+	–
MRSA	WCH 11, 12, 13, 16, 17,18,19, 20, 22, 23, 24, 27 WCH 30, 32, 39, 42, 47, 48, 50, 51, 53, 54, 55, 56 WCH 56, 57, 58, 59, 60, 69, 70, 71, 72, 73, 75, 76 WCH 77, 78, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90 WCH 91, 92, 93, 94, 95, 96, 97, 98, 100, 101, 106 WCH 111, 118, 119, 64, 80, 107, 113, 116, 126 WCH 127, 128, 130, 131, 133, 136	+	+	+

of a strain population are of an identical type. An index of 0.5 means that if one strain is chosen at random from a strain population, then there is a 50% probability that the next strain chosen at random is indistinguishable from the first [14, 15].

RESULTS

Identification and Discrimination of *S. aureus* References and Isolates

Among the 79 strains of the clinical isolates and 6 strains of the enterotoxigenic references of *S. aureus*, 12 strains (6 clinical isolates and all of the 6 reference strains) were found to be susceptible to methicillin (methicillin-sensitive *S. aureus*, MSSA), as shown in Table 1. The rest of the 73 clinical isolates were identified as methicillin-resistant *S. aureus* (MRSA), where the minimal inhibitory concentration of oxacillin against them was 128 μg/ml or more. The classical phenotypic typing methods used in this study could not discriminate the MRSA and MSSA strains any further.

The molecular genotypic typing of the 79 strains of the clinical isolates and 6 strains of the enterotoxigenic references by the three PCR-based methods allowed for a comparison of the ability of each genotypic typing method to discriminate different strain types. After normalization and alignment of the different DNA profiles, the relative

Table 2. PCR-based genotypic characteristics of *S. aureus* strains used in this study.

Designation		ERIC-PCR type	REP-PCR type	ITS-PCR type	Global type
This study	Strain				
	MSSA				
1	KN556	E1	R1	I1	G1 (E1 R1 I1)
2	H288a	E3	R2	I10	G2 (E3 R2 I10)
3	ATCC 19095	E2	R4	I2	G3 (E2 R4 I2)
4	1634/93	E9	R32	I6	G4 (E9 R32 I6)
5	ATCC 27664	E6	R34	I14	G5 (E6 R34 I14)
6	H390	E7	R33	I10	G6 (E7 R33 I10)
7	WCH29	E10	R5	I12	G7 (E10 R5 I12)
8	WCH31	E4	R3	I14	G8 (E4 R3 I14)
9	WCH45	E12	R8	I10	G9 (E12 R8 I10)
10	WCH66	E10	R5	I12	G7 (E10 R5 I12)
11	WCH104	E10	R5	I12	G7 (E10 R5 I12)
12	WCH137	E5	R31	I11	G10 (E5 R31 I11)
	MRSA				
13	WCH11	E12	R11	I8	G11 (E12 R11 I8)
14	WCH12	E18	R36	I13	G12 (E18 R36 I13)
15	WCH13	E12	R8	I8	G13 (E12 R8 I8)
16	WCH16	E10	R5	I12	G7 (E10 R5 I12)
17	WCH17	E12	R12	I9	G14 (E12 R12 I9)
18	WCH18	E12	R9	I9	G15 (E12 R9 I9)
19	WCH19	E12	R10	I8	G16 (E12 R10 I8)
20	WCH20	E12	R11	I4	G17 (E12 R11 I4)
21	WCH22	E12	R6	I4	G18 (E12 R6 I4)
22	WCH23	E13	R11	I4	G19 (E13 R11 I4)
23	WCH24	E19	R11	I4	G20 (E19 R11 I4)
24	WCH27	E15	R15	I4	G21 (E15 R15 I4)
25	WCH30	E15	R11	I4	G22 (E15 R11 I4)
26	WCH32	E15	R15	I4	G21 (E15 R15 I4)
27	WCH39	E13	R11	I4	G19 (E13 R11 I4)
28	WCH42	E13	R17	I4	G23 (E13 R17 I4)
29	WCH47	E13	R17	I4	G23 (E13 R17 I4)
30	WCH48	E13	R18	I4	G24 (E13 R18 I4)
31	WCH50	E13	R17	I4	G23 (E13 R17 I4)
32	WCH51	E8	R35	I7	G25 (E8 R35 I7)
33	WCH53	E13	R11	I4	G19 (E13 R11 I4)

Table 2. Continued.

Designation		ERIC-PCR type	REP-PCR type	ITS-PCR type	Global type
This study	Strain				
34	WCH54	E13	R17	I4	G23 (E13 R17 I4)
35	WCH55	E13	R17	I4	G23 (E13 R17 I4)
36	WCH56	E13	R17	I4	G23 (E13 R17 I4)
37	WCH57	E13	R17	I4	G23 (E13 R17 I4)
38	WCH58	E13	R17	I4	G23 (E13 R17 I4)
39	WCH59	E13	R17	I4	G23 (E13 R17 I4)
40	WCH60	E15	R17	I4	G26 (E15 R17 I4)
41	WCH69	E11	R19	I4	G27 (E11 R19 I4)
42	WCH70	E13	R11	I4	G19 (E13 R11 I4)
43	WCH71	E11	R17	I3	G28 (E11 R17 I3)
44	WCH72	E13	R15	I4	G29 (E13 R15 I4)
45	WCH73	E13	R11	I4	G19 (E13 R11 I4)
46	WCH75	E13	R11	I4	G19 (E13 R11 I4)
47	WCH76	E13	R17	I4	G23 (E13 R17 I4)
48	WCH77	E12	R26	I3	G30 (E12 R26 I3)
49	WCH78	E12	R23	I5	G31 (E12 R23 I5)
50	WCH81	E15	R22	I4	G32 (E15 R22 I4)
51	WCH82	E15	R20	I4	G33 (E15 R20 I4)
52	WCH83	E13	R13	I4	G34 (E13 R13 I4)
53	WCH84	E11	R19	I4	G27 (E11 R19 I4)
54	WCH85	E15	R13	I4	G35 (E15 R13 I4)
55	WCH86	E11	R25	I4	G36 (E11 R25 I4)
56	WCH87	E13	R15	I4	G29 (E13 R15 I4)
57	WCH88	E11	R20	I4	G37 (E11 R20 I4)
58	WCH89	E11	R27	I4	G38 (E11 R27 I4)
59	WCH90	E13	R11	I4	G19 (E13 R11 I4)
60	WCH91	E11	R7	I4	G39 (E11 R7 I4)
61	WCH92	E15	R16	I4	G40 (E15 R16 I4)
62	WCH93	E11	R11	I4	G41 (E11 R11 I4)
63	WCH94	E15	R27	I4	G42 (E15 R27 I4)
64	WCH95	E15	R28	I4	G43 (E15 R28 I4)
65	WCH96	E16	R29	I4	G44 (E16 R29 I4)
66	WCH97	E16	R29	I4	G44 (E16 R29 I4)
67	WCH98	E16	R8	I4	G45 (E16 R8 I4)
68	WCH100	E16	R8	I4	G45 (E16 R8 I4)
69	WCH101	E16	R8	I4	G45 (E16 R8 I4)
70	WCH106	E14	R16	I4	G46 (E14 R16 I4)
71	WCH111	E16	R8	I4	G45 (E16 R8 I4)
72	WCH118	E16	R11	I4	G47 (E16 R11 I4)
73	WCH119	E16	R8	I4	G45 (E16 R8 I4)
74	WCH64	E17	R13	I5	G48 (E17 R13 I5)
75	WCH80	E14	R15	I4	G49 (E14 R15 I4)
76	WCH107	E14	R11	I4	G50 (E14 R11 I4)
77	WCH113	E16	R14	I4	G51 (E16 R14 I4)
78	WCH116	E16	R24	I4	G52 (E16 R24 I4)
79	WCH126	E16	R11	I4	G47 (E16 R11 I4)
80	WCH127	E16	R14	I4	G51 (E16 R14 I4)
81	WCH128	E14	R8	I4	G53 (E14 R8 I4)
82	WCH130	E14	R21	I4	G54 (E14 R21 I4)
83	WCH131	E14	R11	I4	G50 (E14 R11 I4)
84	WCH133	E17	R8	I4	G55 (E17 R8 I4)
85	WCH136	E17	R30	I4	G56 (E17 R30 I4)

genetic similarity among the strains was calculated and visualized by a cluster analysis. The estimated relationships among the strains on the basis of each fingerprint were indicated on the respective dendrogram. For each method, the discriminatory power of the typing system was assessed by counting the number of different strain types detected as a proportion of the total number of strains, and by calculating Simpson's index (*D*) of diversity based on the probability that two unrelated strain samples from the test population are placed into different typing groups. All the 79 isolates and 6 references tested could be typed by the three PCR-based methods.

Analysis of ERIC-PCR Fingerprinting Patterns

The ERIC-PCR of the genomic DNA from the 85 *S. aureus* strains generated multiple DNA fragments from three to six major amplification products, ranging in size from 0.18 to 1.13 kb (Table 3). Three common major bands of about 0.18 kb, 0.21 kb, and 0.54 kb were present in most of the MRSA isolates tested (results not shown). A dendrogram was constructed (not shown) and a cluster analysis of the banding patterns revealed 19 distinct ERIC types (Table 3) for the 85 epidemiologically unrelated strains tested. The two most common ERIC types were types 13 and 16 (21 and 12 isolates, respectively) that were all MRSA isolates and comprised 39% of all strains, whereas the remaining ERIC types contained between one and ten strains per type (Table 2). Type 13 differed from type 16 in one unique faint band of approximately 0.93 kb in size (Table 3). Only one isolate belonged to types 1–9, 18, and 19 (Table 3). Twenty-seven % (strains 18, 19, and 32) of them were MRSA, and the rest (strains 1–6, 8, and 12) were MSSA. The Simpson's index (*D*) of discrimination calculated for the 85 epidemiologically unrelated isolates and references was 0.88 for the ERIC-PCR (Table 6).

Analysis of REP-PCR Fingerprinting Patterns

With the REP-PCR method, the profiles of the 85 *S. aureus* strains revealed amplified bands ranging from 0.33 to 2.62 kb with various intensities (Table 4). Four common major bands of about 0.33 kb, 0.91 kb, 2.18 kb, and 2.62 kb were present in most of the MRSA isolates tested (results not shown). A dendrogram was constructed (not shown) and the 85 epidemiologically unrelated strains were grouped into 36 distinct patterns of fingerprints by a cluster analysis (Table 4). All the types, except for type 36, shared a very intense band of approximately 0.91 kb in size, whereas type 36 did not share any other band. REP types 11 and 17 were the most common (16 and 12 isolates, respectively) constituting 33% of all strains tested and all being MRSA isolates, whereas the rest of the REP types contained between one and nine strains per type (Table 2). Type 11 differed from type 17 in three faint bands of approximately 0.39 kb, 0.51 kb, and 0.68 kb in size (Table 4). Only one isolate belonged to types 1–4, 6, 7, 9, 10, 12, 18, 21–26, 28, and 30–36 (Table 4). Thirty-three % (strains 1–6, 8, and 12) of them were MSSA, and the rest (strains 14, 17–19, 21, 30, 32, 48–50, 55, 60, 64, 78, 82, and 85) were MRSA. The Simpson index (*D*) of discrimination calculated

Table 3. Representative fingerprinting patterns for all 19 ERIC-PCR types of the 85 *S. aureus* strains analyzed in this study. Variations in intensity are not indicated.

Type	No. of strains	Presence of fragments with the following sizes (kb)												
		1.13	1.02	0.93	0.80	0.61	0.54	0.47	0.41	0.38	0.32	0.24	0.21	0.18
E1	1	–		–	–	–		–			–		–	–
E2	1	–			–						–		–	–
E3	1	–			–			–			–		–	–
E4	1	–			–		–	–			–		–	–
E5	1	–		–				–					–	–
E6	1	–					–						–	–
E7	1	–		–	–								–	–
E8	1	–		–									–	–
E9	1						–						–	–
E10	4	–			–		–						–	–
E11	8	–					–				–		–	–
E12	10	–		–			–						–	–
E13	21	–		–			–			–	–		–	–
E14	6	–		–			–			–			–	–
E15	10	–					–			–			–	–
E16	12	–					–			–	–		–	–
E17	3	–		–			–		–	–			–	–
E18	1		–		–			–	–			–		
E19	1	–					–		–				–	–

Table 4. Representative fingerprinting patterns for all 36 REP-PCR types of the 85 *S. aureus* strains analyzed in this study. Variations in intensity are not indicated.

Type	No. of strains	Presence of fragments with the following sizes (kb)																
		2.62	2.43	2.18	2.02	1.94	1.72	1.48	1.31	1.02	0.91	0.79	0.68	0.62	0.59	0.51	0.39	0.33
R1	1		+						+		+	+		+	+			
R2	1	+	+						+		+	+					+	
R3	1	+					+	+	+		+							
R4	1	+							+	+	+	+					+	
R5	4			+		+	+		+		+	+	+		+		+	+
R6	1			+							+	+				+	+	+
R7	1	+	+	+			+		+		+	+					+	+
R8	9	+		+			+		+		+	+				+	+	+
R9	1	+		+		+	+		+		+	+			+	+	+	+
R10	1			+			+		+		+	+	+			+	+	+
R11	16	+		+			+	+	+		+	+						+
R12	1	+		+	+		+	+	+		+	+	+				+	+
R13	3	+	+	+			+	+	+		+	+	+			+	+	+
R14	2	+		+			+	+	+	+	+	+	+			+	+	+
R15	5	+		+			+	+	+		+	+					+	+
R16	2	+		+		+	+	+	+		+	+						+
R17	12	+		+			+	+	+		+	+	+			+	+	+
R18	1	+		+			+		+		+	+	+			+	+	+
R19	2	+		+			+		+		+	+	+		+	+	+	+
R20	2	+		+			+	+	+		+	+	+		+	+	+	+
R21	1	+		+			+	+	+		+				+	+	+	+
R22	1	+		+			+	+	+		+	+	+				+	+
R23	1	+		+			+		+		+	+	+					+
R24	1	+		+		+	+	+	+		+	+	+			+	+	+
R25	1	+		+		+	+		+		+	+	+		+	+	+	+
R26	1	+		+							+	+	+					
R27	2	+		+	+		+	+	+		+	+	+		+	+	+	+
R28	1	+		+	+		+		+		+	+	+		+	+		+
R29	2	+		+	+		+		+		+	+	+		+	+	+	+
R30	1	+		+	+	+	+		+		+	+	+		+	+		+
R31	1	+	+	+					+	+	+	+	+		+		+	
R32	1				+		+				+	+			+		+	+
R33	1		+		+			+	+		+	+	+		+		+	+
R34	1	+	+	+	+				+		+							
R35	1	+		+							+	+						
R36	1															+	+	

for the 85 epidemiologically unrelated isolates and references was 0.93 for the REP-PCR (Table 6).

Analysis of ITS-PCR Fingerprinting Patterns

When a dendrogram was constructed (not shown), the ITS-PCR using the pair of primers 16S-2 and 23S-7 produced fourteen distinguishable patterns for the 85 *S. aureus* strains. The PCR fragments ranged from 0.59 to 1.01 kb, with various band intensities (Table 5). The most common ITS type was type 4 (61 isolates), which were all MRSA isolates and constituted 72% of all strains tested. The rest of the ITS types contained between one and four strains per type (Table 5). Three common major bands of about 0.66 kb, 0.78 kb, and 0.87 kb were present in type 4 isolates (Table 5). Only one isolate belonged to types 1, 2, 6, 7, 11, and 13 (Table 5). Sixty-seven % (strains 1, 3, 4, and 12) of them were MSSA, and the rest (strains 14 and 32) were MRSA. The Simpson's index (*D*) of discrimination calculated for the 85 epidemiologically unrelated isolates and references was 0.48 for the ITS-PCR (Table 6).

Comparison of Three Different Typing Methods

Simpson's index (*D*) of diversity has been adapted for use as a method for deriving a numerical index reflecting the discriminatory ability of single or combined typing systems [5]. The numbers derived are presented as a discrimination index. For each typing method, the discrimination index represents the percentage of occasions

Table 5. Representative fingerprinting patterns for all 14 ITS-PCR types of the 85 *S. aureus* strains analyzed in this study. Variations in intensity are not indicated.

Type	No. of strains	Presence of fragments with the following sizes (kb)																
		1.01	0.98	0.92	0.91	0.89	0.87	0.85	0.82	0.80	0.78	0.76	0.74	0.72	0.68	0.66	0.63	0.59
I1	1				–				–		–			–		–		
I2	1				–						–			–				
I3	2							–								–		
I4	61						–				–					–		
I5	2	–			–							–					–	
I6	1				–	–			–					–				
I7	1		–				–		–				–					
I8	3						–		–							–		
I9	2								–							–		
I10	3				–				–						–			
I11	1								–						–			
I12	4			–						–					–			
I13	1										–				–			–
I14	2							–						–				

that two strains sampled randomly from a population fall into different types. Table 6 shows the discriminatory power of the different PCR typing methods used in this study. When the methods were taken separately, the REP-PCR exhibited the highest discriminatory power with a D of 0.93 for the total strains tested. In contrast, the ITS-PCR method provided the lowest degree of discrimination (below 0.5). Globally, the highest D was obtained when the three PCR methods were taken together with a D of 0.98. With combinations of just two PCR methods, the Ds of an ERIC- and REP-PCR, ERIC- and ITS-PCR, and REP- and ITS-PCR increased to 0.97, 0.90, and 0.94, respectively. There was no strong correlationship between the three PCR methods used in this study for the total 85 strains when compared with each other. This finding implies that these methods measure genetic events independently, thereby making the interpretation of the relationships between strains even more complex. It is clear that no single method is sufficient to unambiguously examine the genetic relatedness among the strains. However, at the same time, there appears to be a tremendous genetic heterogeneity in the epidemic *S. aureus* population that is reflected in the large number of types associated with nosocomial or community-acquired diseases, as studied here.

Table 6. Discrimination indices for PCR-based typing methods of *S. aureus* strains used in this study.

Typing method	No. of types	Size (%) of largest type	Discrimination index
ERIC	19	24.7	0.88
REP	36	18.8	0.93
ITS	14	71.8	0.48
ERIC+REP	54	11.8	0.97
ERIC+ITS	26	24.7	0.90
REP+ITS	41	17.7	0.94
ERIC+REP+ITS	56	11.8	0.98

DISCUSSION

To the best of our knowledge, PCR-based molecular epidemiological typing for MRSA has never previously been reported in Korea. During the last decade, other approaches at the molecular level, such as pulsed-field gel electrophoresis (PFGE) and ribotyping, have been used to assess the relatedness of a variety of epidemic organisms. Despite of the broad applicability of these techniques, their use in clinical microbiology laboratories has been limited, because they are both time-consuming and labor-intensive. However, the PCR techniques described here are easy to perform and can be interpreted with a high degree of discrimination. In addition, they need only 1 day to obtain results, plus the equipment cost and cost per test are moderate with a good reproducibility [25]. In particular, the PCR-based typing methods described here are of special concern when working with pathogenic bacteria, because direct manipulation with live organisms (which takes place when traditional biochemical typing techniques are used) can be avoided. In contrast, although biochemical tests can be used to discriminate among *S. aureus* strains, such tests have obvious drawbacks for unambiguous classification.

The ERIC-PCR patterns are generally less complex than the REP-PCR patterns, yet both provide a good discrimination at the strain level [25]. The application of both ERIC- and REP-PCR to strains to be typed increases discriminatory power over that of either technique used

alone. However, both ERIC- and REP-PCR techniques have considerably better discriminatory power than the ITS-PCR technique. The data presented in Table 6 strongly support the previous comparative findings related to the three different PCR-based methods.

Accordingly, it would appear that the three PCR-based typing described here may be suitable for widespread use in clinical microbiology laboratories for the epidemiological typing of *S. aureus* strains. Furthermore, PCR-generated patterns are suitable for analysis with computer software and storage, provided that 1) the PCR is performed with isolated chromosomal DNA and not with lysed cells, and 2) the gel electrophoresis conditions are standardized. It is expected that consistent results can be obtained when the methods are performed in different laboratories. The interlaboratory reproducibility of the methods is currently under investigation. Stored ERIC-, REP-, and ITS-PCR patterns and the interlaboratory exchange of digitized fingerprints by electronic mail could play an important role in the analysis of future nosocomial or community-acquired MRSA outbreaks, and in monitoring the international spread of strains with high epidemic potentials. Therefore, the molecular typing methods described herein could be used in track-back investigations aimed at determining the source of nosocomial or community-acquired infections and provide opportunities for prevention or remediation. Thus, they will be useful for the epidemiological investigation, prevention, and control of human diseases resulting from infections from MRSA.

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