AAC Journals.ASM.org

A Novel Staphylococcal Cassette Chromosomal Element, SCC*fusC*, Carrying *fusC* and *speG* in Fusidic Acid-Resistant Methicillin-Resistant *Staphylococcus aureus*

Yu-Tzu Lin,[a] Jui-Chang Tsai,[b,c] Hsiao-Jan Chen,[a] Wei-Chun Hung,[a] Po-Ren Hsueh,[d] Lee-Jene Teng[a,d]

Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University College of Medicine, Taipei, Taiwan[a]; Center for Optoelectronic Medicine, National Taiwan University College of Medicine, Taipei, Taiwan[b]; Department of Surgery, Division of Neurosurgery, National Taiwan University Hospital, Taipei, Taiwan[c]; Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan[d]

A high prevalence of *fusC* (16/46, 59%) was found in fusidic acid-resistant methicillin-resistant *Staphylococcus aureus* isolates collected from 2008 to 2010. Nucleotide sequencing of *fusC* and flanking regions revealed a novel staphylococcal cassette chromosome (SCC) structure, SCC*fusC*, which was integrated into *rlmH* and located upstream from SCC*mec*. The SCC*fusC* element contained *speG*, which may contribute to the polyamine resistance.

Fusidic acid is an antibiotic that is used in several countries, including Taiwan, to treat infections caused by *Staphylococcus aureus*, mainly skin and soft tissue infections (1, 2). However, fusidic acid is not licensed in the United States. Resistance to fusidic acid in *S. aureus* has been reported, and the frequency of fusidic acid resistance in *S. aureus* has increased (3). We previously identified the fusidic acid resistance determinants in 45 methicillin-resistant *S. aureus* (MRSA) and 26 methicillin-susceptible *S. aureus* (MSSA) isolates collected from 2002 to 2007 and found that the *fusA* mutation was the major determinant responsible for resistance in MRSA (4). However, another group in central Taiwan found that *fusC* was the predominant determinant (5). To obtain more-up-to-date information about the prevalence of resistance determinants in MRSA, we examined the 46 fusidic acid-resistant (MIC, ≥2 μg/ml) isolates of MRSA collected during a 3-year period (2008 to 2010) by the Bacteriology Laboratory, National Taiwan University Hospital. The isolates were reconfirmed as MRSA on the basis of detection of the *nuc* and *mecA* genes by PCR (6).

The fusidic acid resistance determinants were detected by PCR as previously described (4). Our results indicated that 19 (41%) isolates had *fusA* mutations and 27 (59%) carried the *fusC* gene (Table 1). Compared to the occurrence of *fusC* in our previous report, the prevalence of *fusC* has increased among the fusidic acid-resistant MRSA isolates (4, 5).

The 46 isolates were genotyped by pulsed-field gel electrophoresis (PFGE) (7), *spa* typing (8–10), multilocus sequence typing (MLST) (11), and staphylococcal cassette chromosome *mec* (SCC*mec*) typing (4), and the results are shown in Fig. 1. The PFGE analysis divided the 46 MRSA isolates into five clusters, and the majority of the isolates (44/46; 96%) were clustered into three pulsotypes, A, B, and D (Fig. 1). All but one of the isolates carrying the *fusC* gene were clustered in pulsotype B, suggesting clonal expansion. When the results of *spa* typing, MLST analysis, and SCC*mec* typing were combined, two major genotypes were found (shown as *spa* type [t]-sequence type [ST]-SCC*mec* type): t037-238-III (38/46 [83%], pulsotype A and B) and t002-ST5-II (5/46 [11%], pulsotype D). We also found that all of the *fusC*-carrying MRSA isolates carried SCC*mec* type III and belonged to ST239, which is also the most prevalent health care-associated MRSA (HA-MRSA) clone in Taiwan (4, 5). Thus, the increased prevalence of *fusC* may reflect an increased prevalence of ST239.

TABLE 1 Distribution of fusidic acid MIC and resistance determinants among the fusidic acid-resistant MRSA isolates

Resistance determinant (no. of isolates with determinant)	No. of isolates with fusidic acid MICs (μg/ml) of:		
	2–16	32–64	≥128
fusA mutation (19)	4	5	10
fusC (27)	27	0	0
Total (46)	31	5	10

We subsequently determined the sequence of *fusC* and its flanking regions from a representative isolate, NTUH-4729, by arbitrary PCR (12) and inverse PCR (13). All nucleotide sequencing was performed at the Second Core Laboratory of NTUH using the ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA). The *fusC* gene has previously been found in association with three SCC elements, SCC_{476} (lacking *mecA*) in an MSSA isolate (14), $SCCmec_{N1}$ (carrying *mecA*), and pseudo-SCC*mec*-SCC_{CRISPR} (carrying a clustered regularly interspaced short palindromic repeat [CRISPR] region and *mecA*) in MRSA isolates (15, 16). Our sequence data revealed that the *fusC* gene was located on a novel SCC structure, which was different from SCC_{476} and was named SCC*fusC* (Fig. 2). SCC*fusC*, located upstream from SCC*mec* type III, was 26.5 kb long, composed of 23 putative open reading frames (ORFs), had a GC content of 30.4%, and was integrated into the *attB*scc sequence at the 3′ end of the *rlmH* gene (previously called *orfX*) (Fig. 2).

We used PCR with 21 pairs of primers covering the whole SCC*fusC* region (Fig. 2; see also Table S1 in the supplemental

Received 16 August 2013 Returned for modification 13 October 2013
Accepted 21 November 2013
Published ahead of print 25 November 2013
Address correspondence to Lee-Jene Teng, ljteng@ntu.edu.tw.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/AAC.01772-13.

doi:10.1128/AAC.01772-13

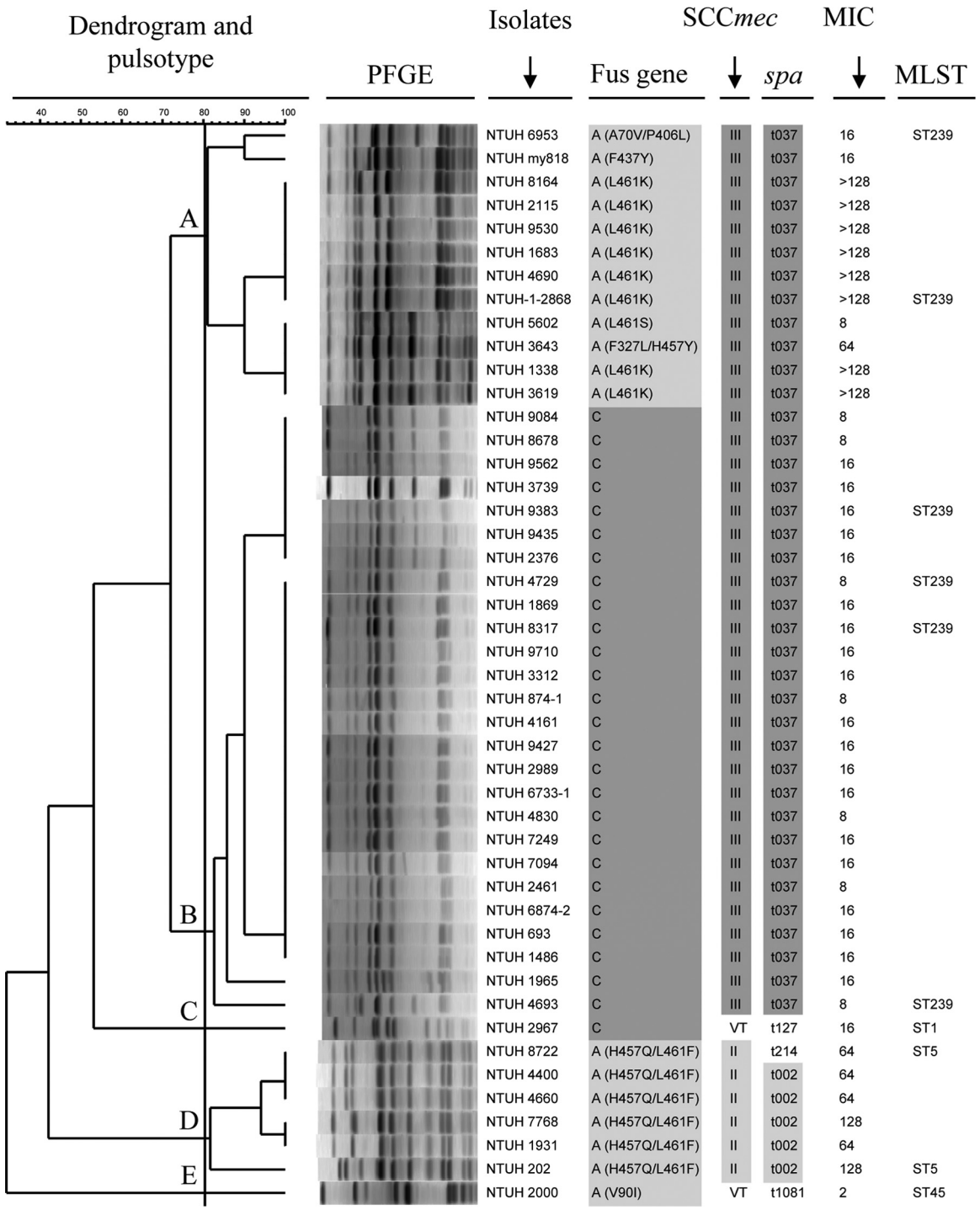

FIG 1 Characteristics of the 46 fusidic acid-resistant MRSA isolates. The dendrogram was generated by using BioNumerics software with the Dice similarity coefficient and unweighted-pair group method (UPGMA) based on the PFGE profiles. The degree of similarity is shown in the scale. To the right are shown Fus gene determinants for resistance to fusidic acid (A, *fusA* point mutation; C, *fusC* determinant), SCC*mec* types (VT, V_T), *spa* types, fusidic acid MICs (μg/ml), and MLST results for selected isolates representing each *spa* type.

material) to detect the prevalence of the SCC*fusC* element in the 26 remaining *fusC*-carrying MRSA isolates. The results showed that all but one (26/27) of the *fusC*-carrying MRSA isolates carried SCC*fusC* and that all isolates belonged to ST239/SCC*mec* type III. One isolate (NTUH-2967) that possessed SCC*mec* type V_T lacked the 5′ region of SCC*fusC* in the *fusC*-carrying structure and was more similar to SCC_{476}.

A comparison between the nucleotide sequences of SCC*fusC* and SCC_{476} indicated that their 3′ regions were very similar (Fig. 2). Both SCC*fusC* and SCC_{476} carry the *ccrA1B1* and *fusC*

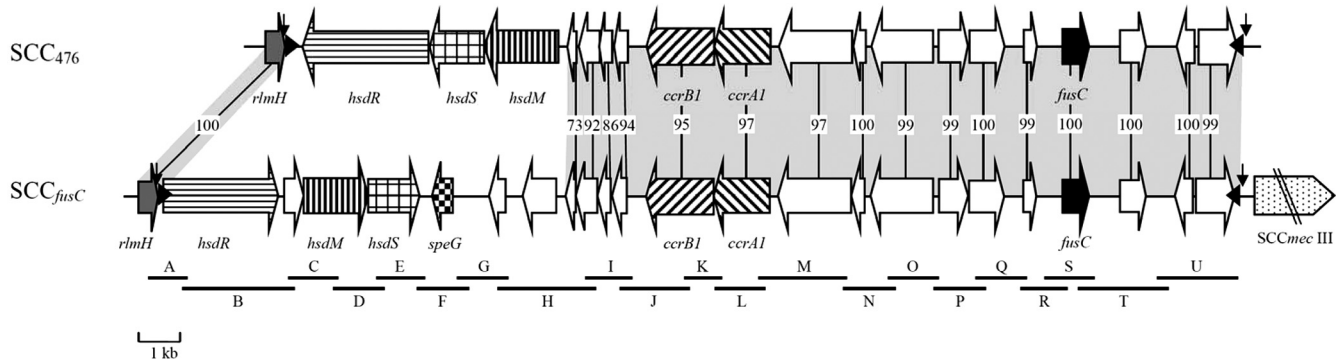

FIG 2 Genetic organization of SCC*fusC* (GenBank accession no. KF527883) compared with that of SCC_{476} (GenBank accession no. NC_002953.3). The patterned arrows represent the putative open reading frames. The genes are drawn according to their sequences and function. *hsdR*, *hsdM*, and *hsdS* encode the R, M, and S subunits of the type I restriction-modification system. *speG* encodes spermidine acetyltransferase. White arrows represent other genes encoding hypothetical proteins. The integration site sequences (ISS) are indicated by the vertical arrows. The inverted repeat sequences are indicated by the thin horizontal arrows. The homologous regions between the two SCCs are shown by shading, and the numbers in the shading indicate the percentages of homology between the corresponding sequences. The horizontal bars at the bottom represent the PCR products used to confirm the presence of the SCC*fusC* element. The identities of *hsdR*, *hsdM*, and *hsdS* between the two elements were below 45%.

genes, and the identities of the 12 ORFs on the 3′ end (from *ccrB1* to the left) were >95%. However, the ORFs on the 5′ end of these two SCCs were much more divergent. Both of the SCCs contain type I restriction-modification system genes, but the directions of their ORFs were different and the sequence identities were low (Fig. 2). Most importantly, SCC*fusC* contains four additional ORFs, including the *speG* gene, which encodes spermidine acetyltransferase.

To detect the presence of the *speG* gene in other *S. aureus* isolates, PCR targeting the *speG* gene was performed with the specific primers speG_7F (5′-CTAAGAGCATTAGAGTATAGTG-3′) and speG_408R (5′-TGTTTTAAATCCTTGTGACTCG-3′). All of the 26 SCC*fusC*-carrying MRSA isolates were *speG* positive. However, the *speG* gene was not found in any *fusC*-negative MRSA isolate or the *fusC*-positive MSSA isolates (data not shown). To detect whether the *speG* gene in SCC*fusC* is functional or not, we determined the MICs of two polyamines, spermine (Spm) and spermidine (Spd), and found that isolates carrying SCC*fusC* had higher MICs for Spm (8 or 16 mM) and Spd (16 mM). Isolates without SCC*fusC* had much lower MICs for Spm (0.5, 1, or 2 mM) and slightly lower MICs for Spd (4 or 8 mM). Polyamines may contribute to anti-inflammation and the promotion of cell proliferation and tissue regeneration (17). It has been reported that in resolving *S. aureus* abscesses, the amounts of polyamines are significantly higher in infected skin tissue than in infected organ tissue (18). *S. aureus* usually lacks the ability to synthesize polyamine and exhibits hypersensitivity toward physiological Spm and Spd concentrations (19). So far, community-associated MRSA (CA-MRSA) USA300 is the only *S. aureus* clone that is resistant to polyamines, due to the presence of arginine catabolic mobile element (ACME)-encoded spermidine acetyltransferase, SpeG (19). In the present study, we found that the *fusC*-carrying structure, SCC*fusC*, also carried a putative virulence gene, *speG* (Fig. 2; see also Table S2 in the supplemental material). The acquisition of both resistance and virulence genes may provide an advantage for bacterial survival in the host.

Conclusions. The *fusC* gene has contributed to fusidic acid resistance in MRSA ST239 in Taiwan in the period from 2008 to 2010. We found that the *fusC* gene in MRSA was located in a novel SCC element, SCC*fusC*, which also carried a virulence gene, *speG*. This is the first report of the presence of *speG* in ST239 HA-MRSA. SCC*fusC* contributed not only to the fusidic acid resistance but also to the polyamine resistance in MRSA.

Nucleotide sequence accession number. The nucleotide sequence of the SCC*fusC* element harbored by isolate NTUH-4729 has been deposited in GenBank under accession number KF527883.

ACKNOWLEDGMENT

This work was supported by grant NSC 100-2320-B-002-014-MY3 from the National Science Council of Taiwan.

REFERENCES

1. **Chen YS.** 2013. Guidelines for the treatment of methicillin-resistant *Staphylococcus aureus* infections in Taiwan. J. Microbiol. Immunol. Infect. **46:**147–150. http://dx.doi.org/10.1016/j.jmii.2013.04.010.
2. **Dobie D, Gray J.** 2004. Fusidic acid resistance in *Staphylococcus aureus*. Arch. Dis. Child. **89:**74–77. http://dx.doi.org/10.1136/adc.2003.019695.
3. **Wang JL, Tang HJ, Hsieh PH, Chiu FY, Chen YH, Chang MC, Huang CT, Liu CP, Lau YJ, Hwang KP, Ko WC, Wang CT, Liu CY, Liu CL, Hsueh PR.** 2012. Fusidic acid for the treatment of bone and joint infections caused by meticillin-resistant *Staphylococcus aureus*. Int. J. Antimicrob. Agents **40:**103–107. http://dx.doi.org/10.1016/j.ijantimicag.2012.03.010.
4. **Chen HJ, Hung WC, Tseng SP, Tsai JC, Hsueh PR, Teng LJ.** 2010. Fusidic acid resistance determinants in *Staphylococcus aureus* clinical isolates. Antimicrob. Agents Chemother. **54:**4985–4991. http://dx.doi.org/10.1128/AAC.00523-10.
5. **Chen CM, Huang M, Chen HF, Ke SC, Li CR, Wang JH, Wu LT.** 2011. Fusidic acid resistance among clinical isolates of methicillin-resistant *Staphylococcus aureus* in a Taiwanese hospital. BMC Microbiol. **11:**98. http://dx.doi.org/10.1186/1471-2180-11-98.
6. **Brakstad OG, Aasbakk K, Maeland JA.** 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. J. Clin. Microbiol. **30:**1654–1660.
7. **Murray BE, Singh KV, Heath JD, Sharma BR, Weinstock GM.** 1990. Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. J. Clin. Microbiol. **28:**2059–2063.
8. **Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN.** 1999. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J. Clin. Microbiol. **37:**3556–3563.

9. **Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, Kreiswirth BN.** 2004. *spa* typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. J. Clin. Microbiol. **42:**792–799. http://dx.doi.org/10.1128/JCM.42.2.792-799.2004.
10. **Harmsen D, Claus H, Witte W, Rothganger J, Turnwald D, Vogel U.** 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. J. Clin. Microbiol. **41:**5442–5448. http://dx.doi.org/10.1128/JCM.41.12.5442-5448.2003.
11. **Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.
12. **O'Toole GA, Kolter R.** 1998. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Mol. Microbiol. **28:**449–461. http://dx.doi.org/10.1046/j.1365-2958.1998.00797.x.
13. **Zimmermann A, Lüthy J, Pauli U.** 2000. Event specific transgene detection in Bt11 corn by quantitative PCR at the Integration Site. Lebensm.-Wiss. Technol. **33:**210–216. http://dx.doi.org/10.1006/fstl.2000.0637.
14. **Holden MT, Feil EJ, Lindsay JA, Peacock SJ, Day NP, Enright MC, Foster TJ, Moore CE, Hurst L, Atkin R, Barron A, Bason N, Bentley SD, Chillingworth C, Chillingworth T, Churcher C, Clark L, Corton C, Cronin A, Doggett J, Dowd L, Feltwell T, Hance Z, Harris B, Hauser H, Holroyd S, Jagels K, James KD, Lennard N, Line A, Mayes R, Moule S, Mungall K, Ormond D, Quail MA, Rabbinowitsch E, Rutherford K, Sanders M, Sharp S, Simmonds M, Stevens K, Whitehead S, Barrell BG, Spratt BG, Parkhill J.** 2004. Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. Proc. Natl. Acad. Sci. U. S. A. **101:**9786–9791. http://dx.doi.org/10.1073/pnas.0402521101.
15. **Ender M, Berger-Bachi B, McCallum N.** 2007. Variability in SCC*mec*$_{N1}$ spreading among injection drug users in Zurich, Switzerland. BMC Microbiol. **7:**62. http://dx.doi.org/10.1186/1471-2180-7-62.
16. **Kinnevey PM, Shore AC, Brennan GI, Sullivan DJ, Ehricht R, Monecke S, Slickers P, Coleman DC.** 2013. Emergence of sequence type 779 methicillin-resistant *Staphylococcus aureus* harboring a novel pseudo staphylococcal cassette chromosome *mec* (SCC*mec*)-SCC-SCC$_{CRISPR}$ composite element in Irish hospitals. Antimicrob. Agents Chemother. **57:**524–531. http://dx.doi.org/10.1128/AAC.01689-12.
17. **Curran JN, Winter DC, Bouchier-Hayes D.** 2006. Biological fate and clinical implications of arginine metabolism in tissue healing. Wound Repair Regen. **14:**376–386. http://dx.doi.org/10.1111/j.1743-6109.2006.00151.x.
18. **Thurlow LR, Joshi GS, Clark JR, Spontak JS, Neely CJ, Maile R, Richardson AR.** 2013. Functional modularity of the arginine catabolic mobile element contributes to the success of USA300 methicillin-resistant *Staphylococcus aureus*. Cell Host Microbe **13:**100–107. http://dx.doi.org/10.1016/j.chom.2012.11.012.
19. **Joshi GS, Spontak JS, Klapper DG, Richardson AR.** 2011. Arginine catabolic mobile element encoded *speG* abrogates the unique hypersensitivity of *Staphylococcus aureus* to exogenous polyamines. Mol. Microbiol. **82:**9–20. http://dx.doi.org/10.1111/j.1365-2958.2011.07809.x.