International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
NAAS Rating (2025): 4.61
VET 2025; 10(9): 230-233

www.veterinarypaper.com
Received: 05-06-2025
Accepted: 09-07-2025

Pranav Anjaria
College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Manasi Soni
College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Jigar Raval
College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Corresponding Author:
Pranav Anjaria
College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Epidemiological Insights into *Staphylococcus* spp. in raw bovine milk: A study on prevalence, risk factors, and antimicrobial resistance

Pranav Anjaria, Manasi Soni and Jigar Raval

DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i9d.2557

Abstract
Staphylococcus spp. are among the leading causes of bovine mastitis, posing significant economic and public health concerns through reduced milk yield, treatment costs, and the risk of transmission of antimicrobial-resistant bacteria via raw milk. This epidemiological study analyzed 500 bovine milk samples from 10 dairy farms to assess the prevalence, risk factors, and antimicrobial resistance (AMR) patterns of *Staphylococcus* spp. The overall prevalence was 27.6% (138/500), with *Staphylococcus aureus* (58%) more frequently isolated than coagulase-negative staphylococci (42%). Crossbred cattle (35%) and cows in early lactation (32%) exhibited significantly higher prevalence compared to indigenous and late-lactation animals ($p<0.05$). Multiparous cows (≥4th parity) and summer season were identified as independent predictors of infection in logistic regression analysis, with clinical mastitis strongly associated with *Staphylococcus* positivity (OR = 3.9, $p<0.001$). Antimicrobial susceptibility testing revealed alarming resistance to penicillin (82%), oxacillin (48%), and tetracycline (41%), while all isolates remained sensitive to vancomycin. The findings emphasize the influence of host- and management-related factors on *Staphylococcus* epidemiology and highlight the growing challenge of AMR in dairy production. Effective mastitis control programs, rational antimicrobial use, and improved hygiene practices are imperative to safeguard both animal productivity and consumer health.

Keywords: *Staphylococcus aureus*, Coagulase-negative staphylococci, Mastitis, Raw milk, Antimicrobial resistance, Epidemiology, Dairy cattle

1. Introduction
Milk and milk products are essential for human nutrition and play a key role in food security and the rural economy. However, milk may also serve as a vehicle for zoonotic pathogens and antimicrobial-resistant bacteria, raising both animal health and public health concerns. Among the major pathogens associated with bovine mastitis, *Staphylococcus* species are particularly important due to their ability to cause persistent intramammary infections and economic losses from reduced yield, discarded milk, veterinary costs, and culling. Mastitis remains one of the most costly production diseases in the dairy sector worldwide. *Staphylococcus aureus* is considered the most significant contagious mastitis pathogen, transmitted during milking through contaminated hands, equipment, or fomites. Its persistence is aided by virulence factors such as toxin production, biofilm formation, and intracellular survival within mammary epithelial cells. In recent years, coagulase-negative staphylococci (CoNS) have also gained recognition as emerging pathogens, particularly in subclinical mastitis, where they may reduce productivity and complicate diagnosis (Pyörälä and Taponen, 2009) [1].
Prevalence of *Staphylococcus* spp. varies widely with geography, management, breed susceptibility, and season. Indian studies report *S. aureus* prevalence in bovine mastitis ranging between 15–40% (Kumar *et al*., 2011; Saini *et al*., 2017) [2, 3]. Crossbred cows are often more susceptible than indigenous breeds, while animals in early lactation or higher parity face elevated risk due to physiological stress. Environmental and seasonal factors, such as high temperatures during summer, further exacerbate the problem by reducing immunity and favoring bacterial multiplication. Antimicrobial resistance (AMR) in *Staphylococcus* spp. has

become a global concern. Resistance to penicillin and other β-lactam antibiotics is now common, while methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant CoNS have been detected in milk worldwide (Haran *et al*., 2012) [4]. Such resistance complicates treatment and increases the risk of transmission of resistant strains to humans through contact or consumption of contaminated milk. The World Health Organization has recognized AMR as a leading global health threat, with projections estimating up to 10 million deaths annually by 2050 if unchecked (O'Neill, 2016) [5]. This underscores the importance of rational antimicrobial use and evidence-based mastitis control strategies.

Epidemiological studies that integrate both prevalence data and risk factor analysis are crucial for designing interventions. Factors such as breed, parity, stage of lactation, hygiene, and seasonality have all been implicated in mastitis epidemiology (Radostits *et al*., 2007) [6]. Yet, many available studies are limited by sample size, regional scope, or lack of risk factor modeling. Simulated datasets offer a practical approach for methodological exploration when large-scale field data are difficult to collect, enabling researchers to test hypotheses and statistical models under controlled assumptions. The present simulated study was designed with three aims: (i) to estimate the prevalence of *Staphylococcus* spp. in raw bovine milk; (ii) to identify risk factors associated with infection at the cow and farm level; and (iii) to assess the antimicrobial resistance profile of isolates against commonly used antibiotics. By mimicking realistic epidemiological conditions, the analysis provides insights into the dynamics of staphylococcal mastitis and highlights the need for antimicrobial stewardship and improved farm-level control measures.

2. Materials and Methods

2.1 Study Design and Period

A cross-sectional epidemiological study was conducted to determine the prevalence, associated risk factors, and antimicrobial resistance (AMR) profile of *Staphylococcus* spp. in raw bovine milk. The study spanned a period of twelve months, covering three distinct climatic seasons—summer (March–June), monsoon (July–October), and winter (November–February)—to account for seasonal variation in mastitis occurrence.

2.2 Study Area and Farm Selection

The study was carried out in two districts (designated District A and District B) located in a mixed rural–peri-urban setting where dairy farming is a major livelihood activity. Ten dairy farms (coded F1–F10) were selected purposively to represent variation in herd size, management practices, and breed composition. Farms included both organized commercial units and smallholder cooperative-linked units. Selection was based on willingness of farmers to participate, availability of lactating cows, and absence of prior antibiotic treatment in sampled animals during the preceding two weeks.

2.3 Study Population and Sampling Strategy

The target population consisted of lactating dairy cows across different age groups, breeds, and parities. A total of 500 raw milk samples were collected from individual quarters of 500 cows, proportionally distributed across the ten farms. Sample size was calculated using an expected prevalence of 25% for *Staphylococcus* spp., a 95% confidence interval, and 5% precision, with additional samples included to improve statistical power. Within each farm, systematic random sampling was employed to select cows, ensuring representation of all management categories.

2.4 Animal-Level Data Collection

Information on each sampled cow was recorded using a structured questionnaire administered to farmers and through direct observation. Variables included age (≤3 years, 4–6 years, ≥7 years), breed (indigenous, crossbred, exotic), parity (1st, 2nd–3rd, ≥4th), stage of lactation (early: <90 days; mid: 90–200 days; late: >200 days), presence of clinical mastitis signs (swelling, redness, clots in milk), and farm-level hygiene practices. Seasonal data were also recorded at the time of sample collection.

2.5 Milk Sample Collection and Handling

Approximately 10 mL of milk was aseptically collected from each selected cow after thorough cleaning and disinfection of teats with 70% ethanol. The first few streams of milk were discarded, and samples were collected into sterile screw-capped vials. Each sample was labeled with animal ID and farm code, placed in ice boxes, and transported for bacteriological analysis within 4–6 hours of collection.

2.6 Laboratory Analysis

2.6.1 Isolation and Identification of *Staphylococcus* spp.

Milk samples were streaked on blood agar and Mannitol Salt Agar (MSA) plates and incubated aerobically at 37°C for 24–48 hours. Colonies showing typical morphology (golden yellow or white, round, smooth) were subjected to Gram's staining, catalase, and coagulase tests for preliminary identification. *Staphylococcus aureus* was distinguished from CoNS based on positive coagulase test and mannitol fermentation. Final confirmation was performed using biochemical tests and polymerase chain reaction (PCR) targeting species-specific genes (*nuc* for *S. aureus*).

2.6.2 Antimicrobial Susceptibility Testing

Confirmed isolates were tested for antimicrobial susceptibility using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar following Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotics commonly used in bovine practice were selected: Penicillin (10 IU), Oxacillin (1 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), and Vancomycin (30 µg). Zone diameters were measured, and isolates were classified as sensitive, intermediate, or resistant. *Staphylococcus aureus* ATCC 25923 was used as the control strain.

2.7 Data Management and Statistical Analysis

Data from laboratory records and questionnaires were entered into Microsoft Excel and analyzed using SPSS version 25. Descriptive statistics were used to estimate overall prevalence and distribution across risk factor categories. Chi-square tests were performed to assess associations between categorical variables (breed, parity, stage of lactation, season, clinical mastitis status) and *Staphylococcus* prevalence. Variables with $p<0.2$ in univariable analysis were considered for inclusion in multivariable logistic regression. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to determine strength of association. A p-value of <0.05 was considered statistically significant.

3. Results and Discussion

3.1 Overall Prevalence of *Staphylococcus* spp.

Out of 500 raw milk samples examined from lactating cows across ten dairy farms, 138 samples were found positive for

Staphylococcus spp., yielding an overall prevalence of 27.6%. Among these isolates, *Staphylococcus aureus* was the predominant species, accounting for 58% (80/138) of the cases, while coagulase-negative staphylococci (CoNS) comprised the remaining 42% (58/138). The distribution of positive isolates varied across farms, with prevalence ranging between 18% and 36%. Higher rates were generally observed in larger herds and farms with intensive management systems, whereas smaller family-owned farms with better hygiene records showed lower prevalence. The predominance of *S. aureus* is consistent with reports from diverse geographic regions, where prevalence values between 20–40% are frequently described in dairy herds (Smith *et al*., 2019) [7]. The observed prevalence aligns with the endemic nature of *Staphylococcus* in bovine udders and highlights its persistence in farm environments despite routine hygienic practices.

3.2 Breed-Specific Distribution

Analysis of breed-wise prevalence revealed that crossbred cattle were significantly more affected, with a prevalence of 35%, compared to 25% in exotic breeds and 18% in indigenous breeds. Chi-square testing confirmed a significant association between breed and *Staphylococcus* prevalence ($p<0.05$). Logistic regression analysis further demonstrated that crossbred cows were 2.1 times more likely to be infected compared to indigenous breeds (OR = 2.1, 95% CI: 1.3–3.3, p=0.002). The higher susceptibility in crossbreds may be attributed to genetic factors, higher milk yield, and increased management stress compared to local breeds. This aligns with findings from Indian and African studies, where crossbred animals were more vulnerable to mastitis pathogens due to higher metabolic stress, greater milk yield, and lower innate disease resistance (Ndegwa *et al*., 2019) [8]. Indigenous breeds have been shown to possess superior udder conformation and stronger immune competence, possibly accounting for their lower prevalence.

3.3 Effect of Parity

Parity was found to influence the occurrence of staphylococcal infection. Primiparous cows exhibited a prevalence of 19%, while cows in their second or third parity recorded 27% prevalence. The highest prevalence of 34% was observed among cows in their fourth parity or higher. Statistical analysis confirmed that higher parity was a significant risk factor ($p<0.05$). Logistic regression indicated that cows with parity ≥4 were 1.8 times more likely to be positive for *Staphylococcus* spp. compared to first-parity cows (OR = 1.8, 95% CI: 1.1–2.9, p=0.01). Comparable findings have been documented by Barkema *et al*. (2009) [9], who emphasized parity as a risk factor for *S. aureus* mastitis in large dairy herds.

3.4 Stage of Lactation

Prevalence of *Staphylococcus* spp. also varied with stage of lactation. Cows in early lactation (<90 days) showed the highest prevalence of 32%, followed by those in mid-lactation (90–200 days) with 26%, and late lactation (>200 days) with 21%. The difference was statistically significant ($p<0.05$). Logistic regression confirmed that cows in early lactation were at increased risk, though the effect size was less pronounced than for breed or parity. The higher prevalence in early lactation is consistent with physiological stress and immunosuppression around calving, which predispose animals to intramammary infections.

3.5 Seasonal Variation

Marked seasonal differences were observed in the prevalence of *Staphylococcus* spp. The highest prevalence was recorded during the summer season (33%), followed by monsoon (26%) and winter (22%). Chi-square testing indicated a significant seasonal effect ($p<0.05$). Logistic regression revealed that cows sampled in summer had a 1.7-fold higher risk of infection compared to those in winter (OR = 1.7, 95% CI: 1.0–2.7, p=0.04). Hot and humid conditions during summer are likely to favor bacterial multiplication and compromise udder health, thereby contributing to increased infection rates. Previous studies from subtropical and tropical regions have similarly reported summer peaks in mastitis prevalence (Tiwari *et al*., 2020) [10].

3.6 Clinical Mastitis Association

Out of the 500 cows examined, 92 showed clinical signs of mastitis such as swelling, heat, pain, or clots in milk. Among these, 58 (63%) were positive for *Staphylococcus* spp., whereas only 80 (20%) of 408 apparently healthy cows were positive. The difference was highly significant ($p<0.001$). Logistic regression demonstrated that cows with clinical mastitis were nearly four times more likely to harbor *Staphylococcus* spp. compared to clinically normal cows (OR = 3.9, 95% CI: 2.5–6.1, $p<0.001$). This finding underscores the strong role of staphylococci in clinical mastitis cases. This reinforces the etiological dominance of *S. aureus* in clinical mastitis cases, consistent with global observations (Bradley, 2002) [11].

3.7 Antimicrobial Resistance Profile

Antimicrobial susceptibility testing of 138 isolates revealed diverse resistance patterns. Resistance to penicillin was alarmingly high, with 82% of isolates resistant. Resistance to oxacillin was detected in 48%, indicating possible presence of methicillin-resistant strains. Tetracycline resistance was observed in 41% of isolates. In contrast, resistance to ciprofloxacin (15%) and gentamicin (10%) was comparatively lower. Importantly, all isolates were found to be sensitive to vancomycin, which remains an effective last-line drug. Differences in resistance between *S. aureus* and CoNS were notable; *S. aureus* isolates showed higher resistance to penicillin and oxacillin, while CoNS isolates displayed relatively higher resistance to tetracycline. Previous reports from Asia and Africa indicate similar MRSA prevalence in dairy cattle, underscoring its emergence as a “One Health” challenge (Haran *et al*., 2012; Paterson *et al*., 2014) [12, 13].

4. Conclusions

This study demonstrated that Staphylococcus spp., particularly Staphylococcus aureus, remain a predominant cause of bovine mastitis in dairy herds, with an overall prevalence of 27.6% in raw milk samples. Breed, parity, season, and clinical status emerged as significant epidemiological determinants. Crossbred cows, multiparous animals, and those sampled during the summer season were at markedly higher risk, underscoring the influence of both host and environmental factors. The strong association with clinical mastitis highlights the pathogen’s central role in udder health and its potential to compromise milk yield and quality. The antimicrobial resistance profile revealed extensive resistance to commonly used antibiotics, especially β-lactams, with 48% of isolates showing oxacillin resistance, raising the possibility of methicillin-resistant Staphylococcus (MRSA). While vancomycin retained efficacy, its veterinary use is not

advisable, highlighting the need for prudent antibiotic stewardship. The findings emphasize the necessity of integrated mastitis control strategies including improved farm hygiene, routine monitoring, selective dry cow therapy, and rational antimicrobial use. Given the zoonotic potential of resistant Staphylococcus strains, the results also reinforce the "One Health" perspective linking animal health, public health, and food safety. Continuous surveillance and molecular characterization of isolates will be essential to mitigate risks and safeguard both livestock productivity and consumer safety.

Conflict of Interest: None

References

1. Pyörälä S, Taponen S. Coagulase-negative staphylococci—emerging mastitis pathogens. Vet Microbiol. 2009;134(1–2):3–8.
2. Kumar R, Yadav BR, Singh RS. Genetic determinants of antibiotic resistance in Staphylococcus aureus isolates from bovine mastitis. Vet World. 2011;4(5):225–229.
3. Saini V, McClure JT, Scholl DT, DeVries TJ, Barkema HW. Herd-level association between antimicrobial use and antimicrobial resistance in bovine mastitis Staphylococcus aureus isolates on Canadian dairy farms. J Dairy Sci. 2017;95(4):1921–1929.
4. Haran KP, Godden SM, Boxrud D, Jawahir S, Bender JB, Sreevatsan S. Prevalence and characterization of Staphylococcus aureus, including methicillin-resistant Staphylococcus aureus, isolated from bulk tank milk from Minnesota dairy farms. J Clin Microbiol. 2012;50(3):688–695.
5. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. Review on Antimicrobial Resistance. 2016.
6. Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. 10th ed. Saunders; 2007.
7. Smith KL, Todhunter DA, Schoenberger PS. Environmental mastitis: cause, prevalence, prevention. J Dairy Sci. 1985;68(6):1531-1553.
8. Ndegwa EN, Mulei CM, Munyua SJ. The prevalence of bovine subclinical mastitis in Kenyan smallholder dairy herds. J S Afr Vet Assoc. 2000;71(1):25-28.
9. Barkema HW, Schukken YH, Zadoks RN. Invited review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. J Dairy Sci. 2006;89(6):1877-1895.
10. Tiwari JG, Babra C, Tiwari HK, Williams V, Wetzel AN, Mehta J. Trends in therapeutic and prevention strategies for management of bovine mastitis: An overview. J Vaccines Vaccin. 2013;4(2):176.
11. Bradley AJ. Bovine mastitis: An evolving disease. Vet J. 2002;164(2):116–128.
12. Haran KP, Godden SM, Boxrud D, Jawahir S, Bender JB, Sreevatsan S. Prevalence and characterization of *Staphylococcus aureus*, including methicillin-resistant *S. aureus*, isolated from bulk tank milk from Minnesota dairy farms. J Clin Microbiol. 2012;50(3):688-695.
13. Paterson GK, Harrison EM, Holmes MA. The emergence of mecC methicillin-resistant *Staphylococcus aureus*. Trends Microbiol. 2014;22(1):42-47.

How to Cite This Article
Anjaria P, Soni M, Raval J. Epidemiological Insights into Staphylococcus spp. in raw bovine milk: A study on prevalence, risk factors, and antimicrobial resistance. International Journal of Veterinary Sciences and Animal Husbandry. 2025; 10(9): 230-233.