TITLE PAGE
- Food Science of Animal Resources -
Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title	Species profiles and antimicrobial resistance of non-*aureus* staphylococci isolated from healthy broilers, farm environments, and farm workers
Running Title (within 10 words)	Non-*aureus* staphylococci in broiler farms
Author	Ji Heon Park[1], Gi Yong Lee[1], Ji Hyun Lim1, Geun-Bae Kim[2], Kun Taek Park[3], and Soo-Jin Yang[1*]
Affiliation	[1]Department of Veterinary Microbiology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul 08826, Republic of Korea; [2]Department of Animal Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea; [3]Department of Biotechnology, Inje University, Gimhae 50834, Republic of Korea
Special remarks – if authors have additional information to inform the editorial office	None
ORCID (All authors must have ORCID) https://orcid.org	Ji Heon Park (https://orcid.org/0000-0002-5843-785X) Gi Yong Lee (https://orcid.org/0000-0001-5308-0065) Ji Hyun Lim (https://orcid.org/0000-0001-6069-5076) Geun-Bae Kim (https://orcid.org/0000-0001-8531-1104) Kun Taek Park (https://orcid.org/0000-0001-6177-0373) Soo-Jin Yang (https://orcid.org/0000-0003-3253-8190)
Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This study was supported by funding from the Research of Korea Centers for Disease Control and Prevention (Projects No. 2017NER54060 & 2021ER220100).
Author contributions (This field may be published.)	Conceptualization: Park KT, Kim GB, Yang S-J Data curation: Jang YS, Choi Y. Formal analysis: Park JH, Lee GY, Lim JH Methodology: Lee GY, Lim JH Investigation: Park JH, Park KT, Yang S-J Writing - original draft: Park JH, Yang S-J Writing - review & editing: Lee GY, Kim GB, Park KT, Yang S-J
Ethics approval (IRB/IACUC) (This field may be published.)	The sampling protocol was reviewed and approved by the IRB/IACUC at Chung-Ang university.

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Soo-Jin, Yang
Email address – this is where your proofs will be sent	soojinjj@snu.ac.kr
Secondary Email address	
Postal address	Department of Veterinary Microbiology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul 08826, Republic of Korea

Cell phone number	+82-10-7679-8001
Office phone number	+82-02-880-1185
Fax number	+82-02-873-1213

Species profiles and antimicrobial resistance of non-*aureus* staphylococci isolated from healthy broilers, farm environments, and farm workers in Korea

Running title: Non-*aureus* staphylococci in broiler farms

Abstract

Non-*aureus* staphylococci (NAS), particularly antimicrobial-resistant NAS, have a substantial impact on human and animal health. In the current study, we investigated (1) the species profiles of NAS isolates collected from healthy broilers, farm environments, and farm workers in Korea, (2) the occurrence of antimicrobial-resistant NAS isolates, especially methicillin resistance, and (3) the genetic factors involved in the methicillin and fluoroquinolone resistance. In total, 216 NAS isolates of 16 different species were collected from healthy broilers (n = 178), broiler farm environments (n = 18), and farm workers (n = 20) of 20 different broiler farms. The two most dominant broiler-associated NAS species were *S. agnetis* (23.6%) and *S. xylosus* (22.9%). Six NAS isolates were *mecA*-positive carrying staphylococcal cassette chromosome *mec* (SCC*mec*) II (n = 1), SCC*mec* IV (n = 1), SCC*mec* V (n = 2), or non-typeable SCC*mec* element (n = 2). While two *mecA*-positive *S. epidermidis* isolates from farm workers had SCC*mec* II and IV, a *mecA*-positive *S. epidermidis* isolate from broiler and a *S. haemolyticus* isolate farm environment carried SCC*mec* V. The occurrence of multidrug resistance (MDR) was observed in 48.1% (104/216 isolates) of NAS isolates with high resistance rates to β-lactams (> 40%) and fusidic acid (FUS, 59.7%). Fluoroquinolone resistance was confirmed in 59 NAS isolates (27.3%), and diverse mutations in the quinolone resistance determining regions (QRDR) of *gyrA*, *gyrB*, *parC*, and *parE* were identified. These findings suggest that NAS in broiler farms may have a potential role in the acquisition, amplification, and transmission of antimicrobial resistance.

250/250 words

Keywords: non-*aureus* staphylococci; broiler; species profiles; antimicrobial resistance

Introduction

Several recent studies have demonstrated that non-*aureus* staphylococci (NAS), including coagulase-negative staphylococci (CoNS), have a substantial impact on human and animal health (Fisher et al., 2018). Nosocomial CoNS are particularly well-known for their ability to form biofilms and acquire resistance to multiple antimicrobial agents (Nadell et al., 2009; O'Gara and Humphreys, 2001; Schilcher and Horswill, 2020). It has been suggested that NAS in livestock farm environments represent a significant reservoir of antimicrobial resistance (AMR) genes, facilitating the horizontal transfer of AMR (Feßler et al., 2018; Shen et al., 2013). Methicillin-resistant *Staphylococcus aureus* (MRSA) has also been proposed to have acquired staphylococcal cassette chromosome *mec* (SCC*mec*) from CoNS, which normally colonize various animal hosts.

The high prevalence of antimicrobial-resistant staphylococci, particularly multidrug-resistant (MDR) staphylococci, in livestock animals and farm environments has become a significant hazard to food safety and public health. Although a relatively large number of studies have focused on livestock-associated MRSA (LA-MRSA) in swine and cattle farms, the prevalence of NAS and their AMR profiles in livestock farms, especially broiler farms, are not well established. Only a few recent studies have investigated the prevalence of antimicrobial-resistant *S. aureus* in broiler farms, poultry slaughterhouses, and retail chicken meat samples in Korea (Kim et al., 2018; Lee et al., 2022). In addition, NAS species, such as *S. agnetis*, *S. saprophyticus*, *S. xylosus*, *S. simulans*, and *S. lentus*, have been reported as opportunistic pathogens in poultry (Boamah et al., 2017; Pyzik et al., 2019; Silva et al., 2022; Szafraniec et al., 2020). However, information on the distribution of NAS species in broiler farms and their AMR profiles is still not available in Korea.

In the present study, we analyzed the species profiles of NAS isolates collected from broiler farms, including healthy broilers, farm environments, and farm workers. In addition, the AMR profiles of the NAS isolates and the major genetic factors involved in the resistance phenotypes, especially methicillin and fluoroquinolone resistance, were examined. To the best of our knowledge, this is the first study to report the species profiles, AMR, and genetic factors associated with fluoroquinolone resistance in NAS isolates from broiler farms in Korea.

Materials and Methods

Collection of samples

All swab samples were obtained from 20 different broiler farms across eight provinces of South Korea in 2019 as previously described (Lee et al., 2022). A total of 916 swab samples were collected from healthy broilers (n = 782; cloacal and throat swabs), farm facilities (n = 71; floor, fence, drinking system, feeder pan, sewage, and ventilators), and farm workers (n = 63; hand and nasal swabs). After sampling, all swabs were transported using ice-cooled containers and processed for the isolation of NAS within 36h of sample collection. The sampling protocol was reviewed and approved by the IRB/IACUC at Chung-Ang university.

Isolation and identification of NAS

Isolation and culture of NAS strains from swab samples were performed as previously described, with minor modifications (Lee et al., 2022). Briefly, all swab samples were inoculated into 4.5 mL of tryptic soy broth (Difco Laboratories, Detroit, MI, USA) containing 10% sodium chloride and incubated at 37℃ for 17-24 h for enrichment. Next, 15-20 μL of the pre-enriched cultures were streaked onto Baird-Parker agar (Difco Laboratories) supplemented with egg yolk and potassium tellurite (Becton Dickinson, Sparks, MD, USA) and incubated for 48 h at 37℃. One or two colonies of presumptive staphylococci per sample were then selected for subsequent identification of species. The species of the NAS strains were first identified by using a matrix-assisted laser desorption ionization (MALDI)-Biotyper classification system (Bruker, Bremen, Germany) and then 16S rRNA sequencing for strains with < 2.0 scores in the MALDI-Biotyper analysis, as previously described (Yang et al., 2022).

Antimicrobial susceptibility tests

To determine the AMR profiles of the NAS strains, standard disc diffusion assays were performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI) (CLSI, 2020a; CLSI, 2020b) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The 14 antimicrobial agents included in the disc diffusion assays were ampicillin (AMP, 10 µg), cefoxitin (FOX, 30 µg), penicillin (PEN, 10 units), gentamicin (GEN, 50 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), clindamycin (CLI, 2 µg), erythromycin (ERY, 15 µg), fusidic acid (FUS, 50 µg), mupirocin (MUP, 200 µg), rifampin (RIF, 5 µg), sulfamethoxazole-trimethoprim (SXT, 23.73- 1.25 µg), quinupristin-dalfopristin (SYN, 15 µg), and tetracycline (TET, 30 µg). All antimicrobial discs were purchased from BD BBL™ (Becton Dickinson, NJ, USA), except for the MUP discs (Oxoid, Hampshire, UK). The minimum inhibitory concentrations (MICs) of vancomycin (VAN), tigecycline (TGC), linezolid (LZD), and teicoplanin (TEC) were determined using standard E-test strips (bioMérieux, France) on Mueller-Hinton agar (Difco Laboratories). Two reference strains, *S. aureus* ATCC29213 and *S. aureus* MW2, were used in antimicrobial susceptibility assays.

Detection of *mecA* and SCC*mec* typing

All NAS strains displaying resistance phenotypes to β-lactam antimicrobial agents were subjected to polymerase chain reaction (PCR) to detect *mecA* gene, as previously described (Geha et al., 1994). The types of staphylococcal cassette chromosome *mec* (SCC*mec*) were then determined in all *mecA*-positive NAS strains using previously described multiplex PCR methods that amplify chromosomal cassette recombinase (*ccr*) genes and *mec* regulatory

elements. The combinations of *ccr* types and *mec* complexes were analyzed to assign the SCC*mec* types of methicillin-resistant NAS strains (Geha et al., 1994; Mendoza et al., 1998).

Mechanisms of fluoroquinolone resistance in the NAS strains

To detect the mutations in fluoroquinolone-resistant strains, quinolone-resistance determining regions (QRDRs) within *gyrA*, *gyrB*, *parC*, and *parE* were PCR-amplified using specific primer sets as previously described (Lee et al., 2020; Sreedharan et al., 1991; Takahashi et al., 1998; Takahata et al., 1997). The PCR products of the QRDRs were then sequenced (Bionics, Seoul, Korea), and mutations were identified using the Cluster Omega server (ebi.ac.uk/Tools/msa/clustalo/). The published sequences of *S. agnetis* (CP009623.1), *S. chromogenes* (CP046028.1), *S. arlettae* (AP019698.1), *S. cohnii* (CP027422.1), *S. condimenti* (CP018776.1), *S. haemolyticus* (CP035291.1), *S. gallinarum* (CP086207.1), *S. saprophyticus* (CP031196.1), *S. simulans* (LT963435.1), and *S. xylosus* (CP013922.1) were used to design QRDR-specific primer sets in each species (Table S1).

Results

Profiles of NAS from broiler farms

A total of 216 NAS strains of 16 different species were isolated from 916 swab samples (216/916, 23.58%) collected from broiler farms during the study period (Fig. 1A-C). Of the 216 NAS strains, 148 (68.5%) CoNS strains displayed 14 different species diversities, whereas 68 coagulase-variable staphylococci (CoVS) were composed of only two species of staphylococci, *S. agnetis* and *S. chromogenes*. As shown in Fig. 1A, 178/216 (82.4%) NAS strains were isolated from healthy broilers, with *S. agnetis* and *S. xylosus* being most frequently associated with healthy broilers (Fig. 1A). Similar to the profiles of NAS strains in broilers, 83.3% (15/18) of NAS strains from farm environments were *S. xylosus* (n = 9), *S. agnetis* (n = 3), and *S. saprophyticus* (n = 3) strains (Fig. 1B). NAS strains from farm workers were distinctive from those of broilers and farm environments in that *S. epidermidis* (11/20, 55%) was most prevalent in the nasal cavities of farm workers (Fig. 1C)

Occurrence of *mecA* in NAS isolated from broilers

Six methicillin-resistant NAS were identified in 216 NAS strains (3.24%) isolated from broiler farms (Table 1). All six strains were *mecA*-positive (Table 1) and were resistant to FOX (Table 2). Analysis of SCC*mec* elements in seven methicillin-resistant NAS revealed that two CoNS (*S. epidermidis* and *S. haemolyticus*) carried SCC*mec* V (Table 1). Two methicillin-resistant *S. epidermidis* strains isolated from farm workers contained SCC*mec* II and SCC*mec* IV. Although two CoNS strains (*S. sciuri* and *S. ureilyticus*) were *mecA*-positive, the SCC*mec* types could not be determined using PCR because of unidentifiable *ccr* or *mec* types.

AMR profiles of NAS isolates from broiler farms

All 216 NAS isolates (both CoVS and CoNS) were susceptible to RIF, VAN, LZD, TEC, and TGC (Fig. 2A and B). CoVS strains (41.2%), particularly *S. agnetis* strains (54.9%), showed higher levels of resistance to GEN than the CoNS strains (4.1%) (Table 2). Both CoVS and CoNS displayed relatively higher levels of resistance to FUS, AMP, PEN, TET, and CIP than to other antimicrobial agents. MDR phenotypes, which show resistance to more than three different classes of antimicrobial agents, were observed in 55.9% and 44.6% of CoVS and CoNS strains, respectively (Table 2). When the two major species of CoVS (*S. agnetis* and *S. chromogenes*) were compared for resistance, *S. agnetis* strains exhibited significantly higher levels of resistance to AMP, PEN, FUS, and GEN, whereas *S. chromogenes* showed higher resistance to CIP, CLI, and TET (Table 2). Among the four major groups of CoNS strains, *S. xylosus* and *S. saprophyticus* strains displayed higher levels of MDR than *S. simulans* and *S. epidermidis* strains. *S. xylosus* and *S. saprophyticus* strains were highly resistant to PEN (> 60%), FUS (> 79.6%) and TET (> 42.9%).

Mutations in the QRDRs of fluoroquinolone-resistant NAS strains

Eighteen CoVS (18/68 strains, 26.5%) and 39 CoNS (39/148 strains, 26.4%) strains displayed resistance to fluoroquinolones (Table 3). All 57 fluoroquinolone-resistant NAS strains had at least one point mutation in the QRDRs of *gyrA*, *gyrB*, *parC*, or *parE* genes. As shown in Table 3, all fluoroquinolone-resistant NAS strains had the S84L mutation in *gyrA*, except for six strains of *S. cohnii* (n = 1), *S. condimenti* (n = 1), *S. lentus* (n = 3) and *S. epidermidis* (n = 1). Mutations in *gyrB* were identified in only three species of CoNS: *S. sciuri*, *S. simulans*, and *S. xylosus* strains. Among the four point mutations in *gyrB* (S567A, A512R,

238 E490G, and S494T), the S567A mutation was most frequently identified in fluoroquinolone-
239 resistant CoNS, especially in *S. xylosus*. Although the S80L mutation was most frequently
240 observed in fluoroquinolone-resistant NAS strains, particularly in *S. agnetis* and *S.*
241 *saprophyticus* strains, high levels of heterogeneity were observed in *parC* mutations of *S.*
242 *epidermidis*, *S. gallinarum*, and *S. xylosus* strains (Table 3). In contrast to mutations in *gyrA*,
243 *gyrB*, and *parC*, which were identified in multiple species of fluoroquinolone-resistant NAS,
244 the T379K mutation in *parE* was detected only in *S. simulans* strains.
245

Discussion

Staphylococci are normal flora on the skin and mucous membranes of poultry and other livestock animals. However, some staphylococci can cause opportunistic infections in poultry and food poisoning in humans (Lee et al., 2022; Mekhloufi et al., 2021; Silva et al., 2022). Although recent studies have demonstrated the important role of CoNS and non-*aureus* CoPS in the transmission of antimicrobial resistance between livestock animals and humans (Huebner and Goldmann, 1999; Silva et al., 2022), limited data are available on the prevalence and species profiles of NAS, as well as their AMR resistance profiles.

Overall, the prevalence rates of CoVS and CoNS among the 216 NAS strains collected from broiler farms were 31.5% (68/216) and 68.5% (148/216), respectively (Table 1). Previous studies conducted in Korea (Lee et al., 2020) and other countries (Marek et al., 2016; Osman et al., 2016; Pyzik et al., 2019) also reported higher prevalence of CoNS compared with CoVS in broiler and retail chicken meat. In a study by Awan and Matsumoto (1998), 77/79 staphylococcal isolates from broilers that showed signs of illness were CoNS, such as *S. simulans*, *S. lentus*, and *S. cohnii*. These results suggest that NAS strains, especially various species of CoNS strains, are colonizing healthy broilers and farm environments, causing opportunistic infections in broilers. In this study, although only two species of CoVS, *S. agnetis* and *S. chromogenes*, were detected, 14 different species of CoNS were identified (Table 1 and Fig. 1A-C). The most frequently isolated species of NAS from healthy broilers were *S. agnetis* (26.4%), *S. xylosus* (21.3%), and *S. simulans* (15.2%) (Fig. 1A). Frequent occurrence of *S. agnetis* (3/18, 16.7%) and *S. xylosus* (9/18, 50.0%) were also observed in the farm environment (Fig. 1B). Recently published studies by our group and others reported that various NAS strains present in retail chicken meat (Martins et al., 2013; Osman et al., 2016; Yurdakul et al., 2013).

In particular, the overall detection rate of NAS in retail chicken was 45.3% in Korea, and the most frequently detected NAS species in retail chicken meat samples were *S. agnetis* (19.4%) and *S. saprophyticus* (19%) (Lee et al., 2020), suggesting *S. agnetis* may have transmitted from broiler farms to retail chicken meat. Although the most common NAS species in the nasal cavities of farm workers in this study were *S. epidermidis* (11/20, 55.0%) and *S. lugdunensis* (2/20, 10.0%), a few strains of NAS species identified in broilers, such as *S. agnetis* (n = 1), *S. xylosus* (n = 2), and *S. saprophyticus* (n = 1), were also isolated from the broiler farm workers (Fig. 1C). These NAS species have been shown to be mainly associated with local and chronic infections in broiler chickens and other poultry (Marek et al., 2016; Szafraniec et al., 2020). It has been reported that *S. agnetis* and *S. xylosus* have various virulence factors involved in host cell adherence, immune evasion, and toxin biosynthesis, which contribute to the persistence and pathogenesis of staphylococci in poultry (Szafraniec et al., 2020). *S. agnetis* has been associated with bacterial chondronecrosis, endocarditis, and septicemia in broiler chickens (Szafraniec et al., 2020). Moreover, exfoliative toxin genes, which contribute to the scalded-skin syndrome in humans, have been found in *S. agnetis* and *S. chromogenes* strains from poultry (Ladhani et al., 1999). These findings suggest that NAS species in broiler farms, especially CoVS species, may be a potential health hazard for broilers and a potential source of chicken meat contamination in Korea.

Recently, methicillin resistance in NAS strains, especially methicillin-resistant CoNS strains, has been reported in livestock farms and in foods of animal origin (Mahato et al., 2017). Analysis of the SCC*mec* types of the six *mecA*-positive NAS strains revealed that two CoNS (*S. epidermidis* and *S. haemolyticus*) from broilers or the surrounding environment carried SCC*mec* V, which is typically associated with methicillin-resistant staphylococci originating from livestock and companion animals (Back et al., 2020; Lee and Yang, 2020). In contrast to SCC*mec* V in broiler-associated NAS and *S. aureus* (Lee et al., 2022) strains, the two strains of

S. epidermidis from farm workers harbored SCC*mec* II or SCC*mec* IV, which has been predominantly found in clinical *S. epidermidis* isolates from human patients (Barbier et al., 2010). Similar to previous studies, which reported heterogeneity of SCC*mec* elements in methicillin-resistant NAS isolates (Rolo et al., 2017), SCC*mec* types of the two *mecA*-positive CoNS strains, one *S. sciuri* and one *S. ureilyticus* strain, could not be determined because of non-typeable *ccr*/*mec* genes. Although the prevalence of SCC*mec* elements in NAS isolates in this study was much less than that of *S. aureus* in a previously reported study (2.8% vs. 21.5%) (Lee et al., 2022) these results indicate that diverse species of NAS may contribute to the transmission of SCC*mec* elements among staphylococci.

It is estimated that approximately 20% of all antibiotics sold in the livestock industry are used for poultry in Korea (Lim et al., 2014). In particular, β-lactams, fluoroquinolones, and tetracyclines have been extensively used to prevent and treat staphylococcal infections in poultry. Correlating to the administration of antibiotics in poultry, relatively high levels of resistance to AMP, PEN, CIP, and TET were identified in both CoVS and CoNS strains (Fig. 2A and B), indicating that the administration of antibiotics facilitated the occurrence of AMR, including the NAS strains in this study. Although FUS has not been listed as a major antibiotic used in poultry farms in Korea, the highest levels of resistance have been identified in several species of CoVS and CoNS, particularly in *S. agnetis* (90.2%), *S. xylosus* (79.6%), and *S. saprophyticus* (95.0%) (Table 2). Previously, it was reported that certain species of NAS, such as *S. agnetis*, *S. saprophyticus*, *S. cohnii*, and *S. ureilyticus*, frequently carry *fusD* and *fusF* for intrinsic resistance to FUS (Chen et al., 2015; O'Neill et al., 2007). The frequent occurrence of antimicrobial-resistant NAS strains, especially MDR strains displaying resistance phenotypes to the antibiotics commonly used in poultry farms, indicates that NAS strains from poultry farms can serve as important reservoirs for AMR.

Frequent occurrence of quinolone resistance has been reported in LA-MRSA and NAS isolates through point mutations in the QRDRs of the two essential enzymes, DNA gyrase and topoisomerase IV (Takahashi et al., 1998; Takahata et al., 1997). In this study, all 57 fluoroquinolone-resistant NAS strains possessed one or more point mutations in the QRDRs of *gyrA*, *gyrB*, *parC*, or *parE* genes (Table 3). In line with previous studies in *S. aureus* and CoNS (Lee et al., 2022; Takahashi et al., 1998), mutations at codon 84 of *gyrA* (S84L and S84F) and codon 80 of *parC* (S80L, T80I, S80I, and S80V) were most frequently found in fluoroquinolone-resistant NAS strains. In addition to the S84L mutation, nine different amino acid substitutions were identified in QRDRs of *gyrA* in CoNS strains. High level of heterogeneity in QRDRs of *parC* was also observed in CoNS strains, especially *S.epidermidis*, *S. gallinarum*, and *S. xylosus* strains. Although none of the fluoroquinolone-resistant CoVS strains had mutations in *gyrB* and *parE*, *S. sciuri*, *S. simulans*, and *S. xylosus* carried mutations in the QRDRs of *gyrB* and *parE* (Table 3). While *S. sciuri* and *S. xylosus* strains had S567A mutation in *gyrB*, *S. simulans* strains had A512R, E490G, and S494T mutations in *gyrB*. As shown in Table 3, only *S. simulans* strains carried the T379K mutation in *parE*. Although the T379K mutation has not been previously described, several other mutations in *parE* have been found in *S. aureus* (Fujimoto-Nakamura et al., 2005) and *Streptococcus pneumoniae* (Jones et al., 2000) in association with quinolone resistance. Further studies are required to determine whether the newly identified mutations in *gyrB* and *parE* can act synergistically with *gyrA* and *parC* mutations to further enhance quinolone resistance in CoNS strains.

In conclusion, our results suggest that (1) there is a relatively high degree of species diversity in NAS isolates, especially CoNS isolates, collected from broiler farms; (2) frequent occurrence of MDR in NAS isolates with high resistance to β-lactams, fluoroquinolones, and fusidane was observed; and (3) fluoroquinolone resistance in broiler-associated NAS isolates was mainly caused by various mutations in the QRDRs of *gyrA* and *parC*.

345 **Acknowledgements**

346

347 This study was supported by funding from the Research of Korea Centers for Disease
348 Control and Prevention (Projects No. 2017NER54060 & 2021ER220100). We thank Soo In
349 Lee and Sun Do Kim for excellent technical assistance with antimicrobial susceptibility assays.

350

References

Awan MA, Matsumoto M. 1998. Heterogeneity of staphylococci and other bacteria isolated from six-week-old broiler chickens. Poult Sci 77:944-9.

Back SH, Eom HS, Lee HH, Lee GY, Park KT, Yang SJ. 2020. Livestock-associated methicillin-resistant *Staphylococcus aureus* in Korea: Antimicrobial resistance and molecular characteristics of LA-MRSA strains isolated from pigs, pig farmers, and farm environment. J Vet Sci 21:e2.

Barbier F, Ruppe E, Hernandez D, Lebeaux D, Francois P, Felix B, Desprez A, Maiga A, Woerther PL, Gaillard K, Jeanrot C, Wolff M, Schrenzel J, Andremont A, Ruimy R. 2010. Methicillin-resistant coagulase-negative staphylococci in the community: High homology of SCC*mec* IVa between *Staphylococcus epidermidis* and major clones of methicillin-resistant *Staphylococcus aureus*. J Infect Dis 202:270-281.

Boamah VE, Agyare C, Odoi H, Adu F, Gbedema SY, Dalsgaard A. 2017. Prevalence and antibiotic resistance of coagulase-negative staphylococci isolated from poultry farms in three regions of Ghana. Infect Drug Resist 10:175-183.

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.

Clinical and Laboratory Standards Institute (CLSI). 2020a. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: Clinical and Laboratory Standards Institute.

Clinical and Laboratory Standards Institute (CLSI). 2020b. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute.

Feßler AT, Wang Y, Wu C, Schwarz S. 2018. Mobile macrolide resistance genes in staphylococci. Plasmid 99:2-10.

Fisher EL, Otto M, Cheung GYC. 2018. Basis of virulence in enterotoxin-mediated staphylococcal food poisoning. Front Microbiol 9:436.

Fujimoto-Nakamura M, Ito H, Oyamada Y, Nishino T, Yamagishi J. 2005. Accumulation of mutations in both *gyrB* and *parE* genes is associated with high-level resistance to novobiocin in *Staphylococcus aureus*. Antimicrob Agents Chemother 49:3810-3815.

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.

Huebner J, Goldmann DA. 1999. Coagulase-negative staphylococci: Role as pathogens. Annu Rev Med 50:223-236.

Jones ME, Sahm DF, Martin N, Scheuring S, Heisig P, Thornsberry C, Kohrer K, Schmitz FJ. 2000. Prevalence of *gyrA*, *gyrB*, *parC*, and *parE* mutations in clinical isolates of S*treptococcus pneumoniae* with decreased susceptibilities to different fluoroquinolones and originating from worldwide surveillance studies during the 1997-1998 respiratory season. Antimicrob Agents Chemother 44:462-466.

Kim YB, Seo KW, Jeon HY, Lim SK, Lee YJ. 2018. Characteristics of the antimicrobial resistance of *Staphylococcus aureus* isolated from chicken meat produced by different integrated broiler operations in Korea. Poult Sci 97:962-969.

Ladhani S, Joannou CL, Lochrie DP, Evans RW, Poston SM. 1999. Clinical, microbial, and biochemical aspects of the exfoliative toxins causing staphylococcal scalded-skin syndrome. Clin Microbiol Rev 12:224-242.

Lee GY, Lee SI, Kim SD, Park JH, Kim GB, Yang SJ. 2022. Clonal distribution and antimicrobial resistance of methicillin-susceptible and -resistant *Staphylococcus*

aureus strains isolated from broiler farms, slaughterhouses, and retail chicken meat. Poult Sci 101:102070.

Lee GY, Yang SJ. 2020. Comparative assessment of genotypic and phenotypic correlates of *Staphylococcus pseudintermedius* strains isolated from dogs with otitis externa and healthy dogs. Comp Immunol Microbiol Infect Dis 70:101376.

Lee SI, Kim SD, Park JH, Yang SJ. 2020. Species distribution, antimicrobial resistance, and enterotoxigenicity of non-*aureus* staphylococci in retail chicken meat. Antibiotics (Basel) 9.

Lim SK, Lee JE, Lee HS, Nam HM, Moon DC, Jang GC, Park YJ, Jung YG, Jung SC, Wee SH. 2014. Trends in antimicrobial sales for livestock and fisheries in Korea during 2003-2012. Korean J Vet Res 54:81-86.

Mahato S, Mistry HU, Chakraborty S, Sharma P, Saravanan R, Bhandari V. 2017. Identification of variable traits among the methicillin resistant and sensitive coagulase negative staphylococci in milk samples from mastitic cows in India. Front Microbiol 8:1446.

Marek A, Stepien-Pysniak D, Pyzik E, Adaszek L, Wilczynski J, Winiarczyk S. 2016. Occurrence and characterization of *Staphylococcus* bacteria isolated from poultry in Western Poland. Berl Munch Tierarztl Wochenschr 129:147-152.

Martins PD, De Almeida TT, Basso AP, De Moura TM, Frazzon J, Tondo EC, Frazzon APG. 2013. Coagulase-positive staphylococci isolated from chicken meat: Pathogenic potential and vancomycin resistance. Foodborne Pathog Dis 10:771-776.

Mekhloufi OA, Chieffi D, Hammoudi A, Bensefia SA, Fanelli F, Fusco V. 2021. Prevalence, enterotoxigenic potential and antimicrobial resistance of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from algerian ready to eat foods. Toxins (Basel) 13.

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 Pt 3:1049-1055.

Nadell CD, Xavier JB, Foster KR. 2009. The sociobiology of biofilms. FEMS Microbiol Rev 33:206-224.

O'gara JP, Humphreys H. 2001. *Staphylococcus epidermidis* biofilms: Importance and implications. J Med Microbiol 50:582-587.

O'neill AJ, Mclaws F, Kahlmeter G, Henriksen AS, Chopra I. 2007. Genetic basis of resistance to fusidic acid in staphylococci. Antimicrob Agents Chemother 51:1737-1740.

Osman K, Badr J, Al-Maary KS, Moussa IM, Hessain AM, Girah ZMA, Abo-Shama UH, Orabi A, Saad A. 2016. Prevalence of the antibiotic resistance genes in coagulase-positive-and negative-*Staphylococcus* in chicken meat retailed to consumers. Front Microbiol 7:1846.

Pyzik E, Marek A, Stepien-Pysniak D, Urban-Chmiel R, Jarosz LS, Jagiello-Podebska I. 2019. Detection of antibiotic resistance and classical enterotoxin genes in coagulase-negative staphylococci isolated from poultry in Poland. J Vet Res 63:183-190.

Rolo J, Worning P, Nielsen JB, Bowden R, Bouchami O, Damborg P, Guardabassi L, Perreten V, Tomasz A, Westh H, De Lencastre H, Miragaia M. 2017. Evolutionary origin of the staphylococcal cassette chromosome *mec* (SCC*mec*). Antimicrob Agents Chemother 61.

Schilcher K, Horswill AR. 2020. Staphylococcal biofilm development: Structure, regulation, and treatment strategies. Microbiol Mol Biol Rev 84.

Shen J, Wang Y, Schwarz S. 2013. Presence and dissemination of the multiresistance gene *cfr* in Gram-positive and Gram-negative bacteria. J Antimicrob Chemother 68:1697-1706.

Silva V, Canica M, Ferreira E, Vieira-Pinto M, Saraiva C, Pereira JE, Capelo JL, Igrejas G, Poeta P. 2022. Multidrug-resistant methicillin-resistant coagulase-negative staphylococci in healthy poultry slaughtered for human consumption. Antibiotics (Basel) 11:365.

Sreedharan S, Peterson LR, Fisher LM. 1991. Ciprofloxacin resistance in coagulase-positive and -negative staphylococci: Role of mutations at serine 84 in the DNA gyrase A protein of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob Agents Chemother 35:2151-2154.

Szafraniec GM, Szeleszczuk P, Dolka B. 2020. A review of current knowledge on *Staphylococcus agnetis* in poultry. Animals 10:1421.

Takahashi H, Kikuchi T, Shoji S, Fujimura S, Lutfor AB, Tokue Y, Nukiwa T, Watanabe A. 1998. Characterization of *gyrA*, *gyrB*, *grlA* and *grlB* mutations in fluoroquinolone-resistant clinical isolates of *Staphylococcus aureus*. J Antimicrob Chemother 41:49-57.

Takahata M, Yonezawa M, Matsubara N, Watanabe Y, Narita H, Matsunaga T, Igarashi H, Kawahara M, Onodera S, Oishi Y. 1997. Antibacterial activity of quinolones against coagulase-negative staphylococci and the quinolone resistance-determining region of the *gyrA* genes from six species. J Antimicrob Chemother 40:383-386.

Yang YJ, Lee GY, Kim SD, Park JH, Lee SI, Kim GB, Yang SJ. 2022. Profiles of non-*aureus* staphylococci in retail pork and slaughterhouse carcasses: Prevalence, antimicrobial resistance, and genetic determinant of fusidic acid resistance. Food Sci Anim Resour 42:225-239.

Yurdakul NE, Erginkaya Z, Unal E. 2013. Antibiotic resistance of enterococci, coagulase negative staphylococci and *Staphylococcus aureus* isolated from chicken meat. Czech J Food Sci 31:14-19.

475

476 **Table 1. SCC*mec* types of methicillin-resistant NAS strains isolated from broiler farms**

477

NAS (n = isolates)	*mecA* positive (%)	*mec* gene	*ccr* gene	SCC*mec* type	Isolated from
CoVS (68)					
S. agnetis (51)	-	-	-	-	
S. chromogenes (17)	-	-	-	-	
CoNS (148)					
S. arlettae (2)	-	-	-	-	
S. cohnii (5)	-	-	-	-	
S. condimenti (1)	-	-	-	-	
S. epidermidis (15)	3 (20)	B	A2B2	IV	worker
		A	A2B2	II	worker
		C2	C	V	broiler
S. gallinarum (12)	-	-	-	-	
S. haemolyticus (1)	1 (100)	C2	C	V	environment
S. lentus (5)					
S. lugdunesis (2)					
S. saprophyticus (20)	-	-	-	-	
S. sciuri (1)	1 (100)	A	-	NT	broiler
S. simulans (27)	-	-	-	-	
S. ureilyticus (3)	1 (33.3)	-	C	NT	broiler
S. warneri (5)	-	-	-	-	
S. xylosus (49)	-	-	-	-	

NAS, non-*aureus* staphylococci; CoVS, coagulase-variable staphylococci; CoNS, coagulase-negative staphylococci; SCC*mec*, staphylococcal cassette chromosome *mec*; NT, non-typeable

478 **Table 2. Antimicrobial resistance profiles of NAS strains isolated from broiler farms**

Species (No of isolates)	Number (%) of isolates resistant to:																		
	AMP	FOX	PEN	CHL	CIP	CLI	ERY	FUS	GEN	MUP	RIF	SXT	SYN	TET	VAN	TEC	LZD	TGC	MDR (%)
CoVS (68)																			
S. agnetis (51)	29 (56.9)	0	28 (54.9)	6 (11.8)	11 (21.6)	1 (2)	1 (2)	47 (90.2)	28 (54.9)	1 (2)	0	0	0	9 (17.6)	0	0	0	0	30 (59)
S. chromogenes (17)	5 (29.4)	0	5 (29.4)	5 (29.4)	7 (41.2)	8 (47.1)	3 (17.6)	0	0	0	0	0	0	6 (35.3)	0	0	0	0	8 (47)
CoVS Total	**34 (50)**	**0**	**33 (48.5)**	**11 (16.2)**	**18 (26.5)**	**9 (13.2)**	**4 (5.9)**	**47 (69.1)**	**28 (41.2)**	**1 (1.5)**	**0**	**0**	**0**	**15 (22.1)**	**0**	**0**	**0**	**0**	**38 (55.9)**
CoNS (148)																			
S. arlettae (2)	2 (100)	0	2 (100)	2 (100)	1 (50)	1 (50)	2 (100)	2 (100)	0	0	0	0	0	2 (100)	0	0	0	0	2 (100)
S. cohnii (5)	1 (20)	0	2 (40)	5 (100)	1 (20)	3 (60)	3 (60)	5 (100)	0	0	0	0	0	4 (80)	0	0	0	0	5 (100)
S. condimenti (1)	0	0	0	0	1 (100)	0	0	0	0	0	0	0	0	1 (100)	0	0	0	0	0
S. epidermidis (15)	9 (60)	3 (20)	9 (60)	0	1 (6.7)	1 (6.7)	3 (20)	6 (40)	2 (13.3)	2 (13.3)	0	2 (13.3)	0	3 (20)	0	0	0	0	4 (26.7)
S. gallinarum (12)	12 (100)	0	12 (100)	2 (16.7)	8 (66.7)	8 (66.7)	8 (66.7)	2 (16.7)	0	0	0	0	0	9 (75)	0	0	0	0	8 (66.7)
S. haemolyticus (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	0	0	0	0	1 (100)	1 (100)	0	0	0	0	1 (100)
S. lentus (5)	0	0	0	3 (60)	3 (60)	1 (20)	3 (60)	5 (100)	0	0	0	1 (20)	0	1 (20)	0	0	0	0	4 (80)
S. lugdunensis (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. saprophyticus (20)	3 (15)	0	12 (60)	1 (5)	6 (30)	3 (15)	8 (40)	19 (95)	0	0	0	1 (5)	0	11 (55)	0	0	0	0	13 (65)
S. sciuri (1)	1 (100)	1 (100)	1 (100)	0	1 (100)	0	1 (100)	1 (100)	0	0	0	1 (100)	0	1 (100)	0	0	0	0	1 (100)
S. simulans (27)	3 (11.1)	0	3 (11.1)	3 (11.1)	5 (18.5)	6 (22.2)	6 (22.2)	0	1 (3.7)	0	0	3 (11.1)	0	3 (11.1)	0	0	0	0	5 (19)
S. ureilyticus (3)	0	1 (33.3)	0	2 (66.7)	0	1 (33.3)	1 (33.3)	3 (100)	0	0	0	0	0	1 (33.3)	0	0	0	0	2 (66.7)
S. warneri (5)	5 (100)	0	5 (100)	0	0	0	1 (20)	1 (20)	0	1 (20)	0	0	0	1 (20)	0	0	0	0	1 (20)
S. xylosus (49)	18 (36.7)	0	35 (71.4)	12 (24.5)	11 (22.4)	2 (4.1)	4 (8.2)	39 (79.6)	3 (6.1)	0	0	0	0	21 (42.9)	0	0	0	0	20 (40.8)
CoNS Total	**55 (37.2)**	**6 (4.1)**	**82 (55.4)**	**31 (20.9)**	**39 (26.4)**	**27 (18.2)**	**41 (27.7)**	**83 (56.1)**	**6 (4.1)**	**3 (2.0)**	**0**	**8 (5.4)**	**1 (0.7)**	**59 (39.9)**	**0**	**0**	**0**	**0**	**66 (44.6)**
TOTAL (216)	**89 (41.2)**	**6 (2.8)**	**115 (53.2)**	**42 (19.4)**	**57 (26.4)**	**36 (16.7)**	**45 (20.8)**	**130 (60.2)**	**34 (15.7)**	**4 (1.9)**	**0**	**8 (3.7)**	**1 (0.5)**	**74 (34.3)**	**0**	**0**	**0**	**0**	**104 (48.1)**

AMP; Ampicillin, FOX; Cefoxitin, PEN; Penicillin, CHL; Chloramphenicol, CIP; Ciprofloxacin,, CLI; Clindamycin, ERY; Erythromycin, FUS; Fusidic acid, GEN; Gentamicin, MUP; Mupirocin, RIF; Rifampin, SXT; Trimethoprim-sulfamethoxazole, SYN; Quinupristin-dalfopristin, TET; Tetracycline, VAN; Vancomycin, TEC; Teicoplanin, LZD; Linezolid, TGC; Tigecycline, MDR; multi-drug resistance

Table 3. Point mutations identified in the QRDRs of *gyrA*, *gyrB*, *parC*, and *parE* genes in fluoroquinolone-resistant NAS strains

	NAS species	No. of FQ-resistant isolates (%)	Mutations in QRDRs				Total No.
			gyrA	*gyrB*	*parC*	*parE*	
CoVS	***S. agnetis* (51)**	11 (21.6)	S84L	-	S80L	-	10
			S84L	-	S80L, Y56H	-	1
	***S. chromogenes* (17)**	7 (41.2)	S84L	-	-	-	6
			S84L	-	R87I, S89T	-	1
CoNS	***S. arlettae* (2)**	1 (50)	S84L	-	T80I	-	1
	***S. cohnii* (5)**	1 (20)	S84F, V158A	-	-	-	1
	***S. condimenti* (1)**	1 (100)	S84F	-	-	-	1
	***S. epidermidis* (15)**	1 (6.7)	S28E, V29C, S84F	-	Q81K, D81H, G107A, S108R, I109L	-	1
	***S. gallinarum* (12)**	8 (66.7)	S84L	-	Y56F, Y74F, S80I, G92D		8
	***S. haemolyticus* (1)**	1 (100)	S84L	-	-	-	1
	***S. lentus* (5)**	3 (60)	T172A	-	S80V	-	1
			T172A	-	S80L	-	2
	***S. saprophyticus* (20)**	6 (43.8)	S84L	-	S80L, R96C	-	6
	***S. sciuri* (1)**	1 (100)	S84L, T172A	S567A	S80I	-	1
	***S. simulans* (27)**	5 (18.5)	S84L	A512R	-	T379K	1
			S84L, D105N, A119F	-	-	T379K	1
			S84L, A132S, A173S	A512R	-	T379K	1
			S84L, A132S	E490G, S494T	-	T379K	1
			S84L, A132S, A173S	E490G, S494T	-	T379K	1
	***S. xylosus* (49)**	11 (22.4)	S84L	S567A	F160L, V165I, Y195F, I209V	-	10
			S84L	S567A	D148H, F160L, L164W, V165I, S168N, D178H, G185R, V187L, Y194F, K207T, Y208S, I209V, D213G	-	1

NAS, non-*aureus* staphylococci; CoVS, coagulase-variable staphylococci; CoNS, coagulase-negative staphylococci; FQ, fluoroquinolone; QRDRs, quinolone resistance determining regions.

Figure legends

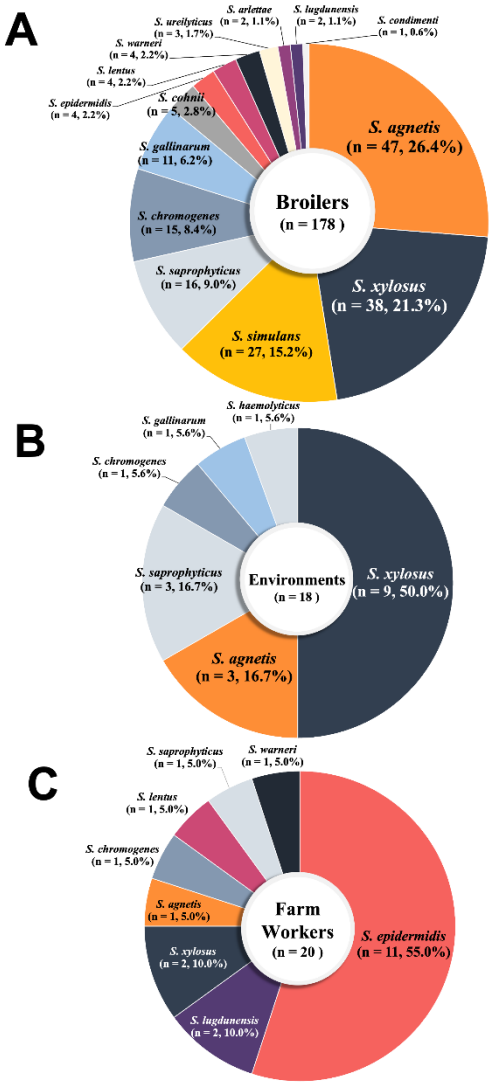

Fig. 1. Profiles of non-*aureus* staphylococci (NAS) isolated from broilers (A), farm environments (B), and farm workers (C) in Korea. A total of 216 NAS isolates of 16 different staphylococcal species were collected from broiler farms in Korea.

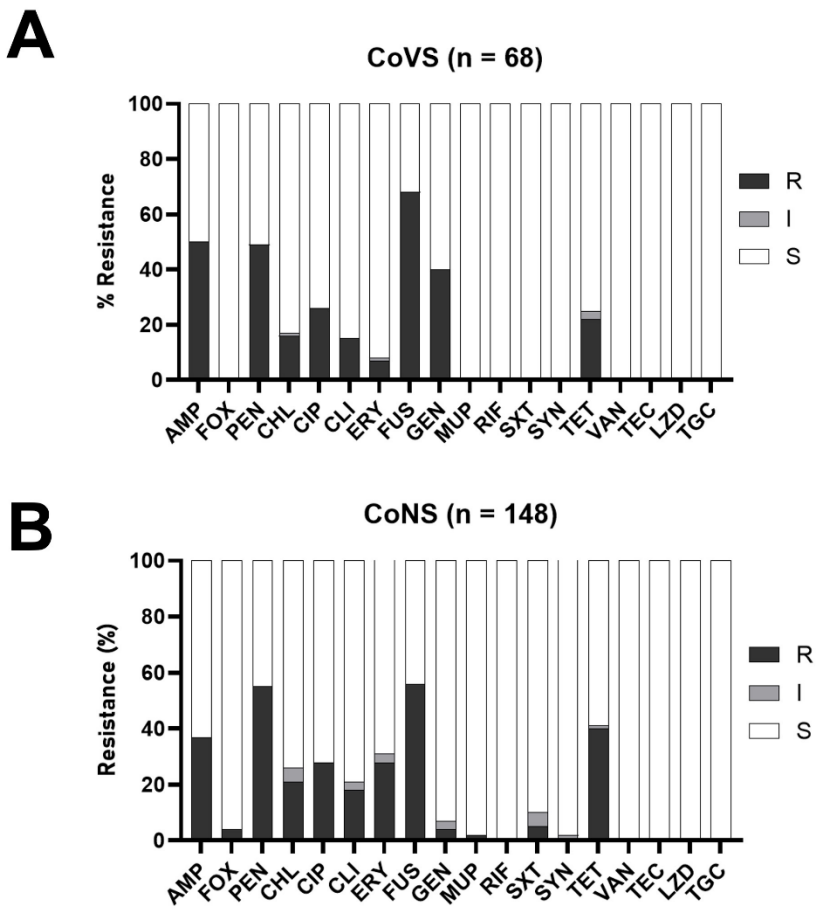

Fig. 2. Antimicrobial resistance profiles of NAS isolates from broiler farms in Korea. Antimicrobial resistance phenotypes of CoVS (A) and CoNS (B) isolates are shown.

AMP, ampicillin; FOX, cefoxitin; PEN, penicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; FUS, fusidic acid; GEN, gentamicin; MUP, mupirocin; RIF, rifampicin; SXT, trimethoprim-sulfamethoxazole; SYN, quinupristin-dalfopristin; TET, tetracycline; VAN, vancomycin; TEC, teicoplanin; LZD, linezolid; TGC, tigecycline.

R, resistant; I, intermediate; S, susceptible

Table S1. Primers for non-*aureus* staphylococci QRDR detection

Target genes	Primer name	Sequence (5'→3')	Amplicon size(bp)
gyrA	gyrA-F	AATGAACAAGGTATGACACC	368
	gyrA-R	GCGATACCTGATGCACCATT	
	PCR condition: 95℃ 5 min + 28× (95℃ 30 sec + 50℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
gyrB	gyrB-agnetis-F	AGTGACACGTCGTAAGTCGG	612
	gyrB-agnetis-R	TGAAGCATCGCACGGTTTTC	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 53℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	gyrB-arlettae-F	TGGCTCGTGTCATTGTCGAA	790
	gyrB-arlettae-R	GTCGCATACACTGCGTTGTC	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 53℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	gyrB-chromogenes-F	GAAACACGGGGACCCTCAAT	545
	gyrB-chromogenes-R	TTCGGATATGGGCACCATCG	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 54℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	gyrB-cohnii-F	AAA AAG CGC GTG AAG TGA CA	696
	gyrB-cohnii-R	GGT TCT CAA CAA CAT CGC CC	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 52℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	gyrB-condimenti-F	CTG CCG GAG GGT CTA CAA AA	534
	gyrB-condimenti-F	TAT CCG CTT CAA TCG CGT CT	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 53℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	gyrB-epidermidis-F	CAG CAT TAG ACG TTT CAA G	250
	gyrB-epidermidis-R	CCA ATA CCC GTA CCA AAT GC	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 55℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	gyrB-haemolyticus-F	ACG TGA AGT GAC ACG TCG TA	410
	gyrB-haemolyticus-F	ATC CCG CTT CGA TTA GTG GT	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 52℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	gyrB-lentus-F	AGAGCTCGTCTAGCAGCGAA	681
	gyrB-lentus-R	CGTTTCGTCAGCTTCTATCGC	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 54℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	gyrB-saprophyticus-F	GAA GTC ACG CGC CGT AAA TC	528

	gyrB-saprophyticus-R	CGA CCA TTT TGG CGT TGG TT	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 53℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	gyrB-gallinarum-F	AGT GAC ACG TCG TAA GTC GG	567
	gyrB-gallinarum-R	TGA TCC GCG TTC ATC TCA CC	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 54℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	gyrB-sciuri-F	TAT CGT TGA GGG TGA CTC TGC	426
	gyrB-sciuri-R	TTC GGC GTT GAG CTA AGT TCT	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 53℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	gyrB-xylosus-F	GGC AGA GAC TCG GAA ACA CA	442
	gyrB-xylosus-R	CCC ACA ATT GGT CTG CGT TC	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 53℃ 30 sec +72℃ 40sec) + 72℃ 5 min		

Target genes	Primer name	Sequence (5'→3')	Amplicon size(bp)
parC	parC-agnetis-F	TTACCTGATGTACGCGACGG	922
	parC-agnetis-R	GTCGACCTTCACTGATCGCT	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 54℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parC-arlettae-F	ACCCGATGTACGTGATGGTT	257
	parC-arlettae-R	ATAGCTGCTGCAGGGTCATT	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 53℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parC-chromogenes-F	CGT CGG GGA TGT CAT TGG AC	162
	parC-chromogenes-R	GTA TAA CGC ATC GCA GCA GG	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 50℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parC-cohnii-F	TTG GCG ACC GAT TTG GTA GAT	309
	parC-cohnii-R	TAG CTG CTG CTG GAT CGT TA	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 52℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parC-condimenti-F	AGT GCC AAA ACA GTC GGT GA	300
	parC-condimenti-R	GTT CGG GAA TCT TGC TGG GA	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 54℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parC-epidermidis-F	TCG CAA TGT ATT CAA GTG GG	245
	parC-epidermidis-R	ATC GTT ATC GAT ACT ACC ATT	

	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 55℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parC-haemolyticus-F	AAG AGT GCG AAG ACA GTC GG	470
	parC-haemolyticus-R	CCG CCA GTA GGG AAA TCT GG	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 54℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parC -lentus-F	ATCCAAGACCGAGCACTTCC	575
	parC -lentus-R	CCGGTAGGGAAATCAGGTCC	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 54℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parC-saprophyticus-F	CGT TCG TGA TGG GCT CAA AC	244
	parC-saprophyticus-R	AGC CGG GTC ATT GTC GAT AC	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 54℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parC-sciuri-F	GCG CTT CCT GAT GTA CGA GA	375
	parC-sciuri-R	CAT CGG CTC CAT CGC TGT AT	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 54℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parC-xylosus-F	CGC ACG GTG ATA CGT CTG TA	428
	parC-xylosus-R	ACC ACC CGT TGG GAA ATC AG	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 54℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
parE	parE-F	CGATTAAAGCACAACAAGCAAG	393
	parE-R	GCGCACCATCAGTATCAG	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 48℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parE-agnetis-F	GGGTGGGTCTGCAAAACTTG	308
	parE-agnetis-R	GTAACGCGATAAACACGCGA	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 52℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parE-arlettae-F	TTAGGTACACCGGAAGCACG	566
	parE-arlettae-R	ACACGTCCTGCCAACACTAA	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 53℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parE-chromogenes-F	TAGGGACACCTGAAGCGAGA	851
	parE-chromogenes-R	ACGACGTGGGGCAACTTTAT	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 53℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parE-cohnii-F	CCA ACA AGC AAG AGA GGC AG	696
	parE-cohnii-R	ATC CAC TCA CGT CTA GGA GC	
	PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 53℃ 30 sec +72℃ 40sec) + 72℃ 5 min		
	parE-condimenti-F	TAC GCT CGT CGA ATT GGT GA	648
	parE-condimenti-R	TAC TTG GAT ATG CGC ACC GT	

PCR condition: 95℃ 5 min + 28× (95 ℃ 30 sec + 53 ℃ 30 sec +72 ℃ 40sec) + 72 ℃ 5 min			
parE-epidermidis-F	AAG CTC AAC AAG CAC GCG AGG CTG	229	
parE-epidermidis-R	TTA AAG TCA GTA CCA ACA CCA GCA C		
PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 48℃ 30 sec +72℃ 40sec) + 72℃ 5 min			
parE-lentus-F	GCA AGA GCT GCC GTA GAT TC	483	
parE-lentus-R	AGC ACC ATC TGT ATC GGC AT		
PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 53℃ 30 sec +72℃ 40sec) + 72℃ 5 min			
parE-saprophyticus-F	GCG TAC AAA AGA CGG GGG TA	701	
parE-saprophyticus-R	CAC CAT CCG TAT CCG CAT CA		
PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 54℃ 30 sec +72℃ 40sec) + 72℃ 5 min			
parE-sciuri-F	GTG AAG CAG CGA GAA AAG CG	374	
parE-sciuri-R	ATG CGC ACC ATC AGT ATC GG		
PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 54℃ 30 sec +72℃ 40sec) + 72℃ 5 min			
parE-xylosus-F	TAG AGA AGC GGC GCG TAA AG	376	
parE-xylosus-R	ATG CGC ACC ATC TGT ATC GG		
PCR condition : 95℃ 5 min + 28× (95℃ 30 sec + 54℃ 30 sec +72℃ 40sec) + 72℃ 5 min			