 DOI: https://doi.org/10.3126/jiaas.v38i1.73081

FIFTEEN-YEAR RETROSPECTIVE STUDY: PREVALENCE OF BACTERIAL ISOLATES IN MASTITIS-SUSPECTED MILK SAMPLES SUBMITTED TO CENTRAL VETERINARY LABORATORY, NEPAL

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ABSTRACT

Bovine mastitis, a prevalent mammary gland inflammation, significantly impacts dairy production worldwide. This study investigated the changing landscape of mastitis-causing bacteria in Nepal. A retrospective analysis of secondary data was conducted, utilizing technical bulletins published by the Central Veterinary Laboratory (CVL) in Kathmandu over a fifteen-year period (2003/2004 to 2017/2018). The analysis focused on 3,445 bovine milk samples screened for mastitis using the California Mastitis Test (CMT).The analysis revealed *Staphylococcus spp.* as the most prevalent bacterial isolate (2,948 positive samples), followed by *E. coli* and *Streptococcus spp.* This finding suggests a potential shift in the dominant mastitis pathogens in Nepal, with *Staphylococcus spp*. potentially surpassing E. coli compared to previous reports. These results highlight the importance of implementing hygiene practices during milking to reduce the spread of contagious pathogens like *Staphylococcus spp*. Additionally, maintaining clean milking environments can help minimize *E. coli* infections. Further research is warranted to understand the factors driving this potential shift in bacterial prevalence and develop targeted prevention and control strategies for mastitis in Nepal.

Keywords: Isolates, Prevalence, Mastitis, *Staphylococcus*

INTRODUCTION

Livestock production plays a vital role in the agricultural sector,forming a key component of the Nepal economy. Livestock provides sustenance for a significant portion of the population and contributes substantially to the nation's Gross Domestic Product (GDP), accounting for 12% and an impressive 27% of the agricultural GDP (Ministry of Livestock Development, 2019). The vast number of milking cattle and buffalo, exceeding 1 million and 1.5 million head respectively in fiscal year 2017/2018 (Ministry of Livestock Development, 2019), further underscores the importance of this sector .Livestock is not only vital for Nepalese livelihoods but also significant contributors to milk production, with cattle and buffalo contributing 40% and 60% to milk production , respectively, in the same fiscal year (Ministry of Livestock Development, 2019). This makes livestock not only a critical source of food and income but also a foundation for rural development and food security in Nepal.

However, challenges such as masitits, a very common mammary gland disease in dairy animals, threaten the sustainability and productivity of the sector. Mastitis has diverse etiologies, characterized by a decline in milk production and poses a substantial economic burden on the dairy sector in both developed and developing nations due to decreased milk production, increased veterinary cost and culling of infected animals (Bradley et al., 2002; Dhakal, 2002). Studies estimate that mastitis represent a significant e burden of cost for dairy farmers worldwide. Particularly, cost of single episode of clinical mastitis have been approximately $107 USD, with over 70% of this cost attributed to decreased milk production and milk withheld from the market. Additionally, over 20% of the cost is associated with veterinary services, drugs, and replacement costs, with the remaining portion attributed to labor expenses (Graves et al., 1993).

Nyman et al. (2009) identified a significant economic burden associated with mastitis in Nepal, with the largest proportion of losses stemming from decreased milk production, amount to approximately Rs. 4287 Nepalese Rupees (approximately USD 63) per buffalo per lactation period. The study further revealed an average milk loss of 11% of the total lactation yield. Furthermore, the cost of medication represents approximately 34% of the total treatment and management expenses associated with mastitis in Nepal.

The increasing prevalence of imported, exotic cross-bred dairy animals in Nepal is likely to exacerbate the mastitis problem as these animals, often selected for higher milk yield nay be more suceptible to mastitis based on their genetic predispositions and local dairy management. Notably, mastitis not only affects lactating animals but can also impact animals during the drying-off (DO) and post-calving (PC) stages (Dhakal, 2002). These stages are thus ctitical for management authority. This necessitates the development of effective prevention and control strategies to mitigate the economic and production losses associated with mastitis in Nepal's growing dairy sector.

Bovine mastitis, a prevalent mammary gland inflammation, can be caused by over 200 infectious agents (Sharma, *et al.*, 2012). In large animals, particularly in Asia, several bacterial pathogens emerge as the most frequent culprits. Studies across the continent have identified *Staphylococcus aureus*, *Streptococcus agalactiae*, other *Streptococcus* species, and *Coliforms* as the primary bacterial culprits (Kader et al., 2002; Sudhan et al., 2005; Chahar et al., 2008; Yong et al., 2009; Sharma and Maiti, 2010).

Kumar et al. (2009) reported a particularly high prevalence (50.00%) of *Streptococcus dysgalactiae* isolated from subclinical mastitis cases in cows, followed by *Staphylococcus aureus* and others. The spectrum of potential causative agents extends beyond these common pathogens. Bovine mastitis can also be associated with *Actinomyces pyogenes*, *Pseudomonas aeruginosa*, *Nocardia asteroides*, *Clostridium perfringens*, and organisms from *Mycobacterium*, *Mycoplasma*, Pasteurella, and Prototheca genera, along with yeasts (Sharma, 2010).

However, it's important to note that the majority of mastitis cases are caused by a relatively small number of these pathogens. *Staphylococcus species*, *Streptococcus species*, *Coliforms*, and *Actinomyces pyogenes* consistently rank among the most frequently isolated bacteria associated with the disease (Sharma, 2010). Understanding the specific bacterial profile of mastitis in a particular region is crucial for implementing effective prevention and control strategies.

MATERIALS AND METHOD

Design of Research

This study employed a retrospective analysis of secondary data **acquired** from the Central Veterinary Laboratory (CVL) located in Tripureshwor, Kathmandu. The data source comprised a collection of technical bulletins published over a fifteen-year period, ranging from fiscal year 2003/2004 to fiscal year 2017/2018.

Data Collection

The analysis focused on a dataset containing 3,445 bovine milk samples submitted to the CVL during the aforementioned timeframe. The California Mastitis Test (CMT) was

employed to screen these samples for the presence of mastitis. This test identified 2,948 samples as positive for mastitis, while the remaining 497 samples tested negative.

Research Model

While a diverse range of bacteria can cause mastitis, this study focused on the five most prevalent bacterial isolates: *Staphylococcus spp*., *Escherichia coli* (*E. coli*), *Streptococcus spp*., *Bacillus spp*., and a category designated as "Others." This "Others" category encompassed less frequently occurring bacteria, including *Proteus spp., Pseudomonas spp., Micrococcus spp., Klebsiella spp.,* and *Moraxella spp*.

To assess the prevalence of the five most prevalent bacterial isolates associated with mastitis in bovine milk samples, a one-way analysis of variance (ANOVA) was employed. This statistical test was used to compare the prevalence of *Staphylococcus spp*., *Escherichia coli* (*E. coli*), *Streptococcus spp*., *Bacillus spp*., and a category designated as "Others" (including *Proteus spp., Pseudomonas spp., Micrococcus spp., Klebsiella spp.,* and *Moraxella spp*.) at ($p < 0.05$). The variable "N" represents the number of observations (milk samples) collected during each year of the study period.

Software of Data Analysis

The research employed IBM SPSS software for data analysis.

Table: 1. Model for comparison prevalence of isolates

	Prevalence of Isolates			
Staphylococcus	E. Coli	Streptococcus	Bacillus	Others
N = 15	N = 15	N = 15	N = 15	N = 15

3. RESULT AND DISCUSSION

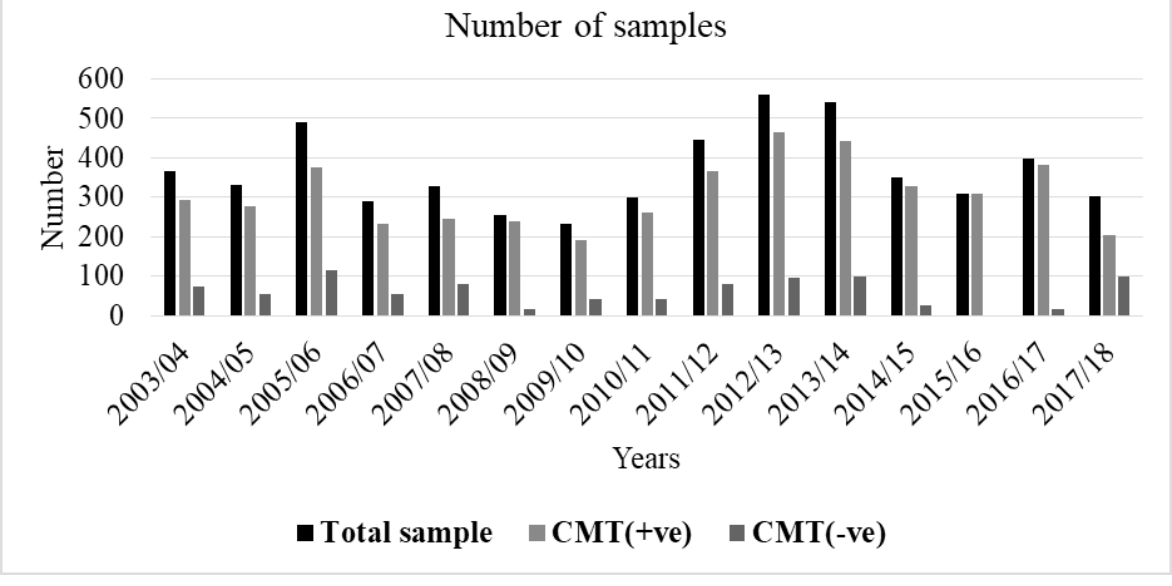

Figure: 1. Total samples submitted to CVL over 15 years (2003//04 - 2017/18)

From a total of 3,445 bovine milk samples submitted to the Central Veterinary Laboratory (CVL) in Nepal over fifteen years, a concerning 85.57% (2,948 samples) tested

positive for mastitis using the California Mastitis Test. This high prevalence underscores the significant challenge mastitis poses to udder health and dairy production.

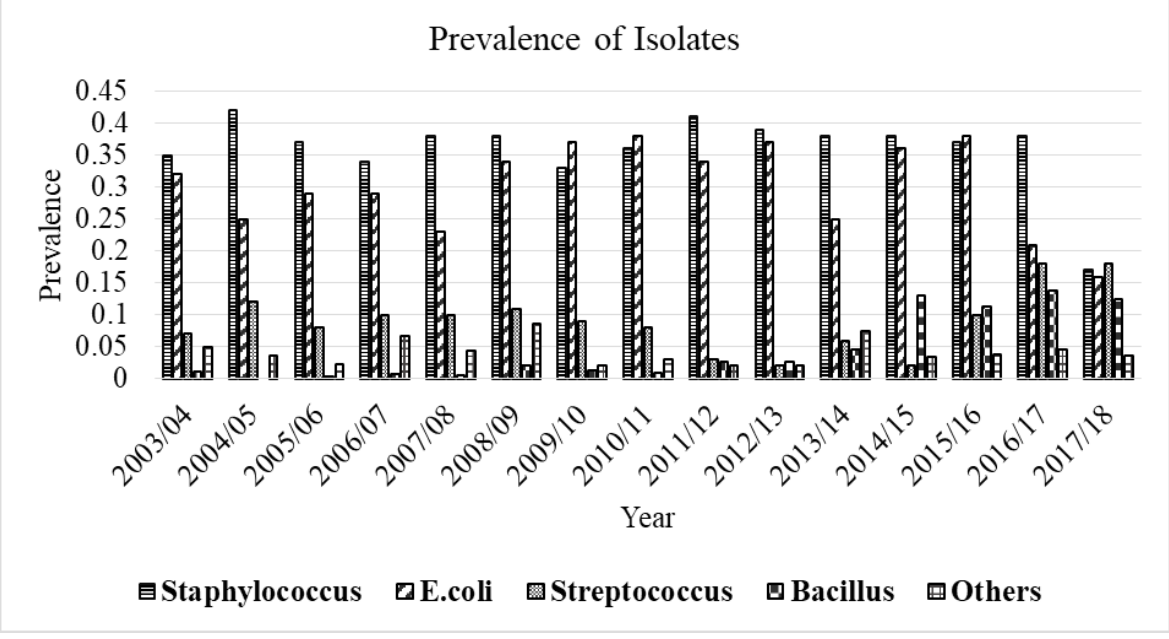

Figure: 2. Prevalence of different bacteria over 15 years (2003//04 - 2017/18)

Result of One-way ANOVA for comparison of the mean of Prevalence

Table 3. ANOVA Table of Prevalence of Isolates

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Isolates	3.501	5	.700	256.664	.000
Error	.191	70	.003		
Total	3.692	75			

The results of the ANOVA revealed a statistically significant difference (F (5, 70) = 256.66, p = 0.000) in the prevalence of these five isolates. This finding indicates that at least one isolate exhibits a prevalence level statistically distinct from the others.

Post-hoc Analysis: Tukey Pairwise Comparisons

To further explore these differences and identify pairwise comparisons between specific isolates, a post-hoc Tukey's Honestly Significant Difference (HSD) test was employed. The results of the Tukey's HSD test revealed that the mean prevalence score for *Staphylococcus spp*. (M = 0.3625, SD = 0.0573) was significantly different from the prevalence of all other isolates: E. coli (M = 0.3029, SD = 0.069), *Streptococcus spp.* (M = 0.0895, SD = 0.048), *Bacillus spp.* (M = 0.0451, SD = 0.052), and Others (M = 0.0417, SD = 0.020). This suggests that *Staphylococcus spp*. had a significantly higher prevalence compared to the remaining four isolates within the study population.

The Tukey's HSD test further revealed that the mean prevalence score of *Escherichia coli* (*E. coli*) (M = 0.3029, SD = 0.069) was statistically different from *Streptococcus spp*. (M = 0.0895, SD = 0.048), Bacillus spp. (M = 0.0451, SD = 0.052), and the "Others" category

(M = 0.0417, SD = 0.020). This indicates that E. coli had a significantly higher prevalence compared to these three groups.

However, the post-hoc analysis did not indicate a statistically significant difference in the mean prevalence scores between *Streptococcus spp*., *Bacillus spp*., and the "Others" category. In other words, the prevalence of Streptococcus spp. (M = 0.0895, SD = 0.048), Bacillus spp. (M = 0.0451, SD = 0.052), and the "Others" category (M = 0.0417, SD = 0.020) were not statistically distinguishable from each other based on the Tukey's HSD test results.

Table 4. Grouping Information Using the Tukey Method and 95% Confidence

Isolates	n	Mean	SE
Staphylococcus	15	0.3607[a]	0.0149
E. Coli	15	0.3027[b]	0.0179
Streptococcus	15	0.0893[c]	0.0125
Others	15	0.0452[c]	0.0052
Bacillus	15	0.0414[c]	0.0135

Means that do not share a letter are significantly different

The findings of this study agrees with previous research conducted by Islam et al. (2011), who identified Staphylococcus spp. as the primary causative agent of both clinical and subclinical mastitis in cows. Their work further highlights S. aureus and Escherichia coli as the most commonly isolated pathogens from cases of clinical mastitis, with Coagulase-Negative Staphylococci (CNS) being the predominant isolate in subclinical mastitis (Islam et al., 2011).

These observations are further supported by the investigations of (Kader *et al.*, 2002; Sudhan *et al.*, 2005; Chahar *et al.*, 2008; Yong *et al.*, 2009; Sharma *et al.*, 2012). Their research across Asia reinforces the prevalence of *Staphylococcus spp*. and *E. coli* as significant bacterial pathogens associated with mastitis in bovine milk samples.

Shifting Bacterial Prevalence in Nepal

The current study also contributes to the understanding of mastitis etiology in the Nepalese context. Dhakal (2006) and Dhakal et al. (2007) reported a previous predominance of *E. coli* as the primary causative agent in Nepal during the period 1994-1998. The present findings, however, suggest a potential shift in this pattern, with *Staphylococcus spp*. emerging as the more prevalent bacteria associated with mastitis cases. This observed change in bacterial distribution necessitates further investigation to understand the potential contributing factors.

The increasing prevalence of exotic cross-bred dairy animals, selected for higher milk yields, may have inadvertently contributed to this shift. These breeds often exhibit reduced immunity to mastitis-causing pathogens, particularly *Staphylococcus spp*., when compared to native breeds (Islam et al., 2011). Farmers should be encouraged to adopt resilient native or cross-bred animals with better adaptability to local conditions while implementing proper herd management practices (Sharma et al., 2012).

Improved management practices are critical to address the spread of contagious pathogens like *Staphylococcus spp.* These include enhanced udder hygiene during milking, use of teat dips, proper sanitization of milking equipment, and segregating infected animals to limit transmission (Chakraborty, 2013). Training programs on biosecurity and milking hygiene can further aid in reducing mastitis prevalence.

The rising use of antibiotics to manage mastitis could also drive changes in bacterial profiles, selecting for antibiotic-resistant strains (Kader et al., 2002; Sudhan et al., 2005). Surveillance programs to monitor antibiotic resistance patterns in mastitis pathogens are crucial. The adoption of evidence-based antibiotic protocols and regular sensitivity testing should be emphasized to avoid inappropriate antibiotic use (Sharma and Maiti, 2010).

Climatic conditions may influence the prevalence of different bacterial pathogens. *Staphylococcus spp*., being contagious, thrives under conditions where poor hygiene prevails, whereas environmental pathogens like *E. coli* may dominate in wet and humid conditions (Chakraborty, 2013). Addressing these factors through barn design improvements, ventilation, and moisture management could mitigate environmental stressors that contribute to bacterial proliferation.

Dominant Mastitis Pathogens in Nepal and Transmission Routes

This study identified *Staphylococcus spp.* as the most prevalent bacteria causing mastitis in Nepal, followed by E. coli and *Streptococcus spp.* This finding aligns with the established contagious nature of *Staphylococcus spp.* (Chakraborty, 2013). These bacteria can persist in infected udders, teat canals, and even on the skin of milkers, including their nails and nasal mucus (Chakraborty, 2013). Unhygienic milking practices, inadequate sanitation of milking personnel, and poor barn hygiene all contribute to the transmission of *Staphylococcus spp*. to susceptible cows.

E. coli, the second most prevalent bacteria in this study, is a well-known culprit in environmental mastitis (Chakraborty, 2013). Its natural habitat includes faeces, soil, mud, animal bedding, and water. During or after milking, particularly when teat sphincters are fatigued or compromised, *E. coli* from these environmental sources can readily enter the teat canal and establish an infection (Chakraborty, 2013).

CONCLUSION

A fifteen-year analysis of bovine milk samples in Nepal revealed *Staphylococcus spp*. as the most prevalent bacteria causing mastitis, followed by *E. coli* and *Streptococcus spp*. This finding suggests a potential shift in bacterial dominance, with *Staphylococcus spp*. surpassing *E. coli* compared to previous reports. These results highlight the importance of implementing hygiene practices during milking to reduce the spread of contagious pathogens and maintaining clean environments to minimize *E. coli* infections, ultimately contributing to improved udder health and reduced economic losses in the Nepalese dairy industry. Further research is warranted to understand the factors driving this potential shift in bacterial prevalence and develop targeted prevention and control strategies for mastitis in Nepal.

ACKNOWLEDGEMENT

The author would like to express sincere gratitude to the Central Veterinary Laboratory (CVL) in Tripureshwor, Kathmandu, Nepal for their invaluable support in this research. Their willingness to provide access to the collection of technical bulletins published over the past fifteen years (2003/2004 to 2017/2018) was instrumental. The data obtained from these bulletins formed the cornerstone of this study and significantly contributed to the investigation into the prevalence of mastitis-causing bacteria in Nepal.

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