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Microbiological Risk Factors for *Staphylococcus aureus* Transmission in Organized vs. Unorganized Dairy Farms

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environmental contamination

ABSTRACT:

Introduction: *Staphylococcus aureus* is an important foodborne pathogen and a major concern in the dairy industry due to its association with bovine mastitis and milk contamination. Mastitis caused by *S. aureus* leads to significant economic losses in dairy production by reducing milk yield and quality, while also posing potential public health risks through contaminated milk and dairy products. The persistence of this pathogen in the farm environment, combined with poor hygiene practices during milking, increases the risk of transmission from animals, farm workers, and environmental sources. Differences in management practices between organized and unorganized dairy farms may influence the prevalence and spread of *S. aureus*. Therefore, monitoring its occurrence in milk and farm-associated sources is essential to identify potential contamination routes and improve milk safety.

Objectives: The present study aimed to assess microbiological hazards associated with the dissemination of Staphylococcus aureus in organized and unorganized dairy production farms.

Methods: A total of 120 samples were collected from both organized and unorganized dairy farms, including raw milk (40), udder swabs (40), floor swabs (20), and milker hand swabs (20). Isolation and identification of *S. aureus* were performed using Baird–Parker agar followed by biochemical confirmation. The presence of mastitis in milk samples was evaluated using the California Mastitis Test (CMT).

Results: The overall prevalence of *S. aureus* was 37.5% in milk samples, 15% in udder swabs, 60% in floor swabs, and 30% in hand swabs. Unorganized farms showed higher prevalence rates (raw milk: 45%, udder swabs: 20%, floor swabs: 100%, hand swabs: 41.6%) compared to organized farms (raw milk: 30%, udder swabs: 10%, floor swabs: 20%, hand swabs: 12.5%). Mastitis was detected in 32.5% of milk samples, with higher occurrence in unorganized farms (35%) than organized farms (30%). Among mastitic samples, *S. aureus* prevalence was 61.5%, with higher detection in unorganized farms (71.4%) than organized farms (50%).

Conclusions: Unorganized dairy farms exhibited higher contamination levels due to risk factors such as mastitis, environmental contamination, and poor hygiene practices, which facilitate the transmission of *S. aureus*. Implementing interventions such as routine mastitis screening and hygiene training for farm workers is essential to reduce *S. aureus* transmission and ensure milk safety.

1. Introduction

In dairy industry, Staphylococcus aureus plays a significant role in causing milk contamination, bovine mastitis, and possible foodborne illness hazards to the general public [1]. Microbiological risk factors, such as contaminated surfaces, human carriers, and infected udders, affect its spread in dairy environments based on the farm management practices [1-4]. unorganized dairy farms frequently involve manual milking, inadequate veterinary care, and uneven sanitation leading to the higher microbial contamination whereas organized dairy farms have automated milking systems, regular udder health monitoring practice, and standardized hygiene protocols that generally show lower microbial contamination [5-9]. Different environments for *S. aureus* transmission are created by these variations in farm structure and practices, especially in raw milk, udder surfaces, floor swabs, and milker hands [3, 9].

Mastitis act as a potential reservoir for *S. aureus*. It is a critical risk factor for *S. aureus* contamination in

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unorganized farms due to unhygiene practice and inadequate monitoring [10-13]. Contaminated environmental surfaces along with human-assisted transmission via milker hands further accelerate the spread of *S. aureus* in poorly sanitized conditions [10, 14-16]. Understandings of these risk factors are necessary while designing targeted strategies with the aim of minimizing *S. aureus* contamination and improving milk safety.

The study focuses on investigating the prevalence and transmission of *S. aureus* via isolation and characterization from milk samples, udder swabs, floor swabs and milkers hand obtained from organized and unorganised dairy farms. Study also focuses on the presence of mastitis in raw milk samples obtained from both settings. The aim of present study is to identify key microbiological risk factors responsible for transmission of *S. aureus* transmission and to provide valuable insights for designing strategies for enhancing dairy farm hygiene and public health safety.

2. Methods

A. Study design and sampling sites

A cross-sectional study design was employed to compare various microbiological risk factors driving *S. aureus* transmission in organized and unorganized dairy farms. Organized dairy farms were defined as large-scale farms with automated milking systems, regular veterinary care, and standardized hygiene protocols whereas unorganized farms were small-scale with manual milking operations and limited infrastructure with poor sanitation practices [3]. A total of four dairy farms i.e. two organized and two unorganized dairy farms were selected from Delhi-NCR, India, based on accessibility and farm types.

B. Sample collection

To determine the prevalence of S.aureus and mastitis presence, four types of samples namely, raw milk, udder swabs, floor swabs, and milker hand swabs were collected from each dairy farm. All samples were collected aseptically according to the standard procedure mentioned by National Mastitis Council, 2017. During routine Milking, 50mL composite milk sample was obtained in sterile container from each lactating cow/buffalo (N = 40) directly from udder. Samples were collected after cleaning teats with 70% ethanol and discarding the first stream of milk. Collected milk samples were stored at 4°C and transported to the laboratory within 6 hrs. For obtaining udder Swabs samples, sterile cotton swabs were moistened with phosphate-buffered saline (PBS) and were used to swab the teat skin of each sampled cow/baffalo (N = 40) Before milking. Floor swab samples were collected from every 100 cm^2 areas of milking parlor floors using sterile swabs moistened with PBS. Five random locations were sampled per farm to address spatial distribution. Hand swabs were obtained from both hands of milking personnel (N = 10) before milking, using sterile swabs moistened with PBS. All the Swab samples were stored in 2 mL PBS at 4°C. A total of 40 raw milk samples, 40 udder swabs, 20 floor swabs, and 20 hand swabs were collected across all dairy farms.

C. Mastitis detection

Presence of mastitis in raw milk samples was determined by using the California Mastitis Test (CMT). For this, 3 mL of milk was mixed with an equal volume of CMT reagent in a paddle, and rotate gently. Formation of gel within 1 minute indicates the presence of mastitis. Gel formation was scored (0 = negative, Trace to 3+ = positive). Milk samples with CMT scores more than Trace were classified as mastitic. Each milk sample was categorized as mastitic or non-mastitic based on CMT Score results.

D. Microbiological analysis

All samples were processed within 24 hours of collection (8 hours in case of raw milk samples) to isolate and identify *S. aureus* according to standard microbiological procedures (ISO 6888-1, 2018). Milk samples (1 mL) were added into 9mL of Mannitol Salt Broth and incubated for overnight at 37°C. Samples positive for *S. aureus* were change its color from red to Yellow. Positive samples were than streak onto Baird-Parker agar supplemented with egg yolk-tellurite emulsion, selective for *S. aureus*. Plates were incubated at 37°C for 24–48 hours. Swab samples in PBS were vortexed for 30 seconds, and 100 µL of the suspension was plated onto Baird-Parker agar [17-19]. Plates were incubated at 37°C for 24–48 hours. Colonies obtained onto the plates were checked for typical *S. aureus* morphology (black, shiny, with a clear halo). Presumptive *S. aureus* colonies were confirmed by Gram staining (Gram-positive cocci), catalase test (positive), and coagulase test (positive).

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3. Results and Discussion

A. Sample collection

A total of 120 samples were collected from organized (58 samples) and unorganized (62 samples) farms, including 40 raw milk samples, 40 udder swabs, 20 floor swabs, and 20 milker hand swabs.

Table 1: Prevalence of *S. aureus* and mastitis by sample type and farm type

Sample Type	Organized Farms (% Positive)	Unorganized Farms (% Positive)	Overall (% Positive)
Raw Milk (*S. aureus*)	6/20 (30%)	9/20 (45%)	15/40 (37.5%)
Udder Swabs	2/20 (10%)	4/20 (20%)	6/40 (15%)
Floor Swabs	2/10 (20%)	10/10 (100%)	12/20 (60%)
Milker Hand Swabs	1/8 (12.5%)	5/12 (41.6%)	6/20 (30%)
Raw Milk (Mastitis)	6/20 (30%)	7/20 (35%)	13/40 (32.5%)

B. Prevalence of Staphylococcus aureus

Overall prevalence of *S. aureus* detected in raw milk samples, udder swabs, floor swabs and milker hand swabs was 37.5%, 15%, 60%, and 30%, respectively. In organized dairy farms, low prevalence of *S. aureus* was seen across all sample types: 30% in raw milk, 10% in udder swabs, 20% in floor swabs, and 12.5% in milker hand swabs. In contrast, unorganized farms exhibited higher prevalence; 45% in raw milk, 20% in udder swabs, 100% in floor swabs, and 41.6% in milker hand swabs (Table 1). Higher prevalence of *S. aureus* in floor swabs (20% organized vs 100% unorganized) and milker hand swabs (12.5% organized vs 41.6% unorganized) indicates a significant environmental contamination and human-mediated transmission risk in unorganized settings.

C. Prevalence of Mastitis

Overall 32.5% (13/40) of raw milk samples were found positive for Mastitis, with 30% (6/20) in organized farms and 35% (7/20) in unorganized farms (Table 1). The slightly higher mastitis prevalence in unorganized farms aligns with limited udder health monitoring and poor sanitization conditions.

D. Association between Mastitis and S. aureus

Among 13 mastitic positive milk samples, 61.5% were positive for *S. aureus* including 50% (3/6) in organized dairy farms and 71.4% (5/7) unorganised dairy farms (Figure 1). These results highlight a stronger association between mastitis and *S. aureus* contamination in unorganized farms. Additionally, out of the 15 milk samples positive for *S. aureus*, 53.3% (8/15) were from obtained from mastitic udders, indicating mastitis as a key microbiological risk factor for transmission of *S. aureus*.

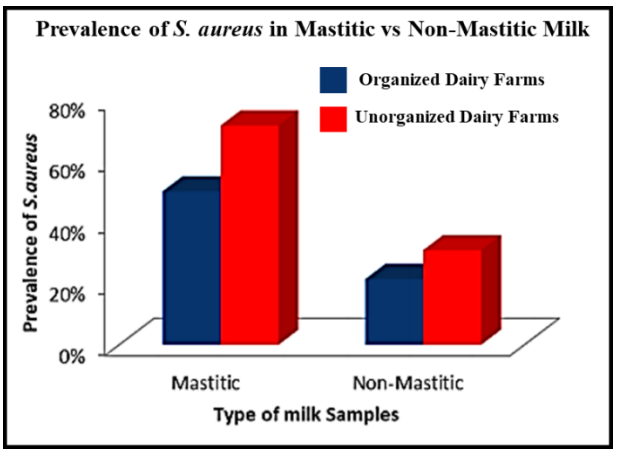

Figure 1: Bar-chart of *S. aureus* prevalence in Mastitic vs. Non-Mastitic Milk

E. Comparative analysis

Higher prevalence of S.aureus in unorganized farms across all sample types compared to organized farms, exhibiting the significant difference (100% vs. 20%) in floor swab samples, indicating the environmental contamination as a major risk factor in unorganized farms. This is likely due to inadequate cleaning practices in unorganized dairy farms. Additionally, the greater prevalence of *S. aureus* on milker hands in unorganized farms (41.6%; 12.5%) represents lack of adherence to hygiene standards as a principal means of transmission. Though the mastitis incidence did not differ between the farms (30% vs. 35%), the higher prevalence of S. aureus in the mastitic milk from unorganized farms (71.4% vs. 50%) indicates udder infections are contributing to the enhanced *S. aureus* abundance in these environments.

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Moreover, the current study indicates that mastitis, environmental contamination, and human-facilitated transmission are the main microbiological risk factors for the transmission of *S. aureus*, and that the prevalence was higher in samples from the unorganized dairy farms than on the organized dairy farms [1, 13]. These results correspond to our research question, emphasizing the impact of *S. aureus* contamination on management of the farm. Mastitis as a major *S. aureus* reservoir, showed 61.5% in mastitic milk samples more precisely 71.4% for unorganized and 50% for organized dairies [3, 13, 20]. The slightly higher occurrence of mastitis in unorganized farms (35 vs 30%) is also an indication of deficient udder health monitoring practices as has been reported earlier.

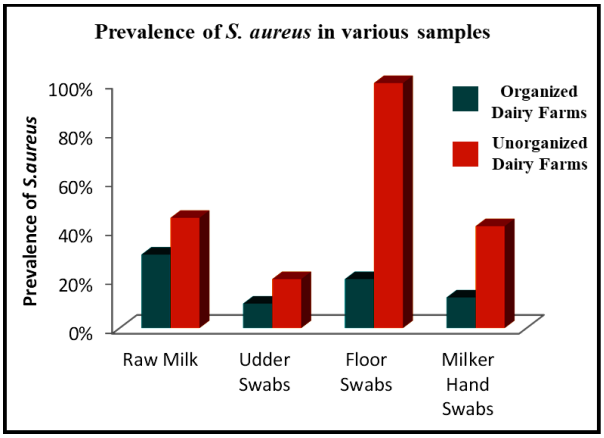

Figure 2: Bar-chart of *S. aureus* prevalence across sample types

In Organized dairy farm's, routine screening minimizes this risk, but 30% mastitis prevalence indicates that challenges continue to be there (Figure 2). Environmental contamination was significantly higher in unorganized farms where 100% floor swabs were positive for *S. aureus*, and 20% in organized farms. This is probably because of bad sanitation and dirt floors, leading to durable reservoirs. Moreover higher *S. aureus* prevalence on milker hands in unorganized farms (41.6% vs. 12.5%) indicates human-mediated transmission due to inadequate hygiene, unlike organized farms' that uses glove and proper sanitization techniques [21-23]. Higher *S. aureus* prevalence in unorganized farms threatens the milk safety, underscoring the importance of interventions like mastitis screening and hygiene training.

Study limitations are that it is based on a relatively small size of sample, we did not do strain typing, and there is no knowledge on factors such as milking practices. Further studies on Antibiotic Resistance and longitudinal trends are warranted to improve control measures and confirm transmission chains.

4. Conclusion

The present study highlights *S. aureus* as a significant microbiological hazard in dairy production systems, particularly in unorganized farms where management practices are comparatively inadequate. The higher prevalence of *S. aureus* in raw milk, udder swabs, floor swabs, and milker hand swabs in unorganized settings underscores the strong association between poor hygiene, environmental contamination, mastitis, and pathogen transmission. The substantial proportion of mastitic milk samples and their high association with *S. aureus* further confirms mastitis as a major risk factor contributing to milk contamination and potential public health threats.

These findings reinforce the importance of systematic mastitis screening, improved udder health management, environmental sanitation, and strict personal hygiene practices among dairy workers. Strengthening biosecurity protocols and routine monitoring can significantly reduce microbial load and limit pathogen dissemination.

Furthermore, integrated environmental monitoring approaches similar to advanced ecological surveillance strategies applied in other biological systems demonstrate how innovative technologies can support risk assessment and management in agricultural ecosystems. In addition, understanding pathogen ecology and control strategies, including biological interventions, provides a broader perspective for sustainable disease mitigation [24-26] Advances in fungal and viral pathogens for biological control of insect pests. Overall, the study confirms that microbiological safety in dairy farms is closely linked to farm management structure, hygiene standards, and animal health practices. Targeted interventions in unorganized farms are particularly crucial to ensure milk quality, consumer safety, and sustainable dairy production.

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Declaration statement.

Author contributions: All authors contributed to the study design. Conception and Content collection were performed by Shweta Sharma. The first draft of the manuscript was written by Shweta Sharma, Dharmsheel Shrivastav and Shilpy Singh commented on previous versions of the manuscript. Final review and editing of the manuscript were done by Shweta Sharma, Dharmsheel Shrivastav, Shilpy Singh, Varun Kumar Sharma. All authors read and approved the final manuscript.

Ethical Statement: The current study was conducted using computational approaches and does not involve any experiments on humans, or animals at any level. Therefore, ethical approval is not required

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Conflict of Interest: The authors declare no conflicts of interest.

Data Availability Statement: All data associated with this manuscript are included in the form of tables and figures within the manuscript.

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