Prevalence of Clinical Mastitis due to *E. coli* in Bovines

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**A B S T R A C T**

Mastitis, an economically important disease causes heavy economic losses in dairy cattle associated with poor quality and quantity of milk, milk discard after treatment and increased cost of veterinary services. *Escherichia coli* is reported to be the major environmental pathogen associated with clinical mastitis. Out of 104 milk samples collected from clinical mastitis cows brought to TVCC, VCRI, Namakkal, the prevalence mastitis due to *E. coli* was found to be 10.57% for *E. coli* identified by isolation on selective media namely eosine methylene blue agar and MacConkey agar and confirmation by employing polymerase chain reaction (PCR).

**K e y w o r d s**

Clinical mastitis, *E. coli*, prevalence, PCR.

**Introduction**

Mastitis is one among those major reasons causing substantial loss to the dairy farmers, worldwide, resulting in 30.0 per cent less productivity per quarter and 15 per cent production per cow (Radostits *et al.*, 2000), and loss of milk yield is estimated to range from 100 to 500 kg/cow per lactation. Globally, mastitis accounts for about 38.0 per cent of the total direct costs of the common production diseases (Kossaibati and Esslemont, 1997). Annual economic losses due to clinical mastitis alone pegged at INR 3014.35 crores (NAAS, 2013).

Clinical mastitis is recognized by abnormal milk, gland swelling and/or systemic illness and the reduction in milk production attributed to clinical mastitis could be 30.0 percent of the total losses (Philpot and Nickerson, 1991 and Philip *et al.*, 1993). Clinical mastitis can be peracute, acute, subacute and chronic, caused by coliforms like *Escherichia coli*, *Klebsiella pneumoniae*, *K. oxytoca*, *Enterobacte raerogenes* and streptococci.

However, *E. coli* is reported to be the commonest pathogens in environmental mastitis resulting in sudden onset of fever, loss of appetite, diarrhea, toxaemia and the infected quarter show swelling, pain with discharge of watery or bloody milk or milk with large thick clots. This paper reports the prevalence of *E. coli* in bovines at cow and
quarter level determined by isolation and identification and confirmation by molecular technique.

Materials and Methods

A total of 136 milk samples from 104 clinical mastitic cases were collected for the period from October 2016 to March 2017 at Teaching Veterinary Clinical Complex (TVCC), Veterinary College and Research Institute, Namakkal.

A first few squirts of milk from each quarter were discarded and the second squirt of milk was collected in a sterile nutrient broth tube and incubated at 37°C for 5-6 hours, the loop was flamed and the sample is streaked in all selective media, Eosin methylene blue and MacConkey agar (Hi-media, Mumbai) and the plates were incubated at 37°C for 24 hours (Quinn et al., 1994). The colonies from the isolates were identified by Gram’s staining technique as recommended by the manufacturer’s kit (Himedia, Mumbai).

The sample DNA was extracted from the isolates of clinical mastitic cases by using polyethylene glycol (PEG) as recommended by Chomczynski et al., (2006). Colonies from the isolates were transferred to 100μl of nuclease free water and then mixed with 900μl of PEG reagent. The mixture was vortexed for 2-3 min, incubated in water bath at 90°C for 15 min. and then centrifuged at 12,000 rpm for 10 min. Then 3μl of this lysate was transferred directly to PCR reaction mixture for amplification. The primers custom synthesized (Bioserve, India) were utilized for amplification of the mastitis causing E. coli targeting 16s ribosomal RNA gene at 585bp (Hassan et al., 2014) and were forward: 5’-GAC CTC GGT TTA GTT CAC AGA-3’ and reverse: 5’-CAC ACG CTG ACG CTG ACC-3’. The cycling conditions were initial denaturation- 95°C/180 sec, denaturation- 94°C/45 sec, annealing 58°C/45 sec, extension-73°C/60 sec with 30 cycles and final extension- 72°C/180 sec. The amplified products were electrophoresed in 1.5 per cent agarose gel and specific bands were visualized under U-V illumination by gel documentation system.

Results and Discussion

The bacterial pathogens from the milk samples were identified by isolation on specific media as isolation and identification of causative pathogens is still considered to be the gold standard (Radostitis et al., 2000).

The colony characteristics of isolates specific to E. coli on EMB agar was identified as bluish green colonies with metallic sheen (Plate 1 and 2) and MacConkey agar as bright pink lactose fermenting colonies. These findings are in accordance with that described by Quinn et al., (1999), and Hogan and Smith (2003).

The prevalence of E. coli in clinical mastitis at cow and quarter levels was identified to be 10.57 and 10.2 per cent, respectively. This finding is in agreement with that (7.69 %) observed by Akhtar et al., (2003), whereas, a high prevalence than that in this study was recorded by Balakrishnan et al., (2004), Das and Joseph (2005), Sumathi et al., (2008) and Chandrasekaran et al., (2014) with 27.5, 17.44, 20.0 and 45.89 per cent, respectively in Tamil Nadu and other states.

The isolates of E. coli from clinical mastitic cases were confirmed by PCR (Figure 1) which is found to be the most appropriate technique for the species identification of mastitic pathogens that are difficult to detect by conventional methods (Mahmmod et al., 2013) with high sensitivity (Steele et al., 2015) and high correlation rates with culture (Cervinkova et al., 2013). The prevalence of E. coli in this report could be associated with poor hygienic conditions as these bacteria
being part of normal bovine intestinal flora, contaminate the environment via faeces and during puerperal period, the cow is especially sensitive to coliform infections due to low immunity at that time (Pyorala, 1995 and Radostitis, 2000).

**Plate.1** Bright pink lactose fermenting colonies of *E. coli* on MacConkey agar

**Plate.2** Greeinsh blue colonies with metallic sheen of *E. coli* on Eosine methylene blue agar
A high prevalence of *E. coli* was detected in right hind (14.8%) and left hind quarter (10.6%) followed by left fore (9.3%) and right fore quarter (6.6%). This finding is in agreement with that of Mekibib *et al.*, (2010) who reported a high prevalence in hind quarter, in contrast, Khanal *et al.*, (2013) and Kavitha *et al.*, (2009) reported a high prevalence in four quarters. The high prevalence in hind quarters might presumably be associated with increased chance of hind quarters being soiled with urine and faces or by the tail leading to poor udder management (Hogan *et al.*, 2003).

It is concluded that *E. coli* is one of the commonest pathogens in clinical mastitis originating from the environment of cow associated with poor hygiene in the house, poor disposal of the litter, udder washing, lack of post teat dipping, indiscriminate usage of antibiotics and lack of awareness, and could lead to severe per acute or acute and chronic mastitis failing to yield response to the treatment. Hence early diagnosis by isolation and selection of antibiotics by antibiogram is essential in the successful control of coliform mastitis due to *E. coli*.

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