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Phenotypic identity of bovine mastitis pathogens obtained from California mastitis positive (CMT) milk samples

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Abstract

Bovine mastitis isolates of 45 numbers from positive milk samples of California Mastitis Test (CMT) in and around Bengaluru area were obtained from the Department of Veterinary Microbiology, Veterinary College, Bengaluru in nutrient broth. Isolates were purified using streaking technique among them 42 number isolates showed the uniform remaining 3 isolates revealed with circular colony morphology on to the streaked plates. Cell morphology of the 45 isolates upon subjecting to the gram staining showed 40 number with purple color with cell arrangement cocci in chains (22), cocci in bunches (18) isolates assigned with Gram positive streptococci, staphylococci respectively, while the remaining 5 isolates showed pink color and short rods under the oil immersion microscope. Thus, in the present study, Gram positive bacterial pathogen were predominant followed by the Gram-negative mastitis pathogens.

Keywords: Mastitis, phenotypic identity, cell morphology, colony morphology

1. Introduction

Mastitis is the inflammation of the mammary gland, characterized by physical, chemical, bacteriological changes in milk and pathological changes in glandular tissues that alter the quality of raw milk. Bacteria play a major role in causing mastitis. Inflammation of the mammary glands exists in two primary forms: clinical and subclinical mastitis. Clinical mastitis, which is less prevalent, is characterized by systemic signs in the milch animals and visible abnormalities in the udder and milk. In contrary, subclinical mastitis is more common and results to reduced milk production without observable clinical signs or abnormalities in the udder or milk. California mastitis test is most common and the gold standard test used to determine the subclinical mastitis. As compared to clinical form, no clinically visible symptoms appear in subclinical mastitis, although change in milk composition can be an indicator. Hence, it is recognized and confirmed by laboratory examination of milk or by animal-side tests such as California Mastitis Test (CMT) followed by laboratory isolation of the causal agent.

2. Materials and Methods**2.1 Materials**

Bovine mastitis isolates of 45 numbers from positive milk samples of California Mastitis Test (CMT) in and around Bengaluru area obtained from the Department of Veterinary Microbiology, Veterinary College, Bengaluru were in nutrient broth (Table 1).

Table 1: Bacterial Isolates obtained from California Mastitis Test (CMT)

Source	Code & Number of isolates
Nelamangala	M1 (1)
Hosakote	M2 (1)
Doddaballapur	M3, M4, M5, M6, M7, M8, M9 (7)
Rajankunte	M10, M11, M12, M13, M14, M15, M16, M17 (8)
Chanapatna	M18, M19, M20, M21, M22, M23, M24, M25, M26, M27 (10)
Ramanagara	M28, M29, M30, M31, M32, M33, M34, M35, M36 (9)
Anekal	M37, M38, M39, M40, M41, M42, M43, M44, M45 (9)
Total	45

2.2 Methods

2.2.1 Purification and maintenance of isolates of Bovine Mastitis

The collected pathogens were purified by streaking technique on to poured plates of using the nutrient agar medium sterile inoculation loop and the plates were incubated for 37 °C/24 h. After incubation, the obtained single pure colony was selected and transferred to nutrient agar slants and incubated for 37 °C/ 24 h. and preserved as stock culture on nutrient agar slant in the refrigerator (Harrigan, 1998)^[1].

2.2.2 Phenotypic identification of isolates of Bovine Mastitis

Purified isolates were subjected to Gram staining to reveal their cell morphology of phenotypic identity. Smears of bacterial isolates were prepared from 18 h old broth cultures on clean grease free slide, air dried treated with crystal violet solution for 1 min followed by Gram's iodine solution for 1 min, immediate gentle rinsing with water, decolorization with ethanol till colour goes off and final counter staining with safranin. Gram's staining helped in declaring the isolates as Gram positive or negative. (Harrigan, 1998)^[1].

3. Results and Discussion

3.1 Phenotypic identity of colony and cell morphology of bacterial isolates of bovine mastitis

Bovine mastitis isolates of 45 numbers from California Mastitis Test (CMT) positive milk samples obtained when streaked on nutrient agar plates, the colonies appeared were either Puntiform or circular and cell morphology under microscopic observation revealed Gram positive cocci in chains, bunches and Gram-negative rods. Among 45 bacterial isolates 42 exhibited uniform colonies while remaining 3 formed circular colonies. Only 5 out of 45 isolates were Gram negative rods whereas remaining 40 were cocci either in chains or bunches. Gram positive cocci in chains were 22 isolates placed as streptococci that predominated followed by

18 isolates of Gram positive cocci in bunches as staphylococci and 5 isolates of Gram negative rods (Plate 1, Figure 1, Table 2).

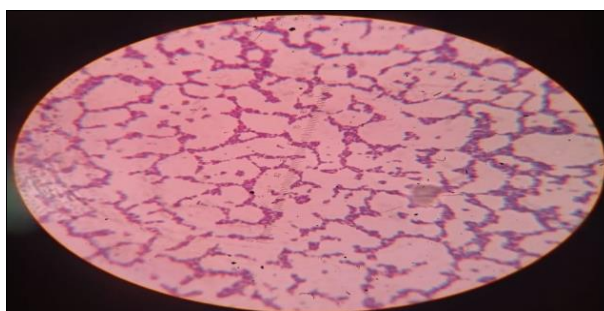


A) Puntiform

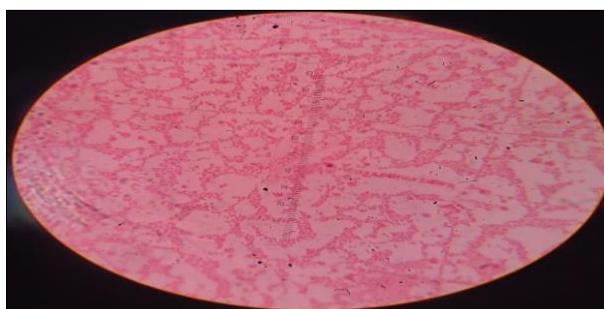


B) Circular

Plate 1: Colony morphology types of bacterial isolates of bovine mastitis



A) Gram positive cocci in bunches



B) Gram negative short rods

Plate 2: Cell Morphology of bacterial isolates of bovine mastitis (100x)

Table 2: Colony and cell morphology of bacterial isolates of bovine mastitis

Code of isolates	Morphology	
	Colony	Cell
M2, M3, M4, M5, M6, M9, M12, M13, M15, M17, M18, M19, M22, M26, M30, M31, M34, M36, M39, M40, M44, M45 (22)	Punctiform, Raised, Entire	Gram positive chains of Cocci – Streptococci
M1, M7, M8, M10, M11, M16, M20, M21, M23, M24, M25, M28, M29, M32, M35, M37, M41, M43 (18)	Punctiform, Raised, Entire	Gram positive bunches of Cocci – Staphylococci
M14, M27, M33 (3)	Circular, Convex, Entire	Gram negative short rods
M38, M42 (2)	Punctiform, Flat, Entire	

In contrast to the present study, Yazdankhah *et al.* (2001) [3] could isolate bacteria from 48 milk samples of mastitis out of 55 while 7 samples did not show any bacterial growth. Jose *et al.* (2021) [2], collected milk samples from 83 lactating dairy cows showing clinical signs of mastitis in Kerala region. Out of the 83 quarter samples examined, 57 samples were positive in culture, of which five had mixed infection with two different bacterial isolates there by accounting for a total of 67 bacterial isolates by streaking on to brain heart infusion agar, followed by incubation of the plates at 37 °C for 24 h.

4. Conclusions

Mastitis is the major hurdle in dairy industry since it's the major economic loss currently in the world. This study concludes that the Gram positive bacterial pathogens are predominant in causing bovine mastitis followed by the Gram negative bacterial isolates. Subclinical mastitis is difficult to identify unless tested, CMT is the most commonly employed test to determine the subclinical mastitis in the dairying animals. Culturing of the CMT positive milk samples would help in isolating the specific bacterial pathogens involved in causing mastitis which would further assist in treating the mastitis disease.

5. Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6. References

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