International Journal of Veterinary Sciences and Animal Husbandry 2024; 9(2): 407-410

International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; 9(2): 407-410
© 2024 VET
www.veterinarypaper.com
Received: 17-12-2023
Accepted: 27-02-2024

Dixit K Parasana
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India

Pushpa M Makwana
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India

Pallavi Maddela
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India

IH Kalyani
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India

Dharmesh R Patel
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India

Sadaf Jan
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India

Corresponding Author:
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India

Molecular detection of coagulase negative *Staphylococcus* species from bovine mastitis

Dixit K Parasana, Pushpa M Makwana, Pallavi Maddela, IH Kalyani, Dharmesh R Patel and Sadaf Jan

Abstract
Mastitis is considered as a major economic condition of a dairy animals. The present study was carried out to investigate role of coagulase-negative staphylococci (CoNS) in bovine mastitis. Total 40 *Staphylococcus* isolates were used for molecular detection of CoNS. Various CoNS were identified up to species level by PCR. The most predominant species identified was *Staphylococcus epidermidis* (20%), followed by *Staphylococcus haemolyticus* (15%)*, Staphylococcus chromogenes* (12.5%)*, Staphylococcus fluerettii* (7.5%), *Staphylococcus sciuri* (2.5%) and *Staphylococcus simulans* (2.5%). Our findings indicate that coagulase-negative staphylococci (CoNS) are also important in the development of mastitis in bovines.

Keywords: CoNS, Mastitis, PCR, *Staphylococcus haemolyticus*, *Staphylococcus sciuri*

Introduction
Bovine mastitis is a multi-etiological condition and occurs based on factors related to the animals and environment, which leads to harmful effects on animal health and decrease profit for dairy farmers (Parasana *et al.* 2022) [1]. Animals with higher milk production are more susceptible to mastitis which remains a serious disease in animals with significant economic loss (Parasana *et al.* 2021) [2].
Staphylococcus species are well-known bacteria that are frequently isolated from the milk samples of most cows infected with clinical and subclinical mastitis (Tenhagen *et al.* 2006) [3]. They are mainly classified into coagulase-positive staphylococci (CoPS) which are more pathogenic and coagulase-negative staphylococci (CoNS) which are considered as minor pathogens (De Buck *et al.* 2021) [4].
CoNS mastitis-causing organisms include *Staphylococcus epidermidis*, *Staphylococcus simulans*, *Staphylococcus chromogenes*, *Staphylococcus xylosus* and *Staphylococcus haemolyticus* (Taponen *et al.* 2006) [5]. Most of the time, CoNS recognition and identification are handled as a collection or group rather than as individual species. Different species of CoNS display diverse virulence traits (Waller *et al.* 2011) [6]. For effective therapeutic treatment and mastitis control identification of CoNS at the species level using more precise methods is considered as a key facor (Idamokoro, 2022) [7].
For identification of CoNS at species level, various commercial biochemical kits are available but it these kits have limitations for identification of all the species of CoNS from animal source (Bes *et al.* 2000) [8]. The use of conventional biochemical tests is more laborious and time consuming (Couto *et al.* 2001) [9] compared to molecular techniques which are considered superior for identification of CoNS upto species level.
The identification of *Staphylococcus* species other than *Staphylococcus aureus* is not routinely performed in veterinary diagnostic laboratories. However, the identification of these species might be important for epidemiological investigations, the assessment of the pathogenic significance of these organisms and the development of specific management practices to prevent mastitis (Sampimon *et al.* 2009) [10]. The use of genotypic methods adds precision in the identification of these species and molecular tests, such as ribotyping and sequencing, have been used to identification of CoNS (Lange *et al.* 2015) [11].

Materials and Methods

Sample collection

In present study 40 isolates of *Staphylococcus* previously confirmed up to genus level recovered from bovine mastitis cases preserved at Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Navsari, Gujarat were used for the species level identification of various Coagulase negative *Staphylococcus* species.

DNA extraction and molecular detection of coagulase negative *Staphylococcus* spp.

For molecular confirmation of various Coagulase negative *Staphylococcus* species primers previously described by Preethirani *et al*. 2015 [12] were used (Table 1). Briefly, DNA extraction from bacterial colony was carried out by manual heating and chilling method as per Chitra *et al*. 2015 [13]. For PCR, reaction mixture was prepared in 25 µl quantity containing 3.0 µl template DNA, 12.5 µl of 2x PCR master mix, 1.0 µl of forward and 1.0 µl of reverse primer and 7.5 µl sterile nuclease-free water. Cycling condition was set as initial denaturation at 95°C for 10 min followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 60°C for 45 s and extension at 72 °C for 45 s with a final extension step at 72 °C for 10 min. PCR product was visualized after electrophoresis using 1.5% agarose gel and with UV Transilluminator (SynGene, UK).

Table 1: Primer sequences of various CoNS

Sr. No.	Organism	Target gene	Primer (5' to 3')	Product size	Reference
1	*Staphylococcus epidermidis*	*rpoB*	F: AGGGCCTGGTGGATTAACAC R: TTGCATGTTTGCTCCCATTA	466 bp	Preethirani *et al.* (2015) [12].
2	*Staphylococcus haemolyticus*	*sodA*	F: GCAGTTGAGGGAACAGATCTTG R: CTAACTGACCATTGTTAACTACTAACC	282 bp	
3	*Staphylococcus chromogenes*	*sodA*	F: GTGACTAAGTTAAACGATGCAG R: CCATTATTTACAACGAGCCATG	303 bp	
4	*Staphylococcus sciuri*	*gap*	F: ATTTCAGCTCCAGCATCAGG R: TGGAACACGTTGAGCTGATC	354 bp	
5	*Staphylococcus simulans*	*gap*	F: CTACACTAGCGACGAAAAAGCAC R: CGTTTACTTCTTCGATTGTTACGTC	482 bp	
6	*Staphylococcus xylosus*	*rpoB*	F: GTCTAGTTATGCCCGTGTGAATG R: AACAATTGCAGCACCTGAGTC	433 bp	
7	*Staphylococcus fluerettii*	*rpoB*	F: ATCAGCTCTTGGACCCGG R: GTCACGAGCAGTTACGTGTTCC	550 bp	

Results

Out of 40 *Staphylococcus*, 8 isolates were amplified *rpoB* gene of *Staphylococcus epidermidis* with specific 466 bp amplicon, 6 isolates were amplified *sodA* gene of *Staphylococcus haemolyticus* with specific 282 bp amplicon and 1 isolate was amplified *gap* gene of *Staphylococcus simulans* with specific 482 bp amplicon (Figure 1).

Out of 40 *Staphylococcus*, 5 isolates were amplified *sodA* gene of *Staphylococcus chromogenes* with specific 303 bp amplicon (Figure 2).

Out of 40 *Staphylococcus*, 3 isolates were amplified *rpoB* gene of *Staphylococcus fluerettii* with specific 550 bp amplicon and 1 isolate was amplified *gap* gene of *Staphylococcus sciuri* with specific 354 bp amplicon (Figure 3). None of the isolate were amplified *rpoB* gene of *Staphylococcus xylosus*. Distribution of various CoNS is shown in Table 2.

Table 2: Percentage wise distribution of CoNS

Organism	Percentage
Staphylococcus epidermidis	8 (20%)
Staphylococcus haemolyticus	6 (15%)
Staphylococcus chromogenes	5 (12.5%)
Staphylococcus fluerettii	3 (7.5%)
Staphylococcus sciuri	1 (2.5%)
Staphylococcus simulans	1 (2.5%)
Other staphylococci	16 (40%)

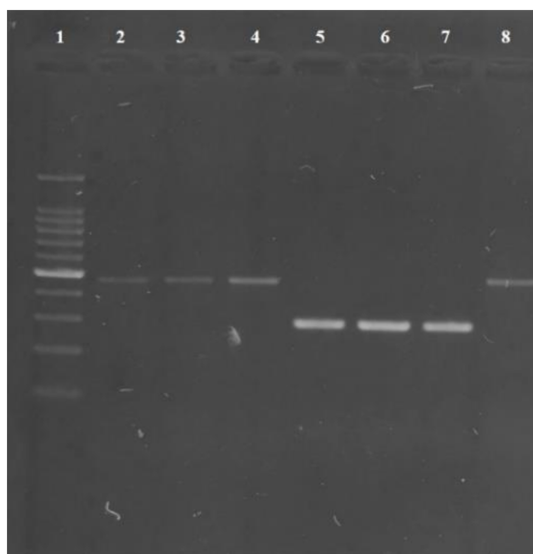

Fig 1: Lane 1- 100 bp Ladder; Lane 2,3,4- *Staphylococcus epidermidis* isolates (466 bp); Lane 5,6,7- *Staphylococcus haemolyticus* isolates (282bp); Lane 8- *Staphylococcus simulans* isolate (482 bp)

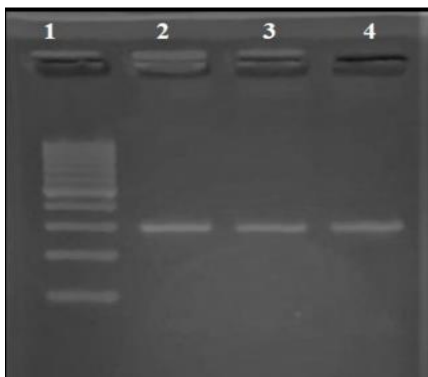

Fig 2: Lane 1- 100 bp Ladder; Lane 2,3,4- *Staphylococcus chromogenes* isolates (303 bp)

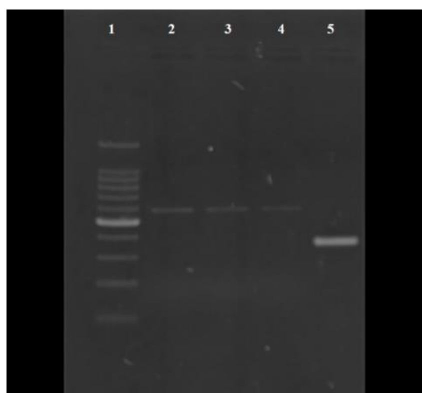

Fig 3: Lane 1- 100 bp Ladder; Lane 2,3,4- *Staphylococcus fluerettii* isolates (550 bp); Lane 5- *Staphylococcus sciuri* isolate (354 bp)

Discussion

The study revealed that *Staphylococcus epidermidis* (20%) exhibited a higher prevalence followed by *Staphylococcus haemolyticus* (15%) and *Staphylococcus chromogenes* (12.5%). Many scientists reported similar results as well. Kenar *et al*. 2012 [14] and Ouakli *et al*. 2022 [15] reported higher prevalence of *Staphylococcus epidermidis*. Becker *et al*. 2014 [16] and Hosseinzadeh *et al*. 2014 [17] found *Staphylococcus haemolyticus* as a most prevalent bacteria from mastitis. Perez and Ancuelo, 2022 [18] reported higher prevalence of *Staphylococcus chromogenes.*

The prevalence of *Staphylococcus sciuri* (2.5%), *Staphylococcus simulans* (2.5%) and *Staphylococcus fluerettii* (7.5%) was lower in our investigation. Similar findings were reported by many scientists. Moser *et al*. 2014 [19] found less prevalence of *Staphylococcus fluerettii.* Ouakli *et al*. 2022 [15] and Perez and Ancuelo, 2022 [18] reported very less prevalence of *Staphylococcus simulans.*

According to the current study's findings, not a single isolate was recognized as a *Staphylococcus xylosus*. In contrary to our results, Soares *et al.* 2012 [20], El-seedy *et al.* 2017 [21] and Klibi *et al.* 2018 [22] reported *Staphylococcus xylosus* as a predominant cause of mastitis.

Coagulase-negative staphylococci were formerly considered as a minor pathogens that had little to no effect on the pathogenicity of mastitis. However, it has been identified to be an emerging pathogen that causes mastitis, according to recent investigations on CoNS by Bhavana and Chaitanya, 2022 [23]; Ouakli *et al.* 2022 [15]; Perez and Ancuelo, 2022 [18].

In summary, our investigation revealed that CoNS are significant pathogen that causes mastitis in bovine. Identification of CoNS upto species level by molecular methods *viz.* PCR and sequencing can be considered superior than biochemical test based identification.

References

1. Parasana DK, Javia BB, Fefar DT, Barad DB, Ghodasara SN. Detection of virulence associated genes in *Streptococcus agalactiae* isolated from bovine mastitis. Iranian Journal of Veterinary Research. 2022;23(3):275-279.
2. Parasana DK, Javia BB, Fefar DT, Barad DB, Ghodasara SN. Molecular characterization and antimicrobial-resistant pattern of *Streptococcus* species isolated from bovine mastitis in and around Junagadh district. Ruminant Science. 2021;10(2):247-252.
3. Tenhagen BA, Koster G, Wallmann J, Heuwieser W. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. Journal of Dairy Science. 2006;89(7):2542-2551.
4. De Buck J, Ha V, Naushad S, Nobrega DB, Luby C, Middleton JR, Barkema HW. Non-*aureus* staphylococci and bovine udder health: current understanding and knowledge gaps. Frontiers in Veterinary Science. 2021;8:658031.
5. Taponen S, Simojoki H, Haveri M, Larsen HD, Pyorala, S. Clinical characteristics and persistence of bovine mastitis caused by different species of coagulase-negative staphylococci identified with API or AFLP. Veterinary Microbiology. 2006;115(1-3):199-207.
6. Waller KP, Aspan A, Nyman A, Persson Y, Andersson UG. CNS species and antimicrobial resistance in clinical and subclinical bovine mastitis. Veterinary Microbiology. 2011;152(1-2):112-116.
7. Idamokoro EM. Coagulase-negative staphylococci as an evolving mastitis causing organism in cows: A review. F1000 Research. 2022;11:824.
8. Bes M, Guerin-Faublee V, Meugnier H, Etienne J, Freney J. Improvement of the identification of staphylococci isolated from bovine mammary infections using molecular methods. Veterinary Microbiology. 2000;71(3-4):287-294.
9. Couto I, Pereira S, Miragaia M, Sanches IS, de Lencastre H. Identification of clinical staphylococcal isolates from humans by internal transcribed spacer PCR. Journal of Clinical Microbiology. 2001;39(9):3099-3103.
10. Sampimon OC, Barkema HW, Berends IM, Sol J, Lam TJ. Prevalence and herd-level risk factors for intramammary infection with coagulase-negative staphylococci in Dutch dairy herds. Veterinary Microbiology. 2009;134(1-2):37-44.
11. Lange CC, Brito MA, Reis DR, Machado MA, Guimaraes AS, Azevedo AL, *et al*. Species-level identification of staphylococci isolated from bovine mastitis in Brazil using partial *16S rRNA* sequencing. Veterinary Microbiology. 2015;176(3-4):382-388.
12. Preethirani PL, Isloor S, Sundareshan S, Nuthanalakshmi V, Deepthikiran K, Sinha AY, *et al*. Isolation, biochemical and molecular identification, and in-vitro antimicrobial resistance patterns of bacteria isolated from bubaline subclinical mastitis in South India. PLoS One. 2015;10(11):e0142717.
13. Chitra MA, Jayanthy C, Nagarajan B. Detection and sequence analysis of accessory gene regulator genes of *Staphylococcus pseudintermedius* isolates. Veterinary World. 2015;8(7):902.
14. Kenar B, Kuyucuoglu Y, Seker E. Antibiotic susceptibility of coagulase-negative staphylococci isolated from bovine subclinical mastitis in Turkey. Pakistan Veterinary Journal. 2012;32(3):390-393.

15. Ouakli N, Djamila BA, Nadia D, Abdelkrim Y, Rachid K. Staphylococcal subclinical mastitis in dairy cows in Blida region (Algeria). Journal of Veterinary Medical Research. 2022;30(1):1-6.
16. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clinical Microbiology Reviews. 2014;27(4):870-926.
17. Hosseinzadeh S, Dastmalchi Saei H. Staphylococcal species associated with bovine mastitis in the North West of Iran: Emerging of coagulase-negative staphylococci. International Journal of Veterinary Science and Medicine. 2014;2(1):27-34.
18. Perez RH, Ancuelo AE. Isolation and Characterization of *Staphylococcus* spp. and the unintended discovery of non-staphylococcal strains associated with bovine mastitis in region IV-A, Philippines. Philippine Journal of Science. 2022;151(5):1793-1805.
19. Moser A, Stephan R, Ziegler D, Johler S. Species distribution and resistance profiles of coagulase-negative staphylococci isolated from bovine mastitis in Switzerland. Schweizer Archive fur Tierheilkunde. 2013;155:333-338.
20. Soares LC, Pereira IA, Pribul BR, Oliva MS, Coelho SM, Souza M. Antimicrobial resistance and detection of *mecA* and *blaZ* genes in coagulase-negative *Staphylococcus* isolated from bovine mastitis. Pesquisa Veterinaria Brasileira. 2012;32(8):692-696.
21. El-Seedy F, Radwan AI, Hassan HW, Shehata A. Coagulase negative staphylococci as an emerging cause of bovine mastitis: prevalence, antimicrobial resistance and biofilm formation. Journal of Veterinary Medical Research. 2017;24(1):1-11.
22. Klibi A, Maaroufi A, Torres C, Jouini A. Detection and characterization of methicillin-resistant and susceptible coagulase-negative staphylococci in milk from cows with clinical mastitis in Tunisia. International Journal of Antimicrobial Agents. 2018;52(6):930-935.
23. Bhavana RN, Chaitanya RK. Identification of coagulase negative staphylococcal species from bovine mastitis in India. Iranian Journal of Veterinary Research. 2022;23(4):358.