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Original Article

Phenotypic and genotypic detection of multi drug resistant coagulase-positive *Staphylococcus* spp. isolates from canine pyoderma

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Abstract

Background: Dermatological infections in dogs are challenging to treat due to antibiotic resistance, which leads to longer recovery time and the need for stronger antibiotics. **Aims:** This study was undertaken to determine the prevalence of antimicrobial resistance in coagulase-positive staphylococcal isolates from pyoderma infection in dogs. This study also aimed to identify isolates with methicillin-resistance and multidrug resistance. **Methods:** 73 coagulase-positive staphylococci isolated from varying degrees of canine pyoderma cases. The samples were analyzed for the presence of *Staphylococcus* spp. using polymerase chain reaction (PCR) and resistance against antibiotics was studied by antimicrobial profile, minimum inhibitory concentration (MIC), and PCR on isolated bacteria. **Results:** Out of 75 bacterial isolates identified, 73 isolates were confirmed as *Staphylococcus* species by PCR. A higher percentage of antibiotic resistance was observed against penicillin-G (46.27%), followed by amoxiclav (38.81%), enrofloxacin (32.84%), cefpodoxime, oxytetracycline (28.36% each), levofloxacin (26.86%), and co-trimoxazole (22.39%). 29 (49.15%) *S. pseudintermedius*, three (50.00%) *S. schleiferi* subsp. *coagulans*, and two (100%) *S. aureus* isolates exhibited multidrug resistance. However, one (1.49%) isolate (*S. pseudintermedius*) revealed low-level mupirocin resistance in the E-test. Also, 12 (20.34%) methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), one (16.67%) methicillin-resistant *S. schleiferi* subsp. *coagulans* (MRSS) and one (50%) methicillin-resistant *S. aureus* (MRSA) were reported using PCR. **Conclusion:** This study helps to understand the increased level and pattern of resistance in coagulase-positive staphylococci isolated from different types of canine pyoderma cases.

Key words: β-lactam, Canine pyoderma, Multidrug resistant, Mupirocin resistance, *Staphylococcus pseudintermedius*

Introduction

Pyoderma is one of the most common and persistent causes of canine skin diseases globally (Bhat, 2010). Pyoderma in dogs is most commonly caused by the coagulase-positive *Staphylococcus pseudintermedius* (Lynch and Helbig, 2021), but it can also be brought on by *Staphylococcus aureus* and *Staphylococcus schleiferi* subsp. *coagulans* (Loeffler and Lloyd, 2018). Other bacteria include certain anaerobes, aerobic coryneform, coagulase-negative staphylococci, *Micrococcus* spp., and α-hemolytic *streptococci*. Dogs with deep pyoderma may have Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Proteus* spp., and *Escherichia coli* (Jane *et al*., 2014).

Antimicrobial resistance amongst bacterial pathogens is a pressing global issue in human and veterinary medicine. Over the past few years, there has been a steady increase in antimicrobial resistance, leading to serious implications for public health. Failure to treat infections caused by resistant bacteria results in the higher rates of morbidity and mortality, and increase in the treatment expenses. In veterinary medicine, the emergence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) has become a significant concern. Multi-drug resistant staphylococcal strains have emerged as a result of the indiscriminate use of various antibiotics over time, which is either due to mutations in the genes that encode target proteins or the acquisition and accumulation of genes that confer antibiotic

resistance (Silva *et al*., 2021). Several authors reported the carrier status of antimicrobial resistance-encoding genes in the *Staphylococcus* genus, the most critical being the methicillin-resistance encoding *mec* (Gonzalez-Dominguez *et al*., 2020). As staphylococcal strains show multidrug resistance, their treatment options were limited, leading to the more frequent use of alternative antimicrobial drugs, like mupirocin (Kizerwetter-Swida *et al*., 2021).

Antibiotic susceptibility testing is commonly conducted through the conventional disk diffusion technique, which relies on breakpoints to determine the clinical effectiveness. In the cases of multidrug-resistant infections, dilution testing and minimum inhibitory concentrations (MICs) by E-test can provide valuable insights (Loeffler and Lloyd, 2018). The transmission of antibiotic-resistant strains of pathogenic staphylococci between pets, their owners, and veterinary staff has been identified as a potential zoonotic risk in recent studies (Bhatt, 2021). Coagulase-positive *Staphylococcus*, which has gained recognition as a significant zoonotic pathogen, requires attention. Notably, around 4-5% of small animal veterinary practitioners in the USA and Italy carry MRSP in their nasal passages, underscoring this fact that *S. pseudintermedius* is not a usual commensal organism in humans (Guardabassi *et al*., 2013).

This study was undertaken to determine the coagulase-positive *Staphylococcus* bacteria associated with canine pyoderma in dogs, with a particular focus on evaluating the extent of methicillin resistance and multidrug resistance amongst the identified species. The findings from this research are expected to provide valuable insights to determine effective antimicrobial treatment strategies for cases of canine pyoderma.

Materials and Methods

Sample collection

Samples were collected from 80 dogs that were affected by varying degrees of pyoderma. These degrees included surface pyoderma (5 cases), superficial pyoderma (52 cases), deep pyoderma (6 cases), and recurrent pyoderma (9 cases). The samples were received from the Veterinary Clinical Complex (VCC), College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India. Amongst various breeds of dogs, the Labrador retriever breed was the most common (24 out of 80), followed by non-descriptive breeds (22 out of 80) and German shepherd (15 out of 80). The pustule contents or swabs from ulcerated lesions were collected aseptically using a sterile HiCulture collection device (PW 003, HiMedia Laboratories, Mumbai). The collected samples were subjected to primary bacterial culture by inoculation into Brain Heart Infusion Broth (BHI broth) for a period of 6 to 8 h at 37°C temperature.

Identification of *Staphylococcus* species

Inoculated BHI broth was sub-cultured on BHI agar, and incubated at 37°C for 48 h to obtain pure culture. Phenotypically, staphylococci were identified based on colony characteristics, Gram's staining and morphology, catalase reaction, colony pigmentation, mannitol fermentation, and hemolysin production, as per the techniques described by Quinn *et al*. (2011). Microscopically, the characteristic patterns of Gram-positive cocci arranged as individuals, pairs, and a bunch of grapes were identified as *S. pseudintermedius*, as reported by Strommenger *et al*. (2018). *Staphylococcus schleiferi* was identified based on the characteristic patterns of Gram-positive cocci arranged as individuals, pairs, small clusters or chains of 3 to 7 cells as reported by Freney *et al*. (1988). Whereas, *S. aureus* was identified by the characteristic pattern of Gram-positive cocci arranged in the bunch of grapes as of another *Staphylococcus* spp.

For the molecular identification of various species of *Staphylococcus*, specific sets of primers were used as described by different authors. The *16s rRNA* gene (Martineau *et al*., 2001) was amplified for the detection of *Staphylococcus* spp., whereas *S. aureus*, *S. pseudintermedius*, and *S. schleiferi* subsp. *coagulans* were identified by amplification of the *Au-nuc*, *Pse-nuc*, and *Sch-nuc* genes, respectively as described by Gonzalez-Dominguez *et al*. (2020) (not mentioned in this article).

Isolation of bacterial genomic DNA

The conventional method (Proteinase K-SDS method) described by Sambrook and Russell (2001) was implemented to extract bacterial genomic DNA from a pure staphylococcal culture. The purity and concentration of isolated DNA were assessed using the µDrop™ Plate in a µDrop plate reader (Thermo Scientific).

The investigation of resistant genes for methicillin (*mecA*), mupirocin (*mupA*, *mupLL*), and vancomycin (*vanA*) antibiotics was done through PCR detection. The details of the primers, including their names, oligonucleotide sequences, and their corresponding product sizes, are described in Table 1. The PCR reaction was performed using a total volume of 25 µL reaction mixture, comprising 12.5 µL 2X master mix (Thermo Scientific, Lithuania), 1 µL of 10 pmol forward and reverse primer each (Eurofins Genomics India Pvt. Ltd., Bangalore), 3 µL genomic DNA, and 7.5 µL Nuclease free water. The cycling conditions for the detection of resistance genes were used as per the authors mentioned 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 carried out in Tris-Acetic acid-EDTA (TAE) buffer at 80 V for 60 min. The amplified product was visualized using a gel documentation system (Bio-PrintST4® VilberLourmat).

Table 1: Oligonucleotide sequences of primers used in PCR for the detection of antibiotic resistance genes among CoPS

Target gene	Primer sequence (5´ to 3´)	Product size (bp)	Reference
mecA	F: CCTAGTAAAGCTCCGGAA R: CTAGTCCATTCGGTCCA	314 bp	Tamakan and Gocmen (2022)
mupA	F: TATATTATGCGATGGAAGGTTGG R: AATAAAATCAGCTGGAAAGTGTTG	458 bp	Sum *et al*. (2020)
*mup*LL	F: CCGGAATTAAGTTTCCCAGC R: CAAAGTTTTCATAGTTGTTAATCGT	450 bp	Abdulgader *et al*. (2020)
vanA	F: ATGAATAGAATAAAAGTTGC R: TCACCCCTTTAACGCTAATA	1032 bp	Mahmood and Flayyih (2014)

F: Forward, and R: Reverse

Antimicrobial susceptibility testing

The antimicrobial susceptibility tests were conducted on all coagulase-positive *Staphylococcus* spp. isolates obtained from cases of canine pyoderma. The disc diffusion method was performed on Mueller-Hinton agar, as recommended by the Kirby-Bauer method (Bauer *et al*., 1966). The zones of inhibition were measured and interpreted according to the standards set by the Clinical and Laboratory Standards Institute guidelines (CLSI, 2017). The antimicrobial drugs used against the isolates, along with their respective disc potencies, were as follows: Methicillin (5 µg), Penicillin-G (10 units), Ampicillin/Sulbactam (10 µg), Amoxiclav (10 µg), Ceftazidime (30 µg), Cefepime (30 µg), Cefpodoxime (10 µg), Ceftriaxone (10 µg), Ceftizoxime (30 µg), Cefoperazone (75 µg), Gentamicin (10 µg), Amikacin (30 µg), Levofloxacin (5 µg), Enrofloxacin (10 µg), Oxytetracycline (30 µg), Aztreonam (30 µg), Chloramphenicol (30 µg), Co-Trimoxazole (Trimethoprim/Sulphamethoxazole) (25 µg), and Clindamycin (10 µg). The isolates showing resistance against ≥3 antimicrobial classes were defined as multidrug-resistant, as described by Magiorakos *et al*. (2012).

Determination of minimum inhibitory concentration (MIC) using the E-test

This method was conducted using commercially available MIC determination paper strips, Ezy MIC™ strips, manufactured by HiMedia Laboratories, Mumbai. These strips contain pre-coated antibacterial drugs in a concentration gradient manner, allowing the determination of MICs when tested against the target organism. The Ezy MIC™ strips used in this study included Mupirocin (EMO87) with a range of 0.064-1024 mcg/ml (mupirocin low-level resistance: 8-256 mcg/ml, mupirocin high-level resistance: ≥512 mcg/ml). The results were interpreted using the guidelines provided by Mostafa and Awad (2020). Additionally, Vancomycin-Cefoxitin (EM0771) strips were employed, with concentrations ranging from VAN: 0.19-16.0 mcg/ml and CX: 0.5-64 mcg/ml. The results were interpreted as per the standards set by HiMedia.

Results

Isolation and identification of bacterial isolates

A total of 75 out of 108 isolates from 80 canine pyodermas were confirmed as *Staphylococcus* spp. This confirmation was based on staining, morphology, growth characteristics, hemolysin production, and various biochemical tests (not shown in this article). Out of 75 *Staphylococcus* isolates, 59 (78.67%) *Staphylococcus pseudintermedius*, 6 (8%) *Staphylococcus schleiferi* subsp. *coagulans*, 2 (2.66%) *Staphylococcus aureus*, and 8 (10.67%) other *Staphylococcus* spp. were identified by using species specific primers. Out of 75 isolates identified, 73 isolates were confirmed as *Staphylococcus* species by PCR using a genus-specific *16S rRNA* gene primer. In the present study, the overall prevalence of canine pyoderma in male (37/72) and female (35/72) dogs was found quite similar. However, male dogs (5/72) were observed more affected by deep pyoderma than female dogs (1/72). Whereas, recurrent pyoderma was noted more in the case of female dogs (6/72) than in male dogs (3/72).

Antibiotic sensitivity testing

Amongst major coagulase positive staphylococci (CoPS) isolates, a higher degree of antibiotic resistance was revealed against penicillin-G (46.27%), followed by amoxyclav (38.81%), enrofloxacin (32.84%), cefpodoxime, oxytetracycline (28.36% each), levofloxacin (26.86%), and Co-Trimoxazole (22.39%). Whereas, a lower degree of antibiotic resistance was observed against ceftazidime (14.92%), methicillin (10.45%), ceftizoxime, clindamycin (8.96% each), amikacin, cefepime, chloramphenicol (7.46% each), cefoperazone (5.97%), ceftriaxone, gentamicin (4.48% each), and ampicillin/sulbactam (2.98%).

Amongst antibiotics tested under the β-lactam group, a higher degree of antibiotic resistance was observed against penicillin-G (46.27%), followed by amoxyclav (38.81%), cefpodoxime (28.36%), ceftazidime (14.92%), methicillin (10.45%), ceftizoxime (8.96%), cefepime (7.46%), cefoperazone (5.97%), ceftriaxone (4.48%) and ampicillin/sulbactam (2.98%). Whereas in non-β-lactam antibiotics, higher antibiotic resistance was detected against enrofloxacin (32.84%), followed by oxytetracycline (28.36%), levofloxacin (26.86%), co-trimoxazole (22.39%), clindamycin (8.96%), amikacin and chloramphenicol (7.46% each), and gentamicin (4.48%). Out of 67 tested major CoPS isolates, 34 (50.74%) isolates comprising of 29 (49.15%) *S. pseudintermedius*, 3 (50.00%) *S. schleiferi* subsp. *Coagulans*, and 2 (100%) *S. aureus* isolates exhibited

Table 2: Antibiotic susceptibility and resistance patterns of major CoPS isolates

Antibiotic group	Antibiotic used	Susceptibility (%)	Intermediate (%)	Resistance (%)
Beta lactams	Methicillin	88.06%	1.49%	10.45%
	Penicillin-G	53.73%	-	46.27%
	Ampicillin/Sulbactam	97.01%	-	2.99%
	Amoxiclav	59.70%	1.49%	38.81%
Cephalosporins	Ceftazidime	82.09%	2.99%	14.92%
	Cefepime	91.05%	1.49%	7.46%
	Cefpodoxime	62.69%	8.95%	28.36%
	Ceftriaxone	88.06%	7.46%	4.48%
	Ceftizoxime	85.07%	5.97%	8.96%
	Cefoperazone	91.04%	2.99%	5.97%
Aminoglycosides	Gentamicin	88.05%	7.46%	4.47%
	Amikacin	91.05%	1.49%	7.46%
Fluoroquinolones	Levofloxacin	70.15%	2.99%	26.86%
	Enrofloxacin	67.16%	-	32.84%
Tetracyclines	Oxytetracycline	70.15%	1.49%	28.36%
Monobactum	Aztreonam	0%	0%	100%
Amphenicol	Chloramphenicol	77.61%	14.93%	7.46%
Sulfa group	Co-Trimoxazole (Trimethoprim/Sulphamethoxazole)	73.13%	4.48%	22.39%
Lincomycin	Clindamycin	74.63%	16.42%	8.95%

Table 3: Comparison of phenotypic and genotypic determinants of methicillin resistance among major CoPS isolates

Sr. No.	Major CoPS spp.	Total No. of isolates (n=67)	Methicillin resistance	
			Phenotype	Genotype (*mecA*)
1	Methicillin resistant *Staphylococcus pseudintermedius* (MRSP)	59	7 (11.86%)	12 (20.34%)
3	Methicillin resistant *Staphylococcus schleiferi* subsp. *coagulans* (MRSS)	6	0	1 (16.67%)
5	Methicillin resistant *Staphylococcus aureus* (MRSA)	2	0	1 (50%)

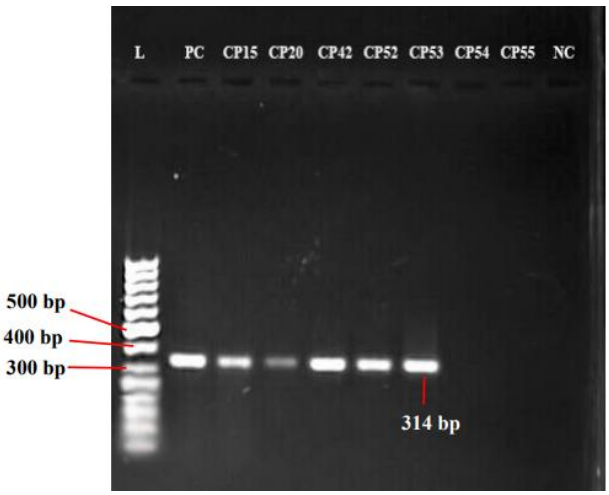

Fig. 1: Detection of *mecA* gene (314 bp *S. schleiferi* subsp. *Coagulans*) through PCR. L: 50 bp plus ladder; PC: Positive control (*S. aureus* ATCC 43300) CP15, CP20, CP42, CP52; CP53: Samples positive for presence of *mecA* gene CP54; CP55: Negative samples, and NC: Negative control (*E. coli* MTCC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

multidrug resistance against 19 selected antibiotics of different classes (Table 2). In *S. aureus*, 100% MDR was observed with the fact that the number of *S. aureus* isolated in the present study was less.

Detection of antibiotic resistance genes

In this study, out of the 67 major CoPS isolates, 14 (20.90%) isolates yielded the desired fragment of the 314 bp amplicon of the *mecA* gene (Fig. 1). Of these, 12 (20.34%) isolates were methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), 1 (16.67%) isolate was identified as methicillin-resistant *S. schleiferi* subsp. *coagulans* (MRSS) and 1 (50%) isolate was confirmed as methicillin-resistant *S. aureus* (MRSA) (Table 3). However, only 7 (10.44%) isolates exhibited methicillin resistance phenotypically amongst the total major CoPS isolates by disc diffusion test. None of the CoPS isolates yielded the desired fragment of amplicon for the mupirocin-resistant gene (*mupA*, *mupLL*), vancomycin-resistant gene (*vanA*), and coagulase gene (*coa*).

Minimum inhibitory concentration (MIC)

The MIC results of the present study reflected 1 (1.49%) isolate (*S. pseudintermedius*) (MupRL) with low-level resistant (8 mcg/ml) to mupirocin (carrying low level mupirocin resistance) and 1 (1.49%) isolate (*S. pseudintermedius*) was resistant (8 mcg/ml) to Cefoxitin (Table 4; Fig. 2), whereas none of the isolates were found resistant to vancomycin.

Discussion

A total of 75 *Staphylococcus* spp. were identified during the study, of these, 78.67% were of *Staphylococcus pseudintermedius*, 8% of *Staphylococcus*

Table 4: Distribution of MDR and E-test resistant isolates among major CoPS spp.

Sr. No.	Major CoPS spp.	Total No. of isolates	No. of MDR isolates (%)	E-test		
				Mupirocin	Vancomycin	Cefoxitin
1	*S. pseudintermedius*	59	29 (49.15%)	1 (1.69%)*	0	1 (1.69%)
2	*S. schleiferi* subsp. *coagulans*	6	3 (50%)	0	0	0
3	*S. aureus*	2	2(100%)	0	0	0
Total		67	34	1 (1.49%)	0	1 (1.49%)

* Low level mupirocin resistance (8-256 mcg/ml)

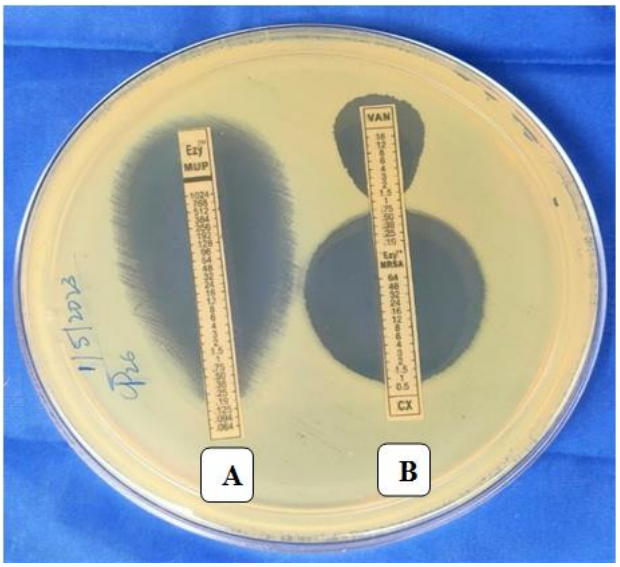

Fig. 2: Determination of minimum inhibitory concentration using E-test. (**A**) Mupirocin Ezy MIC™ strip, and (**B**) Vancomycin-Cefoxitin Ezy MIC™ strip

schleiferi subsp. c*oagulans*, 2.66% of *Staphylococcus aureus* and 10.67% were of other *Staphylococcus* spp. Comparatively similar findings were also observed by Ankita and Gandge (2018), Abusleme *et al*. (2022) as well as Rana *et al*. (2022) in their studies, which indicates its major involvement in canine pyoderma. Ravens *et al*. (2014), Chaudhary *et al*. (2019) as well as Tamakan and Gocmen (2022) in their studies observed a higher percentage of incidence of these organisms in cases of pyoderma compared to present findings. Differences in results could be due to attributes of various factors like age, health status, and other concurrent infections as well as seasons. However, a lower percentage of incidence was reported by Shah *et al*. (2017), who reported 69.49% *S. pseudintermedius*, 25.42% *S. aureus*, and 5.08% other *Staphylococcus* spp. Contrary to the present results, Senapati *et al*. (2014) reported *S. aureus* in 85.8% of samples and *S. pseudintermedius* in 22.3% of samples. The variation in results may be due to the involvement of breed susceptibility, geographical differences, and hygienic practices.

Nearly the same number of male and female dogs were found affected by surface and superficial pyoderma, respectively. However, male dogs were found more affected by deep pyoderma than female dogs. This may be because male dogs are preferred to be kept as pets than female dogs (Khinchi, 2019). Whereas, female dogs were observed more affected by recurrent pyoderma than male dogs. This may be because female dogs are attributed to various stress factors such as whelping, lactation, and cyclicity, which lower the immune status of these animals, making them more susceptible to these infections (Janardhan *et al.*, 2022).

In the present study, a higher degree of antibiotic resistance was observed against β-lactam antibiotics (Table 2). In concurrence with this study, Dziva *et al*. (2015) reported that 49.2% and 20% of CoPS isolates were resistant to penicillin and amoxicillin/clavulanic acid, respectively. Park *et al*. (2018) found that 96.4% of *S. pseudintermedius* isolates were resistant to penicillin. Similar results were also reported by Gonzalez-Dominguez *et al*. (2020), Prior (2021), and Lai *et al*. (2022). Similar kind of resistance gained by the same antibiotics may be due to common factors like similar management and drug usage practices in different geographical areas. Contrary to the present findings were also observed by Huerta *et al*. (2011), Hariharan *et al*. (2014), and Chaudhary *et al*. (2019) which may be due to strain variation prejudiced use of antibiotics in treating such cases.

During the study, few antibiotics amongst non-β-lactam groups were found for the development of a higher degree of resistance. The researchers Dziva *et al*. (2015), Gonzalez-Dominguez *et al*. (2020), and Abusleme *et al*. (2022) also reported higher resistance against various non-β-lactam group of antibiotics which were in concurrence with the present findings. This similarity in resistance could be because of the common choice of antibiotics among clinicians for the non-β-lactam group of antibiotics for the treatment of canine skin disorders in different regions. Contrarily to these results, Reddy *et al*. (2016) and Lai *et al*. (2022) reported a higher degree of resistance amongst various antibiotics of the non-β-lactam group, which may be attributed to the indiscriminate or under-dose uses of antibiotics in that particular geographical region for the treatment of various skin ailments of canines.

In this study, a significant proportion (50.7%) of CoPS isolates exhibited multidrug resistance (MDR), demonstrating resistance to a range of 3 to 15 drugs out of the 19 antibiotics that were used. In concurrence with the present study, different studies have highlighted the emergence of multidrug resistance amongst CoPS. Our findings are in support with the study of Perez-Sancho *et al*. (2020), and Gomez-Sanz *et al*. (2013). Different studies have also detected MDR amongst MSSP isolates from companion animals (Couto *et al*., 2014). Lee *et al*. (2019) reported that 27.5% (90/327) of the most

commonly identified isolates were resistant to three or more antimicrobial classes and were considered multidrug-resistant. The present study has reported a higher degree of MDR, which is a disturbing finding. The trend for increased resistance to more classes of antimicrobials is truly alarming. Phenotypically by E-test, low-level mupirocin resistance was observed in 1.49% of isolates (*S. pseudintermedius* (MupRL)), and 1.49% of the *S. aureus* isolate showed resistance to cefoxitin. Similar findings were documented by various scientists in humans (Kumar *et al*., 2020; Mostafa and Awad, 2020; Taha *et al*., 2022) because mupirocin is not a drug of choice for the treatment of canine pyoderma.

By studying the antibiogram pattern, it has been observed that *S. pseudintermedius* isolates retrieved from deep pyoderma had low-level resistance to mupirocin, resistance to cefoxitin, and resistance to 15 out of the 19 antibiotics that were used in this study. There might be progression from superficial pyoderma to deep pyoderma because of treatment failure. This is a matter of concern because of the zoonotic potential of this multidrug-resistant *S. pseudintermedius* isolates, which may get transmitted from dogs to humans, leading to treatment failure with lifesaving antibacterial drugs in humans. Therefore, instead of directly using antibiotics indiscriminately, bacterial culture and antibiotic susceptibility testing should be done before therapy to prevent the development of antibiotic resistance.

The low-level resistance to mupirocin might be due to the capacity of staphylococci to transfer plasmids between the species. *S. pseudintermedius* might have acquired the resistance plasmid by harboring a mupirocin resistance gene from *S. aureus* of human carriers or vice versa, which may be due to persistent usage of mupirocin for the decolonization of MRSA (Godbeer *et al*., 2014; Park *et al*., 2018). Another probable reason is that, as mupirocin is a topical antibiotic, high concentration or sustained sub-inhibitory concentration is maintained at the site of the infection. This causes point mutations in the tRNA synthetase chromosomal gene (*ileS*), which is stable and non-transferable, conferring low-level mupirocin resistance (Mahmoudi *et al*., 2019).

Although 14 major CoPS isolates were found to carry the *mecA* gene in our study, only 1 isolate was found phenotypically resistant to cefoxitin. This may be because the E-test for determining the MIC of cefoxitin does not reliably detect the presence of *mecA* in *S. pseudintermedius* isolates, so laboratories should undertake oxacillin disc or MIC analysis if they encounter *S. pseudintermedius* isolates (Wu *et al*., 2016).

In this study, 20.90% of the isolates were found to carry the *mecA* gene amongst 67 CoPS isolates. Of these, 20.34%, 16.67%, and 50% of the isolates were confirmed as MRSP, MRSS, and MRSA, respectively. However, only 10.44% of the major CoPS isolates were methicillin-resistant phenotypically by the disc diffusion test, which may be because the *mecA* gene can be heterogeneously expressed, and hence all methicillin-resistant staphylococcal strains may be undetectable by phenotypic approaches (Duran *et al*., 2012). Over the past 15 years, numerous studies have demonstrated the emergence and increasing prevalence of methicillin and multidrug resistance in staphylococci obtained from not only humans but also from several animal species, including horses and dogs (Magiorakos *et al*., 2012; Priyantha *et al*., 2016). This is in accordance with Gonzalez-Dominguez *et al*. (2020), Prior (2021), and Abusleme *et al*. (2022) who also documented similar or little higher methicillin-resistance staphylococcus species isolated from canine pyoderma, whereas lower resistance was reported by Dziva *et al*. (2015), and Rana *et al*. (2022) in their respective studies. Contrary to the present study, Tamakan and Gocmen (2022) reported 0% MRSA isolates. The variation in finding resistance may be due to the different antibiotic usage patterns in a particular area and its different antibiotic usage policies.

Public health significance

The prevalence of MRSP infections has been rising recently. MRSP has been regarded as a One Health problem (Prior, 2021). As humans are not natural hosts for *S. pseudintermedius*, there is an illustration that, these bacteria being reservoirs, can spread the antimicrobial resistant genes to commensal skin flora of humans because of the close interaction of humans and animals. Since MRSP can persist in the environment for a long time, bacteria may spread from dogs to humans and vice versa. The risk factors for the acquisition of MRSP are the same as those of human MRSA, such as repetitive antibiotic therapy, frequent hospital visits, and invasive procedures which is a risk factor for dog owners with weak immune systems or households with multiple pets. According to Somayaji *et al*. (2016), 92.1% of the patients audited for their study who had *S. pseudintermedius* infection, either lived with dogs or had regular contact with dogs. Globally, as the prevalence of MRSP-associated infections in humans and dogs continues to rise, it is considered an emerging zoonotic agent (Prior, 2021).

This study was undertaken because *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* are known to be the primary etiological agents causing canine pyoderma and little research has been done in India to detect their presence. We have observed a high occurrence of both methicillin resistance and multidrug resistance amongst the isolated species of staphylococci. Moreover, we have noticed an increased level of resistance amongst certain β-lactam and non β-lactam antibiotics compared to previous studies. These findings compel clinicians to review their approach to designing a treatment regimen for canine skin infections. Our current findings challenge the conventional belief that *S. aureus* is the most commonly reported *Staphylococcus* species in cases of canine skin infection. The MRSP, MRSS, and MRSA have been reported in this study as well as reported throughout the world, which represents a serious threat to the dog and human health. A low-level of mupirocin resistance of *S. pseudintermedius* also indicates further studies.

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Conflict of interest

The authors declare no conflicts of interest.

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