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Prevalence Of *Staphylococcus Aureus* in Clinical Cases of Buffaloes with Mastitis and Broiler Chicken with Gangrenous Dermatitis

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ABSTRACT
The emergence of *Staphylococcus aureus* (*S. aureus*) as a significant pathogen in livestock and poultry necessitates comprehensive surveillance to understand its prevalence and implications for animal health and productivity. This study focuses on the prevalence of *S. aureus* in two critical animal health contexts: mastitis in buffaloes and gangrenous dermatitis (GD) in broiler chickens across twelve districts of Andhra Pradesh, India. Milk samples were collected from buffaloes diagnosed with clinical mastitis, and skin swabs were taken from broilers exhibiting signs of GD. Microbiological identification involved culturing on selective media such as Mannitol Salt Agar and performing biochemical assays, including catalase, coagulase, and hemolysis tests on blood agar. Molecular confirmation was achieved through PCR amplification targeting specific *staur* genes.
Out of 130 milk samples from buffaloes with mastitis, *S. aureus* was detected in 78.09% of cases. Among the 86 skin swabs from GD-afflicted broilers, *S. aureus* prevalence was 100%. These findings underscore the significant burden of *S. aureus* infections in both dairy and poultry production systems. The high prevalence highlights the pathogen's role in economic losses, reduced productivity, and potential zoonotic transmission risks. This study provides essential baseline data for developing targeted strategies to manage and mitigate *S. aureus*associated infections in livestock and poultry, contributing to improved animal health and food safety.

Keywords: *Staphylococcus aureus*, Buffalo mastitis, Gangrenous dermatitis, Livestock infections, Zoonotic potential

Introduction

S. aureus known for its pathogenicity and endemic prevalence, has increasingly been recognized as an emerging threat to both human and animal health (Kumar, 2009; Sheela et al., 2016). Its role as a primary etiological agent of mastitis in buffaloes and GD in poultry necessitates a comprehensive evaluation and understanding of its impact on livestock. Mastitis, a prevalent and economically devastating disease, leads to significant losses in milk production and quality, thereby affecting the livelihoods of dairy farmers. Moreover, GD in poultry exacerbates economic losses by reducing bird health and productivity.
In this context, the present investigation was designed to address the growing concerns associated with *S. aureus* infections in buffaloes (mastitis) and broiler chicken (GD). Clinical samples were meticulously collected from cases of mastitis in buffaloes and gangrenous dermatitis in broiler chicken. These samples underwent a rigorous process of isolation to identify *S. aureus* strains. Following isolation, a detailed analysis of the biochemical and molecular characteristics of the strains was conducted. This involved an array of advanced techniques aimed at elucidating the pathogenic mechanisms, resistance profiles, and virulence factors of *S. aureus*.
The biochemical characterization included tests for catalase and coagulase activity, which are hallmark indicators of *S. aureus*. Additionally, molecular characterization involved polymerase chain reaction (PCR) to confirm the *S. aureus* strains
By isolating and characterizing *S. aureus* strains from clinical cases, this study aims to deepen our understanding of the pathogen's behavior in livestock environments. Such knowledge is crucial for developing effective control measures and therapeutic strategies to mitigate the impact of *S. aureus* on the dairy and poultry industries. This research underscores the importance of continuous surveillance and advanced diagnostic approaches in managing and controlling bacterial infections that threaten animal health and economic stability.

Materials & Methods

Sample Size and Method of Collection of Clinical Samples

This study aimed to investigate the prevalence of *Staphylococcus aureus* in buffaloes with clinical mastitis and broiler chickens exhibiting gangrenous dermatitis (GD) across twelve districts of Andhra Pradesh, India. A total of 130 milk samples were collected aseptically from buffaloes diagnosed with mastitis. Teats were cleaned with alcohol swabs, and the first few streams of milk were discarded to minimize contamination. Similarly, 86 skin swabs were obtained from GD-affected broiler chickens by carefully swabbing necrotic lesions using sterile cotton applicators. All samples were placed in sterile containers and transported to the laboratory at 4°C, where they were processed within 24 hours.

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Isolation and Morphological Confirmation

The isolation of *S. aureus* was carried out by culturing the samples on Mannitol Salt Agar (MSA), a selective medium that promotes the growth of *S. aureus*. Plates were incubated at 37°C for 24–48 hours. Colonies exhibiting yellow pigmentation, indicative of mannitol fermentation, were presumptively identified as *S. aureus*. Morphological confirmation was conducted through Gram staining. Smears were prepared from selected colonies, fixed, and stained. The presence of Gram-positive cocci arranged in clusters was consistent with *S. aureus*.

Biochemical Confirmation

Further confirmation of *S. aureus* isolates involved a series of biochemical tests. The catalase test was performed by adding 3% hydrogen peroxide to bacterial colonies on a glass slide, with the production of bubbles indicating a positive reaction. The coagulase test, used to identify the production of the coagulase enzyme, was conducted by mixing a suspension of bacterial colonies with rabbit plasma on a glass slide. The formation of visible clumps confirmed the presence of coagulase-positive *S. aureus*. Hemolysis was assessed by streaking the isolates onto 5% sheep blood agar plates and incubating at 37°C for 24 hours. Colonies exhibiting betahemolysis, characterized by clear zones around the colonies, were further considered as *S. aureus*.

Molecular Confirmation

Molecular confirmation of *S. aureus* was achieved through PCR amplification targeting the *staur* gene. Genomic DNA was extracted using the boiling lysis method. A loopful of bacterial culture was suspended in 200 µL of sterile distilled water and subjected to boiling at 95°C for 10 minutes. The suspension was centrifuged at 10,000 rpm for 5 minutes, and the supernatant containing DNA was collected and stored at -20°C until further use. PCR was performed in a 25 µL reaction mixture containing 2.5 µL of 10X PCR buffer, 2.0 µL of MgCl2 (25 mM), 0.5 µL of dNTPs (10 mM each), 0.5 µL each of forward and reverse primers (10 pmol), 0.25 µL of Taq DNA polymerase (5 U/µL), and 2.0 µL of template DNA, with the remaining volume made up with nuclease-free water. The thermocycling conditions included an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes.

The PCR products were analyzed using 1.5% agarose gel electrophoresis prepared with Trisacetate-EDTA (TAE) buffer. Five microliters of each PCR product were mixed with loading dye and loaded into the gel wells alongside a 100 bp DNA ladder. Electrophoresis was conducted at 100 volts for 45 minutes, and the amplified bands were visualized under UV light using a gel documentation system.

Results

Isolation and Phenotypic Identification of *S. aureus*

Out of 130 milk samples collected from buffaloes with mastitis, 105 (80.7%) were confirmed to harbor *S. aureus*. For broiler chickens, among 86 skin swabs collected from GD-affected birds, 86 (100%) isolates tested positive for *S. aureus*. Initial isolation on Mannitol Salt Agar (MSA) yielded colonies with characteristic yellow pigmentation, indicative of mannitol fermentation (Figure 1). This was observed in all presumptive isolates, aligning with the typical morphology of *S. aureus*.

Morphological confirmation through Gram staining revealed Gram-positive cocci arranged in grape-like clusters in all isolates. Further biochemical tests substantiated these results. The catalase test was positive for all isolates, with the release of oxygen bubbles upon exposure to hydrogen peroxide (Figure 2). The coagulase test showed distinct clumping in rabbit plasma in all the 105 milk and 86 broiler chicken isolates, confirming coagulase-positive *S. aureus* (Figure 2). Hemolytic activity on 5% sheep blood agar demonstrated alpha-hemolysis in 42 isolates, beta-hemolysis in 26 isolates characterized by clear zones around the colonies, further supporting the identification of *S. aureus*, and 15 as both while 22 were non-hemolytic (Figure 3).

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Figure 1. Mannitol Fermentation by *S. aureus* on MSA media plates

Figure 2. Positive Catalase test and Coagulase test result showing positive reactions

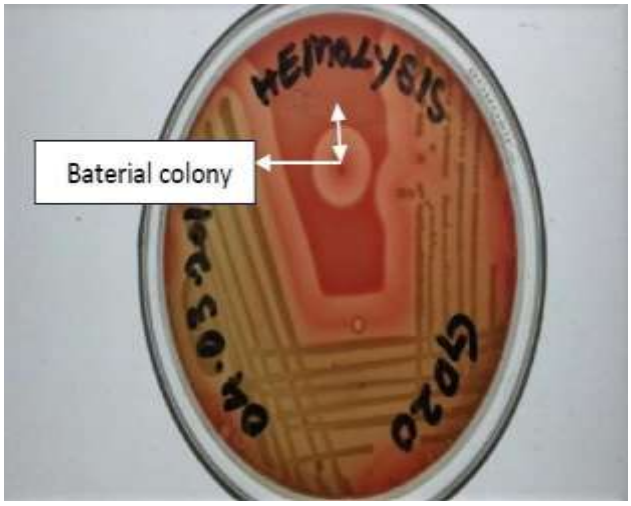

Figure 3. *S. aureus* hemolytic profile on Blood Agar plate

Molecular Confirmation

All phenotypically identified *S. aureus* isolates underwent molecular confirmation using PCR targeting the *staur* gene. DNA was successfully extracted from all presumptive isolates using the boiling lysis method. PCR amplification produced

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distinct amplicons of the expected size for the *staur* gene, confirming the identity of 102 buffalo and 86 broiler chicken isolates as *S.*
aureus. (Figure 4)

Figure 4. Agarose gel electrophoresis patterns of *staur* 4 and *staur* 6 of *S. aureus* genes

Lanes 1,2,3,4,5,6,7,9,10,11,12,13,14 positive isolates. Lane 8 negative control.

Prevalence in Buffaloes
Of the 130 milk samples from buffaloes with mastitis, 78% were confirmed to harbor *S. aureus*. This high prevalence underscores the significant role of *S. aureus* in mastitis, a major disease in dairy animals. The results emphasize the economic implications of *S. aureus* infections in dairy production, with mastitis leading to decreased milk yield and quality.

Prevalence in Broiler Chickens
Among the 86 skin swabs collected from GD-affected broiler chickens, *S. aureus* was detected in 100% of cases. This high prevalence highlights the importance of *S. aureus* in the pathogenesis of gangrenous dermatitis, which is associated with severe economic losses in poultry due to high mortality rates and poor growth performance.

Discussion
The prevalence of *Staphylococcus aureus* (S. aureus) in food products, particularly dairy and poultry, poses significant public health risks and economic challenges for the agricultural industry. This study revealed a notably high prevalence of *S. aureus* in milk and broiler chicken samples collected from Andhra Pradesh, India. Among 130 milk specimens analyzed, 105 samples underwent preliminary screening using mannitol salt agar (MSA), a selective medium that supports the growth of *Staphylococci* by inhibiting non-halophilic bacteria. Of these, 102 isolates (78%) were confirmed as *S. aureus* through subsequent biochemical tests. Similarly, all 86 broiler chicken samples exhibited 100% prevalence for mannitol fermentation and were molecularly verified as *S. aureus*.
This widespread prevalence in milk and broiler chicken aligns with findings from other regions. Studies, including Chavan et al. (2007), documented *S. aureus* as a predominant pathogen in mastitis cases in Hissar, India, with 38.66% of isolates being coagulase-positive, a key virulence factor associated with pathogenicity. Sharma et al. (2011) and Roychoudhary and Dutta (2009) also highlighted the dominance of *S. aureus* in mastitis and gangrenous dermatitis (GD) cases, emphasizing its impact on milk quality, animal health, and poultry production.
Historical data further corroborate these findings, with Char et al. (1983), Saini et al. (1994), and Unnerstad et al. (2009) consistently reporting *S. aureus* as a leading pathogen in mastitis cases globally. Adwan et al. (2010) similarly documented its prevalence in raw milk in Northern Palestine, highlighting the pathogen's adaptability and resilience in diverse environments and hosts. Despite such robust evidence, the scarcity of region-specific data from Andhra Pradesh remains a notable gap.
Given the state's reliance on dairy and poultry industries, this lack of localized research is a critical concern. Region-specific studies are essential for understanding the epidemiology of *S. aureus*, as its pathogenicity and antibiotic resistance

profiles can vary significantly with geographic location and farming practices. Kalorey et al. (2007) and Otter and French (2010) emphasized the importance of tailoring control measures based on localized data, which can inform public health policies and improve disease management strategies. Addressing this gap will enhance the ability to design targeted interventions, mitigate infection risks, and ensure food safety.

Conclusion

This study highlights the high prevalence of *S. aureus* in milk and broiler chicken samples from Andhra Pradesh, indicating the pathogen's significant presence in these food sources and its implications for public health and agriculture. While global and national studies have extensively documented *S. aureus* prevalence in dairy and poultry, the lack of data specific to Andhra Pradesh underscores the need for focused regional research. Such investigations are critical for developing targeted management strategies, improving food safety standards, and supporting the economic sustainability of the dairy and poultry sectors in this region

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