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Epidemiology of Mastitis Pathogens in Dairy Cattle in Cauvery Delta Region of Tamil Nadu

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ABSTRACT

The study was conducted during the year 2024 from January to December for a period of one year at Veterinary Clinical Complex, Orathanadu. The aim of the study was to identify the most common etiology, prevalence and risk factors associated with the mastitis in the dairy cattle in Cauvery Delta region of Tamil Nadu. A total of 16049 cattle and 275 buffalo were presented to VCC, VC&RI, Orathanadu, of which 529 cases (cattle-527, buffalo-2) were mastitis. A total of 130 milk samples were collected from 124 mastitis cases for bacterial culture. The *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Coagulase negative staphylococci, Streptococcus* spp and *Bacillus* spp were isolated and confirmed by PCR. Among the bacterial isolates, the *Klebsiella pneumoniae* (62.50%) was the predominant bacteria followed by *Coagulase- negative staphylococci* (16.34%), *Bacillus* spp (5.77%), *Staphylococcus aureus* (4.81%), *Streptococcus* spp (4.81%), *Escherchia coli* (2.88%), and *Pseudomonas aeruginosa* (2.88%). The prevalence of mastitis in cattle and buffalo were 3.28 and 0.73%, respectively. The Crossbred Jersey cows were more susceptible than Crossbred Holstein Friesian and Indigenous breeds. The lactation stage had highly influenced the occurrence of disease. The animals in second and third lactation, especially in the early stage of lactation at 5–7 years were highly susceptible to mastitis. The prevalence of disease was high during the rainy season (33.07%) followed by autumn (24.0%), winter (22.87%) and summer (20.06%). In this study, the environmental mastitis pathogens was highly prevalent than contagious pathogen. It could be controlled by reducing stress and increasing host resistance by supplementation of vitamin E and selenium.

KEYWORDS: Epidemiology, *Klebsiella pneumoniae*, mastitis, prevalence, season, *Staphylococcus* spp.

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

India is a largest milk producing country and contributes 23% of global milk production (Mouli et al., 2023; Deepika Kumari, 2024) dairy farming is a major source of income for small and marginal farmers (Banerjee et al., 2017). However, mastitis remains major problem affect the economy of rural farmers. Mastitis is an inflammation of mammary gland, disrupts blood milk barrier affects the milk production and compositional changes in the milk (Lakshmi et al., 2024). It is a multi- etiological disease of mammary gland caused by bacteria, fungi, virus and algae, however, it is predominantly caused by bacteria (EI-Sayed and Kamel, 2021). The intramammary infection in dairy cattle is most commonly caused by contagious pathogens and environmental pathogens. The contagious pathogen (*Staphylococcus aureus*, *Streptococcus agalactiae* and *Mycoplasma bovis*) are usually resides in the udder and transmitted from infected to another quarter of mammary gland during milking process (Morales-Ubaldo et al., 2023). Environmental pathogens (*Escherchia coli*, *Klebsiella* spp, *Enterobacter* spp, *Citrobacter* spp, *Enterococcus* spp, *Streptococcus uberis* and *Serratia* spp) are primarily found in cow's environment such as soil, bedding materials, pasture, soil, faeces, sewage and drinking water. The cow's teat orifice was constantly exposed to environmental pathogens and enters through teat canal between milking processes (Klaas and Zadoks, 2018). The most frequently reported mastitis pathogens in dairy cattle throughout the world were *Staphylococcus aureus, Streptococcus agalactiae,* Coagulase-negative staphylococci (CoNS), *Escherichia coli* and *Klebsiella pneumoniae* (Pyorala and Taponen, 2009; Chandrasekaran et al., 2015; Sugiyama et al., 2022; Langhorne et al., 2023).

Based on clinical symptoms, the mastitis is classified into subclinical and clinical mastitis. In subclinical mastitis, there are no visible changes in mammary gland and milk secretion, but compositional change in milk and milk quality were observed. Whereas in clinical mastitis, the major visible changes like hot and painful swelling of udder, lameness on the affected side of the quarter, fever, anorexia or reduced feed intake and presence of clots or flakes in milk or yellowish serous fluid secretion were observed (Adkins and Middleton, 2018; Goulart and Mellata, 2022; Dego and Vidlund, 2024). Mastitis is most commonly occurred in early lactation is due to increased oxidative stress and decreased defence mechanism of mammary gland during the transition period and adversely affects the milk yield (Cheng and Han, 2020). The mastitis is not only affecting the milk yield during disease conditions, but also has a long term effect on milk yield, because the affected animal does not regain the peak milk yield after recovery in their remaining part of lactation (Rajala-Schultz et al., 1999). The identification of etiological agent and implementation of treatment in right time is utmost important to improve the clinical cure rate in mastitis. So, the present study focused on epidemiology of mastitis pathogens in clinical mastitis of dairy cattle in Cauvery delta region of Tamil Nadu, India.

2. MATERIALS AND METHODS

The study was conducted during the year 2024 at Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu for a period of one year (January, 2024 to December, 2024). A Orathanadu was a place of Cauvery delta region located 33 km distance from Thanjavur at 10.6277° N, 79.2525° E direction. A total of 16049 cattle and 275 buffalo were presented to Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu from various places of Thanjavur, Thiruvarur and Pudukottai districts for treatment, of which 527 cow and 2 buffalo suffered from mastitis during the study period. A total of 130 (128 cow and 2 buffalo) milk samples from 124 mastitis cases were collected for bacterial culture to identify the most common etiology of mastitis in dairy cattle in the Cauvery delta region of Tamil Nadu.

The epidemiological data like herd size, species, breed, age, parity, lactation stage, milk yield, feeding practices, milking hygiene and previous history of mastitis were collected using survey questionnaires.

The milk samples were cultured as per standard procedures (Anonymous, 2017). Briefly, the milk samples were inoculated into sterile nutrient broth and incubated at 37°C for 12–24 hrs for the appearance of turbidity. The broth culture was streaked into Nutrient agar (NA), Mannitol salt agar (MSA), MacConkey agar (MA), Eosin and Methylene Blue (EMB) agar, Blood agar and Cetrimide agar by using a sterile inoculation loop and incubated at 37°C for 24–48 hrs. The plates were examined for bacterial growth, colony morphology and colour changes in the medium. The biochemical test was also performed to identify the bacterial species. The polymerase chain reaction (PCR) was performed to confirm the bacteria.

3. RESULTS AND DISCUSSION

3.1. Identification of bacteria

Out of 130 milk samples, the bacterial growth was observed in 104 samples. In bacterial culture, *K. pneumoniae* (Figure 1 and 2), *E. coli, P. aeruginosa, S. aureus,* CoNS (Figure 3 and 4), *Streptococcus* spp and *Bacillus* spp were identified based on colony character and biochemical test, the isolates were confirmed by PCR (Figure 5 and 6). The Gram negative bacteria (68.27%) were more prevalent than the Gram positive bacteria (31.73%). Among the bacterial isolates, the *K. pneumoniae* (62.50%) was most frequently isolated followed by CoNS (16.34%), *Bacillus* spp (5.77%), *S. aureus*

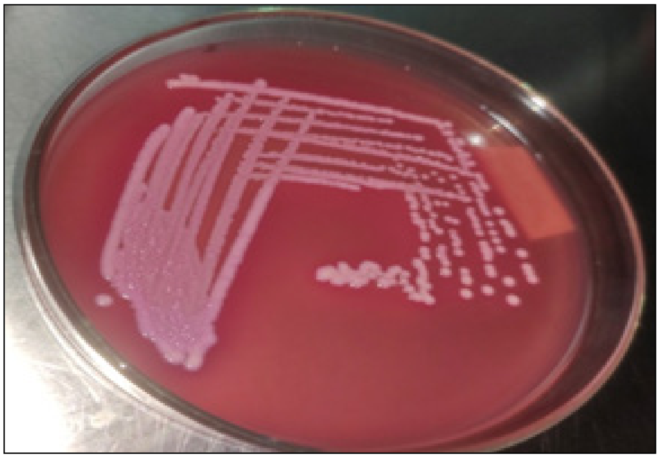

Figure 1: MacConkey agar - Pink mucoid Colonies- *Klebsiella pneumoniae*

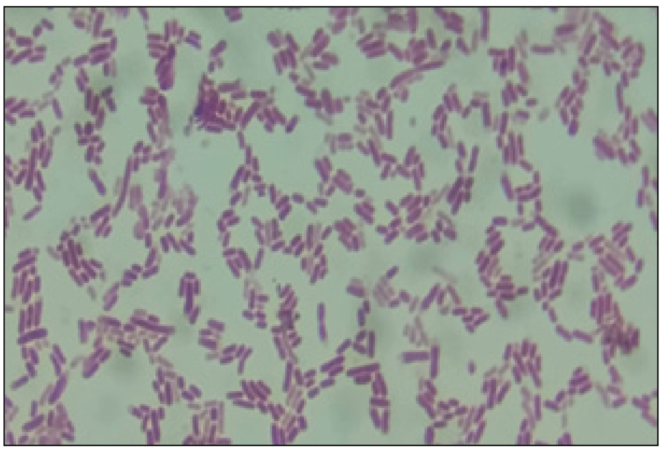

Figure 2: Gram negative rods- *Klebsiella pneumoniae* in Gram's staining (1000X)

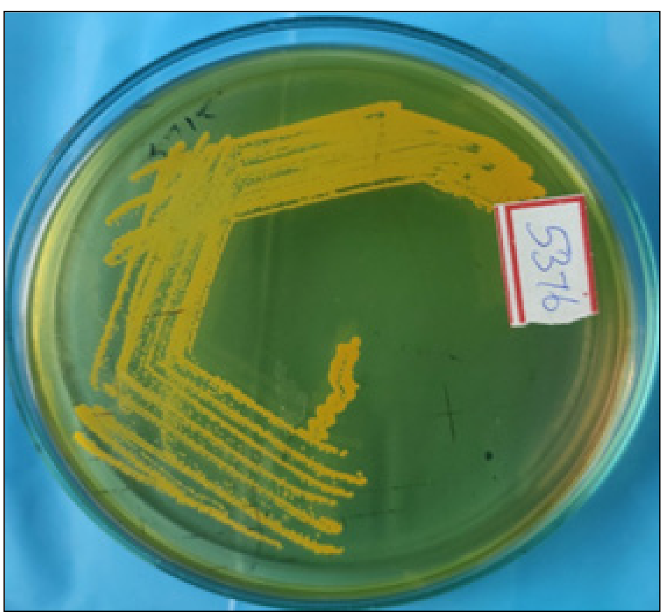

Figure 3: Mannitol salt agar-Golden yellow colonies- *Staphylococci* spp

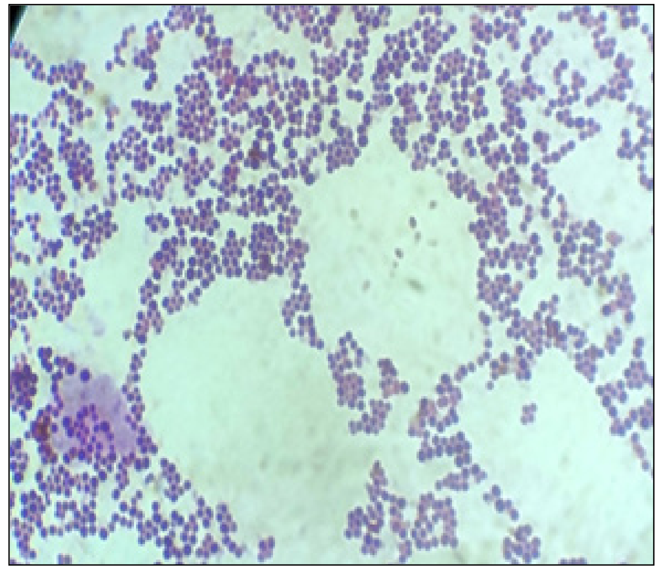

Figure 4: Gram positive cocci- *Staphylococci* spp; Gram's staining (1000X)

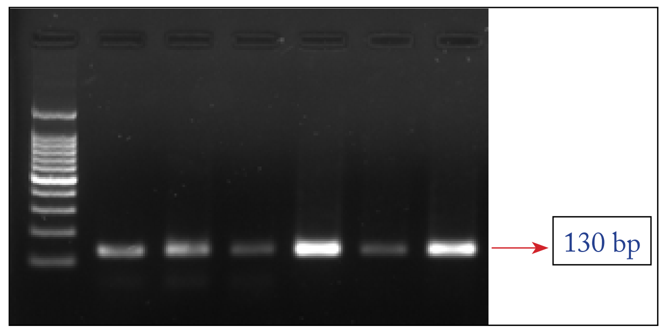

Figure 5: PCR amplification of *Klebsiealla Pneumoniae*

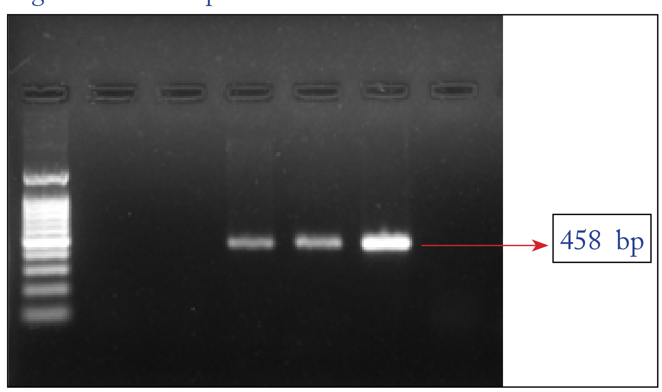

Figure 6: PCR amplification of *Staphylococcus aureus*

(4.81%), *Streptococcus* spp (4.81%), *E. coli* (2.88%), and *Pseudomonas* spp (2.88%).

3.2. Prevalence of mastitis pathogens

In this study, a high prevalence of *K. pneumoniae* followed by CoNS and with a low prevalence of *S. aureus, Bacillus* spp, *Streptococcus* spp, *E. coli* and *P. aeruginosa* were observed in dairy cattle. The prevalence of *K. pneumoniae* (62.50%) was found to be higher than the reported prevalence of

Sugiyama et al. (2022). who opined that the *K. pneumoniae* was the most frequently isolated mastitis pathogen (38.8%) followed by *E. coli* (37.4%) in acute bovine mastitis in Japan. The *K. pneumoniae* was the most detrimental environmental mastitis pathogen, because of poor response to treatment as a result of its strong biofilm forming ability. The biofilm formation leads to prolonged and persistent infection and induces severe inflammatory response in the mammary gland and systemic signs of fever, anorexia with severe udder swelling. The animals survive out of systemic signs; the milk production does not return normal levels as that of before infection (Cheng et al., 2019; Sugiyama et al., 2022: Cao et al., 2023).

In the present study, the CoNS (16.34%) was the second most prevalent mastitis pathogen in clinical mastitis of dairy cattle. This result was in concordance with the report of Zigo et al. (2022), who reported that the CoNS (16.5%) was the predominant mastitis pathogens in clinical mastitis during the early stage of lactation in the Slovak Czech Republic.

The lower prevalence of *S. aureus* (4.81%) was observed, which was coinciding with the report of Zigo et al. (2022), who reported that the 7.8% of *S. aureus* infection in the early stage of lactation. Cobirka et al. (2020) reported that the prevalence of contagious mastitis was decreased with a relative increase in environmental mastitis.

The *Streptococcus* spp was another bacteria most commonly isolated from organized dairy farm with clinical mastitis. The prevalence of *Streptococcus* spp (4.81%) was lower than the reported prevalence of Sumathi et al. (2008). who reported that 16% of clinical mastitis in Karnataka was caused by *Streptococci* spp. The *Streptococci* was a major problem in organized dairy farm, because it causes low grade to persistent chronic intramammary infection (Kabelitz et al., 2021) and intermittent episodes of recurrent clinical mastitis. It was difficult to control due to persistence in the mammary gland (Hogeveen et al., 2011).

In this study, *E. coli* (2.88%) was the least isolated bacteria in milk samples of periparturient cows and immediately after calving with the development of peracute gangrene in the mammary gland along with systemic signs of persistent fever, anorexia and recumbency. However, the prevalence rate was lower than the report of Chandrasekaran et al. (2015), who reported that 50.64% of *E. coli* infections in clinical mastitis cases. *E. coli* was most the common coliform bacteria mostly occurring in the early lactation and usually fatal in hyperacute form (Burvenich et al., 2003).

In this study, the epidemiological factors like breed, lactation stage, parity, age, feeding practices and season highly influenced the occurrence of clinical mastitis in dairy cattle.

3.3. Species prevalence

The prevalence of clinical mastitis in cattle and buffalo were 3.28% and 0.73%, respectively, but it was lower than the reported prevalence of mastitis in cattle (13.01%) and buffalo (7.30%) in organized farms of Tamil Nadu (Thirunavukkarasu and Prabaharan, 1999). The lowest prevalence of mastitis in buffalo was attributed to the presence of a strong smooth muscle sphincter around the teat orifice and reduces the susceptibility to intramammary infection (Uppal et al., 1994).

3.4. Breed prevalence

The higher prevalence rate of mastitis was recorded in Crossbred Jersey (83.5%) followed by Crossbred Holestein Friesian (14.8%) and Indigenous breed (1.7%) (Figure 7). It was in concordance with the report of Fesseha et al. (2021), but Chandrasekaran et al. (2015) reported that the Crossbred Holstein Friesian highly susceptible than Crossbred Jersey and non-descriptive animals.

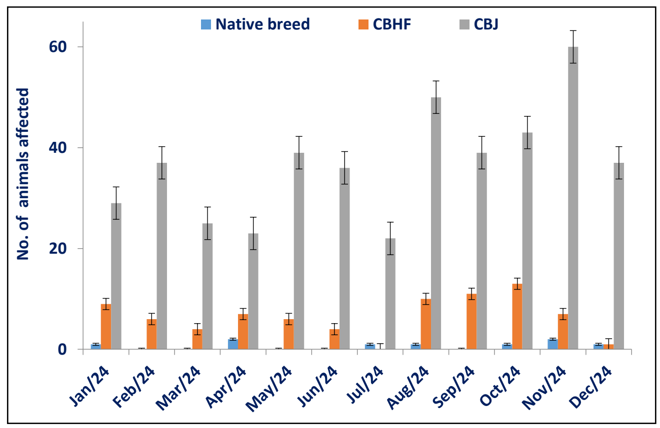

Figure 7: Breed wise and month wise prevalence of mastitis in dairy cattle

3.5. Lactation stage

In this study, the mastitis was most commonly recorded in animals in second (30.43%) followed by third lactation (27.78%) and first lactation (24.19%) (Table 1). The lactation stage had a higher influence on the occurrence of clinical mastitis. The prevalence of disease was higher in early lactation (43.30%) followed by mid (29.86%) and late lactation (26.84%) (Table 1). These findings were corroborated with the report of Lal et al. (2022), who reported that the animals in early stage of lactation (35.25%) were more susceptible than mid (29.69%) and late stage (18.82%). Moosavi et al. (2014) reported that the mastitis was higher during the late lactation period in summer months, but the higher prevalence of mastitis was observed in the early lactation period in the spring and winter seasons.

3.6. Age and parity

The disease was most commonly recorded in multiparous

Table 1: Influence of lactation, stage of lactation, age and parity on occurrence of mastitis in dairy cattle

Category	No. of animals affected	Mastitis (%)
Lactation		
First	128	24.19
Second	161	30.43
Third	147	27.78
Fourth	50	9.45
Fifth	20	3.78
Sixth	14	2.64
Seventh	7	1.32
Ninth	2	0.38
Stage of lactation		
Early (0-3 months)	229	43.30
Mid (4-6 months)	158	29.86
Late (> 6 months)	142	26.84
Age		
3-4 yrs	117	22.11
5-7 yrs	303	57.27
>7 yrs	109	20.60
Parity		
Primiparous	112	21.17
Multiparous	417	78.82

(78.82%) than primiparous (21.17%) animals, especially between 5–7 years of age (57.27%) followed by young at 3–4 yrs (22.11%) and older cows >7 yrs (20.60%) (Table 1). The similar findings were reported by Fesseha et al. (2021), who reported that the mastitis was most frequently occurred in animals between 6–9 yrs than young age. The incidence of mastitis was significantly increased with the increases of parity (Chen et al., 2023). The prevalence of CoNS mastitis is higher in primiparous cows than in older cows (Table 1). The *S. chromogenes* is the major CoNS species affecting nulliparous and primiparous cows, whereas *S. simulans* has been isolated more frequently from older cows. Multiparous cows generally become infected with CoNS during late lactation, whereas primiparous cows develop infection before or shortly after calving (Pyorala and Taponen, 2009).

3.7. Feeding practices

In the present study, feeding of cotton seed milk during the transition period increased the incidence of peracute gangrenous *E. coli* mastitis in dairy cattle. The overfeeding of grain has been implicated as a major risk factor for the occurrence of mastitis in heifer (Fox, 2009). Ma et al. (2021) reported that feeding of a high concentrate diet in dairy cattle, induces the oxidative stress and inflammatory response in the mammary gland due to impaired immune response during the transition period.

3.8. Seasonal prevalence

The occurrence of mastitis was higher in the rainy season (33.07%) followed by autumn (24.00%), winter (22.87%) and summer (20.06 %) season (Table 2). It was contradictory to the report of Ranjan et al. (2011), who opined that the highest incidence of mastitis in winter (47.37%) followed by summer (42.26%) and least in rainy season (7.37%). However, the highest incidence of mastitis was recorded in the autumn season in China (Chen et al., 2023). In the present study, there was an increased trend of *Staphylococcus* mastitis in summer and *Klebsiella* mastitis in the rainy, autumn and winter seasons were observed during the study period. These findings were in concordance with the report of Acharya et al. (2021). They reported that there were more cases of *Staphylococcus aureus* mastitis in summer months, coagulase negative staphylococci and *E. coli* mastitis in spring and summer months (Acharya et al., 2021). Song et al. (2020) reported that there was a higher proportion of *Staphylococcus* spp in summer and a lower percentage of *Streptococcus* spp in the autumn season.

Table 2: Seasonal prevalence of mastitis in dairy cattle during 2024

Season	Month	No. of mastitis cases	Percentage
Summer	March	29	5.50
	April	32	6.05
	May	45	8.51
Sub total (a)	106	20.06	
Rainy (Monsoon)	June	40	7.56
	July	23	4.34
	August	62	11.72
	September	50	9.45
Sub total (b)	175	33.07	
Autumn	October	58	10.96
	November	69	13.04
Sub total (c)	127	24.00	
Winter	December	39	7.37
	January	39	7.37
	February	43	8.13
Subtotal (d)	121	22.87	
Grand total (a+b+c+d)	529	100	

The prevalence of mastitis was higher during the month of November, followed by August, October and September and the least prevalence was recorded in July (Figure 7). These findings were dissimilar to the report of Ali et al. (2021), they reported that the higher prevalence of mastitis in both cattle and buffalo was during the months of July, August and September, and the lowest in May and November.

4. CONCLUSION

The environmental mastitis pathogen (*K. pneumoniae*) was most frequently isolated in dairy cattle. The *K. pneumoniae* caused the severe reduction of milk yield or complete loss of milk yield in the diseased lactation, which resulted in increased culling percentage. It could be controlled by reducing stress during transition period and increasing host resistance and implementation of mastitis control programme.

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