

Molecular detection of major coagulase positive *Staphylococcus* isolates from the canine pyoderma cases

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ABSTRACT

Pyoderma is a one of the most common dermatological condition frequently observed in small animal practice. The genus *Staphylococcus* is responsible for approximately 90% of pyoderma cases in dogs, caused by bacterial infection. The purpose of the present study was to investigate the prevalence of major coagulase positive *Staphylococcus* bacteria causing different types of canine pyoderma in dog breeds. The samples were collected from the clinical cases of canine pyoderma with varying grade from the different breeds of dogs for the molecular based detection of major coagulase-positive *Staphylococcus* species. Out of total bacterial isolates, majority of them were molecularly identified as *Staphylococcus* spp. by 16S rRNA gene. Of these *Staphylococcus* spp., most common isolated organism was *S. pseudintermedius* (80.82%) followed by *S. schleiferi* subsp. *coagulans* (8.22%) and *S. aureus* (2.74%), using *Pse-nuc*, *Sch-nuc*, and *Au-nuc* genes, respectively.

Keywords: Canine pyoderma, *Staphylococcus aureus*, *Staphylococcus pseudintermedius*, *Staphylococcus schleiferi* subsp. *coagulans*

Nowadays, dogs are treated as members of the family in this fast-changing socio-economic scenario of the world. As we interact with dogs every day by touching and fondling them, abnormalities of the skin can easily be recognized. In canine practice, over 20% of the cases are about dermatological issues, which are most common next to preventive health care (Hill *et al.* 2006). In dogs, flea infestations, bacterial infections, allergic skin diseases, anal sac problems and neoplasia are the most common dermatological conditions (Khoshnegah *et al.* 2013). Generally, bacterial infections occur as secondary complications of other pathological conditions like allergies, atopic dermatitis and adverse food reactions, when skin barrier dysfunction occurs (DeBoer and Marsella 2001). Pyoderma is the most common bacterial skin infection in dogs, caused by many Gram-positive or Gram-negative bacteria, which are normal resident microflora of the skin. Dogs of any age or gender are affected by pyoderma (Nocera *et al.* 2021) and are usually caused by *Staphylococcus* spp. (Nakaminami *et al.* 2021). 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 coryneforms, 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). There are many methods for the detection of bacteria which are adopted by scientists, but most convenient and rapid method for the detection of CoPS is multiplex-PCR (M-PCR) method. Sasaki *et al.* (2010), performed a sequence analysis of *nuc* genes in CoPS and related species and developed a M-PCR method for the species identification of the CoPS-Targeting *nuc* gene locus. Conventionally, identification of bacteria is usually performed by cultural isolation, biochemical tests, and sugar fermentation pattern, but it takes more than 72 h to identify the bacteria up to the genus and species level. To overcome this limitation, the most convenient technique of molecular approach is used.

MATERIALS AND METHODS

Sample collection: This study consisted of a descriptive study, including sampling of dogs with clinical symptoms typical of various types of canine pyoderma including surface pyoderma (Intertrigo), superficial spreading pyoderma, deep pyoderma and recurrent pyoderma (n=80) attending the Veterinary Clinical Complex, Veterinary College, Junagadh, Gujarat, India. The inclusion criteria for the dogs of the study were: clinical findings compatible with canine pyoderma; isolation of bacterial cocci by culturing skin swabs on bacteriological media;

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and growth of colonies compatible with *Staphylococcus* species on sheep blood agar and brain heart infusion agar. The skin swabs were collected aseptically by gentle rolling of the sterile cotton swab moistened with sterile 0.9% saline solution, across the border of the skin lesion from dogs.

Isolation and biochemical characterization: The samples collected from the canine pyoderma cases were enriched into brain heart infusion broth (BHI broth) for 6 to 8 h at 37°C. Then it was transferred onto Brain heart infusion (BHI) agar and incubated at 37°C for 48 h for pure culture. The bacteria were identified up to genus level based on colony characteristics, Gram's staining, microscopic morphology, and growth on Mannitol Salt Agar (MSA) (HiMedia laboratory, Mumbai) for individual primary isolate. Further, these isolates were subjected to study haemolytic pattern on sheep blood agar and primary biochemical tests such as catalase, oxidase and coagulase tests were conducted as per the standard procedure (Quinn *et al.* 2014).

Isolation of bacterial genomic DNA: Isolation of bacterial genomic DNA from pure staphylococcal cultures was carried out using the conventional method (Proteinase K-SDS method) as per Sambrook and Russell (2001). The purity and concentration of isolated DNA was assessed using µDrop™ Plate in µDrop plate reader (Thermo Scientific).

Detection of *Staphylococcus* genus and species using PCR: The sets of primers for the detection of genus and species of coagulase positive staphylococci were used as described by different authors. The detail about name and oligonucleotide sequence of primers with targeted gene along with their product size are given in Table 1. The composition of reaction mixture was as described by Martineau *et al.* (2001) and Gonzalez- Dominguez *et al.* (2020). The cycling conditions for PCR for *Staphylococcus* genus specific 16SrRNA gene was kept at initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 55°C for 40 s and an extension at 72°C for 45 s with a final extension step at 72°C for 7 min. The cycling condition for *Staphylococcus* species specific genes (*Au-nuc*, *Pse-nuc*, and *Sch-nuc*) identification was kept at initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 30 s and an extension

at 72°C for 30 s with a final extension step at 72°C for 5 min. The amplification reactions were carried out using a programmable thermal cycler (Verity, Applied Biosystems by life technology, Singapore). The PCR products (5 µL) were analyzed by gel electrophoresis on agarose (1.5% w/v) gel followed by visualization of product using gel documentation system (Bio-PrintST4® Vilber Lourmat).

RESULTS AND DISCUSSION

Identification and biochemical characterization: A total of 75 isolates were confirmed as *Staphylococcus* spp. among 108 bacterial isolates recovered from 80 dogs with canine pyoderma based on Gram's staining, morphology, growth characteristics and various biochemical tests, which included 59 (78.67%) *Staphylococcus pseudintermedius*, 6 (8%) *Staphylococcus schleiferi* subsp. *coagulans*, 2 (2.66%) *Staphylococcus aureus* and 8 (10.67%) other *Staphylococcus* spp. Similar results were obtained by Abusleme *et al.* (2022) who reported, 84.48% *S. pseudintermedius* and 15.52% *S. aureus*; Rana *et al.* (2022) reported, 78.16% *S. pseudintermedius*, 19.71% *S. aureus* and 2.11% other *Staphylococcus* spp. Whereas higher per cent of incidence was reported by Chaudhary *et al.* (2019) who reported, 87% *S. intermedius*, 8% *S. aureus* subsp. *aureus*, 3% other *Staphylococcus* spp. and 2% *S. schleiferi* subsp. *coagulans*; Tamakan and Gocmen (2022) reported, 87.5% *S. pseudintermedius*, 9.37% *S. aureus* and 3.12% *S. schleiferi*. However lower per cent 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% samples and *S. pseudintermedius* in 22.3% samples.

Molecular detection of various species of coagulase positive staphylococci: Primers targeting 16S rRNA gene previously designed by Martineau *et al.* (2001) were employed for the 16S rRNA gene confirmation in the *Staphylococcus* isolates. Out of 75 phenotypically identified isolates, 73 isolates were confirmed as *Staphylococcus* species. The primers targeting species specific thermonuclease (*nuc*) genes of *S. pseudintermedius*, *S. schleiferi* subsp. *coagulans* and *S. aureus* previously designed by Gonzalez-Dominguez *et al.* (2020) were used for identification of major coagulase

Table 1. Oligonucleotide sequences of primers used for identification of major coagulase positive staphylococci by targeting genus and species-specific target gene

Primer sequence (5' to 3')	Target gene	Product size (bp)	Reference
F: GGCCGTGTTGAACGTGGTCAAATCA R: TTACCATTTTCAGTACCTTCTGGTAA	16SrRNA (<i>Staphylococcus</i> spp.)	370bp	Martineau <i>et al.</i> (2001)
F: CAGAAACGGTGAAACCGAAT R: CCATAGCGGTCTTGCTTTTC	<i>Au-nuc</i> (<i>S. aureus</i>)	127bp	Gonzalez- Dominguez <i>et al.</i> (2020)
F: TGATGCAGCTTTTCCGTATG R: AAAGATGGGCAAGATGAACG	<i>Pse-nuc</i> (<i>S. pseudintermedius</i>)	99bp	
F: TAAACGACGGAAGGCAGT R: CCAATCATACGCACACGTTTC	<i>Sch-nuc</i> (<i>S. schleiferi</i> subsp. <i>coagulans</i>)	115bp	

F, Forward; R, Reverse.

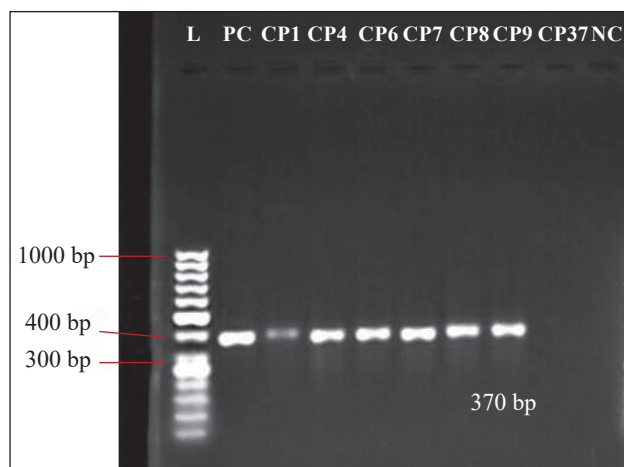


Fig. 1. Genus specific PCR of *Staphylococcus* spp. for 16S rRNA gene (370 bp) [L: 50 bp plus ladder, PC: Positive control (*S. aureus* ATCC 43300), CP1, CP4, CP6, CP7, CP8, CP9: Samples positive for *Staphylococcus* spp. isolated from canine pyoderma, CP37: Negative sample, and NC: Negative control (*E. coli* MTCC 722)].

positive *Staphylococcus* species. During the study a standard strain of *Staphylococcus aureus* (ATCC 43300), *S. pseudintermedius* (ATCC 49444), *S. schleiferi* subsp. *coagulans* (ATCC 49545) were used as positive control and *E. coli* (MTCC-722) was used as negative control for the detection of *Staphylococcus* species specific *nuc* genes.

During the study, 73 isolates were confirmed as *Staphylococcus* species by PCR using genus specific 16sRNA gene primer (Fig. 1). Out of 73 molecularly confirmed *Staphylococcus* isolates, 59 (80.82%) isolates yielded desired fragment of 99 bp amplicon of *Pse-nuc* gene, specific for *S. pseudintermedius*, 6 (8.22%) isolates yielded desired fragment of 115 bp amplicon of *Sch-nuc* gene specific for *S. schleiferi* subsp. *coagulans*, 2 (2.74%) isolates yielded desired fragment of 127 bp amplicon of *Au-nuc* gene specific for *S. aureus* and 6 (8.22%) isolates did not yield any fragment to fall into above categories of *Staphylococcus* spp.

A total of 97.33% isolates were confirmed molecularly as *Staphylococcus* spp. Similarly, Viegas *et al.* (2022) reported 99.23% and Igbinosa *et al.* (2016), Mathapati *et al.* (2016), Chaudhary *et al.* (2021), Fadhil and Mohammed (2022), Kadhim and Abdullah (2022) reported 100% *Staphylococcus* isolates positive for the presence of 16S rRNA gene.

During study, 59/73 isolates were confirmed as *S. pseudintermedius*. Similar results were reported by Abusleme *et al.* (2022) and Rana *et al.* (2022), who reported 84.48% and 78.16% *S. pseudintermedius* isolates, respectively. Whereas, higher per cent of positivity was obtained by Soedaranto *et al.* (2011) and Schmidt *et al.* (2014), who reported 100% and 89.21% *S. pseudintermedius* isolates, respectively. However, lower per cent of positivity was obtained by Gonzalez-Dominguez *et al.* (2020) and Hritcu *et al.* (2020) who reported 30% and 69.27% *S. pseudintermedius* isolates, respectively. However, 6/73

isolates were confirmed as *S. schleiferi* subsp. *coagulans*. Similar results were obtained by Kawakami *et al.* (2010), who reported 10.5% *S. schleiferi* subsp. *coagulans* isolates. In contrary to this study, Sasaki *et al.* (2010), Dziva *et al.* (2015) and Gonzalez-Dominguez *et al.* (2020) reported 1.33%, 3.1% and 2% *S. schleiferi* subsp. *coagulans* isolates, respectively (lower percentage than *S. aureus*). During the study, 2/73 (2.74%) isolates were confirmed as *S. aureus*. In comparison to this finding, little higher percentage were obtained by Lautz *et al.* (2006), Schmidt *et al.* (2014) and Gonzalez-Dominguez *et al.* (2020) who reported 8.16%, 10.78% and 6% *S. aureus* isolates, respectively. A very high percentage was observed by Dziva *et al.* (2015), Abusleme *et al.* (2022) and Rana *et al.* (2022), who reported 58.5%, 15.52% and 19.71% *S. aureus* isolates, respectively.

This study concludes the fact that, *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* are known to be the primary etiological agents causing canine pyoderma. As humans are not natural hosts for *S. pseudintermedius*, there is illustration that, these bacteria being reservoir, can spread the antimicrobial resistant genes to the human commensal skin flora because of close interaction of humans and dogs. Since MRSP can persist in the environment for a long time, bacteria may spread from dogs to humans and vice-versa. Globally, as the prevalence of MRSP associated infections in humans and dogs continues to rise, it is considered as an emerging zoonotic agent.

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