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Coliform mastitis in dairy cows in Thanjavur region, Tamil Nadu

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Abstract

Bovine mastitis are caused by wide range of microorganisms. A high proportion of all clinical mastitis is caused by Gram-negative bacteria, mostly coliforms, including *Escherichia coli*, *Enterobacter* and *Klebsiella*. The present study investigated the prevalence of coliform mastitis causing organisms in dairy cows with mastitis brought to the Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu. Milk samples were collected in sterile containers from 140 cows with mastitis for isolation and identification of coliforms followed by antibiotic resistance study. Briefly, the collected milk samples were enriched and streaked onto selective medium and identified using biochemical tests. The study revealed *E. coli* and *Klebsiella* as common etiological agents in this region with prevalence of 25.7% and 18.6%, respectively. *E. coli* isolates were maximum resistant to methicillin whereas *Klebsiella* isolates were maximum resistant to ampicillin. Since coliforms are considered as environmental contaminants, good hygiene practices during milking process, teat dipping, fly control and dry cow therapy could reduce the prevalence of coliform mastitis.

Keywords: Coliform mastitis, *E. coli*, *Klebsiella*, dairy cows

Introduction

Bovine mastitis is characterized by inflammation of mammary gland. It is one of the most common and expensive health problem and leading cause of antibiotic usage in dairy cows [1, 2]. It results in increased production costs due to low production and quality, discarded milk, culling, treatment of affected animals and delayed genetic progress [3, 4]. It is caused by wide range of microorganisms [5]. A high proportion of clinical mastitis is caused by Gram-negative bacteria, mostly coliforms, including *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Serratia marcescens* [6-8] and they are considered as environmental mastitis pathogens [9]. They are normal inhabitants of gastrointestinal tract of warm blooded animals [10] and are abundant in faecal material, soil and manure. Coliforms occurring at high concentration ($\geq 10^6$ /g) in the bedding increase the occurrence of clinical mastitis in dairy cows [11].

Coliform mastitis results in a higher incidence of cow death and or agalactia-related culling (30 to 40%) in dairy cows [12] and it also results in subclinical infections that persist for longer periods of time [13]. Antimicrobial agents used inappropriately like use of wrong drug, dose and duration results in increase in antimicrobial resistance of coliforms mastitis with decreased outcome for treatment [14]. In addition, *Escherichia coli* and *Klebsiella* are listed as “critical mastitis” pathogens by World Health Organization (WHO) to tackle antimicrobial resistance problem [15, 16]. Identification of prevalence of bovine mastitis etiological agents is essential to design effective treatment and control strategies for mastitis. So far, the prevalence of coliform mastitis in dairy cows in Orathanadu region of Thanjavur is not reported. Therefore, the objective of the present study was to determine the prevalence of coliform mastitis in dairy cows with mastitis brought to the Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu, Thanjavur district, Tamil Nadu.

Materials and Methods

Sample collection, microbiological analysis and biochemical characterization

Bovine mastitis milk samples were collected from 140 cows with signs of mastitis for isolation and identification of coliforms. A volume of 1 ml of milk samples were transferred to nutrient broth and incubated at 37 °C overnight. After incubation, it was streaked on Sorbitol MacConkey (SMA) and Eosin Methylene Blue (EMB) agar for isolation of coliforms. All the

isolates were purified by sub-culturing in nutrient broth and further streaking on nutrient agar. The presumptive *Klebsiella* and *E. coli* isolates were subjected to Grams staining, catalase test, indole test, oxidase test, methyl red test, VP test, Simmon's citrate utilization tests and triple sugar iron utilization tests.

Antimicrobial susceptibility testing.

All the isolated *E. coli* and *Klebsiella* organisms were tested for antimicrobial susceptibility using disc diffusion methods [17]. The agents used in this study include Methicillin (5 mcg), Vancomycin (30 mcg), Erythromycin (15 mcg), Streptomycin (10 mcg), Gentamicin (120 mcg), Doxycycline (30 mcg), Tetracycline (30 mcg), Amoxicillin-clavulanic acid (20 and 10 mcg) and Ampicillin (10 mcg). Pure bacterial cultures were enriched in brain–heart infusion broth at 37°C for 6–8 h. The cultures were streaked on Mueller Hinton agar plates (Himedia, India) using a sterile cotton swab and the antibiotic discs were dispensed using a disc dispenser (Himedia, India) with sufficient space in between each disc to avoid overlapping. The agar plates were incubated at 37°C for 16–18 h and the zones of inhibition for each antibiotic were measured.

Results and Discussion

Mastitis is widespread in dairy animals. Many studies have implicated Gram-negative coliforms as the main etiological agents of clinical mastitis [18, 19]. In this study, 140 mastitis milk samples were collected and the prevalence of coliform mastitis in dairy cows in Thanjavur region was determined based on colony morphology, Gram's staining and biochemical characterization. *E. coli* and *Klebsiella* were common etiological agents in this region with prevalence of 25.7% and 18.6% respectively (Figure 1). *E. coli* had pink colonies on SMA agar and bluish green colonies with metallic sheen on EMB agar. They were Grams negative rod shaped bacteria on Gram's staining, positive for Indole and Methyl red tests and negative for Voges-Proskaur and citrate utilization tests and acidic butt and acidic slant in TSI utilization test (Figure 2). *Klebsiella* colonies isolated from the samples were mucoid on SMA agar, Grams negative rod shaped organism in Gram's staining, negative for indole and methyl red tests and positive for Voges-Proskaur and citrate utilization tests (Figure 3).

Previously published articles have reported variable prevalence of *E. coli* and *Klebsiella* [7, 19-28]. A variable *E. coli* prevalence of 17.19% [20], 21.05% [21], 26.7% [7], 32% [22], 34.48% [23], 34.7% [24] and 39.9% [25] were reported. Similarly, variable *Klebsiella* prevalence of 4.2% [26], 6.57% [21] and 35.6% [19] were reported.

In this study, *E. coli* (25.7%) was found to be the most common coliforms followed by *Klebsiella* (18.6%). Similarly, Schukken *et al.*, 2011 [27] found *E. coli* as the most common coliforms species followed by *Klebsiella* spp. and *Enterobacter* spp in milk samples from cows affected with clinical mastitis.

The common sources of coliform organisms include bedding, feedstuffs, soil, faeces, water and other organic matter present around the dairy animals. The incidence of coliform mastitis is directly proportional to number of organisms present in the bedding material. Reducing the number of organisms in bedding by use of inorganic bedding materials would be beneficial in reducing clinical mastitis caused by coliforms [10].

Inappropriate uses of antimicrobial agents have resulted in the development of antimicrobial resistance (AMR) among coliform organisms and they pose hurdle in treatment of clinical mastitis [28]. The isolated *E. coli* and *Klebsiella* were tested for antimicrobial resistance pattern. In this study, 70% of isolated *E. coli* were resistant to methicillin, erythromycin, streptomycin, ampicillin and vancomycin and whereas 70% of *Klebsiella* isolates were resistant to ampicillin, methicillin and vancomycin (Figure 4). Antimicrobial resistance is major public health problem in both medical and veterinary science. To tackle AMR problem, the World Health Organization (WHO) recently endorsed a global action plan and published a list of priority pathogens for research and development of new antibiotics. This list includes several mastitis causing pathogens such as *Escherichia coli*, *Klebsiella* as “critical” organisms and *Staphylococcus aureus* as “high priority” organisms for tackling problems associated with AMR [15, 16]. Hence to prevent the emergence of antimicrobial resistance, antimicrobial agents should be used at correct dosage for correct duration after testing the *in vitro* sensitivity testing [20].

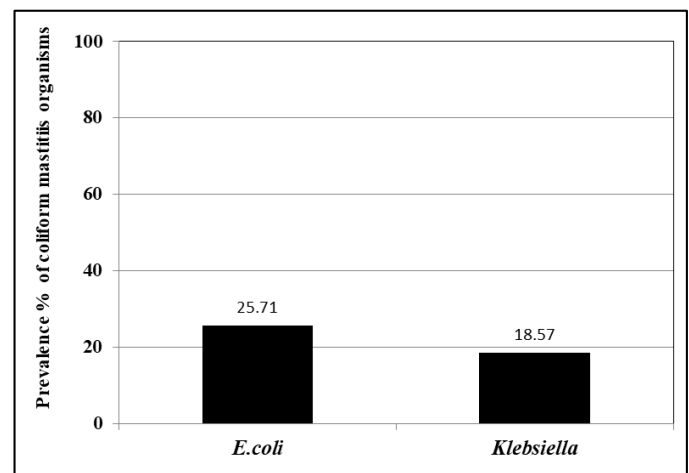


Fig 1: Prevalence of coliform mastitis causing organisms in dairy cows with mastitis



Fig 2: *E. coli* on SMA agar (pink colonies), IMViC (+/+/-/-) and TSI utilization (acidic slant and acidic butt)



Fig 3: *Klebsiella* on SMA agar (mucoid colonies), IMViC (-/-/+ /+) and TSI utilization (acidic slant and acidic butt)

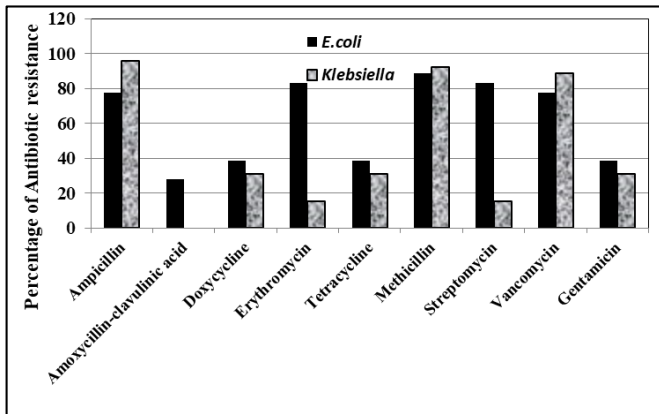


Fig 4: Percentage of antibiotic resistance among the *E. coli* and *Klebsiella* isolated from dairy cows with mastitis

Conclusion

The study results revealed the prevalence of *E. coli* and *Klebsiella* as major coliform pathogens involved in mastitis in the Orathanadu region. These pathogens could be originating from the cow's environment associated with poor prevailing hygienic conditions, lack of proper disposal of litter and dung, absence of teat dipping after milking and indiscriminate use of antimicrobial agents. Following good hygienic practices during milking process, teat dipping, fly control and dry cow therapy could reduce the prevalence of coliform mastitis

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