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Association with Elevated Somatic Cell Counts and Characterization of *Aerococcus viridans* Isolates from Bovine Mastitis Milk in South Korea

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Abstract

Aerococcus viridans, an emerging pathogen, is responsible for the recent increase in cases of bovine mastitis. However, its specific effects on mastitis remain largely unexplored. In this study, we examined the correlation between *A. viridans*-induced mastitis infections and somatic cell counts (SCCs), and characteristics of *A. viridans* isolates from bovine mastitis milk. Among 1774 mastitis milk samples collected between 2016 and 2021 in South Korea, 69 (3.9%) *A. viridans* isolates were obtained. Mastitis milk samples containing *A. viridans* exhibited significantly higher SCCs than did non-mastitis samples. Most isolates (80.5%) were associated with subclinical mastitis (200–1200 × 10^3 cells/mL), whereas 19.5% were associated with clinical mastitis (> 1.2 × 10^6 cells/mL). In pulsed-field gel electrophoresis analysis, *A. viridans* isolates displayed substantial genetic diversity, with no dominant clones identified. Antimicrobial susceptibility testing revealed high resistance rates to ceftiofur (46.4%) and oxacillin + 2% NaCl (44.9%) among β-lactams, followed by tetracycline (36.2%) and erythromycin (10.1%), with 21.7% isolates being multidrug-resistant. Fifty-four isolates (78.3%) were able to form biofilms, with all recent isolates being biofilm-positive, in contrast to several earlier non-producers. Our findings suggest the necessity for targeted management strategies and continuous monitoring for mitigating *A. viridans*-induced mastitis in dairy cows.

Introduction

Bovine mastitis characterized by an inflammation of the mammary gland is a highly important disease in dairy cows worldwide, which leads to substantial economic losses owing to decreased milk production, reduced milk quality, and increased veterinary costs, along with labor and treatment expenses [1–3]. Mastitis can be classified into two categories, clinical and subclinical mastitis, based on symptoms [3]. Clinical mastitis is characterized by observable symptoms in cow's udder, including swelling, fever, and alterations in consistency of milk quality such as clotting, discoloration, and changes in texture [3]. In contrast, subclinical mastitis does not present visible symptoms; however, it can be detected by changes in milk composition, such as elevated somatic cell counts (SCCs), and results in higher economic losses than that caused by clinical mastitis [3].

Various bacteria, including *Staphylococcus aureus*, *Streptococcus* spp., and *Escherichia coli*, cause bovine mastitis [4]. *Aerococcus viridans* has been recently identified as an emerging pathogen associated with bovine mastitis [5–9]. *Aerococcus* belongs to the family Aerococcaceae, within the order Lactobacillales, and is classified as a catalase-negative, environmental, and Gram-positive coccus [10, 11]. Accurate identification of *Aerococcus* species has been challenging, often resulting in their misidentification as alpha-hemolytic streptococci, staphylococci, or enterococci, which leads to an underestimation of their pathogenic potential [10, 12]. However, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF MS) has enabled rapid and accurate

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identification of *Aerococcus* species, with several studies evaluating its role as a pathogen [10, 12, 13]. *A. viridans* is increasingly recognized as an opportunistic pathogen capable of infecting a wide range of animal species. It has been implicated in urinary tract infections and endocarditis in humans, reproductive and systemic infections in pigs, and gaffkemia, a fatal septicemic disease, in European lobsters (*Homarus gammarus*) [10–14]. In South Korea, *A. viridans* has been identified as a causative agent of bovine mastitis [15, 16]. Nevertheless, the specific role of this bacterium in the pathogenesis of bovine mastitis remains poorly understood [6].

Focused studies on identifying pathogen-specific characteristics for emerging pathogens are essential for defining risk factors and management strategies, and establishing appropriate antibiotic usage [4]. In particular, biofilm formation by pathogens associated with bovine mastitis is a significant virulence factor, which can resist host immune defense, thereby leading to recurrent or persistent cases of mastitis [1, 17]. Studies of the bacterial genome using pulsed-field gel electrophoresis (PFGE) provide crucial information on the clonal origin of isolates during an infectious epidemic, and for monitoring the source, route of transmission, and mode of infection propagation [18].

In this study, we aimed to examine the correlation between *A. viridans*-induced mastitis and SCCs. Additionally, we aimed to characterize the antimicrobial resistance, biofilm-forming ability, and genetic diversity of mastitis-associated isolates from dairy farms in South Korea.

Materials and Methods

Collection of Quarter Milk Samples

Between 2016 and 2021, 1774 quarter milk samples of lactating cows with mastitis and 1,648 quarter milk samples of those without mastitis were collected from 75 dairy farms in South Korea, as described by the National Mastitis Council [19]. The 75 dairy farms were located in the following provinces: Gyeongsangbuk-do (46), Gyeonggi-do (18), Chungcheongbuk-do (7), Chungcheongnam-do (2), Jeollabuk-do (1), and Jeollanam-do (1) (Fig. 1). SCCs of the samples were assessed using a Fossmatic System 400 (Foss Electric, Hillerød, Denmark), according to the manufacturer's instructions. Milk samples were considered indicative of mastitis if the SCCs exceeded 2.0×10^5 cells/mL, or if clinical symptoms of mastitis were observed [3, 7].

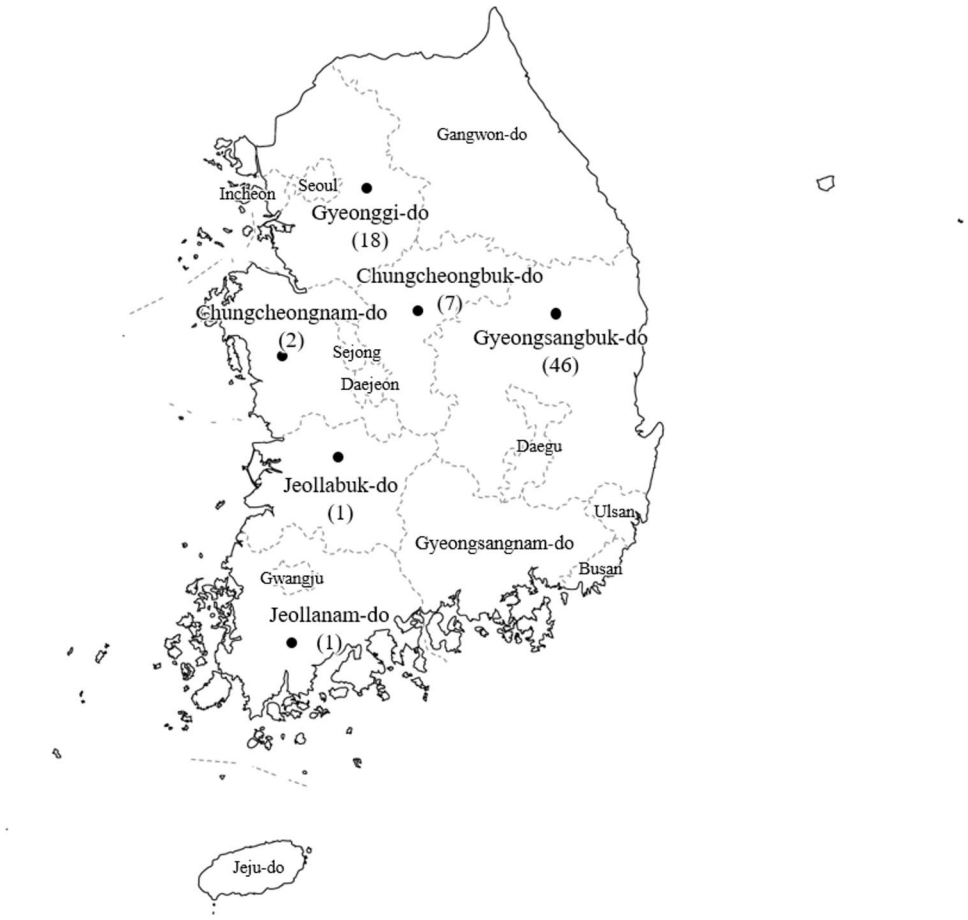


Fig. 1 A map of South Korea indicating the number of dairy farms surveyed by regions

Table 1 Comparison of somatic cell counts (SCCs) between *Aerococcus viridans*-induced mastitis and non-mastitis milk samples

Status	Number of milk samples	SCC ($\times 10^3$ cells/mL) Mean ± standard deviation	*P* value
Non-mastitis cows	121	64.68 ± 49.20	< 0.001
Cows with *A. viridans*-induced mastitis	41	1220.85 ± 2057.21	

SCCs in quarter milk were categorized as follows: 200–400, 400–1200, 1200–5000, and > 5000 ($\times 10^3$ cells/mL). Subclinical mastitis was defined as SCCs ranging between 2.0 $\times 10^5$ and 1.2 $\times 10^6$ cells/mL, and clinical mastitis was defined as SCCs > 1.2 $\times 10^6$ cells/mL or the presence of clinical symptoms [20].

Isolation and Identification of *A. viridans*

Milk samples from lactating cows exhibiting clinical signs of mastitis or having SCCs ≥ 2.0 $\times 10^5$ cells/mL were collected, and bacteria were isolated from these samples as described by the National Mastitis Council [19, 21]. Briefly, 10 µL sample was streaked onto blood agar plates (Komed, Gyeonggi, Republic of Korea) and incubated for 24 h at 37 °C. Samples yielding > 10 colony-forming units (CFUs) were considered positive for bacterial growth. Those with ≤ 10 CFUs were excluded to reduce the risk of misclassifying environmental contamination as true infection. Samples showing three or more distinct colony morphologies were classified as contaminated and were excluded from analysis. Suspected colonies were purified and identified using MALDI–TOF MS (Biomerieux, Marcy L'Étoile, France) with the VITEK MS v.3.2 library, following the manufacturer's instructions.

PFGE Analysis

PFGE was performed as previously described [14]. Genomic DNA was digested with *Sma*I (TaKaRa Bio Inc., Otsu, Japan) for 6 h at 25 °C. The fragments were separated by PFGE with initial and final switch times of 1 and 30 s, respectively, using a gradient of 6 V/cm for 21 h in 0.5 × Tris–borate-ethylenediaminetetraacetic acid on a CHEF Mapper XA System (Bio-Rad Laboratories Inc., Hercules, CA, USA). The isolates were analyzed for similarity using the Dice coefficient, with the tolerance and optimization settings of 1%. Dendrograms were constructed using unweighted pair group method with arithmetic averages (UPGMA) in BioNumerics v.5.10 (Applied Maths, Sint-Martens-Latem, Belgium).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of *A. viridans* isolates was tested using the broth microdilution and disk diffusion methods for *Streptococcus* spp., according to the CLSI guideline M100 [22]. The minimum inhibitory concentration (MIC) was determined using cation-adjusted Mueller–Hinton broth with 5% lysed horse blood and a Sensititre mastitis plate (CMV1 AMAF; Trek Diagnostics, Cleveland, OH, USA), following the manufacturer's instructions. The Sensititre mastitis plate contained 10 antimicrobials at varying concentrations (µg/mL): ampicillin (0.12–8), cephalothin (2–16), ceftiofur (1–4), penicillin (0.12–8), penicillin–novobiocin combination (1/2–8/16), pirlimycin (0.5–4), sulfadimethoxine (32–256), erythromycin (0.25–4), oxacillin + 2% NaCl (2–4), and tetracycline (1–8). In addition, susceptibility to vancomycin (30 µg) was tested using the disk diffusion method on Mueller–Hinton agar with 5% sheep blood. The susceptibility data were analyzed as previously described [5, 23–25]. *Streptococcus pneumoniae* ATCC 49619 and *S. aureus* ATCC 29213 strains were used for quality control.

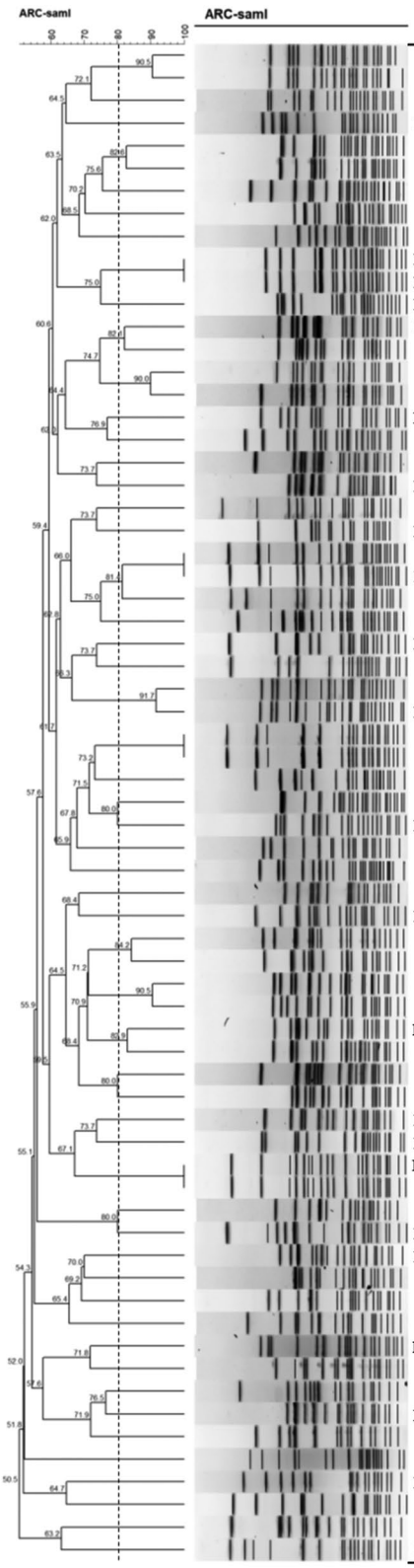
Biofilm Formation Assay

Biofilm formation by *A. viridans* was quantified using a 96-well microtiter plate assay, as previously described [26]. Briefly, the isolates were cultured overnight at 37 °C in tryptic soy broth (TSB) with 0.5% glucose. Then, 20 µL culture was mixed with 180 µL TSB containing 0.5% glucose in flat-bottom 96-well plates and incubated for 48 h at 37 °C. After incubation, the culture was discarded, and the wells were washed thrice with 200 µL phosphate-buffered saline (PBS). Biofilms were fixed with 200 µL methanol for 10 min, dried for 15 min, and stained with 160 µL of 1% crystal violet for 5 min. Excess dye was removed by washing with PBS, and biofilms was dissolved in 200 µL acetone–ethanol (20/80, vol/vol) for 10 min. A 100-µL aliquot was transferred to a new 96-well microplate, and the optical density (OD) at 550 nm was measured to quantify biofilm formation. The isolates were classified as follows: non-adherent [OD ≤ OD negative control

Table 2 Distribution of SCCs in milk samples (n = 41) positive for *A. viridans* as the sole isolate

SSCs in milk ($\times 10^3$ cells/mL)	Number of mastitis milk samples positive for *A. viridans* (%)	Interpretation[a]
200–400	17 (41.5)	Subclinical mastitis
400–1200	16 (39.0)	
1200–5000	5 (12.2)	Clinical mastitis
> 5000	3 (7.3)	

[a]Bekuma and Galmessa[20]



Strain number	Year	Province	Farm	SCC	Resistance pattern	MDR	Biofilm Production	Cluster (80%)
M20-L-BTN4	2020	GG	A	6,769	OXA TET XNL	MDR	W	1
M20-L-BTN7	2020	GG	A	299	OXA TET XNL	MDR	W	1
M20-G-BHG8-2	2020	GG	B	1,255	ERY XNL		W	2
M16-L-BHB22-2	2016	GG	B	448	TET		N	3
M19-G-BGS1	2019	GB	C	536	OXA XNL		W	4
M19-R-BDM29	2019	GG	E	2,644			W	4
M19-W-BUN7	2019	JB	F	492	OXA TET XNL	MDR	W	5
M17-AP-B68J-2	2017	GB	G	7,093	ERY OXA TET XNL	MDR	N	6
M16-N-BDM2-3	2016	GB	D	1,372			N	7
M16-Q-BHG53-1	2016	GG	B	1,672	OXA		N	8
M16-Q-BHG80-2	2016	GG	B	504	OXA PEN XNL		N	8
M16-Q-BHG65-2	2016	GG	B	2,301	OXA XNL		N	9
M16-L-BHB10-1	2016	GG	B	3,886	OXA		N	10
M17-E-BDH	2017	CN	I	287	OXA TET		W	10
M19-W-BUN70	2019	JB	F	2,663	OXA TET XNL	MDR	W	11
M19-W-BUN64	2019	JB	F	223	OXA TET XNL	MDR	W	11
M16-T-BHM14-1	2016	CB	J	645	XNL		N	12
M16-U-BDH21	2016	CN	I	225	OXA		W	13
M16-K-BHJ6-2	2016	GG	B	Not tested	ERY XNL		N	14
M16-Q-BHG26-1	2016	GG	B	316			W	15
M16-V-BG31-2	2016	GB	H	4,702	OXA TET XNL	MDR	W	16
M16-Q-BHG48-2	2016	GG	B	658	ERY		N	17
M21-AO-BCH3	2021	GB	K	8,965	OXA		W	18
M17-C-BHG13-2	2017	GG	B	370			W	18
M16-V-BG30-2	2016	GB	H	667	OXA XNL		W	19
M21-DX-BGR1	2021	GB	L	413	OXA		M	20
M16-Q-BHG32-1	2016	GG	B	754			W	21
M17-C-BHG2	2017	GG	B	398			W	22
M16-K-BHJ11-3	2016	GG	B	Not tested	OXA TET XNL	MDR	W	23
M16-Q-BHG15-1	2016	GG	B	248	OXA TET XNL	MDR	W	23
M21-GH-BNM2	2021	GB	M	336	XNL		W	24
M21-GH-BNM4	2021	GB	M	1,147	XNL		W	24
M21-V-BCS2	2021	GB	N	421	TET		W	25
M21-AT-BHG6	2021	GG	B	1,296	ERY OXA TET XNL	MDR	W	26
M16-Q-BHG19-2	2016	GG	B	414			W	26
M16-Q-BHG9-2	2016	GG	B	392	OXA XNL		W	27
M21-AT-BHG7	2021	GG	B	8,378			W	28
M16-J-BCC6	2016	GG	O	587	TET		N	29
M16-U-BDH16-1	2016	CN	I	1,081	OXA TET		W	30
M21-GH-BNM1	2021	GB	M	333	OXA XNL		W	31
M19-W-BUN49	2019	JB	F	557	OXA TET XNL	MDR	W	31
M19-W-BUN1	2019	JB	F	430	TET		M	32
M19-W-BUN22	2019	JB	F	254	TET		M	32
M18-J-BYM-49-2	2018	JB	F	668	TET		W	33
M19-W-BUN19	2019	JB	F	465	OXA TET XNL	MDR	W	33
M16-N-BDM3-2	2016	GB	D	730			N	34
M19-W-BUN36	2019	JB	F	1,737	OXA TET XNL	MDR	W	34
M16-Q-BHG30-1	2016	GG	B	282	OXA		W	35
M18-D-BDH11-2	2018	CN	I	482			W	36
M17-AP-BSB68H	2017	GB	G	6,126	XNL		N	37
M17-C-BHG8	2017	GG	B	990	XNL		W	37
M20-G-BHG14	2020	GG	B	216			W	38
M16-Q-BHG20-1	2016	GG	B	404	ERY OXA TET XNL	MDR	W	38
M16-Q-BHG31-1	2016	GG	B	380	ERY		W	39
M19-W-BUN69	2019	JB	F	206	TET		W	40
M20-L-BTN9	2020	GG	A	237	OXA TET XNL	MDR	W	41
M20-G-BHG13	2020	GG	B	387	OXA XNL		W	42
M16-N-BDM12-2	2016	GB	D	212			W	43
M20-G-BHG1	2020	GG	B	452			W	44
M16-O-BSJ3-1	2016	GB	P	596			W	45
M16-T-BHM16-1	2016	CB	J	3,058			N	46
M16-T-BHM8-2	2016	CB	J	1,271			W	47
M21-GU-BGS1	2021	GB	Q	535	XNL		W	48
M16-U-BDH24-2	2016	CN	I	380	TET XNL		W	49
M17-C-BHG4	2017	GG	B	2,549	OXA		W	50
M20-AV-BSY2	2020	GG	R	760			W	51
M20-AW-BNM2	2020	GB	M	709	XNL		W	52
M16Q-BHG38-1	2016	GG	B	500			N	Not typable
M16Q-BHG62-1	2016	GG	B	768			W	Not typable

◂**Fig. 2** Dendrogram showing *Sma*I-pulsed-field gel electrophoresis (PFGE) patterns and summary of history, antimicrobial resistance pattern, and biofilm-forming ability of *A. viridans* isolates from bovine mastitis milk. The similarities between isolates were evaluated using the Dice coefficient and UPGMA clustering method. Clusters were based on ≥80% similarity. SCC, somatic cell count ($\times 10^3$ cells/mL); GG, Gyeonggi-do; CB, Chungcheongbuk-do; CN, Chungcheongnam-do; GB, Gyeongsangbuk-do; JB, Jeollabuk-do; JN, Jeollanam-do; OXA, oxacillin +2% NaCl; TET, tetracycline; XNL, ceftiofur; ERY, erythromycin; PEN, penicillin; MDR, multidrug resistance; W, weak; M, moderate; Not Tested, somatic cell counts were not tested owing to the presence of excessive clots in milk samples. Not typable, no band patterns observed after *Sma*I digestion

(NC)], weak (OD NC < OD ≤ 2 × OD NC), moderate (2 × OD NC < OD ≤ 4 × OD NC), and strong (OD > 4 × OD NC) [27].

Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics for Windows, v.20.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including means and standard deviations, were calculated for SCC values. An independent-samples *t*-test was used to compare SCC values between milk samples, in which only *A. viridans* was isolated (*A. viridans*-only) and those of non-mastitis. Pearson's correlation coefficient (r) was calculated to assess the strength and direction of the relationship between *A. viridans*-only isolation and SCC levels. Statistical significance was set at $p < 0.05$.

Results

SCCs in Milk Samples with *A. viridans*-Induced Mastitis

Among the 3422 samples, 1774 were classified as mastitis-positive and 1648 as normal (non-mastitis) samples. A total of 69 isolates of *A. viridans* (3.9%) were obtained in the 1774 mastitis milk samples, of which 41 were identified as single-species and 28 as mixed infections.

SCCs were assessed in milk samples from individual quarters. For comparison, 28 samples with co-infections were excluded to eliminate the potential influence of other pathogens. Instead, 41 samples, in which *A. viridans* was identified as the sole pathogen in samples from 13 farms, were included in the analysis. Among the 1648 non-mastitis samples, 121 were selected from the same farms where *A. viridans*-positive cows were detected in order to minimize potential baseline differences in SCC levels between farms. The difference in SCCs between *A. viridans*-only and non-mastitis samples is summarized in Table 1. The mean SCC of the *A. viridans*-positive samples (1220.85×10^3 cells/mL) was significantly higher than that of the non-mastitis samples (64.68×10^3 cells/mL; $p < 0.001$). Pearson's correlation analysis revealed a significant positive correlation between *A. viridans*-only samples and SCC levels ($r = 0.441$, $p < 0.001$), indicating that *A. viridans*-positive samples had significantly elevated SCCs. This result confirmed the association between the presence of *A. viridans* and elevation of SCCs in bovine mastitis.

The distribution of SCCs in the 41 *A. viridans*-positive milk samples revealed that 80.5% had SCCs ranging between 200 and 1200×10^3 cells/mL, indicating subclinical mastitis, whereas 19.5% had $> 1.2 \times 10^6$ cells/mL, corresponding to clinical mastitis (Table 2). Specifically, the percentages of SCCs ranging between 200–400, 400–1200, and 1200–5000, and that > 5000 ($\times 10^3$ cells/mL) were 41.5, 39.0, 12.2, and 7.3%, respectively.

PFGE Analysis

Molecular fingerprinting of *A. viridans* isolates was performed using PFGE. The PFGE dendrogram describing genetic relationships of the isolates is shown in Fig. 2. Of the 69 isolates, 67 showed interpretable banding patterns following *Sma*I digestion. Based on an 80% similarity threshold, these 67 isolates were classified into 52 distinct PFGE clusters. Among them, 37 consisted of a single isolate, and 15 comprised two isolates. No clusters contained more than two isolates, indicating a high level of genetic diversity among the isolates.

Antimicrobial Susceptibility

Of the 69 isolates, 19 (27.5%) were susceptible to all tested antibiotics. The isolates exhibited the highest resistance to ceftiofur (46.4%), followed by those to oxacillin +2% NaCl (44.9%), tetracycline (36.2%), erythromycin (10.1%), and penicillin (1.4%) (Table 3). In contrast, all isolates were susceptible to ampicillin, cephalothin, and vancomycin. The isolates had 50 and 90% MIC values ≤32 for sulfadimethoxine. The most common resistance profile of the isolates followed the ceftiofur/oxacillin +2% NaCl/tetracycline (17.4%) pattern (Fig. 2). Moreover, 15 (21.7%) isolates were multidrug-resistant (MDR). Twelve MDR isolates were resistant to three antibiotic classes, and three were resistant to four antibiotic classes.

Table 3 Distribution of the minimum inhibitory concentrations (MICs) of *A. viridans* (n = 69) isolated from bovine mastitis milk

Antimicrobial class	Antimicrobials	Distribution (%) of isolates (μL/mL)[a]													MIC_{50}[b]	MIC_{90}[c]	Resistance
		≤0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	> 256	(μL/mL)	(μL/mL)	rate (%)[d]
Cephems	Cephalothin					95.7		2.9	1.4						≤ 2	≤ 2	0
	Ceftiofur			11.6	17.4	4.3	20.3	46.4							4	> 4	46.4
Sulphonamides	Sulphadimethoxine									95.7	2.9			1.4	≤ 32	≤ 32	–
Lincosamide	Pirlimycin			97.1	2.9										≤ 0.5	≤ 0.5	0
Macrolides	Erythromycin		89.9					10.1							≤ 0.25	> 4	10.1
Penicillins	Ampicillin	98.6			1.4										≤ 0.12	≤ 0.12	0
	Penicillin	29.0	59.4	7.2		2.9	1.4								0.25	0.5	1.4
	Oxacillin + 2% NaCl					15.9	39.1	44.9							4	> 4	44.9
Penicillins/Aminocoumarin	Penicillin/Novobiocin				100										≤ 1/2	≤ 1/2	0
Tetracyclines	Tetracycline				63.8			5.8	30.4						≤ 1	> 8	36.2
Glycopeptide	Vancomycin[e]														–	–	0

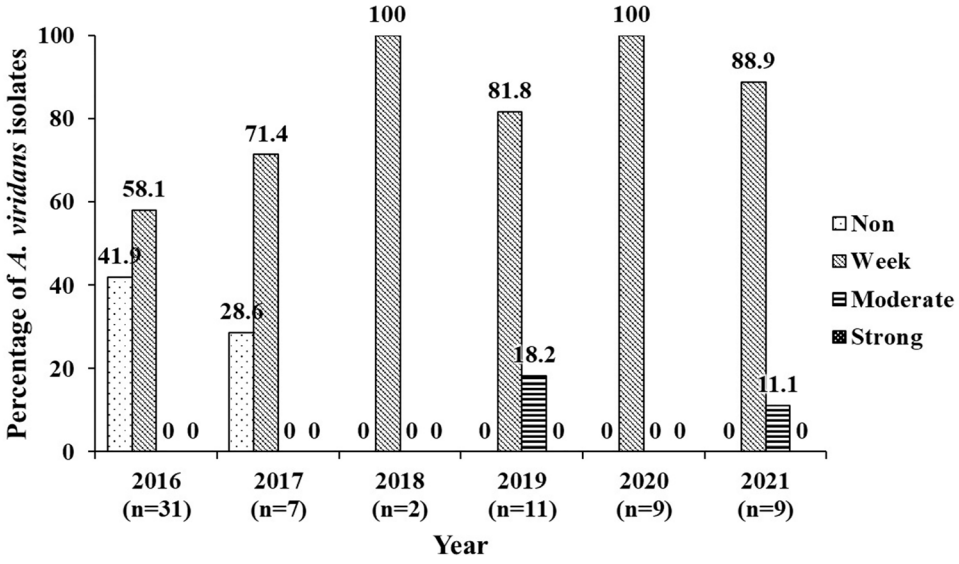


Fig. 3 Biofilm-forming capacity of *A. viridans* isolated from bovine mastitis milk

Biofilm Formation

Of the 69 total isolates, 54 (78.3%) were capable of forming biofilms, whereas 15 (21.7%) did not form biofilms (Fig. 2). The percentages of weak and moderate biofilm producers were 73.9 and 4.3%, respectively, and no strong biofilm producers were identified. Among those isolated in 2016 and 2017, 41.9 and 28.6% isolates, respectively, were unable to form biofilms. However, all isolates obtained between 2018 and 2021 demonstrated weak to moderate biofilm-forming ability (Fig. 3).

Discussion

Aerococcus viridans is emerging as an important but poorly understood cause of bovine mastitis in South Korea. In this study, we investigated the correlation between *A. viridans*-derived mastitis and SCCs in mastitis milk samples while examining characteristics such as antimicrobial resistance, biofilm-forming ability, and genetic diversity.

Changes in SCCs arise owing to the recruitment of white blood cells into milk, primarily in response to inflammation triggered by bacterial infiltration of the mammary glands, which makes SCCs a well-established indicator of mastitis [20, 28]. The findings of this study suggested that *A. viridans* was associated with elevated SCCs in bovine milk. The positive correlation between the presence of *A. viridans* and SCC levels indicated that this bacterium might have induced an inflammatory response in the mammary gland. Notably, 80.5% of the *A. viridans*-positive samples fell within the range indicative of subclinical mastitis, whereas 19.5% exhibited SCCs characteristic of clinical mastitis. These findings suggest that *A. viridans* may be primarily associated with subclinical mastitis, potentially contributing to persistent infections rather than acute clinical cases. These results are consistent with those of previous reports indicating that *A. viridans* influences SCC levels and serves as an etiological agent of subclinical mastitis [7, 9].

PFGE is widely regarded as the gold standard for bacterial typing, owing to its ability to generate unique DNA fingerprints for isolates, thereby making it a critical tool for tracing transmission pathways for infection control [18]. This study demonstrated substantial genetic diversity among *A. viridans* isolates, as all 52 clusters formed at 80% similarity comprised either one or two isolates. These findings align with those of previous reports showing high genetic diversity among *A. viridans* isolates from mastitis cases in Japan and China [5, 6]. This diversity indicates that *A. viridans*, a ubiquitous environmental bacterium, may cause mastitis through diverse pathways, including processed manure and bedding materials as potential reservoirs [6, 29]. Collectively, these results indicate that *A. viridans* isolates from bovine mastitis milk exhibit considerable genetic diversity, with no evidence of dominant epidemic clones. Future studies should explore the genomic characteristics of *A. viridans* and its potential transmission between animals and the environment for further elucidating its role in mastitis epidemiology.

Antimicrobial therapy remains the cornerstone for treating bovine mastitis, and empirical treatments are commonly employed [2, 3]. Therefore, the updated data on antibiotic susceptibility are vital for informed veterinary decision making [2]. Animal-derived *A. viridans* is generally susceptible to β-lactams and vancomycin [7, 8, 30]. The susceptibility rates of *A. viridans* to penicillin and ampicillin have been reported as 91% in dog oral swab isolates, and 61 and 83%, respectively, in cat isolates from Algeria [31].

However, swine fetal isolates in South Korea have shown relatively low susceptibility rates, 23.1% for penicillin and 53.8% for ampicillin [11]. Our study demonstrated that *A. viridans* isolates from bovine mastitis showed high susceptibility to β-lactams such as penicillin and ampicillin. However, notable resistance to ceftiofur and oxacillin + 2% NaCl was also observed. These findings are further supported by those of previous reports documenting methicillin-resistant *A. viridans* isolates from bovine mastitis cases [30]. Taken together, these results suggest that antimicrobial resistance in *A. viridans* may vary depending upon the host species and geographical region, potentially influenced by factors such as antimicrobial usage practices, herd management, and environmental conditions [3, 5]. These findings highlight the need for ongoing surveillance for informed treatment of mastitis and for tracking the resistance trends.

Biofilm formation is a critical virulence factor in mastitis, which enables the persistence of pathogens within the mammary gland, evasion of the immune responses, and resistance to antimicrobial treatment, thereby promoting chronic infections [1, 32]. In this study, most *A. viridans* isolates were able of forming biofilms, with 100% of the recent isolates demonstrating this characteristic. Although comparative data from non-mastitis milk samples were not available in our study, the high prevalence of biofilm-forming *A. viridans* isolates suggests their potential for persistence within the mammary gland and contribution to persistent intramammary infection. While studies specifically addressing biofilm production by *A. viridans* are limited, highly virulent strains isolated from subclinical mastitis milk have been reported to adhere to and invade bovine mammary epithelial cells [33]. Continued surveillance and further studies are necessary for elucidating the pathogenic significance and underlying mechanisms of biofilm formation by *A. viridans*.

Conclusion

A. viridans is associated with increased SCC levels in bovine mastitis, predominantly in subclinical cases. The significant correlation between *A. viridans* infection and SCC further supports its role in mastitis pathogenesis. Additionally, high genetic diversity, resistance to certain β-lactam antibiotics, and biofilm-forming ability suggest that *A. viridans* may persist in dairy farm environments, complicating the efforts of controlling mastitis. Therefore, continuous monitoring of its prevalence, antimicrobial resistance patterns, and virulence factors is essential for developing effective prevention and treatment strategies.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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