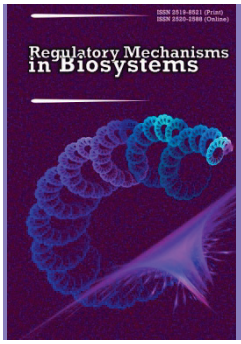

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Biofilm forming ability of coagulase-positive staphylococci isolated from animals in Ukraine

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Staphylococcal biofilms are an important virulence factor that allows for effective infectious effects and colonization of the animal body. This study was devoted to the evaluation of the biofilm forming ability of different strains of *Staphylococcus pseudintermedius* and *Staphylococcus aureus* isolated from animals in Ukraine. In addition, the presence of extracellular adhesin genes *icaA* and *icaD* in *S. pseudintermedius* strains was determined. The density of the biofilms was determined by culturing microorganisms in 96-well plates and staining the resulting structures with crystal violet. The genes responsible for biofilm formation were identified by classical polymerase chain reaction (PCR) using primers selected from the literature. The data obtained from this study showed a direct correlation between the density of the biofilm and the number of viable cells involved in its formation. Thus, 23.1% of *S. pseudintermedius* strains and 25% of *S. aureus* strains isolated from dogs demonstrated the ability to form a dense biofilm, while 46.2% of *S. pseudintermedius* strains and 50% of *S. aureus* strains formed a weak biofilm. The origin of the isolates had no significant effect on the biofilm characteristics. Coagulase-positive staphylococci obtained from cats did not form dense biofilms. 42.9% of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from cows with mastitis had moderate to strong biofilm forming properties. Isolates that showed resistance to three or more antibiotic groups tended to form denser biofilms. In addition, 73.3% of the studied *S. pseudintermedius* strains were found to contain the *icaA* gene, and 90% – the *icaD* gene. A genotypic profile combining both *icaA* and *icaD* genes was present in 66.7% of the bacteria, while one strain lacked both genes. Understanding the biofilm forming properties of staphylococcal isolates is important in the context of developing optimal treatment strategies and effective antibiotic use, which will contribute to better control of infections caused by these microorganisms.

Keywords: bacterial biofilms; biofilm formation genes; MRSA; antibiotic resistance; coagulase-positive staphylococci; optical density.

Introduction

To effectively colonize various biotic and abiotic surfaces, bacteria form complex consortiums – biofilm. This is an ordered structure formed by one or more species of microorganisms. They synthesize extracellular polymeric substances (EPS) that surround the bacteria and create a protective barrier around them (Rather et al., 2021). The composition of EPS, as well as the structure of the biofilm, can vary depending on the type of bacteria (Yan & Bassler, 2019). *Staphylococcus aureus* EPS includes various compounds: proteins, polysaccharides, nucleic acids, and lipids (Karygianni et al., 2020).

Within the biofilm, bacteria constantly interact with each other. Schilcher & Horswill (2020) draw attention to an extensive system of regulatory mechanisms that ensures the response of bacteria to environmental conditions (Palchykov et al., 2019; Zazharskyi et al., 2020). A number of staphylococcal quorum sensing systems (SaeRS, SigB, and CodY) are responsible for the synthesis of adhesins and the attachment of bacterial cells to surfaces, while other systems (Agr, Rot, and MgrA) weaken bacterial adhesion and promote their escape from the structure. These features help staphylococci to effectively protect themselves from environmental conditions and spread in the environment. In the context of the infectious process, biofilm formation significantly complicates the effect of the immune system on microorganisms. The study by Abdul Hamid et al. (2021) found that the immune response to planktonic and biofilm forms of bacteria has some differences. Two hours after infection, the number of polymorphonuclear lymphocytes was higher after biofilm culture, and 48 hours after infection, lymphocytes had higher activity. In addition, phagocytes were more mobile, and cells responding to biofilm infection were delayed at the periphery of the structure. Arima et al. (2018) confirm these findings, the inflammatory process is more intense than the reaction to strong biofilm producers. In addition to physical protection, the structural elements of the biofilm, staphylococci produce numerous pathogenicity factors that affect the course of the immune response and reduce its effectiveness (De Vor et al., 2020). Idrees et al. (2021) indicate that bacteria of this family can differentiate gene expression, depending on the type of lymphocytes involved in the immune response. This creates additional opportunities for adaptation to the effects of host immunity.

Bacteria in biofilms acquire additional mechanisms of antibiotic tolerance. The extracellular matrix makes it difficult for antibiotics to penetrate microbial cells. Inside the biofilm, the microenvironment is changed, which affects the mechanism of action of antimicrobial agents. It also contains persistent cells. These are metabolically inactive bacteria, which means that antibiotics cannot affect them (Sharma et al., 2019).

Antimicrobials show lower activity even against susceptible strains of *S. pseudintermedius* and *S. aureus* in biofilms (Rather et al., 2021). As noted by Tuon et al. (2023), for the effective treatment of staphylococcal infections associated with biofilm formation, it is necessary to use antibiotics in higher concentrations than in conventional treatment. For some drugs, it is impossible to achieve the required concentrations during medical use. Biofilms are also a favorable environment for the functioning of the horizontal gene transfer mechanism, which simplifies the acquisition of resistance by susceptible bacteria (Kranjec et al., 2021).

Staphylococci obtained from different animal and human species differ in biofilm formation activity. Vitale et al. (2019) concluded that human isolates of *S. aureus* have a higher level of biofilm forming properties. Also, Silva et al. (2022) noted that staphylococcal isolates from different animal species have differences in the potential to form biofilms.

A significant number of infections in which biofilm formation plays an important role are associated with various medical devices, such as central venous catheters, joint prostheses, and urinary catheters (Jamal et al., 2018). There are differences in the development of bacterial biofilms caused by *S. pseudintermedius* on abiotic and biotic surfaces. Bacteria proliferate much faster on structures made of synthetic materials (Kher et al., 2023).

Bacteria in the form of biofilms are often present in chronic wounds. They inhibit healing mechanisms and secrete pathogenicity factors that constantly affect the tissues at the site of injury. However, their involvement in the transition from acute to chronic wound process has not yet been established (Gajula et al., 2020; Thaarup et al., 2022). Biofilms also play an important role in the formation and development of numerous chronic infections in animals and can lead to relapse after treatment (Nesse et al., 2023).

The aim of the study was to investigate the ability of coagulase-positive staphylococci isolated from companion animals and cows to form biofilms and to identify genes associated with biofilm formation in *S. pseudintermedius* isolates.

Material and methods

The experimental studies were conducted in compliance with the relevant requirements and standards, in particular, they meet the requirements of DSTU ISO/IEC 17025:2005 (2006). All manipulations were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Convention, 1986).

In total, 22 cultures of *S. pseudintermedius* and 6 cultures of *S. aureus* from diseased animals were studied, divided into 5 groups according to their origin: eye, ear, nose, body (skin lesions and wound infections) and milk. All cultures were tested for resistance to 8 antibacterial agents by agar diffusion method (Table 1). Cultures that showed intermediate resistance in this study were classified as resistant. We also studied 8 *S. pseudintermedius* and 4 *S. aureus* isolates from healthy animals, and methicillin resistance was determined for these cultures. One isolate of *S. pseudintermedius* from healthy animals was methicillin-resistant, all others were sensitive. Selection, identification, and determination of antibiotic resistance of isolates from sick and healthy animals have been described previously (Shevchenko et al., 2023).

Table 1
Antibiotic resistance of the isolates used in the study

Antibiotic	S. *pseudintermedius*, n	*S. aureus*, n
Penicillin (10 OD)	4	3
Gentamicin (10 μg)	1	0
Trimethoprim-sulfamethoxazole (1.25/23.7 5 μg)	12	3
Tetracycline (30 μg)	1	0
Erythromycin (15 μg)	4	1
Ciprofloxacin (5 μg)	2	2
Sensitive to antibiotics	7	1

We also studied 8 methicillin-resistant *S. aureus* isolates from cows with mastitis. All isolates were resistant to benzylpenicillin (1 IU) and cefoxitime (30 μg), 3 isolates were resistant to kanamycin (30 μg), 2 isolates were resistant to clindamycin (2 μg), 2 isolates were resistant to tetracycline (30 μg) and 2 isolates were resistant to ciprofloxacin (5 μg). Antibiotic resistance was determined by the disc diffusion method, and MRSA strains were considered to be resistant to the antibiotic cefoxitin (Shevchenko & Andriichuk, 2023).

20 μL of a suspension of each bacterial strain diluted to 0.5 by McFarland's standard was added to a 96-well flat-bottom microplate. 180 μL of trypsin soy broth (Merck, Germany) containing 1% glucose was added to each well and incubated at 37 °C for 24 hours. Sterile trypsin soy broth (TSB) was used as a negative control. After incubation, the wells were washed three times with 300 μL of phosphate-buffered saline (PBS) to remove planktonic bacteria. The biofilm was fixed with methyl alcohol, dried, and stained with 1% crystal violet alcohol solution (Macrochem, Ukraine) for 15 min. Excess stain was washed off with distilled water. After drying, a mixture of ethyl alcohol and acetone in a ratio of 8:2 was added to the well and left for 10 min. Then the absorbance at 492 nm was measured using a Rayto RT-2100C microplate reader (China) (Cafiso et al., 2007). Each culture was inoculated in triplicate and the average was calculated. The minimum optical density limit (ODc) was calculated relative to the control. Three standard deviations (mean $OD_{control}$ + 3 * SD) were added to the mean optical density of the negative control. All isolates were classified according to their biofilm formation ability into non-biofilm forming strains (OD ≤ ODc), strains with low biofilm formation activity (ODc < OD ≤ 2ODc), medium biofilm formation activity (2ODc < OD ≤ 4ODc), and strong biofilm formation activity (4ODc < OD) (Jantorn et al., 2021).

Biofilm formation genes were determined by classical PCR.

For DNA extraction, a bacterial suspension was prepared at a concentration of 4 according to the McFarlend standard. 200 μL of the suspension was transferred to a separate tube. IndiSpin Pathogen Kit (Indical, Germany) was used for DNA extraction. The amplification reaction was performed in a 25 μL reaction mixture, which included: 12.5 μL of One-Taq® 2X Master Mix with Standard Buffer (New England Biolabs, USA), 7.5 μL of deionized water, 1 μL of oligonucleotides and 3 μL of DNA. The amplification was performed in a GeneAmp PCR System 2400 thermal cycler (Applied Biosystems, USA). Primers used in the study were selected from the literature: *icaA* R 5'ACTGTTTCGGGAC AAGCAT'3. *icaA* F 5'ATTGAGGCTGTAGGGCGTTG'3 product size 134 bp; *icaD* R 5'CGTTAATGCCTTCTTTCTTATTGCG'3 *icaD* F 5'ATTAGCGCACATTCGGTGTT'3 product size 166 bp (Meroni et al., 2019), primers synthesized by YURiA-PHARM LLC (Ukraine).

The results were detected in a 2% agarose gel with the addition of 0.5% ethidium bromide. The reaction was analyzed by the presence of a specific band of the appropriate size opposite the well with the positive control and the absence of a corresponding band opposite the well with the negative controls.

Statistical processing of the results was performed in the program Jamovi ver. 2.3 (2023, Australia). The Shapiro-Wilk test and Q-Q plot were used to determine the normality of the distribution. To confirm a statistically significant difference between the two groups, the Mann-Whitney U test was used for data with a non-normal distribution. The Kruskal-Wallis test was used for analysis of variance.

Results

The results of the study showed that staphylococcal strains differed in their ability to form biofilm. Of the 22 studied isolates of *S. pseudintermedius* selected from sick animals, 10 (45.5%) were identified as weak biofilm producers. This group included 6 (54.5%) isolates isolated from the body, 1 (25%) isolate from the ear and all 3 isolates from the eye. 60% of the isolates in this group were resistant to one or more antibacterial agents. 7 isolates of *S. pseudintermedius* (31.9%) had moderate biofilm forming properties. This group included 3 (27.2%) isolates from the body, 1 (25%) isolate from the ear, 2 (66.7%) isolates from the nose and one from milk. 85.7% of the isolates were resistant to one or more antibiotics. A strong biofilm forming activity was determined in 5 (22.7%) isolates of *S. pseudintermedius*. Two (50%) were obtained from the ear, 2 from the body (18.2%) and 1 (33.3%) from the nose. 60% of the isolates were resistant to one or more antibiotics (Table 2). Isolates that were resistant to 3 and 5 antibacterial agents formed the highest density biofilms.

After dye extraction, the optical density in the wells with *S. pseudintermedius* with weak biofilm forming properties was 0.337 ± 0.052 OD_{492} (median 0.342), in the group with moderate biofilm forming properties – 0.486 ± 0.021 (median 0.454). In the study of strong biofilm producers, the optical density was in the range of 1.06 ± 0.32 (median 0.96).

Of the *S. aureus* isolates studied, two isolates had a weak biofilm production capacity (33.3%) and were isolated from the body. Two isolates with medium biofilm forming properties were isolated from the body and one from the ear (50% in total). One isolate with the ability to form a dense biofilm (16.7%) was obtained from the body. The optical density of

the solution for the study of *S. aureus* isolates with weak biofilm forming properties was 0.531 ± 0.066 OD_{492} (median 0.520, Table 3).

Table 2
Ability to form a biofilm in canine and feline isolates of *S. pseudintermedius* selected from sick animals

Animal species	Source of isolation	Resistance profile	Biofilm formation genes	OD, (n = 3) mean ± SD	Biofilm formation activity
Dog	body	SXT	*icaD*	0.295 ± 0.027	weak
Dog	body	SXT	*icaA/D*	0.511 ± 0.088	medium
Dog	nose	SXT+ERY	*icaA/D*	0.454 ± 0.085	medium
Dog	ear	SXT	*icaA/D*	0.958 ± 0.106	strong
Dog	nose	GEN+SXT+PEN+TET+ERY	*icaA/D*	1.618 ± 0.579	strong
Dog	body	SA	*icaD*	0.259 ± 0.017	weak
Dog	eye	SXT	*icaA/D*	0.286 ± 0.022	weak
Dog	body	SA	*icaA*	0.360 ± 0.085	weak
Dog	body	SA	*icaA/D*	0.368 ± 0.011	weak
Dog	milk	SXT	*icaA/D*	0.444 ± 0.103	medium
Dog	ear	SA	*icaA/D*	0.538 ± 0.100	medium
Dog	ear	CIP	*icaA/D*	0.867 ± 0.149	strong
Dog	body	SXT+PEN+ERY	*icaA/D*	0.987 ± 0.119	strong
Dog	eye	SXT	*icaA/D*	0.386 ± 0.045	weak
Dog	body	CIP	*icaA/D*	0.392 ± 0.058	weak
Dog	body	PEN+ERY	*icaA/D*	0.453 ± 0.092	medium
Dog	body	SXT+PEN	*icaD*	0.572 ± 0.136	medium
Dog	body	SA	*icaA/D*	0.850 ± 0.102	strong
Cat	eye	CIP	*icaD*	0.323 ± 0.060	weak
Cat	body	SA	*icaA/D*	0.292 ± 0.070	weak
Cat	ear	SXT	*icaD*	0.406 ± 0.121	weak
Cat	nose	SXT+TET	*icaA/D*	0.432 ± 0.079	medium

Note: SXT – trimethoprim-sulfamethoxazole, GEN – gentamicin, PEN – penicillin, TET – tetracyclin, ERY – erythromycin, CIP – ciprofloxacin, SA – sensitive to antibiotics.

Table 3
Ability to form a biofilm in canine and feline isolates *S. aureus* selected from sick animals

Animal species	Source of isolation	Resistance profile	OD, (n = 3) mean ± SD	Biofilm formation activity
Dog	body	SXT+PEN+ERY	1.191 ± 0.049	strong
Dog	body	PEN	0.471 ± 0.090	medium
Dog	body	SXT	0.272 ± 0.010	weak
Dog	body	PEN+CIP	0.302 ± 0.037	weak
Cat	body	SA	0.601 ± 0.144	medium
Cat	ear	SXT+CIP	0.502 ± 0.019	medium

Note: SXT – trimethoprim-sulfamethoxazole, PEN – penicillin, ERY – erythromycin, CIP – ciprofloxacin, SA – sensitive to antibiotics.

The optical density of bacteria from different parts of the body (χ^2 = 6.12, P = 0.190) did not differ significantly. No statistically significant differences were found when comparing the optical density of isolates from cats and dogs (P = 0.604). There was also no statistically significant difference between different pathogens (P = 0.764).

A total of 8 isolates of *S. pseudintermedius* were studied, 4 weak (50%), three moderate (37.5%) and 1 strong (12.5%) biofilm producers were identified. Of the 4 isolates of *S. aureus*, two produced weak biofilm (50%), one moderate (25%) and one strong (25%). The optical density of the solution of weak producers of *S. pseudintermedius* was 0.279 ± 0.043 OD_{492} (median 0.265). One isolate (25%) of *S. aureus* had medium and strong biofilm forming properties, and two weak (50%, Table 4).

Among the methicillin-resistant *S. aureus* obtained from cows, three strong (42.9%), three moderate (42.9%) and one weak (14.2%) biofilm producers were identified (Fig. 5). The optical density in the study of moderate biofilm producers was 0.533 ± 0.072 OD_{492}, and strong biofilm producers were 1.120 ± 0.203 OD_{492}.

The extracellular adhesin genes were found in 29 (96.7%) isolates of *S. pseudintermedius*, *icaA* in 22 (73.3%) and *icaD* in 27 (90%, Fig. 1). The genotypic profile of *icaA+icaD* was present in 20 (66.7%) of the studied cultures, *icaA* alone in 2 (6.7%), and *icaD* alone in 7 (23.3%) isolates.

Table 4
Ability to form a biofilm in canine *S. pseudintermedius* and *S. aureus* selected from healthy animals (n = 3)

Animal species	Source of isolation	Biofilm formation genes	OD, x ± SD	Biofilm formation activity
MRSP	nose	*icaA/D*	0.862 ± 0.041	strong
S. pseudintermedius	ear	*icaA/D*	0.262 ± 0.043	weak
S. pseudintermedius	ear	*icaA*	0.244 ± 0.042	weak
S. pseudintermedius	ear	*icaD*	0.742 ± 0.082	medium
S. pseudintermedius	ear	*icaD*	0.513 ± 0.033	medium
S. pseudintermedius	ear	*icaA/D*	0.413 ± 0.038	medium
S. pseudintermedius	nose	icaNG	0.267 ± 0.036	weak
S. pseudintermedius	nose	*icaA/D*	0.341 ± 0.069	weak
S. aureus	ear	–	0.261 ± 0.014	weak
S. aureus	ear	–	0.434 ± 0.038	medium
S. aureus	nose	–	0.796 ± 0.052	strong
S. aureus	nose	–	0.348 ± 0.036	weak

Table 5
Indicators of biofilm density and the number of live cells of MSRA isolates collected from cows (n = 3)

Resistance profile	OD, x ± SD	Biofilm formation activity
AMX+CEF+CLD	0.448 ± 0.017	medium
AMX+CEF+CLD+KAN+CIP	1.092 ± 0.063	strong
AMX+CEF+KAN	0.225 ± 0.011	weak
AMX+CEF+TET+KAN	1.382 ± 0.018	strong
AMX+CEF+TET	0.542 ± 0.132	medium
AMX+CEF	0.886 ± 0.022	strong
AMX+CEF+CIP	0.626 ± 0.019	medium

Note: AMX – amoxicillin and clavulanic acid, CEF – cefoxitin, TET – tetracyclin, CLD – clindamycin, KAN – kanamycin.

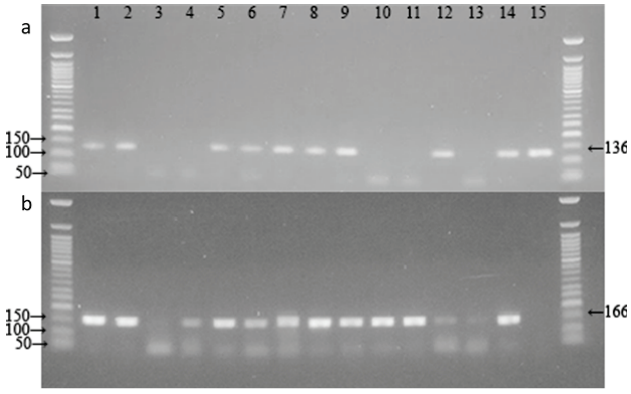

Fig. 1. PCR results of gene detection: *a* – PCR results of *icaA* gene detection (136 bp); *b* – PCR results of *icaD* gene detection (166 bp); 1,2,5,6,7,8,9,12,14 – *icaA/D*; 3,4,10,11,13 – *icaD*; 15 – *icaA*; no differences were found between the groups with *icaA/D* genes and the optical density of the solution in the biofilm study (χ^2 = 0.685, P = 0.710)

Discussion

Biofilm formation plays a key role in the pathogenicity of bacteria. The research community is actively studying the phenomenon of biofilm formation by different species of staphylococci. Therefore, in this publication, we have studied the ability to form biofilms in *S. pseudintermedius* and *S. aureus* strains obtained in our previous studies.

Notcovich et al. (2018) reported a strong biofilm formation ability in 27.1% of the studied milk-derived *S. aureus* cultures. In the study by Shah et al. (2019), 20% of isolates had a high biofilm formation ability. At the same time, MRSA isolates were significantly more likely (65%) to have a high level of biofilm production than methicillin-susceptible isolates (5%). In a study by Wu et al. (2019), 20.7% of strains had the ability to form a dense biofilm. Oxacillin-susceptible isolates more often did not form a biofilm than resistant strains, which is consistent with the results of the previous team. Thus, MRSA isolated from cows more often have the potential to form a dense biofilm than MSSA. Garkavenko et al. (2020),

studied *Staphylococcus aureus* isolated from food. According to their data, 57.1% of MRSA strains showed a high ability to form biofilms, as did bacteria obtained from patients with bovis mastitis. We observed the same trends as the previously mentioned authors: isolates resistant to β-lactam antibiotics were more prone to form a dense biofilm. In our study, 42.9% of MRSA strains had a high biofilm formation capacity. We did not study the biofilm forming properties of methicillin-susceptible isolates obtained from cow's milk. However, 20% of *S. aureus* isolates from companion animals had a high degree of biofilm forming properties, which is consistent with the data from studies of milk isolates.

The data on the biofilm forming potential of *S. pseudintermedius* provided by some authors are similar to those obtained in the study of *S. aureus*. Meroni et al. (2019) found that the number of isolates with strong biofilm forming properties was 28.8%. Wang et al. (2022) also reported similar results: 24.1% of the studied strains had a high biofilm formation capacity. Our results are consistent with the data of these researchers: 23.4% of *S. pseudintermedius* isolates had a high biofilm formation capacity.

The results of Jantorn et al. (2021) are different. Strong biofilm forming properties were found in 41.5% of *S. pseudintermedius*, which is higher than the previously described values. The authors did not find a statistically significant difference between MRSP and MSSP. However, there is a strong correlation between biofilm forming properties and resistance of isolates to several antibiotics. We detected only one MRSP isolate, so we cannot compare the effect of the methicillin resistance factor on biofilm formation activity. In our study, isolates resistant to 3 and 5 antibiotics had the highest optical density values.

Chan et al. (2019) did not find any strong biofilm producers among clinical isolates of *S. pseudintermedius*. Based on their own research and the results of other authors, it can be argued that the biofilm forming properties of different staphylococcal strains may differ slightly. Therefore, there must be genetic factors that contribute to the formation of a denser biofilm. We studied the presence of genes that are part of the *ica* operon. This cluster of genes is responsible for the synthesis of polysaccharide intercellular adhesin (PIA/PNAG). These compounds play a crucial role in the formation of biofilms, as they ensure the adhesion of bacteria to biotic and abiotic surfaces. The operon consists of four *icaABCD* genes responsible for a synset of enzymes that control different steps of PIA biosynthesis and modeling. *IcaA* is responsible for the initiation of polysaccharide synthesis, *icaD* enhances the activity of the *icaA* enzyme and helps to form the polysaccharide chain (François et al., 2023).

Vishovan et al. (2021), *ica* genes were found in staphylococci isolated from different sources. Of the two isolates of coagulase-positive staphylococci from companion animals, one had the *icaA* gene. In a study by Wang et al. (2022), these genes were found in 96.6% of isolates. At the same time, they were present in all isolates collected from animals with keratitis and absent in one isolate from a healthy dog, and had the same *icaA*+ *icaD* profile. A study by Meroni et al. (2020) found fewer (69.6%) isolates with the *icaA* gene than *icaD* (100%). According to our data, the *icaD* gene was present in more isolates (90%) than the *icaA* gene (73.3%). In one isolate, the gene was not detected at all, it had the lowest biofilm density, but higher than in the negative control. There are also reports of detection of these genes in all the studied isolates that were submitted to the laboratory by Phophi et al. (2023). In contrast, Hritcu et al. (2020) found only one isolate of *S. pseudintermedius* that expressed the *icaD* gene. However, the primer set they used for the study was originally designed to detect genes in *S. aureus*. In the original study by the authors who developed the *icaA* primer, there is evidence of cross-hybridization with *S. pseudintermedius*. The absence of a positive reaction does not allow us to assert the absence of these genes in the studied strains (Cramton et al., 1999).

In this study, we investigated the degree of biofilm formation properties of *S. aureus* and *S. pseudintermedius* isolates. We and other authors have noted slightly higher biofilm formation properties among methicillin-resistant bacteria. Such trends arise because resistant bacteria have higher biofilm forming activity, or vice versa, because biofilm forming bacteria are more likely to acquire resistance to antibacterial agents. Intercellular adhesin genes are not associated with biofilm forming activity. More detailed studies are needed to compare the genetic characteristics of isolates that differ in their biofilm forming potential.

Conclusion

Coagulase-positive staphylococci form biofilms of different densities. Among the isolates of *S. pseudintermedius* taken from sick animals, 45.5% formed a low density biofilm, 31.8% of medium density and 22.7% of high density. Among the pathogenic *S. aureus*, 33.3% of isolates formed a weak biofilm, 50% of isolates formed a medium biofilm and 16.7% of isolates formed a dense biofilm. Isolates collected from healthy animals more often formed a weak biofilm 62.5% of *S. pseudintermedius* and 50% of *S. aureus*. MRSA isolates collected from cows mainly formed a dense and medium biofilm in 42.9% of isolates. The *icaD* gene was more frequent 90% than *icaA* 73%. Both genes were present in 67% of the studied microorganisms, one isolate did not have both genes. The presence of *ica* genes does not correlate with biofilm indicators, additional studies of other operons associated with biofilm formation are needed.

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