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Original article

First report of *Lactococcus garvieae* from bovine mastitis in Türkiye characterizing some adhesion-related virulence genes and antimicrobial resistance profiles

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Abstract

This research explored the distribution of adhesion-associated virulence determinants and analyzed the antimicrobial susceptibility characteristics of *Lactococcus garvieae* isolates recovered from bovine mastitis milk samples. Samples were obtained from dairy cows diagnosed with mastitis between 2020 and 2025 and were subsequently sent to the Microbiology Laboratory at the Bornova Veterinary Control Institute (Türkiye) for bacteriological analysis. Polymerase chain reaction (PCR) was performed to detect the presence of adhesion-associated virulence genes, including p*avA, psaA, LP1-P4, AC1, AC2* and *AF* in the *L. garvieae* strains. Antibiotic susceptibility of the isolates was examined using the disk diffusion method, and multidrug-resistant strains that were resistant to at least three antimicrobial classes were identified. Molecular analyses revealed that *pavA, psaA*, *LP3, AC1* and *AC2* genes were present in all isolates (100%). The *AF, LP1*, and *LP4* genes were each detected in two isolates (20%), while *LP2* was identified in only one isolate (10%). According to antibiotic susceptibility results, all isolates were susceptible to florfenicol and ampicillin (100%). The highest resistance rate was observed against ciprofloxacin (90%), followed by oxytetracycline and sulfamethoxazole/trimethoprim (80%), penicillin and enrofloxacin (70%), spectinomycin (60%), and erythromycin (40%). Multidrug resistance was detected in nine of the ten isolates (90%), whereas one isolate was resistant only to β-lactams and fluoroquinolones. This study provides the first data on the virulence characteristics of *L. garvieae* strains isolated from bovine mastitis milk samples in Türkiye. The findings demonstrate that these isolates harbor adhesion-related virulence genes and exhibit a high prevalence of multidrug resistance. These results highlight the pathogenic potential of *L. garvieae* in bovine mastitis; however, further studies are needed to better elucidate its pathogenic mechanisms, genetic diversity, and resistance traits.

Keywords: adhesion-related genes, antimicrobial resistance, bovine mastitis, *Lactococcus garvieae*, virulence factors

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Introduction

Mastitis represents one of the most frequent and economically burdensome diseases in dairy farming worldwide. Beyond its impact on milk yield, the disorder also increases treatment expenditures, leads to milk disposal, and causes discomfort that compromises animal welfare, as reflected in altered behavior (Lin et al. 2023). The condition is multifactorial in origin, with bacteria being the predominant cause. These pathogens are generally grouped into two categories: major and minor. *Staphylococcus aureus, Escherichia coli, Klebsiella spp., Streptococcus agalactiae, S. dysgalactiae, S. uberis* and *Enterococcus* spp. are recognized as the primary causative organisms of mastitis, while non-aureus staphylococci, *Corynebacterium* spp., and *Lactococcus* spp. are typically considered secondary contributors (Xie et al. 2023). Research attention has historically concentrated on major pathogens, whereas minor organisms have received limited focus.

Lactococcus garvieae is a significant pathogen that causes septicemic-hemorrhagic infections (lactococcosis) in both freshwater and marine fish, resulting in substantial economic losses in aquaculture. It was first isolated from rainbow trout in Japan in the late 1950s, and later infections caused by this pathogen were also reported in freshwater shrimp, octopus, and various fish species, as well as dolphins (*Tursiops truncatus*) (Salogni et al. 2024, Wu et al. 2024). Beyond aquatic organisms, the increasingly broad host spectrum, including humans (Francés-Cuesta et al. 2022), buffalo (Carvalho et al. 1997), cattle (Rodrigues et al. 2016), and pigs (Tejedor et al. 2011) suggests that *L. garvieae* may represent a potential zoonotic pathogen (López-Campos et al. 2015, Wu et al. 2024). The association of lactococci with bovine mastitis was reported long ago, as *Streptococcus garvieae*, originally described from intramammary infections, was subsequently reclassified as *L. garvieae* (Devriese et al. 1999). Their close phenotypic similarity to streptococci has led to frequent misidentification over the years. *Lactococcus* isolates have often been misidentified as *Streptococcus uberis* or other streptococci, which has prevented a full assessment of the true contribution of these bacteria to mastitis cases (Lin et al. 2023, Krukowski et al. 2025). With the application of molecular techniques, the pathogenic importance of *Lactococcus* species in mastitis has become more evident, with *L. garvieae* emerging as a significant pathogen. This Gram-positive, facultatively anaerobic, catalase-negative coccus was first reported as a causative agent of bovine mastitis in the early 1980s (Rodrigues et al. 2016). Although it is primarily recognized as an agent of infections in fish populations, *L. garvieae* has also been linked to severe human diseases, including endocarditis, peritonitis, bacteremia, and sepsis. The bacterium has also been isolated from a wide range of hosts, including ruminants, companion animals, and reptiles, as well as from environmental reservoirs and food products such as raw milk and cheese (Rodrigues et al. 2016, Krukowski et al. 2025). Veterinary reports have identified *L. garvieae* in both clinical and subclinical cases of bovine and buffalo mastitis. Its presence has been confirmed in several countries, including Belgium, Spain, Poland, and China (Rodrigues et al. 2016, Lin et al. 2023, Krukowski et al. 2025). These findings indicate that *L. garvieae* is more widespread and important than previously recognized.

The mechanisms of pathogenicity in *L. garvieae* have not yet been fully clarified, but several virulence traits have been described. These include hemolysins, adhesins, and capsular polysaccharides that support bacterial survival and evasion of host immune responses. Furthermore, the emergence of antimicrobial resistance poses significant concerns for both veterinary treatment strategies and public health (Xie et al. 2023, Krukowski et al. 2025). Interestingly, not all *Lactococcus* members are pathogenic: *Lactococcus lactis* is widely applied as a starter culture in the dairy industry and as a probiotic with beneficial functions in animals and humans (Lin et al. 2023). This duality highlights the paradoxical role of the genus, encompassing both beneficial and potentially harmful species.

In the context of mastitis pathogenesis, bacterial adhesion to the mammary epithelium is regarded as a crucial initial step. For this reason, the present study focused on several adhesion-related virulence genes. Among the genes examined, *pav*A was first identified in *Streptococcus pneumoniae* as encoding an adhesin protein that mediates binding to host cells (Holmes et al. 2001), and while its role has been demonstrated in *S. pneumoniae*, comparative genomic analyses suggest that it may also contribute to adhesion in *L. garvieae* isolates (Miyauchi et al. 2012). Similarly, psaA encodes a surface-expressed manganese-binding protein that contributes to both adhesion and resistance against host immune defenses, thereby playing a crucial role in pathogenesis (Holmes et al. 2001). LP1-LP4 represent LPxTG motif-containing surface proteins, which are covalently anchored to the peptidoglycan layer of Gram-positive bacteria and facilitate attachment to epithelial surfaces (Miyauchi et al. 2012). Similarly, AC1 and AC2 encode adhesin proteins that promote attachment to epithelial cells. Finally, although supported by more limited data, the AF gene has also been described as an additional adhesion factor that may contribute to host-pathogen interactions (Xie et al. 2023).

Taken together, these findings suggest that adhesion-associated genes may play a central role in the pathogenesis of *L. garvieae* in bovine mastitis. In conclusion, *L. garvieae*, once considered a minor mastitis agent, is now acknowledged as an emerging pathogen due to its broad host spectrum, zoonotic potential, and growing detection in dairy herds. However, knowledge gaps remain regarding its epidemiology, resistance patterns, and virulence factors. In this context, the present study aimed to characterize *L. garvieae* strains isolated from bovine mastitis in Türkiye, with emphasis on adhesion-related virulence genes and antimicrobial resistance profiles. To our knowledge, this is the first report on the determination of some virulence properties of *L. garvieae* isolated from bovine mastitis cases in Türkiye.

Materials and Methods

Sampling

This research analyzed 308 bovine mastitis milk samples collected from the Aegean Region, submitted for bacteriological examination to the Microbiology Laboratory at the Bornova Veterinary Control Institute in Izmir, Türkiye, during the period from 2020 to 2025. The sample size was calculated using Thrusfield's (2018) prevalence study formula, assuming an expected prevalence of 3.3%, a 95% confidence level, and a 2% margin of error. The study was approved by the Local Ethics Committee of İzmir/Bornova Veterinary Control Institute (Approval No: E-71705440-550-19125472; 05.05.2025), and the approval document has been submitted separately.

Bacterial isolation and identification using VITEK 2 identification system

For this purpose, milk samples delivered to the laboratory under cold chain conditions were inoculated onto Columbia Agar (5% sheep blood, Liofilchem) and incubated at 37°C for 24-48 hours. Following incubation, isolates showing Gram-positive, catalase-negative, and oxidase-negative coccus morphology were identified using the Vitek 2 GP ID card (bioMérieux, France) (Funke et al. 1998). Furthermore, sucrose fermentation testing (Liofilchem) was conducted additionally. Isolates that did not ferment sucrose were presumptively identified as *L. garvieae* (Satıcıoğlu et al. 2023).

DNA extraction and molecular confirmation of the strains

Genomic DNA isolation from the isolates was performed using a commercial silica-membrane purification kit (High Pure PCR Template Preparation Kit, Roche Diagnostics GmbH, Mannheim, Germany). Species confirmation was achieved by amplifying the 16S rRNA gene region with *Lactococcus garvieae* specific primers pLG-1 (5′-CATAACAATGAGAATCGC-3′) and pLG-2 (5′-GCACCCTCGCGGGTTG-3′). Following optimization, PCR reactions were prepared to a total volume of 25 µL, consisting of 12.5 µL of Xpert Fast Hotstart Mastermix (2×, GRiSP, Portugal), 2 µL each of forward and reverse primers, 5 µL of extracted DNA, and 3.5 µL nuclease-free water. Thermal cycling included an initial denaturation at 94°C for 3 min; 40 cycles of 94°C for 15 s, 55°C for 30 s, and 72°C for 15 s; and a final extension at 72°C for 3 min, conducted in a Techne TC-412 thermocycler (Keison Products, United Kingdom). PCR products were separated by electrophoresis on 1% agarose gel and visualized, and amplicons of ~1100 bp were considered indicative of *L. garvieae*. The reference strain *L. garvieae* ATCC 43921 served as the positive control (Zlotkin et al. 1998, Feito et al. 2022).

Determination of virulence factors of *L. garvieae* isolates

For isolates confirmed as *L. garvieae* nine adhesion-associated virulence genes were screened by PCR. Each reaction was adjusted to a final volume of 25 µL, consisting of 12.5 µL Xpert Fast Hotstart Mastermix (2×, GRiSP, Portugal), 2 µL of each primer (forward and reverse), 5 µL of DNA template, and 3.5 µL nuclease-free water. The amplification protocol included an initial denaturation step at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 15 s, annealing at the temperatures shown in Table 1 for 30 s, and extension at 72°C for 15 s, with a final elongation phase of 72°C for 3 min. Reactions were conducted in a Techne TC-412 thermocycler (Keison Products, UK). PCR products were separated on 2% agarose gels and subsequently visualized. Primer sequences and expected product sizes for the investigated targets are listed in Table 1 (Türe and Altınok 2016).

Antimicrobial susceptibility analyses of *L. garvieae* isolates

Antibiotic susceptibility testing was conducted on bacteria identified as *Lactococcus garvieae* utilizing the Kirby-Bauer disk diffusion method. The tests were performed on Mueller–Hinton agar supplemented with 5%

Table 1. Primer sequences and amplicon sizes of virulence genes.

Target Gene	Gene Abbreviation	Primer Name	Primer Sequence (5′ to 3′)	Amplicon Size (bp)	Annealing Temp. (°C)
Adhesin Pav	*pav*	AP F	CCTGTCGGGCGCTTTTATTG	232	56
		AP R	TCCCGGAAGAAGAGTACGGT		
Adhesin PsaA	*PsaA*	APSA F	GTTGCAACAGCTGGACACAG	180	54
		APSA R	ATACGGTTGAGTTGGGCTGG		
LPxTG-1	*LP1*	LP1-F	GTGAACGTGGAGCTTCCAGA	878	54
		LP1-R	CCACTCACATGGGGGAGTTC		
LPxTG-2	*LP2*	LP2 F	GCCAGTGAGAGAACCGTTGA	767	54
		LP2 R	CAGGTTCAAGTGCAACTGCC		
LPxTG-3	*LP3*	LP3 F	TTAAGCACAACGGCAACAGC	231	54
		LP3 R	CACGCGAAATGATGGTGCAT		
LPxTG-4	*LP4*	LP4-F	GGGAGCACCGGATTCACTTT	928	52
		LP4-R	ACAAAGCCGCAGACCTTACA		
Adhesin cluster 1	*AC1*	AC1 F	TTGGGCACATCAGACTGGAC	264	54
		AC1 R	AGCATCATCAGCTGCCAAGT		
Adhesin cluster 2	*AC2*	AC2 F	CTGCGAGTGGCATCTCCATT	160	52
		AC2 R	TCAACACTGCGACCTTCTGT		
Adhesin	*AF*	AF F	CAGCCAGCACCAGGTTATGA	358	54
		AF R	CTCCTGCGTTGACATGGACT		

bp – Base pair, Temp – Temperature

Table 2. Antibiotic susceptibility treshold levels of each type of antibiotic.

Antibiotic	Disc content	S	R
FFC	30 µg	≥29	≤22
OT	30 µg	≥19	≤14
PG	10 U	≥15	≤14
ENR	5 µg	≥23	≤16
SH	100 µg	≥14	≤10
SXT	25 µg	≥16	≤10
CIP	5 µg	≥26	≤21
AML	10 µg	≥17	≤13
E	15 µg	≥23	≤13

S – Susceptible, R – Resistant, FFC – Florphenicol, OT – Oxytetracycline, PG – Penicillin, ENR – Enrofloxacin, SH – Spectinomycin, SXT – Sulfamethoxazole/Trimethoprim, CIP – Ciprofloxacin, AML – Ampicillin, E – Erythromycin

sheep blood, as recommended for this species. Bacterial inocula were adjusted to a turbidity of 0.5 according to the MacFarland standard and spread onto the agar surface in 100 µl volumes. Plates were incubated at 37°C for 24 hours. After incubation, the diameters of the inhibition zones were measured in millimeters (Miller and Harbottle 2018, Sezgin et al. 2023, Salogni et al. 2024). Zone diameter interpretations were based on predefined breakpoints in Table 2, and results were categorized as susceptible (S), intermediate (I), or resistant (R).

Results

Isolation and identification of bacterial strains using VITEK 2 identification system

A total of 308 milk samples were examined, and catalase- and oxidase-negative Gram-positive cocci were isolated from 10 samples. All isolates were identified using the VITEK 2 identification system, and sucrose fermentation testing was additionally performed. All isolates examined in this study were found to be negative for sucrose fermentation. This phenotypic

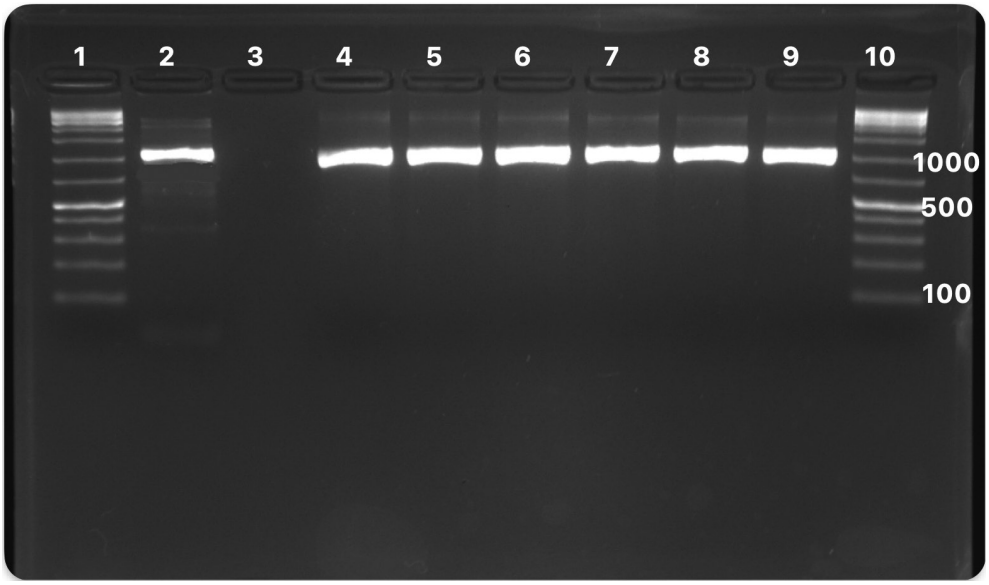

Fig. 1. Agarose gel electrophoresis image of the 1100 bp PCR amplicons obtained from *L. garvieae* isolates. In the image, M denotes the molecular size marker (GRS Universal Ladder, GRiSP), P the positive control (*L. garvieae* ATCC 43921), N the negative control (DNase/RNase-free water), and lanes 1-10 the sample isolates.

characteristic is considered an important criterion for distinguishing *L. garvieae* from *L. petauri*, as the former does not ferment sucrose, whereas the latter does, as noted in the literature. This finding indicated that the isolates obtained in the present study were consistent with *L. garvieae* (Satıcıoğlu et al. 2023). Among the ten milk samples collected from cows diagnosed with mastitis, *L. garvieae* was identified. The bacterium was isolated as the sole pathogen in eight samples, while co-isolation with *Escherichia coli* and *Staphylococcus* spp. was observed in two samples.

Molecular confirmation of *L. garvieae* strains

Based on the findings obtained from the VITEK 2 identification system and the sucrose fermentation results of the strains, all ten strains identified as *L. garvieae* tested positive for the species-specific gene region of *L. garvieae*. Consequently, these strains were conclusively confirmed as *L. garvieae* through PCR analysis (Fig 1).

Determination of virulence factors of *L. garvieae* isolates

In this study, the presence of the virulence genes *pav*, *psaA*, *LP1*, *LP2*, *LP3*, *LP4*, *AC1*, *AC2*, and *AF* was evaluated in 10 different *L. garvieae* isolates. Following PCR analyses, all isolates (100%) were found to carry the pavA, psaA, LP3, AC1, and AC2 genes. The LP1 and LP4 genes were detected in two isolates (20%), while LP2 was identified in only one isolate (10%). The AF gene was found to be positive in two isolates (20%). The results indicated that *L. garvieae* strains exhibited genetic diversity in terms of adhesion-related virulence factors (Table 3).

Antimicrobial susceptibility profiles of *L. garvieae* isolates

Based on the disk diffusion results, all *L. garvieae* isolates demonstrated susceptibility to florfenicol and ampicillin (10/10; 100%). The susceptibility rates for the other antimicrobial agents were as follows: erythromycin 6/10 (60%), spectinomycin 4/10 (40%), penicillin 3/10 (30%), enrofloxacin 3/10 (30%), oxytetracycline 2/10 (20%), sulfamethoxazole/trimethoprim 2/10 (20%), and ciprofloxacin 1/10 (10%). Resistance was most prevalent to ciprofloxacin (9/10; 90%), followed by oxytetracycline and sulfamethoxazole/trimethoprim (each 8/10; 80%), penicillin and enrofloxacin (each 7/10; 70%), spectinomycin (6/10; 60%), and erythromycin (4/10; 40%). No resistance was observed to florfenicol or ampicillin. Multidrug resistance (MDR), characterized by resistance to three or more antimicrobial classes, was identified in 9 out of 10 isolates (90%), involving between three and six classes; only isolate 9 was not MDR, exhibiting resistance to two classes (β-lactams and fluoroquinolones). The numbers of susceptible and resistant strains, as well as information on multidrug resistance (MDR), are presented in Table 4.

Discussion

Lactococcus garvieae, considered a minor environmental pathogen for bovine mastitis, has limited research data on bovine mastitis to date (Lin et al. 2023,

Table 3. Distribution and percentages of virulence genes in *L. garvieae* strains.

Strain No	pav	PsaA	LP1	LP2	LP3	LP4	AC1	AC2	AF
1	+	+	-	+	+	-	+	+	+
2	+	+	-	-	+	+	+	+	+
3	+	+	-	-	+	-	+	+	-
4	+	+	-	-	+	-	+	+	-
5	+	+	+	-	+	-	+	+	-
6	+	+	-	-	+	-	+	+	-
7	+	+	-	-	+	+	+	+	-
8	+	+	-	-	+	-	+	+	-
9	+	+	+	-	+	-	+	+	-
10	+	+	-	-	+	-	+	+	-
%	100	100	20	10	100	20	100	100	20

Table 4. Antibiotic susceptibility patterns and multidrug resistance profiles of *L. garvieae* isolates.

Antibiotic	S	R	MDR isolates n (%)
FFC	10	0	-
OT	2	8	-
PG	3	7	-
ENR	3	7	-
SH	4	6	-
SXT	2	8	-
CIP	1	9	-
AML	10	0	-
E	6	4	-
Overall MDR	-	-	9 (90)

S – Susceptible, R – Resistant, FFC – Florphenicol, OT – Oxytetracycline, PG – Penicillin, ENR – Enrofloxacin, SH – Spectinomycin, SXT – Sulfamethoxazole/Trimethoprim, CIP – Ciprofloxacin, AML – Ampicillin, E – Erythromycin

Wu et al. 2024). In a study conducted in China by Xie et al. (2023), a total of 49 *L. garvieae* isolates were identified from 1,441 clinical mastitis samples (3.40%). Similarly, in another study conducted in China by Lin et al. (2023), 39 *L. garvieae* isolates (1.35%) were obtained from 2,899 milk samples of clinical mastitis cases. In a study conducted in Brazil by de Oliveira et al. (2022) using subclinical mastitis samples, a total of 72 bacterial isolates were obtained from 321 milk samples. Five of these isolates were identified as *L. garvieae*. In another recent study from Poland, which reported the first identification of *L. garvieae* as an etiological agent of bovine mastitis in the country, a total of 118 milk samples from clinical and subclinical mastitis cases were examined, and three isolates obtained from subclinical mastitis samples were identified as *L. garvieae* (overall prevalence 2.54%) (Krukowski et al. 2025). In Türkiye, both early and recent studies have also reported the isolation of *L. garvieae* from subclinical mastitis cases in cows. Hadimli et al. (2013) detected *L. garvieae* at a rate of 0.96% among 104 catalase-negative Gram-positive cocci and demonstrated that this species is a rare but identifiable mastitis agent in Türkiye. In a recent study conducted in Türkiye by Ötkün et al. (2024), thirteen *Lactococcus* strains were identified from 108 mastitis milk samples. These included twelve samples from subclinical and one from clinical mastitis milk. Eight (7.4%) of these isolates were identified as *L. garvieae.* In the present study, *L. garvieae* was detected in 10 of 308 milk samples (3.25%). No distinction was made between clinical and subclinical mastitis samples. Scillieri Smith et al. (2020) examined 8,868 mastitis samples obtained from dairy farms in New York and identified *Lactococcus* species among 473 isolates alongside other bacteria. The species distribution was determined as *L. lactis* (24%) and *L. garvieae* (3%). It was reported that *Lactococcus* isolates showed lower somatic cell count normalization and bacteriological cure rates compared to *Streptococcus dysgalactiae* and

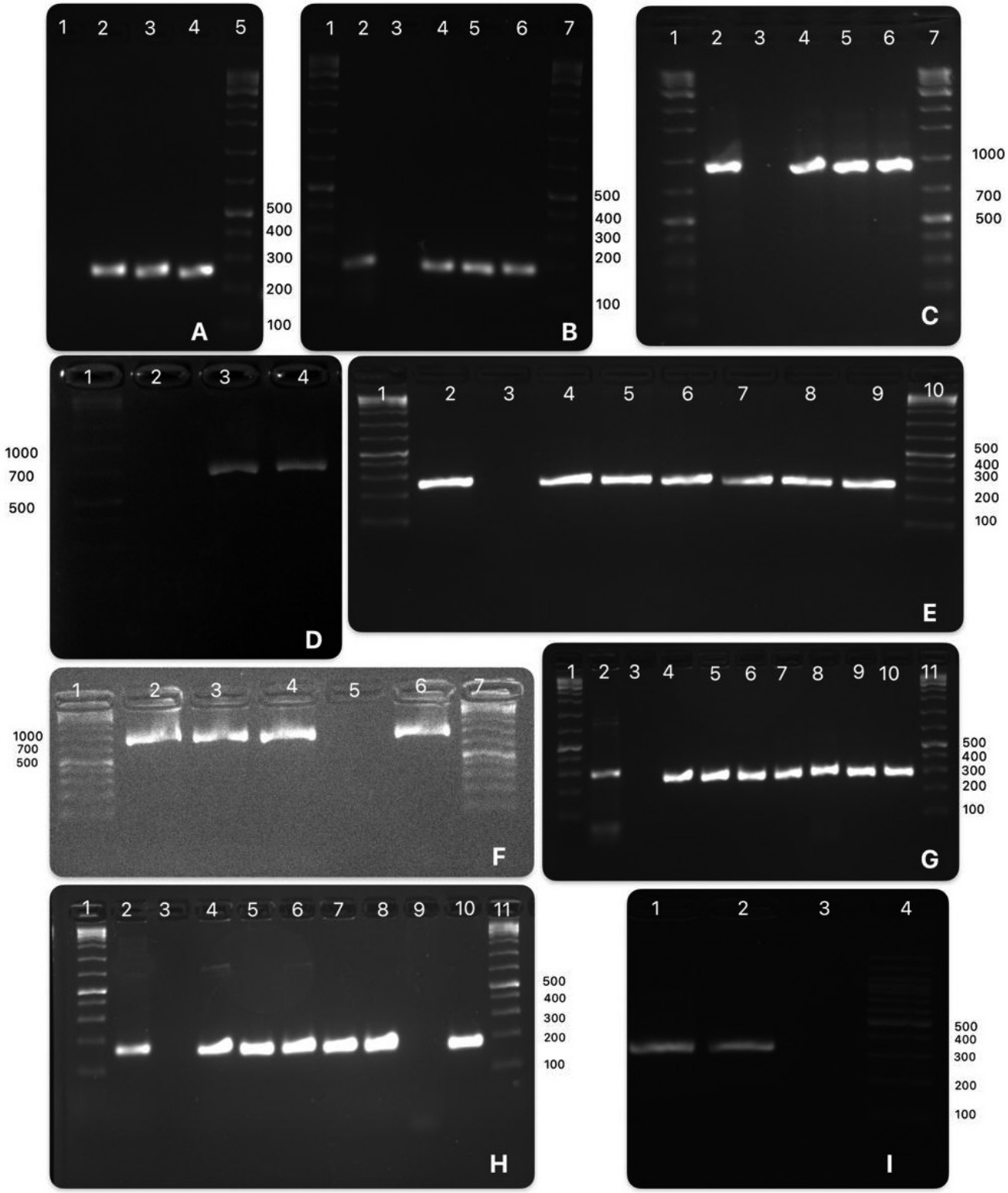

Fig. 2. Agarose gel electrophoresis images of PCR amplicons for the *pav, PsaA, LP1, LP2, LP3, LP4, AC1, AC2,* and *AF* genes, shown sequentially. In each image, M denotes the molecular size marker (GRS Universal Ladder, GRiSP), P the positive control, N the negative control, and lanes 1-10 the sample isolates.

S. uberis. Rowe et al. (2021) reported that cows infected with various Gram-positive bacteria, including *Lactococcus*, before the dry period had an increased risk of developing clinical and subclinical mastitis in the subsequent lactation and exhibited reduced milk yield. These findings support that *L. garvieae* may be a causative agent of mastitis in dairy cattle. When these data are evaluated together, it is observed that *L. garvieae* can be isolated from both clinical and subclinical mastitis cases, but the isolation rates tend to be rela-

tively higher in subclinical cases. This suggests that *L. garvieae* has been increasingly identified in different regions of the world, and that prevalence rates may vary depending on mastitis status and geographical differences.

In the current study, *L. garvieae* was isolated alone in eight mastitis milk samples, together with *Esherichiae coli* in one sample, and *Staphylococcus* spp. in one sample. This suggests that *L. garvieae* may play a role as both a primary and secondary pathogen in bovine mastitis. Similar findings were reported by Rodrigues et al. (2016) in Portugal, who demonstrated that *L. garvieae* strains isolated from cows with clinical mastitis were genetically highly homogeneous and exhibited clonal spread within the herd. Tejedor et al. (2011) characterized *Lactococcus garvieae* isolates obtained from trout, cattle, and pigs using PFGE and found low genetic relatedness among strains isolated from different host species. Eraclio et al. (2018), in a study conducted on two dairy farms in Minnesota, isolated *L. garvieae* from sand bedding material and from milk samples of cows with mastitis. Based on genetic typing analyses, the sand- and milk-derived isolates were shown to share a common clonal origin but to belong to a genetically distinct cluster compared with previously reported reference strains isolated from other sources. The isolates identified in this study represent a novel, farm-specific genetic variant of *L. garvieae*, distinct from strains previously reported from fish or other sources. More recently, Lin et al. (2023) analyzed *L. garvieae* isolates from clinical mastitis cases across different regions of China at the genomic level, revealing significant genetic diversity, virulence genes, and antibiotic resistance profiles. The researchers reported that these characteristics contribute to the persistence of the bacterium in diverse ecosystems by increasing its environmental adaptation and host diversity. In this context, the present findings indicate that *L. garvieae* may be a potential mastitis agent not only in fish but also in dairy cattle. The strain's isolation, both alone and in association with other bacteria, suggests a variable pathogenicity pattern depending on environmental conditions and host factors (Rodrigues et al. 2016, Lin et al. 2023).

The adhesion of microorganisms to host cells is the first step in the colonization of host surfaces (Döpfer et al. 2000). Bacterial adhesion is regarded as a crucial virulence factor that promotes bacterial colonization and tissue invasion, playing a significant role in pathogenesis. Bacteria perform this function through various cell surface proteins. These proteins are adhesion-related proteins and are basically classified into four categories: lipoproteins covalently bound to membrane lipids, cytoplasmic membrane proteins, proteins containing a carboxyl-terminal LPxTG-like motif, and proteins with specific domains that recognize certain cell wall components (Teker et al. 2020). Mastitis pathogenesis recognizes the attachment of microorganisms to mammary epithelial cells as a critical first step in enabling persistent colonization within glandular tissue. It has been reported that the binding of various pathogenic bacteria to epithelial surfaces through specific adhesins or surface structures plays an important role in the initiation of intramammary infection (Döpfer et al. 2000, Hensen et al. 2000, Kerro Dego et al. 2002, Keane 2019). Similarly, the presence of adhesion factors in *L. garvieae* is considered an important virulence mechanism suggesting that this species may act as an agent in mastitis cases (Lin et al. 2023, Xie et al. 2023). Surface proteins such as adhCI-II, adhPavA, adhPsaA, and LPxTG (I–III) synthesized by this bacterium facilitate bacterial attachment to host tissues, promoting colonization and initiating the infection process (Balta et al. 2025).

Comparative genomic analyses have been a critical tool in elucidating genetic markers associated with virulence potential in *L. garvieae.* López-Campos et al. (2015) compiled existing genomic and omics studies, emphasizing the importance of these approaches in explaining the genomic diversity, environmental adaptability, and presence in diverse hosts of *L. garvieae*. Studies using different molecular approaches to examine the genetic diversity in *L. garvieae* have reported that the population of this species can be separated into independent genomic lineages, but this separation is not directly related to ecological origin or host (Eraclio et al. 2018). Morita et al. (2011) investigated adhesins, *pavA*, *psaA*, and the *LPxTG* motif surface proteins in fish isolates through experimental and comparative analyses, demonstrating that these genes are potential virulence determinants. Miyauchi et al. (2012) identified candidate virulence genes associated with adhesion, capsule formation, hemolysins, and the oxidative stress response by comparative genome analysis of fish, human, and food-borne *L. garvieae* isolates. Subsequently, Türe and Altınok (2016) investigated these genes using PCR-based methods in field strains isolated from trout in Türkiye, suggesting that different combinations of genes may be responsible for *L. garvieae* virulence.

Although studies on the phenotypic and genetic diversity of *L. garvieae* are available (Vela et al. 2000, Tejedor et al. 2011), research on the virulence characteristics of the agent has largely focused on fish isolates; only a limited number of evaluations have been conducted on strains obtained from humans and other hosts. However, data on the distribution of virulence genes in strains isolated from bovine mastitis remain quite

limited (Lin et al. 2023, Xie et al. 2023). In the present study, the presence of nine adhesion-related genes (*adhPav, adhPsaA, LPxTG-1, LPxTG-2, LPxTG-3, LPxTG-4, AC1, AC2*, and *AF*) was investigated in 10 mastitis isolates. All strains were found to carry adhPav, adhPsaA, *LPxTG-3*, *AC1,* and *AC2* genes, whereas the remaining gene regions were detected in only one or two isolates. *LPxTG*-motif surface proteins encode adhesins that covalently bind to the peptidoglycan layer in many Gram-positive bacteria, including *L. garvieae* (Morita et al. 2011, Miyauchi et al. 2012). The distribution of these genes has been reported to vary among host sources. Specifically, the *LPxTG-3* and *LPxTG-4* genes identified in fish isolates were reported to be absent in human-derived strains (Miyauchi et al. 2012, Gibello et al. 2016). Teker et al. (2020) examined similar genes in a total of 21 *L. garvieae* isolates of fish, milk, and human origin, and reported that *adhPsaA*, *adhCI*, and *LPxTG-1* genes were conserved in all isolates. In contrast, *adhPav* and *adhCII* genes could not be amplified in some milk and human strains; the *LPxTG-2* gene was detected only in six isolates (mostly fish-derived), while *LPxTG-3* was found exclusively in fish isolates. The researchers indicated that *LPxTG-3* might represent a virulence factor specific to fish hosts, whereas *LPxTG-4* was not considered a reliable host-specific marker. Similarly, in the study conducted by Türe and Altınok (2016) on fish isolates, *adhPav*, *adhPsaA*, *adhCII*, and *LPxTG-2* genes were found in all isolates, whereas *LPxTG-1*, *LPxTG-3*, *LPxTG-4*, and *adhCI* genes were missing in some strains. The authors emphasized that *LPxTG-2* could represent a conserved surface adhesin in fish hosts and may play a role in host specificity. In the comparative genomic analysis conducted by Lin et al. (2023), 39 *L. garvieae* strains isolated from dairy cows were compared with isolates of fish, human, and food origin. The researchers, based on previously defined virulence genes (Morita et al. 2011, Miyauchi et al. 2012, Gibello et al. 2016), constructed a database and analyzed hemolysins, iron uptake genes, capsule gene clusters, adhesion genes (*adhPavA*, *adhPsaA*, *the adhesin gene*), *LPxTG*-motif proteins (1–6), and various enzymatic virulence genes. The study reported that *adhPavA*, *adhPsaA*, *AC1*, and *AC2* genes were present in all milk isolates, indicating that these genes may play an important role in epithelial adherence. Likewise, Xie et al. (2023) obtained similar findings in genomic and molecular analyses of 49 bovine mastitis isolates collected from different regions of China. The researchers detected *adhPav*, *adhPsaA*, *AC1*, and *AC2* genes in all isolates (100%), whereas the *AF* gene was not found in any of them (0%). In the same study, only the *LPxTG-3* gene (22.45%) was found positive within the *LPxTG* gene cluster, while *LPxTG-1*, *LPxTG-2*, and *LPxTG-4* were not detected. These findings indicate that certain adhesion-related genes are highly conserved in milk-derived isolates, while variation in the *LPxTG* gene family may be linked to host adaptation. Similarly, in the present study, the presence of *adhPav*, *adhPsaA*, *AC1*, and *AC2* gene regions was confirmed in all ten mastitis isolates. The conservation of these genes across all isolates demonstrates that the genetic structure mediating epithelial cell adhesion in milk-derived *L. garvieae* strains is highly stable. The findings are consistent with those reported by Lin et al. (2023) and Xie et al. (2023) revealing that similar adhesion gene profiles are maintained across isolates of different geographical origins.

Antimicrobial resistance (AMR) trends indicate that *L. garvieae* has developed distinct adaptive patterns over time in both fish- and milk-derived isolates. In fish isolates, macrolide and tetracycline resistance has persisted since 1986, carried on transferable R-plasmids (Maki et al. 2008). Similarly, Öztürk et al. (2024) reported a high prevalence of multidrug resistance (MDR) among *L. garvieae* strains isolated from fish in different countries, with particularly elevated resistance rates to macrolides (erythromycin) and tetracyclines, and notable resistance to florfenicol and oxytetracycline, likely influenced by environmental antibiotic exposure. These findings suggest that *L. garvieae* can maintain and disseminate resistance determinants under prolonged antimicrobial pressure in aquatic environments. Comparable resistance patterns have also been documented among bovine mastitis isolates. Lin et al. (2023) reported that all 39 *L. garvieae* isolates from clinical bovine mastitis cases in China were resistant to chloramphenicol and clindamycin, with high resistance to amikacin (90 %), cefpodoxime (82.5%), cefazolin (45%), and gentamicin (37.5%), while retaining full susceptibility to β-lactam and fluoroquinolone antibiotics. Xie et al. (2023) found that all 49 mastitis isolates were susceptible to penicillin, ampicillin, ceftiofur, and cefquinome, although 12.24% were resistant to cephalexin. All isolates were resistant to lincomycin and rifaximin, 73.47% were resistant to oxytetracycline, while complete susceptibility was observed for marbofloxacin and vancomycin. Krukowski et al. (2025) reported benzylpenicillin, clindamycin, and rifampicin resistance among three *L. garvieae* isolates in Poland, whereas full susceptibility to macrolides, glycopeptides, and aminoglycosides was maintained. Likewise, de Oliveira et al. (2022) found high resistance to β-lactams (ampicillin, oxacillin, and penicillin) and erythromycin (80%), moderate resistance to tetracycline and cephalexin (60%), and high susceptibility to gentamicin and vancomycin (80%) in Brazilian sub-

clinical mastitis isolates. When compared with Turkish data, the resistance pattern observed in the present study was largely consistent with that reported by Ötkün et al. (2024). Both studies demonstrated consistently high susceptibility to β-lactam antibiotics alongside increasing resistance to fluoroquinolones and macrolides. Ötkün et al. (2024) identified eight *L. garvieae* isolates from bovine mastitis cases, all of which were fully susceptible to penicillin, ampicillin, and gentamicin, but resistant to enrofloxacin (100 %) and to amoxicillin–clavulanic acid and tylosin (87.5 %). Similarly, in the present study, all isolates were fully susceptible to florfenicol and ampicillin but exhibited high resistance to ciprofloxacin (90%) and erythromycin (40%). This pattern reflects a shared trend of preserved β-lactam susceptibility combined with increasing fluoroquinolone and macrolide resistance, suggesting comparable selective pressures acting on *L. garvieae* populations in Türkiye. Overall, *L. garvieae* isolates in this study maintained susceptibility to β-lactam antibiotics while showing increased resistance to fluoroquinolones, macrolides, and, in some cases, tetracyclines. These findings highlight the remarkable adaptive capacity of *L. garvieae* to environmental antimicrobial exposure and its potential role in inter-host dissemination of resistance determinants. Given its zoonotic potential, the occurrence of *L. garvieae* in both dairy and aquaculture systems may contribute to shared reservoirs of resistance genes across production chains, underscoring the importance of AMR surveillance within the *One Health* framework.

Conclusion

In conclusion, the obtained genetic profile indicates that some adhesion-related genes in *L. garvieae* (especially *adhPav, adhPsaA, AC1,* and *AC2*) are widely conserved in both fish- and milk-derived isolates. Conversely, variation in genes belonging to the LPxTG family across hosts suggests that this group of genes may play a selective role in host adaptation. These findings highlight the need for further studies on gene expression, functional protein analysis, and host-bacteria interactions in large-scale isolates from different host species to better understand the host-specific pathogenicity mechanisms of *L. garvieae*. The evaluation of antimicrobial resistance profiles of *L. garvieae* reveals that resistance events can vary significantly across geographical regions, which may be related to local antibiotic use practices and ecological conditions. Therefore, it is important to monitor resistance trends in *L. garvieae* strains isolated from bovine mastitis and to develop strategies for rational antibiotic use.

Author Declarations

The study was approved by the Local Ethics Committee of İzmir/Bornova Veterinary Control Institute (Approval No: E-71705440-550-19125472; 05.05.2025). The approval document has been submitted separately.

Use of generative artificial intelligence

The authors declare that generative artificial intelligence tools were used only for language editing, grammar correction, and improvement of the readability of the manuscript. No generative artificial intelligence tool was used for data generation, data analysis, interpretation of results, or preparation of scientific content.

Conflict of interest

The authors declare no conflicts of interest.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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