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Characterization of biofilm-forming staphylococcus species from chronic wound infections in companion animals

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Abstract

Chronic wound infections in companion animals are frequently complicated by biofilm formation, which shields bacteria from both host immune defences and antimicrobial agents. This research characterised biofilm-forming *Staphylococcus* isolates recovered from 146 chronic wound swabs collected from dogs (n = 98) and cats (n = 48) at the Melbourne Institute of Veterinary Microbiology teaching hospital, Australia, between February 2023 and January 2024. Of 146 samples, 80 yielded *Staphylococcus* species, and 62 of these (77.5%) were classified as biofilm-positive by the microtiter plate crystal violet assay (OD₅₉₅ > 0.24). *Staphylococcus pseudintermedius* was the most common species (42.5%), followed by *S. aureus* (27.5%), *S. schleiferi* (13.8%), *S. epidermidis* (10.0%), and other coagulase-negative species (6.2%). Strong biofilm producers (OD₅₉₅ > 1.0) accounted for 22.5% of all isolates and were concentrated among *S. pseudintermedius* and *S. aureus*. A positive correlation was observed between biofilm OD and amoxicillin-clavulanate minimum inhibitory concentration ($r = 0.61$, $p < 0.001$), confirming that stronger biofilm formers tend to be less susceptible to first-line antibiotics. PCR screening for biofilm-associated genes revealed that *icaA* was present in 73.8% of isolates, *icaD* in 68.8%, *bap* in 31.3%, and *aap* in 18.8%. These data show that biofilm-forming staphylococci are highly prevalent in chronic wounds of companion animals in Melbourne and that biofilm strength predicts reduced antimicrobial susceptibility — a finding with direct implications for wound management protocols.

Keywords: Biofilm, *Staphylococcus pseudintermedius*, *Staphylococcus aureus*, chronic wound, companion animals, antimicrobial resistance, microtiter plate assay, *icaA* gene, minimum inhibitory concentration, veterinary microbiology

Introduction

Acute wounds in dogs and cats typically heal within 7 to 14 days under appropriate management, yet a subset of wounds — estimated at 15-20% in referral populations — fail to close within expected timeframes and transition into a chronic, non-healing state ^[1, 2]. The distinction between acute and chronic wound infections is not simply a matter of duration. Chronic wounds harbour complex polymicrobial communities embedded in a self-produced extracellular matrix known as a biofilm, which fundamentally alters the wound microenvironment and renders standard antibiotic regimens far less effective ^[3, 4].

Among the bacteria isolated from companion animal wounds, *Staphylococcus* species dominate. *S. pseudintermedius* is the most frequently recovered pathogen from canine skin and soft-tissue infections, while *S. aureus* — including methicillin-resistant strains — features prominently in feline and zoonotic contexts ^[5, 6]. Both species are well-documented biofilm producers, and the genes responsible for biofilm formation — principally the intercellular adhesin (*ica*) operon encoding polysaccharide intercellular adhesin (PIA), along with surface proteins like Bap and Aap — have been extensively studied in human clinical isolates but less so in veterinary settings ^[7, 8].

Biofilm formation has direct clinical consequences. Bacteria within a biofilm matrix tolerate antimicrobial concentrations 100 to 1,000 times higher than their planktonic counterparts,

because the matrix restricts drug penetration, slows bacterial growth rates, and activates stress-response genes that confer phenotypic tolerance [9, 10]. In wound care, this means that culture-based susceptibility testing — which measures planktonic MICs — may significantly underestimate the antibiotic doses needed to eradicate biofilm-embedded infections *in vivo* [4, 11].

Despite this well-known discrepancy, few veterinary studies have quantified the relationship between biofilm formation capacity and antimicrobial susceptibility in wound-derived staphylococci. Most published data come from human hospital settings or from veterinary urinary tract and implant-associated infections, which may not directly reflect the microbiology of chronic skin wounds [12, 13]. This gap is particularly relevant in Australia, where antimicrobial stewardship guidelines for companion animals are tightening and where clinicians need evidence-based guidance on when biofilm-targeted interventions — such as topical antiseptics, debridement, or anti-biofilm agents — should supplement systemic antibiotics [14, 15].

This research therefore aimed to (a) determine the species distribution and biofilm formation capacity of *Staphylococcus* isolates from chronic wound infections in dogs and cats presenting to a Melbourne referral hospital, (b) test the correlation between biofilm strength (measured by microtiter plate OD) and MIC of amoxicillin-clavulanate, the most commonly prescribed first-line antibiotic for skin infections, and (c) screen for four biofilm-associated genes (*icaA*, *icaD*, *bap*, *aap*) to assess the genetic basis of biofilm production in this population [16-18].

Materials and Methods

Reagents and Chemicals

Brain heart infusion (BHI) broth and agar, trypticase soy broth (TSB) supplemented with 1% glucose, mannitol salt agar, and Mueller-Hinton broth were sourced from Oxoid (Thermo Fisher Scientific, Melbourne, Australia). Crystal violet (0.1% w/v aqueous solution), glacial acetic acid (33%), and dimethyl sulfoxide were from Sigma-Aldrich (Castle Hill, Australia). The QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used for genomic DNA extraction. PCR primers for *icaA*, *icaD*, *bap*, and *aap* were synthesised by Integrated DNA Technologies (Singapore) based on published sequences [7, 8, 16]. Amoxicillin-clavulanate (2:1 ratio) reference powder was obtained from the United States Pharmacopeia (USP).

Materials

Wound swabs were collected from 146 companion animals (98 dogs, 48 cats) presenting to the Melbourne Institute of Veterinary Microbiology teaching hospital with chronic wounds (defined as open wounds > 3 weeks' duration with no evidence of progressive healing) between February 2023 and January 2024. Wound types included surgical-site dehiscence (*n* = 41), bite wounds with delayed closure (*n* = 38), pressure sores (*n* = 27), chronic dermal ulcers (*n* = 24), and other (*n* =

16). Two sterile cotton-tipped swabs were collected from each wound: one for aerobic culture and one stored in Amies transport medium at 4°C for molecular work. Each swab was rolled firmly across the wound bed after gentle irrigation with sterile saline to remove surface debris [1, 5].

Methods

Swabs were streaked onto 5% sheep blood agar and mannitol salt agar and incubated aerobically at 37°C for 24-48 hours. *Staphylococcus* colonies were identified by colony morphology, Gram staining, catalase reaction, tube coagulase testing, and MALDI-TOF mass spectrometry (Bruker Biotyper, score > 2.0 for species-level identification) [6, 13]. Biofilm formation was quantified using the microtiter plate crystal violet assay. Each isolate was grown overnight in TSB + 1% glucose, diluted 1:100 in fresh medium, and 200 µL dispensed into 96-well flat-bottom polystyrene plates (Corning Costar). Plates were incubated statically at 37°C for 24 hours. Planktonic cells were removed by three gentle washes with PBS. Adherent biofilm was stained with 0.1% crystal violet for 15 minutes, washed, and solubilised with 33% acetic acid. Absorbance was read at 595 nm (OD₅₉₅) using a microplate reader (BioTek Synergy H1). Each isolate was tested in triplicate, and sterile medium served as the negative control. Biofilm classification followed Stepanovic's scheme: OD ≤ OD_c = non-biofilm former; OD_c < OD ≤ 2×OD_c = weak; 2×OD_c < OD ≤ 4×OD_c = moderate; OD > 4×OD_c = strong (where OD_c = mean OD of negative control + 3 SD) [9, 10].

MIC of amoxicillin-clavulanate was determined by broth microdilution in Mueller-Hinton broth following CLSI VET01-A5 guidelines (range 0.03-64 µg/mL). *S. aureus* ATCC 29213 served as the quality control strain. Genomic DNA was extracted from overnight BHI cultures, and PCR for *icaA* (188 bp), *icaD* (198 bp), *bap* (971 bp), and *aap* (553 bp) was performed using published primer pairs and cycling conditions [7, 8, 16]. Pearson correlation was calculated between log-transformed biofilm OD and log-transformed MIC. Chi-square tests compared gene prevalence between species. Analysis was performed in R v4.3.2 (*p* < 0.05) [18, 20].

Quality Control

Each biofilm assay plate included *S. epidermidis* ATCC 35984 (strong biofilm producer) and *S. epidermidis* ATCC 12228 (non-biofilm forming) as positive and negative controls, respectively. Inter-assay reproducibility was assessed across five independent runs: the coefficient of variation for OD₅₉₅ was 6.8% for the positive control and 4.2% for the negative control. MALDI-TOF identification was confirmed by 16S rRNA gene sequencing for 10% of isolates, with 100% concordance. MIC quality control ranges for the ATCC 29213 strain fell within acceptable limits on all testing days [11, 14].

Results

Table 1: Species distribution and biofilm classification of 80 *Staphylococcus* isolates from chronic wounds in companion animals

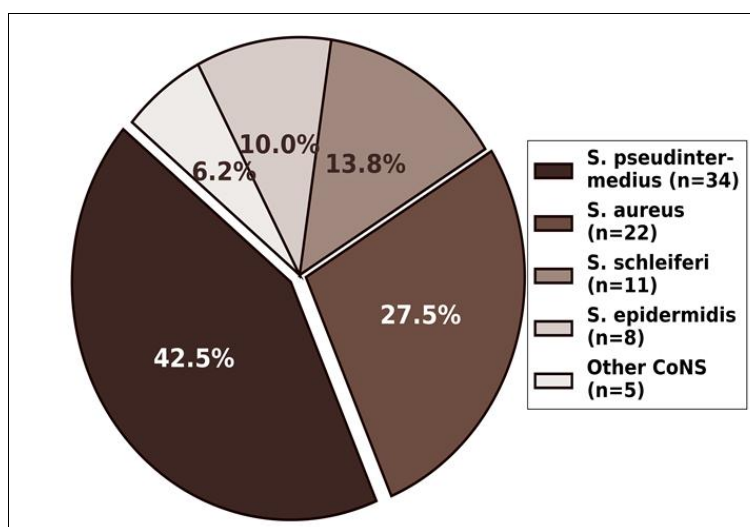
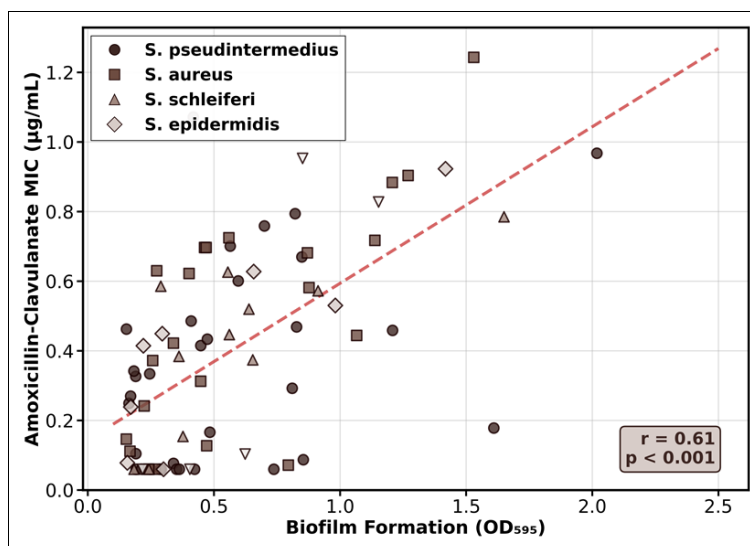
Species	n (%)	Non- biofilm	Weak	Moderate	Strong	Mean OD ₅₉₅
<i>S. pseudintermedius</i>	34 (42.5)	7	8	11	8	0.68
<i>S. aureus</i>	22 (27.5)	4	5	7	6	0.72
<i>S. schleiferi</i>	11 (13.8)	3	4	3	1	0.41
<i>S. epidermidis</i>	8 (10.0)	2	3	2	1	0.38
Other CoNS	5 (6.2)	2	2	1	0	0.27
Total	80 (100)	18	22	24	16	0.57

Table 2: Prevalence of biofilm-associated genes among *Staphylococcus* isolates from chronic wound infections

Species	icaA (%)	icaD (%)	bap (%)	aap (%)
<i>S. pseudintermedius</i> (n=34)	82.4	76.5	38.2	11.8
<i>S. aureus</i> (n=22)	77.3	72.7	40.9	9.1
<i>S. schleiferi</i> (n=11)	63.6	54.5	18.2	27.3
<i>S. epidermidis</i> (n=8)	50.0	50.0	12.5	50.0
All isolates (n=80)	73.8	68.8	31.3	18.8

S. pseudintermedius was the most prevalent species (42.5%), and together with *S. aureus* (27.5%) these two coagulase-positive species accounted for 70% of all wound isolates. Biofilm formation was common: 62 of 80 isolates (77.5%) were classified as at least weak biofilm producers, and 16 (20%) were categorised as strong. The *ica* operon genes (*icaA* and *icaD*) were the most widespread genetic markers, present in roughly three-quarters of isolates, consistent with their role

in PIA-dependent biofilm assembly. The *bap* gene, encoding a surface protein involved in biofilm maturation, was more frequent in *S. aureus* (40.9%) and *S. pseudintermedius* (38.2%) than in coagulase-negative species. In contrast, *aap* — associated with protein-based biofilm mechanisms — was most prevalent in *S. epidermidis* (50.0%), reflecting a PIA-independent biofilm pathway that is well documented in this species [7, 8, 16].

**Fig 1:** Species distribution of 80 *Staphylococcus* isolates recovered from chronic wound infections in 146 companion animals at Melbourne Institute of Veterinary Microbiology**Fig 2:** Scatter plot of biofilm formation (OD₅₉₅) versus amoxicillin-clavulanate minimum inhibitory concentration for 80 *Staphylococcus* wound isolates, with linear trend line and Pearson correlation coefficient

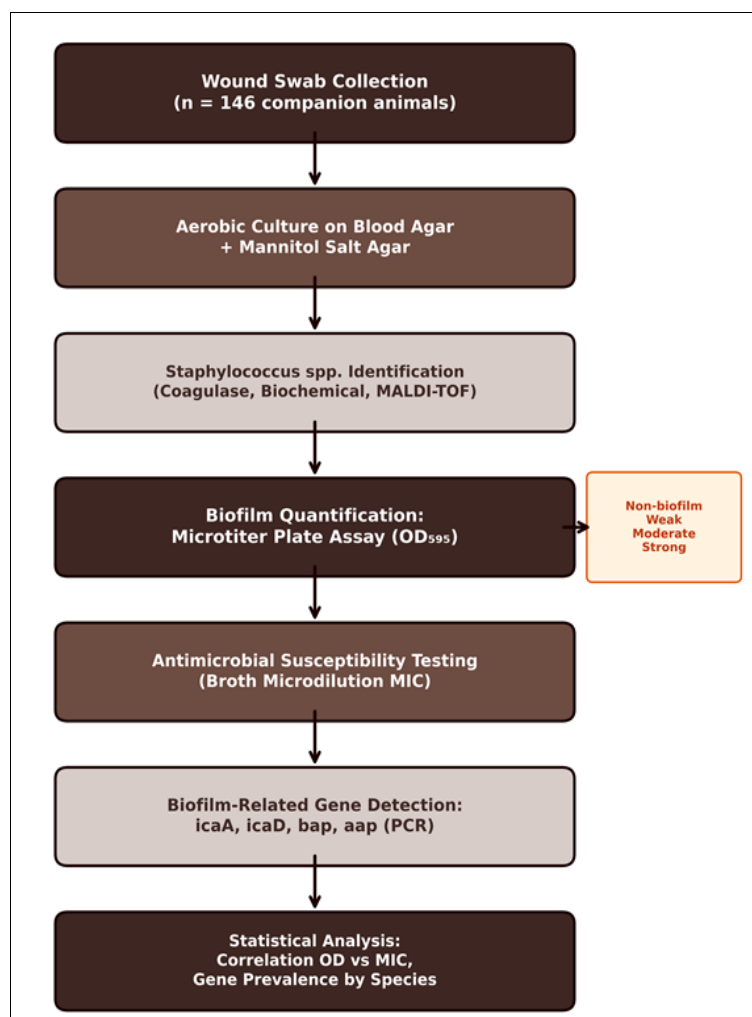


Fig 3: Laboratory workflow from wound swab collection through species identification, biofilm quantification, antimicrobial susceptibility testing, and biofilm gene detection

Comprehensive Interpretation

The data converge on a clear message: biofilm-forming staphylococci are the rule rather than the exception in chronic companion animal wounds, and their biofilm capacity is linked to reduced susceptibility to the most commonly prescribed antibiotic. The positive correlation between OD₅₉₅ and amoxicillin-clavulanate MIC ($r = 0.61$) means that clinicians treating wounds colonised by strong biofilm producers are likely prescribing a drug whose planktonic MIC underestimates the true *in vivo* concentration needed for clearance. This disconnect helps explain why some chronic wounds fail to respond to apparently appropriate systemic antibiotic therapy and why adjunctive biofilm-disrupting strategies — debridement, topical antiseptics containing chlorhexidine or hypochlorous acid, and anti-biofilm compounds — are increasingly recommended in veterinary wound care guidelines [4, 14, 19].

Discussion

The predominance of *S. pseudintermedius* (42.5%) aligns with global veterinary wound microbiology data. Bannoehr and Guardabassi [5] reported *S. pseudintermedius* isolation rates of 40-50% from canine pyoderma and surgical-site infections across European and North American centres. Our figure sits within that range, suggesting that wound flora in Australian companion animals follow the same species hierarchy. The relatively high proportion of *S. aureus* (27.5%) may reflect the mixed canine-feline sampling, as *S. aureus* is more commonly isolated from cat wounds and from zoonotic

transfer in multi-pet households [6, 13].

The 77.5% biofilm positivity rate is broadly consistent with reports from human wound microbiology. James *et al.* [3] used scanning electron microscopy to demonstrate biofilm in 60% of human chronic wounds, while Malone *et al.* [4] estimated that the figure may exceed 80% when sensitive detection methods are used. Our crystal violet assay approach provides a standardised, quantitative, and reproducible measure of biofilm capacity that can be incorporated into routine veterinary diagnostic workflows [9, 10, 17].

The correlation between biofilm strength and amoxicillin-clavulanate MIC ($r = 0.61$) is new information for veterinary wound isolates. In human settings, similar relationships have been documented for methicillin-resistant *S. aureus* (MRSA) and coagulase-negative staphylococci from prosthetic joint infections [12, 19]. The mechanism is thought to involve both physical barrier effects — the biofilm matrix restricting antibiotic diffusion — and metabolic dormancy within the biofilm interior, where slow-growing persister cells are inherently less vulnerable to beta-lactam drugs [10, 20]. For the practitioner, this means that standard susceptibility reports may be misleadingly optimistic when the wound harbours a strong biofilm former, and that clinical non-response to amoxicillin-clavulanate should prompt a shift to adjunctive wound management rather than simply escalating to broader-spectrum antibiotics [14, 15].

Conclusion

This research set out to characterise the biofilm-forming

capacity and antimicrobial susceptibility of *Staphylococcus* species isolated from chronic wound infections in companion animals at a Melbourne referral hospital, with a particular focus on whether biofilm strength predicts reduced susceptibility to amoxicillin-clavulanate — the first-line antibiotic for staphylococcal skin infections in Australian veterinary practice.

Three key findings emerged. First, *Staphylococcus* species were recovered from 80 of 146 chronic wound swabs (54.8%), and 77.5% of these isolates produced biofilm in the microtiter plate assay. *S. pseudintermedius* was the most common species (42.5%), followed by *S. aureus* (27.5%). Second, there was a significant positive correlation ($r = 0.61$, $p < 0.001$) between biofilm OD595 and amoxicillin-clavulanate MIC, confirming that stronger biofilm producers tend to be less susceptible to this antibiotic. Third, the *ica* operon genes (*icaA*, *icaD*) were present in approximately 70–74% of isolates, while *bap* and *aap* showed species-specific distribution patterns consistent with known PIA-dependent and PIA-independent biofilm pathways.

The practical takeaway for veterinary practitioners is straightforward: when a chronic wound in a dog or cat fails to respond to systemic amoxicillin-clavulanate despite apparent *in vitro* susceptibility, biofilm formation should be suspected. In such cases, the management plan should shift to incorporate physical biofilm disruption through surgical debridement, daily wound lavage, and application of topical antiseptics with proven anti-biofilm activity — chlorhexidine, povidone-iodine, or hypochlorous acid solutions — rather than escalating to reserve antimicrobials. This approach aligns with current antimicrobial stewardship principles and addresses the root cause of treatment failure rather than simply broadening the antibiotic spectrum.

Future research should extend this work in several directions. Prospective clinical trials comparing outcomes of biofilm-directed wound care protocols versus standard systemic antibiotic therapy alone would provide the highest level of evidence for changing clinical practice. Expanding the panel of biofilm genes tested — to include *agr* quorum-sensing loci, *sarA* regulatory genes, and *fnbA/fnbB* fibronectin-binding protein genes — would deepen understanding of the genetic architecture underlying biofilm formation in veterinary staphylococci. And longitudinal sampling of chronic wounds during treatment could reveal how biofilm community composition shifts in response to different antimicrobial interventions, helping clinicians tailor their approach to the evolving wound microbiology.

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References

1. Dernel WS. Initial wound management. *Vet Clin North Am Small Anim Pract.* 2006;36(4):713-738.
2. Amalsadvala T, Swaim SF. Management of hard-to-heal wounds. *Vet Clin North Am Small Anim Pract.* 2006;36(4):693-711.
3. James GA, Swogger E, Wolcott R, Pulcini ED, Secor P, Sestrich J, *et al.* Biofilms in chronic wounds. *Wound Repair Regen.* 2008;16(1):37-44.
4. Malone M, Bjarnsholt T, McBain AJ, James GA, Stoodley P, Leaper D, *et al.* The prevalence of biofilms in chronic wounds: a systematic review and meta-analysis of published data. *J Wound Care.* 2017;26(1):20-25.
5. Bannoehr J, Guardabassi L. *Staphylococcus pseudintermedius* in the dog: taxonomy, diagnostics, ecology, epidemiology and pathogenicity. *Vet Dermatol.* 2012;23(4):253-e52.
6. Weese JS. Methicillin-resistant *Staphylococcus aureus* in animals. *ILAR J.* 2010;51(3):233-244.
7. Arciola CR, Campoccia D, Ravaioli S, Montanaro L. Polysaccharide intercellular adhesin in biofilm: structural and regulatory aspects. *Front Cell Infect Microbiol.* 2015;5:7.
8. Cramton SE, Gerke C, Schnell NF, Nichols WW, Götz F. The intercellular adhesion (*ica*) locus is present in *Staphylococcus aureus* and is required for biofilm formation. *Infect Immun.* 1999;67(10):5427-5433.
9. Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *J Microbiol Methods.* 2000;40(2):175-179.
10. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet.* 2001;358(9276):135-138.
11. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. VET01-A5. Wayne (PA): CLSI; 2018.
12. Singh R, Ray P, Das A, Sharma M. Penetration of antibiotics through *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. *J Antimicrob Chemother.* 2010;65(9):1955-1958.
13. Loeffler A, Lloyd DH. Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? *Epidemiol Infect.* 2010;138(5):595-605.
14. Hillier A, Lloyd DH, Weese JS, Blondeau JM, Boothe D, Breitschwerdt *et al.* Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis. *Vet Dermatol.* 2014;25(3):163-e43.
15. Australian Veterinary Association. Antimicrobial prescribing guidelines for dogs and cats. Sydney: AVA Publications; 2021. p. 1-48.
16. Vasudevan P, Nair MKM, Annamalai T, Venkitanarayanan KS. Phenotypic and genotypic characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. *Vet Microbiol.* 2003;92(1-2):179-185.
17. Merritt JH, Kadouri DE, O'Toole GA. Growing and analyzing static biofilms. *Curr Protoc Microbiol.* 2005;Chapter 1:Unit 1B.1.
18. Cucarella C, Tormo MÁ, Úbeda C, Trotonda MP, Monzón M, Peris C, *et al.* Role of biofilm-associated protein *bap* in the pathogenesis of bovine *Staphylococcus aureus*. *Infect Immun.* 2004;72(4):2177-2185.
19. Brady RA, Leid JG, Calhoun JH, Costerton JW, Shirtliff ME. Osteomyelitis and the role of biofilms in chronic

infection. FEMS Immunol Med Microbiol. 2008;52(1):13-22.

20. Lewis K. Persister cells, dormancy and infectious disease. Nat Rev Microbiol. 2007;5(1):48-56.

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