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Fusidic Acid Resistance Determinants in *Staphylococcus aureus* Clinical Isolates▽†

Hsiao-Jan Chen,[1] Wei-Chun Hung,[1] Sung-Pin Tseng,[1] Jui-Chang Tsai,[2,3] Po-Ren Hsueh,[4,5] and Lee-Jene Teng[1,4]*

Department of Clinical Laboratory Sciences and Medical Biotechnology[1] and Center for Optoelectronic Biomedicine,[2] National Taiwan University College of Medicine and Department of Surgery, Division of Neurosurgery,[3] Department of Laboratory Medicine,[4] and Department of Internal Medicine,[5] National Taiwan University Hospital, Taipei, Taiwan

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A total of 71 fusidic acid-resistant *Staphylococcus aureus* (45 methicillin-resistant and 26 methicillin-susceptible) isolates were examined for the presence of resistance determinants. Among 45 fusidic acid-resistant methicillin-resistant *S. aureus* (MRSA), isolates, 38 (84%) had *fusA* mutations conferring high-level resistance to fusidic acid (the MIC was ≥128 μg/ml for 22/38), none had *fusB*, and 7 (16%) had *fusC*. For 26 fusidic acid-resistant methicillin-susceptible *S. aureus* (MSSA), only 3 possessed *fusA* mutations, but 15 (58%) had *fusB* and 8 (31%) had *fusC*. Low-level resistance to fusidic acid (MICs ≤ 32 μg/ml) was found in most *fusB*- or *fusC*-positive isolates. For 41 isolates (38 MRSA and 3 MSSA), with *fusA* mutations, a total of 21 amino acid substitutions in EF-G (*fusA* gene) were detected, of which R76C, E444K, E444V, C473S, P478S, and M651I were identified for the first time. The nucleotide sequencing of *fusB* and flanking regions in an MSSA isolate revealed the structure of partial IS*257-aj1*-LP-*fusB-aj2-aj3*-IS*257*-partial *blaZ*, which is identical to the corresponding region in pUB101, and the rest of *fusB*-carrying MSSA isolates also show similar structures. On the basis of *spa* and staphylococcal cassette chromosome *mec* element (SCC*mec*) typing, two major genotypes, *spa* type t037-SCC*mec* type III (t037-III; 28/45; 62%) and t002-II (13/45; 29%), were predominant among 45 MRSA isolates. By pulsed-field gel electrophoresis analysis, 45 MRSA isolates were divided into 12 clusters, while 26 MSSA isolates were divided into 15 clusters. Taken together, the distribution of fusidic acid resistance determinants (*fusA* mutations, *fusB*, and *fusC*) was quite different between MRSA and MSSA groups.

Fusidic acid has been used as a topical agent for skin infection and for some systemic infections caused by *Staphylococcus aureus* (12). Fusidic acid-resistant *S. aureus* has been reported in many countries, with the prevalence ranging from 0.3 to 52.5%, and the occurrences of resistance determinants were remarkably different among different countries (5, 6). The rate of fusidic acid resistance in *S. aureus* in our hospital each year is about 3 to 6%. Although this frequency is not very high, the understanding of fusidic acid resistance mechanisms still is very important.

Two major fusidic acid resistance mechanisms have been reported in *S. aureus*: the alteration of the drug target site (4, 24, 25), which is due to mutations in *fusA* (encoding elongation factor G [EF-G]) (24, 28) or *rplF* (or FusE, encoding ribosome protein L6) (19, 25), and the protection of the drug target site by FusB family proteins, including *fusB*, *fusC*, and *fusD* (27, 30). Point mutations in *fusA* occur mainly in domain III of EF-G and usually permit normal colony size and growth rate, conferring the FusA class resistance (20, 25). Fusidic acid-resistant small-colony variant (SCV) isolates, referred to as the FusA-SCV class, were due mostly to mutations in domain V of EF-G (19, 25). Some *fusA* point mutations may compromise fitness during growth *in vivo* and *in vitro*, but these costs may be partly or fully compensated for by acquiring additional amino acid substitutions (24). Another subclass of mutations, located in *rplF* (32), is referred to as the FusE class, which confers fusidic acid resistance to SCV isolates.

Acquired fusidic acid resistance genes found in *Staphylococcus* spp. include *fusB*, *fusC*, and *fusD*. The genes *fusB* and *fusC* were found in *S. aureus* and coagulase-negative staphylococci (22, 27, 30, 34), and *fusD* was an intrinsic factor causing fusidic acid resistance in *Staphylococcus saprophyticus* (30). The *fusB* determinant originally was found on the plasmid pUB101 in *S. aureus* (26). Later, the *fusB* determinant also was found on a transposon-like element (27) or in a staphylococcal pathogenicity island (29).

In this study, we analyzed fusidic acid resistance determinants among methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) isolates for which fusidic acid had a MIC of ≥2 μg/ml. The distribution of fusidic acid resistance determinants was found different in MRSA and MSSA groups. Furthermore, to understand the phylogenetic relationship of resistance determinant-containing isolates, genotyping also was performed.

MATERIALS AND METHODS

Bacterial strains. Seventy-one fusidic acid-resistant *S. aureus* isolates (MIC ≥ 2 μg/ml) were tested in this study, including 45 MRSA and 26 MSSA isolates. Isolates were collected between October 2002 and January 2007 in the Bacteri-

* Corresponding author. Mailing address: Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University College of Medicine, 1 Chang-Te Street, Taipei 100, Taiwan. Phone: 886-2-23123456, ext. 66918. Fax: 886-2-23711574. E-mail: ljteng@ntu.edu.tw.

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ology Laboratory, National Taiwan University Hospital, a 2,500-bed teaching hospital in northern Taiwan. The 45 MRSA isolates were chosen randomly from each month, representing about half of the collection. The 26 fusidic acid-resistant MSSA isolates were selected from all MSSA isolates in the collection period. Only one isolate per patient was included. The sources of 71 isolates included blood (59), external ear (2), pus (2), sputum (2), wound (2), synovial fluid (1), abscess (1), ascites (1), and burn (1).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed by standard agar dilution according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Bacterial inocula were prepared by direct colony suspension to a turbidity of 0.5 McFarland standards. A bacterial density of 10^4 CFU/spot was inoculated on Mueller-Hinton agars with various concentrations of fusidic acid (0.03 to 128 μg/ml) by using a Steers replicator, and the plates were incubated at 33 to 35°C for 16 to 20 h. *S. aureus* ATCC 29213 was used as the control organism. The breakpoint of fusidic acid resistance was 2 μg/ml (8).

Detection of fusidic acid resistance determinants by PCR. To detect *fusA* mutations, the DNAs were amplified with primers fusA-F and fusA-R (see Table S1in the supplemental material) and then sequenced by fusA −68_−49, fusA 404_425, fusA 946_968, and fusA down 47_25 (Table S1). The presence of acquired fusidic acid resistance determinants (*fusB*, *fusC*, and *fusD*) was detected by PCR (Table S1). PCRs were carried out using a DNA thermal cycler (MJ Research, Watertown, MA) with 30 cycles of denaturation (30 s at 94°C), annealing (30 s; at 45°C for *fusA*, 50°C for *fusB* and *fusC*, and 57°C for *fusD*), and extension (72°C for 2 min for *fusA* and 30 s for the others), followed by a final extension step (72°C for 10 min). The expected amplicons were 492 bp for *fusB*, 411 bp for *fusC*, and 465 bp for *fusD*. PCR products were separated by electrophoresis in 1.5% agarose gels.

Southern blotting. To clone and sequence the *fusB* fragment in MSSA, Southern blotting was used to estimate the fragment sizes digested by restriction enzymes and to perform further cloning procedures. Southern blot analysis was performed with the DNA from a representative isolate, NTUH-5020, using restriction enzymes (BamHI, EcoRI, HindIII, PstI, SalI, and XbaI) (New England BioLabs, Ipswich, MA) and detected with a digoxigenin (DIG)-labeled *fusB*-specific probe prepared by PCR amplification (see Table S1 in the supplemental material). The hybridization assay was performed by using a commercial kit (Roche Diagnostics GmbH, Penzberg, Germany).

Nucleotide sequencing of *fusB* and flanking regions. To determine the sequence of *fusB* and its flanking regions, an LA PCR *in vitro* cloning kit (Takara Shuzo Co. Ltd., Japan) was used. The LA PCR was carried out with the XbaI-digested DNA fragments. After ligating the XbaI-digested DNA fragments with cassette adapters, the amplification was performed with cassette primers (C1 for the first PCR and C2 for the nested PCR) supplied by the manufacturer and target gene-specific primers (fusB 437-465F for the first PCR and fusB 531-559F for the nested PCR) (see Table S1 in the supplemental material). The *fusB* upstream sequences were amplified with a pair of primers, IS 257 518-499R and fusB 283-254R (Table S1). Amplification products subsequently were sequenced on an Applied Biosystems 3100 DNA sequencer (Applied Biosystems, Foster City, CA) using the *Taq* BigDye deoxy terminator cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions.

SCC*mec* typing. The staphylococcal cassette chromosome *mec* element (SCC*mec*) types were determined by the detection of *ccr* genes and the types of *mec* complexes (7). The detection of *ccrAB* was carried out by a multiplex PCR using a mixture of four primers: a degenerate forward primer (β2) and three reverse primers (α2, α3, and α4) specific to *ccrA1B1* (SCC*mec* type I) (0.7 kb), *ccrA2B2* (SCC*mec* type II or type IV) (1 kb), and *ccrA3B3* (SCC*mec* type III) (1.6 kb) (13). The determination of the presence of *ccrC* (SCC*mec* type V) was carried out by PCR with primers γF and γR (520 bp) (14). Another PCR amplification was performed to detect the *ccrC2* gene (257 bp; SCC*mec* type VII, previously named SCC*mec* type V_T) (33). *mec* complex class A was determined by the amplification of the *mecI* gene by mecI-1 and mecI-2 (481 bp) (10). A 1,287-bp fragment amplified by mecRA1 (located in mecR1) and mDA2 (located in IS*1272*) was used to identify *mec* complex class B (17). Transposase C of Tn*554* was detected by amplification with the primer pair Tn554C F and Tn554C R (2). The primers mentioned above are listed in Table S1 in the supplemental material.

***spa* typing and multilocus sequence typing.** The *spa* typing was performed as previously described (31). An amplification of the staphylococcal protein A gene (*spa*) was carried out with the primer pair spa-1095F and spa-1517R (see Table S1). Since this pair of primers couldn't produce a *spa* PCR product in NTUH-2803, another forward primer, spa-1063F, was used (18). The *spa* type was determined by using the Ridom Spaserver website (http://www.spaserver.ridom.de) (11). The multilocus sequence typing (MLST) was analyzed in eight MRSA isolates, representing different *spa* types according to a method described previously (9).

Pulsed-field gel electrophoresis. The genotyping of fusidic acid-resistant MRSA and MSSA was performed by pulsed-field gel electrophoresis (PFGE). Genomic DNAs were prepared and digested with SmaI (New England BioLabs) (23) and then separated in a CHEF-DR II apparatus (Bio-Rad Laboratories). PFGE was carried out at 200 V and 13°C for 20 h, with pulse times ranging from 5 to 60 s. The pulsotypes were analyzed by BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium). The dendrogram of pulsotype relationships was produced by the unweighted pair group method using arithmetic averages (UPGMA) based on Dice similarity indices.

TABLE 1. Fusidic acid resistance determinants detected among MRSA and MSSA isolates

Species (no. of isolates showing fusidic acid MIC ≥ 2 μg/ml)	No. (%) of isolates with different fusidic acid resistance determinants			
	fusA mutation	*fusB*	*fusC*	*fusD*
MRSA (45)	38 (84)	0 (0)	7 (16)	0 (0)
MSSA (26)	3 (12)	15 (58)	8 (31)	0 (0)
Total (71)	41 (58)	15 (21)	15 (21)	0 (0)

RESULTS

MIC of fusidic acid. Among 45 fusidic acid-resistant MRSA isolates, the MICs of fusidic acid ranged from 4 to >128 μg/ml, with a 50% minimum inhibitory concentration (MIC_{50}) of 32 μg/ml and MIC_{90} of >128 μg/ml. The fusidic acid MICs for 26 fusidic acid-resistant MSSA isolates ranged from 2 to 32 μg/ml, giving a MIC_{50} of 8 μg/ml and MIC_{90} of 16 μg/ml. The results indicated that the level of fusidic acid resistance was higher in MRSA than in MSSA.

Prevalence of fusidic acid resistance determinants. To determine the prevalence of fusidic acid resistance determinants among 45 MRSA and 26 MSSA isolates, the entire *fusA* gene was sequenced, and other fusidic acid resistance genes (*fusB*, *fusC*, and *fusD*) were detected by PCR. Point mutations in *fusA* were found in 38/45 (84%) MRSA isolates, while 3 of 26 fusidic acid-resistant MSSA isolates possessed *fusA* point mutations (Table 1). Amplifications with primers specific for *fusB*, *fusC*, and *fusD* (see Table S1 in the supplemental material) revealed that none of the 45 MRSA isolates possessed either *fusB* or *fusD*, but seven MRSA isolates carried *fusC* (16%). The *fusB* gene was prevalent in MSSA isolates (15/26; 58%). Eight MSSA isolates (31%) carried the *fusC* determinant. Neither MRSA nor MSSA carried the *fusD* determinant (Table 1). The presence of *fusB* and *fusC* also was confirmed by Southern blotting with specific probes (data not shown).

Relationship of MIC to fusidic acid resistance determinants. The correlation of MIC to fusidic acid resistance determinants among MRSA and MSSA isolates was analyzed (Table 2). Isolates with *fusA* mutations usually had higher levels of fusidic acid resistance (the MIC for more than half of the isolates [22/41] was ≥128 μg/ml), while isolates with other determinants (*fusB* or *fusC*) had lower levels of resistance to fusidic acid (MICs ≤ 32 μg/ml).

Mutations in *fusA*. We determined the nucleotide sequences of *fusA* from all MRSA and MSSA isolates. Point mutations in *fusA* were detected in 38 MRSA and 3 MSSA isolates. A total of 22 different nucleotide substitutions causing 21 amino acid

TABLE 2. Distribution of fusidic acid MIC and resistance determinants among fusidic acid-resistant *S. aureus* isolates

Resistance determinant (no. of isolates)	No. of isolates with different fusidic acid MIC (μg/ml)		
	2–16	32–64	≥128
fusA point mutation (41)	15	4	22
fusB (15)	14	1	0
fusC (15)	15	0	0
Total (71)	44	5	22

exchanges at 17 different positions in EF-G were found (Table 3). Single-amino-acid substitutions were found in 21 MRSA and 3 MSSA isolates, and two amino acid substitutions were found in the other 17 MRSA isolates. Most amino acid substitutions occurred in domain III of EF-G (14/21; 67%), followed by domain I (5/21; 24%), while only one substitution was found in domain II and domain V. Six different amino acid substitutions were not previously reported, including R76C (in domain I), E444V (domain III), E444K (domain III), C473S (domain III), P478S (domain III), and M651I (domain V) (Table 3). A high frequency of amino acid substitutions arose at His-457 and Leu-461 of EF-G. Even a single-amino-acid substitution, such as H457Y or L461K, could result in a high level of fusidic acid resistance (MIC ≥ 128 μg/ml).

Genetic structure of *fusB*-containing regions in MSSA. Since most of the fusidic acid-resistant MSSA carried *fusB*, we determined the sequences of a 4,765-bp fragment containing *fusB* and its flanking regions in an MSSA isolate, NTUH-5020. The nucleotide sequences revealed the organization of partial IS*257-aj1*-LP-*fusB-aj2-aj3*-IS*257*-partial *blaZ*. The genetic structures of the *fusB* fragments in the other 15 clinical isolates were further tested by PCR mapping with two pairs of primers, IS 257 518-499R/fusB 283-254R for the upstream region and fusB 531-559F/IS 257 33-52F (see Table S1 in the supplemental material) for the downstream region of *fusB*. In addition, we confirmed the structure of *fusB* fragment by Southern blotting (data not shown). The results indicated that the genetic structures of *fusB* elements among our MSSA isolates were very similar.

Genotyping of MRSA by *spa* type, SCC*mec* type, and MLST. To understand the phylogenetic relationships between the fusidic acid-resistant MRSA isolates, we determined *spa* types and SCC*mec* types of the MRSA isolates. The 45 MRSA isolates belonged to five different *spa* types. The majority (42/45) of MRSA isolates belonged to two major *spa* types, t037 (29/45; 64%) and t002 (13/45; 29%). The remaining three isolates belonged to the *spa* types t437, t036, and t037*. The *spa* type t037* (repeat ID based on Ridom Spaserver website, 190-12-16-2-25-17-24) differ from t037 with only one nucleotide difference.

Three SCC*mec* types (II, III, and IV) were identified among the 45 fusidic acid-resistant MRSA isolates. The majority (29/45; 64%) contained SCC*mec* type III. Thirteen (29%) were SCC*mec* type II, and only two (4%) were SCC*mec* type IV. One isolate, NTUH-4257 (*spa* type t037), possessing *mec* complex A, *ccrC*, and transposase C of Tn*554*, was tentatively classified as SCC*mec* III* (Fig. 1). The 38 MRSA isolates with *fusA* mutations belonged to SCC*mec* types II, III, or IV, while all (seven) strains carrying *fusC* were restricted to SCC*mec* type III.

TABLE 3. FusA (EF-G) alternation sites detected in 41 *S. aureus* isolates

Amino acid substitution (domain[a])	Nucleotide substitution	No. of isolates	Fusidic acid MIC (μg/ml)
P404L[b]	CCA→CTA	2 (1 MRSA, 1 MSSA)	8, 16
P406L[b]	CCA→CTA	1	16
E444K[f]	GAA→AAA	1	16
G451V[b]	GGT→GTT	2	8, 16
M453I[d]	ATG→ATA	2	8
H457Q[g]	CAC→CAA	1	8
H457Y[b]	CAC→TAC	5	128, >128
L461K[b]	TTA→AAA	6	>128
L461S[b]	TTA→TCA	2	4, 8
P478S[f]	CCA→TCA	1 (MSSA)	8
M651I (domain V)[f]	ATG→ATA	1 (MSSA)	2
M16I (domain I)[c], H457Y[b]	ATG→ATA, CAC→TAC	3	16, 32, 64
A67T (domain I)[e], H457Y[b]	GCA→ACA, CAC→TAC	3	128, >128
A70V (domain I)[c], H457Y[b]	GCA→GTA, CAC→TAC	1	>128
A71V (domain I)[c], P404L[b]	GCT→GTT, CCA→CTA	1	8
R76C (domain I)[f], H457Y[b]	CGT→TGT, CAC→TAC	1	>128
A376V (domain II)[c], L456F[b]	GCT→GTT, CTT→TTT	1	32
E444V[f], L461F[b]	GAA→GTA, TTA→TTT	1	32
H457Q[g], L461F[b]	CAC→CAA, TTA→TTT	4	128
H457Q[g], L461K[b]	CAC→CAG, TTA→AAA	1	>128
L461K[b], C473S[f]	TTA→AAA, TGT→AGT	1	>128

[a] No domain specification indicates domain III of EF-G.
[b] Previously reported for *S. aureus* (4, 24).
[c] The amino acid substitutions have been identified as compensatory mutations in *S. aureus* (24).
[d] Reported for *Salmonella typhimurium* (15).
[e] The amino acid substitution has been reported to have no impact on fusidic acid resistance or compensation for fitness in *S. aureus* (3).
[f] The amino acid substitutions were first identified among *S. aureus* isolates.
[g] The amino acid substitution H457Q was recently reported by Castanheira et al. (5).

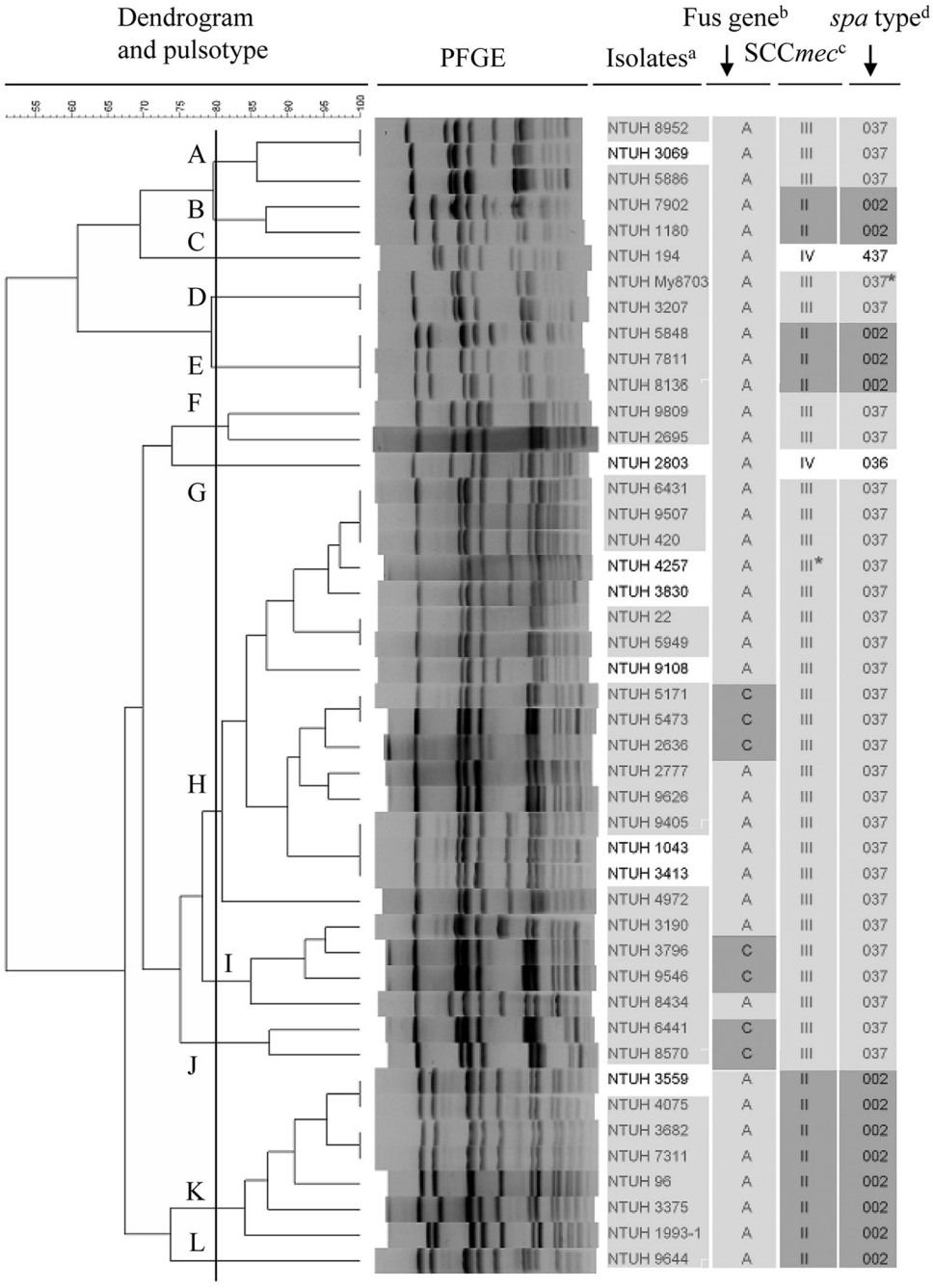

FIG. 1. Genotypes and fusidic acid resistance determinants among 45 fusidic acid-resistant MRSA isolates. The dendrogram was produced by BioNumerics software, showing distance calculated by the Dice similarity index of SmaI-digested DNA fragments. The degree of similarity is shown in the scale. Footnotes: a, light gray indicates isolates collected from blood specimens; b, resistance determinants of resistance to fusidic acid (A, *fusA* point mutation [light gray]; C, *fusC* determinant [dark gray]); c, SCC*mec* type based on *ccr* gene and *mec* complex (light gray, SCC*mec* type III; dark gray, SCC*mec* type II; SCC*mec* type III*, undetermined but possibly SCC*mec* type III [*ccrA3B3* PCR failed], which contained *mec* complex A, *ccrC*, and transposase C of Tn*554*); d, *spa* type based on data from the Ridom Spaserver website (http://www.spaserver.ridom.de) (light gray, *spa* type t037 [repeat ID, 15-12-16-2-25-17-24] and *spa* type t037* [repeat ID, 190-12-16-2-25-17-24; one nucleotide different from t037]; dark gray, *spa* type t002).

The MLST was analyzed in eight MRSA isolates, with two representing *spa* t002 and SCC*mec* type II (NTUH-7811 and NTUH-4075), four representing *spa* t037 (including three SCC*mec* type III [NTUH-420, NTUH-5171, and NTUH-1043] and one undetermined but possible SCC*mec* III, NTUH-4257), one representing *spa* t437 (SCC*mec* type IV; NTUH-194), and one representing t036 (SCC*mec* type IV; NTUH-2803). The sequence type (ST) for four isolates of *spa* type t037 was ST239. The ST of two isolates with *spa* type t002 was ST5. The STs for two isolates containing SCC*mec* type IV were ST59 for

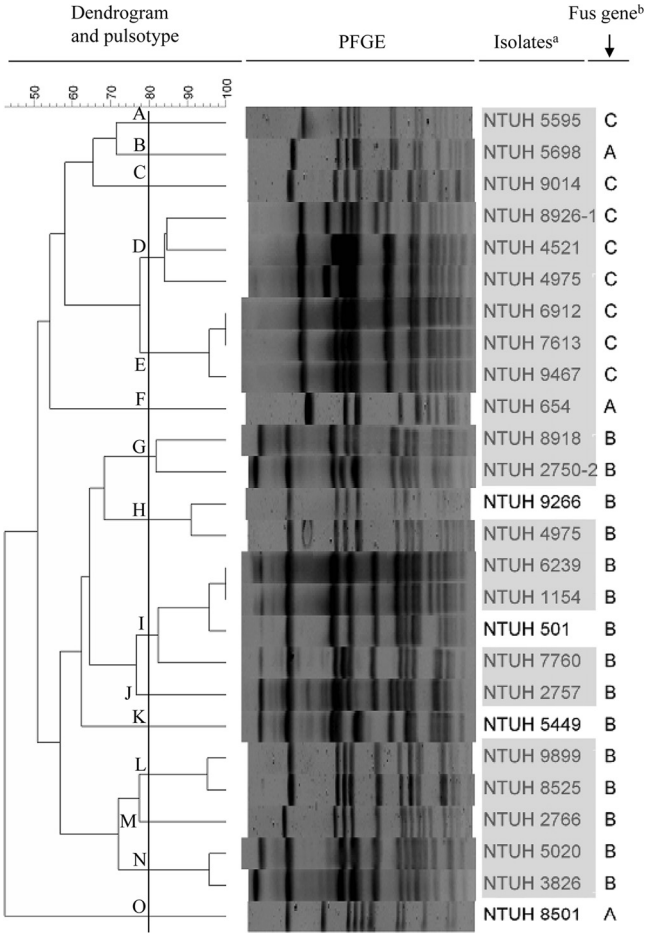

FIG. 2. Genotypes and fusidic acid resistance determinants among 26 fusidic acid-resistant MSSA isolates. The dendrogram was produced by BioNumerics software, showing distance calculated by the Dice similarity index of SmaI-digested DNA fragments. The degree of similarity is shown in the scale. Footnotes: a, light gray, isolates collected from blood specimens; b, resistance determinants to fusidic acid (B, *fusB*; C, *fusC*).

spa type t437 (NTUH-194) and ST254 for *spa* type t036 (NTUH-2803), respectively.

PFGE analysis in MRSA and MSSA. To determine the genetic diversity among 45 fusidic acid-resistant MRSA and 26 MSSA isolates, we performed PFGE and assigned pulsotypes to clusters with >80% similarity with BioNumerics software, based on the Dice similarity index in the dendrogram created by the UPGMA algorithm. The 45 MRSA isolates were divided into 12 clusters (Fig. 1), while 26 MSSA isolates were divided into 15 clusters (Fig. 2). Pulsotype H was the most frequent pulsotype in MRSA (17/45; 38%). Some isolates belonging to a closely related pulsotype carried different *fusA* mutations, such as NTUH-My8703 (P406L) and NTIH-3207 (A71V and P404L) in pulsotype D. Seven MRSA containing *fusC* distributed in three PFGE types (Fig. 1). MSSA isolates containing *fusB* or *fusC* clustered separately (Fig. 2).

DISCUSSION

Previous studies have shown geographical differences in the prevalence of fusidic acid resistance determinants among *S. aureus*. Lannergard et al. reported that approximately equal frequencies of the FusA, FusB, and FusC classes were found in bacteremia isolates of *S. aureus* (19). In U.S. and European collections, *fusC* was more prevalent than *fusB* in *S. aureus* strains (5, 6). However, the previous studies did not compare the difference between MRSA and MSSA. In the present study, we found that the prevalence of fusidic acid resistance determinants was quite different between MRSA and MSSA groups. The predominant determinants in MRSA were *fusA* mutations, followed by *fusC*, and none carried *fusB* determinant. In contrast, most MSSA carried the acquired resistance gene *fusB* followed by *fusC*, with only three isolates showing *fusA* mutations. In our isolates, the *fusA* mutations and acquired FusB-family determinants were not detected in a same strain, which might be due to the inability of different determinants to interact synergistically (27).

In agreement with previous reports, isolates with *fusA* mutations usually displayed higher levels of resistance to fusidic acid (MICs ranging from 4 to >128 μg/ml). Previous reports and the findings presented here indicate that single-amino-acid substitutions in EF-G can lead to high levels of resistance to fusidic acid (for example, H457Y and L461K). The MIC of fusidic acid in MSSA carrying *fusB* ranged from 4 to 32 μg/ml, which was much lower than those in MRSA with *fusA* mutations. The MIC of fusidic acid in MRSA and MSSA isolates carrying *fusC* ranged from 8 to 16 μg/ml, which is similar to findings in other reports (30).

Among 41 isolates (38 MRSA and 3 MSSA) with *fusA* mutations, a total of 22 types of nucleotide changes causing 21 amino acid substitutions were found. Of these, most (14 mutations) occurred in domain III, followed by domain I (5 mutations), one in domain II, and one in domain V. Mutations with M16I, A70V, A71V in domain I, and A376V in domain II have been reported as compensatory mutations in *S. aureus* (24). Nine amino acid changes in domain III have previously been reported to be associated with fusidic acid resistance in *S. aureus* (4, 24), and one (M453I) has been identified in *Salmonella typhimurium* (15). The amino acid substitution, A67T, has been reported as having no impact on fusidic acid resistance or compensation for fitness in *S. aureus* (3). The importance of three mutations of *fusA* (P406L, H457Y, and L461K) for fusidic acid resistance in *S. aureus* has been directly proved by site-directed mutagenesis (4). Among the *fusA* mutations detected in the present study, six (R76C, E444K, E444V, C473S, P478S, and M651I) were first reported (Table 3). Of these, mutations of E444V, E444K, C473S, P478S located in domain III, and M651I located in domain V of EF-G were very possibly a cause of fusidic acid resistance. The amino acid substitution R76C was found to be accompanied with another *fusA* mutation (Table 3). Since A70V and A71V have been reported as fitness compensation mutations, it is possible the R76C play the same role. Amino acid alteration at position 457 (His) replaced by tyrosine has been reported previously to produce a MIC of 64 μg/ml (4). Substitution at His-457 with glutamine had not been reported when we prepared the first version of the manuscript. However, it was just recently published by Castanheira et al. (5, 6). This result indicated that different mutations at the same amino acid position result in different levels of resistance. The role of the six newly found amino acid

substitutions in *fusA* is unknown and needs further investigation.

In agreement with previous reports, the *fusB* genes in our MSSA isolates are located in a genetic structure identical to that of pUB101 (26, 34). However, the MICs of fusidic acid for our MSSA isolates were lower than that for pUB101 isolates (26). Why was the *fusB* gene detected only in MSSA and not in MRSA isolates? PFGE analysis demonstrated the heterogeneity of pulsotypes among the *fusB*-containing MSSA isolates, not in a single clone. However, isolates with *fusB* or *fusC* formed separate clusters. Thus, the horizontal and clonal spreading may have both contributed to the fusidic acid resistance in *S. aureus* isolates.

On the basis of *spa* and SCC*mec* typing, a major genotype, *spa* type t037-SCC*mec* type III (t037-III; 28/45; 62%), corresponding to PFGE types A, D, F, H, I, and J, was found to be dominant in fusidic acid-resistant MRSA, followed by t002-II (13/45; 29%), corresponding to PFGE types B, E, K, and L. The MLST analysis with representatives of different *spa* types indicated that isolates of *spa* t037, t002, t437, and t036 were identified as ST239, ST5, ST59, and ST254, respectively. Previous studies have shown that *spa* type t037 with SCC*mec* type III belonged to ST239 or ST241, while *spa* type t002 with SCC*mec* type II belonged to ST5 (21). ST239-SCC*mec* type III (ST239-III) or ST241-III, which was known as the Brazil/Hungary clone, and ST5-II, which was known as the New York/Japan clone, were common in Asia (1, 16). In general, the MRSA isolates with *fusA* mutations or carrying *fusC* were distributed among diverse PFGE types. The MSSA isolates containing *fusB* or *fusC* formed separate clusters. However, different types of *fusA* mutations could be found in MRSA isolates with identical SCC*mec* type, *spa* type, and closely related PFGE types (found in pulsotypes A, D, E, H, and K), suggesting that they originate from the same clone but were independently selected by antibiotic pressure.

In conclusion, different resistance determinants were responsible for fusidic acid resistance in MRSA and MSSA isolates. Resistance to fusidic acid in MRSA was associated mostly with *fusA* point mutations. The acquired FusB-family determinants were responsible for fusidic acid resistance in MSSA. Phylogenetic analysis indicated that fusidic acid-resistant MRSA and MSSA belonged to different lineages.

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