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Species Distribution, Antimicrobial Resistance, and Enterotoxin Profiles of Non-*aureus* Staphylococci Isolated from Poultry Slaughterhouses in Korea

Running title: Non-*aureus* staphylococci in poultry slaughterhouses

Abstract

Although non-*aureus* staphylococci (NAS), such as coagulase-negative staphylococci, can substantially affect human and animal health, information on NAS species distribution in poultry slaughterhouses and their antimicrobial resistance (AMR) profiles is limited. In this study, we analyzed the prevalence of NAS species and AMR profiles of NAS isolates collected from poultry slaughterhouses, including chicken carcasses and facility environments. In total, 100 NAS isolates were collected from six poultry slaughterhouses in Korea. The AMR patterns of the NAS species and the major genetic elements associated with AMR phenotypes, particularly methicillin and fluoroquinolone resistance, were determined. In addition, the prevalence of classical staphylococcal enterotoxin (SE, *sea-see*) and toxic shock syndrome toxin-1 (*tst-1*) genes among NAS isolates was examined. Among the 10 NAS species, coagulase-negative *Staphylococcus simulans* (n = 49, 49%) was the most dominant species, followed by *Staphylococcus agnetis* (n = 16, 16%). The multiple drug resistance phenotype was identified in 67% (n =67) of the NAS isolates, with the highest resistance to erythromycin (66%) and clindamycin (62%). Furthermore, fluoroquinolone resistance was confirmed in 34 (34%) NAS isolates. Fifteen NAS isolates were *mecA*-positive, harboring SCC*mec* I (n = 2), SCC*mec* IV (n = 1), or non-typeable SCC*mec* types (n = 12). Carriage of SE genes was detected in none of the NAS isolates, and toxic shock syndrome toxin 1 gene (*tst1*) was detected in only two CoNS strains. Our results suggest that NAS in poultry slaughterhouses can have potential role in the maintenance and transmission of AMR

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244 words

Key words non-*aureus* staphylococci, poultry slaughterhouse, species profiles, antimicrobial resistance, fluoroquinolone resistance

Introduction

Staphylococci are commensal bacteria that colonize on the skin and mucous membranes of humans and animals (Becker et al., 2014; Casey et al., 2007). However, they are occasionally implicated in local and systemic infections such as scalded skin syndrome, gastroenteritis, and toxic shock syndrome (Ladhani et al., 2004; Lowy, 1998). Although *Staphylococcus aureus* is most frequently associated with disease outbreaks, recent studies have revealed that non-*aureus* staphylococci (NAS) substantially affect human and animal health (Adkins et al., 2018; Osman et al., 2017; Wuytack et al., 2020). The consumption of or close contact with raw or undercooked meat and other food products contaminated with bacteria are the most common transmission routes from livestock to humans (Osman et al., 2017; Osman et al., 2016; Podkowik et al., 2012). As a zoonotic bacterial pathogen, *S. aureus* is characterized by the (i) production of coagulase, which converts fibrinogen to fibrin, and (ii) secretion of toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxins (SEs), which cause staphylococcal food poisoning (SFP) (Argudín et al., 2010; Dellaripa, 2000). Although some NAS strains, particularly coagulase-negative staphylococci (CoNS), have one or more genes encoding various SEs, their pathophysiological roles in SFP remain unclear (Podkowik et al., 2013; Wisniewski et al., 2023). However, antimicrobial resistance (AMR) genes in NAS can be horizontally transferred to confer AMR phenotype in other staphylococci. Notably, studies have revealed a high prevalence of methicillin-resistant *S. aureus* (MRSA) and MR-NAS in livestock farms, slaughterhouses, and retail meat (Huber et al., 2011; Lim et al., 2010; Schnitt et al., 2021; van Cleef et al., 2010). The *mecA*-containing staphylococcal cassette chromosome *mec* (SCC*mec*) and other mobile genetic elements (MGEs) carrying AMR genes can be transferred between *S. aureus* and NAS, which

normally co-colonize in livestock such as cattle, pigs, goats, sheep, and poultry (Bhargava and Zhang, 2012; Pyzik et al., 2019; Ray et al., 2016).

Poultry carcasses have been associated with various foodborne pathogens such as *Salmonella* spp., *Campylobacter* spp., and *Staphylococcus aureus* (Crețu et al., 2015; Crețu et al., 2012; Crețu et al., 2011). Moreover, poultry is one of the principal reservoirs for antimicrobial-resistant staphylococci owing to the excessive use of antibiotics in poultry meat production (Apata, 2009; Diarra and Malouin, 2014). High fluoroquinolone (FQ) resistance in chicken-associated staphylococci has led to therapeutic dilemmas in both human and veterinary medicine (Dalhoff, 2012). Although the AMR profiles of *S. aureus* isolates from poultry and retail chicken meat are annually monitored in several countries including Korea, the USA, and the European Union (Abdalrahman et al., 2015; Fessler et al., 2011; Lee et al., 2022; Normanno et al., 2007), the AMR data for NAS isolates are relatively limited. Several previous studies have revealed various NAS species with AMR, including *Staphylococcus gallinarum*, *Staphylococcus xylosus*, *Staphylococcus simulans*, *Staphylococcus arlettae*, *Staphylococcus chromogenes*, *Staphylococcus epidermidis*, *Staphylococcus hyicus*, and *Staphylococcus lentus* in the poultry food chain (Osman et al., 2016; Pimenta et al., 2021; Pyzik et al., 2019). In addition to AMR, most genes encoding SEs located on MGEs can be transferred between NAS and *S. aureus*, thereby increasing the morbidity and mortality rates of staphylococci (Alibayov et al., 2014).

Previously, we reported the AMR and SE profiles of NAS isolates from healthy broilers (Park et al., 2023) and retail chicken meat (Lee et al., 2020) in Korea. However, NAS species distribution in poultry slaughterhouses and their AMR profiles remain unreported. Therefore, in the present study, we analyzed the species prevalence, AMR phenotypes, and SE gene distribution of NAS isolates obtained from poultry slaughterhouses, including chicken carcasses and facility environments. Furthermore, the major genetic factors associated with

methicillin and FQ resistance phenotypes were examined using SCC*mec* typing and quinolone-resistance determining region (QRDR) sequencing.

Materials and Methods

Sample preparation

In total, 270 swab samples were collected from six poultry slaughterhouses located in six Korean provinces from March to December 2019. Swab samples were obtained from chicken carcasses (n = 240) within 8 h of slaughter before a chilling process; and slaughterhouse environments (n = 30), including cutting boards, sewage, floors, and tables. All swabs were kept at 4℃ and delivered to the laboratory within 24 h of sample collection to isolate staphylococci.

Isolation and species identification of staphylococci

For NAS isolation, swabs collected were inoculated into 3 mL of tryptic soy broth (Difco Laboratories, Detroit, MI, USA) supplemented with 10% sodium chloride (TSB-NaCl) and cultured at 37℃ for 18-24 h. Next, 10-μL aliquots of pre-enriched TSB-NaCl cultures were streaked onto Baird-Parker agar (Difco Laboratories) containing egg yolk supplements and potassium tellurite. After 24-48 h incubation at 37℃, presumptive staphylococcal colonies were picked from each agar plate and re-streaked on Baird-Parker agar for subsequent identification. Individual isolates were subcultured on tryptic soy agar (Difco Laboratories) at 37℃ for 18 h to extract genomic DNA using a Genmed DNA kit (Seoul, Korea) based on the manufacturer's protocols. NAS species were identified using 16S rRNA sequencing and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Daltonics GmbH, Bremen, Germany).

Detection of *mecA* and SCC*mec* elements typing

All staphylococcal isolates exhibiting cefoxitin resistance were screened for the presence of the *mecA* gene using polymerase chain reaction (PCR), as described previously (Geha et al., 1994). For *mecA*-positive isolates, SCC*mec* types were determined using multiplex PCR, which amplified the chromosomal cassette recombinase genes and *mec* regulatory elements (Kondo et al., 2007).

Antimicrobial susceptibility assays

To determine the antimicrobial susceptibility phenotype of each NAS isolate, the standard disc diffusion method was used according to the Clinical and Laboratory Standards Institute's (CLSI) and CLSI VET01S guidelines (CLSI, 2015; CLSI, 2022). Fourteen different antibiotic discs were utilized for the disc diffusion assay on Mueller-Hinton agar (Difco Laboratories): cefoxitin (FOX, 30 µg), penicillin (PEN, 10 µg), ampicillin (AMP, 10 µg), gentamicin (GEN, 50 µg), ciprofloxacin (CIP, 5 µg), chloramphenicol (CHL, 30 µg), erythromycin (ERY, 15 µg), fusidic acid (FUS, 50 µg), clindamycin (CLI, 2 µg), mupirocin (MUP, 200 µg), rifampicin (RIF, 5 µg), sulfamethoxazole-trimethoprim (SXT, 23.75- 1.25 µg), tetracycline (TET, 30 µg), and quinupristin-dalfopristin (SYN, 15 µg).

Susceptibilities to vancomycin (VAN), linezolid (LZD), tigecycline (TEG), and teicoplanin (TEC) were examined using a standard Etest (bioMérieux, France). Two reference strains, *Staphylococcus aureus* ATCC 29213 and *S. aureus* MW2, were included for the disc diffusion assay and Etest.

Detection of quinolone-resistance determining regions (QRDRs) mutations

Fluoroquinolone (FQ)-resistant NAS isolates frequently carry point mutations within the QRDRs of DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) (Ito et al., 1994; Ng et al., 1996). Genomic DNA from each FQ-resistant isolate was subjected to

PCR amplification using QRDR-specific primer sets (Table S1). The QRDRs-specific primer sets were designed based on the following published sequences of reference genomes: *Staphylococcus agnetis* (NCTC7887, NCBI GenBank accession number UHAH01000002.1), *S. chromogenes* (17A, NCBI GenBank accession number NZ_CP031274.1), *S. arlettae* (P2, NCBI GenBank accession number AP019698.1), *S. lentus* (H29, NCBI GenBank accession number CP059679.1), *Staphylococcus nepalensis* (JS1, NCBI GenBank accession number NZ_CP017460.1), *S. simulans* (MR2, NCBI GenBank accession number NZ_CP016157.1). Then, the PCR products were sequenced at Bionics (Seoul, Korea). Differences in the amino acid sequences of the QRDRs were determined using the nucleotide sequences of the PCR results. Finally, multiple sequence alignments of *gyrA*, *gyrB*, *parC*, and *parE* genes were analyzed using the CLUSTALW server (www.genome.jp/tools-bin/clustalw).

Staphylococcal enterotoxin (SE) gene detection

Multiplex PCR was performed as previously described (Omoe et al., 2005) to examine the carriage of the toxic shock syndrome toxin-1 gene (*tst1*) and five classical SE genes (*sea*, *seb*, *sec*, *sed*, and *see*) in the NAS isolates. Five *S. aureus* reference strains (N315, MW2, COL, FRI472, and FRI913) were used as positive controls for the SE gene detection.

Results

Species profiles of the non-*aureus* staphylococci (NAS) isolates from poultry slaughterhouses

During the study period, 100 NAS isolates of 10 different species were obtained from 270 swab samples (37.04%, 100/270) collected from six different poultry slaughterhouses (Figs. 1A and B). Out of the 100 NAS isolates, 91 NAS isolates were obtained from chicken carcasses, and nine were isolated from the slaughterhouse environments. Based on their ability to produce coagulase, these NAS isolates were divided into coagulase-variable (CoVS) (n = 19 isolates) and coagulase-negative staphylococci (CoNS) (n = 81) (Table 1). While the 19 CoVS isolates comprised only two species of NAS, *S. agnetis* (n = 17) and *S. chromogenes* (n = 2), the 81 CoNS isolates had eight different *Staphylococcus* species: *S. simulans* (n = 49), *S. lentus* (n = 11), *S. arlettae* (n = 8), *S. xylosus* (n = 4), *Staphylococcus sciuri* (n = 4), *Staphylococcus warneri* (n = 3), *S. epidermidis* (n = 1), and *Staphylococcus urealyticus* (n = 1). The two most prevalent NAS species isolated from the chicken carcasses were *S. simulans* (49.5%, 45/91 isolates) and *S. agnetis* (17.6%, 17/91 isolates) (Fig. 1A). Similarly, the nine NAS isolates from the facility environments were *S. simulans* (n = 4 isolates), *S. arlettae* (n = 2), *S. lentus* (n = 2), and *S. agnetis* (n=1) (Fig. 1B).

Detection of *mecA* and staphylococcal cassette chromosome *mec* (SCC*mec*) types of methicillin-resistant NAS

PCR analysis revealed that none of the CoVS isolates were positive for *mecA* gene (Table 1). Among the 81 CoNS isolates, 15 (18.5%) were positive for *mecA*: *S. simulans* (n = 8), *S. lentus* (n = 6), and *S. epidermidis* (n = 1). Furthermore, SCC*mec* element typing of the 15 *mecA*-positive NAS isolates revealed that eight *S. simulans* isolates had either non-

typeable SCC*mec* (n = 6) or SCC*mec* I (n = 2). In addition, all six methicillin-resistant *S. lentus* strains contained non-typeable SCC*mec* elements. One methicillin-resistant *S. epidermis* isolate from a chicken carcass sample possessed SCC*mec* IV.

AMR profiles of the NAS isolates

Compared with the other antibiotics, the CoVS isolates exhibited higher resistance to FUS (84.2%, 16/19 isolates), PEN (84.2%, 16/19 isolates), AMP (84.2%, 16/19), GEN (78.9%, 15/19), and CIP (63.2%, 12/19) (Table 2 and Fig. 2). Although a higher resistance level to these antibiotics was also observed in CoNS isolates, CoNS exhibited lower resistance rates to the five antibiotics compared with CoVS isolates. In addition, CoVS isolates exhibited higher levels of multidrug resistance (MDR) phenotypes (resistant to three or more classes/subclasses of antimicrobial agents) than CoNS isolates (84.2% vs. 63.0%, respectively). When the AMR of the dominant CoVS species (*S. agnetis*) and the two major species of CoNS (*S. simulans* and *S. lentus*) were compared, *S. agnetis* (82.4%) and *S. lentus* (90.9%) exhibited significantly higher degrees of MDR than *S. simulans* (57.1%) (Table 2). Fig. 3 illustrates the heterogeneity of the AMR profiles within the four major species of NAS isolates, *S. simulans*, *S. agnetis*, and *S. lentus*. *S. agnetis* isolates tended to show high resistance to AMP, GEN, PEN, CIP, and FUS (Fig. 3A). Interestingly, *S. lentus*, and *S. simulans* isolates exhibited rather distinct AMR profiles (Figs. 3B and C). However, both *S. lentus* and *S. simulans* displayed comparatively high levels of resistance to CHL, CIP, CLI, and ERY. Notably, *S. agnetis* and *S. lentus* isolates exhibited higher levels of CIP resistance (61.1% and 63.6%, respectively) than *S. simulans* isolates.

Mutations in the QRDRs of FQ-resistant NAS isolates

Among the 100 NAS isolates, 12 CoVS (10 *S. agnetis* and two *S. chromogenes*) and 22 CoNS (eight *S. simulans*, seven *S. arlettae*, and seven *S. lentus*) isolates exhibited resistance to CIP (Table 3). Sequencing analysis of QRDRs within *gyrA*, *gyrB*, *parC*, and *parE* revealed that mutations at codon 84 in *gyrA* (S84L and D84N) and codon 80 in *parC* (S80L, T80I, T80R, S80F, and S80Y) were most frequently associated with the CIP-resistant phenotype of the NAS isolates (Table 3). Unlike point mutations in *gyrA* and *parC*, a high degree of mutation heterogeneity was observed in *gyrB* and *parE* within and between different NAS species (Table 3). Nevertheless, none of the FQ-resistant *S. lentus* (n = 7) isolates carried point mutations in *gyrB*. Similarly, none of the FQ-resistant *S. agnetis* isolates harbored point mutations in *parE*.

Detection of SE and TSST-1 genes in NAS isolates

Among the 100 NAS isolates, only two CoNS isolates (one *S. simulans* and one *S. xylosus*) from chicken carcasses were positive for TSST-1 gene. None of the 19 CoVS isolates were positive for the five classical SE genes and *tst1*.

Discussion

Many recent studies have revealed that NAS are important reservoirs of AMR and enterotoxin genes, which can be transmitted to other pathogenic bacteria, including more pathogenic strains of *S. aureus*. In our previous research, we suggested that NAS in retail chicken meat can play an important role in the transmission of AMR and enterotoxin genes (Lee et al., 2020). However, not many studies have investigated the species profiles of antimicrobial-resistant NAS and their significance in food-related public health.

In the present study, we investigated NAS species distribution and their AMR profiles in chicken carcasses, facility environments, and poultry slaughterhouse workers in Korea. Similar to the findings of our previous studies on NAS profiles in broiler farms (Park et al., 2023) and retail chicken meat (Lee et al., 2020), the prevalence of CoNS (81.0%, 81/100) was considerably higher than that of CoVS (19.0%, 19/100) (Table 1). A higher prevalence of CoNS compared with CoVS has also been reported in both healthy broilers and broilers with signs of illness from other countries. In the present study, *S. simulans* (49.0%, 49/100) and *S. agnetis* (17.0%, 17/100) were the most frequently identified NAS species in poultry slaughterhouses; this is consistent with the previously reported high prevalence of *S. agnetis*, *S. simulans*, *S. haemolyticus*, and *S. xylosus* in broiler farms (Park et al., 2023). Sample origin did not affect the species distribution of NAS isolates in chicken carcasses and facility environments in poultry slaughterhouses (Figs. 1A and B). The four major NAS species, *S. simulans*, *S. agnetis*, *S. lentus*, and *S. arlettae*, were commonly identified in both chicken carcasses and facility environments. These results suggest that broiler-associated NAS species (Park et al., 2023), both CoVS and CoNS species are potential sources of contaminants in chicken carcasses and facility environments in poultry slaughterhouses.

Approximately 20% of all the antibiotics sold in the livestock industry, particularly FQs, β-lactams, and tetracyclines, are consumed in poultry farming in Korea (Lim et al., 2014). Thus, recent studies on livestock-associated antimicrobial-resistant NAS, particularly methicillin-resistant NAS isolates, have raised significant concerns regarding the transmission of resistance genes to more pathogenic bacteria in the food production system. In a previous study in Switzerland (Huber et al., 2011), researchers reported that 52.8% (38/72) of *mecA*-positive NAS isolates, i.e., *S. lentus* (n = 30), *S. sciuri* (n = 6), and *S. epidermidis* (n = 2), were obtained from chicken carcasses. In the present study, *mecA*-positive NAS isolates (15.0%, 15/100) were present at relatively low levels in poultry slaughterhouses. As summarized in Table 1, eight *mecA*-positive but FOX-susceptible NAS isolates (two *S. lentus* and six *S. simulans*) carrying non-typeable SCC*mec* elements were identified. Recent studies revealed the *mecA*-positive but FOX-susceptible phenotype of staphylococci (Goering et al., 2019; Ho et al., 2020). This discrepancy could be owing to the altered expression of *mecA* because of dysregulation in *mecR1* (encoding the inducer protein MecR1), *mecI* (encoding the repressor protein MecI), or *bla* regulatory system (*blaR1-blaI*) (Liu et al., 2016). In addition, a single-nucleotide insertion within *mecA*, resulting in the premature termination of *mecA* expression, has been identified in *S. aureus* strains (Kime et al., 2019).

Largely correlating with the sales amounts of antibiotics in the poultry industry in Korea, relatively high levels of resistance to β-lactams, GEN, TET, and CIP were identified in NAS isolates, particularly in CoVS isolates. This suggests that antibiotic selective pressure affect the prevalence of antimicrobial-resistant NAS isolates in poultry slaughterhouses (Table 2). Furthermore, the high prevalence of FUS resistance in some of NAS isolates, particularly *S. agnetis*, *S. arlettae*, *S. lentus*, and *S. xylosus*, may be because of intrinsic FUS resistance owing to the frequent carriage of *fusD* and *fusF* (Hung et al., 2015). Notably, the

high prevalence of NAS isolates with MDR phenotypes to the antibiotics extensively consumed in poultry farms suggests that antibiotic selective pressure facilitates the proliferation of antimicrobial-resistant NAS in broiler farms, and thus, its transmission to chicken carcasses in slaughterhouses.

The World Health Organization has categorized FQs as the highest-priority critically important antibiotics (CIAs) (Scott et al., 2019). However, FQ-resistant *S. aureus* and NAS strains have been frequently isolated in livestock farms, particularly poultry farms, in Korea. In this study, 34 FQ-resistant NAS isolates (12 CoVS and 22 CoNS) were identified to harbor multiple point mutations in the QRDRs of DNA gyrase and topoisomerase IV, except for one *S. arlettae* isolate carrying a single point mutation in *parC* (Table 3). Consistent with the findings of previous studies of FQ-resistant staphylococci (Takahashi et al., 1998; Wang et al., 2013), all 34 FQ-resistant NAS isolates possessed point mutations in *gyrA* (codon 84) or *parC* (codon 80). In contrast to the mutations in *gyrA* and *parC*, which are frequently concentrated in codons 84 and 80, respectively, point mutations in *gyrB* and *parE* were identified at various locations in QRDRs. Moreover, previous studies on FQ-resistant staphylococci (Nakaminami et al., 2014; Yamada et al., 2008) revealed a high degree of heterogeneity in the point mutations in *gyrB* and *parE*. Although major mutations, including S84L at *gyrA*, S80L at *parC*, and D84N at *parC*, were previously known to be associated with FQ resistance (Li et al., 1998; Takahashi et al., 1998; Vila et al., 1997), the precise role of other minor mutations in FQ resistance should be elucidated in the future research.

SEs produced by coagulase-positive *S. aureus* are a major cause of SFP (Argudín et al., 2010; Fisher et al., 2018). However, many recent studies have indicated that NAS isolates, including those from livestock and food products, carry SE genes and are potentially enterotoxigenic (Mekhloufi et al., 2021; Podkowik et al., 2013). In this study, our results demonstrate that CoVS isolates from poultry slaughterhouses do not harbor the classical SE

nor *tst-1* genes. Although previous studies have revealed the highest prevalence of *sec* and *tst1* in CoNS strains (H jek, 1978; Valle et al., 1990), only two *tst1*-positive (one *S. simulans* and one *S. xylosus*) NAS isolates were detected in the present study. Since SEA and TSST-1 were first detected in CoNS strains isolated from patients with toxic shock syndromes (Crass and Bergdoll, 1986), potential enterotoxigenic or pathogenic NAS strains carrying SE genes have been detected in humans (Achek et al., 2018; Banaszkiewicz et al., 2022), livestock (Ruiz-Ripa et al., 2020; Ü nal and Ç inar, 2012), and ready-to-eat foods (Chajęcka-Wierzchowska et al., 2020; Crass and Bergdoll, 1986; Cunha et al., 2006; Rall et al., 2010; Zell et al., 2008). Despite the proposed instability of SE genes in CoNS isolates (Banaszkiewicz et al., 2022), the carriage of SE and TSST-1 genes in NAS isolates from poultry slaughterhouses should be monitored as a significant threat to food safety.

In summary, this is the first report on the species diversity, AMR patterns, and genetic characteristics of methicillin- and FQ-resistant NAS isolates obtained from poultry slaughterhouses in Korea. Our findings suggest that (i) the overall species distribution of NAS isolates in poultry slaughterhouses is similar to that in broiler farms (Park et al., 2023) in Korea; (ii) MDR phenotypes with relatively high resistance levels of resistance to PEN, AMP, GEN, CIP, and TET are observed in NAS isolates; (iii) FQ-resistance in NAS isolates is caused by point mutations occurring at specific locations (*gyrA* and *parC*) or rather various locations (*gyrB* and *parE*) of QRDRs; and (iv) the carriage of *tst1* only in CoNS isolates, indicating the extremely low prevalence of the TSST-1 and SE genes in NAS isolates. In addition, this study underscores a potential role of poultry slaughterhouses in transmission of antimicrobial-resistant NAS strains to human food chains.

Top combat AMR in poultry industry, a multisectoral approach, including poultry farms, slaughterhouses, and human workers, is necessary. Improper use of antibiotics and indiscriminate prescription of antibiotics should be avoided to prevent occurrence and

amplification of antimicrobial-resistant bacteria in poultry farms. Furthermore, importance of hygiene measures in slaughterhouses should be emphasized along with a multisectoral surveillance networks to better detect and mitigate spread of AMR.

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Table 1. Profiles of NAS and SCC*mec* types of methicillin-resistant NAS strains isolated from poultry slaughterhouses

NAS[1] (n = isolates)	*mecA* positive (%)	*mec* gene	*ccr*[2] gene	SCC*mec*[3] type
CoVS[4] (19)				
Staphylococcus agnetis (17)	-	-	-	-
Staphylococcus chromogenes (2)	-	-	-	-
CoNS[5] (81)				
Staphylococcus arlettae (8)	-	-	-	-
Staphylococcus epidermidis (1)	1 (100)	B	A2B2	SCC*mec* IV
Staphylococcus lentus (11)	6 (54.5)	Multi Multi Multi Multi Multi Multi	- - - - - Multi	NT[6]
Staphylococcus simulans (49)	8 (16.3)	B B B B B - E A	A1B6 A1B1 - A1B1 - A1B6 - A1B1	NT SCC*mec* I NT SCC*mec* I NT NT NT NT
Staphylococcus sciuri(4)	-	-	-	-
Staphylococcus urealyticus (1)	-	-	-	-
Staphylococcus warneri (3)	-	-	-	-
Staphylococcus xylosus (4)	-	-	-	-

[1]NAS, non-*aureus* staphylococci; [2]*ccr*, chromosomal cassette recombinase; [3]SCC*mec*, staphylococcal cassette chromosome *mec*; [4]CoVS, coagulase-variable staphylococci; [5]CoNS, coagulase-negative staphylococci; [6]NT, non-typeable.

Table 2. Antimicrobial resistance profiles of NAS strains isolated from poultry slaughterhouses

NAS[2] (n=isolates)	Number of Antimicrobial Resistance[1] (%)														
	AMP	**FOX**	**PEN**	**CHL**	**CIP**	**CLI**	**ERY**	**FUS**	**GEN**	**MUP**	**RIF**	**SXT**	**SYN**	**TET**	**MDR[3] (%)**
CoVS[4] (19)															
Staphylococcus agnetis (17)	14 (83.3)	0	14 (83.3)	2 (16.7)	10 (61.1)	6 (38.9)	6 (38.9)	15 (88.2)	14 (77.8)	0	0	0	0	4 (27.8)	14 (82.4)
Staphylococcus chromogenes (2)	2 (100)	0	2 (100)	1 (50)	2 (100)	2 (100)	2 (100)	1 (50)	1 (50)	0	0	0	0	1 (50)	2 (100)
CoVS Total	**16 (84.2)**	**0**	**16 (84.2)**	**3 (15.8)**	**12 (63.2)**	**8 (42.1)**	**8 (42.1)**	**16 (84.2)**	**15 (78.9)**	**0**	**0**	**0**	**0**	**5 (26.3)**	**16 (84.2)**
CoNS[5] (81)															
Staphylococcus arlettae (8)	8 (100)	0	8 (100)	7 (85.7)	7 (85.7)	6 (75)	8 (100)	8 (100)	0	0	0	0	0	3 (37.5)	8 (100)
Staphylococcus epidermidis (1)	1 (100)	1 (100)	1 (100)	0	0	0	1 (100)	1 (100)	0	0	0	0	0	0	1 (100)
Staphylococcus lentus (11)	6 (54.5)	4 (36.4)	6 (54.5)	7 (63.6)	7 (63.6)	7 (63.6)	7 (63.6)	8 (72.7)	0	0	0	5 (45.5)	0	2 (18.2)	10 (90.9)
Staphylococcus sciuri (4)	2 (50)	0	2 (50)	1 (25)	0	0	0	4 (100)	0	0	0	0	0	0	0
Staphylococcus simulans (49)	5 (10.2)	2 (4.1)	4 (8.2)	26 (53.1)	8 (16.3)	37 (75.5)	38 (77.6)	3 (6.1)	0	0	0	10 (20.4)	0	4 (8.2)	28 (57.1)
Staphylococcus urealyticus (1)	0	0	0	1 (100)	0	1 (100)	1 (100)	1 (100)	0	0	0	0	0	0	1 (100)
Staphylococcus warneri (3)	0	0	0	0	0	3 (100)	3 (100)	0	0	0	0	0	3 (100)	0	3 (100)
Staphylococcus xylosus (4)	3 (75)	0	4 (100)	0	0	0	0	4 (100)	0	0	0	0	0	0	0
CoNS Total	**25 (30.9)**	**7 (8.6)**	**25 (30.9)**	**42 (51.9)**	**23 (28.4)**	**54 (66.7)**	**58 (71.6)**	**29 (35.8)**	**0**	**0**	**0**	**15 (18.5)**	**3 (3.7)**	**9 (11.1)**	**51 (63)**
TOTAL (100)	**41 (41)**	**7 (7)**	**41 (41)**	**45 (45)**	**35 (35)**	**62 (62)**	**66 (66)**	**45 (45)**	**15 (15)**	**0**	**0**	**15 (15)**	**3 (3)**	**14 (14)**	**67 (67)**

[1]AMP, ampicillin; FOX, cefoxitin; PEN, penicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; FUS, fusidic acid; GEN, gentamycin; MUP, mupirocin; RIF, rifampin; SXT, trimethoprim-sulfamethoxazole; SYN, quinupristin-dalfopristin; TET, tetracycline; [2]NAS, non-*aureus* staphylococci; [3]MDR, multi-drug resistance; [4]CoVS, coagulase-variable staphylococci; [5]CoNS, coagulase-negative staphylococci.

Table 3. Point mutations within QRDRs of *gyrA, gyrB, parC,* and *parE* genes associated with quinolone resistance in the study strains

	NAS[2] species	No. of FQ[3]-resistant isolates (%)	Mutations in QRDRs[1]				Total	Zone of inhibition (mm)
			gyrA	*gyrB*	*parC*	*parE*		
CoVS[4]	*Staphylococcus agnetis* (17)	10 (58.8)	S84L	-	S80L	-	6	12.8± 1.2
			S84L	D562Y	S80L	-	2	13.5± 2.1
			S84L	F522V, K550R, D562Y	S80L	-	1	13
			S84L	F522V, E560Q, D562Y, E566Q, E597D	S80L	-	1	13
	Staphylococcus chromogenes (2)	2 (100)	S84L	I359V, G375A, I379V, T514K	H77L, G78A, S80L	T348P, D356E, S357A, S360A, D565T	1	6
			S84L, I131L	H511I, T514N	S80L, D84Y	K568N	1	13
CoNS[5]	*Staphylococcus arlettae* (8)	7 (87.5)	S84L	-	T80I	-	3	6
			-	-	T80I	-	1	6
			S84L	-	T80I	A531Q	1	6
			S84L		T80I	A360S	1	6
			S84L	K554N, R561Q, K565Q, K567T	T80I	K375T, V382G, K383E, R395G, R406Q, K407E, G413S, A418P	1	6
	Staphylococcus lentus (11)	7(63.6)	S84L, T172A	-	S80L	-	2	6
			S84L, T172A	-	S80L	Y497T	3	6
			S84L, A162S, T172A	-	-	Y497T	1	10
			S84L, A162S, T172A	-	S80L	Y497T	1	9
	Staphylococcus simulans (49)	8 (16.3)	S84L, A173S	-	F74Y, S80F	-	1	14
			S84L, A173S	-	S80Y, D84N	-	1	6
			S84L, A173S	A512R	S80Y, D84N	-	1	6
			S84L, A173S	A512P	S80F, D84N	-	1	6
			S84L, A173S	-	S80Y, D84N	L377I	1	6
			S84L, A173S	-	S80F, D84N	N498K, R499S	1	6
			S84L, A132S, A173S	E489G, S494T	S80I	V359I, F365Y, E467D	2	6

[1]QRDRs, quinolone resistance determining regions; [2]NAS, non-*aureus* staphylococci; [3]FQ, fluoroquinolone; [4]CoVS, coagulase-variable staphylococci; [5]CoNS, coagulase-negative staphylococci.

FIGURE LEGENDS

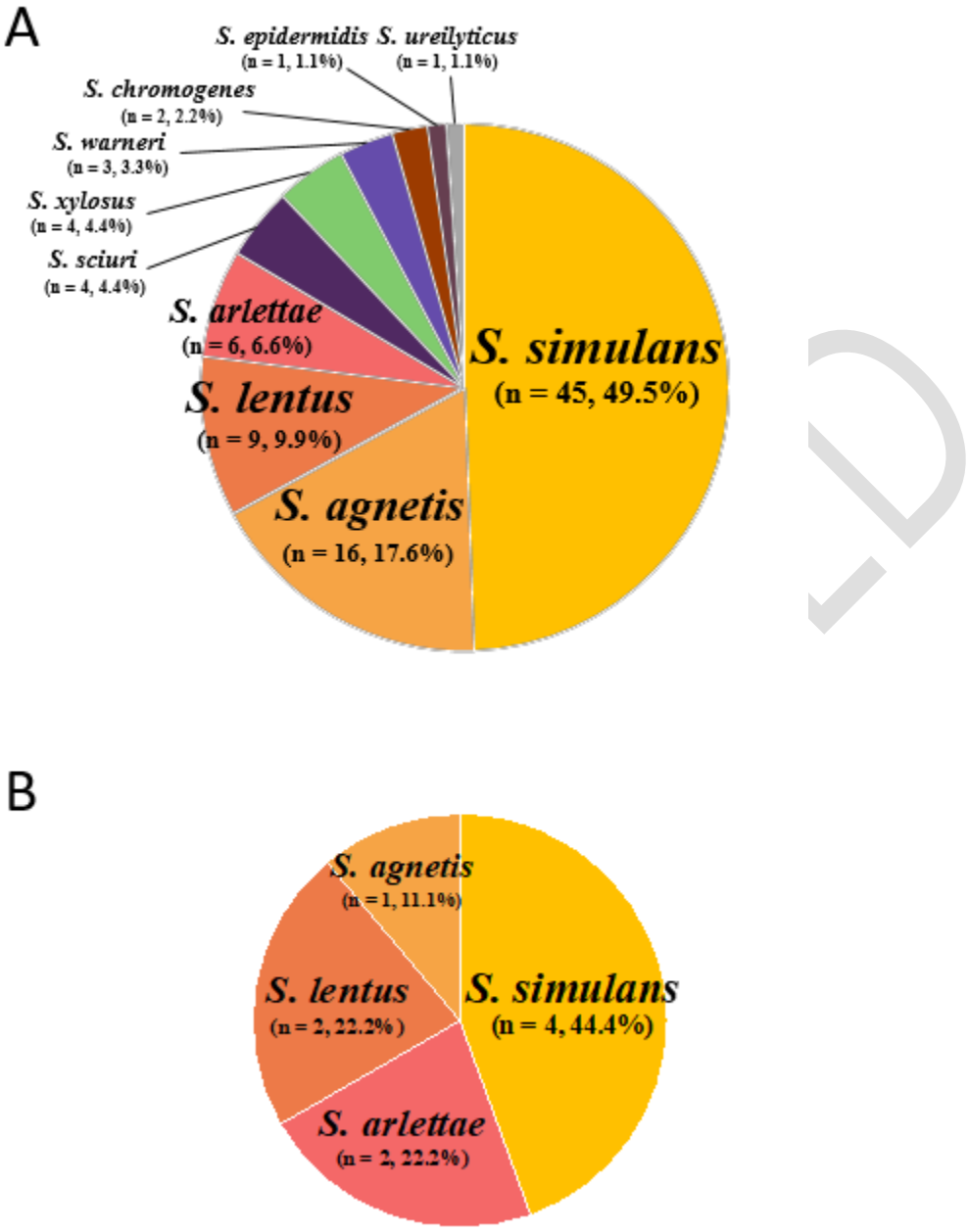

FIG 1. Species distribution of non-*aureus* staphylococci (NAS) isolated from chicken carcasses (**A**) and slaughterhouse environments (**B**) in Korea. In total, 100 NAS isolates from 10 different species were identified in poultry slaughterhouses in Korea.

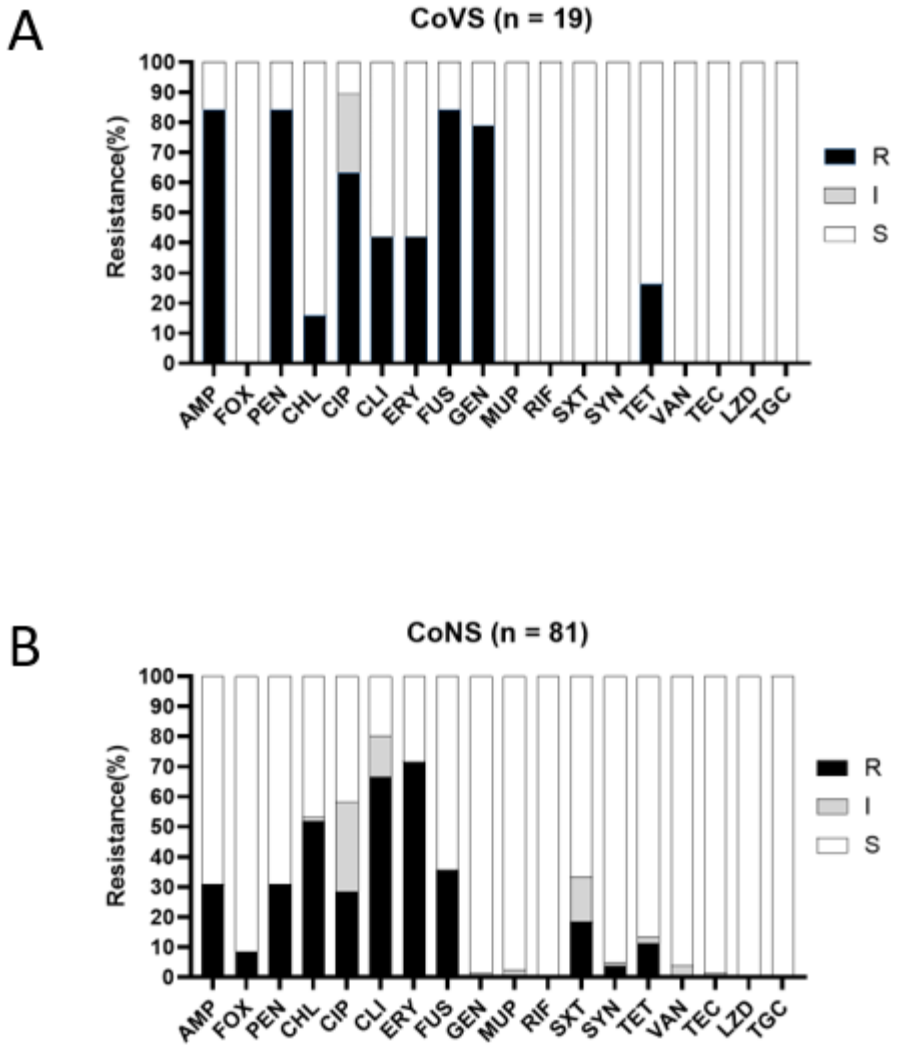

FIG 2. Antimicrobial resistance patterns of the non-*aureus* staphylococci (NAS) isolates collected from the slaughterhouses in Korea. Antimicrobial resistance phenotypes of coagulase-variable staphylococci (CoVS) (**A**) and coagulase-negative staphylococci (CoNS) (**B**) isolates.

R, resistant; I, intermediate; S, susceptible

AMP, ampicillin; PEN, penicillin; CHL, FOX, cefoxitin; FUS, fusidic acid; chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; CLI, clindamycin; ERY, erythromycin; MUP, mupirocin; SXT, trimethoprim-sulfamethoxazole; RIF, rifampicin; SYN, quinupristin-

dalfopristin; TET, tetracycline; VAN, vancomycin; LZD, linezolid; TEC, teicoplanin; TGC, tigecycline.

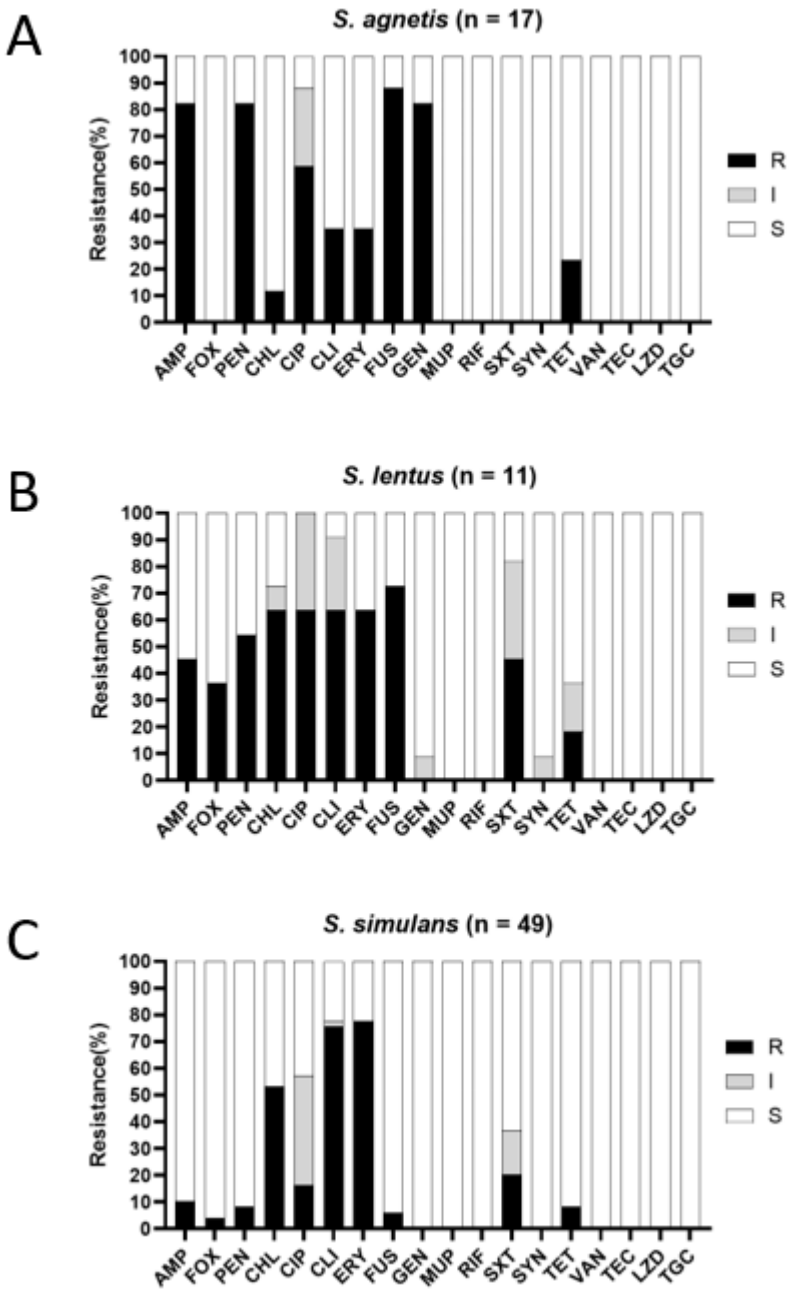

FIG 3. Antimicrobial resistance patterns of the major non-*aureus* staphylococci (NAS) collected from poultry slaughterhouses. Antimicrobial resistance profiles of *S. agnetis* (**A**), *S lentus* (**B**), and *S. simulans* (**C**) isolates.

Table S1. Primers for detecting QRDRs mutations in fluoroquinolone-resistant NAS

Target gene	Species		Primer sequence ((5'→3')	Amplicon size (bp)	PCR condition
gyrA	All species	**F**	AATGAACAAGGTATGACACC	368	95℃ 5 min + 28X (95 ℃ 30 sec + 50 ℃ 30 sec +72 ℃ 40sec) + 72 ℃ 5 min
		R	GCGATACCTGATGCACCATT		
gyrB	*Staphylococcus agnetis*	**F**	AGTGACACGTCGTAAGTCGG	612	95℃ 5 min + 28X (95 ℃ 30 sec + 53 ℃ 30 sec +72 ℃ 40sec) + 72 ℃ 5 min
		R	TGAAGCATCGCACGGTTTTC		
	Staphylococcus arlettae	**F**	TGGCTCGTGTCATTGTCGAA	790	
		R	GTCGCATACACTGCGTTGTC		
	Staphylococcus nepalensis	**F**	AAAAAGCGCGTGAAGTGACA	696	
		R	GGTTCTCAACAACATCGCCC		
	Staphylococcus chromogenes	**F**	GAAACACGGGGACCCTCAAT	545	95℃ 5 min + 28X (95 ℃ 30 sec + 54 ℃ 30 sec +72 ℃ 40sec) + 72 ℃ 5 min
		R	TTCGGATATGGGCACCATCG		
	Staphylococcus lentus	**F**	AGAGCTCGTCTAGCAGCGAA	681	
		R	CGTTTCGTCAGCTTCTATCGC		
	Staphylococcus simulans	**F**	CCTCTCGTGCACGTATCGCA	300	95℃ 5 min + 28X (95 ℃ 30 sec + 48 ℃ 30 sec +72 ℃ 40sec) + 72 ℃ 5 min
		R	TGATATGCGCACCATCCACA		
parC	*Staphylococcus agnetis*	**F**	TTACCTGATGTACGCGACGG	922	95℃ 5 min + 28X (95 ℃ 30 sec + 54 ℃ 30 sec +72 ℃ 40sec) + 72 ℃ 5 min
		R	GTCGACCTTCACTGATCGCT		
	Staphylococcus lentus	**F**	ATCCAAGACCGAGCACTTCC	575	
		R	CCGGTAGGGAAATCAGGTCC		
	Staphylococcus arlettae	**F**	ACCCGATGTACGTGATGGTT	257	95℃ 5 min + 28X (95 ℃ 30 sec + 53 ℃ 30 sec +72 ℃ 40sec) + 72 ℃ 5 min
		R	ATAGCTGCTGCAGGGTCATT		
	Staphylococcus chromogenes	**F**	CGTCGGGGATGTCATTGGAC	162	95℃ 5 min + 28X (95 ℃ 30 sec + 50 ℃ 30 sec +72 ℃ 40sec) + 72 ℃ 5 min
		R	GTATAACGCATCGCAGCAGG		
	Staphylococcus nepalensis	**F**	TTGGCGACCGATTTGGTAGAT	309	
		R	TAGCTGCTGCTGGATCGTTA		
	Staphylococcus simulans	**F**	GTGCCAAAACAGTCGGTGAT	364	95℃ 5 min + 28X (95 ℃ 30 sec + 52 ℃ 30 sec +72 ℃ 40sec) + 72 ℃ 5 min
		R	AAGTTGTGCGGCGGAATATC		

Target gene	Species		Primer sequence ((5'→3')	Amplicon size (bp)	PCR condition
parE	*Staphylococcus agnetis*	**F**	GGGTGGGTCTGCAAAACTTG	308	95℃ 5 min + 28X (95 ℃ 30 sec + 52 ℃ 30 sec +72 ℃ 40sec) + 72 ℃ 5 min
		R	GTAACGCGATAAACACGCGA		
	Staphylococcus nepalensis	**F**	AGCCCAACAAGCAAGAGAAG	649	
		R	TGTCTCTGGGTTCATTGTCGT		
	Staphylococcus arlettae	**F**	TTAGGTACACCGGAAGCACG	566	95℃ 5 min + 28X (95 ℃ 30 sec + 53 ℃ 30 sec +72 ℃ 40sec) + 72 ℃ 5 min
		R	ACACGTCCTGCCAACACTAA		
	Staphylococcus chromogene	**F**	TAGGGACACCTGAAGCGAGA	851	
		R	ACGACGTGGGGCAACTTTAT		
	Staphylococcus simulans	**F**	CGCGTCGCATTGGTGAATTA	628	
		R	CCATCTGTATCGGCATCGGT		
	Staphylococcus lentus	**F**	CGATTAAAGCACAACAAGCAAG	393	
		R	GCGCACCATCAGTATCAG		

Table S2. Antimicrobial resistance profiles of 100 NAS strains isolated from poultry slaughterhouses

NAS species	Strain	AMR[1] profiles
Staphylococcus agnetis (n = 17)	CCSM-112	AMP-PEN-CIP-FUS-GEN
	CCSM-151	CIP-FUS
	CCSM-1101	CHL-FUS
	CCSM-1112	CHL-FUS
	CSSM-231	AMP-PEN-FUS-GEN
	CSSM-241	AMP-PEN-FUS-GEN
	CSSM-2101	AMP-PEN-FUS-GEN
	CSSM-2111	AMP-PEN-CIP-FUS-GEN
	CGSM-1131EA	AMP-PEN-CIP-CLI-ERY-FUS-GEN
	CGSM-132EB	AMP-PEN-CLI-ERY-GEN-TET
	CGSM-162EA	AMP-PEN-CIP-CLU-ERY-FUS-GEN
	CGSM-172EA	AMP-PEN-CIP-CLI-ERY-FUS-GEN-TET
	CGSM-1102EA	AMP-PEN-CIP-FUS-GEN
	CGSM-1102EA	AMP-PEN-CIP-CLI-ERY-FUS-GEN-TET
	CGSM-1122EA	AMP-PEN-CIP-FUS-GEN
	CGSM-1172EA	AMP-PEN-CIP-FUS-GEN
	CGSE-103EB	AMP-PEN-CLI-ERY-GEN-TET
Staphylococcus chromogenes (n = 1)	CCSM-1181	AMP-PEN-CHL-CIP-CLI-ERY-TET
	CGSM-191EA	AMP-PEN-CIP-CLI-ERY-FUS-GEN
Staphylococcus alrettae (n = 8)	CSSE-103	AMP-PEN-CHL-CIP-CLI-ERY-FUS-TET
	CSSM-161	AMP-PEN-CHL-CIP-CLI-ERY-FUS
	CSSM-172	AMP-PEN-CHL-CIP-CLI-ERY-FUS-TET
	CSSM-111	AMP-PEN-CHL-CIP-ERY-FUS
	CSSM-1132	AMP-PEN-CHL-CLI-ERY-FUS-TET
	CSSM-1142	AMP-PEN-CIP-CLI-ERY-FUS
	CSSM-1192	AMP-PEN-CHL-CIP-CLI-ERY-FUS
	CSSE-102	AMP-PEN-CHL-CIP-ERY-FUS
Staphylococcus epidermidis (n = 1)	CJSM-112	AMP-FOX-PEN-ERY-FUS
Staphylococcus lentus (n = 11)	CSSM-191	PEN-CHL-CIP-CLI-ERY-FUS
	CSSM-1112	AMP-FOX-PEN-CHL-CIP
	CSSM-1122	CHL-CIP-CLI-ERY-TET
	CSSE-104	FUS
	CSSM-221	AMP-FOX-PEN-CIP-ERY-FUS-SXT
	CSSM-2131	AMP-PEN-CLI-ERY-FUS
	CSSM-2141	AMP-FOX-PEN-CHL-FUS-SXT
	CGSM-1111-10	CIP-CLI-ERY-SXT-TET
	CGSM-1181	CHL-CIP-CLI-FUS-SXT
	CGSE-102-10	CHL-CLI-ERY-FUS-SXT
	CSSM-151	AMP-FOX-PEN-CHL-CIP-CLI-ERY-FUS
Staphylococcus sciuri (n = 4)	CSSM-181	AMP-PEN-FUS
	CCSM-172	FUS
	CSSM-122	CHL-FUS
	CSSM-141	AMP-PEN-FUS
Staphylococcus warneri (n = 3)	CSSM-1181	CLI-ERY-SYN
	CSSM-112	CLI-ERY-SYN
	CSSM-1102	CLI-ERY-SYN
Staphylococcus xylosus (n = 4)	CSSM-1182	AMP-PEN-FUS
	CSSM-1171	AMP-PEN-FUS
	CSSM-132	AMP-PEN-FUS

NAS species	Strain	AMR profiles
Staphylococcus simulans (n = 49)	CSSM-131	CHL-CIP-CLI-ERY-TET
	CCSM-162	-
	CCSM-1191	-
	CSSM-1101	CIP-CLI-ERY
	CSSM-1151	CHL-CLI-ERY
	CSSM-162	CIP-TET
	CSSM-182	AMP-PEN-CLI-ERY
	CSSM-1162	CIP-TET
	CSSM-1202	AMP-PEN-CLI-ERY
	CSSE-101	AMP-PEN-CHL-CLI-ERY
	CSSM-251	AMP-FOX
	CSSM-291	CHL-CIP
	CSSM-2161	CHL-CIP-ERY-TET
	CSSM-2171	AMP-PEN
	CSSM-2181	CHL
	CSSM-2191	FOX-FUS
	CSSM-2201	-
	CGSM-141EB	CIP-CLI-ERY
	CGSM-151	CLI-ERY
	CGSM-151-10	CLI-ERY
	CGSM-161	CHL-CLI-ERY-TET
	CGSM-161-10	CHL-CIP-ERY-FUS-SXT
	CGSM-181	CLI-ERY
	CGSM-181-10	CHL-CLI-ERY-FUS
	CGSM-191EB	CLI-ERY
	CGSM-1101	CHL-CLI-ERY
	CGSM-1121-10	CHL-CLI-ERY
	CGSM1131-10	CHL-CLI-ERY
	CGSM-1141-10	CLI-ERY
	CGSM-1151-10	CHL-CLI-ERY-SXT
	CGSM-1151EA	CHL-CLI-ERY
	CGSM-1161-10	CLI-ERY
	CGSM-1171-10	CHL-CLI-ERY-SXT
	CGSM-1181-10	CHL-CLI-ERY-SXT
	CGSM-1191-10	CHL-CLI-ERY-SXT
	CGSM-1191	CHL-CLI-ERY
	CGSM-1201-10	CHL-CLI-ERY
	CGSM-112-10	CLI-ERY
	CGSM-142EA-10	CHL-CLI-ERY-SXT
	CGSM-142	CLI-ERY
	CGSM-162EA-10	CLI-ERY
	CGSM-192EB	CHL-CLI-ERY
	CGSM-1142-10	CHL-CLI-ERY
	CGSM-1152	CHL-CLI-ERY-SXT
	CGSM-1182	CHL-SXT
	CGSE-102	CHL-CLI-ERY-SXT
	CGSE-103-10	CLI-ERY
	CGSE-105	CHL-CLI-ERY-SXT
	CSSM-152	CIP-CLI-ERY

CSSM-1152	PEN-FUS	*Staphylococcus ureilyticus* (n = 1)	CSSM-261	CHL-CIP-CLI-ERY-FUS

[1]AMR, antimicrobial resistance; AMP, ampicillin; FOX, cefoxitin; PEN, penicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; FUS, fusidic acid; GEN, gentamycin; MUP, mupirocin; RIF, rifampin; SXT, trimethoprim-sulfamethoxazole; SYN, quinupristin-dalfopristin; TET, tetracycline.