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# Molecular Detection of Virulence Genes of *E. coli* in Urinary Tract Infections in Dogs

**Sandeep Siwach, Ruchi Tiwari*, Ajay Pratap Singh, Rashmi Singh, Ambika Arun, Ranjana Singh, Meenakshi Singh**

Department of Veterinary Microbiology, DUVASU Mathura
*Email: ruchi.vet@gmail.com

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**ABSTRACT**

Urinary tract infections (UTIs) are the most common bacterial infections in dogs, with *E. coli* being the predominant etiological agent. In this study, 18 *E. coli* isolates obtained from canine UTI cases were investigated for the presence of selected virulence-associated genes and their antimicrobial resistance profile. Standard bacteriological methods confirmed the isolates, and polymerase chain reaction (PCR) was used to detect the fimH, hlyA, and papC virulence genes. Antibiotic susceptibility test was used using the disc diffusion method. Molecular analysis revealed the presence of *fimH* in the majority of isolates**;** *papC and hlyA* were detected in a few isolates, indicating varying virulence potentials among the strains. Antibiogram profiling indicated a high level of resistance to β-lactam antibiotics, cephalosporins, and aminoglycosides**,** highlighting the emergence of multidrug-resistant *E. coli* in canine UTIs. The findings emphasized the role of virulence genes in the pathogenesis of canine UTIs and underlined the need for routine molecular surveillance and judicious antimicrobial use in veterinary care.

**Keywords**: *E. coli*, urinary tract infection, virulence genes, antibiotic resistance

## INTRODUCTION

*Escherichia coli* is the most predominant etiological agent of urinary tract infections (UTIs) in animals, accounting for the majority of community-acquired and hospital-associated infections. Uropathogenic *E. coli* (UPEC) strains possess a diverse array of virulence factors that enable colonization of the urinary tract, evasion of host immune defences, and persistence within the host. Among these, adhesins and toxins play a pivotal role in the pathogenesis of UTIs. The type 1 fimbrial adhesin (fimH) mediates bacterial attachment to urothelial cells, facilitating colonization of the bladder and initiation of infection, which causes cystitis. The pyelonephritis-associated pili (papC) encode P-fimbriae, which are strongly associated with ascending infections and renal involvement, contributing to inflammation and recurrent UTIs. Additionally, α-hemolysin (hlyA) is a pore-forming cytotoxin that damages the host epithelial cells, induces inflammatory responses, and modulates immune signalling, thereby enhancing bacterial survival and tissue invasion. The presence and combination of these virulence genes are considered as the key markers of UPEC pathogenicity and are closely linked with the disease severity, recurrence, and antimicrobial resistance patterns. Therefore, molecular detection of virulence genes such as fimH, papC, and hlyA would provide valuable insights into the pathogenic potential of *E. coli* strains involved in UTIs and aid in understanding their epidemiology and clinical significance (Johnson, 1991; Mulvey *et al.*, 2001; Bien *et al.*, 2012).

## MATERIALS AND METHODS

**Bacterial Isolates:** A total of 18 *E. coli* isolates obtained from the canine urinary tract infected cases were collected from the Veterinary Clinical Complex (VCC), Mathura,

and the nearby private veterinary clinics. The isolates were subjected to cultural and biochemical characterization and confirmed as *E. coli* using standard methods. Genomic DNA was extracted using a commercial kit (Qiagen) as per the manufacturer's instructions. The confirmed isolates were preserved as glycerol stocks (20% glycerol) at -80 °C until further use.

**Antibiotic Susceptibility Testing:** Antimicrobial susceptibility of the confirmed *E. coli* isolates was determined by the Kirby–Bauer disc diffusion method on Mueller-Hinton agar, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2022). The isolates were tested against a panel of common antimicrobial agents, including amikacin (AK), gentamicin (GEN), norfloxacin (NX), penicillin-G (P), ciprofloxacin (CIP), chloramphenicol (C), doxycycline (DO), vancomycin (VA), ceftriaxone (CTR), cefotaxime (CTX), cotrimoxazole (COT), amoxicillin–clavulanic acid (AMC), azithromycin (AZM), cefepime (CPM), clindamycin (CD), ampicillin (AMP), cloxacillin (COX), imipenem (IPM), streptomycin (HLS), cefuroxime (CFX), nalidixic acid (NA), nitrofurantoin (NIT), and enrofloxacin (EN). Interpretation of inhibition zone diameters was performed as per CLSI breakpoints.

## RESULTS AND DISCUSSION

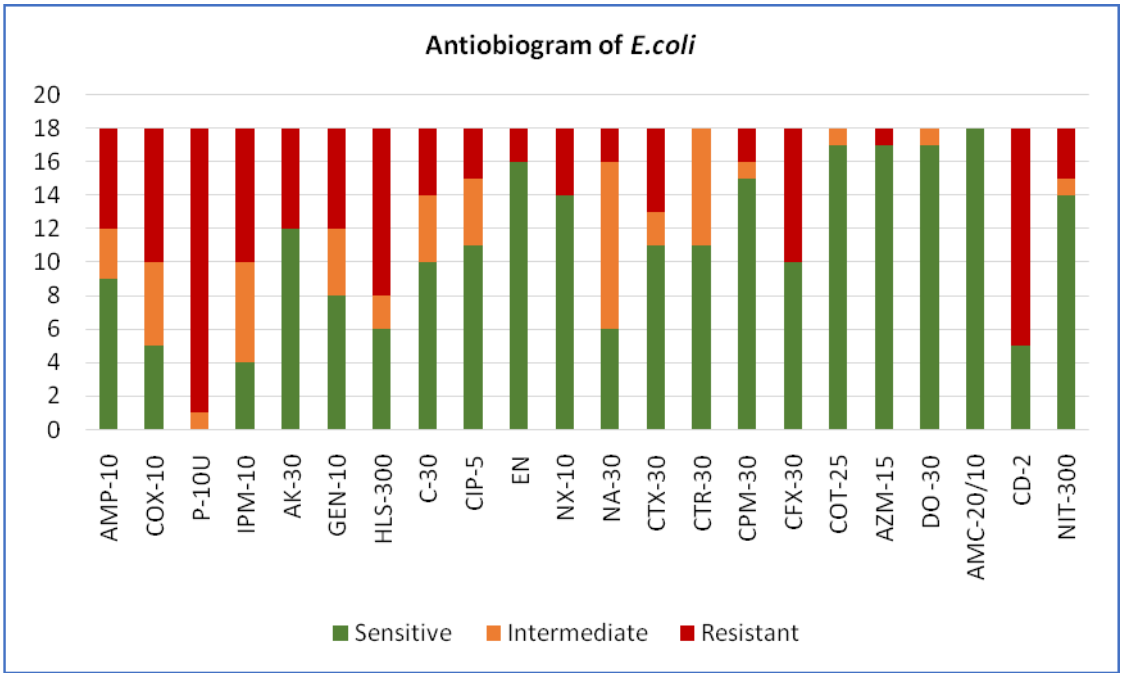
This study attempted to demonstrate the high prevalence of virulence-associated genes among *E. coli* isolates from canine urinary tract infections, indicating the predominance of uropathogenic *E. coli* (UPEC) strains in the study population. Molecular analysis revealed the presence of the fimH gene (508 bp) in all isolates (100%), highlighting its critical role in the initial colonization of the urinary tract (Figure 1). The universal detection of fimH underscores its importance in mediating bacterial adhesion to urothelial cells and facilitating bladder persistence, making it a key determinant in the pathogenesis of canine UTIs. The hlyA gene (172 bp), encoding α-hemolysin, was detected in 72.22% of the isolates. This toxin is known to induce host cell damage, trigger inflammatory responses, and promote tissue invasion, thereby contributing to disease severity. The relatively high occurrence of hlyA suggested its involvement in exacerbating clinical manifestations of UTIs. Similarly, the papC gene (308 bp), responsible for the expression of P-fimbriae and commonly associated with ascending infections and pyelonephritis, was detected in 83.33% of isolates, indicating a strong association with recurrent and complicated UTIs.

The co-occurrence of multiple virulence genes further highlighted the pathogenic potential of the isolates. The simultaneous presence of fimH and papC in 83.33% of isolates suggested enhanced adhesive and invasive capacity, facilitating persistence and recurrence of infection. Notably, the combined presence of fimH, hlyA, and papC in 72.22% of isolates indicated a synergistic effect of adhesins and toxins, which might contribute to increased tissue damage, immune evasion, and chronicity of infection. Such co-expression of virulence factors has been associated with more severe clinical outcomes and treatment failure in UTIs. The prevalence observed in this study was higher than that reported by Tiba *et al.* (2008), who documented lower frequencies of these virulence genes, and Sriram and Gopinath (2016), who reported only 60% positivity for hlyA, suggesting possible regional or host-specific variations in virulence gene distribution.

Antibiotic susceptibility testing revealed that nitrofurantoin exhibited the highest sensitivity (77.77%), supporting its continued usefulness in the treatment of canine UTIs. In contrast, clindamycin showed minimal

effectiveness, reflecting intrinsic or acquired resistance among UPEC strains. While amoxicillin–clavulanic acid and cotrimoxazole demonstrated good activity against several isolates, notable resistance was observed against methicillin and imipenem, raising concerns regarding the emergence of multidrug-resistant strains. These findings partially aligned with Villarroel *et al.* (2002), who reported sensitivity of canine UPEC isolates to fluoroquinolones, gentamicin, and ampicillin; however, reduced ampicillin susceptibility in this study indicated evolving resistance patterns. Likewise, the high susceptibility to fluoroquinolones observed in this study is consistent with Dey et al. (2016), who reported 85.70% sensitivity among canine UTI isolates from Kolkata.

**Figure 1: Results of the Antibiotic Susceptibility Testing**




***Antibiogram profiling of E. coli isolates: sensitive (green), intermediate (yellow), resistant (red) percentage of isolates***

Overall, the high occurrence and co-occurrence of virulence genes, coupled with emerging antimicrobial resistance, emphasized the clinical importance of molecular characterization of *E. coli* in canine UTIs, besides the need for routine virulence profiling and antimicrobial susceptibility testing to guide effective therapeutic strategies and mitigate recurrent or complicated infections.

## SUMMARY

The present study revealed a high prevalence of virulence-associated genes among *E. coli* isolates from canine UTIs, with universal detection of fimH and frequent occurrence of papC and hlyA genes. To summarize, the molecular analysis of 18 isolates revealed the presence of the *fimH* gene in all isolates (100%) with an amplicon size of 508 bp. The *hlyA* gene (172 bp) was detected in 72.22% of isolates, while the *papC* gene (308 bp) was present in 83.33%. Co-occurrence of *fimH* and *papC* was observed in 83.33% of isolates, while *fimH*, *hlyA*, and *papC* were detected together in 72.22% of isolates. Co-occurrence of multiple virulence genes indicated enhanced pathogenic potential and possible association with recurrent and severe infections. Antibiotic susceptibility testing

showed a higher sensitivity to nitrofurantoin and fluoroquinolones, while notable resistance was observed against several commonly used antimicrobials. These findings emphasized the clinical significance of molecular virulence profiling and antimicrobial susceptibility testing for effective management of canine UTIs.

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