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Original scientific paper

# Detection of antibiotic resistance genes among *Staphylococcus schleiferi* subsp. *coagulans* and its antibiogram profile from canine pyoderma isolates

**Shobha Khambam[1], Sanjay Nanjibhai Ghodasara[2*], Dilip Balubhai Barad[2], Bhavesh Bhagvanjibhai Javia[2], Dhaval Tribhuvanbhai Fefar[3] and Jaysukh Bachubhai Kathiriya[4]**

[1]MVSc Student in Veterinary Microbiology, Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India

[2]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India

[3]Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India

[4]Department of Veterinary Public Health and Epidemiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India

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**ABSTRACT**

Pyoderma, or bacterial infection of the skin, is the most common dermatological problem encountered in dogs. Recently, *Staphylococcus schleiferi* is an emerging zoonotic pathogen that colonizes the skin and mucosal surfac- es of small animals and humans. The purpose of the present study was to identify the presence of *Staphylococcus schleiferi* subsp. *coagulans* and to study their resistance pattern against various antimicrobial agents phenotypically, as well as genotypically, from canine pyoderma in dog breeds. A total of 80 skin swabs were collected aseptically from dogs of various breeds affected with different types of canine pyoderma. A total of 108 bacteria were isolated from 80 skin swabs, of which 73 (97.33%) isolates were molecularly confirmed as *Staphylococcus* genus. Out of the 73 molecularly confirmed isolates, 8.22% (6/73), isolates were identified as *S. schleiferi* subsp. *coagulans*, using *Sch-nuc* gene amplification. An antimicrobial resistance study of β-lactam antibiotics showed 50% and 16.67% resis- tance against penicillin-G and amoxyclav, respectively, and among non-β-lactam antibiotics, 33.33% resistance was observed against enrofloxacin, oxytetracyclin and Co-Trimoxazole, while 16.67% resistance was observed against levofloxacin and chloramphenicol. Fifty percent (3/6) of *S. schleiferi* subsp. *coagulans* isolates showed multidrug resistance among 19 selected antibiotics classified under different classes of antimicrobials, and one (16.67%) isolate was identified as methicillin resistant *S. schleiferi* subsp. *coagulans* (MRSS) genotypically. This study helps to un- derstand the increased level and pattern of resistance in *S. schleiferi* subsp. *coagulans* isolated from different types of canine pyoderma.

**Key words:** *S. schleiferi* subsp. *coagulans*; antimicrobial agents; canine pyoderma; methicillin resistance; β-lac- tam antibiotics

* Corresponding author:
Sanjay Nanjibhai Ghodasara, Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India, phone: 09662055459, e-mail: snghodasara@gmail.com.

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## Introduction

Dogs frequently encounter pyoderma, which is a bacterial infection affecting the skin. It is the most prevalent dermatological issue observed in canines (NESBIT and ACKERMAN, 1998). The staphylococci are frequently associated with superficial pyoderma. It is important to note that Gram-negative bacteria can also lead to secondary infection, especially in cases of deep pyoderma (ROSSER, 2000). The initial diagnosis of pyoderma is typically made through medical history and physical examination. Complementary tests, including Gram staining, culture, and molecular methods are then conducted to confirm the diagnosis.

Recent studies have shown potential zoonot- ic risks associated with the transmission of anti- biotic resistant strains of pathogenic staphylococci between pets, their owners, and veterinary staff (BHAT, 2021). *S. schleiferi*, which can exhibit coagulase negative (CNS: subsp. *schleiferi*) or coagulase positive (CPS: subsp. *coagulans*) characteristics, has typically been linked to skin infections in dogs and cats. However, many studies have identified it as a pathogen capable of causing infections in humans as well (TZAMALIS et al., 2013). *Staphylococcus schleiferi* is an emerging zoonotic pathogen that colonizes the skin and mucosal surfaces of small animals (GRIFFETH et al., 2008).

In both human and veterinary medicine, antimicrobial resistance among bacterial pathogens is one of the main global issues. They have been rising over recent decades, and have major implications in health, as failure in treatment results in higher morbidity, mortality, and treatment costs for diseases. Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) are now a significant concern in veterinary medicine. Methicillin resistance has also been shown by *S. schleiferi.* Many of these (*S. pseudintermedius*, *S. aureus, S. schleiferi)* also demonstrate resistance to fluoroquinolone antimicrobials (JANE et al., 2014). Multi-drug resistant staphylococcal strains have emerged as a result of the indiscriminate use of various antibiotics, due to mutations in the genes that encode target proteins (SILVA et al., 2021). Several authors have reported the carrier status of antimicrobial resistance-encoding genes in the *Staphylococcus* genus, where the most critical is the methicillin-resistance encoding *mec* gene which encodes an alternative penicillin-binding protein (PBP2a) with a strong affinity to β-lactamic antibodies (GONZALEZ-DOMINGUEZ et al., 2020).

Antibiotic sensitivity testing is most often performed using the traditional disk diffusion method with breakpoints guiding predictions on clinical efficacy. Dilution testing and minimum inhibitory concentrations (MICs) will be helpful in treating multidrug-resistant infections, where a borderline MIC may still be overcome with the use of high doses of an authorized antimicrobial drug rather than choosing a less safe drug (LOEFFLER and LLOYD, 2018). Many methods are used for the identification and detection of bacteria, including conventional and molecular approaches. Conventionally, identification of bacteria takes more time in order to discover the most effective antibiotics using disk diffusion methods (AMIN et al., 2011). To overcome this limitation, the molecular approach for identification, characterization and study of antibiotic resistance genes among the bacteria is the most convenient technique.

The objectives of this study were to identify the bacteria associated with canine pyoderma in dogs around the Junagadh region of Gujarat, particularly with regard to the emerging pathogen *Staphylococcus schleiferi* subsp. *coagulans,* and to determine the extent of antimicrobial drug resistance. The study also examined the presence of resistance, as well as the patterns of antimicrobial resistance. The findings from this research are expected to provide valuable insights for determining suitable antimicrobial treatment strategies. The PCR method was evaluated in identification of species-specific sequences of *Staphylococcus schleiferi* subsp. *coagulans.*

## Materials and methods

*Sample collection.* Samples were collected from 80 dogs affected with varying degrees of pyoderma including surface pyoderma (5), superficial pyoderma (52), deep pyoderma (6) and recurrent pyoderma (9), that were brought to the Veterinary

Clinical Complex (VCC), of the College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh. The majority of the dogs were of Labrador retrievers (24/80), non-descript breeds (22/80) and German shepherds (15/80), and others breeds of dogs encountered with a low- er percentage of pyoderma infection. The pustule contents or swabs applied to ulcerated lesions were obtained aseptically, using a sterile, Hiculture collection device (PW 003, HiMedia Laboratories, Mumbai), and the organisms were cultured in Brain Heart Infusion Broth (BHI broth) for 6 to 8 hrs at 37°C.

*Identification of Staphylococcus species*. The organisms in cultured BHI broth were transferred to BHI agar and incubated for 48 hrs at 37°C for pure culture. The staphylococci were identified on the basis of colony characteristics, gram staining and morphology, catalase reaction, colony pigmentation, mannitol fermentation and haemolysin production, according to the techniques described by QUINN et al. (2011). *Staphylococcus schleiferi* were tentatively identified by the characteristic pattern of Gram-positive cocci arranged as individuals, pairs, small clusters or chains of 3 to 7 cells, as reported by FRENEY et al. (1988).

*Isolation of bacterial genomic DNA.* Isolation of bacterial genomic DNA from the pure staphylococcal culture was carried out using the conventional method (Proteinase K-SDS method) according to SAMBROOK and RUSSELL (2001). The purity and concentration of the isolated DNA was assessed using a µDrop™ Plate in µDrop plate reader (Thermo Scientific).

Detection of *S. schleiferi* subsp. *coagulans* and its resistant genes using PCR: The detection of *Staphylococcus schleiferi* subsp. *coagulans* (*Sch-nuc*) and their resistant genes for Methicillin (*mecA*), Mupirocin (*mupA*, *mupLL*) and Vancomycin (*vanA*) antibiotics were studied in the present research. The details of the names and oligonucleotide sequences of the primers with targeted genes, along with their product sizes are given in Table 1. The PCR reaction was carried out in a total of 25 µl reaction mixture composed of 12.5 µl 2X master mix (Thermo Scientific, Lithuania), 1 µl of 10pmol forward and reverse primer (Eurofins Genomics India Pvt. Ltd., Bangaluru), 3 µl genomic DNA and 7.5 µl Nuclease free water. The cycling conditions for PCR of *S. schleiferi* subsp. c*oagulans* (*Sch- nuc*) and resistance genes were used according to the authors described in Table 1. The amplification reactions were carried out using a programmable thermal cycler (Verity, Applied Biosystems by life technology, Singapore). To identify the amplicon of the targeted sequence, 10 µl PCR product was loaded with gel loading dye in 1.5% w⁄v agarose gel containing 0.5 µg/ml ethidium bromide with a DNA ladder, and electrophoresis was done in 1x Tris-Acetic acid-EDTA (TAE) buffer at 80 V for 60 min. The amplified product was visualized using gel documentation system (Bio-PrintST4® VilberLourmat).

*Antimicrobial susceptibility testing*. All the isolates of *S. schleiferi* subsp. *Schleiferi* obtained from the cases of canine pyoderma were tested for antimicrobial susceptibility tests using the disk diffusion method on Mueller-Hinton agar, as recommended by Kirby-Bauer (BAUER et al., 1966). Zones of inhibition were measured and interpreted as per the Clinical and Laboratory Standards Institute standards (CLSI, 2017). The antimicrobial drugs used against isolates, and the disk potencies were as follows: Methicillin (5µg), Penicillin-G (10 units), Ampicillin/Sulbactam (10/10µg), Amoxyclav (20/10µg), Ceftazidime(30µg), Cefepime (30µg), Cefpodoxime (10µg), Ceftriaxone (10µg), Ceftizoxime (30µg), Cefoperazone (75µg), Gentamicin (10µg), Amikacin (30µg), Levofloxacin (5µg), Enrofloxacin (10µg), Oxytetracycline (30µg), Aztreonam (30µg), Chloramphenicol (30µg), Co-Trimoxazole (Trimethoprim/ Sulphamethoxazole) (25µg) and Clindamycin (10µg).

*Determination of minimum inhibitory concentration (MIC) by E- test*. The MIC of *S. schleiferi* subsp. *Schleiferi* was determined by E-test by using the commercial MIC determination paper strips, Ezy MIC™ strips (HiMedia Laboratories, Mumbai). These strips contain pre-coated antibacteri- als in a concentration gradient capable of showing MICs upon testing against the test organism. The Ezy MIC™ strips used, along with their range of MICs were Mupirocin (EMO87): 0.064-1024 mcg/ml (Mupirocin Low level resistance: 8-256 mcg/

Table 1. Oligonucleotide sequences of primers used for identification of *S. schleiferi* subsp. *coagulans* and their resistance genes

| Primer sequence (5' to 3') | Target gene | Product size (bp) | Reference |
|---|---|---|---|
| F: TTAAAACGACGGAAGGCAGT<br>R: CCAATCATACGCACACGTTC | *Sch-nuc** | 115bp | GONZALEZ-DOMINGUEZ et al. (2020) |
| F:CCTAGTAAAGCTCCGGAA<br>R:CTAGTCCATTCGGTCCA | *mecA* | 314 bp | TAMAKAN and GOCMEN, (2022) |
| F:TATATTATGCGATGGAAGGTTGG<br>R: AATAAAATCAGCTGGAAAGTGTTG | *mupA* | 458 bp | SUM et al. (2020) |
| F:CCGGAATTAAGTTTCCCAGC<br>R:CAAAGTTTTCATAGTTGTTAATCGT | *mupLL* | 450 bp | ABDULGADER et al. (2020) |
| F:ATGAATAGAATAAAAGTTGC<br>R:TCACCCCTTTAACGCTAATA | *vanA* | 1032 bp | MAHMOOD and FLAYYIH, (2014) |
| F:<br>GTAGATTGGGCAATTACATTTTGGAGG<br>R: CGCATCAGCTTTGTTATCCCATGTA | *coa* | 214bp | MOON et al. (2007) |

*- gene for *S. schleiferi* subsp. *coagulans*; F: Forward; R: Reverse

ml, Mupirocin High level resistance: ≥512 mcg/ml), and the results were interpreted according to MOSTAFA and AWAD (2020). Vancomycin-Cefoxitin (EM0771): VAN: 0.19-16.0 mcg/ml CX: 0.5-64 mcg/ml and the results were interpreted according to the HiMedia standards. Those isolates that demonstrated resistance against three or more classes of antibiotics were considered to be multidrug resistance isolates, as described by MAGIORAKOS et al. (2012).

## Results

Isolation and identification of bacterial isolates: A total of 73 isolates were confirmed as *Staphylococcus* spp. from 108 bacterial isolates recovered from 80 dogs with canine pyoderma, on the basis of staining, morphology, growth characteristics, haemolysin production and various biochemical tests (Table 2). Among these 73 *Staphylococcus* spp. isolates, six (8.22%) were confirmed as *Staphylococcus schleiferi* subsp. *coagulans* and the rest of the isolates were other species of *Staphylococcus*. All the six isolates of *Staphylococcus schleiferi* subsp. *coagulans* were confirmed as *Staphylococcus* species by the genus specific 16sRNA gene primer.

Molecular detection of *S. schleiferi* subsp. *coagulans*. and its resistant genes: In this study, out of 73 molecularly confirmed *Staphylococcus* isolates, six (8.22%) isolates yielded the desired fragment of 115 bp amplicon of the *Sch-nuc* gene specific for *S. schleiferi* subsp. *coagulans* (Fig. 1A). Out of these six isolates, one (16.67%) isolate yielded the desired fragment of amplicon in the *mecA* gene (Fig. 1B) primer considered as methicillin resistant *S. schleiferi* subsp. *coagulans* (MRSS), and five (83.33%) isolates did not yield the desired fragment of *mecA* gene amplicon, considered as methicillin sensitive *S. schleiferi* subsp. *coagulans* (MSSS). None of the *S. schleiferi* subsp. *coagulans* isolates yielded the desired fragment of amplicon for the mupirocin resistant gene (*mupA*, *mupLL*), the vancomycin resistant gene (*vanA*) or the coagulase gene (*coa*) (Table 1).

Antimicrobial susceptibility testing: Among β-lactam antibiotics, 50% (3/6), and 16.67% (1/6) of

Table 2. Phenotypic and genotypic detection of various resistance genes of *S. schleiferi* subsp. *coagulans* from clinical cases of canine pyoderma

| Sr. no. | Sample no. | Phenotypic methods | | | | | | Genotypic methods | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | KOH | Catalase | Oxidase | Coagulase | MSA | Pattern of hemolysis | *Sch-nuc* | *mecA* | *mupA, mupLL, vanA, Coa* |
| 1 | **CP 8** | - | + | - | + | NF | γ | + | - | - |
| 2 | **CP 12** | - | + | - | + | NF | α | + | - | - |
| 3 | **CP 20** | - | + | - | + | NF | β | + | + | - |
| 4 | **CP 44b** | - | + | - | + | NF | β | + | - | - |
| 5 | **CP 51a** | - | + | - | + | NF | α | + | - | - |
| 6 | **CP 80a** | - | + | - | + | NF | β | + | - | - |

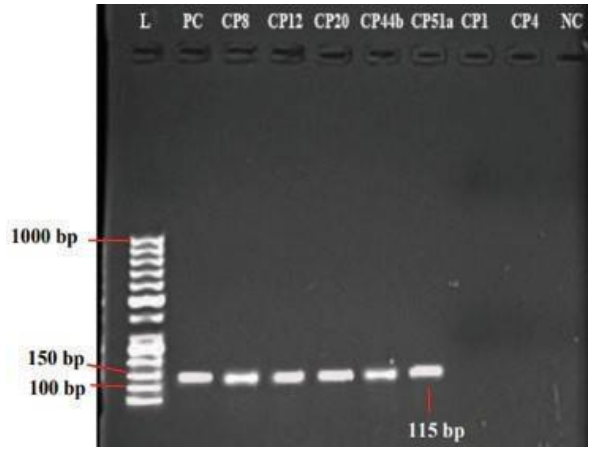


Fig. 1A. Species specific PCR of *S. schleiferi* subsp. *coagulans* for *Sch-nuc* gene (115 bp). L: 50 bp plus ladder, PC: Positive control (*S. schleiferi* subsp. *coagulans* ATCC 49545), CP8, CP12, CP20, CP44b, CP51a: Samples positive for *S. schleiferi* subsp. *coagulans*, CP1, CP4: Negative samples, NC: Negative control (*E. coli* MTCC 722).

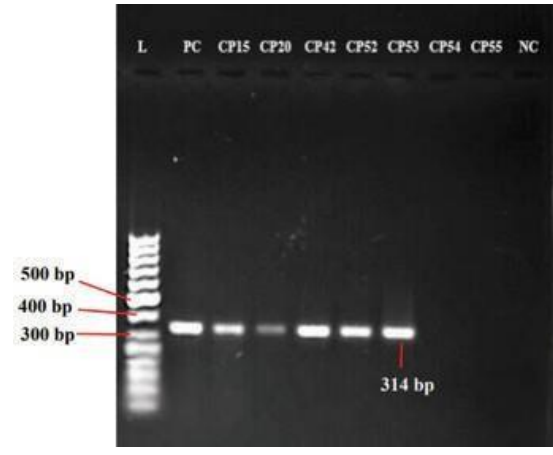


Fig. 1B. Detection of *mecA* gene (314 bp *S. schleiferi* subsp. *coagulans* through PCR. L: 50 bp plus ladder PC: Positive control (*S. aureus* ATCC 43300) CP15, CP20, CP42, CP52, CP53: Samples positive for presence of *mecA* gene CP54, CP55: Negative samples NC: Negative control (*E. coli* MTCC 722).

the isolates showed resistance against penicillin-G and amoxyclav, respectively, whereas no other antibiotics of this group showed resistance against *S. schleiferi* subsp. *coagulans* isolates. However, among the non β-lactam antibiotics, 33.33% (2/6) resistance was observed against enrofloxacin, oxytetracyclin and Co-Trimoxazole, while 16.67% (1/6) resistance was observed against levofloxacin and chloramphenicol. Out of the six isolates, three (50.00%) *S. schleiferi* subsp. *coagulans* isolates showed multidrug resistance against 19 selected antibiotics. None of the isolates showed resistance against Mupirocin (EMO87) and Vancomycin-Cefoxitin (EM0771) by E-test. One isolate was genotypically methicillin resistant, but was phenotypically negative by the disk diffusion method.

Sensitive isolates of canine pyoderma: Antibiotic susceptibility/resistance in bacterial isolates varies by region due to differences in the choice of antibiotic, antibiotic availability and usage patterns in treating canine pyoderma, therefore, isolates of

canine pyoderma were analyzed to determine the status or to obtain information on sensitive patterns among the strains in a particular region during this investigation. Overall, 72% (57/80) of the cases of canine pyoderma were superficial pyoderma, followed by recurrent pyoderma (12%, 9/80), and surface pyoderma (10%, 8/80). Among the coagulase positive *Staphylococcus*, *S. pseudintermedius* followed by *S. schleiferi* subsp. *coagulans* was the most common bacteria isolated and ampicillin/sulbactam, amikacin, cefepime and cefoperazone were found to be the most sensitive antibiotics against different types of pyoderma (Table 3).

Table 3. Antibiotic resistance profiles and antibiotic resistance gene detection from canine pyoderma associated with *S. schleiferi* subsp. *coagulans*

| Group of antibiotic | Antibiotics | No. of isolates (%) | | | Resistance determinants by gene amplification |
|---|---|---|---|---|---|
| | | S | I | R | |
| B-lactam group of antibiotics | Methicillin (5µg) | 6 (100%) | | | *mecA* gene (1) |
| | Penicillin-G (10 units) | 3 (50%) | | 3 (50%) | |
| | Ampicillin/Sulbactam (10/10µg) | 6 (100%) | | | |
| | Amoxyclav (20/10µg) | 5 (83.33%) | | 1 (16.67%) | |
| Cephalosporins | Ceftazidime (30µg) | 6 (100%) | | | |
| | Cefepime (30µg) | 6 (100%) | | | |
| | Cefepime (30µg) | 6 (100%) | | | |
| | Cefpodoxime (10µg) | 6 (100%) | | | |
| | Ceftriaxone (10µg) | 6 (100%) | | | |
| | Ceftizoxime (30µg) | 6 (100%) | | | |
| | Cefoperazone (75µg) | 6 (100%) | | | |
| Aminoglycosides | Gentamicin (10µg) | 5 (83.33%) | 1 (16.67%) | | |
| | Amikacin (30µg) | 6 (100%) | | | |
| Fluoroquinolones | Levofloxacin (5µg) | 4 (66.66%) | 1 (16.67%) | 1 (16.67%) | |
| | Enrofloxacin (10µg) | 4 (66.66%) | | 2 (33.33%) | |
| Tetracyclines | Oxytetracycline (30µg) | 3 (50%) | 1 (16.67%) | 2 (33.33%) | |
| Monobactum | Aztreonam (30µg) | 6 (100%) | | | |
| Amphenicol | Chloramphenicol (30µg) | 4 (66.66%) | 1 (16.67%) | 1 (16.67%) | |
| Sulfa group | Co-Trimoxazole (Trimethoprim/ Sulphamethoxazole) (25µg) | 4 (66.66%) | | 2 (33.33%) | |
| Lincomycin | Clindamycin (10µg) | 6 (100%) | | | |

**Discussion**

In the current investigation, 8.22% isolates were identified as *Staphylococcus schleiferi* subsp. *coagulans*, and the remaining percentage of isolates were attributed to other species within the *Staphylococcus* genus. Similarly, ELIZABETH et al. (2005) isolated *Staphylococcus schleiferi* subsp. *coagulans* from the ears and skin of dogs with otitis and pyoderma. COSTA et al. (2021) isolated 32% isolates of *Staphylococcus schleiferi* subsp *coagulans* corresponding to all canine pyoderma-related isolations. RAVENS et al. (2014) reported 11.11% *S. schleiferi* from pyodema. Slightly lower percentages of isolates were reported by CHAUDHARY et al. (2019), TAMAKAN and GOCMEN (2022) and GONZALEZ-DOMINGUEZ et al. (2020), who reported 2%, 3.12%, and 2% of *S. schleiferi* subsp. *coagulans* isolates, respectively.

In *S. schleiferi* subsp. *coagulans*, all six (100%) isolates were mannitol non fermenters. This may be because *S. schleiferi* subsp. *coagulans* does not acidify maltose, mannitol or sucrose. Similar results were obtained by FRANK et al. (2003), who reported 100% mannitol non-fermentative *S. schleiferi* subsp. *coagulans* isolates.

All the isolates of *S. schleiferi* subsp. *coagulans* were coagulase positive by tube coagulation test. Similarly, various authors reported varying percentages of coagulase positive isolates, including CHAUDHARY et al. (2019), HRITCU et al. (2020) and TAMAKAN and GOCMEN (2022), who reported 58 (97%), 115 (69.27%) and 32 (64%) CoPS, respectively.

However, no isolates were amplified for the presence of *coa* gene. The reason could be that there was no coagulase gene or related protein responsible for this activity that was well defined except in *S. aureus*. Therefore, primers specific for the *coa* gene of *S. aureus* could not amplify the desired fragment of *S. schleiferi* subsp. *coagulans* in this study. Similar kind of results were also documented by many researchers in many studies, and they mentioned diversity in the size of the fragments amplified using *coa* primers, and speculated that this could be related to the presence of a coagulase gene structurally distinct from *S. aureus* (SEWID et al., 2018; SILVA et al., 2003)

Genotypically, one (16.67%) isolate found methicillin resistance to *S. schleiferi* subsp. *coagulans* (MRSS) by the *mecA* gene, but it was negative by the disk diffusion method against methicillin (ABST) and cefoxitin (E-test), this might be due to the fact that the methicillin gene could not be expressed phenotypically. None of the isolates showed resistance phenotypically against mupirocin, vancomycin and cefoxitin by the E-test. Similarly, KAWAKAMI et al. (2010) and LEE et al. (2019) reported 30.0% and 24% MRSS isolates, respectively. ELIZABETH et al. (2005) reported One of the *S. schleiferi* subsp *schleiferi* isolates from ears, 2 of the *S. schleiferi* subsp *coagulans* isolates from ears, and 1 of the *S. schleiferi* subsp *coagulans* isolates from the skin were resistant to methicillin. SUM et al. (2020), BATHOORN et al. (2012) reported zero percent resistance against mupirocin by E-test, whereas 2% (1/50) of *S. schleiferi* isolates showed intermediate resistance to vancomycin (MOREIRA et al., 2020). In contrast to this study, 98% percentage of resistance for vancomycin was reported by GONZALEZ-DOMINGUEZ et al. (2020). The rise in methicillin-resistant *Staphylococcus* spp. in recent years raises concern, not only for animals but also humans. This is due to the fact that domesticated animals can play a role in the dissemination of these extremely resistant strains within the confines of a household (MORRIS et al., 2012).

Fifty percent (3/6) of the *S. schleiferi* subsp. *coagulans* isolates exhibited multidrug resistance (MDR). Similarly, BURKE and DOMENICO (2023) reported that 44.9% isolates were MDR-SS and demonstrated methicillin resistance (OXA), showing an association with the multidrug resistance of *S. schleiferi*. This is consistent with previous research findings, which indicates that MR strains also have the ability to acquire multidrug resistance genes through alternative genetic mechanisms (CAIN, 2013). The exposure to systemic antibiotics prior to culture was also considered a high risk for multidrug resistance in both *S. pseudintermedius* and *S. schleiferi* (BURKE and DOMENICO, 2023).

Penicillin-G (50%) and amoxyclav (16.67%) antibiotics of the β-lactam group showed resistance

against the isolated species. Similar results have been reported by many scientists. KAWAKAMI et al. (2010) reported 44.7% CoPS isolates resistant to amoxicillin/clavulanic acid. DURAN et al. (2012) reported 89.7%, 28.9% and 17.7% *Staphylococcus* isolates resistant to penicillin, amoxicillin/clavulanic acid and methicillin, respectively. GONZALEZ-DOMINGUEZ et al. (2020) reported 6% *S. schleiferi* isolates resistant to ampicillin/sulbactam. Contrary to the present findings, CHAUDHARY et al. (2019) reported 1.66% *Staphylococcus* isolates resistant to amoxicillin/clavulanic acid.

However, among the non β-lactam groups of antibiotics, 33.33% resistance was found against enrofloxacin, oxytetracyclin and Co-Trimoxazole, while 16.67% resistance was observed against levofloxacin and chloramphenicol. The higher resistance among non β-lactam antibiotics was attributed to the frequent use of these antibiotics in the treatment of common bacterial ailments in this region. Similarly, LEE et al. (2019) observed higher resistance among non β-lactam antibiotics in MRSS, and reported 40% resistance to enrofloxacin. DURAN et al. (2012) reported that 35.6% and 32.2% *Staphylococcus* isolates were resistant to tetracycline, and trimethoprim/sulphamethoxazole, respectively. GONZALEZ-DOMINGUEZ et al. (2020) reported that 74% of *S. schleiferi* isolates were resistant to trimethoprim/sulphamethoxazole. Contrary to the present findings, LAI et al. (2022) reported 20.12% *S. schleiferi* isolates were found to be resistant to enrofloxacin.

DAVIS et al. (2013) reported that 16% of the isolates were resistant to tetracycline, marking an increase from 6% in 2005. Notably, 14% of the isolates displayed resistance to trimethoprim-sulfamethoxazole, whereas no resistance was observed in 2005. In the current investigation, an increase in resistance was detected in *S. schleiferi* when compared to the findings reported by Davis and his colleagues in 2013. This indicates a growing trend of resistance within the *S. schleiferi* species over a decade. These findings indicate that the growing antimicrobial resistance is restricting the available treatment options for clinical *S. schleiferi* infections in animals.

## Conclusions

Over the past 15 years, numerous studies have demonstrated the emergent and increasing prevalence of methicillin and multidrug resistance in staphylococci obtained not only from humans, but also from several veterinary species, includ- ing horses and dogs (BEEVER et al., 2015; PRIYANTHA et al., 2016). This work describes the frequency and source of *S. schleiferi* subsp. *coagulans* from canine pyoderma. The prevalence of both methicillin resistance and multi drug resistance among the isolated species of *S. schleif- eri* subsp. *Coagulans*, with increased levels of resistance among some β-lactam and non β-lactam antibiotics compared to previous studies is causing clinicians to rethink about designing a regime for the treatment of canine skin infections. In our study, we have presented compelling evidence indicating that *S. schleiferi* subsp. *coagulans* is the predominant *Staphylococcus* species, second only to *S. pseudintermedius*, in our specific environment. This finding challenges the conventional notion that *S. aureus* is the most commonly reported *Staphylococcus* species from canine skin infections. In the context of the present investigation, the accurate performance of bacterial culture and antimicrobial profiling, alongside the molecular identification of methicillin resistance genes in *Staphylococcus* isolates, constitutes a comprehensive methodology that greatly benefits the clinical diagnosis, treatment, and overall prognosis of patients suffering from canine pyoderma.

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## Declaration of competing interests

There are no conflicts of interest.

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**KHAMBAM, S., S. N. GHODASARA, D. B. BARAD, B. B. JAVIA, D. T. FEFAR, J. B. KATHIRIYA: Otkrivanje gena za rezistenciju na antibiotike i antibiogram bakterije *Staphylococcus schleiferi* subsp. *coagulans* u izolatima pasa s piodermijom. Vet. arhiv 95, 437-447, 2025.**

**SAŽETAK**

Piodermija ili bakterijska infekcija kože, najčešći je dermatološki problem u pasa. Odnedavno se smatra da infekciju kod malih životinja i ljudi prati bakterija *Staphylococcus schleiferi* koja kao emergentni zoonotski patogen naseljava kožu i površinske sluznice. Cilj je rada bio istražiti prisutnost bakterije *Staphylococcus schleiferi* subsp. *coagulans* u pasa s piodermijom te istražiti fenotipske i genotipske obrasce njezine rezistencije na različite antimikrobne tvari. Aseptičkim postupkom prikupljeno je 80 obrisaka kože pasa različitih pasmina s piodermijom. Iz tih je 80 uzoraka izolirano 108 bakterija među kojima je molekularnim metodama potvrđeno da 73 izolata (97,33%) pripadaju rodu *Staphylococcus*. Od navedenog broja, uz primjenu molekularne metode genske amplifikacije *Sch--nuc* 8,22% izolata (6/73) identificirano je kao *S. schleiferi* subsp. *coagulans*. Istraživanje antimikrobne rezistencije β-laktamskih antibiotika pokazalo je 50%-tnu rezistenciju na penicillin G i 16,67%-tnu rezistenciju na amoksiklav, a među ne-β-laktamskim antibioticima 33,33%-tna rezistencija uočena je za enrofloksacin, oksitetraciklin i kotrimoksazol, dok je 16,67%-tna rezistencija zapažena kod levofloksacina i kloramfenikola. Pedeset posto (3/6) izolata bakterije *S. schleiferi* subsp. *coagulans* pokazalo se multirezistentnima među 19 odabranih antibiotika svrstanih u različite skupine antimikrobnih lijekova, a jedan je izolat (16,67%) genotipski je identificiran kao rezistentan na meticilin *S. schleiferi* subsp. *coagulans* (MRSS). Rezultati ovog istraživanja doprinos su boljem razumijevanju porasta antimikrobne rezistencije bakterije *S. schleiferi* subsp. *coagulans* izolirane iz pasa različitih pasmina s piodermijom.

**Ključne riječi:** *S. schleiferi* subsp. *coagulans*; antimikrobne tvari; piodermija u pasa; rezistencija na meticilin; β-laktamski antibiotik