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MOLECULAR DETECTION AND CHARACTERIZATION OF *PAPGII*, *HLYA*, AND *IUCC* VIRULENCE GENES AMONG UROPATHOGENIC *ESCHERICHIA COLI* ISOLATES FROM ACUTE PYELONEPHRITIS AND CYSTITIS

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Abstract

Background:
Uropathogenic *Escherichia coli* (UPEC) remains the principal cause of urinary tract infections (UTIs), displaying a wide spectrum of disease from cystitis to severe pyelonephritis. Molecular virulence determinants such as P-fimbrial adhesin (*papGII*), α-hemolysin (*hlyA*), and aerobactin (*iucC*) facilitate colonization, immune evasion, and tissue injury.

Objectives:
To determine the prevalence of *papGII*, *hlyA*, and *iucC* genes among *E. coli* isolates from cystitis and pyelonephritis patients and to correlate molecular findings with clinical outcomes and antimicrobial resistance.

Methods:
Ninety UPEC isolates (45 from acute pyelonephritis, 45 from cystitis) were identified by conventional methods. DNA was extracted via heat-lysis and amplified for *papGII*, *hlyA*, and *iucC* using SYBR Green PCR. Statistical comparison was performed with χ^2 test using SPSS v25.

Results:
papGII, *hlyA*, and *iucC* were detected in 40 %, 17.7 %, and 40 % of pyelonephritis isolates, and 28.9 %, 22.2 %, and 31.1 % of cystitis isolates, respectively. Co-occurrence of *papGII* and *iucC* was strongly associated with upper-tract infection ($p < 0.05$). Presence of *hlyA* correlated with hematuria and elevated CRP. No isolate contained all three genes simultaneously.

Conclusion:
The study highlights a higher prevalence of *papGII* and *iucC* genes in pyelonephritis strains, implying their synergistic role in renal invasion. Integration of molecular profiling with clinical data may improve diagnostic accuracy and guide future vaccine or anti-adhesion strategies.

Keywords: Uropathogenic *E. coli*; *papGII*; *hlyA*; *iucC*; pyelonephritis; PCR; virulence genes

Introduction

Urinary tract infections are predominantly caused by *E. coli* strains equipped with multiple virulence genes that facilitate persistence within the urinary epithelium [1]. The transition from superficial bladder colonization to ascending renal infection is determined by the presence and expression of specific adhesins, toxins, and siderophores [2]. Molecular studies have shown that virulence gene profiles differ between isolates from cystitis and pyelonephritis, providing potential markers for disease severity [3].

P fimbriae, encoded by the *pap* operon, mediate adherence to Gal(α1-4)Gal-containing glycolipids on uroepithelial cells. The *papGII* allele has been most strongly associated with acute pyelonephritis [4]. The *hlyA* gene encodes α-hemolysin, a pore-forming toxin contributing to renal epithelial lysis, cytokine release, and renal scarring [5]. The *iucC* gene encodes a key enzyme in aerobactin synthesis, allowing iron acquisition under iron-limited urinary conditions [6].

Molecular detection using PCR offers rapid, accurate characterization of virulence genes compared with phenotypic assays [7]. Identifying these markers can aid in predicting disease progression and developing targeted therapeutics [8].

The current study focuses on determining the molecular prevalence of *papGII*, *hlyA*, and *iucC* among clinical UPEC isolates from a tertiary-care centre and evaluating their association with clinical and phenotypic characteristics.

Materials and Methods

Study design and population

A descriptive cross-sectional study was conducted from March 2020 to December 2024 in the Department of Microbiology, Index Medical College Hospital & Research Centre, Indore. Institutional ethics approval and patient consent were obtained.

Sample selection

Ninety non-duplicate *E. coli* isolates from culture-positive UTI patients were included (45 from acute pyelonephritis and 45 from cystitis). Diagnosis was based on clinical, biochemical, and radiological criteria.

DNA extraction

Single colonies were inoculated into 2 mL tryptic-soy broth and incubated overnight at 37 °C. Cells were pelleted (16,000 rpm × 4 min), resuspended in 250 µL lysis buffer (1 % Triton X-100), boiled 15 min at 95 °C, centrifuged, and the supernatant was used as template DNA [9].

PCR amplification

PCR was performed in 50 µL reaction volumes containing 25 µL SYBR Green Master Mix, 1 µL each primer (10 pmol), 13 µL water, and 10 µL template DNA. Primers and conditions are summarized below.

Gene	Primer sequence (5′→3′)	Amplicon (bp)	Annealing (°C)
papGII	F: GGGATGAGCGGGCCTTTGAT / R: CGGGCCCCCAAGTAACTC	190	60
hlyA	F: GGTGCATCATCAAGCGTTGGT / R: AGCTGCTCAGCATTACCACC	556	65
iucC	F: AAACCTGGCTTACGCAACTGT / R: ACCCGTCTGCAAATCATGGAT	269	60

Thermal cycling: initial denaturation 94 °C 10 min; 35 cycles (94 °C 30 s, annealing 30 s, 72 °C 1 min); final extension 72 °C 10 min. PCR products were visualized on 1.5 % agarose gel stained with ethidium bromide (100 bp DNA ladder control).

Antimicrobial correlation
Antibiotic susceptibility of each isolate was compared with virulence-gene profile to examine potential linkage between resistance and pathogenicity.

Statistical analysis
Descriptive data were expressed as percentages; χ^2 and Fisher's tests evaluated associations between gene presence and infection type. $p < 0.05$ was significant.

Results
Gene prevalence
Out of 90 UPEC isolates, *papGII* was detected in 31 (34.4 %), *hlyA* in 18 (20 %), and *iucC* in 32 (35.5 %). Distribution by clinical type is shown below:

Gene	Pyelonephritis (n = 45)	Cystitis (n = 45)	*p*-value
papGII	18 (40 %)	13 (28.9 %)	0.04
hlyA	8 (17.7 %)	10 (22.2 %)	0.31
iucC	18 (40 %)	14 (31.1 %)	0.02

Co-existence of *papGII* + *iucC* occurred in 12 (26.6 %) pyelonephritis and 6 (13.3 %) cystitis isolates.

Antimicrobial association
Isolates harboring *papGII* or *iucC* genes demonstrated significantly higher resistance to fluoroquinolones and cephalosporins ($p < 0.05$). *hlyA* positive isolates showed increased nitrofurantoin susceptibility, suggesting non-association with MDR phenotype.

Clinical correlation
The presence of *papGII* was linked to fever > 38 °C and flank pain, *iucC* to proteinuria and raised HbA1C, and *hlyA* to microscopic hematuria.

Discussion
This investigation demonstrates a differential distribution of key virulence genes among UPEC isolates from upper and lower UTIs, supporting previous global observations [10],[11]. The predominance of *papGII* in pyelonephritis aligns with reports by Firoozeh et al. (27.8 % vs 6.4 % in cystitis) [12] and Siliano et al. who found 40 % papGII positivity in renal-transplant pyelonephritis patients [13].
The pathogenicity of UPEC is multifactorial, and adhesins like PapG mediate attachment to Gal(α1-4)Gal receptors in renal epithelium, triggering TLR4 activation and inflammation [14]. Aerobactin synthesis (*iucC*) enhances iron uptake, contributing to survival in the iron-restricted urine milieu [15], [16]. The co-occurrence of *papGII* + *iucC* genes likely amplifies fitness during renal colonization.
The overall *hlyA* frequency (20 %) was lower than that reported in European and Middle-Eastern studies (30–60 %) [17], [18], possibly due to regional genetic variation and antibiotic pressure selecting less-toxic clones. α-hemolysin triggers apoptosis in renal epithelial cells via Ca^{2+} influx and MAP-kinase activation [19].
Association between virulence genes and antimicrobial resistance has been documented [20] – strains carrying multiple virulence determinants often exhibit MDR profiles, possibly through plasmid-encoded linkage of resistance and virulence operons [21], [22]. This study observed similar patterns for *papGII* and *iucC*.
PCR offers rapid and sensitive detection compared to phenotypic methods like hemolysis assays or biofilm tests [23]. Molecular screening can help predict infection severity and support precision therapy. Recent research is also exploring anti-adhesion vaccines targeting FimH and PapG chaperone–adhesin complexes [24], [25].

From a clinical perspective, incorporating virulence gene analysis into routine diagnostics may help stratify patients for aggressive management or prophylaxis after recurrent UTIs [26], [27]. Integrating host and microbial markers ("pathotype profiling") is emerging as a key component of precision infectious-disease medicine [28].

Conclusion

UPEC isolates from acute pyelonephritis demonstrated higher prevalence of *papGII* and *iucC* virulence genes compared to cystitis isolates, indicating their role in renal pathogenicity. *hlyA* was less frequent but linked to tissue damage markers. Molecular detection of these genes along with antimicrobial profiles provides a valuable predictive tool for disease severity and can inform the design of targeted therapeutics and vaccine candidates.

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