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Article

Antimicrobial Resistance and Prevalence of Methicillin-resistant *Staphylococcus aureus* in Bovine Mastitis Milk from Conventional and Organic Dairy Farms in South Korea

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Abstract: Bovine mastitis (BM) has been causing great economic and financial losses in the dairy industry worldwide and one of its major pathogenic agents is *S. aureus*. BM treatment still relies on antibiotics. The extensive use of antimicrobials gives rise to bacterial strains such as methicillin-resistant *S. aureus* (MRSA) and mupirocin-resistant *S. aureus* (MuRSA). This study aimed to investigate and compare the prevalence of MRSA and MuRSA and antimicrobial susceptibility in *S. aureus* isolates from bovine mastitis milk in conventional and organic dairy farms. Milk samples were taken from mastitis-infected cattle in 6 conventional and 7 organic dairy farms from South Korea. According to the Food Codex, 163 suspected *S. aureus* colonies were isolated from the pooled milk samples from each farm. Further rapid coagulase test confirmed 11 out of 74 isolates from 4 conventional farms (CF1, CF2, CF3, CF4) while 17 out of 89 isolates from 3 organic farms (OF1, OF2, OF3) exhibited coagulase activity. Multiplex PCR amplification showed that the *nuc* gene marker for *S. aureus* was found in all coagulase-positive isolates from OF 1 and at least one isolate from CF1, CF2, CF3, and CF4. Conversely, only 2 isolates from CF2 contained *mecA* gene for MRSA while none had the *mupA* gene for MuRSA. Antimicrobial susceptibility testing revealed that conventional farm isolates were more resistant to antibiotics particularly, against ampicillin and tetracycline, and, in turn, could imply a potential to develop multidrug resistance if stringent measures to control antimicrobial use in dairy farms is not implemented properly.

Keywords: *Staphylococcus aureus*; antimicrobial resistance; bovine mastitis; organic dairy farm; conventional dairy farm

1. Introduction

Bovine mastitis (BM) is one of the major concerns in dairy industry, causing considerable economic and financial losses worldwide [1]. BM is the inflammation of mammary gland caused mainly by bacteria, fungi, some microscopic algal species, viruses, and occasionally by physical trauma [2]. BM's pathogenic agents consist of different of gram-positive and gram-negative bacteria, and are classified as contagious (e.g., *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma* spp., *Corynebacterium bovis*) or environmental (e.g., *Escherichia coli*, *Enterococcus* spp., coagulase-negative *Staphylococcus*, *Streptococcus uberis*) [1,2]. Contagious pathogens live in the cow's udder and teat skin

and grow and colonize at the teat canal. Mastitis caused by contagious pathogens are often characterized by sub-clinical intramammary infections with no visible signs, increased somatic cell count and can be transmitted from cow-to-cow, particularly during milking. On the other hand, environmental mastitis pathogens are found in the cow's environment and can enter the teat during milking or when the cow's inherent or natural immunity becomes weak. Environmental pathogens are opportunistic microorganisms that cause clinical mastitis which can be easily noticed and detected, as characterized by evident swelling of the teat and with occasional pus included in the milk [3,4]. And among these pathogenic agents, the most related to both clinical and sub-clinical mastitis is *S. aureus* [5] which has also been noted to cause several human infections and, in turn, raises the concern of cross-infection between humans and animals through contaminated food and other factors. Although the risk of getting MRSA through pasteurized milk consumption is low, raw milk and milk products may still pose a threat to the public when exposed to viable bacteria [5,6].

The general measures applied to reduce infection and prevent spread of mastitis include improving sanitation (e.g., enhanced milking hygiene), teat disinfection after milking, and maintenance of milking machines [1,2]. However, these protocols can only reduce the degree of contamination and do not necessarily eliminate the existing infection. Treatment of active mastitis still relies greatly on antibiotics [5,6]. The continuous and extensive use of different antibiotics causes some bacterial strains like *S. aureus* to become resistant against antimicrobial agents, particularly on β -lactams. Hence, the prevalence and rise of *S. aureus* strains, coagulase-negative staphylococci (CoNS) species, and other bacterial strains which have exhibited resistance to different antibiotics such as, penicillin, clindamycin, tetracycline, methicillin, mupirocin, and vancomycin over the years [6-8], particularly in conventional farms. Consequently, organic farming has been introduced to combat the emergence of antibiotic-resistant mastitis pathogens. Organic livestock production practices ban the use of non-therapeutic antibiotics and growth promoters. Only when animal safety is endangered that antibiotics use is allowed, which is very seldom [9]. However, there are still some instances where these antibiotic-resistant microorganisms are found in organic farms as well [10]. In Korea, sales of antibiotics used in livestock doubled from 55 tons in 2011 to 97 tons in 2020 according to the 2020 National Antibiotics Use and Resistance and Monitoring, with the resistance level of livestock-isolated *S. aureus* for penicillin, ampicillin, and oxacillin to be 69.4%, 57.7%, and 2.3%, respectively [11].

Consumers are becoming more health-conscious and are leaning towards "organic" or naturally sourced food commodities. Increased awareness and concern for food safety and health issues drive the consumers to choose high quality products often labeled as organic, antibiotic-free, and animal welfare-approved milk products. Organic milk sales in Korea increased significantly from 5 billion won in 2008 to 104 billion won in 2020 [12]. With the increasing demand for organic livestock products market, there is a need to check the safety of the said products.

As far as the authors know, only a few studies had been conducted that focus on the comparative antimicrobial resistance of bacterial isolates from conventional and organic dairy farms, and most of the milk samples used in these studies were taken from bulk tank milk samples, especially in South Korea. In this manuscript we tried to isolate *S. aureus* from pooled milk samples of cattle already infected with mastitis (clinical and sub-clinical) from conventional and organic farms in South Korea and checked for presence for MRSA and MuRSA, as well as their corresponding antimicrobial susceptibility.

2. Materials and Methods

2.1. Materials and Reagents

Tryptic soy broth (TSB) and Baird-Parker agar (BPA) were purchased from BD Difco™ (NJ, USA). Egg-yolk tellurite emulsion was bought from Oxoid (New Hampshire, England). Muller-Hinton agar (MHA), nutrient agar (NA), and nutrient broth (NB) were distributed by MBCCell (Seoul, Korea). Antibiotics used, ampicillin (AMP) was obtained from GA BioChem (GA, USA) while oxacillin (OX), gentamicin (CN), tetracycline (TE), erythromycin (E), chloramphenicol (C),

ciprofloxacin (CIP), vancomycin (VA), and teicoplanin, (TEC) were supplied by LiofilChem® (Roseto degli Abruzzi, Italy). Pastorex Staph-plus latex agglutination test kit was from Bio-Rad (CA, USA). PCR premix and oligonucleotide primers were secured from Bioneer (Daejeon, Korea). Bacterial DNA extraction kit was provided by Takara Bio (Shiga, Japan). Sodium chloride (NaCl) was ordered from Sigma-Aldrich (St. Louis, MO, USA). All other reagents, media, and materials used were HPLC- or analytical-grade.

2.2. Methods

2.2.1. Sample collection and isolation of *S. aureus* from bovine mastitis milk samples

Pooled raw milk samples from mastitis-infected (clinical and sub-clinical) cattle were aseptically collected from 6 conventional dairy farms and 7 organic-certified dairy farms from southern area of Gyeonggi-do and Chungcheongnam-do, South Korea in late 2021 to the first quarter of 2022. For each infected cattle, teat surface was disinfected with 70% ethanol and after a few streams of milk were discarded, 10 ml of milk was collected in sterile polyethylene screw-capped bottles from each teat. Milk samples from individual cattle were pooled per farm and kept in icebox (at 4°C) and immediately transported to laboratory for analysis.

S. aureus was isolated according to the Korean Food Standards Codex [13]. For each sample, 25 g of milk was transferred in sample filter bag and mixed with 225 ml of TSB containing 10% NaCl using stomacher (BagMixer 400CC, InterScience, France). The homogenized mixture was then incubated at 36±1 °C for 18-24 h. The enriched media was plated onto BPA supplemented with 5% egg-yolk tellurite emulsion and incubated at 36±1°C for another 18-24 h. Each black, glossy colony surrounded by transparent bands was separated and cultured to NA for further isolation. Gram-positive cocci with staphylococcal arrangement were verified using gram-staining. Further confirmation was done by rapid coagulase test using Pastorex™ Staph-plus latex agglutination test kit (Bio-Rad, CA, USA). Confirmed *S. aureus* cultures were stocked in sterile glycerol solution and stored at -20°C until further analysis. Re-testing and subculture onto NA were done whenever storage period exceeded 7 days.

2.2.2. Antimicrobial susceptibility testing of isolated *S. aureus* cultures using disc diffusion method

Table 1. Class, concentrations, and inhibition zones of antibiotics that were used in this study.

Compound Class	Antibiotic Name	Code	Disc concentration (ug)	Zone diameter (mm)		
				R [†]	I [†]	S [†]
β-lactams	Ampicillin	AMP	10	15	16-21	22
	Oxacillin	OX	10	17	18-24	25
Aminoglycosides	Gentamicin	CN	10	18	19-27	28
Glycopeptides	Teicoplanin	TEC	30	14	15-21	22
	Vancomycin	VA	30	16	17-21	22
Macrolides	Erythromycin	E	15	21	22-30	31
Phenicol	Chloramphenicol	C	10	18	19-26	27
Quinolones	Ciprofloxacin	CIP	5	21	22-30	31
Tetracyclines	Tetracycline	TE	30	23	24-30	31

[†] R, I, S means resistant, intermediate, and susceptible, respectively.

Antimicrobial susceptibility testing was done following the guidelines of CLSI (Clinical and Laboratory Standards Institute) [14] using disc diffusion test against nine antibiotics (ampicillin, oxacillin, gentamicin, teicoplanin, vancomycin, erythromycin, chloramphenicol, ciprofloxacin, and tetracycline) as described in Table 1 with their corresponding concentration and inhibition zones.

Bacterial suspension was made following the density of McFarland 0.5 turbidity standard (1.5×10⁸ CFU/ml) and swabbed evenly onto the entire surface of MHA plates. Antibiotic discs were applied firmly onto the inoculated MHA plate with enough space for the inhibition zones. The agar plates were then incubated at 36±1 °C for 18-24 h and inhibition zones measured using vernier caliper. Isolates were tested for each antibiotic on duplicate plates and classified (resistant, intermediate, susceptible) according to the average of their corresponding inhibition zones.

2.2.3. Bacterial DNA extraction, PCR amplification, and gel electrophoresis

Glycerol stock of isolated *S. aureus* samples were plated on NA and incubated at 36±1 °C for 18-24 h. Bacterial DNA was obtained following Takara MiniBEST bacteria genomic DNA extraction kit (Takara Bio, Shiga, Japan). First, lysis was done by making 1 ml of bacterial suspension (0.5-2.0×10⁹ CFU/ml) and centrifugation at 12,000 rpm for 2 min. Supernatant was discarded and 50 µl buffer BS and 50 µl lysozyme were added to the bacteria pellet. The mixture was homogenized and incubated in a 37°C-waterbath for 1 h, with occasional inversion mixing every 20 min. The incubated solution was centrifuged at 12,000 rpm for 5 min and supernatant was discarded. Then, 180 µl buffer GL, 20 µl proteinase K, and 10 µl RNase A were added, mixed thoroughly, and incubated at 56°C-waterbath for 10-30 min, with pipet mixing every 5 min until solution becomes clear. Buffer GB (200 µl) and 100% ethanol (200 µl) were added and mixed well. The solution was then transferred to the spin column with a collection tube and centrifuged at 12,000 rpm for 2 min. Flow-through was discarded and buffer WA (500 µl) was added to the spin column and centrifuged (12,000 rpm, 1 min). Flow-through was removed and spin column was washed with 700 µl buffer WB and centrifuged (12,000 rpm, 1 min). Flow-through was discarded and spin column was washed with buffer WB once more and centrifuged (12,000 rpm, 2 min). After, spin column was transferred to a new 1.5 ml centrifuge tube and 50 µl elution buffer and let stand at room temperature for 5 min before centrifugation (12,000 rpm, 2 min) to elute the DNA.

Extracted DNA samples were then subjected to PCR amplification using the following primers (Table 2):

Table 2. Primers used in PCR amplification of *S. aureus* isolates from bovine mastitis milk in conventional and organic farms.

Target gene	Primer	Oligonucleotide Sequence (5' - 3')	Amplicon Size (bp)
<i>mecA</i>	<i>mecA</i> 1	GTAGAAATGACTGAACGTCCGATAA	310
	<i>mecA</i> 2	CCAATTCACATTGTTTCGGTCTAA	
<i>nuc</i>	<i>nuc</i> 1	GCGATTGATGGTGATACGGTT	279
	<i>nuc</i> 2	AGCCAAGCCTTGACGAAGTAAAGC	
<i>mupA</i>	<i>mupA</i> 1	TATATTATGCGATGGAAGGTTGG	457
	<i>mupA</i> 2	AATAAAATCAGCTGGAAAGTGTTG	

Multiplex PCR (M-PCR) assay was performed according to [7] with slight modifications. An aliquot of 1 µl bacterial DNA template was added to 20-µl PCR premix containing the reaction buffer with 1.5 mM MgCl₂, 250 µM each of deoxynucleoside triphosphate (dATP, dUTP, dGTP, and dCTP), 1.0 U of Top DNA polymerase (Bioneer, Daejeon, Korea), and 1 µL of each primer for *mecA* (1.2 µM), *nuc* (0.4 µM), and *mupA* (0.5 µM). Amplification was done using Q-Cycler II gradient thermal cycler (QuantaBiotech, Surrey, UK) and carried out as follows: initial denaturation step at 94°C for 5 min; 10 cycles of 94°C for 40 s, 58°C for 40 s, and 72°C for 1 min; 25 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min; and final extension step at 72°C for 10 min. Amplified samples were stored at 4°C prior to gel electrophoresis. PCR amplicons were loaded on 2.0% agarose gel (Bioneer, Daejeon, Korea) containing 0.5 µg/ml mango blue dye and visualized under UV light box using digital image system (GDS-200D, MDM, Korea).

2.2.4. Statistical Analysis

The connection between type of farm (conventional or organic) and isolate activity (% resistance) for each antibiotic were analyzed using Chi-Square analysis for independent variance with Statistical Packages for the Social Sciences (IBM SPSS Statistics 23.0) software (SPSS Inc., USA). Significant difference among data were identified with $p < 0.05$ values. The same treatment was done for the relationship between farm type and resistant categories.

3. Results

3.1. Isolation of *S. aureus* from bovine mastitis milk samples in conventional and organic dairy farms

Following the Korea Food Codex [13], 163 suspected *S. aureus* colonies were isolated from all the bovine mastitis milk samples, wherein, 74 colonies were from 6 conventional dairy farms while 89 colonies were from 7 organic dairy farms. Additional rapid coagulase test confirmed that only 11 out of 74 (15%) of the separated colonies from 4 conventional dairy farm samples while 17 out of 89 (19%) of the isolates from 3 organic dairy farm samples exhibited coagulase activity which is a characteristic of *S. aureus*.

3.2. Antimicrobial susceptibility of isolated *S. aureus* cultures

Coagulase-positive *Staphylococci* (CoPS) isolates in BM milk samples from conventional and organic farms were further tested for antibiotic susceptibility against 9 antimicrobial substances (see Table 3).

Overall, the CoPS isolates exhibited less than 50% resistance against the following antibiotics used in this study: gentamicin (46.4%), tetracycline (46.4%), ampicillin (39.3%), erythromycin (21.4%), oxacillin (14.3%), and chloramphenicol (14.3%).

Table 3. Antimicrobial resistance (number and % of resistant isolate) of coagulase-positive *Staphylococcus* from conventional and organic farms.

Antibiotic Name	Code	Conventional Farm (n=11)		Organic Farm (n=17)		Total (n=28)	
		Number	%	Number	%	Number	%
Ampicillin	AMP	9	81.8	2	11.8	11	39.3
Oxacillin	OX	4	36.4	0	0.0	4	14.3
Gentamicin	CN	4	36.4	9	52.9	13	46.4
Teicoplanin	TEC	0	0.0	0	0.0	0	0.0
Vancomycin	VA	0	0.0	0	0.0	0	0.0
Erythromycin	E	5	45.5	1	5.9	6	21.4
Chloramphenicol	C	2	18.2	2	11.8	4	14.3
Ciprofloxacin	CIP	0	0.0	0	0.0	0	0.0
Tetracycline	TE	9	81.8	4	23.5	13	46.4

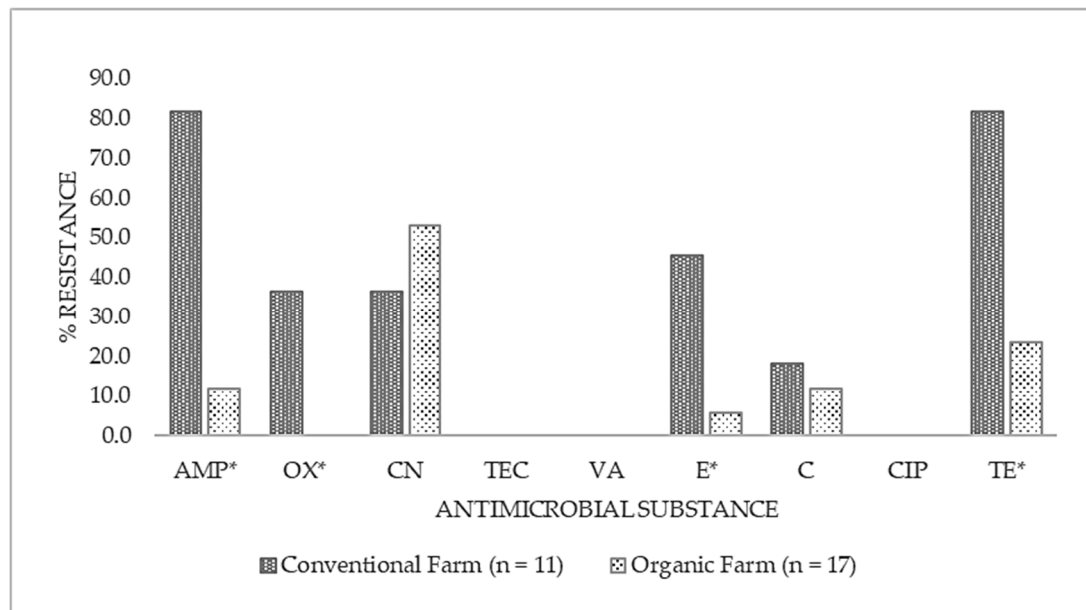


Figure 1. Antibiotic resistance (%) of *S. aureus* isolated from bovine mastitis (BM) milk in conventional and organic dairy farms (n = number of coagulase-positive *Staphylococcus* isolates tested). Antimicrobial substances used were ampicillin (AMP), oxacillin (OX), gentamicin (CN), teicoplanin (TEC), vancomycin (VA), erythromycin (E), chloramphenicol (C), ciprofloxacin (CIP), and tetracycline (TE). Asterisk (*) denoting significant difference ($p < 0.05$) between conventional and organic farm samples.

Figure 1 showed that conventional farm isolates demonstrated more noticeable antibiotic resistance than the organic farm isolates. Resistance against ampicillin and tetracycline was detected in 81.8% of the conventional isolates while 45.5% were resistant to erythromycin, 36.4% were resistant to oxacillin, and 18.2% were resistant to chloramphenicol. Conversely, organic farm isolates exhibited lower resistance against ampicillin (11.8%), erythromycin (5.9%), chloramphenicol (11.8%), and tetracycline (23.5%). However, higher resistance against gentamicin was noted in organic farm isolates at 52.9% compared to conventional farm isolates at 36.4%. Additionally, the isolates from both dairy farms were discovered to be sensitive to the quinolone antibiotic, ciprofloxacin at 85.7% (data not shown). Furthermore, organic farm milk isolates also showed susceptibility against the β -lactam antibiotics, ampicillin, and oxacillin at 76.5% and 82.4% (data not shown).

All CoPS isolates were grouped and categorized according to the number of antimicrobial substances/s they were resistant against (shown in Figure 2). CoPS isolates from conventional farm samples were all resistant to at least one antibiotic while 23.5% of the organic farm isolates were classified as not resistant to any of the antimicrobials tested in this study. Most of the CoPS isolates from conventional farms (63.6%) were resistant to three or more substances, followed by both 18.2% of isolates resistant to one and two antimicrobial substances, respectively. Organic farm CoPS isolates were mainly composed of 52.9% resistant to one antibiotic while 17.6% were resistant to two antimicrobials. Lastly, there was a small percentage (5.9%) of organic farm isolates that were resistant to three or more substances.

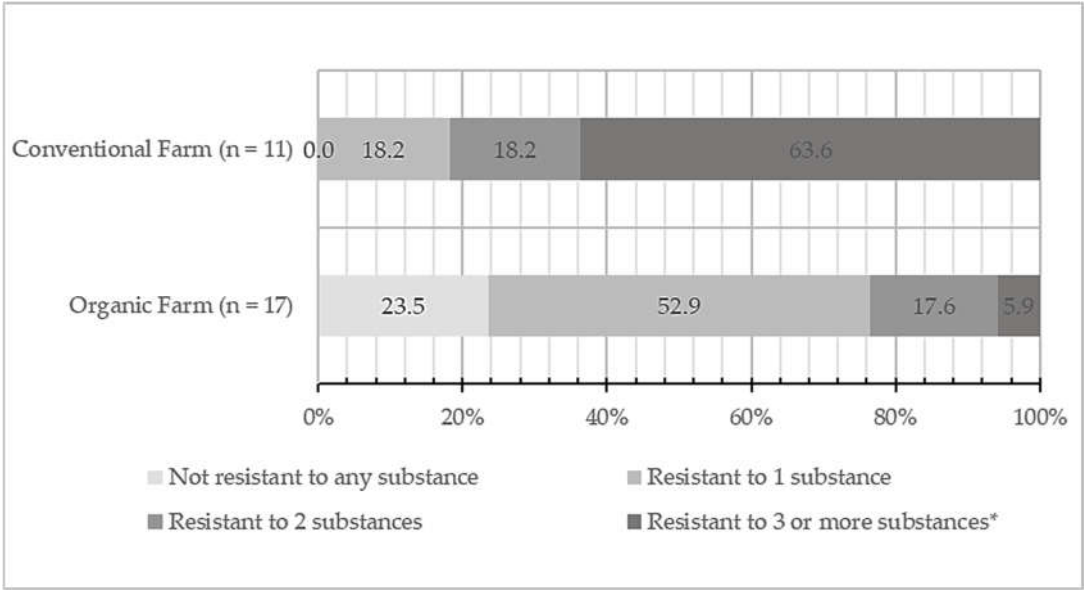


Figure 2. Percent isolate (%) classification of coagulase-positive *Staphylococcus* (CoPS) isolates from bovine mastitis milk in conventional and organic dairy farms according to the number of antimicrobial substances resistant against (n = number of CoPS isolates tested). Antimicrobial agents tested were ampicillin, oxacillin, gentamicin, teicoplanin, vancomycin, erythromycin, chloramphenicol, ciprofloxacin, and tetracycline. Asterisk (*) denoting significant difference ($p < 0.05$) between conventional and organic farm samples.

3.3. DNA extraction and PCR amplification of isolated *S. aureus* isolates

DNA of *S. aureus* isolates were extracted using a bacterial DNA extraction kit and subjected to PCR amplification. The corresponding agarose gel visualization confirmed which isolates from organic (Figure 3) and conventional (Figure 4) farms contained the *nuc*, *mecA*, and *mupA* genes.

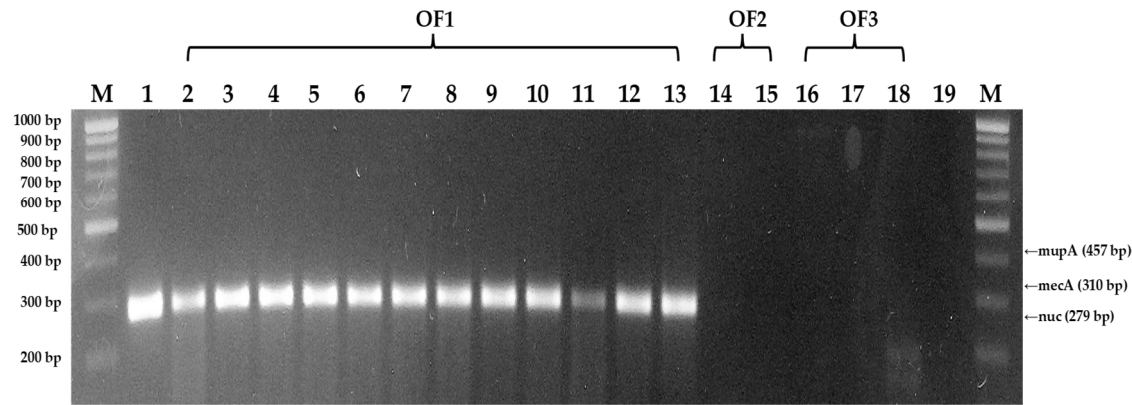


Figure 3. Agarose gel visualization of M-PCR detecting *mecA*, *mupA*, and *nuc* genes in bovine mastitis milk *S. aureus* isolates from organic farms (Lanes 2-18; OF1, OF2, OF3 are organic farms 1, 2, and 3, respectively) with negative template control (Lane 19; no dna template added), and *nuc* gene control *Staphylococcus aureus* (Lane 1).

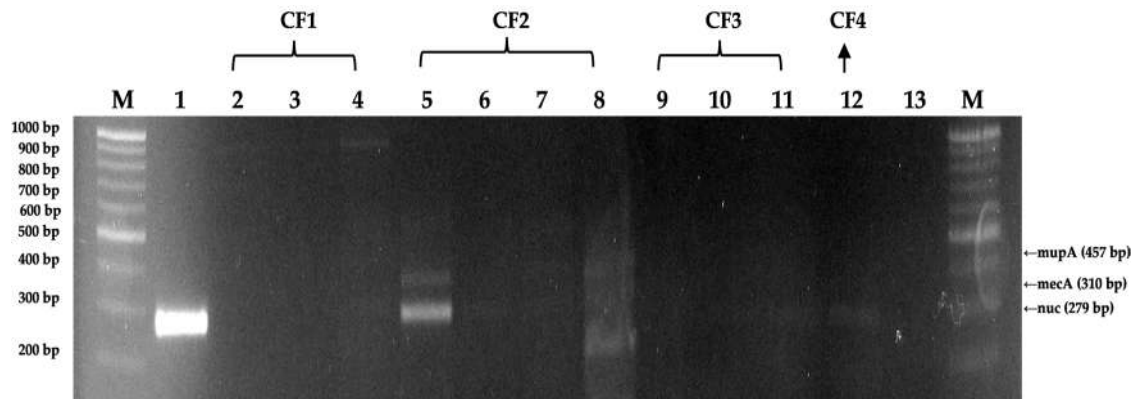


Figure 4. Agarose gel visualization of M-PCR detecting *mecA*, *mupA*, and *nuc* genes in bovine mastitis milk *S. aureus* isolates from conventional farms (Lanes 2-12; CF1, CF2, CF3, CF4 are conventional farms 1, 2, 3, and 4, respectively) with negative template control (Lane 13; no dna template added), and *nuc* gene control *Staphylococcus aureus* (Lane 1).

Of the 3 organics farm samples, 12 isolates that were all from the organic farm 1, OF1, contained the *nuc* gene which is specific gene marker for *S. aureus* [7,15]. Meanwhile, at least one isolate from conventional farms, CF1, CF2, CF3, and CF4, contained the *nuc* gene. Additionally, only two isolates from conventional farm 2 (CF2) were confirmed to have the *mecA* gene which is a determinant of methicillin resistance [7,16] while none of the organic farm isolates was noted with the *mecA* gene. On the other hand, the *mupA* gene, which is a determinant of mupirocin resistance [7,16], was not detected in any of the isolates from organic and conventional farms.

4. Discussion

In this study, higher counts of suspected *S. aureus* were isolated from BM milk in organic farms (89 colonies) than from the conventional farm (74 colonies) samples. Tikofsky et al. [9] also found more *S. aureus* isolated from milk samples from organic farms (144) than conventional farms (117) in US. Roesch et al. [17] observed a similar trend with more *S. aureus* and other mastitis pathogens isolated from organic farms (93) than conventional farms (65) in Switzerland. Sato et al. [18] also discovered more *S. aureus* from organic farms than conventional farms in Wisconsin (73 and 64, respectively). However, dairy herds in Denmark showed an opposite trend with slightly higher *S. aureus* isolate count in conventional farms than organic farms at 77 and 75, correspondingly [18]. The higher *S. aureus* isolate count found in organic farms could be due to the fact these farms have very limited use of antibiotics, which is only for emergency cases when the cattle's life is in danger [9,10]. Use of non-therapeutic antibiotics and growth promoters are prohibited in organic farms, except for critical situations wherein animal safety is compromised that antibiotics use is allowed [9,10,19]. Even so, CoPS isolates were present in more than half of the conventional farm samples (4 out of 6) compared to organic farm samples (3 out of 7). This result was confirmed by the M-PCR amplification and shown through the agarose gel electrophoresis wherein some the organic farm milk isolates did not contain the *nuc* gene marker of *S. aureus* but were positive for the coagulase activity. It is also because of the restricted use of antimicrobials in organic farms that more varied species of microorganisms thrive in organic farms [9,17-19].

For antimicrobial susceptibility testing, conventional CoPS isolates were found to be significantly more resistant against ampicillin, oxacillin, erythromycin, and tetracycline than the organic CoPS isolates. Similar observations were noted in a study on MRSA from organic and conventional herds in Germany and their corresponding antimicrobial resistance wherein conventional farm isolates exhibited higher resistance against the penicillin (100%), ceftiofur (100%), tetracycline (97.2%), erythromycin (44.4%), gentamicin (11.1%), and chloramphenicol (8.3%) [20]. Ray et al. [21] checked the antimicrobial susceptibility of *Salmonella* from milk samples and discovered that conventional farm isolates were more resistant against streptomycin and sulfamethoxazole.

Antibiotic use had long been linked to the subsequent rise of antimicrobial resistance, in which the resistance strengthens or compounds with each exposure to the antimicrobial substance due to selective pressure [22-24]. The most widely used antimicrobials for the treatment of mastitis are β -lactam compounds such as ampicillin and oxacillin [2,25,26] and Korea is no exception to this practice [11]. β -lactams function as an antimicrobial agent by inactivating the enzymes called penicillin-binding proteins (PBPs) through covalent bonding. PBPs are critical to the cell cycle-related, morphogenetic synthesis of cell wall peptidoglycan. Accordingly, β -lactams can effectively inhibit the transpeptidation of PBPs, disturb the cross-linking of the bacterial peptidoglycan and, in turn, result to weakening of the cell wall, freeing the cytoplasmic contents, and eventual cell death when exposed to an appropriate concentration [27-30]. However, this antimicrobial activity can be bypassed due to the expression of a unique PBP, known as PBP2a, which has low affinity for all β -lactam antibiotics and can be found in MRSA [26,29,30]. PBP2a does not easily attach to the β -lactams and cannot be inactivated. And since it has similar structural motifs as the innate PBPs 1-4 in *S. aureus*, it can keep transpeptidation, support the cross-linking of peptidoglycan and allow the survival of MRSA [29,30]. On the other hand, erythromycin and tetracycline were not allowed for consumption in cattle livestock industry in Korea but similar compounds as erythromycin (spiramycin and tylosin, which belonged to macrolides antibiotics), and tetracycline (chlortetracycline and oxytetracycline) were used instead [11]. The topmost sold antibiotic classes for use in livestock and fisheries in Korea are the following: β -lactams > tetracyclines > phenicols > macrolides > sulfonamides > aminoglycosides [11].

Although resistance levels were less than 50% for most antimicrobials, the implication of multidrug resistance was evident in both types of farm isolates. A considerably higher count of multidrug resistant CoPS isolates was found in conventional farms than in organic farm samples. Microbial isolates resistant to three or more classes of antimicrobials were classified as "multidrug-resistant" [31]. This could also be attributed to the regular exposure of infected cattle in conventional farms to antibiotics as one of their treatment methods for BM. However, the exposure parameters like dose, administration route, and dose frequency, were not specified as antibiotic usage was difficult to monitor and quantify in especially in conventional farms [18]. Grobbel et al. [31] investigated and compared the antimicrobial resistance of *Escherichia coli* from organic and conventional poultry farms in Germany where they found that conventional farm isolates were resistant to three or more antibiotics. Meissner et al. [10], on the other hand, noted an almost similar percentage of multidrug-resistant isolates of extended-spectrum β -lactamase-producing *E. coli* from conventional and organic pig fattening farms.

The isolates from organic farms 2 and 3 (OF2 and OF3) that did not contain the *nuc* gene could belong to other CoPS species but further tests are needed to be done to properly identify them. Aside from *S. aureus*, the other *Staphylococcus* spp. that exhibit coagulase activity are the following: *S. intermedius*, *S. schleiferi* subsp. *coagulans*, *S. hyicus*, *S. lutrae*, *S. delhini*, and *S. pseudintermedius*, which also demonstrate antibiotic resistance and biofilm formation [32,33]. CoPS are opportunistic pathogens, meaning they can easily adapt accordingly to their surroundings or hosts, causing a lot of potent virulence factors [32].

Two isolates from conventional farm 2 were confirmed to have the *mecA* gene which is a known determining factor of methicillin resistance [7,16]. MRSA contains the *mecA* gene which is responsible for encoding PBP 2a [27,29]. This result was further confirmed with the antimicrobial resistance. On the other hand, the *mupA* gene, which is a determinant of mupirocin resistance [7], was not detected in any of the conventional and organic farm isolates. Mupirocin is an antibiotic that could effectively inhibit bacterial synthesis and has high antimicrobial activity against *S. aureus* [8,34]. Mupirocin acts as an antimicrobial by binding to the enzyme isoleucyl-transfer RNA synthetase preventing isoleucine incorporation during bacterial protein synthesis, which makes it effective against MRSA and some methicillin-resistant CoNS [7,8,34].

The presence of mupirocin-resistant isolate was investigated to check for any possibility of inter-host-adapted (human-to-cattle and vice versa) variants of *S. aureus*. Different serovars of host-adapted *S. aureus* could develop through host-switching and gene acquisition, loss and diversification [5]. Nonetheless, this non-detection of *mupA* gene in any of the isolates could imply

that mupirocin and other mupirocin-related compounds could still be effective against *S. aureus* that would be isolated from conventional and organic dairy farms in South Korea.

5. Conclusions

This study showed that there is variation in *S. aureus* isolated from mastitis-infected cow's milk from organic and conventional dairy farms in South Korea. Although a higher isolate count was found from organic farms, these isolates have low antimicrobial resistance and did not contain the *mecA* gene. On the other hand, conventional farm isolates were more resistant to antimicrobials, particularly against ampicillin and tetracycline. Moreover, *mecA* gene was found in conventional farm isolates through multiplex PCR. Consequently, these findings could imply a potential to develop multidrug resistance in BM- and livestock-related bacterial isolates if stringent measures to control antimicrobial use in dairy farms are not implemented properly. Additional analyses are needed to further confirm the other *Staphylococcus* species of the other isolates that did not contain *mecA*, *mupA*, or even *nuc* gene.

Supplementary Materials: Not applicable.

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