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# Prevalence and characteristics of livestock-associated methicillin-susceptible *Staphylococcus aureus* in the pork production chain in Korea

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## ABSTRACT

The emergence and prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) in livestock animals have become a worldwide public health concern. While the prevalence and genetic profiles of MRSA strains in pigs and pork meat have been actively studied, livestock-associated MSSA strains have only been characterized in a few small-scale studies. In this investigation, we assessed the nationwide prevalence of MSSA in the Korean pig production chain, including pig farms, slaughterhouses, and retail markets. Among the 41 MSSA strains, the predominant clonal lineages were sequence type (ST) 398 (n = 15, 37%) and ST5 (n = 13, 32%). Although the overall prevalence of MSSA (2.58%) was low and mostly restricted to pig farms, ST398 MSSA strains showed higher level of multidrug resistance phenotype versus non-ST398 MSSA strains. In addition to the MDR phenotype, all of the ST398 MSSA strains exhibited resistance to tetracycline as they harbored the *tet*(K), *tet*(L), and/or *tet*(M) genes. However, ST398 MSSA strains did not exhibit increased resistance to zinc compared with the non-ST398 strains. This study is the first to provide evidence of ST398 MSSA emergence in livestock animals in Korea. Further studies are necessary to elucidate the potential of ST398 MSSA strains for human transmission. Our findings suggest that the MDR phenotype and high levels of tetracycline resistance may have played an important role in the emergence and prevalence of ST398 MSSA in pig farms in Korea.

**Keywords:** MSSA; ST398; pig; antimicrobial resistance; zinc resistance

## INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of septicemia in hospital-associated (HA) and community-associated settings [1]. Recently, an increase in the incidence of livestock-associated (LA)-MRSA infections has been reported in various animal species, particularly in the incidence of sequence type (ST) 398 MRSA in pigs in European countries and North America [2,3]. ST398 LA-MRSA strains have also been isolated from people in close contact with pigs, indicating the transmission of LA-MRSA between pigs and humans [2]. The prevalence of ST398 LA-MRSA strains in pig farms seems to be associated with the use of antibiotics, particularly tetracycline compounds [4,5]. ST398 LA-MRSA

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isolates tend to exhibit multidrug resistance (MDR) phenotypes to various antimicrobial agents [6,7]. Over the past decade, increased number of infections caused by ST398 methicillin-susceptible *S. aureus* (MSSA) has also been reported in humans [8,9]. Bouiller et al. [10] reported that clonal complex (CC) 398 MSSA bloodstream infections were more highly associated with fatal outcomes than non-CC398 MSSA infections.

Although the prevalence of ST398 MRSA in pigs and pork meat samples has been investigated extensively [11-13], limited information is available on the prevalence and characteristics of ST398 MSSA isolates in the pork production chain in Korea. In this study, we investigated national prevalence of MSSA in the pork production chain, including pig farms, slaughterhouses, and retail markets. In addition, we analyzed the multilocus sequence types (MLST), accessory gene regulator (*agr*) types, staphylococcal protein A (*spa*) types, and staphylococcal enterotoxin (SE) types to genetically characterize the MSSA strains. Furthermore, antimicrobial resistance and zinc resistance profiles were examined to identify potential correlations with the prevalence of the ST398 MSSA isolates.

## MATERIALS AND METHODS

### *S. aureus* strains and culture conditions

The 41 MSSA isolates used in this study are listed in **Table 1**. A total of 1587 swab samples were obtained from pig farms (n = 19), slaughterhouses (n = 7), and retail markets (n = 35) in 8 different provinces of Korea over the period of 14-months between 2017 and 2018. Pig farm samples were obtained from finishing pigs (n = 760), the farm environment (n = 114), and farm workers (n = 135); slaughterhouse samples were obtained from pig carcasses (n = 280), the facility environments (n = 18), and slaughterhouse workers (n = 13); and retail market samples were obtained from fresh pork meat samples (n = 260), the facility environments (n = 3), and market workers (n = 4) were collected.

Within 24-h of sampling, all swab samples were inoculated into 5 mL of tryptic soy broth (TSB; Difco Laboratories, USA) supplemented with 10% NaCl and cultured at 37°C. Pork meat samples (25 g) were homogenized in 225 mL of 10% NaCl-TSB and incubated at 37°C. After 24 h, 10 µL aliquots of the pre-enriched media were streaked onto Baired Parker agar (BPA; Difco Laboratories) and incubated for 16–18 h at 37°C. All *S. aureus* strains were identified using the Vitek 2 system (bioMérieux, France) and 16S rRNA sequencing [14]. All MSSA strains were *mecA*-negative and susceptible to cefoxitin and oxacillin (data not shown).

### Susceptibility assays

Susceptibilities to antimicrobial agents were determined using the disk diffusion methods according to the 2017 Clinical and Laboratory Standards Institute guidelines [15]. The antimicrobial agents used were chloramphenicol (30 µg), clindamycin (2 µg), erythromycin (15 µg), ampicillin (10 µg), cefoxitin (30 µg), gentamicin (30 µg), sulfamethoxazole-trimethoprim (23.73–1.25 µg), dalfopristin (15 µg), rifampin (5 µg), tetracycline (30 µg), ciprofloxacin (5 µg), and mupirocin (200 µg, Oxoid, UK). All antibiotic discs were purchased from BD BBL, unless stated otherwise. The minimum inhibitory concentrations (MICs) to oxacillin, tetracycline, linezolid, vancomycin, and teicoplanin were determined using the standard E-test (bioMérieux) on Meuller-Hinton agar (MHA) plates. The MICs to zinc chloride were determined using the standard agar dilution assay (range, 0.25–16 mM) as described previously [16]. All the susceptibility tests were repeated three times.

**Table 1.** Summary of methicillin-susceptible *Staphylococcus aureus* investigated in this study

| Strain | MLST | Source | Staphylococcal protein A type | Accessory gene regulator type | Antimicrobial resistance | *tet*-R genes* | TET MICs (μg/mL)† | *czrC* | Zinc MICs (mM/mL)‡ | SEs |
|---|---|---|---|---|---|---|---|---|---|---|
| PJFA-443 | ST398 | Pig | t1451 | I | AMP, CHL, CIP, CLI, ERY, GEN, STX, SYN, TET | *tet(K)*, *tet(L)* | 16 | – | 4 | - |
| PJFA-463 | ST398 | Pig | t1451 | I | AMP, CHL, CIP, CLI, ERY, TET | *tet(M)* | 16 | + | 4 | - |
| PJFA-493 | ST398 | Pig | t1451 | I | AMP, CHL, CIP, CLI, ERY, TET | *tet(M)* | 16 | – | 5 | - |
| PJFA-413 | ST398 | Pig | NT | I | AMP, CHL, CIP, CLI, ERY, TET | *tet(M)* | 16 | – | 5 | - |
| PJFA-514 | ST398 | Pig | t664 | I | AMP, CHL, CLI, ERY, GEN, SYN, TET | *tet(L)* | 16 | + | 8 | - |
| PCFH-311 | ST398 | Farm worker | t571 | I | AMP, TET | *tet(M)* | 16 | – | 2 | - |
| PSFH-111 | ST398 | Farm worker | t571 | I | AMP, CHL, CIP, TET | *tet(M)* | 16 | – | 2 | - |
| PSFH-121 | ST398 | Farm worker | t571 | I | AMP, CHL, CIP, TET | *tet(M)* | 16 | – | 2 | - |
| PSFH-321 | ST398 | Farm worker | t571 | I | AMP, CHL, CIP, CLI, ERY, GEN, SYN, TET | *tet(M)*, *tet(L)* | 16 | – | 4 | - |
| PSFH-331 | ST398 | Farm worker | t571 | I | AMP, CHL, CIP, CLI, ERY, GEN, SYN, TET | *tet(M)*, *tet(L)* | 16 | – | 4 | - |
| PSFH-341 | ST398 | Farm worker | t571 | I | AMP, CHL, CIP, CLI, ERY, GEN, SYN, TET | *tet(M)*, *tet(L)* | 16 | – | 4 | - |
| PSFH-351 | ST398 | Farm worker | t571 | I | AMP, CHL, CIP, CLI, ERY, GEN, STX, SYN, TET | *tet(M)*, *tet(L)* | 16 | – | 4 | - |
| PJFH-511 | ST398 | Farm worker | t571 | I | AMP, CHL, CIP, CLI, ERY, MUP, STX, TET | *tet(M)*, *tet(K)* | 16 | + | 4 | *sec* |
| PSFH331 | ST398 | Farm worker | t18103 | I | AMP, CHL, CIP, CLI, ERY, GEN, STX, SYN, TET | *tet(K)*, *tet(L)* | 16 | – | 4 | - |
| PJFE-306 | ST398 | Farm environment | t571 | I | AMP, CHL, CIP, CLI, ERY, TET | *tet(M)* | 16 | + | 2 | - |
| PKFA-581 | ST5 | Pig | t002 | I | AMP, CHL, GEN, SYN | - | 1 | – | 4 | - |
| PCFA-241 | ST5 | Pig | t002 | III | AMP, CHL, CLI | - | 0.5 | + | 4 | - |
| PKFA-521 | ST5 | Pig | t002 | II | AMP, CHL | - | 1 | – | 4 | - |
| PKFA-512 | ST5 | Pig | t002 | II | AMP, CHL | - | 1 | – | 4 | - |
| PKFA-532 | ST5 | Pig | t002 | II | AMP, CHL | - | 1 | – | 4 | - |
| PKFA-592 | ST5 | Pig | t010 | II | AMP, CHL | - | 1 | – | 4 | - |
| PKFA-513 | ST5 | Pig | t002 | II | AMP, CHL, GEN | - | 2 | – | 4 | - |
| PKFA-523 | ST5 | Pig | t002 | II | AMP, CHL | - | 1 | – | 4 | - |
| PKFA-563 | ST5 | Pig | t002 | II | AMP, CHL | - | 1 | – | 4 | - |
| PKFA-584 | ST5 | Pig | t7083 | II | AMP, CHL | - | 1 | – | 4 | - |
| PGFH-222 | ST5 | Farm worker | t899 | II | AMP | - | 1 | + | 4 | - |
| PJSH-311 | ST5 | Slaughterhouse worker | t5440 | II | AMP | - | 1 | – | 4 | - |
| PKFE-503 | ST5 | Farm environment | t002 | II | AMP, CHL, GEN | - | 8 | – | 4 | - |
| PKFH-511 | ST9 | Farm worker | t1939 | II | AMP, CHL, CLI, GEN, TET | *tet(L)* | 64 | – | 4 | - |
| PKFH-521 | ST9 | Farm worker | t1939 | II | AMP, CHL, CLI, GEN, TET | *tet(L)* | 64 | – | 4 | - |
| PKFH-531 | ST9 | Farm worker | t1939 | II | AMP, CHL, CLI, GEN, TET | *tet(L)* | 64 | – | 4 | - |
| PGSM131 | ST9 | Slaughterhouse carcass | t337 | II | AMP, CHL, MUP | *tet(L)* | 4 | – | 2 | - |
| PCFH-351 | ST188 | Farm worker | t8275 | I | AMP, CHL | - | 1 | – | 4 | - |
| PSMH-616 | ST188 | Retail market worker | t189 | I | AMP | - | 1 | – | 4 | - |
| PCFH-211 | ST433 | Farm worker | t021 | II | AMP | - | 1 | – | 4 | - |
| PSFH-211 | ST433 | Farm worker | t021 | III | AMP | - | 1 | + | 4 | - |
| PKFA-542 | ST403 | Pig | t002 | II | AMP, CHL | - | 2 | – | 4 | - |
| PGFH-221 | ST554 | Farm worker | t002 | II | AMP, TET | *tet(L)* | 32 | + | 4 | - |
| PSMH-611 | ST1 | Retail market worker | t18104 | III | AMP, ERY | - | 2 | – | 4 | - |
| PKFA552 | ST2115 | Pig | t002 | II | AMP, CHL, MUP | *tet(L)* | 4 | – | 4 | - |
| PKFA-553 | NT | Pig | t664 | II | AMP, CHL, CLI, STX, TET | *tet(L)* | 64 | + | 4 | - |

AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; MUP, mupirocin; SXT, trimethoprim-sulfamethoxazole; SYN, quinupristin-dalfopristin; TET, tetracycline; NT, non-typeable.
*Tetracycline resistance genes; *tet(K)*, *tet(L)*, *tet(M)*, *tet(O)* and *tet(S)*; †Tetracycline MICs ≥ 16 μg/mL indicate resistance; ‡MIC values of > 2 mM indicate zinc resistance.

## Molecular characterization and typing

Multilocus sequence typing (MLST) was performed on all confirmed MSSA strains as described previously [17]. Briefly, 7 PCR-amplified housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) were sequenced and the sequence types (STs) were assigned according to the *S. aureus* MLST database (http://pubmlst.org/saureus/). The *agr* types (I-IV) of the MSSA strains were determined by multiplex PCR assays, as described previously [18]. Determinations of the *spa* types on all MSSA strains were performed using a specific primer

set [19]. The PCR products were sequenced, and the *spa* type was determined based on the variable number tandem repeats in the SpaServer database (http://spa.ridom.de/). Sequencing of all PCR products was performed at Cosmo Genetech (Korea).

Tetracycline resistance (*tetK, tetL, tetM, tetO*, and *tetS*) and zinc resistance (*czrC*) genes were detected by PCR with specific primer sets, as described previously [16,20].

### Detection of SE and Panton-Valentine leukocidin (PVL) genes

The presence of five different staphylococcal enterotoxin genes (*sea, seb, sec, sed*, and *see*), PVL gene, and the toxic shock syndrome toxin-1 gene (*tst1*) in all MSSA strains was examined by using multiplex-PCR assays as described previously [21]. Genomic DNA samples from reference *S. aureus* strains were included as positive controls (FRI913: *sea*, *sec*, *see*, *tst1*; COL: *seb*; and FRI472: *sed*) [22].

### Statistical analysis

Data were analyzed using the Mann-Whitney U test (GraphPad Software Inc., USA, www.Graph Pad.com), with a $p$ values of $< 0.05$ considered statistically significant.

## RESULTS

### Prevalence of MSSA in pig farms, slaughterhouses, and retail outlets

A total of 41 MSSA strains (2.58%) were isolated from 1,587 samples collected from pig farms, slaughterhouses, and retail markets during the 14-months of study period (**Table 1**). As shown

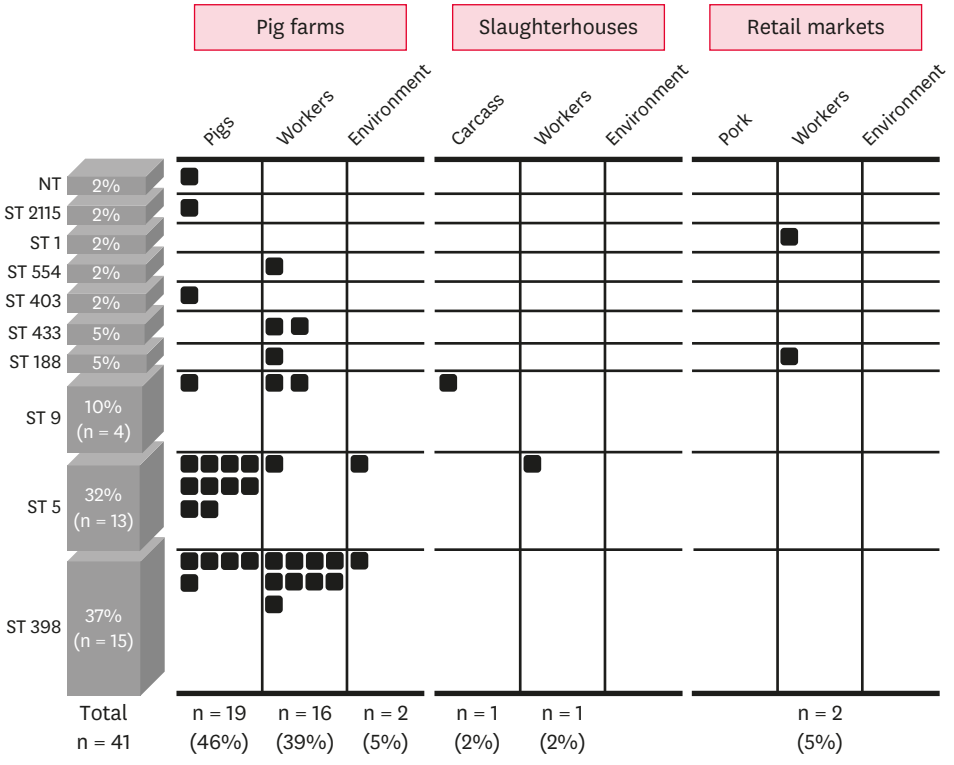


**Fig. 1.** Prevalence and genetic profiles of MSSA isolates recovered from pig farms, slaughterhouses, and retail markets. Each square represents an individual MSSA isolate.
NT, non-typeable for MLST; MSSA, methicillin-susceptible *Staphylococcus aureus*.


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in **Fig. 1**, 37 of the MSSA strains (90%) were isolated from pig farms and 4 MSSA strains were isolated from slaughterhouses and retail markets (2 strains each). Among the 37 MSSA strains isolated from pig farms, 19 and 16 strains were isolated from pigs and farm workers, respectively, whilst 2 strains were isolated from the environment (one from a pig pen and one from a toilet). No MSSA strains were isolated from pork meat samples.

## Genetic profiles of the MSSA strains

MLST analyses of the 41 MSSA strains revealed 9 different ST types, with one non-typeable strain isolated from a pig (**Fig. 1**). The most significant clonal lineage among the 9 ST types was ST398 (n = 15, 37%), followed by ST5 (n = 13, 32%), ST9 (n = 4, 10%), ST188 (n = 2, 4.9%), ST433 (n = 2, 4.9%), ST403 (n = 1, 2.4%), ST554 (n = 1, 2.4%), ST1 (n = 1, 2.4%), and ST2115 (n = 1, 2.4%). All ST398 MSSA strains were isolated only from pig farms (pigs, farm workers, and farm environment), with none isolated from the slaughterhouse or retail market samples. Similarly, all 12 ST5 MSSA strains were isolated from pig farms, except for one which was isolated from a slaughterhouse worker. Sequence analyses of *spa* in the 41 MSSA strains revealed 15 different *spa* types (**Table 1**). Interestingly, 3/5 ST398 MSSA strains isolated from pigs had t451 type, while 9/10 ST398 MSSA strains isolated from farm workers/farm environment were t571 type. This difference in *spa* types between the swine-associated MSSA strains and the MSSA strains from farm/slaughterhouse workers was also observed in the ST5 MSSA strains (t002 vs. t899/t5440). Conversely, all ST398 MSSA strains belonged to *agr* type I regardless of the sample source, and no differences were observed in the *agr* type of the ST5 MSSA strains depending on the sample sources.

## Antimicrobial resistance profiles of the MSSA strains

All 41 MSSA strains displayed resistance to ampicillin, and they were all susceptible to rifampin (**Fig. 2**). As shown in **Table 1**, ST398 MSSA strains tended to have higher level of multidrug resistance (MDR) phenotype (resistant to more than three antimicrobial classes) than the other ST types of strains. Specifically, as demonstrated by the results in **Fig. 2A and B**, 14/15 ST398 MSSA strains (93%) and 10/26 non-ST398 MSSA strains (38.5%) exhibited an MDR phenotype. The ST389 MSSA strains displayed higher levels of resistance to chloramphenicol, clindamycin, erythromycin, gentamycin, sulfamethoxazole-trimethoprim, quinupristin-dalfopristin, tetracycline, and ciprofloxacin (**Fig. 2C and D**). Notably, all the ST398 MSSA strains harbored one or two tetracycline resistance genes (*tetM*, *tetK*, or *tetL*) and displayed 100% resistance to tetracycline (**Table 1**). However, none of the 41 MSSA strains harbored *tetO* or *tetS* for tetracycline resistance phenotype. All the MSSA strains were susceptible to linezolid, vancomycin, teicoplanin, and daptomycin based on the E-test results (data not shown).

## Detection of *czrC* and zinc chloride MICs

It has been proposed that zinc resistance is associated with specific clonal lineages of MRSA, such as the ST398 and ST541 MRSA strains [23]. Although only 9 of the MSSA strains (22%) were positive for *czrC* gene, all 41 MSSA strains displayed zinc chloride MICs of > 2 mM, indicating a *czrC*-independent zinc resistance phenotype. Among the nine MSSA strains, *czrC* carriage was not associated with specific ST types, *spa* types, or *agr* types.

## Staphylococcal enterotoxin genes

None of the MSSA strains carried genes for SEs or TSST-1, except for a single ST398 MSSA strain from a pig farm worker, which was positive for *sec* (**Table 1**). All MSSA strains were negative for the PVL gene.


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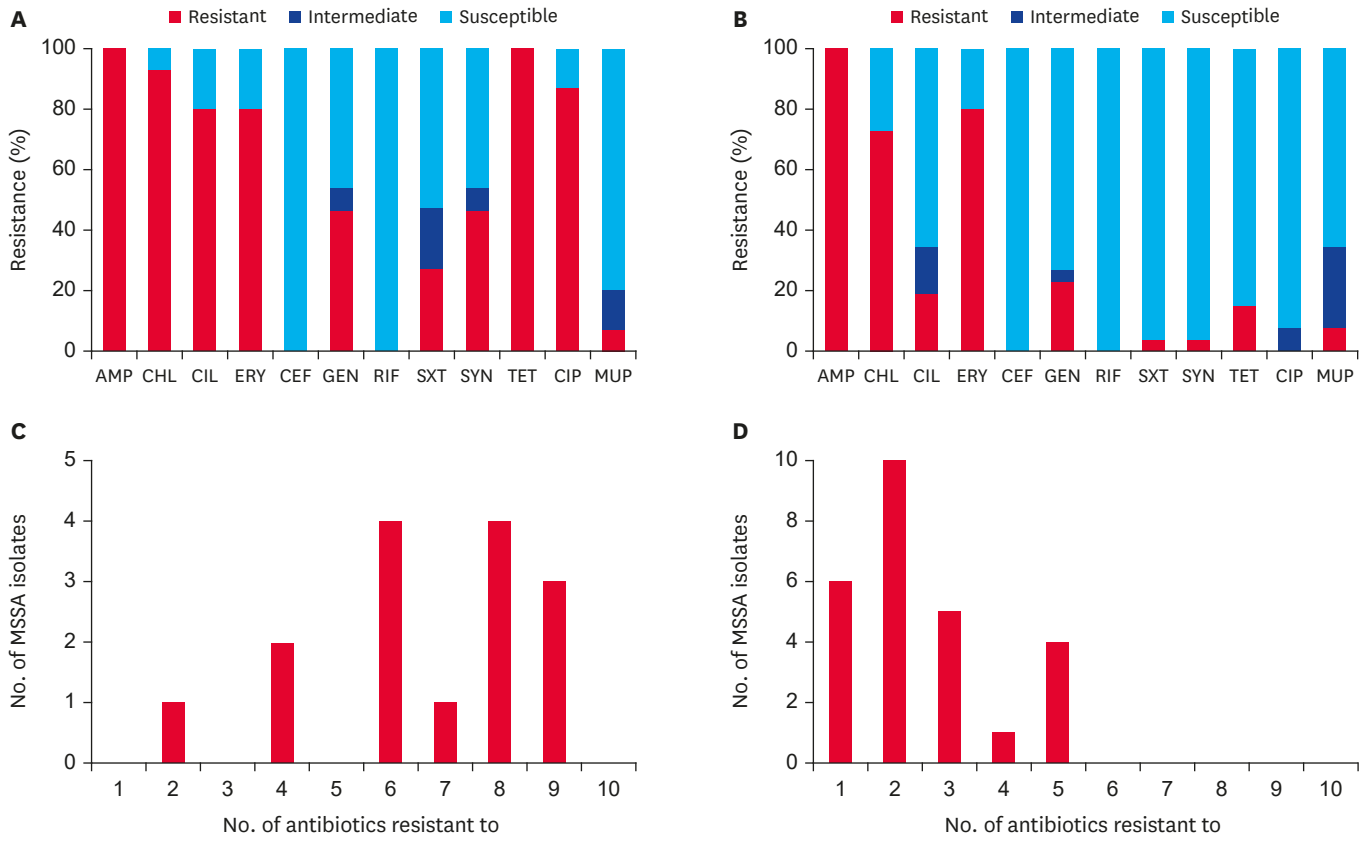


**Fig. 2.** Antimicrobial susceptibility and frequency of MDR phenotype. Antimicrobial susceptibility of (A) the CC398 and (B) non-CC398 MSSA isolates and frequency of MDR phenotype in (C) the CC398 and (D) non-CC398 MSSA isolates. Susceptibility assays were performed using the disc diffusion methods according to the 2017 Clinical and Laboratory Standards Institute guidelines [15].
AMP, ampicillin; CHL, chloramphenicol; CLI, clindamycin; ERY, erythromycin; CEF, cefoxitin; GEN, gentamicin; RIF, rifampicin; SXT, trimethoprim-sulfamethoxazole; SYN, quinupristin-dalfopristin; TET, tetracycline; CIP, ciprofloxacin; MUP, and mupirocin; MDR, multidrug resistance.

## DISCUSSION

ST398 MRSA has frequently been reported in livestock animals, particularly in pigs [24], and a growing number of incidents are being reported in which humans are infected with ST398 MRSA via direct or indirect exposure [9,25]. Although still at a significantly lower prevalence than in most European countries, the ST398 clonal lineage has become a major LA-MRSA clone in Korea [26-28]. Furthermore, recent studies have demonstrated that ST398 MSSA is an emerging zoonotic pathogen, able to colonize both humans and animals [2,8,9]. Although the detailed mechanisms of pathogenicity in ST398 MSSA remain to be elucidated, this clonal lineage of MSSA has been reported to cause fatal infections in humans with/without direct animal contact [29,30].

In the current study, we investigated the genotypic and phenotypic characteristics of MSSA strains isolated from the pork production chain, including pig farms, slaughterhouses, and retail markets.

The overall carriage of MSSA in pigs, farm workers, and the farm environment was 2.4%, 50%, and 1.6%, respectively. The prevalence of MSSA in pigs observed in this study was somewhat lower than the MRSA prevalence (3.2%–4.4%) reported by previous studies in Korea [27,28].


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However, 17/34 (50%) of the pig farm workers carried MSSA in their anterior nasal cavity, higher than the previously reported MRSA prevalence (16.7%) in pig farm workers [28]. This study is the first to investigate the prevalence and molecular characteristics of MSSA strains from pigs and pig farm workers in Korea. Therefore, continuous surveillance programs are required to monitor prevalence of MSSA as well as MRSA in the pork production chain.

MLST analyses revealed that ST398 (37%) and ST5 (32%) were the two major clonal lineages in Korean pig farms (**Table 1** and **Fig. 1**). ST398 MSSA has been identified as a predominant clone in pigs from other Asian countries, such as Japan and China [31,32]. In agreement with these previous reports [31-33], ST398 MSSA with *spa* type t571 (ST398 MSSA-t571) was mainly associated with human hosts (**Table 1**). Unlike these previous studies, which reported the prevalence of ST398 MSSA-t034 in pigs and pork meat samples, the ST398 MSSA strains isolated from pigs in this study had *spa* types of t1451 (n = 4) or t664 (n = 1) *spa* types. ST398 MSSA-t1451 has previously been reported in human bloodstream infections in New York [34]. In addition to t571, t1451, and t664, an ST398 MSSA-t18103 strain was isolated from a pig farm worker, suggesting that a rapid evolutionary change occurred in response to the new host environment. As shown in **Table 1**, the second most prevalent clonal lineage in the MSSA strains was ST5, in particular ST5 MSSA-t002 (n = 9). ST5 MRSA has been well recognized as one of the most globally disseminated HA-MRSA and LA-MRSA lineages [35]. However, little attention has been paid to its prevalence in livestock animals. The ST5 MSSA-t002 clone was identified among MSSA strains collected from hospitals in Denmark and UK in 1957 [36], indicating that this clonal type has successfully adapted to both human and pig populations. Interestingly, although nine different *spa* types (t1451, t664, t571, t18103, t002, t010, t7083, t899, and t5440) were identified in the two most prevalent ST types, ST398 and ST5, none of the *spa* types were shared by the two ST types. Importantly, the *spa* types of the ST398 and ST5 MSSA strains differed depending on whether the strains were isolated from humans or pigs, indicating rapid evolutionary adaptation to different hosts. Furthermore, all ST398 and ST5 MSSA strains were isolated from pig farms except for one ST5 strain isolated from a slaughterhouse worker, suggesting that the two major ST types of MSSA strains are mostly associated with pigs, farm workers, or the farm environment.

Recent studies have shown that ST398 MRSA tends to exhibit an MDR phenotype with 100% resistance to tetracycline [5,37]. Similarly, we found that more ST398 MSSA strains exhibited an MDR phenotype than the non-ST398 MSSA strains (93% and 38.5%, respectively; **Fig. 2A-D**). Importantly, all of the ST398 MSSA strains were resistant to tetracycline by harboring one or two of *tet*(K), *tet*(L), and/or *tet*(M) genes. This high level of tetracycline resistance phenotype mediated by *tet*(K), *tet*(L), and/or *tet*(M) genes has been well recognized in CC398 (ST398 and ST541) LA-MRSA strains [38]. In addition to the ST398 MSSA strains, all four ST9 MSSA strains and the three other MSSA strains (one ST544, one ST2115, and one non-typeable strain) carried *tet*(L), although one of the ST9 MSSA strains and the ST2115 strain did not exhibit a tetracycline resistance phenotype. These data combined with the MDR phenotype in ST398 MSSA suggest that ST398 MSSA strains may have been selected for by various antimicrobial agents, especially tetracycline. In contrast, all the ST5 MSSA strains were susceptible to tetracycline and did not possess the tetracycline resistance genes, warranting further characterization of ST5 MSSA to elucidate how these MSSA strains are transmitted between pigs despite exhibiting lower antimicrobial resistance than the ST398 MSSA strains.

Previous studies have suggested that resistance to zinc and other metals may co-select for CC398 MRSA strains carrying the *czrC* gene in staphylococcal cassette chromosome *mec*


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(SCC*mec*) type V [16,23]. The 41 MSSA strains displayed zinc chloride MICs of 2–8 mM, and nine of them were positive for the *czrC* gene. Neither the zinc chloride MIC nor presence of the *czrC* gene was associated with specific genotypes of the MSSA strains. Unlike the previous studies of CC398 MRSA [39,40], these data suggested that the use of zinc in animal feed might not contribute towards the prevalence of the ST398 and ST5 MSSA strains in pig farms in Korea.

It should be noted that our current study has several limitations. Firstly, since our data were generated based on a limited number of MSSA strains; therefore, further studies with a larger number of MSSA strains are required. Secondly, information on antibiotic usage in pig farms, particularly tetracycline usage, was not available; however, to the best of our knowledge, this is the first study to characterize MSSA strains collected from the Korean pork production chain and report the prevalence of ST398 LA-MSSA in pig farms in Korea.

In conclusion, the two major clonal lineages of MSSA in the pork production chain in Korea were ST398 and ST9 MSSA, which were mainly isolated from pig farms (pigs and farm workers). The prevalence of ST398 MSSA in pig farms appears to be associated with their MDR phenotype, especially their resistance to tetracycline. Since the increasing number of fatal infections caused by ST398 MSSA has posed a major public health concern, comprehensive surveillance should be continued for MSSA in livestock animals.

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