

## Emergence of Quinolone Resistance in *E. coli*-associated Coliform Mastitis: A Case Study

Hima, K.M., A.Janus\*, J.Alex, P.M.Deepa, R.L.Rathish, Biju P.Habeeb and F.A.Lali

Dept. Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Pookode – 673576, Kerala

\*Email: janusa@kvasu.ac.in

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### ABSTRACT

A four-year-old Holstein Friesian crossbred cow with coliform mastitis showed poor response to enrofloxacin. *Escherichia coli* isolated from milk was multidrug resistant, with an enrofloxacin MIC >1024 µg/mL and QRDR mutations in *gyrA* (Ser83Leu and Asp87Asn) and *parC* (Ser80Ile). Gentamicin therapy resulted in complete recovery.

**Keywords:** Coliform mastitis, Quinolone resistance and mutation

### INTRODUCTION

Coliform mastitis is a severe bovine mammary infection caused by environmental pathogens such as *Escherichia coli* (*E. coli*), *Klebsiella* spp., and *Enterobacter* spp., occurring mainly during early lactation and resulting in systemic illness, reduced milk yield and significant economic losses (Abegewi *et al.*, 2022). Fluoroquinolones are the antibiotics, commonly used in food-producing animals because of their broad-spectrum activity, favorable pharmacokinetic properties and high tissue penetration. However, the emergence of quinolone-resistant *E. coli* has increasingly compromised therapeutic efficacy, leading to treatment failures and prolonged disease courses. The present case report aims to evaluate quinolone resistance in an *E. coli* isolate recovered from a case of coliform mastitis with poor clinical response to enrofloxacin therapy. The study correlates clinical history, antimicrobial susceptibility testing, minimum inhibitory concentration (MIC) determination, and molecular analysis of the mechanism causing quinolone resistance.

### CASE HISTORY AND OBSERVATIONS

A four-year-old Holstein Friesian crossbred cow was presented to the Teaching Veterinary Clinical Complex, Pookode, Wayanad, with a complaint of mastitis, which was unresponsive to treatment. The animal was in her third lactation and calved five days ago. The animal was treated with an injection of enrofloxacin for the last three days. Clinical examination revealed that the animal was anorectic and febrile, with reduced rumen motility (one to two contractions per four minutes), while the heart rate and respiratory rate remained within normal limits. Milk from the udder was straw coloured. Palpation of the udder revealed hardness and pain in the left fore quarter.

The milk sample was collected aseptically and streaked on Brain Heart Infusion Agar (BHIA) and incubated the plates at 37 ± 0.5°C for 24 hours. The isolate was identified based on colony morphology, Gram staining and cultural characteristics. The isolate was confirmed by biochemical reactions (Quinn *et al.*, 2013) and polymerase chain reaction (PCR) (Bej *et al.*, 1991) (Figure 1). Phenotypic antibiotic sensitivity/resistance of the isolate was identified by using the Kirby-Bauer disc diffusion method and MIC determination by broth microdilution assay as per the Clinical and Laboratory Standards Institute guidelines (CLSI, 2023). The isolate was resistant to enrofloxacin, ciprofloxacin, norfloxacin, levofloxacin, nalidixic acid, amoxicillin-clavulanate, cefoperazone sodium, ceftriaxone-tazobactam, cefpodoxime, tetracycline and sulphadiazine-trimethoprim and was sensitive to gentamicin and amikacin. The MIC of enrofloxacin was greater than 1024

µg/mL, and the MIC of gentamicin was also determined to guide effective therapy and was found to be 0.0625 µg/mL. Genotypic profiling of quinolone resistance was assessed by PCR amplification of the quinolone resistance-determining regions (QRDRs) of the *gyrA* (Fig. 2) and *parC* genes (Figure 3) (Balakrishnan et al., 2018). The *gyrA* and *parC* genes were

sequenced (GeneSpec Labs, Angamaly), and the consensus *gyrA* and *parC* sequences were aligned with reference *E. coli* K-12 substr. MG1655 sequences from GenBank using ClustalW in MEGA 12, translated into amino acids, and analysed to identify QRDR point mutations, such as Ser83Leu and Asp87Asn in *gyrA* (Figure 4) and Ser80Ile in *parC* (Figure 5).

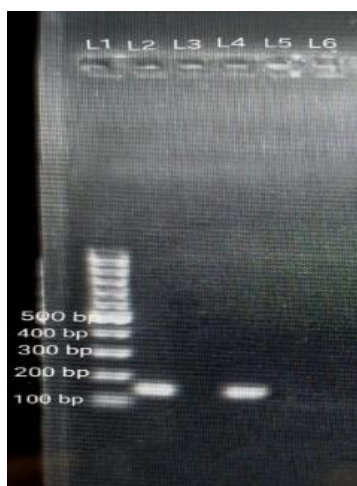
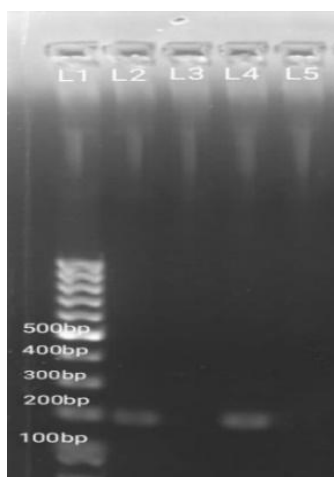


Figure 1: Agarose gel electrophoresis of *uidA* gene-specific PCR of *E. coli*; L1: DNA marker 100 bp, L2: Positive control, L3: Negative control, L4: Sample (147 bp)



Figures 2 and 3: Agarose gel electrophoresis of PCR-amplified *gyrA* and *parC* QRDRs from *E. coli*, L1: DNA marker 100 bp, L2 and L3: Positive and negative controls and L4: Sample

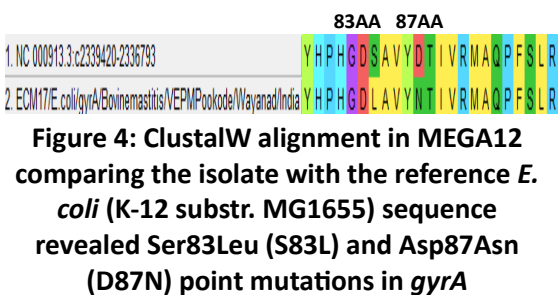
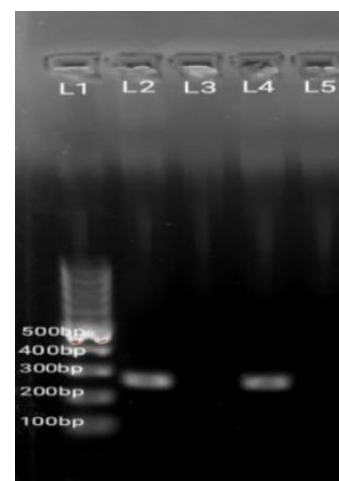


Figure 4: ClustalW alignment in MEGA12 comparing the isolate with the reference *E. coli* (K-12 substr. MG1655) sequence revealed Ser83Leu (S83L) and Asp87Asn (D87N) point mutations in *gyrA*



Figure 5: ClustalW alignment in MEGA12 comparing the isolate with the reference *E. coli* (K-12 substr. MG1655) sequence revealed Ser80Ile (S80I) point mutation in *parC*

## TREATMENT AND DISCUSSION

The animal had been previously treated with enrofloxacin without clinical improvement. Broth microdilution revealed an MIC above the therapeutic range, confirming the treatment failure. Based on susceptibility results, therapy was switched to gentamicin with flunixin meglumine and supportive fluids, resulting in complete clinical recovery within five days. The animal was treated with

antibiotic Gentamicin @ 4 mg/Kg OD based on the antibiotic sensitivity test, Flunixin meglumine @ 2.2 mg/Kg OD and fluid therapy for five days. The animal showed recovery after five days of treatment. Quinolone antimicrobial resistance poses a significant global challenge, as bacterial sensitivity to these agents has diminished (Urban-Chmiel et al., 2022). This resistance predominantly develops through target, plasmid and chromosome-mediated mechanisms, notably

involving QRDR mutations in the *gyrA* and *parC* genes that disrupt drug–enzyme binding and elevate MIC values (Balakrishnan *et al.*, 2016). In *E. coli*, amino acid substitutions at *gyrA* (Ser83Leu and Asp87Asn) and *parC* (Ser80Ile) are reported (Balakrishnan *et al.*, 2016). In this study, gentamicin and amikacin were effective. The *E. coli* isolate from bovine mastitis exhibited multidrug resistance to four antimicrobial classes, which is in agreement with Janus *et al.* (2024).

### SUMMARY

This report documents coliform mastitis caused by *E. coli* with poor response to enrofloxacin, where phenotypic testing and MIC determination confirmed quinolone resistance and molecular analysis identified QRDR mutations in *gyrA* (Ser83Leu, Asp87Asn) and *parC* (Ser80Ile), highlighting the value of combined phenotypic and genotypic approaches for effective antimicrobial selection in bovine mastitis.

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