RESEARCH ARTICLE

F.U. Vet. J. Health Sci.
2026; 40 (2): 136 - 141
http://www.fusabil.org

Investigation of Biofilm-Forming Capacity and Antimicrobial Resistance in *Staphylococcus aureus* Isolates from Canine Skin Lesions

Zeynep YERLİKAYA [1, a]
Özden DELLAL [2, b]
Müzeyyen DEMİR [2, c]
Sadiye Dilşa ATEŞ [2, d]
Osman Yaşar TEL [2, e]
Songül ÖTKÜN [3, f]

[1] FıratUniversity,
Faculty of Veterinary Medicine,
Department of Microbiology,
Elazığ, TÜRKİYE

[2] Harran University,
Faculty of Veterinary Medicine,
Department of Microbiology,
Şanlıurfa, TÜRKİYE

[3] Siirt University,
Faculty of Veterinary Medicine,
Department of Microbiology,
Siirt, TÜRKİYE

[a] ORCID: 0000-0001-6895-2727
[b] ORCID: 0009-0002-5616-8796
[c] ORCID: 0000-0002-7082-4473
[d] ORCID: 0000-0003-2740-7313
[e] ORCID: 0000-0001-7848-3899
[f] ORCID: 0000-0003-2736-953X

Canine bacterial skin infections are frequently associated with staphylococci, and biofilm formation together with antimicrobial resistance (AMR) may compromise treatment outcomes. This study investigated biofilm production, carriage of the biofilm-associated genes *icaA* and *icaD*, and antimicrobial susceptibility in canine *S. aureus* isolates from skin infections. Swabs (n=130) from dogs with skin lesions were cultured, and *S. aureus* was confirmed by 23S rRNA PCR. Biofilm formation was quantified using a microtiter plate assay, *icaA/icaD* were detected by PCR, and antimicrobial susceptibility was determined by disk diffusion according to CLSI guidelines. 72 (55.4%) samples were confirmed as *S. aureus*. Most isolates produced biofilm, and 23.6% were classified as high biofilm producers. Overall, 70.8% of isolates carried at least one of *icaA* or *icaD*, but *icaA* positivity was significantly less frequent among high than low biofilm producers (p=0.002). *icaD* showed no significant association with biofilm category. Multidrug resistance was common (56.9%), and high biofilm producers showed a higher proportion of non-susceptibility to amoxicillin-clavulanate than low producers (p=0.049). These findings indicate a substantial burden of biofilm-associated, antimicrobial-resistant *S. aureus* in canine skin infections and a complex, partly *ica*-independent relationship between biofilm phenotype and genotype with implications for empirical therapy.

Key Words: *Staphylococcus aureus, biofilm, icaA, icaD, antimicrobial resistance*

Köpeklerde Deri Lezyonlarından İzole Edilen Staphylococcus aureus Suşlarında Biyofilm Oluşturma Kapasitesi ve Antimikrobiyal Direncin Araştırılması

Köpeklerde görülen bakteriyel deri enfeksiyonları sıklıkla stafilokoklarla ilişkilidir ve biyofilm oluşumu ile antimikrobiyal direncin (AMR) birlikteliği tedavi başarısını olumsuz etkileyebilmektedir. Bu çalışmada, deri lezyonu bulunan köpeklerden izole edilen *S. aureus* suşlarında biyofilm oluşturma kapasitesi, biyofilmle ilişkili *icaA* ve *icaD* genlerinin varlığı ve antimikrobiyal duyarlılık profilleri değerlendirildi. Bu amaçla 130 deri svabı kültüre edildi, *S. aureus* izolatları 23S rRNA PCR ile doğrulandı, biyofilm oluşumu mikrotitre plakta kristal viyole yöntemiyle ölçüldü. *icaA* ve *icaD* PCR ile araştırıldı ve antimikrobiyal duyarlılık CLSI kriterlerine göre disk difüzyon yöntemiyle belirlendi. 72 örnekte (%55.4) *S. aureus* saptandı. İzolatların çoğu biyofilm üreticisi olup %23.6'sı yüksek biyofilm oluşturan grup olarak sınıflandırıldı. Genel olarak izolatların %70.8'inde en az bir *icaA* veya *icaD* geni varlığı saptandı. *icaA* pozitifliği, yüksek biyofilm üreticilerinde düşük biyofilm üreticilerine kıyasla anlamlı derecede daha düşüktü (p=0.002). *icaD* ile biyofilm kategorisi arasında ise anlamlı bir ilişki bulunmadı. Çoklu ilaç direnci yaygındı (%56.9) ve yüksek biyofilm oluşturan izolatlarda amoksisilin-klavulanata duyarlı olmayanların oranı düşük biyofilm grubuna göre daha yüksekti (p=0.049). Elde edilen bulgular, köpek deri enfeksiyonlarına ait *S. aureus* izolatlarında biyofilmle ilişkili AMR yükünün belirgin olduğunu ve biyofilm fenotipi ile *ica* genotipi arasındaki ilişkinin kısmen *ica* bağımsız mekanizmaları da içeren karmaşık bir yapıda olabileceğini düşündürmektedir.

Anahtar Kelimeler: *Staphylococcus aureus, biyofilm, icaA, icaD, antimikrobiyal direnç*

Introduction

Canine bacterial skin infections are a frequent reason for presentation in small-animal practice, and pyoderma is a major indication for systemic antimicrobial therapy (1). Staphylococci are the principal pathogens in superficial and deep canine pyoderma, with *Staphylococcus pseudintermedius* recognised as the primary aetiological agent (2). However, *Staphylococcus aureus* is increasingly reported in skin and soft-tissue infections of companion animals, including dogs (3, 4). Close contact between dogs and their owners facilitates bidirectional transmission of *S. aureus*, including methicillin-resistant lineages, which are now regarded as relevant One Health-associated zoonotic hazards (5).

Biofilm formation is a key virulence trait of *S. aureus*, promoting persistent colonisation, evasion of host defences, and tolerance to antimicrobial therapy (6). In staphylococci, a major biofilm matrix component is polysaccharide intercellular adhesin (PIA), a PNAG polymer synthesised by the *icaADBC* operon, and co-expression of *icaA* and *icaD* has been linked to strong biofilm formation (7). The microtiter plate crystal violet assay remains a standard method for quantifying staphylococcal biofilm biomass

Received : 08.02.2026
Accepted : 07.04.2026

Correspondence

Zeynep YERLİKAYA
Fırat University,
Faculty of Veterinary Medicine,
Department of Microbiology
Elazığ – TÜRKİYE

zyerlikaya@firat.edu.tr

in human and veterinary research (8, 9). However, PIA or PNAG is not the sole determinant of the biofilm phenotype, and *ica*-independent mechanisms involving surface proteins, extracellular DNA, and other matrix components can also mediate biofilm formation in *S. aureus* (10-12).

Systemic antimicrobial therapy is central in the management of canine pyoderma, and drugs such as amoxicillin–clavulanate, first-generation cephalosporins and clindamycin are commonly used as first-line options (13). However, increasing reports of AMR and multidrug resistance (MDR) among staphylococci from canine skin and soft-tissue infections raised concerns for treatment success and antimicrobial stewardship (14, 15). Biofilm formation further complicates this picture, as biofilm-embedded *S. aureus* cells show reduced susceptibility to multiple drug classes and can act as reservoirs for recurrent infection (16). Although the interplay between biofilm production and AMR has been examined in staphylococci studies jointly assessing biofilm-forming capacity, *ica* biofilm-associated genes, and antimicrobial susceptibility in *S. aureus* from canine skin infections remains limited.

In this context, this study investigated *S. aureus* isolates recovered from canine skin lesions, focusing on their frequency, biofilm-forming capacity, *icaA/icaD* status, and antimicrobial susceptibility. Associations between biofilm production, *ica* gene carriage and multidrug resistance, particularly non-susceptibility to amoxicillin–clavulanate, were also evaluated.

Materials and Methods

Research and Publication Ethics: Ethical review and approval were waived for this study in accordance with national regulations and institutional requirements. The Animal Experiments Local Ethics Committee of Harran University (Meeting No. 2023/005, Decision No. 01-10, document date and number 15.09.2023–260686) concluded that diagnostic skin swab sampling in dogs is not subject to formal ethical approval.

Animals and Sample Collection: This study used clinical samples from dogs with cutaneous lesions presented to Harran University Animal Hospital, Şanlıurfa, Türkiye, between 2022 and 2025. Skin swabs were collected from 130 dogs with suspected bacterial infection using sterile swabs, transported under cold-chain conditions, and cultured on the day of sampling.

Bacterial Culture and Identification: Samples were streaked onto blood agar with 5% defibrinated sheep blood and mannitol salt agar (MSA; Oxoid, Basingstoke, UK) and incubated aerobically at 37 °C for 24-48 h. Colonies with staphylococcal morphology and growth on MSA that were Gram-positive and catalase-positive were regarded as *Staphylococcus* spp. Preliminary identification of *S. aureus* relied on colony morphology, mannitol fermentation on MSA, and a latex agglutination test (Phadebact Staph Aureus Test, Bactus AB, Sweden) with *S. aureus* ATCC 25923 as the positive control.

Molecular Assays: Genomic DNA was extracted from phenotypically identified Staphylococcus isolates using a classical phenol–chloroform method, and the purified DNA was resuspended in nuclease-free water and stored at −20°C until PCR analysis.

Species-level confirmation of *S. aureus* was performed targeting the 23S rRNA gene using the primer pair Sau-327/Sau-1645 as previously described (Table 1) (17). Amplifications were carried out in 25 µL reaction mixtures containing standard PCR buffer, $MgCl_2$, dNTPs, Taq DNA polymerase, primers and 2 µL of template DNA, under the following cycling conditions: initial denaturation at 94°C for 2 min; 35 cycles, each composed of 94°C for 45 s, 64°C for 1 min and 72°C for 2 min; and a final extension at 72°C for 10 min.

Table 1. The sequence of primers used in this research

Target gene	Primer sequence (5'-3')	Amplicon size (bp)	Reference
23S rRNA	F: GGACGACATTAGACGAATCA R: CGGGCACCTATTTTCTATCT	1318	(17)
icaA	F: ACACTTGCTGGCGCAGTCAA R:TCTGGAACCCAACATCCAACA	188	(18)
icaD	F:ATGGTCAAGCCCAGACAGAG R:AGTATTTTCAATGTTTAAAGCAA	198	(18)

The presence of the biofilm-associated genes *icaA* and *icaD* was assessed using gene-specific primers (Table 1) (18) with the same reaction mixture and a cycling profile of 95°C for 5 min; 33 cycles of 95°C for 30 s, 62°C for 30 s and 72°C for 30 s; and a final extension at 72°C for 5 min. PCR products were separated on 2% agarose gels alongside a FastRuler Middle Range DNA Ladder (Thermo Scientific, USA) and visualised under UV illumination. *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 35984 were used as positive controls for the 23S rRNA and *icaA/icaD* assays, respectively.

Phenotypic Assessment of Biofilm Formation: Biofilm formation was evaluated using a 96-well microtiter plate crystal violet assay as described by Christensen et al., with minor modifications (19). Briefly, overnight cultures grown in Tryptic Soy Broth (TSB) were adjusted to a 0.5 McFarland standard, diluted 1:100 in TSB supplemented with 1% (w/v) glucose and dispensed (200 µL) in triplicate into sterile, flat-bottom polystyrene microtiter plates. Plates were incubated statically at 37°C for 24 h, then gently washed with phosphate-buffered saline to remove non-adherent cells, air-dried, stained with 0.1% crystal violet, and rinsed with distilled water. Bound stain was solubilised with 95% ethanol, and the optical density (OD) at 570 nm was measured in a microplate reader (VersaMax, Molecular Devices, San Jose, CA, USA). The mean OD of triplicate wells was used for each isolate. Biofilm production was interpreted using an optical-density cut-off (ODc), calculated as the mean OD of negative-control wells plus three standard deviations (ODc=mean OD_NC + 3 × SD_NC). Isolates with OD ≤ ODc were classified as non-biofilm producers, those with ODc < OD ≤ 2 × ODc as weak, 2 × ODc < OD ≤ 4 × ODc as moderate, and OD >

4 × ODc as strong biofilm producers. *Staphylococcus aureus* ATCC 35556 was included as a positive control, and wells containing sterile TSB (Oxoid) served as negative controls.

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method in accordance with CLSI guidelines (20). Standardised bacterial suspensions (0.5 McFarland) were spread onto Mueller-Hinton agar supplemented with 5% defibrinated sheep blood. Commercial disks (Oxoid, Basingstoke, UK) containing penicillin (2 U), amoxicillin–clavulanic acid (30 µg), erythromycin (15 µg), tetracycline (5 µg), clindamycin (2 µg), enrofloxacin (5 µg), and trimethoprim–sulfamethoxazole (25 µg) were applied. Plates were incubated aerobically at 37°C for 18-24 h, and inhibition zone diameters were measured and interpreted as susceptible, intermediate, or resistant according to CLSI breakpoints for *S. aureus*.

Statistical Analysis: Statistical analyses were performed using GraphPad Prism version 10.4.1 (GraphPad Software, San Diego, CA, USA). Associations between categorical variables were assessed using Fisher's exact test (two-tailed), with $p<0.05$ considered statistically significant. For antimicrobial susceptibility, isolates with intermediate susceptibility were grouped with resistant isolates as non-susceptible, and biofilm production was dichotomised as low (non/weak) versus high (moderate/strong) for comparative analyses.

Results

Characterization of *Staphylococcus aureus* Isolates and Biofilm Production: Of the 130 skin swab samples from dogs with cutaneous lesions, 95 (73.1%) yielded bacterial growth, and 84 (64.6%) isolates were phenotypically consistent with *S. aureus* based on mannitol fermentation and latex agglutination (Table 2). PCR targeting the staphylococcal 23S rRNA gene confirmed 72 (55.4%) *S. aureus* isolates, which were included in subsequent biofilm and antimicrobial susceptibility analyses (Figure 1A).

According to OD-based categories, 1 (1.4%) isolate was a non-biofilm producer, 54 (75.0%) weak, 13 (18.1%) moderate, and 4 (5.6%) strong producers; thus 55/72 (76.4%) were classified as low (non/weak) and 17/72 (23.6%) as high (moderate/strong) biofilm formers.

Among the 72 *S. aureus* isolates, 21 (29.2%) were negative for both *icaA* and *icaD*, whereas 51 (70.8%) carried at least one of these genes. Of these, 27 (37.5%) harboured only *icaA*, 14 (19.4%) only *icaD* and 10 (13.9%) both genes (Table 2, Figure 1B, C), giving overall frequencies of 37/72 (51.4%) for *icaA* and 24/72 (33.3%) for *icaD*.

Antimicrobial Susceptibility Profiles: Antimicrobial susceptibility testing was performed for 72 PCR-confirmed *S. aureus* isolates against seven antimicrobial agents, and the results are summarised in Table 3.

Non-susceptibility to β-lactams was 44.4% for penicillin and 47.2% for amoxicillin–clavulanic acid (Figure 2, Table 3). Among non-β-lactam agents, the highest non-susceptibility rate was observed for clindamycin (83.3%), followed by tetracycline (52.8%), trimethoprim–sulfamethoxazole (45.8%), and erythromycin (34.7%), whereas enrofloxacin showed the lowest rate (26.4%). Overall, 41 of 72 isolates (56.9%) were multidrug resistant, being non-susceptible to at least three antimicrobial classes.

Correlation Between Biofilm Production, *ica* Genes and Antimicrobial Resistance: The distribution of *ica* genes differed between low and high biofilm categories, mainly due to the pattern observed for *icaA*. Only 3/37 (8.1%) of *icaA*-positive isolates were classified as high biofilm producers, compared with 14/36 (38.9%) of *icaA*-negative isolates ($p=0.002$) (Figure 3A), indicating an inverse association between *icaA* carriage and high-level biofilm formation. In contrast, 9/24 (37.5%) of *icaD*-positive isolates and 8/48 (16.7%) of *icaD*-negative isolates were high biofilm producers, but this difference did not reach statistical significance ($p=0.076$) (Table 4).

High biofilm-forming isolates showed a slightly higher proportion of multidrug resistance than low biofilm producers (64.7% vs 54.5%), but this difference was not statistically significant ($p=0.579$). Non-susceptibility rates were broadly similar between low and high biofilm producers for most agents. However, high biofilm producers had a significantly higher non-susceptibility rate to amoxicillin–clavulanic acid than low producers (70.6% vs 40.0%; $p=0.049$) (Figure 3B), while no significant differences were observed for the remaining antimicrobials. Multidrug resistance was also similarly frequent among *icaA*-positive and *icaA*-negative isolates (62.2% vs 51.4%; $p=0.476$), and among *icaD*-positive and *icaD*-negative isolates (62.5% vs 54.2%; $p=0.616$), indicating no clear association between *ica* gene carriage and MDR in this collection.

Table 2. Summary of sample processing and characterization of *S. aureus* isolates

Parameter	Number (*n*)	Percentage (%)
Total skin swab samples collected	130	–
Samples with bacterial growth	95	73.1
Phenotypically presumptive *S. aureus*	84	64.6
PCR-confirmed *S. aureus* isolates	72	55.4
Isolates positive for at least one biofilm-associated gene (*icaA* and/or *icaD*)	51	70.8
Isolates negative for both *icaA* and *icaD*	21	29.2
icaA-positive only	27	37.5
icaD-positive only	14	19.4
Positive for both *icaA* and *icaD*	10	13.9
Total *icaA*-positive isolates	37	51.4
Total *icaD*-positive isolates	24	33.3

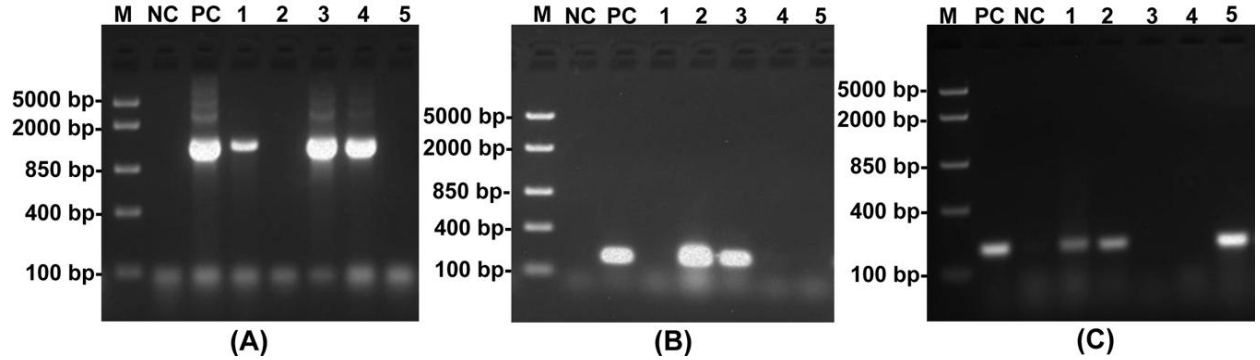

Figure 1. Representative agarose gel electrophoresis of PCR products used for species confirmation and detection of biofilm-associated genes in canine *S.aureus* isolates. **(A)** Species-specific amplification of the *S. aureus* 23S rRNA gene (1318 bp). Lanes: M, DNA ladder; NC, no-template control; PC, *S. aureus* ATCC 25923; 1–5, clinical isolates tested for 23S rRNA amplification showing positive or negative PCR results. **(B)** Detection of the *icaA* gene (188 bp). Lanes: M, DNA ladder; NC, no-template control; PC, *S. epidermidis* ATCC 35984; 1–5, PCR-confirmed clinical *S. aureus* isolates showing positive or negative amplification for *icaA*. **(C)** Detection of the *icaD* gene (198 bp). Lanes: M, DNA ladder; PC, *S. epidermidis* ATCC 35984; NC, no-template control; 1–5, PCR-confirmed clinical *S. aureus* isolates showing positive or negative amplification for *icaD*

Table 3. Distribution of antimicrobial susceptibility categories (n, %) in 72 *Staphylococcus aureus* isolates recovered from canine skin lesion swabs

Antimicrobial agent	Susceptible *n* (%)	Intermediate *n* (%)	Resistant *n* (%)	Non-susceptible* *n* (%)
Penicillin (2 U)	40 (55.6)	3 (4.2)	29 (40.3)	32 (44.4)
Amoxicillin–clavulanic acid (30 µg)	38 (52.8)	2 (2.8)	32 (44.4)	34 (47.2)
Erythromycin (15 µg)	47 (65.3)	8 (11.1)	17 (23.6)	25 (34.7)
Clindamycin (2 µg)	12 (16.7)	9 (12.5)	51 (70.8)	60 (83.3)
Tetracycline (30 µg)	34 (47.2)	17 (23.6)	21 (29.2)	38 (52.8)
Enrofloxacin (5 µg)	53 (73.6)	4 (5.6)	15 (20.8)	19 (26.4)
Trimethoprim–sulfamethoxazole (25 µg)	39 (54.2)	13 (18.1)	20 (27.8)	33 (45.8)

*Non-susceptible: intermediate + resistant.

Table 4. Distribution of *icaA* and *icaD* among low and high biofilm-forming *S. aureus* isolates

Variable	Low biofilm (*n* = 55) *n* (%)	High biofilm (*n* = 17) *n* (%)	*p*-value*
icaA-positive	34 (61.8)	3 (17.6)	0.002
icaA-negative	21 (38.2)	14 (82.4)	
icaD-positive	15 (27.3)	9 (52.9)	0.076
icaD-negative	40 (72.7)	8 (47.1)	

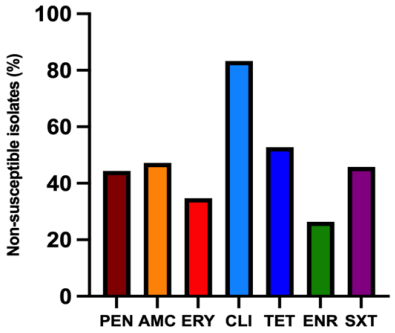

Figure 2. Non-susceptibility rates to selected antimicrobials among 72 canine *S. aureus* isolates. Bars indicate the percentage of isolates classified as non-susceptible (intermediate or resistant) to penicillin G (PEN), amoxicillin–clavulanic acid (AMC), erythromycin (ERY), clindamycin (CLI), tetracycline (TET), enrofloxacin (ENR) and trimethoprim–sulfamethoxazole (SXT).

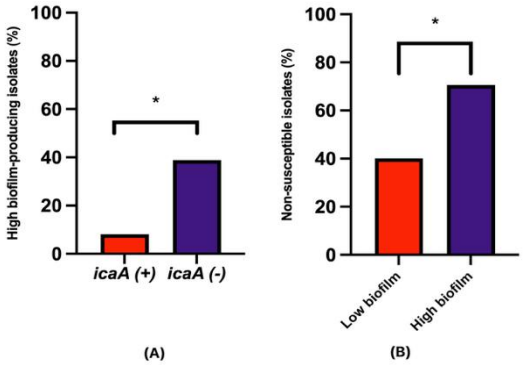

Figure 3. Associations between biofilm category, *icaA* status, and amoxicillin–clavulanic acid non-susceptibility in canine *S. aureus* isolates. **(A)** Proportion of high biofilm-forming isolates among *icaA*-positive and *icaA*-negative strains (8.1% vs 38.9%; *p*=0.002, Fisher's exact test). **(B)** Non-susceptibility to amoxicillin–clavulanic acid in low and high biofilm producers (40.0% vs 70.6%; *p*=0.049, Fisher's exact test). Asterisks indicate statistically significant differences.

Discussion

Bacterial skin infections in dogs are predominantly associated with staphylococci, with *S. pseudintermedius* recognised as the primary pathogen, whereas *S. aureus* is less frequent but noteworthy because of its zoonotic and One Health relevance (1, 21). Biofilm formation and antimicrobial resistance are key traits that promote persistence and treatment failure in staphylococcal infections (22, 23). However, most studies on biofilm and biofilm-associated genes have focused on *S. pseudintermedius* or non-canine *S. aureus*, and integrated data on biofilm phenotype, *ica* status, and resistance profiles in canine *S. aureus* isolates remain scarce. In this context, the present study examined canine *S. aureus* isolates to characterise their biofilm phenotype, *icaA/icaD* carriage and antimicrobial susceptibility, and to explore relationships between these traits.

S. aureus was recovered from 72 of 130 (55.4%) canine skin swab samples, indicating that this species is an important component of the bacterial flora of canine skin lesions in our setting. Almost all isolates produced measurable biofilm and 23.6% were classified as high (moderate/strong) biofilm formers, suggesting that biofilm formation is common among canine *S. aureus* skin isolates. This high prevalence of biofilm production is consistent with reports of frequent biofilm formation by coagulase-positive staphylococci in companion-animal and human skin infections (21, 24). The study included a broad case mix of skin lesions with suspected bacterial involvement rather than only confirmed pyoderma, which may partly explain the relatively high proportion of *S. aureus* compared with studies restricted to canine pyoderma.

In this collection, 70.8% of the isolates carried at least one biofilm-associated gene, with *icaA* and *icaD* detected in 51.4% and 33.3% of isolates, respectively. Although co-expression of *icaA* and *icaD* has traditionally been linked to strong biofilm formation and many studies report a positive association between *ica* genes and biofilm production (25), *icaA* positivity in our study was significantly less frequent among high than low biofilm producers, and *icaD* status was not associated with biofilm category. This discrepancy with the classical *ica*-dependent model aligns with evidence that *S. aureus* can form biofilm through *ica*-independent mechanisms involving surface proteins, extracellular DNA, and other matrix components, and suggests that *ica* carriage alone does not reliably predict biofilm-forming capacity in canine *S. aureus* skin isolates (11, 26, 27).

In addition to widespread biofilm formation, the isolates showed a substantial burden of antimicrobial resistance, with more than half meeting the criteria for multidrug resistance. Non-susceptibility to several first-line drugs for canine skin infections, including amoxicillin–clavulanate, tetracycline, clindamycin and fluoroquinolones, indicates that empirical use of these agents may not always provide adequate coverage in our setting. The MDR proportion appears higher than in some reports on staphylococci from canine pyoderma but comparable to others (28, 29), and is consistent with the global trend of increasing multidrug resistance among staphylococci from companion animals. This pattern may reflect the referral nature of our veterinary teaching hospital and prior antimicrobial exposure, which could not be systematically documented, and underlines the need for culture and susceptibility-based therapy, particularly in recurrent or non-responsive cases, as well as the integration of antimicrobial stewardship principles into the management of canine skin infections (13).

In our study, high biofilm-forming isolates showed a higher frequency of multidrug resistance than low producers, although this was not statistically significant. In contrast, non-susceptibility to amoxicillin–clavulanate was significantly more common in high biofilm producers (p=0.049), suggesting particular relevance of biofilm for reduced susceptibility to this first-line agent. While some studies have reported links between biofilm formation, *ica* genes, and resistance in staphylococci, others have not found consistent correlations (30-32), and our data likewise indicate that the interplay between biofilm phenotype, *ica* status, and antimicrobial resistance in canine *S. aureus* is complex and cannot be inferred from any single parameter.

This study has limitations. It was conducted in a single veterinary teaching hospital with a moderate number of isolates, especially in the high biofilm subgroup, which may limit statistical power. Dogs were included based on skin lesions suggestive of bacterial infection rather than confirmed pyoderma, and the culture-based design does not clarify the clinical role of *S. aureus* (pathogen vs coloniser) in individual cases. Molecular typing and detailed characterisation of methicillin resistance were not performed, so clonal relationships and wider epidemiology could not be assessed. Nevertheless, the high frequency of biofilm formation and multidrug resistance among canine *S. aureus* skin isolates highlights their clinical and One Health relevance, and supports culture and susceptibility-guided therapy alongside antimicrobial stewardship in canine skin infections.

References

1. Wang Q, Chen S, Ma S, et al. Antimicrobial resistance and risk factors of canine bacterial skin infections. Pathogens 2025; 14: 309.

2. Faccin M, Wiener DJ, Rech RR, et al. Common superficial and deep cutaneous bacterial infections in domestic animals: A review. Vet Pathol 2023; 60: 796-811.

3. Costa SS, Ribeiro R, Serrano M, et al. *Staphylococcus aureus* causing skin and soft tissue ınfections in companion animals: Antimicrobial resistance profiles and clonal lineages. Antibiotics (Basel) 2022; 11: 599.

4. Lan Anh NT, Hong Loan NVT, NT YV, et al. Prevalence, species identification, and antibiotic resistance of

Staphylococci in dogs visiting veterinary clinics in Vietnam. PLoS One 2025; 20: e0328472.

5. Cuny C, Layer-Nicolaou F, Werner G, et al. A look at staphylococci from the one health perspective Int J Med Microbiol 2024; 314: 151604.
6. Francois P, Schrenzel J, Gotz F. Biology and regulation of Staphylococcal biofilm. Int J Mol Sci 2023; 24: 5218.
7. Arciola CR, Campoccia D, Ravaioli S, et al. Polysaccharide intercellular adhesin in biofilm: Structural and regulatory aspects. Front Cell Infect Microbiol 2015; 5:7.
8. Stepanovic S, Vukovic D, Dakic I, et al. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods 2000; 40: 175-179.
9. Allkja J, van Charante F, Aizawa J, et al. Interlaboratory study for the evaluation of three microtiter plate-based biofilm quantification methods. Sci Rep 2021; 11: 13779.
10. Fitzpatrick F, Humphreys H, O'Gara JP. The genetics of staphylococcal biofilm formation--will a greater understanding of pathogenesis lead to better management of device-related infection? Clin Microbiol Infect 2005; 11: 967-973.
11. O'Gara JP. Ica and beyond: Biofilm mechanisms and regulation in *Staphylococcus epidermidis* and *Staphylococcus aureus*. FEMS Microbiol Lett 2007; 270: 179-188.
12. Brooks JL, Jefferson KK. Phase variation of poly-N-acetylglucosamine expression in *Staphylococcus aureus*. PLoS Pathog 2014; 10: e1004292.
13. Loeffler A, Cain CL, Ferrer L, et al. Antimicrobial use guidelines for canine pyoderma by the International Society for Companion Animal Infectious Diseases (ISCAID). Vet Dermatol 2025; 36: 234-282.
14. Sigudu TT, Oguttu JW, Qekwana DN. Antimicrobial resistance of *Staphylococcus* spp. isolated from canine specimens submitted to a veterinary diagnostic laboratory in South Africa. Vet World 2025; 18: 1421-1432.
15. Saraiva S, Calouro R, de Sousa T, et al. Bacterial agents and antimicrobial-resistance patterns in canine otitis externa. Animals (Basel) 2025; 15: 3317.
16. Fidelis CE, Orsi AM, Freu G, et al. Biofilm formation and antimicrobial resistance of *Staphylococcus aureus* and *Streptococcus uberis* isolates from bovine mastitis. Vet Sci 2024; 11: 170.
17. Riffon R, Sayasith K, Khalil H, et al. Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. J Clin Microbiol 2001; 39: 2584-9.
18. Kroning IS, Iglesias MA, Sehn CP, et al. *Staphylococcus aureus* isolated from handmade sweets: Biofilm formation, enterotoxigenicity and antimicrobial resistance. Food Microbiol 2016; 58: 105-111.
19. Christensen GD, Simpson WA, Younger JJ, et al. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: A quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol 1985; 22: 996-1006.
20. CLSI. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved Standard. 4th Edition (CLSI document VET01-A4). CLSI. 2013.
21. Andrade M, Oliveira K, Morais C, et al. Virulence potential of biofilm-producing *Staphylococcus pseudintermedius*, *Staphylococcus aureus* and *Staphylococcus coagulans* Causing skin infections in companion animals. Antibiotics (Basel) 2022; 11: 1339.
22. Bertelloni F, Cagnoli G, Ebani VV. Virulence and antimicrobial resistance in canine *Staphylococcus* spp. Isolates. Microorganisms 2021; 9: 515.
23. Dancova N, Kiraly J, Hajduckova V, et al. Characterization and antimicrobial resistance profiles of biofilm forming strains of *Staphylococcus aureus* isolated from skin lesions. Microorganisms 2025; 13: 2449.
24. Kwiecinski J, Kahlmeter G, Jin T. Biofilm formation by *Staphylococcus aureus* isolates from skin and soft tissue infections. Curr Microbiol 2015; 70: 698-703.
25. Bamneshin K, Poudineh M, Alibabaei RH, et al. Prevalence of *icaADBC* genes, and correlation with biofilms and antibiotic resistance in *S. aureus*: A systematic review and meta-analysis. Germs 2024; 14: 387-401.
26. Peng Q, Tang X, Dong W, et al. A review of biofilm formation of *Staphylococcus aureus* and its regulation mechanism. Antibiotics (Basel) 2022; 12: 12.
27. Dengler V, Foulston L, DeFrancesco AS, et al. An electrostatic net model for the role of extracellular DNA in biofilm formation by *Staphylococcus aureus*. J Bacteriol 2015; 197: 3779-87.
28. Prosic I, Vejnovic B, Misic D, et al. Antibiotic resistance patterns of bacteria ısolated from canine skin and ear ınfections in Serbia. Antibiotics (Basel) 2025;15: 21.
29. Hritcu OM, Schmidt VM, Salem SE, et al. Geographical variations in virulence factors and antimicrobial resistance amongst Staphylococci ısolated from dogs from the United Kingdom and Romania. Front Vet Sci 2020; 7: 414.
30. Silva V, Correia E, Pereira JE, et al. Exploring the biofilm formation capacity in *S. pseudintermedius* and Coagulase-Negative Staphylococci species. Pathogens 2022; 11: 689.
31. Pajohesh R, Tajbakhsh E, Momtaz H, et al. Relationship between biofilm formation and antibiotic resistance and adherence genes in strains ısolated from raw cow milk in Shahrekord, Iran. Int J Microbiol 2022; 2022: 6435774.
32. Darwish SF, Asfour HA. Investigation of biofilm forming ability in Staphylococci causing bovine mastitis using phenotypic and genotypic assays. Scientific World Journal 2013; 2013: 378492.