Profiles of Non-*aureus* staphylococci in retail pork and slaughterhouse carcasses: prevalence, antimicrobial resistance, and genetic determinant of fusidic acid resistance

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Running title: Fusidic acid resistance in staphylococci from pork production chains

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Abstracts

As commensal colonizers in livestock, there has been little attention on staphylococci, especially non-*aureus* staphylococci (NAS), contaminating meat production chain. To assess prevalence of staphylococci in retail pork and slaughterhouse carcass samples in Korea, we collected 578 samples from Korean slaughterhouses (n = 311) and retail markets (n = 267) for isolation of staphylococci and determined antimicrobial resistance phenotypes in all the isolates. The presence of and prevalence of *fusB*-family genes (*fusB, fusC, fusD,* and *fusF*) and mutations in *fusA* genes were examined in fusidic acid resistant isolates. A total of 47 staphylococcal isolates of 4 different species (*S. aureus*, n = 4; *S. hyicus*, n = 1; *S. epidermidis*, n = 10; *M. sciuri*, n = 32) were isolated. Fusidic acid resistance were confirmed in 9/10 *S. epidermidis* and all of the 32 *Mammaliicoccus sciuri* (previously *Staphylococcus sciuri*) isolates. Acquired fusidic acid resistance genes were detected in all the resistant strains; *fusB* and *fusC* in *S. epidermidis* and *fusB/C* in *M. sciuri*. MLST analysis revealed that ST63 (n =10, 31%) and ST30 (n =8, 25%) genotypes were most prevalent among fusidic acid resistant *M. sciuri* isolates. In conclusion, the high prevalence of *fusB*-family genes in *S. epidermidis* and *M. sciuri* strains isolated from pork meat indicated that NAS might act as a reservoir for fusidic acid resistance gene transmissions in pork production chains.

224/250 words

Keywords: Non-*aureus* staphylococci, antimicrobial resistance, retail pork, slaughterhouse carcass

Introduction

Staphylococci are frequent inhabitants of the normal microbiota of skin and mucous surfaces in humans and animals (Davis, 1996). Although less frequent than the coagulase-positive staphylococci such as *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS) can cause many nosocomial and community-associated infections including skin and soft tissue infections (SSTIs), urinary tract infections, endocarditis, and blood stream infections (Piette et al., 2009). Moreover, several recent studies have reported the potential role of non-*aureus* staphylococci (NAS) in transmission of antimicrobial resistance by acting as a reservoir for antimicrobial resistance genes (Archer et al., 1994; Nemeghaire et al., 2014a).

Fusidic acid is a bacteriostatic steroid antibiotic originated from *Fusidium coccineum*, previously used to treat staphylococcal skin infections since the early 1960s (Godtfredsen et al., 1962). It targets elongation factor G (EF-G) that functions as ribosomal translocase and interact with ribosomal recycling factor to release ribosomal complexes (Fernandes, 2016). However, fusidic acid resistance can occur due to spontaneous mutations in *fusA*, which encodes EF-G (Turnidge et al., 1999). Point mutations in *fusE*, encoding ribosomal protein L6, are also associated with fusidic acid resistance in staphylococci (Norström et al., 2007). In addition, FusB-family proteins bind to EF-G and protect them from fusidic acid binding (O'Neill et al., 2006). The FusB-family proteins are produced by the *fusB*, *fusC*, *fusD*, and *fusF* genes and frequently mediate low-level resistance to fusidic acid (Fernandes, 2016). These *fusB*-family genes have been reported in *S. aureus* and NAS isolates, either carried on a plasmid, phage-associated resistance islands, or staphylococcal cassette chromosome (SCC) (O'Neill et al., 2006; O'Neill et al., 2007; Chen et al., 2015). It has been well known

89 that *Staphylococcus saprophyticus* and *Staphylococcus cohnii* subsp. *urealyticus* possess
90 *fusD* and *fusF* genes for their intrinsic fusidic acid resistance (Chen et al., 2015; O'Neill et
91 al., 2007).

92 The significance of food-producing animals as carriers of foodborne zoonotic
93 pathogens and antimicrobial resistance genes has been demonstrated in many countries
94 including Korea (Hung et al., 2015; Nam et al., 2011; Nemeghaire et al., 2014a). In contrast
95 to coagulase-positive *S. aureus*, well recognized as a major causative pathogen of
96 staphylococcal food poisoning and antimicrobial resistance, occurrence of NAS in foods of
97 animal origin, their antimicrobial resistance phenotypes, and the genetic factors associated
98 with the resistant phenotypes have not been well investigated. Therefore, we aimed to
99 investigate i) the profiles of NAS in pork and carcass samples collected from retail markets
100 and slaughterhouses in Korea, ii) the antimicrobial resistance phenotypes of the
101 staphylococcal isolates, and iii) the occurrence and distribution of *fusB*-family genes (*fusB*,
102 *fusC*, *fusD*, and *fusF*), and iv) the point mutations in *fusA* genes by sequencing analyses.
103

Materials and Methods

Sample collection and isolation of staphylococci

We obtained a total of 578 swab or pork meat samples from seven slaughterhouses (311 carcass samples) and 35 retail markets (267 pork meat samples) across eight Korean provinces in 2018. Slaughterhouse carcass samples were obtained from a single visit at seven different slaughterhouses: Gyeonggi (46 swabs), Gangwon (46 swabs), Chuncheong (46 swabs), Jeolla (two slaughterhouses, 40 and 42 swabs), and Gyeongsang (two slaughterhouses, 45 and 46 swabs). Each carcass swab was prepared on an area of (10×10 cm) per site on the back and chest of pig carcasses within 8 h of slaughter. Fresh pork meat samples were collected from four to five retail markets in each province. All samples were kept at 4℃ and processed for isolation of staphylococci within 24 h of collection.

Swab samples from slaughterhouses were inoculated into 6 mL of tryptic soy broth (TSB; Difco Laboratories, Detroit, MI, USA) containing 10% sodium chloride (NaCl) for enrichment at 37℃. Each pork meat sample (25 g) was homogenized in 225 ml of 10% NaCl-TSB. After overnight incubation, 15 μl aliquots of the pre-enriched NaCl-TSB cultures were streaked onto Baired-Parker agar (BPA\; Difco Laboratories) supplemented with potassium tellurite and egg yolk, and then grown at 37℃ for up to 48h. Next, up to two presumptive staphylococcal colonies from each plate were re-streaked on BPA plate for identification. For genomic DNA isolation, individual isolates were inoculated into fresh TSB, cultured at 37℃ for 18 - 24h, and bacterial cell pellets were subjected to the Genmed DNA extraction kit (Genmed, Seoul, Korea) according to the manufacturer's protocols. Identification of staphylococcal species was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS; Bruker Daltonik,

Bremen, Germany) and 16S rRNA sequencing. For bacterial identification using MALDI-Biotyper Realtime Classification system, presumptive staphylococci were placed on a target plate coated with specific energy-absorbent agent, the matrix. The sample within the matrix was then ionized in an automated mode with a laser beam as recommended by the manufacturer. Next, peptides in bacterial sample converted into protonated ions and the peptide mass fingerprints were used to identify bacterial species based on the spectral database (Bruker Daltonics, MALDI Biotyper 3.1). Score values ≥2.0 were used for an identification of staphylococcal species. For 16S rRNA sequencing analyses, 16S rRNA genes were PCR-amplified using a universal primer set (16S_27F: 5'- AGA GTT TGA TCC TGG CTC AG -3' and 16S_1492R: 5'- TAC GGY TAC CTT GTT ACG ACT T - 3') (De Lillo et al., 2006). PCR amplifications were performed as follows: denaturation at 94℃ for 30 s followed by 28 cycles of 94℃ for 30 s, 52℃ for 30 s, and 72℃ for 1 min. After a final 10 min extension at 72℃, the samples were purified using PCR purification kit (Bionics, Seoul, Korea), and then sequenced at Bionics.

Antimicrobial susceptibility assays

Standard disc diffusion methods were used to determine the antimicrobial susceptibility of each isolate according to Clinical and Laboratory Standards Institute's (CLSI) recommendations for the following antimicrobial agents: penicillin (PEN, 10 μg), ampicillin (AMP, 10 μg), cefoxitin (FOX, 30 μg), chloramphenicol (CHL, 30 μg), clindamycin (CLI, 2 μg), erythromycin (ERY, 15 μg), fusidic acid (FA, 10 μg), ciprofloxacin (CIP, 5 μg), mupirocin (MUP, 200 μg), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 μg) tetracycline (TET, 30 μg), rifampicin (RIF, 5 μg), gentamycin (GEN,

10 μg), quinupristin/dalfopristin (SYN, 15 μg). The minimum inhibitory concentrations (MICs) to fusidic acid (liofilchem, Roseto degli Abruzzi, Italy) and oxacillin (OXA; bioMérieux, Marcy l'Etoile, France) were determined by standard E-test. OXA E-test was performed on MH BBL II agar (Becton Dickinson, Sparks, MD, USA) supplemented with 2% NaCl. The breakpoint for fusidic acid resistance (> 1 μg/ml) was based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST, 2021) guidelines. The *S. aureus* ATCC 25923 strain was included as a reference for the antimicrobial susceptibility analyses.

mecA detection and SCC*mec* type determination

All the strains showing resistance to oxacillin or cefoxitin were examined for the presence of *mecA* gene using the PCR method as described previously (Geha et al., 1994)

The *mecA* positive staphylococcal strains were subjected to SCC*mec* typing as previously described (Kondo et al., 2007). A series of multiplex PCR reactions were employed to amplify *mec* regulatory elements (*mec*) and chromosomal cassette recombinase (*ccr*) genes. The combinations *mec* complexes and *ccr* types were used to determine the SCC*mec* types of the staphylococcal strains. The PCR was preceded as previously described (Kondo et al., 2007).

Detection of fusidic acid resistance determinants

The carriage of acquired fusidic acid resistance genes *fusB, fusC*, *fusD*, and *fusF* were detected by PCR methods as described before (Chen et al., 2015, 2010; Mclaws et al., 2008). The primers used to amplify *fusB*-family genes are listed in Table 1. For detection of *fusB*-family gene homologues in *Mammaliicoccus sciuri* (previously *Staphylococcus sciuri*) strains,

a specific primer set was designed based on the published sequences of 7 *M. sciuri* strains (https://www.ncbi.nlm.nih.gov) carrying the *fusB/C*-family genes (Table 1). The PCR conditions for detecting *fusB/C* in *M. sciuri* were as follows: denaturation at 95℃ for 2 min, followed by 28 cycles of denaturation at 94℃ for 35 s, annealing at 53℃ for 40 s, extension at 72℃ for 45 s, and final extension at 72℃ for 10 min.

To detect point mutations within *fusA* genes in fusidic acid resistant isolates, DNA sequencing analyses were performed. Using the specific primer set (AF and AR) as shown in Table 1, *fusA* gene was amplified from genomic DNA samples purified from *Staphylococcus epidermidis* and *M. sciuri* strains. The PCR amplicons were sequenced with AF and two additional primers A-S1 and A-S2 at Bionics (Seoul, Korea). The *fusA* sequence data were then compared with the published sequences of *S. epidermidis* (GenBank: NZ_CP035288.1) and *M. sciuri* (GenBank: CP071138.1).

Multi-locus sequence type (MLST) analysis

Except one *Staphylococcus hyicus* strain, whose MLST scheme has not yet been developed, MLST was performed on all *S. aureus*, *S. epidermidis*, and *M. sciuri* isolates as described previously (Enright et al., 2000; Schauer et al., 2021; Thomas et al., 2007). For MLST analyses, internal fragments of seven housekeeping genes from each strain were PCR amplified and sequenced. The seven genes amplified from each species of staphylococci are: *arcC, aroE, gmk, glpF, pta, tpi,* and *yqiL* for *S. aureus* (Enright et al., 2000); *arcC, aroE, gtr, pyrR, mutS, yqiL,* and *tpiA* for *S. epidermidis* (Thomas et al., 2007); *ack, aroE, glpK, ftsZ, gmk, pta1,* and *tpiA* for *M. sciuri* (Schauer et al., 2021). The alleles and sequence types (STs) of each staphylococcal species were assigned according to the MLST databases (https://www.pubmlst.org/).

Results

Profiles of staphylococci isolated from pork meat and carcass samples

As presented in Table 2, 44 staphylococci (44/311, 14.1%) of four different species were isolated from slaughterhouse carcass samples, and only three strains of *M. sciuri* (3/267, 1.1%) were isolated from retail pork samples obtained over the 10-month study period. The most frequent staphylococci identified in the carcasses were *M. sciuri* (previously *S. sciuri*), comprising ~66% (29/44) of the staphylococcal isolates from slaughterhouse samples. While four different species (*S. aureus*, *S. hyicus*, *S. epidermidis*, and *M. sciuri*) were identified in the slaughterhouse samples, only three strains of *M. sciuri* were cultured from retail pork meat samples. All of the 47 isolates used in this investigation were obtained from different carcass or meat samples.

MLST analyses of the *S. aureus* and *S. epidermidis* isolates revealed four and six different sequence types (STs), respectively, with four non-typeable *S. epidermidis* strains (Fig. 1). The 32 strains of *M. sciuri* were assigned to three different STs: ST63 (n = 10, 31.3%), ST30 (n = 8, 3%), and ST96 (n = 1, 3.1%) with 13 non-typeable strains under the current *M. sciuri* MLST scheme.

Methicillin-resistant staphylococci in retail pork and pork carcass

Seven methicillin-resistant staphylococci, two methicillin-resistant *S. aureus* (MRSA) and five methicillin-resistant *S. epidermidis* (MRSE) strains, were identified among the 47 staphylococcal isolates, indicating 14.9% methicillin resistance prevalence (Table 2). All seven methicillin-resistant staphylococcal strains were *mecA* positive and exhibited resistant phenotype to OXA, OXA MIC ≥ 4μg/ml (*S. aureus*), OXA MIC ≥ 0.5μg/ml (*S. epidermidis*)

(Table 3). Each of the five MRSE strains were assigned to five different sequence types (STs) of ST172, ST130, ST80, ST173, and ST768. The two MRSA strains were ST398 with SCC*mec* V and ST2084 with SCC*mec* IV, respectively (Table 2).

Although all 32 *M. sciuri* strains showed OXA MICs of ≥ 0.5 μg/ml, none of the strains were cefoxitin-resistant (Table 3). Furthermore, all the *M. sciuri* strains were negative for the *mecA* gene.

Antimicrobial resistance profiles

All 47 isolates were susceptible to rifampin, gentamicin and quinupristin-dalfopristin (Table 4). Multidrug resistance was observed in 17 staphylococcal isolates (36.2%), which showed resistance to ≥ 3 different antimicrobials agent classes. As shown in Table 4, 41/47 (87.2%) isolates were resistant to fusidic acid, displaying the highest frequency of resistance for fusidic acid. Among the 47 isolates, 9/10 (90%) *S. epidermidis* strains and all 32 *M. sciuri* strains were fusidic acid-resistant. However, all the fusidic acid resistant *S. epidermidis* and *M. sciuri* strains displayed fusidic acid MICs ranging from 6 - 24 μg/ml, indicating low-level resistance (Fernandes, 2016).

Genetic determinants of fusidic acid resistance

All *S. epidermidis* and *M. sciuri* isolates showing resistance phenotype to fusidic acid were examined for the presence of *fusB*, *fusC*, *fusD*, and *fusF*. All nine fusidic acid-resistant *S. epidermidis* strains were *fusB* positive, with one carrying the *fusC* gene (Table 3). Similarly, all 32 *M. sciuri* isolates were positive for a *fusB*-family homolog, *fusB/C*. None of the fusidic acid resistant isolates were carrying *fusD* nor *fusF*.

247 Besides the *fusB*-family genes, point mutations within the *fusA* open reading frame
248 (ORF) encoding EF-G have been associated with fusidic acid resistance (Turnidge et al.,
249 1999). As shown in Table 3, a V599I mutation was confirmed in the *fusA* in only one of the
250 *S. epidermidis* (SSE9) isolates. No mutation was identified in the *fusA* gene of *M. sciuri*
251 isolates from the sequencing analyses.
252
253

Discussion

High prevalence of antimicrobial resistance is a significant threat to public health as it undermines treatment options for bacterial infections (Sugden et al., 2016). Since staphylococci are frequently associated with commensal microbiota of skin and mucous surface of various food-producing animals, the development and spread of antimicrobial resistance among staphylococci in the food chain is considered an important threat to food safety (Founou et al., 2016). While coagulase-positive *S. aureus* has been well investigated for its ability to develop antimicrobial resistance and zoonotic potentials to infect human and animal hosts, relatively few studies have focused on the role of NAS, such as CoNS, in antimicrobial resistance development and transmission. Indeed, several recent studies demonstrated that the antimicrobial resistance in CoNS has been increasing over the past decades (Piette et al., 2009), and they act as reservoir for resistance genes that can be transferred to other bacteria (Von Wintersdorff et al., 2016).

In this study, we assessed prevalence of staphylococci in retail pork meat and slaughterhouse carcass samples collected from eight provinces of Korea. Overall, the prevalence of staphylococci in the retail pork and slaughterhouse carcasses was 1% and 16.5%, respectively. As shown in Table 2, only 4/44 (9.1%) staphylococci from slaughterhouse carcass samples were *S. aureus*, and this high proportion of NAS over *S. aureus* was similar to previous report (Fijałkowski et al., 2016). Among the three different species of NAS from slaughterhouses, *M. sciuri* displayed the highest prevalence (65.9%) followed by *S. epidermidis* (22.7%). While previous studies reported much higher levels of *S. aureus* prevalence in retail pork meat samples in China (18.6%) (Wu et al., 2018), Denmark (60%) (Tang et al., 2017), and USA (16-66%) (Hanson et al., 2011; O'Brien et al., 2012), no *S. aureus* was detected from retail pork meat samples in this study. At least several factors

279 such as sample treatment, enrichment/isolation method, and geographical location may have
280 affected the differences in prevalence of *S. aureus* and other staphylococci. It should also be
281 noted that the use of different sampling methodology (swab samples on ~100 cm^2 surface
282 versus 25g of pork meats) to isolate staphylococci from slaughterhouse carcass samples and
283 retail pork meat samples would have affected the overall prevalence and proportion of each
284 species presented in this investigation.

285 The occurrence and prevalence of antimicrobial-resistant NAS in retail pork and
286 slaughterhouse carcass samples have not been well investigated in Korea. Recent reports of
287 methicillin-resistant CoNS from food-producing animals have raised concerns regarding
288 transmission of these antimicrobial-resistant staphylococci through the meat production chain
289 (Huber et al., 2011; Nemeghaire et al., 2014b). In this study, 7/44 (15.9%) staphylococci
290 from slaughterhouse carcass samples were methicillin-resistant staphylococci (two MRSA
291 and five MRSE strains) (Table 2). Out of the two MRSA strains, only one strain of *S. aureus*
292 (SSA1) was ST398 carrying SCC*mec* V, which has been frequently reported *S. aureus*
293 genotype in pigs and pork meat worldwide (Chuang et al., 2015; Golding et al., 2010; Lozano
294 et al., 2009). Consistent with previous reports (Garza-González et al., 2010; Ruppé et al.,
295 2009), 4/5 MRSE carried SCC*mec* IV for methicillin resistance. As presented in Table 3, all
296 the *M. sciuri* isolates displayed a low-level of the OXA resistance phenotype (0.5 - 2 μg/ml).
297 However, all of these OXA-resistant isolates were susceptible to FOX (Tables 3 and 4), and
298 none of them were positive for the *mecA* gene. Previously, it has been reported that CoNS
299 isolates other than *S. epidermidis* strains that displays OXA MICs of 0.5 – 2 μg/ml may lack
300 *mecA* (Feßler et al., 2010), and have been defined as methicillin-susceptible strains (CLSI,
301 2015). It has been suggested that the *mecA*–negative OXA-resistant CoNS may overexpress
302 penicillinase (Kolbert et al., 1995).

For the last ten years, over a two-fold increase (from 15 to 34%) in fusidic acid resistance in clinical isolates of *S. aureus* has been reported in Korea (Hong et al., 2016). More recently, it has been reported that ~27% of *Staphylococcus pseudintermedius* isolates from canine pyoderma and otitis were resistant to fusidic acid (Lim et al., 2020). In the current study, as shown in Table 4, 9/10 *S. epidermidis* strains and the 32 *M. sciuri* strains displayed fusidic acid resistance. Similarly, the high rates of resistance to fusidic acid in *S. saprophyticus*, *S. xylosus*, and *M. sciuri* isolates collected from ready-to-eat foods have been reported in Taiwan (Wang et al., 2019). Coagulase-negative staphylococci isolated from meat products also displayed fusidic acid resistance rates of 79.2% and 43% in Nigeria (Okoli et al., 2018) and Poland (Fijałkowski et al., 2016)respectively. Recent studies from Taiwan and the UK reported 14% and 46% prevalence, respectively, of fusidic acid resistance in clinical isolates of *S. epidermidis* (Chen et al., 2011; Mclaws et al., 2008). The widespread occurrence of fusidic acid resistance in non-*aureus* staphylococcal isolates indicate that NAS such as *S. epidermidis* and *M. sciuri* could become a significant public health concern, serving as a reservoir of antimicrobial resistance through food chains. In line with previous reports (Chen et al., 2011; Lee et al., 2018; Mclaws et al., 2008), fusidic acid resistance in *S. epidermidis* isolates from slaughterhouse carcasses in this study was mediated by the *fusB* gene (Table 3). Although one strain of *S. epidermidis* (SSE3) was double positive for *fusB* and *fusC* genes, this strain showed a fusidic acid MIC of 8 μg/ml, indicating that the presence of the two *fusB*-family genes does not confer a high-level fusidic acid resistance phenotype (≥ MIC of 128 μg/ml). None of the *S. epidermidis* isolates displaying fusidic acid resistance were positive for *fusD* nor *fusF* (Table 3). Sequencing analyses of *fusA* in *S. epidermidis* revealed that the SSE9 strain had V599I mutation within the linker site between domain IV and V of EF-G. Amino acid sequence substitutions in EF-G have frequently been

associated with high-levels of fusidic acid resistance (Fernandes, 2016). However, the location of V599I mutation within EF-G and the relatively low-level fusidic acid resistance (MIC of 24 μg/ml) indicate that the point mutation in V599I is not causing fusidic acid resistance in the *S. epidermidis* strain.

The high prevalence of fusidic acid resistance in *M. sciuri* isolated from ready-to-eat-foods (99%) (Wang et al., 2019), healthy chickens (100%) (Nemeghaire et al., 2014b), and livestock (100%) (Bagcigil et al., 2007) has recently been reported. Similar to these reports, all of the *M. sciuri* isolates from retail pork (n = 29) and slaughterhouse carcasses (n = 3) exhibited fusidic acid resistance (Tables 3 and 4). To determine genetic factors involved in the fusidic acid resistance in *M. sciuri* isolates, a specific primer set detecting homologues of *fusB*-family genes (*fusB/C*) was designed in the current study using the published sequences of seven *M. sciuri* strains in NCBI databases. All the *M. sciuri* isolates carried the *fusB/C* gene for the fusidic acid resistance phenotype (Table 3), and the sequencing analyses of the amplified PCR products confirmed the sequences of *fusB/C* genes (data not shown). As shown in Table 5 and Fig. 2, FusB/C protein from *M. sciuri* displayed 42 - 47% of similarity to amino acid sequences of previously characterized FusB-family proteins. None of the 32 *M. sciuri* strains had point mutation in the *fusA* gene, correlating with the low-level fusidic acid resistance phenotypes observed in the *M. sciuri* strains.

These results combined with the MLST analyses suggests that various genetic lineages of *S. epidermidis* and *M. sciuri* strains contribute to the high prevalence of fusidic acid resistance in NAS isolated from retail pork and slaughterhouse carcass samples in Korea.

In summary, our results suggest that i) a relatively higher level of NAS than *S. aureus* are present in pork production chains, particularly in slaughterhouse carcass samples, ii) there is a high prevalence of fusidic acid resistance in NAS isolates, especially in *S.*

epidermidis and *M. sciuri* isolates, and iii) *fusB*-family genes, rather than *fusA* mutations, caused the high occurrence of fusidic acid-resistant *S. epidermidis* and *M. sciuri*. Our results demonstrate a high prevalence of fusidic acid-resistant NAS in pork meat production chains, which may act as reservoirs for fusidic acid resistance. To the best of our knowledge, this is the first study to report the genetic determinants and prevalence of fusidic acid resistance in NAS collected from pork meat production chains in Korea.

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References

Archer GL, Niemeyer DM. 1994. Origin and evolution of DNA associated with resistance to methicillin in staphylococci. Trends Microbiol 2:343–347.

Bagcigil FA, Moodley A, Baptiste KE, Jensen VF, Guardabassi L. 2007. Occurrence, species distribution, antimicrobial resistance and clonality of methicillin- and erythromycin-resistant staphylococci in the nasal cavity of domestic animals. Vet Microbiol 121:307–315.

Chen HJ, Hung WC, Lin YT, Tsai JC, Chiu HC, Hsueh PR, Teng LJ. 2015. A novel fusidic acid resistance determinant, *fusF*, in *Staphylococcus cohnii*. J Antimicrob Chemother 70:416–419.

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.

Chen HJ, Tsai JC, Hung WC, Tseng SP, Hsueh PR, Teng LJ. 2011. Identification of *fusB*-mediated fusidic acid resistance islands in *Staphylococcus epidermidis* isolates. Antimicrob Agents Chemother 55:5842–5849.

Chuang YY, Huang YC. 2015. Livestock-associated meticillin-resistant *Staphylococcus aureus* in Asia: An emerging issue?. Int J Antimicrob Agents 45:334–340.

CLSI (Clinical and Laboratory Standards Institute). 2015. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. 3rd ed. Wayne, PA, USA.

Davis CP. 1996. Normal flora. In: Baron S, ed. medical microbiology. 4th ed. University of Texas Medical Branch at Galveston, Galveston, TX, USA. Chapter 6.

Enright MC, Day NPJ, 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.

EUCAST. 2021. European committee on antimicrobial susceptibility testing (EUCAST). Available from: https://www.eucast.org/clinical_breakpoints/. Accessed at Aug 2. 2021.

Fernandes P. 2016. Fusidic acid: A bacterial elongation factor inhibitor for the oral treatment of acute and chronic staphylococcal infections. Cold Spring Harb Perspect Med 6:1–18.

Feßler AT, Billerbeck C, Kadlec K, Schwarz S. 2010. Identification and characterization of methicillin-resistant coagulase-negative staphylococci from bovine mastitis. J Antimicrob Chemother 65:1576–1582.

Fijałkowski K, Peitler D, Karakulska J. 2016. Staphylococci isolated from ready-to-eat meat – Identification, antibiotic resistance and toxin gene profile. Int J Food Microbiol 238:113–120.

Founou LL, Founou RC, Essack SY. 2016. Antibiotic resistance in the food chain: A developing country-perspective. Front Microbiol 7:1–19.

Garza-González E, López D, Pezina C, Muruet W, Bocanegra-García V, Muñoz I, Ramírez C, Llaca-Díaz JM. 2010. Diversity of staphylococcal cassette chromosome *mec* structures in coagulase-negative staphylococci and relationship to drug resistance. J Med Microbiol 59:323–329.

Geha DJ, Uhl JR, Gustaferro CA, Persing DH. 1994. Multiplex PCR for identification of methicillin-resistant staphylococci in the clinical laboratory. J Clin Microbiol 32:1768–1772.

Godtfredsen WO, Jahnsen S, Lorck H, Roholt K, Tybring L. 1962. Fusidic acid: A new antibiotic. Nature 193:987.

412 Golding GR, Bryden L, Levett PN, McDonald RR, Wong A, Wylie J, Graham MR, Tyler S,
413 van Domselaar G, Simor AE, Gravel D, Mulvey MR. 2010. Livestock-associated
414 methicillin- resistant *Staphylococcus aureus* sequence type 398 in humans, Canada.
415 Emerg Infect Dis 16:587–594.
416 Hanson BM, Dressler AE, Harper AL, Scheibel RP, Wardyn SE, Roberts LK, Kroeger JS,
417 Smith TC. 2011. Prevalence of *Staphylococcus aureus* and methicillin-resistant
418 *Staphylococcus aureus* (MRSA) on retail meat in Iowa. J. Infect. Public Health 4: 169–
419 174.
420 Hong SN, Kim J, Sung H. 2016. A study on changes in antimicrobial resistant
421 *Staphylococcus aureus* from wound isolates in a south korean university hospital for the
422 past 10 years (2006, 2016). Korean J Clin Lab Sci 48:335–342.
423 Huber H, Ziegler D, Pflüger V, Vogel G, Zweifel C, Stephan R. 2011. Prevalence and
424 characteristics of methicillin- resistant coagulase-negative staphylococci from livestock,
425 chicken carcasses, bulk tank milk, minced meat, and contact persons. BMC Vet Res 7:6.
426 Hung WC, Chen HJ, Lin YT, Tsai JC, Chen CW, Lu HH, Tseng SP, Jheng YY, Leong KH,
427 Teng LJ. 2015. Skin commensal staphylococci may act as reservoir for fusidic acid
428 resistance genes. PLoS One 10:1–15.
429 Kolbert CP, Connolly JE, Lee MJ. 1995. Detection of the staphylococcal *mecA* gene by
430 chemiluminescent DNA hybridization. J Clin Microbiol 33:2179–2182.
431 Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, Hiramatsu K. 2007.
432 Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type
433 assignment: Rapid identification system for *mec*, *ccr*, and major differences in junkyard
434 regions. Antimicrob Agents Chemother 51:264–274.
435 Lee JYH, Monk IR, Gonçalves da Silva A, Seemann T, Chua KYL, Kearns A, Hill R,

Woodford N, Bartels MD, Strommenger B, Laurent F, Dodémont M, Deplano A, Patel R, Larsen AR, Korman TM, Stinear TP, Howden BP. 2018. Global spread of three multidrug-resistant lineages of *Staphylococcus epidermidis*. Nat Microbiol 3:1175–1185.

Lim YJ, Hyun JE, Hwang CY. 2020. Identification of fusidic acid resistance in clinical isolates of *Staphylococcus pseudintermedius* from dogs in Korea. Vet Dermatol 31:267-e62.

Lozano C, López M, Gómez-Sanz E, Ruiz-Larrea F, Torres C, Zarazaga M. 2009. Detection of methicillin-resistant *Staphylococcus aureus* ST398 in food samples of animal origin in Spain. J Antimicrob Chemother 64:1325–1326.

Mclaws F, Chopra I, O'Neill AJ. 2008. High prevalence of resistance to fusidic acid in clinical isolates of *Staphylococcus epidermidis*. J Antimicrob Chemother 61:1040–1043.

Mendoza M, Meugnier H, Bes M, Etienne J, Freney J. 1998. Identification of *Staphylococcus* species by 16S-23S rDNA intergenic spacer PCR analysis. Int J Syst Bacteriol 48:1049–1055.

Nam HM, Lee AL, Jung SC, Kim MN, Jang GC, Wee SH, Lim SK. 2011. Antimicrobial susceptibility of *Staphylococcus aureus* and characterization of methicillin-resistant *Staphylococcus aureus* isolated from bovine mastitis in Korea. Foodborne Pathog Dis 8:231–238.

Nemeghaire S, Argudín MA, Feßler AT, Hauschild T, Schwarz S, Butaye P. 2014a. The ecological importance of the *Staphylococcus sciuri* species group as a reservoir for resistance and virulence genes. Vet Microbiol 171:342–356.

Nemeghaire S, Argudín MA, Haesebrouck F, Butaye P. 2014b. Molecular epidemiology of methicillin-resistant *Staphylococcus sciuri* in healthy chickens. Vet Microbiol 171:357–363.

460 Norström T, Lannergård J, Hughes D. 2007. Genetic and phenotypic identification of fusidic
461 acid-resistant mutants with the small-colony-variant phenotype in *Staphylococcus*
462 *aureus*. Antimicrob Agents Chemother 51:4438–4446.
463 O'Brien AM, Hanson BM, Farina SA, Wu JY, Simmering JE, Wardyn SE, Forshey BM,
464 Kulick ME, Wallinga DB, Smith TC. 2012. MRSA in conventional and alternative retail
465 pork products. PLoS One 7: 3–8.
466 Okoli CE, Njoga EO, Enem SI, Godwin EE, Nwanta JA, Chah KF. 2018. Prevalence,
467 toxigenic potential and antimicrobial susceptibility profile of *Staphylococcus* isolated
468 from ready-to-eat meats. Vet. World 11: 1214–1221.
469 O'Neill AJ, Chopra I. 2006. Molecular basis of *fusB*-mediated resistance to fusidic acid in
470 *Staphylococcus aureus*. Mol Microbiol 59:664–676.
471 O'Neill AJ, McLaws F, Kahlmeter G, Henriksen AS, Chopra I. 2007. Genetic basis of
472 resistance to fusidic acid in staphylococci. Antimicrob Agents Chemother 51:1737–1740.
473 Piette A, Verschraegen G. 2009. Role of coagulase-negative staphylococci in human disease.
474 Vet Microbiol 134:45–54.
475 Ruppé E, Barbier F, Mesli Y, Maiga A, Cojocaru R, Benkhalfat M, Benchouk S, Hassaine H,
476 Maiga I, Diallo A, Koumaré AK, Ouattara K, Soumaré S, Dufourcq JB, Nareth C,
477 Sarthou JL, Andremont A, Ruimy R. 2009. Diversity of staphylococcal cassette
478 chromosome *mec* structures in methicillin-resistant *Staphylococcus epidermidis* and
479 *Staphylococcus haemolyticus* strains among outpatients from four countries. Antimicrob
480 Agents Chemother 53:442–449.
481 Schauer B, Szostak MP, Ehricht R, Monecke S, Feßler AT, Schwarz S, Spergser J,
482 Krametter-Frötscher R, Loncaric I. 2021. diversity of methicillin-resistant coagulase-
483 negative *Staphylococcus* spp. and methicillin-resistant *Mammaliicoccus* spp. isolated

484 from ruminants and new world camelids. Vet Microbiol 254.
485 Sugden R, Kelly R, Davies S. 2016. Combatting antimicrobial resistance globally. Nat
486 Microbiol p 16187.
487 Tang Y, Larsen J, Kjeldgaard J, Andersen PS, Skov R, Ingmer H. 2017. Methicillin-resistant
488 and -susceptible *Staphylococcus aureus* from retail meat in Denmark. Int. J. Food
489 Microbiol. 249: 72–76.
490 Thomas JC, Vargas MR, Miragaia M, Peacock SJ, Archer GL, Enright MC. 2007. Improved
491 multilocus sequence typing scheme for *Staphylococcus epidermidis*. J Clin Microbiol
492 45:616–619.
493 Turnidge J, Collignon P. 1999. Resistance to fusidic acid. Int J Antimicrob Agents 12:Suppl 2
494 Von Wintersdorff CJH, Penders J, Van Niekerk JM, Mills ND, Majumder S, Van Alphen LB,
495 Savelkoul PHM, Wolffs PFG. 2016. Dissemination of antimicrobial resistance in
496 microbial ecosystems through horizontal gene transfer. Front Microbiol 7:1–10.
497
498 Wang YT, Lin YT, Wan TW, Wang DY, Lin HY, Lin CY, Chen YC, Teng LJ. 2019.
499 Distribution of antibiotic resistance genes among *Staphylococcus* species isolated from
500 ready-to-eat foods. J Food Drug Anal 27:841–848.
501 Wu S, Huang J, Wu Q, Zhang J, Zhang F, Yang X, Wu H, Zeng H, Chen M, Ding Y, Wang J,
502 Lei T, Zhang S, Xue L. 2018. *Staphylococcus aureus* isolated from retail meat and meat
503 products in China: Incidence, antibiotic resistance and genetic diversity. Front.
504 Microbiol. 9: 1–14.
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508 **Table 1. Primers used to detect fusidic acid resistance determinants in this study**

Primer name	Sequence (5'→3')	Product size (bp)	Target gene	References
BF	CTA TAA TGA TAT TAA TGA GAT TTT TGG	431	*fusB*	(Mclaws et al., 2008)
BR	TTT TTA CAT ATT GAC CAT CCG AAT TGG			
CF	TTA AAG AAA AAG ATA TTG ATA TCT CGG	332	*fusC*	(Mclaws et al., 2008)
CR	TTT ACA GAA TCC TTT TAC TTT ATT TGG			
B/C-F	CTT AAA AGC TAC GTC GTC CCA	299	*fusB/C*	this study
B/C-R	CCA TCA CCT TTA GAT TTC GTC GTA			
AF	GCT CAT TAC CGT TGG TAA GAT AGA A	2.5k	*fusA*	this study
AR	TTG GCA TGT GTT TTT GAG CGA			
A-S1	TTA ATT GAA GCT GTT GCT GA	Sequencing only		
A-S2	TGC ACA AGT TCA AGG TAA ATT CTC	Sequencing only		
DF	AAT TCG GTC AAC GAT CCC	465	*fusD*	(Chen et al., 2010)
DR	GCC ATC ATT GCC AGT ACG			
FF	CTA AAA TAG ACA TTT ATC AGC AG	427	*fusF*	(Chen et al., 2015)
FR	GGT ATA TTG TCC ATC ACC AG			

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Table 2. Profiles of staphylococci isolated from pork meat and carcass samples

Bacterial species	No. of methicillin-resistant strains	SCC*mec* type (No. of strains)
staphylococci from slaughterhouses (44/311 samples)		
S. aureus (n = 4/311, 1.29%)	2	SCC*mec* IV (1), SCC*mec* V (1)
S. hyicus (n = 1/311, 0.32%)	0	-
S. epidermidis (n = 10/311, 3.22%)	5	SCC*mec* IV (4), SCC*mec* V (1)
M. sciuri (n = 29/311, 9.32%)	-	-
staphylococci from retail markets (3/267 samples)	0	-
M. sciuri (n = 3/267, 1.12%)	0	-
Total 47 strains	7	

Table 3. Genotypes, antimicrobial resistance phenotypes, and fusidic acid resistance determinants of staphylococci isolated from pork meat and carcass samples

ID	species	Resistance profiles	MICs to			*mecA*	SCC*mec* type	FA resistance genes	*fusA* mutation	MLST	Score
			OXA (µg/ml)	TET (µg/ml)	FA (µg/ml)						Value[c]
SSA1	MRSA	OXA-AMP-FOX-PEN-CHL-CIP-OXA-TET	6	64	0.25	+	V	-	[a]NA	ST398	2.304
SSA2	MRSA	OXA-AMP-FOX-PEN	192	0.5	0.125	+	IV	-	NA	ST2084	2.144
SSA3	MSSA	AMP-PEN	0.5	1	0.19	-	NA	-	NA	ST5	2.204
SSA4	MSSA	AMP-PEN-CHL-MUP	0.5	4	0.125	-	NA	-	NA	ST9	2.279
SSE1	*S. epidermidis*	OXA-AMP-FOX-PEN-ERY-FA	64	1	24	+	IV	*fusB*	-	ST172	2.362
SSE2	*S. epidermidis*	OXA-AMP-PEN-FA	64	4	24	+	IV	*fusB*	-	ST130	2.32
SSE3	*S. epidermidis*	OXA-AMP-FOX-PEN-FA	32	0.5	8	+	IV	*fusB, fusC*	-	ST80	2.293
SSE4	*S. epidermidis*	OXA-AMP-PEN-CHL-ERY-FA-MUP-SXT	8	4	32	+	V	*fusB*	-	ST173	2.436
SSE5	*S. epidermidis*	AMP-PEN-FA	0.25	2	8	-	NA	*fusB*	-	[b]NT	2.375
SSE6	*S. epidermidis*	AMP-PEN-FA	0.25	4	8	-	NA	*fusB*	-	NT	2.249
SSE7	*S. epidermidis*	OXA-FA-MUP-TET	16	32	24	+	IV	*fusB*	-	ST768	2.293

SSE8	*S. epidermidis*	AMP-PEN-FA	0.25	4	12	-	NA	*fusB*	-	NT	2.376
SSE9	*S. epidermidis*	AMP-PEN-FA	0.25	2	24	-	NA	*fusB*	V599I (GTT→ATT)	NT	2.264
SSE10	*S. epidermidis*	AMP-PEN	0.25	0.5	0.25	-	NA	*fusB*	-	ST1017	2.247
SSH1	*S. hyicus*	AMP-PEN-CLI-SXT-TET	0.25	16	0.19	-	NA	-	NA	NA	2.138
RMS1	*M. sciuri*	OXA-CLI-FA	2	0.5	12	-	NA	*fusB/C*	-	ST63	2.273
RMS2	*M. sciuri*	OXA-FA	0.5	0.25	16	-	NA	*fusB/C*	-	NT	2.377
RMS3	*M. sciuri*	OXA-FA	1	0.25	16	-	NA	*fusB/C*	-	ST30	2.274
SMS4	*M. sciuri*	OXA-FA-TET	1	16	8	-	NA	*fusB/C*	-	ST63	2.227
SMS5	*M. sciuri*	OXA-FA	1	1	16	-	NA	*fusB/C*	-	NT	2.433
SMS6	*M. sciuri*	OXA-FA	1	0.5	12	-	NA	*fusB/C*	-	NT	2.324
SMS7	*M. sciuri*	OXA-FA	1	0.5	16	-	NA	*fusB/C*	-	NT	2.212
SMS8	*M. sciuri*	OXA-FA	1	0.5	24	-	NA	*fusB/C*	-	ST63	2.012
SMS9	*M. sciuri*	OXA-CLI-FA	2	0.5	16	-	NA	*fusB/C*	-	ST63	2.01
SMS10	*M. sciuri*	OXA-FA	0.5	0.25	12	-	NA	*fusB/C*	-	NT	2.343
SMS11	*M. sciuri*	OXA-FA	2	1	8	-	NA	*fusB/C*	-	NT	2.257
SMS12	*M. sciuri*	OXA-FA	1	1	12	-	NA	*fusB/C*	-	NT	2.13
SMS13	*M. sciuri*	OXA-CLI-FA	0.5	0.25	16	-	NA	*fusB/C*	-	ST63	2.248

SMS14	*M. sciuri*	OXA-FA	0.5	0.25	8	-	NA	*fusB/C*	-	NT	2.17
SMS15	*M. sciuri*	OXA-FA	0.5	0.25	12	-	NA	*fusB/C*	-	NT	2.028
SMS16	*M. sciuri*	OXA-CLI-FA	0.5	0.25	6	-	NA	*fusB/C*	-	NT	2.077
SMS17	*M. sciuri*	OXA-FA	1	0.25	8	-	NA	*fusB/C*	-	ST63	2.214
SMS18	*M. sciuri*	OXA-PEN-FA	2	0.25	8	-	NA	*fusB/C*	-	NT	2.056
SMS19	*M. sciuri*	OXA-FA	0.5	0.25	12	-	NA	*fusB/C*	-	NT	2.203
SMS20	*M. sciuri*	OXA-FA	1	8	12	-	NA	*fusB/C*	-	ST63	2.29
SMS21	*M. sciuri*	OXA-FA-TET	1	16	8	-	NA	*fusB/C*	-	ST63	2.185
SMS22	*M. sciuri*	OXA-FA	1	0.5	12	-	NA	*fusB/C*	-	ST63	2.203
SMS23	*M. sciuri*	OXA-FA	1	0.25	8	-	NA	*fusB/C*	-	NT	2.336
SMS24	*M. sciuri*	OXA-FA-TET	1	16	8	-	NA	*fusB/C*	-	ST30	2.218
SMS25	*M. sciuri*	OXA-FA-TET	1	16	12	-	NA	*fusB/C*	-	ST30	2.277
SMS26	*M. sciuri*	OXA-FA-TET	1	16	8	-	NA	*fusB/C*	-	ST30	2.272
SMS27	*M. sciuri*	OXA-FA-TET	1	16	12	-	NA	*fusB/C*	-	ST30	2.085
SMS28	*M. sciuri*	OXA-FA-TET	1	16	12	-	NA	*fusB/C*	-	ST30	2.081
SMS29	*M. sciuri*	OXA-FA	1	0.25	12	-	NA	*fusB/C*	-	ST30	2.398
SMS30	*M. sciuri*	OXA-FA	1	0.25	8	-	NA	*fusB/C*	-	ST30	2.325

SMS31	*M. sciuri*	OXA-FA	1	0.5	12	-	NA	*fusB/C*	-	ST63	2.295
SMS32	*M. sciuri*	OXA-PEN-FA	1	8	8	-	NA	*fusB/C*	-	ST96	2.282

[a]NA, not applicable

[b]NT, Not-typable stain

[c]Strains with score value of ≥2.000 were used in this study

Table 4. Prevalence of antimicrobial resistance in staphylococci isolated from pork meat and carcass samples

	Frequency of resistance					Total (n = 47)
	MRSA (n = 2)	**MSSA (n = 2)**	***S. hyicus* (n = 1)**	***S. epidermidis* (n = 10)**	***M. sciuri* (n = 32)**	
OXA	2	-	-	5	**32**	39, 83.0%
AMP	2	2	1	9	-	14, 29.8%
FOX	2	-	-	2	-	4, 8.5%
PEN	2	2	1	9	2	16, 34.0%
CHL	1	1	-	1	-	3, 6.4%
CIP	1	-	-	-	-	1, 2.1%
CLI	-	-	1	1	4	6, 12.8%
ERY	-	-	-	2	-	2, 4.3%
FA	-	-	-	**9**	**32**	**41, 87.2%**
MUP	-	1	-	2	-	3, 6.4%
SXT	-	-	1	1	-	2, 4.3%
TET	1	-	1	1	7	10, 21.3%
RIF	-	-	-	-	-	-
GEN	-	-	-	-	-	-
SYN	-	-	-	-	-	-

525 **Table 5. Similarities of FusB-family protein amino acid sequences among staphylococci and mammaliicocci**

526

FusB-family proteins	Species	Accession number	Amino acid size	Amino acid sequence similarity				
				FusB	FusC	FusD	FusF	MS-FusB/C
FusB	*S. aureus*	CAL23838.1	213	-	45%	47%	53%	44%
FusC	*S. aureus*	WP_001033157.1	212	45%	-	41%	42%	47%
FusD	*S. saprophyticus*	BAE19310.1	213	47%	41%	-	68%	42%
FusF	*S. cohnii*	AVL76727.1	214	53%	42%	68%	-	46%
MS-FusB/C	*M. sciuri*	QYG31551.1	214	44%	47%	42%	46%	-
MF-FusB/C	*M. fleurettii*	WP_078357269.1	214	44%	46%	43%	47%	92%
MV-FusB/C	*M. vitulinus*	QQT15456.1	214	44%	44%	43%	48%	87%
ML-FusB/C	*M. lentus*	QMU10254.1	214	41%	43%	41%	46%	81%

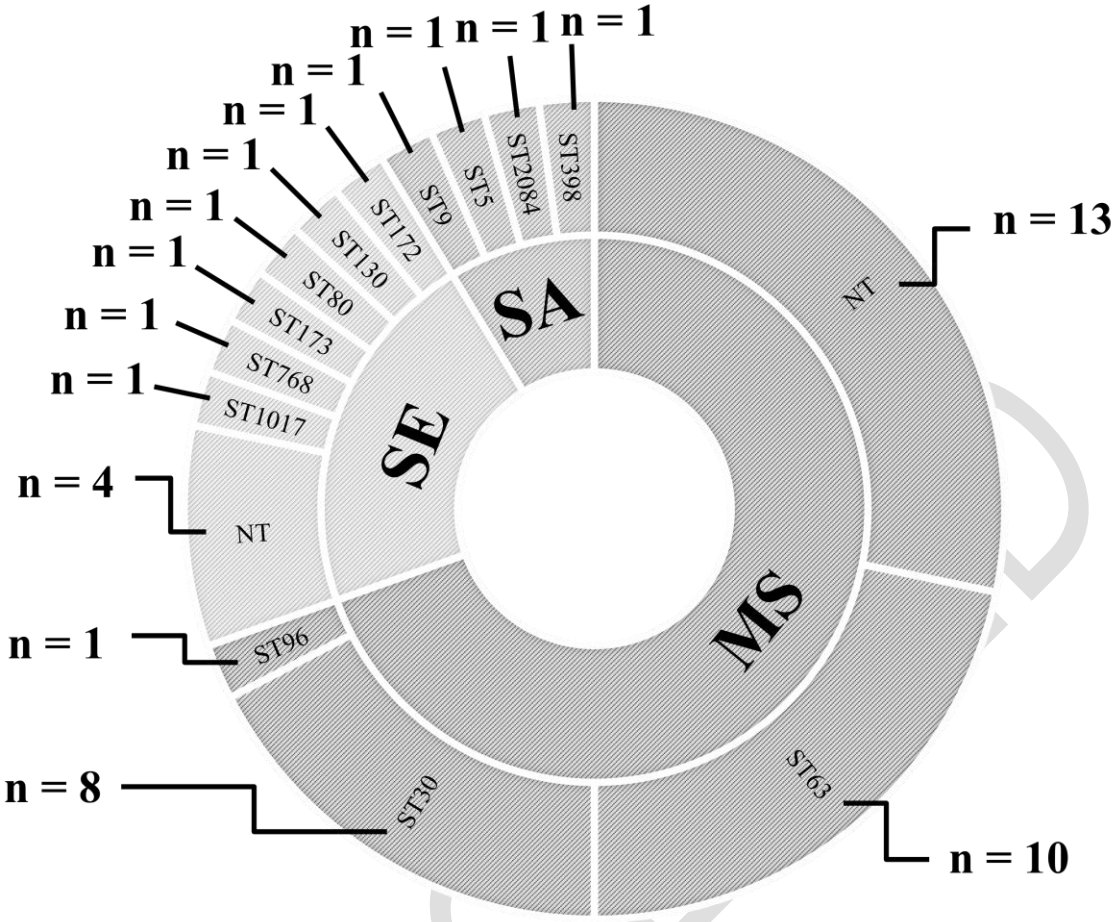

Fig. 1. MLST profiles of staphylococci isolated from retail pork and carcass samples in Korea.

SA, *S. aureus*; SE, *S. epidermidis*; MS, *M. sciuri*

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MS-FU  1    MNNYIKPYQFVSIKEKSEQLFNVYKSVNDIKTIDTFQAITYDHISQLFEEKHSEIEDFTK  60
Fus B  1    .KTM.Y.H.YNY.RSVILR.K....T.NDKE.VKVI.SE..NDINEI.GHIDDD..ESL.  60
Fus C  1     M.K.EV.K..KV.QLVY..IKL.RT-NDMNSHK.QKDFLLNEINDI.K..DID.S..IT  58
Fus D  1    .EKQLY....NY...RVAH.V.A.N..NDPN..ASIKDV.R.EILST.NSRNTT.RSNVE  60
Fus F  1    .EKQ.Y....NY...RIAH.L.A....NDLN..ASIKET.KIDIY.Q.HQIDDTLTEAIE  60
MF-FU  1    ........................NDF.............I....S...T...E..N  60
MV-FU  1    .....Q........Q.....S......NDL.............I....S.......E..N  60
ML-FU  1    ...F.Q...Y.....H..K........NDP..........N..IE...T..Y..VDN.IN  60

MS-FU  61   IIMNKKISRAQIEKLLFGLKSYVIPFEQPSSKQLEKIFKKTKKLKHFEWDNIDLKESSYI  120
Fus B  61   VL..IRL.NKE..AILNKFLE..V.F.L..PQK.Q.VF.KVKKIKIPQFEEY...V..FV  120
Fus C  59   S.DDV.LTKKKA.H.LNE..V.IQDF.I...S.....FRKVKK.KRPDINL..T..I..L  118
Fus D  61   KL..VQLTKE.AQ.ILTTIQM..K.F.H..N..VTNLF.KVKK.KTPLISDEV.QT.T..  120
Fus F  61   KL..IR.TKV.VD.ILET.QT....F.H..K..V..TFRKIKK.KSPLISDEI.L..T..  120
MF-FU  61   .....R.........L....N....F..........LFIK.KK.KQPD............  120
MV-FU  61   .....R.....V..VL.........F.M........LF.K.KK.KQPD.ES.........  120
ML-FU  61   ....P..N.S....VLL...V....FQ......I..VF.K.KK.KQPD........T...  120

MS-FU 121   GWNDSGKQKKFIVLY-E-D-DK-LIGVSGDLSPTIKPGICSTCKKESNVSMFLSTTK-SKGDGTY  180
Fus B 121   GWNELASNR.Y.IY.-D-E-K.Q.KGLYGEI.NQVVK.F.TI.N......LFMKKS.-TNS.GQY  181
Fus C 119   GWN.NSSNR.Y..YK-NL.-..-FEGIYGEI..NKVK.F.KI.NQ..DT.LF.NK..HN.SSG.Y  180
Fus D 121   GWN.IASNR...IY.-D-NFG.-.NG.YG.I.NQTVKSF..I.N...R.ALFMRK.R-TGN.GQY  181
Fus F 121   GWN.IASNR...IY.N.-Q-GT-.TGFYG.IANQTVK.Y.AI.N.....ALFMRK.R-TS..GQY  181
MF-FU 121   GWN.........IM.-.-N-..-.TG..GE.......P.F..I.........F.....-....G.Y  180
MV-FU 121   GWN.........IM.-.-N-GI-.TG..G........P.F..I.........F.....-....G.Y  180
ML-FU 121   GWN.......Y.IM.-.-.-..-.TG.VG...T...P.V..I.........F.....-....G.Y  180

MS-FU 181   TKNCNYICHDSEHCMQQLDQLNGLYEFIHTVKTK  214
Fus B 182   V.KGDYI.RDSI..NK..TDI.QF.NF.  209
Fus C 181   ..KGDYI.YDSFK.N.N..DI.N...F.VKI.  212
Fus D 182   ..KGDYI.FDSTL.NH.ISD.SHFHHFLNKIQ  213
Fus F 182   ..KGDYI.FDSIK.N...SDITQF.QFV  209
MF-FU 181   ..KG.YI..DS.R.N......D....F.......  214
MV-FU 181   ..KG.YI..DSQR.N......D...DF.....A.  214
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ML-FU 181   ..KG.YI..DSIR.N......E...DF.R...M.  214
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Fig. 2. Amino acid sequence alignment of FusB/C from mammaliicocci and FusB-family proteins. GenBank accession numbers are FusB/C, (in *M. sciuri*, QYG31551.1; *M. fleurettii*, WP_078357269.1; *M. vitulinus*, QQT15456.1; *M. lentus*, QMU10254.1); FusB, CAL23838.1; FusC, WP_001033157.1; and FusD, BAE19310.1. Amino acids highlighted are conserved sequences. (The key residues associated with the interaction with EF-G are underlined.) Sequences were pairwised with dots for identities.