SHORT COMMUNICATION

Isolation, Molecular Detection and Antibiogram of *E. coli* Isolated from the Mastitis Milk of Crossbred Jersey Cows in Puducherry

Kirouchendraji Sandhiya[1]*, Sivachandiran. R.[2], BhanuRekha V.[1], K.M. Venkatesh[3]

Abstract

Though mastitis is caused by multiple etiological agents, bacteria are the most common etiological agents. The molecular detection of an organism is less time-consuming and more accurate than conventional methods, hence the present study was aimed at conventional as well as molecular detection and the antibiogram study of *E. coli* from the suspected subclinical (15) and clinical (15) mastitis milk samples collected in Puducherry's Aryankuppam region. All the collected samples were positive for the California Mastitis Test. Subsequently, 19 *E. coli* isolates were isolated, and they were investigated using the PCR technique (uspA gene) as well as biochemical tests. Twelve samples were found positive by PCR (uspA gene). Furthermore, an antibiogram study revealed that penicillin (100%) was highly resistant, followed by nalidixic acid (78%). Notably, the highest susceptibility was observed for ciprofloxacin (92%), followed by gentamicin (85%), and ceftriaxone (71%). The probable isolates obtained need to be additionally investigated using more mastitis milk samples.

Key words: Antibiogram, Crossbred cow, *E. coli*, Mastitis, Milk, uspA gene

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Introduction

Milk, in addition to being a wholesome food, provides an ideal environment for the growth and multiplication of bacterial organisms that affect the human gut environment (Donkar *et al.,* 2007). Mastitis is the most expensive dairy animal disease, posing a significant economic risk to dairy animal welfare and farming practises (Essa *et al.,* 2023). Besides the damage it causes, mastitis is the primary cause of antimicrobial resistance (AMR) in dairy cows due to the repeated and extensive use of antibacterial drugs (Erskine *et al.,* 2002). To put it simply, AMR is a serious problem that needs to be tackled, other wise infections in animals may become challenging to treat or control in the near future. To address this issue, big steps must be taken in animal husbandry practices, which necessitate the development of an AMR database or information in a particular region or state. However, India doesn't have many resources on AMR, and there is a need to study AMR region- or state-wide to make use of them via a proper plan of action for the betterment of human as well as animal life (Nukala *et al.,* 2022).

Mastitis is caused by multiple etiological agents, but *E. coli* is the main cause of it (Mishra *et al.,* 2017). They have six universal stress protein (usp) genes, namely A, C, D, E, F, and G. Their expression is regulated by diverse environmental stresses. Among these genes, uspA is an essential gene that helps with cellular development, motility, and adhesion that is required for the survival of *E. coli* (Mishra *et al.,* 2017). Previous studies have identified *E. coli* from mastitis milk samples using the PCR method by amplifying the uspA gene (Mishra *et al.,* 2017; Ramasamy *et al.,* 2021). Thus, the current study aims at the molecular detection and antibiogram study of *E. coli* isolates extracted from suspected mastitis milk samples to shed light on trends in the same.

[1]Department of Veterinary Public Health and Epidemiology, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry - 605009, India

[2]Department of Veterinary Public Health and Epidemiology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600007, India

[3]Sathyabama Institute of Science and Technology, Chennai-600119, India

Corresponding Author: Kirouchendraji Sandhiya, Department of Veterinary Public Health and Epidemiology, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry - 605009, India
e-mail: nksandhiya1999@gmail.com

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Materials and Methods

Collection of Samples

A total of thirty suspected milk samples were collected from crossbred Jersey cows in the Aryankuppam region of Puducherry that had both clinical and subclinical mastitis. The

suspected milk samples were collected aseptically after being subjected to the California Mastitis Test (CMT). Then they were immediately transferred to the laboratory for further examinations at the Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry.

Isolation, Identification, Antibiogram, and Biochemical Tests for *E. coli*

The colonies of *E. coli* were cultured, isolated, and identified using Luria broth, MacConkey's agar (MAC), and Eosin Methylene Blue (EMB) agar plates as described by Vignesh *et al.* (2022). Following that, the biochemical tests (catalase, oxidase, indole, citrate utilization, methyl red, and vogs-proskauer) were performed using the methods described in Krieg and Holt (1984). Further, antimicrobial sensitivity testing was carried out for all the *E. coli* isolates by the disc diffusion method as described by Bauer *et al.* (1966). The antibiogram discs (Himedia, India) used in the present study were gentamicin (GEN-50 µg), cefotaxime (CTX-10 µg), ciprofloxacin (CIP-10 µg), penicillin (P-2 µg), tetracycline (TE-10 µg), chloramphenicol (C-25 µg), nalidixic acid (NA-30 µg) and trimethoprim (TR-10 µg). Subsequently, the results were recorded and interpreted as per standard guidelines (CLSI, 2019).

DNA Extraction and Amplification of the uspA Gene of *E. coli*

The genomic DNA of the bacteria was isolated from the loopful of bacterial colonies using the method followed by Ramasamy *et al.* (2021). After the isolation of DNA, a pair of primers (F-5'-CCGATACGCTGCCAATCAGT-3' and R-5'-ACGCAGACCGTAAGGGCCA GAT-3') was used to amplify the target region (884 bp) of the uspA gene of *E. coli*, as described by Mishra *et al.* (2017). The amplicon covers the complete CDS (796 bp) of the gene and is species-specific. The thermal profile of the PCR was followed as described by Mishra *et al.* (2017). After PCR was performed, the amplified product was analyzed, recorded, and documented as described by Shrivastava *et al.* (2022).

Results and Discussion

In the present study, all the suspected mastitis milk (fifteen each of clinical and subclinical mastitis) samples tested were positive for CMT. From the total 30 suspected milk samples, 19 (63.3%) were found positive for *E. coli* contamination. *E. coli* colonies were identified by their bright pink colour in MAC agar. Further, EMB agar was used as a definitive medium, and colonies were characterised by a smooth, circular, and metallic sheen appearance. Gram staining from bacterial isolates had pink colour characteristics and was organised in singles and pairs, which is a phenotypic characteristic of Gram-negative bacteria. Environmental mastitis caused by *E. coli* is a major challenge faced by farmers in the dairy sector due to significant economic loss (Essa *et al.,* 2023; Singh *et al.,* 2018). These pathogens may be present in bedding material, soil, walkways, or pasture. Controlling environmental mastitis has become a main task among farmers today. It is always advisable to manage the milking routines to reduce the risk of both contagious and environmental mastitis.

The antibiogram (Fig. 1) in the current study revealed that penicillin had the highest resistance (100%), followed by nalidixic acid (78%). On the other hand, ciprofloxacin had the highest antibiotic susceptibility (92%), followed by gentamicin (85%), ceftriaxone (71%), chloramphenicol (64%), and tetracycline (50%). These results are consistent with the findings of the previous study, in which ciprofloxacin and gentamicin were most sensitive (Singh *et al.,* 2018; Ramasamy *et al.,* 2021), and tetracycline and penicillin were resistant to *E. coli* isolated from the mastitis milk (Ramasamy *et al.,* 2021). However, the resistance was mostly shown for penicillin and nalaxidic acid; some other antibiotics, such as tetracycline and chloramphenicol, showed intermediate to complete resistance apart from being sensitive to some of the samples. This implies that they will become resistant in the near future if the overuse or indiscriminate usage of these drugs continues in veterinary practises in the study regions.

The genomic DNA was extracted from each bacterial isolate and used as a template for the PCR assay. PCR primers targeting the uspA gene of *E. coli* amplified 884 bp fragments (Fig. 2) of DNA. Among the 19 *E. coli* positive samples (conventional method), 12 samples were found positive by PCR. The PCR results of the current study were consistent with the previous study that amplified the uspA gene of *E. coli* from clinical and subclinical mastitis (Mishra *et al.,* 2018).

Fig. 1: Antibiogram results of the *E. coli* isolates.

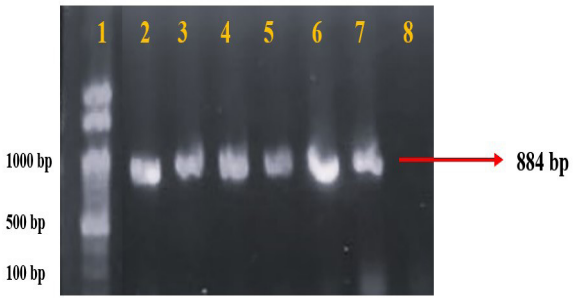

Fig. 2: PCR Amplification of uspA gene of *E. coli*
From left, lane 1: 200 bp marker and lanes 2-8: PCR product uspA gene- 884 bp

Conclusion

In the present study, ciprofloxacin was the most sensitive drug in the Puducherry region for therapeutic use in clinical and subclinical mastitis, followed by gentamicin and ceftriaxone. The highest resistance was observed against penicillin and nalixidic acid. Further, the results need to be warranted by large sample size including the minimum inhibitory concentration (MIC) and characterization of the resistance genes of the antibiotics specific to the organism need to be assessed in future studies.

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