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Microbiological and molecular genetic characterization of *Staphylococcus aureus* and *Staphylococcus pseudintermedius*

Shevchenko M., **Tsarenko T.**

Bila Tserkva National Agrarian University

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Coagulase-positive staphylococci are an important infectious agent causing numerous infections in animals. *Staphylococcus aureus* and *Staphylococcus pseudintermedius* share a number of similar cultural and biochemical characteristics, which makes their differentiation difficult. Since these species have different zoonotic potential, it is advisable to develop rapid and specific schemes for species differentiation of pathogens.

We have studied the cultural and biochemical characteristics of *Staphylococcus* spp. isolated from dogs, cats and cows. In total, 103 halophilic coccal cultures were isolated from companion animals and 45 from cows, of which 55 and 30 cultures were coagulase-positive staphylococci, respectively. The reactions that can be used to differentiate S. *pseudintermedius* and S. *aureus* were studied. Growth inhibition zones around the disk with polymyxin B antibiotic for S. *pseudintermedius* were statistically higher ($p<0.001$) than for S. *aureus*. The determination of acetone production to differentiate between these pathogens has less specificity, as 30% of S. *pseudintermedius* showed a false-positive reaction. The belonging of two isolates to the species *Staphylococcus pseudintermedius* was confirmed by MALDI-TOF.

The virulence of staphylococci is due to the presence of genes that regulate the synthesis of various pathogenicity factors and cause antibiotic resistance. Molecular genetic methods can detect the presence of gene specificity and help to assess the risk of a particular strain causing infection. Using classical and real-time PCR, the mecA gene was detected in 8 S. *aureus* strains and 1 S. *pseudintermedius* strain that showed phenotypic resistance to methicillin. The pathogenicity genes *lukF* and siet were present in 100%, and the *lukS* gene in 90% of the studied *Staphylococcus pseudintermedius*.

The study highlights a number of aspects of the diagnosis and differentiation of coagulase-positive staphylococci. The possibility of using the Neonatal FAST well D-ONE microculture system for use in veterinary laboratories was also studied. The data obtained can be used to develop methodological approaches to the identification of pathogenic staphylococci using a combination of different methods.

Key words: *S. pseudintermedius*, resistance to methicillin, pathogenicity genes, MALDI-TOF MS.

Problem statement and analysis of recent research. *Staphylococcus* spp. is an important pathogen that causes numerous infections in various animal species and is an urgent problem for veterinary medicine. Currently, the *Staphylococcus* spp. family includes more than 45 species that differ in a set of pathogenic factors [1].

The group of coagulase-positive staphylococci is considered to be the most virulent. S. *aureus* is well studied and is a common pathogen among all animal and human species. S. *pseudintermedius* and S. *cougulans* [2] are microorganisms associated with dogs, and S. *intermedius* can be detected in sick companion animals, so in mastitis in cows, S. *hyicus* is a species-specific pathogen for pigs [3].

Coagulase is an enzyme that causes blood plasma to clot and form a clot. However, the mechanism of plasma coagulation initiation differs for different

staphylococci. The coa gene, which encodes classical coagulase, is present only in staphylococci of the S. *aureus* complex. In other CoPS species, coagulase activity is associated with the von Willebrand factor binding protein in plasma [4].

Hemolysin is another important pathogenicity factor in staphylococci. The results of the effect of this enzyme on erythrocytes are used as a diagnostic criterion for the identification and differentiation of staphylococci. The vast majority of S. *aureus* strains produce β-hemolysin, unlike other less pathogenic staphylococci [5]. Other CoPS are also capable of producing hemolysins [6].

Some authors have reported that a small number of S. *aureus* and S. *pseudintermedius* isolates may not cause erythrocyte hemolysis [7, 8]. Because of this, they may be misidentified as coagulase-negative staphylococci (CoNS) if microbiological schemes provide for further stages of testing only after establishing hemolytic activity on blood agar.

In addition to the above enzymes used for species differentiation, staphylococci synthesize a wide range of other compounds that determine pathogenicity and the ability to suppress the immune response. These pathogenicity factors determine the species specificity of bacteria [9].

In the context of human health, staphylococci pose a danger not only as an infectious pathogen, but also as a source of enterotoxins (SE) as they can cause food poisoning. S. *aureus* strains in different geographical regions and animal populations differ in the genes encoding the synthesis of enterotoxins [10]. Other members of the CoPS group are also capable of producing these toxins, and it is important to understand what factors influence this activity [11, 12].

Determination of antibiotic resistance requires accurate species identification of staphylococcal isolates, as this affects the interpretation of the results. For example, according to the EUCAST and CLSI standards, the antibiotic cefoxitin should be used to detect MRSA isolates, while oxacillin is used for MRSP. The growth retardation zones of SIG representatives around the cefoxitin disk do not correlate with the presence of the mecA gene [13]. However, tests using this drug have higher sensitivity and specificity for detecting MRSA [14].

Accurate identification and differentiation of CoPS is important for establishing a definitive diagnosis, studying the prevalence of different staphylococci, characterizing antibiotic resistance of strains and determining their zoonotic potential. However, the use of selective and rapid biochemical tests is not sufficient to differentiate *Staphylococcus* intermedius group (SIG) from the *Staphylococcus aureus* complex [15]. There is also a problem of differentiating SIG staphylococci from each other, as it requires the use of additional reactions [16]. Currently, much attention is being paid to the possibility of using modern methods based on molecular genetic studies or mass spectrometry to differentiate different staphylococci [15, 17].

The aim of our work was to study the cultural and biochemical properties of *Staphylococcus* spp. isolates obtained from different animals, to detect the mecA gene and genes associated with pathogenicity factors.

Material and methods. The research was conducted at the research laboratory of veterinary and sanitary expertise and laboratory diagnostics of the Institute of Postgraduate Training of Veterinary Medicine Managers and Specialists of Bila Tserkva NAU, within the framework of the initiative topic of the Department of Epizootology and Infectious Diseases of the FVM BNAU "Study of the role of opportunistic pathogens in the etiology and pathogenesis of animal diseases" No. state registration 0121U110291.

Sampling was carried out in June 2021 and from June 2022 to May 2023. We collected 105 ear and 58 nasal samples from healthy dogs. 109 samples were collected from sick animals: 107 samples of skin and mucous membranes of different parts of the body and 2 samples of milk.

Milk samples in the amount of 90 pieces were received by the laboratory in 2021-2022.

Identification and phenotypic antibiotic resistance of isolates from companion animals [18] and milk [19] have been described previously.

Materials from companion animals were inoculated into primary enrichment medium: nutrient broth (Himedia, USA) or trephine-soy broth (Merck, Germany). Then, they were inoculated on the surface of mannitol salt agar (Merck, Germany) or Bid-Parker agar (Himedia, USA), and Colombian agar with 5% sheep blood (Biomerieux, France). After gram staining, coccal Gram+ colonies were tested for the presence of oxidase, catalase, and coagulase.

The final confirmation of the genus of the isolates to *Staphylococcus* spp. and the differentiation of S. *aureus* and S. *pseudintermedius* was performed using classical PCR.

The isolates of S. *aureus* and S. *pseudintermedius* were additionally tested for resistance to the antibiotic Polymyxin B 300 U (Himedia, USA) and acetone production.

Catalase reaction on glass. A bacillus of pure culture was added to a drop of hydrogen peroxide (Viola, Urkaina). Foaming indicated a positive reaction.

Detection of cytochrome oxidase. The bacterial culture was added to the test zone of the OXItest strip (Erba, USA). No color change indicates a negative reaction.

To determine the hemolytic activity, cultures were inoculated on the surface of the CBA and cultured for 24 hours at 37°C. The zone of enlightenment around the colonies indicated hemolytic activity.

To detect DNA polymerase activity, the cultures were inoculated on the surface of DNA agar (Himedia, USA) and cultured for 24 hours at 37°C. After cultivation, a 1H sulfuric acid solution was applied to the agar surface. The formation of a zone of enlightenment indicated the presence of DNAase [20].

The Voges-Proskauer reaction was used to detect the formation of acetone. Pure cultures were grown overnight in Clark's glucose-phosphate broth (Farmaktiv, Ukraine). After cultivation, 0.6 α-naphthol and 0.2 ml of 40% KOH were added. Reddening of the medium within 10 min indicated a positive reaction.

Resistance to Polymyxin B was determined by the agar diffusion method. A bacterial suspension diluted to 0.5 according to the MacFarland standard was applied to the surface of Muller-Hinton agar (Himedia, USA). The suspension was evenly distributed over the surface of the agar, the excess volume was removed, and the dish was dried. After that, a disk with Polymyxin B was placed on the surface of the medium and cultured for a day at 37°C. After cultivation, the growth inhibition zone was measured.

The study was performed using the NEONATAL FAST well d-one microculture platform (CPM SAS, Italy). 20 µL of bacterial suspension diluted to 0.5 by MacFarland's standard was added to the accumulation medium (included in the kit) and cultured for 2 hours at 37°C. After that, 150 µL of the medium was resuspended in 10 ml of sterile saline. 150 µL of the resulting suspension was added to the wells of the kit. According to the instructions, two drops of sterile paraffin were added to some wells. The identification panel was cultured for 24 hours at 37°C.

For DNA extraction, a bacterial suspension was prepared at a concentration of 4 according to the McFarlenda standard.

The RT-PCR reaction was performed on 22 isolates of S. *pseudintermedius* and 6 S. *aureus* from sick animals, and 4 S. *pseudintermedius* from healthy animals. In the classical PCR reaction, 30 isolates of S. *pseudintermedius*, 10 S. *aureus* from companion animals and 8 S. *aureus* from cows were tested.

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. Amplification was performed in a GeneAmp PCR System 2400 thermal cycler (Applied Biosystems, USA). Primers for the study were selected from the literature (Table 1) [21].

Real-time PCR was performed using the DNA-technology multipltx REAL-TIME PCR Detection Kit (DNA technology). 10 µL of polymerase and 5 µL of test DNA were added to the wells of the kit. Temperature conditions were set according to the instructions. In addition, negative and positive controls were added. The reaction was read in CFX96 (Bio-Rad, USA).

Table 1– **Oligonucleotides used in the study**

Name	Oligonucleotide (5'-3')	Annealing temperature, °C	Product size, bp
siet-F	ATGGAAAATTTAGCGGCATCTGG	50	359
siet-R	CCATTACTTTTCGCTTGTTGTGC		
Luk F-F	CCTGTCTATGCCGCTAATCAA	50	572
Luk F-R	AGGTCATGGAAGCTATCTCGA		
Luk S-F	TGTAAGCAGCAGAAAATGGGG	51	503
Luk S-R	GCCCGATAGGACTTCTTACAA		
mecA-F	TCCAGATTACAACTTCACCAGG	49	532
mecA-R	CCACTTCATATCTTGTAACG		

Identification of cultures by MALDI-TOF MS was performed in the laboratory of EC Biolights LLC (Ukraine).

Statistical processing was performed in Jamovi ver. 2.3.28. Mann-Whitney U test was used to determine a statistically significant difference.

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).

Results of the study. As a result of the study of materials obtained from animals, cultures of coagulase-positive staphylococci were isolated. In total, 103 coccal cultures were isolated from companion animals, which grew on a medium with a salt content of 4.5%. 100 cultures were catalase-positive and 3 were catalase-negative. All catalase-negative cultures were obtained from dogs. Mannitol was fermented by 72 cultures obtained from companion animals and 35 cultures from cows (Table 2).

The biochemical characteristics of 30 isolates of S. *pseudintermedius* and 10 S. *aureus* were studied. All studied *S. aureus* and 30% of *S. pseudintermedius* produced acetoin (Fig. 1, A). The zone of growth retardation around the disk with Polymyxin B antibiotic was within 9.5±0.6 mm (Fig. 1, B1) for *S. aureus* isolates and 15.2±0.8 mm (Fig. 1, B2) for *S. pseudintermedius isolates*. All coagulase-positive staphylococci caused b-hemolysis on blood agar (Fig. 1, C).

All tested S. *aureus* and 29 S. *pseudintermedius* isolates produced DNAase (Fig. 1, D).

Two isolates of S. *pseudintermedius* were tested using the Neonatal FAST well D-ONE kit. After culturing, we found that some of the wells changed color. The reactions were not specific for S. *aureus*, which the kit is designed to detect (Fig. 3). The reactions in wells 6, 7, 15 and 19 differed between different isolates of S. *pseudintermedius* (Fig. 2).

Both isolates tested were resistant to all antibiotics in the kit. One tested isolate was methicillin resistant and the other methicillin sensitive. The MRSA test showed a positive reaction for MRSP. When testing the S. *aureus* isolate, we got the expected result with no false negatives. The microculture kit is not sufficiently informative for the identification of S. *pseudintermedius*, and its use in veterinary medicine requires additional research.

Table 2 – **Cultural and biochemical characteristics of *Staphylococcus* spp. isolates**

Characteristics of the pathogen	Dogs		Cats	Cows
	Healthy	Sick	Sick	Sick
Halophilic Gr+ cocci	59	31	13	45
Mannitol fermentation	44	22	6	35
Presence of catalase	56	31	13	45
Oxidative activity	0	0	0	0
Blood plasma coagulation	27	22	6	30
CoPS	27	22	6	30
CoNS	29	9	7	15
Other cocci	3	0	0	0

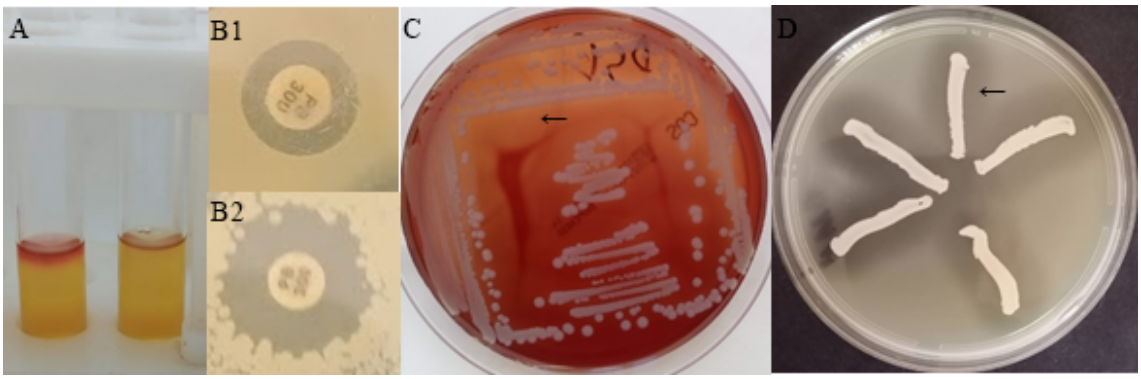

Fig. 1. **Cultural and biochemical characteristics of *Staphylococcus* spp. isolates.**
A. VP test results. 1 - weakly positive, 2 - negative; b. Zone of growth inhibition around the disk with Polymyxin B. 1-10 mm S. *aureus*, 2-16 mm S. *pseudintermedius*; C. Beta-hemolysis of S. *pseudintermedius* on blood agar; D. Zone of enlightenment on DNA agar around 5 strains of S. *pseudintermedius*.

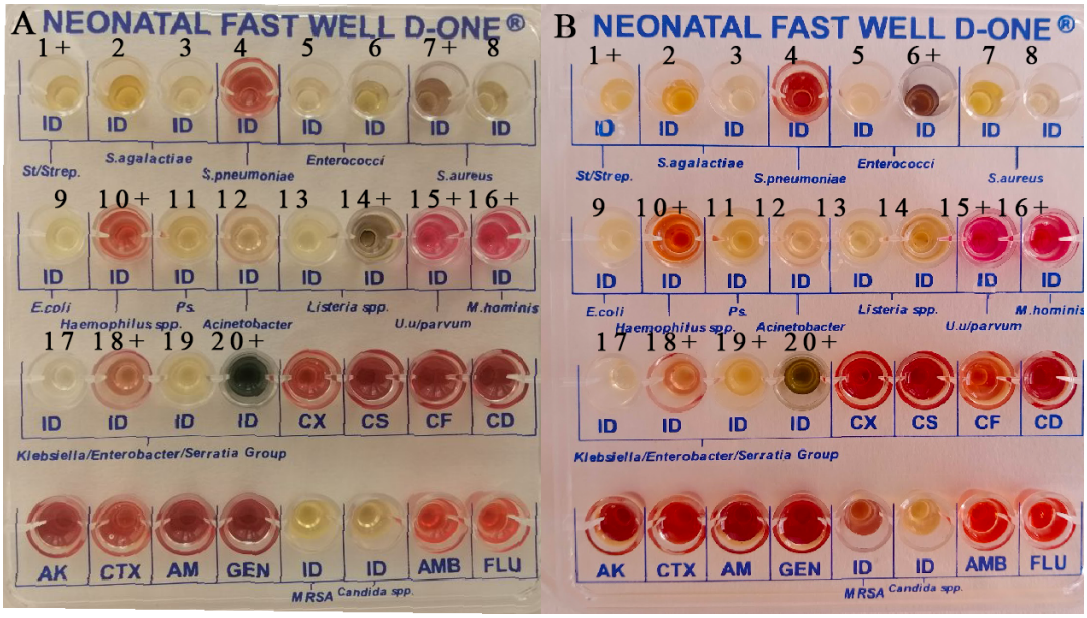

Fig. 2. **Neonatal FAST well D-ONE plates after 24 hours of thermostatting. Positive reactions are marked with a "+" symbol. *S. pseudintermedius* isolates № 1; B. *S. pseudintermedius* isolates № 2.**

To finally confirm that the staphylococci belonged to the S. *pseudintermedius* species, identification was performed using MALDI-TOF MS (Fig. 3).

X-axis: mass of one charge in daltons (m/z, Da), y-axis: signal intensity (conventional units).

Both tested cultures were identified as S. *pseudintermedius*.

According to the results of the study, methicillin resistance genes were found in 7 isolates of S. *aureus* from cows and 1 isolate of S. *pseudintermedius* from a healthy dog. MRSP testing of the isolates was performed using classical PCR and real-time PCR (Fig. 4). The data from both studies are consistent.

Results of the RT-PCR test. Blue line - positive control. Orange lines - test samples. Green line - MRSP strain.

The presence of genes associated with pathogenicity was also determined (Fig. 5). The *lukS* gene was found in 90% of the isolates, while the *lukF* and siet genes were found in 100% of the isolates.

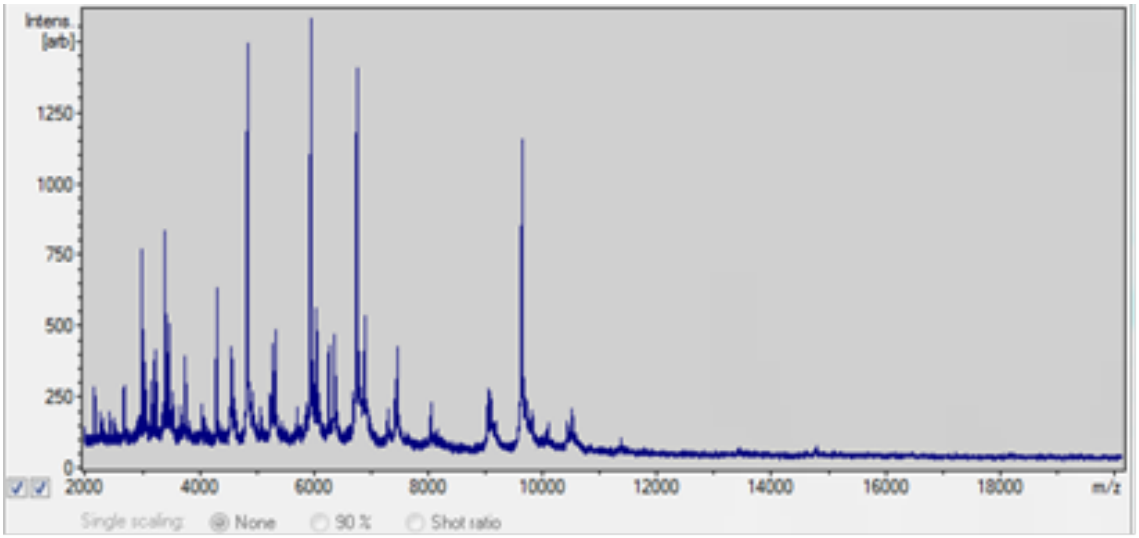

Fig. 3. **Histogram obtained as a result of S. *pseudintermedius* identification using the MALDI-TOF MS platform.**

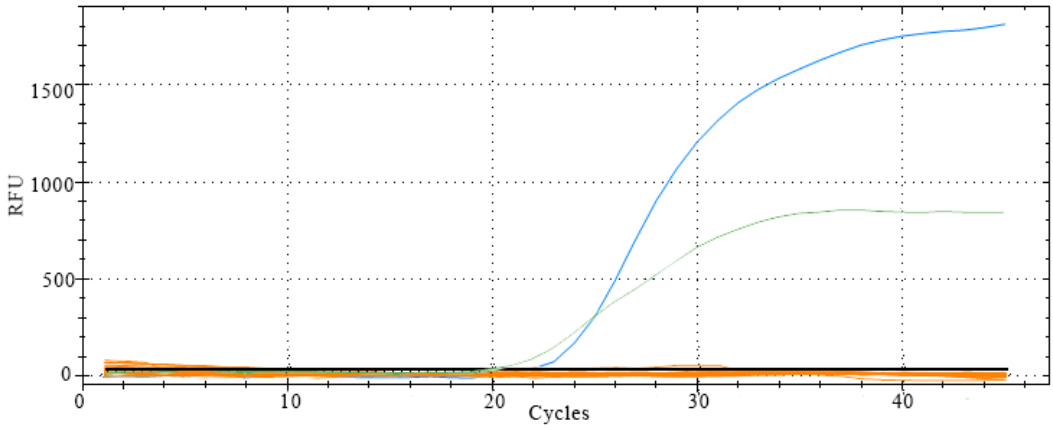

Fig. 4. **Detection of the mecA gene in the MRSP strain.**

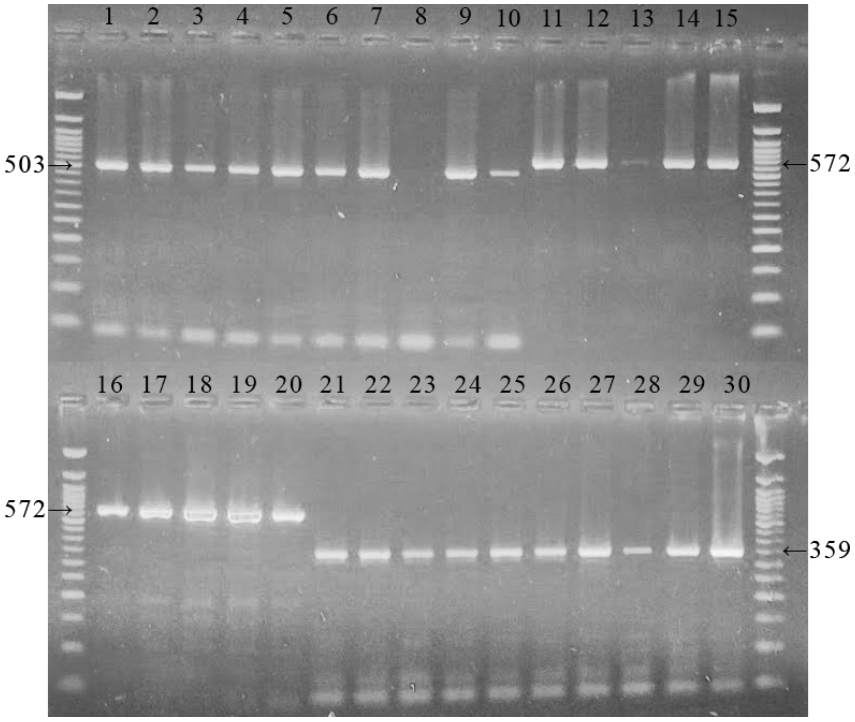

Fig. 5. **Results of electrophoretic detection of pathogenicity genes in the classical PCR reaction.**

1-7, 9, 10 - 503 bp fragments of the *lukS* gene, 8 - no *lukS* fragments; 11-20 - 572 bp fragments of the *lukF* gene; 12-30 - 359 bp fragments of the siet gene

Discussion. Representatives of the CoPS group have a number of similar biochemical and cultural properties, which makes it difficult to identify and differentiate them by microbiological methods. Solving this problem requires a combination of different methods and approaches. Previously, we described the possibility of using PCR and sequencing for definitive identification of S. *pseudintermedius* and S. *aureus* [22]. In this study, strains of S. *pseudintermedius* previously identified by Sanger sequencing were investigated by MALDI-TOF MS. This method also has high sensitivity and specificity, and can be considered the "gold standard" for the identification of bacterial cultures [23, 24].

In our study, we characterized some cultural and biochemical features of the studied staphylococcal isolates.

The use of salt-containing media, as well as rapid biochemical reactions for the detection of catalase and oxidase, allow for the clear identification of *Staphylococcus* species. The use of the rabbit plasma coagulation reaction also solves the problem of differentiating between more pathogenic and less pathogenic isolates. However, the use of this reaction to differentiate S. *aureus* from S. *pseudintermedius* and other members of the SIG group is not informative. According to the literature, S. *aureus* and SIG are resistant to Polymyxin B, in our study, the zone of growth inhibition around the disk with antimicrobial preparation of S. *pseudintermedius* was larger ($p<0.001$). This reaction can be used to differentiate these pathogens. According to the literature, S. intermedius and S. *pseudintermedius* do not produce acetone, unlike S. *aureus*. However, in our study, 10% of S. *pseudintermedius* produced acetoin, although the reaction was weaker than that of S. *aureus*. Other authors also determined the variability of this reaction. In the study by Rusenova et. all (2020), 12% of positively reacting cultures of S. *pseudintermedius* were found [25]. According to Sasaki et al. (2007), 66.3% of the strains tested by the same method as in our study showed a nonspecific reaction. But when using the commercial ID 32 Staph kit, only 10% of isolates showed a positive reaction to acetoin [26].

We have studied the pattern of positive reactions of S. *pseudintermedius* using the Neonatal FAST well D-ONE microculture system. This panel contains 20 wells with reagents for bacterial identification and 9 wells for antibiotic resistance testing. The kit contains one well with a positive reaction, which indicates the presence of *Staphylococcus* spp. This reaction was positive for both S. *pseudintermedius* and S. *aureus* strains tested. For the detection of S. *aureus*, the kit contains 2 wells. None of the tested S. *pseudintermedius* showed a specific reaction and could not be identified as S. *aureus*. We obtained a precise and sensitive reaction for the S. *aureus* isolate. We did not detect resistance to clindamycin and amikacin by the disc diffusion method. Cefoxitin, colistin, cefazolin, and ampicillin, according to the EUCAST standard, are not used to determine the resistance of S. *pseudintermedius*. In addition, the antibiotics cefoxitin, cefazolin, and ampicillin belong to the b-lactam class, and one of the isolates tested showed sensitivity to other compounds of this class when using the agar diffusion method. At the same time, this isolate did not lead to a positive reaction in the MRSA detection well, while the MRSP isolate did. Since the kit is convenient for setting up the reaction and helps to save time in routine diagnostics, it is advisable to adapt the microculture platform for use in veterinary laboratories.

We have identified the presence of genetic elements responsible for the formation of methicillin resistance and the synthesis of certain pathogenicity factors. The study was conducted with cultures that showed resistance to beta-lactam antibiotics in our previous studies [18, 19].

We did not detect MRSA isolates among companion animals. All cultures of S. *aureus* with the mecA gene were isolated from milk of cows with mastitis. Cattle-associated methicillin-resistant staphylococci (LA MRSA) can be dangerous for humans because they may have zoonotic potential and thus pose risks to human health in the non-hospital environment [27]. The literature describes that after the detection of MRSA isolates among cows with subclinical mastitis, S. *aureus* with a genotype identical to the milk isolates was detected in a veterinarian and other people who came into contact with the cattle [28]. Other authors have also reported the detection of MRSA isolates in milk from cows in the European region. Papadopoulos et al. (2018) in two studies conducted at different time intervals found that 25% and 33.3% of S. *aureus* isolates were resistant to methicillin. They also found resistant strains in farm workers. MRSA strains were found in 38% of tanker milk samples taken from Czech farms. In all of the above studies, methicillin resistance was confirmed by the presence of the mecA gene [29]. The study Vishovan et al, (2020) also describes the spread of staphylococci among companion animals. The authors found no coagulase-positive isolates among cats and two isolates in dogs [30]. Resistance to oxacillin was shown by 1 CoPS from dogs and cows. The mecA gene was found in one isolate isolated from milk. Coagulase-negative staphylococci also showed hemolytic activity, and more than half of the isolates from both cats and dogs fermented mannitol.

According to a meta-analysis by Abdullahi et al., (2022) of colonization in healthy dogs MRSA can be in the range of 2.4-3.2%, and MRSP 2.5-3.7%. We examined 58 nasal swabs from healthy dogs, and MRSP was found in 1 (1.7%) [31]. This figure is lower than that reported in the meta-analysis. No coagulase-positive *Staphylococcus aureus* that was resistant to methicillin was found in the sick animals. Other authors reported that 6 out of 101 S. *pseudintermedius* strains isolated over 8 years from sick dogs were resistant to methicillin [32]. In the study by Fàbregas et al. (2023), nasal carriage of MRSP was found in 3 out of 9 dogs. In one other dog, the MRSP strain was isolated from

another body site. The same genotypes colonized the nasal cavity and other body sites in all studied animals [33].

S. pseudintermedius has a number of important factors that determine its pathogenic and zoonotic potential. Staphylococcal leukotoxin (Luk-I) is a toxin that causes macrophage lysis, thereby suppressing the immune response. It consists of two components, lukF and lukS, encoded by separate genes [34]. Exfoliative (ET) toxin targets the epidermis and causes tissue separation. This pathogenicity factor is encoded by the siet gene.

Glajzner et al. (2023) identified genes responsible for the synthesis of Luk-I and ET in all isolates from humans and dogs [35]. Pitchenin et al, (2018) also reported the detection of these genes in the vast majority of isolates, *lukF* and siet were detected in 91%, and *lukS* in 95% of the studied strains [36]. In our study, *lukS* genes were detected in 90% of S. *pseudintermedius*, and *lukF* and siet in all studied strains. This is in line with the data of the previously mentioned groups. However, there are reports of a much lower prevalence of *lukF*/S among S. *pseudintermedius* isolates. Kmieciak & Szewczyk, (2018) found *lukF*/S genes in only 10.2% of the studied strains, but the siet gene was detected in all studied isolates [37].

Combining different research methods helps to solve the problem of identifying and differentiating different types of staphylococci. To understand the potential hazards of different staphylococci, it is important to consider the genetic characteristics of the strains, such as pathogenicity factors and antibiotic resistance genes.

Conclusions. S. *aureus* and S. *pseudintermedius* have similar culture and biochemical properties, some of which differ between different genotypes of the same species. The most accurate methods for species identification of staphylococci are molecular genetic studies and MALDI-TOF MS. Isolates identified by sequencing as S. *pseudintermedius* were further confirmed by MALDI-TOF MS.

Determination of the growth retardation zone around the disk with the antibiotic Polymyxin B could be used to tentatively differentiate between S. *aureus* (band gap 9.5±0.6) and S. *pseudintermedius* (band gap 15.2±0.8), the difference was statistically significant ($p<0.001$).

The presence of the mecA 9 gene of methicillin-resistant CoPS, which showed phenotypic resistance to β-lactam antibiotics, was established.

The staphylococcal leukotoxin gene *lukF* was found in 100%, and the other - *lukS* in 90% of S. *pseudintermedius* isolates. The gene for the exfoliative toxin siet was found in all strains studied.

The Neonatal FAST well D-ONE panel is a convenient and effective method for the identification of S. *aureus*, but due to the variability of biochemical reactions of S. *pseudintermedius*, it cannot be used for its identification. It is advisable to consider the possibility of adapting this type of panel for use in veterinary laboratories.

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Мікробіологічна та молекулярно-генетична характеристика *Staphylococcus aureus* і *Staphylococcus pseudintermedius*

Шевченко М. В., Царенко Т. М.

Коагулазопозитивні стафілококи – важливий інфекційний агент, що зумовлює численні інфекції у тварин. *Staphylococcus aureus* та *Staphylococcus pseudintermedius* мають ряд однакових культуральних та біохімічних ознак, що ускладнює їх диференціацію. Оскільки ці види мають різний зоонозний потенціал, доцільним є розроблення швидких та специфічних схем видової диференціації збудників.

Було вивчено культуральні та біохімічні особливості *Staphylococcus spp.,* ізольованих від собак, котів та корів. Загалом було ізольовано 103 галофільних кокових культури від тварин-компаньйонів та 45 – від корів, з них коагулазопозитивними стафілококами виявились 55 та 30 культур відповідно. Вивчені реакції, які можуть бути використані для диференціації *S. pseudintermedius* та *S. aureus.* Зона затримки росту навколо диску з антибіотиком polymyxin B для *S. pseudintermedius* статистично вища ($p<0,001$) ніж *S. aureus.* Визначення продукції ацетоїну з метою диференціації цих патогенів, має меншу специфічність, оскільки 30 % *S. pseudintermedius* проявили хибнопозитивну реакцію. Належність двох ізолятів до виду *Staphylococcus pseudintermedius* підтверджено за допомогою MALDI-TOF.

Вірулентність стафілококів обумовлена наявністю генів, що регулюють синтез різних чинників патогенності та обумовлюють стійкість до антибіотиків. Молекулярно-генетичні методи можуть виявити наявність специфічних генів, та допомогти оцінити ризики щодо небезпеки конкретного штаму який спричинює інфекцію. За допомогою класичного ПЛР та ПЛР в реальному часі, у 8 штамів *S. aureus* та 1 *S. pseudintermedius*, що проявили фенотипову стійкість до метицеліну виявлений ген mecA. Гени патогенності lukF та siet були наявні у 100 %, а ген lukS у 90 % досліджених *Staphylococcus pseudintermedius*.

У дослідженні висвітлено низку аспектів діагностики та диференціації коагулазопозитивних стафілококів. Також вивчено можливість використання мікрокультуральної системи Neonatal FAST well D-ONE у ветеринарних лабораторіях. Отримані дані можуть бути використані для розробки методичних підходів щодо ідентифікації патогенних стафілококів із застосуванням комплексу різних методів.

Ключові слова: *S. Pseudintermedius*, стійкість до метицеліну, гени патогенності, MALDI-TOF MS.

ORCID iD:
Shevchenko M. https://orcid.org/0000-0002-7002-1494
Tsarenko T. https://orcid.org/0000-0003-4373-5958