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Molecular Insights into Virulence and Resistance in *Escherichia coli* and *Klebsiella pneumoniae*:The Dual Menace of Uropathogens.

Shanmathy K. R., Madhumala Shanmugasundaram, Muthu Lakshmi Backiasubramanian, Shanthi Mariappan, Uma Sekar
Department of Microbiology, Sri Ramachandra Institute of Higher Education and Research, Chennai, India.

Email: shanthi.m@sriramachandra.edu.in

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ABSTRACT

Background: *Escherichia coli and Klebsiella pneumoniae* are the most common uropathogenic Enterobacterales causing community and hospital acquired urinary tract infection. The knowledge of virulence factors of *E.coli* and *K.pneumoniae* and their antibiotic susceptibility pattern will help in better understanding of the treatment of UTI. **Materials And Methods:** A total of 209 clinically significant, non - repetitive, consecutive *E. coli(*143 isolates*)* and *Klebsiella pneumoniae*(66 isolates*)* isolated from urine was included in this study. Polymerise chain reaction was performed to detect the presence of virulence genes such as *fimH*, *iutA*, and *sat* of *Escherichia coli*, and *fimH*, *entB*, and *uge* of *Klebsiella pneumoniae* as well as carbapenemase-encoding genes such as *blaNDM* and *blaOXA-48*. Antibiotic susceptibility testing was done by disc diffusion technique according to CLSI. Univariate analysis was done to find the association between virulence genes and mortality. **Results:** Among *E.coli* isolates,90 carried one or more of the virulence genes, most commonly *fimH*(33.5%) followed by *iutA*(32.8%) and *sat* gene(10.4%). In the study, *iutA* showed statistically significant association with ESBL producing *E.coli* isolate Among *K.pneumoniae* isolates, 39 carried one or more of the virulence genes,most commonly *fimH gene*(50%) followed by *entB* (37.8%) and *uge* gene(3.02%). In this study, *entB* showed statistically significant association with Carbapenem resistant *K.pneumoniae* isolates. **Conclusion:** The study highlights a high prevalence of antimicrobial resistance in *E. coli* and *K.pneumoniae* urinary isolates, especially to commonly used antibiotics such as ampicillin, cefotaxime, and ciprofloxacin. The presence of multiple virulence traits contribute to more severe or persistent infections. A significant association was found between *iutA* and ESBL production in *E. coli*, and between *entB* and carbapenem resistance in *K. pneumoniae*. The presence of *sat* gene in *E. coli* and *uge* gene in *K. pneumoniae* was associated with higher mortality, indicating their potential role in disease severity.

INTRODUCTION:

Urinary tract infections (UTIs) are one of the most frequently encountered bacterial infections in both community and hospital settings, affecting individuals across all age groups. They represent a significant burden on healthcare systems worldwide, contributing to increased morbidity, hospital admissions, and antibiotic usage.[1] The most common causative agents of UTIs are Gram-

Journal of Molecular Science
Volume 35 Issue 2, Year of Publication 2025, Page 246-254
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negative bacilli, with *Escherichia coli* and *Klebsiella pneumoniae* being the predominant uropathogens implicated in both uncomplicated and complicated infections.[2]

Urinary tract infections (UTIs) are one of the common bacterial illnesses that affect humans. According to the location of the infection, UTIs are categorized generally as pyelonephritis (the kidney) and cystitis (the bladder). Uropathogenic Escherichia coli (UPEC) is the most common cause of UTI.UPEC harbours specialized virulence genes that enable it to colonize the urinary tract and cause disease. These include Adhesins (type 1fimbriae (*fimH*), Pfimbriae(*pap*), Toxins (hemolysin(*hlyA*), cytotoxic necrotizingfactor1(*cnf1*), siderophores(*iutA*,*fyuA*), capsules and lipopolysaccharides for immune evasion. These virulence determinants enable UPEC to adhere to uroepithelial cells, evade host defenses, cause tissue injury, and establish infection in both the lower and upper urinary tract.[3]

Klebsiella pneumoniae possesses a variety of virulence factors such as Polysaccharide capsule (especially K1 and K2 serotypes), Fimbriae (especially type 1 fimbriae encoded by *fimH*), Lipopolysaccharides (LPS encoded by *uge*), Siderophores for iron acquisition. Hypervirulent strains, often associated with the Mucoviscosity-associated gene A(magA) and K2 serotype(*k2A)* genes, have been implicated in severe infections such as liver abscess and meningitis,urinary tract infections. [4]

Capsular polysaccharides that are referred to as K antigens, they are important in the defense of phagocytosis of the organism through capsules. Among the 77 capsular serotypes identified, K1 and K2 most commonly are associated with severe infections in humans. [4]

The K1 capsular serotype of Klebsiella pneumoniae is specifically associated with the *magA* whereas the *k2A* is specifically associated with the capsule-associated gene A (k2A). K1 and K2 serotypes are also usually associated with liver abscesses Lipopolysaccharides (LPS) are important in the protection of bacteria against lysis by complements.They are synthesized under control of the uge gene which encodes uridine diphosphate galacturonate 4-epimerase. Strains of Klebsiella pneumoniae that have lost this gene are less capable of causing urinary, pulmonary infections. *FimH*1 gene encodes components of fimbriae which mediate bacterial adhesion. Most of the Klebsiella pneumoniae strains (90 percent) contain the type 1 fimbriae that allow the bacteria to attach to all kinds of epithelial cells with an especially good attachment to bladder epithelial cells 4).[4]

The emergence of multidrug-resistant (MDR) and carbapenem-resistant Enterobacteriaceae (CRE) significantly challenges treatment. Resistance is mediated by carbapenemase genes such as: *bla*KPC (Klebsiella pneumoniae carbapenemase), *bla*NDM(NewDelhi metallo-β-lactamase), *bla*VIM, *bla*IMP and *blaOXA-48* like enzymes.5 These genes confer resistance to most β-lactam antibiotics, including penicillins, cephalosporins, and carbapenems, and are often plasmid-mediated, facilitating rapid dissemination.[6]

The presence of virulence factors and antimicrobial resistance genes in *Escherichia coli* and *Klebsiella pneumoniae* poses a significant clinical threat, particularly in urinary tract infections. Molecular characterization of these determinants is essential to understand the underlying pathogenic mechanisms and guide targeted therapeutic strategies. Identifying resistance genes is also crucial for selecting effective antimicrobial therapy and implementing appropriate infection control measures. This study was undertaken to characterize the virulence and carbapenem resistance genes in *E. coli* and *K. pneumoniae* isolated from UTI cases in a tertiary care university teaching hospital.

MATERIALS AND METHODS:

The study was conducted in a 1600-bedded university teaching hospital from June 2023 to May2024.The study included 209 clinically significant,consecutive,Non repetitive isolates of *Escherichia coli* and *Klebsiella penumoniae*,collected over a period of one year from June 2023 to May 2024.Species identification utilized conventional biochemical methods/VITEK®MS MALDI-TOF(Biomerieux). The significance of the isolates was based on clinical history, presence of the organism in the Gram stain and pure growth in culture with a significant colony count.

Antimicrobial susceptibility testing:

Susceptibility to various classes of antibiotics was determined by the disc diffusion method in accordance with clinical laboratory standard institute(CLSI 2023) guidelines. The antibiotics tested were Amikacin(30 mcg),Gentamicin(10mcg),Fosfomycin(200mcg),Ampicillin(10mcg), Cefotaxime (30mcg), Ciprofloxacin (5 mcg), Cotrimoxazole (1.25/23.75), Nitrofurantoin (300 mcg), Piperacillin tazobactam (100/10), Imipenem(10mcg). The antibiotic discs were procured from Himedia Laboratories (Mumbai, Maharashtra, India)

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 2, Year of Publication 2025, Page 246-254
DoI-17.4687/1000-9035.2025.034

DNA extraction:
The DNA of the collected isolates were extracted using boiling and lysis procedure. The suspension of individual colonies in 400µl of TE buffer or distilled water. It was then boiled at 95°C for 10 mins and immediately freezed at -20°C for another 10 minutes, this was then centrifuged at 12000 rpm for 10 minutes. The suspension was collected and the pellet was discarded. 2µl of this suspension was used as template for amplification. The sequence was stored at -20°C for further analysis [7].

Detection of virulence encoding genes
PCR for virulence encoding genes: primers used are given in Table 1.

PCR conditions:
Detection of virulence determinants of *Escherichia coli:*
1.PCR conditions for detection of FimH, Iut A: Initial denaturation at 95°C for 4 minutes, followed by 32 cycles at 94°C for 30 seconds, annealing temperature at 63°C for 30 seconds, extension at 72 °C for 50 seconds, and a final extension for one cycle at 72°C for 5 minutes.

2. PCR conditions for detection of Sat: Initial denaturation at 95°C for 4 minutes, followed by 32 cycles at 94°C for 30 seconds, annealing temperature at 55°C for 30 seconds, extension at 72 °C for 50 seconds, and a final extension for one cycle at 72°C for 5 minutes.

Detection of virulence determinants of *Klebsiella pneumoniae:*
1.PCR conditions for detection of FimH: Initial denaturation at 94°C for 4 minutes, followed by 30 cycles at 94°C for 30 seconds, annealing temperature at 55°C for 40 seconds, extension at 72 °C for 60 seconds, and a final extension for one cycle at 72°C for 10 minutes.

2.PCR conditions for detection of Uge: Initial denaturation at 94°C for 5 minutes, followed by 30 cycles at 94°C for 60 seconds, annealing temperature at 54°C for 45 seconds, extension at 72 °C for 60 seconds, and a final extension for one cycle at 72°C for 7 minutes.

3.PCR conditions for detection of EntB: Initial denaturation at 94°C for 5 minutes, followed by 30 cycles at 94°C for 60 seconds, annealing temperature at 54°C for 45 seconds, extension at 72 °C for 60 seconds, and a final extension for one cycle at 72°C for 7 minutes

Table 1: Primers used for virulence genes of *Escherichia coli and Klebsiella pneumoniae.*

Virulence genes	Primers (5'- 3')	Amplicon Size (Base pair)	References
Virulence genes of *Escherichia coli*			
fimH	F: TGCAGAACGGATAAGCCGTGG R: GCAGTCACCTGCCCTCCGGTA	508	3
iutA	F: GGCTGGACATCATGGGAACTGG R: CGTCGGGAACGGGTAGAATCG	302	
Sat	F: ACTGGCGGACTCATGCTGT R: AACCCTGTAAGAAGACTGAGC	387	
Virulence genes of *Klebsiella pneumoniae*			
fimH	F: ATGAACGCCTGGTCCTTTGC R: GCTGAACGCCTATCCCCTGC	688	8,9
EntB	F: GTCAACTGGGCCTTTGAGCCGTC R: TATGGGCGTAAACGCCGGTGAT	400	
uge	F: TCTTCACGCCTTCCTTCACT R: GATCATCCGGTCTCCCTGTA	534	

Detection of genes encoding for Carbapenem resistance:
PCR for metallo-beta-lactamase genes: *bla*NDM**,** *bla*OXA-48 primers used are given in Table 2.

NDM: Initial denaturation at 94°C for 10 minutes, followed by 36 cycles at 94°C for 30 seconds, annealing temperature at 52°C for 40 seconds, extension at 72 °C for 50 seconds, and a final extension for one cycle at 72°C for 5 minutes.
Oxa-48: Initial denaturation at 94°C for 10 minutes, followed by 36 cycles at 94°C for 30 seconds, annealing temperature at 52°C for 40 seconds, extension at 72 °C for 50 seconds, and a final extension for one cycle at 72°C for 5 minutes

Table 2: Primers used for Carbapenemase encoding genes

Carbapenemase gene	Primers(5'-3')	Amplicon size	Reference
***bla*NDM**	F: GGTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	621	10
***bla*OXA-48**	F: GCGTGGTTAAGGATGAACAC R: CATCAAGTTCAACCCAACCG	438	

DNA Sequencing:

Journal of Molecular Science
Volume 35 Issue 2, Year of Publication 2025, Page 246-254
DoI-17.4687/1000-9035.2025.034

PCR positive amplicons for each virulence genes were purified and sequenced strains for each virulence gene served as positive controls. Sequencing was done by using Sanger AB 13730 XL DNA analyzing instrument(Immugenixbio, Tamil Nadu, India). Using Bio edit sequence programme(product version 7.0.5.3), the nucleotide sequences were aligned, and they were then compared with basic alignment search tool offered on National Centre for Biotechnology Information website (www.ncbi.nlm.nih.gov). The nucleotide sequences of these virulence genes were submitted to GenBank and the following accession numbers were obtained MZ420493,MZ501821,MZ198898 ,PV940690,PV940691,PV940689.

RESULTS:

This study involved the analysis of 209 isolates of *Escherichia coli* and *Klebsiella pneumoniae*, which were obtained from both outpatient and hospitalized patients. Of these isolates, *E. coli* accounted for 68.5% (143 isolates), while *K. pneumoniae* comprised 31.5% (66 isolates).

A total of 209 isolates were included in this study, of them 38.75% (81/209) were from males and 61.24 (128/209) were from female patient.

Antibiotic susceptibility pattern of Escherichia coli:

The Kirby-Bauer disc diffusion method revealed the resistance pattern as follows: Amikacin 20(13.9%), Gentamicin 32(22.3%), Fosfomycin 13(9%), Ampicillin113(79.02%), Cefotaxime106(74.1%), ciprofloxacin 95(66.4%), cotrimoxazole 86(60.1%),Nitrofurantoin 35(24.4%), Piperacillin- tazobactum 36(25.1%), Imipenem 13(9 %)

Molecular methods:

Gene profile:

PCR analysis revealed that of the 143 *E. coli* included in the study, virulence genes were detected in 90(62.93%) .The most common gene was detected was *fim*H *in* 33.56%, followed by *iut A* gene in 32.86%, *sat* gene in 10.48%

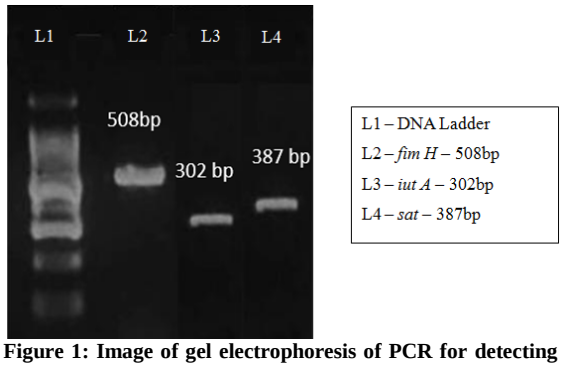

Figure 1: Image of gel electrophoresis of PCR for detecting virulence gene in uropathogenic *E. coli*. Band at 508 bp (L1) represents the presence of fimH gene, band at 302 bp (L2) represents presence of *iutA* gene, band at 387bp (L3) represents presence of *sat* gene

Only a single virulence gene was detected in 49.6%(n=71) isolates. The virulence genes that occurred singly were *fim H*(n=32), *iut A* (n=31), *sat* (n=8) The most common 2 genes combination were: *fimH* and *iutA* (n=13), followed by *fimH* and *sat* (n=4), and *iutA* and *sat* (n= 4).All 3 virulence genes were detected in 1 isolate

Association of Antibiotic resistance and Virulence genes in *E.coli* isolates:

fimH was present in 42.8% of Nitrofurantoin-resistant isolates, 23.1% of Fosfomycin-resistant isolates and 34.7% of Ciprofloxacin-resistant isolates. ***iutA*** appeared in 45.7% of Nitrofurantoin-resistant isolates, 38.5% of Fosfomycin-resistant isolates and 40% of ciprofloxacin-resistant isolates, ***sat*** was seen in 22.8% of Nitrofurantoin-resistant isolates,7.6% of Fosfomycin-resistant isolates and 11.5% of ciprofloxacin-resistant isolates.

Table 3: Association of Antibiotic resistance and Virulence genes in *E.coli* isolates

Antimicrobial agent	No. of isolates resistant	*fimH*	*iutA*	*sat*
Amikacin	20	9	7	1
Fosfomycin	13	3	5	1
Cefotaxime	106	36	40	13
Ciprofloxacin	95	33	38	11
Cotrimoxazole	86	30	31	10
Nitrofurantoin	35	15	16	8
Piperacillin-tazobactum	36	16	11	6
Imipenem	13	7	3	2

Table 4: Distribution of virulence genes among the carbapenem susceptible and resistant isolates of *E.coli*

Virulence gene	No. of isolates	Carbapenem susceptible (130)	%	Carbapenem resistant (13)	%	P value
fimH	48	41	31.53%	7	53.84%	0.1044
iutA	47	44	33.84%	3	23.1%	0.4306
sat	15	13	10%	2	15.38%	0.5458

p- value was calculated with significance level at 0.05. Virulence genes of *E.coli* did not show a significant difference in distribution between susceptible and resistant groups.

Journal of Molecular Science
Volume 35 Issue 2, Year of Publication 2025, Page 246-254
DoI-17.4687/1000-9035.2025.034

Table 5: Distribution of virulence genes among ESBL producers and non ESBL producers of *E.coli* isolates

Virulence gene	No. of isolates	ESBL producers(106)	%	Non ESBL producers (37)	%	P value
fimH	48	36	33.9%	12	32.4%	0.8652
iutA	47	40	37.7%	7	18.9%	0.0359
sat	15	13	12.2%	2	5.4%	0.2411

Only *iutA* showed a statistically significant association with ESBL producers ($p < 0.05$). The other genes did not show a significant difference in distribution between ESBL and Non-ESBL Producers.(Table 5)

Antibiotic susceptibility pattern of *Klebsiella pneumoniae:*

The Kirby–Bauer disc diffusion method revealed the resistance pattern for the antibiotics tested were as follows: Amikacin 29(43.9%), Gentamicin 28(42.4%), Fosfomycin 31(46.9%), Cefotaxime 37(56%), ciprofloxacin 40(65.1%), cotrimoxazole 37(56%), Nitrofurantoin 43(65.1%), Piperacillin-tazobactum 28(42.4%), Imipenem 28(42.4%).

Molecular Methods:

Gene profile:

PCR analysis revealed that of the 66 *K.pneumoniae* included in the study, virulence genes were detected in 59%. Overall, *fim H* gene was detected in 50% followed by *entB* gene in 37.87%,*uge* gene in 3.02%.

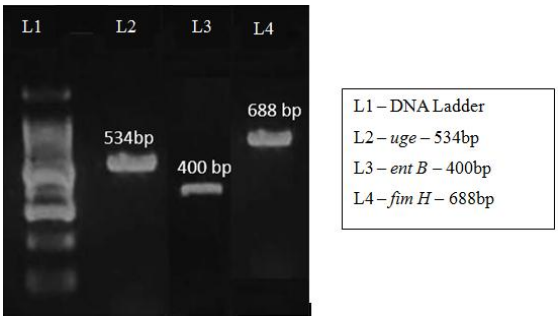

Fig 2:Image of gel electrophoresis of PCR for detecting virulence gene in uropathogenic *K.pneumoniae* Band at 534 bp (T1) represents the presence of *uge* gene, band at 400 bp (T2) represents presence of *entB* gene, band at 688bp (T3) represents presence of *fimH* gene

Only a single virulence gene was detected in 30.3%(n=20) isolates. The virulence genes that occurred singly were *fim H(n=14)*, *ent b (n=6)*, uge did not occur solely but it coexisted with virulence genes like *fimH and ent B.* The most common 2 genes combination were: *fimH* and *entB* (n=19), followed by *fimH* and *uge* (n=2), and *entB* and *uge* (n= 2).All 3 virulence genes were detected in 2 isolate

Association of Antibiotic resistance and Virulence genes of *K.pneumoniae* isolates

fimH was present in 53.5% of Nitrofurantoin-resistant isolates, 54.8% of Fosfomycin-resistant isolates and 57.5% of Ciprofloxacin-resistant isolates. ***entB*** appeared in 48.8% of Nitrofurantoin-resistant isolates, 35.5% of Fosfomycin-resistant isolates and 50% of ciprofloxacin-resistant isolates, ***uge*** was seen in 4.65% of Nitrofurantoin-resistant isolates, 6.6% of Fosfomycin-resistant isolates and 5% of ciprofloxacin-resistant isolates(Table 6)

Table 6: Association of antibiotic resistant and virulence genes of *K.pneumoniae* isolates

Resistant drug	No. of isolates	*fimH*	*entB*	*uge*
Amikacin	29	18	17	2
Fosfomycin	31	17	11	2
Cefotaxime	37	20	18	2
Ciprofloxacin	40	23	20	2
Cotrimoxazole	37	23	19	2
Nitrofurantoin	43	23	21	2
Piperacillin-tazobactam	28	18	18	2
Imipenem	28	17	17	2

Table 7: Distribution of virulence genes among the carbapenem susceptible and resistant isolates of *K.pneumoniae*

Virulence gene	No. of isolates	Carbapenem susceptible (38)	%	Carbapenem resistant (28)	%	P value
fimH	33	16	42.1%	17	60.7%	0.1350
EntB	25	8	21.1%	17	60.7%	0.00103
uge	2	-	-	2	7.1%	-

Presence of *entB* exhibited a statistically significant association with carbapenem resistance ($p<0.05$). The other genes did not show a significant difference in distribution between susceptible and resistant groups.(Table 7)

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 2, Year of Publication 2025, Page 246-254
DoI-17.4687/1000-9035.2025.034

Table 8: Distribution of virulence genes among the ESBL producers and Non ESBL Producers of *K.pneumoniae* isolates

Virulence gene	No. of isolates	ESBL producers (37)	%	Non ESBL producers (29)	%	P value
fimH	37	20	54.1%	17	58.6%	0.7106
EntB	27	18	48.6%	9	31.03%	0.1485
uge	2	-	5.4%	-	6.8%	-

Detection of Carbapenem resistant genes

PCR analysis revealed that of the 41 Carbapenem resistant *E.coli* and *Klebsiella pneumoniae* included in the study, genes encoding the carbapenem resistance (NDM, OXA-48) were detected in 60.97% (26/41) of the isolates. Among 13 Carbapenem resistant *Escherichia coli* isolates 8 (61.5%) carried NDM gene, 2(15.3%) isolates carried OXA-48 gene and 5(38.5%) carried both NDM and OXA-48. Among 28 Carbapenem resistant *Klebsiella pneumoniae* isolates 5 carried both NDM and OXA-48 (17.8%), 9(32.1%) carried NDM gene, and 7(25%) isolate carried OXA-48 genes

DISCUSSION:

This study involved the analysis of 209 isolates of *Escherichia coli* and *Klebsiella pneumoniae*, which were obtained from both outpatient and hospitalized patients. Most of the isolates were from females with the distribution of 61% among females and 39% among males. This is similar to the findings in the study by **Singh et al** with a prevalence of 57 % in females and 46% males. The frequency of occurrence of UTI is more in females due to the presence of short urethra. Similar findings of increased female predilection of UTI were observed in a study by **Shah et al** with a distribution of 53% in Females;47 % in males whereas in a study by **Ruiz et al** there was higher prevalence in male accounting to 64% in males and 36% in females.[11-13]

Virulence Gene Detection:

Genomic DNA was extracted from all UTI *E. coli* subjected to polymerase chain reaction (PCR). PCR analysis revealed that of the 143 *E. coli* included in the study, virulence genes were detected in 62.93% (90/143).53 isolates did not have any genes. In the present study *Fim H* was the most common gene (33.56 %). Overall, *fim H* gene was detected in 33.56% (48/ 143) followed by *iut A* gene in 32.86% (47/143), *sat* gene in 10.48 % (15/143).

Fim H adhesion, which is positioned at the tip of type 1 *fim*briae and mediates attachment to the urothelial cell surface, initiates bacterial invasion and inhibits bacterial washout by urine flow. (14) The presence of the *fimH* gene was confirmed by PCR, and the results showed that the *fim H* gene was present in 33.5% of isolates**.** This demonstrated that the *fimH* gene was present in the majority of the UPEC strains, and the findings of the present study was in agreement with previous studies. According to **Tarchouna et al.,** the *fimH* gene was the most common virulence gene found in the UTI isolates studied. **Garofalo et al** studied 18 UPEC isolates found that the *fimH* gene was the most common virulence factor[15,16].

The attributed characteristic is important for the pathogenesis of urosepsis isolates. According to the current findings, **32.8%** of UPEC carry the *iut* A gene. This finding are in concordance with the studies in which *iutA* prevalence ranging between **30–70%** in UPEC strains(**Miranda et al**) According to the current study, *IutA* is the second most common and is similar to the other relatable studies by **Zhao et al, ki Wook yen et al**.[17-19]

In the literature, the presence of the *Sat* gene was frequently associated with pyelonephritis, corresponding to the virulence potential of isolates carrying it. In this study **10.48%** carry the sat gene which is similar to the study from Malaysia (**Maniam et al**) identified *sat* in only **7.5%** of isolates, which closely resembles the prevalence in our findings.[20]

Virulence Gene Combinations:

Of the 90 isolates that carried the virulence genes, 68(60.71%) carried more than one gene in various combinations. Among the various gene combinations, *Fim H+IutA* is the most common one followed by *FimH+sat, iutA+sat and FimH+ sat+iutA.* **Malekzadegan et al**. noted that their most frequent virulence gene pattern included both *fimH and iutA.*[21]

Antibiotic Resistance profile:

The highest resistance was observed to Ampicillin, with 113 isolates (79%) classified as resistant. Cefotaxime resistance was observed in 74%(106/143) isolates. Fluoroquinolone resistance was also elevated, about 66%(95/143) of isolates were ciprofloxacin-resistant. Co-trimoxazole (trimethoprim-sulfamethoxazole) resistance was noted in 60%(86/143). **Chakraborty et al.** observed similar high resistance rates among UPEC in India, including prevalent resistance to ampicillin, piperacillin-tazobactum and fluoroquinolones.(22)From the finding of this study, nitrofurantoin and imipenem continue to remain susceptible against isolates of *E.coli* that produce ESBL.[23]

Journal of Molecular Science
Volume 35 Issue 2, Year of Publication 2025, Page 246-254
DoI-17.4687/1000-9035.2025.034

The study by **Maryam et al** with a resistance of around 7.8%, aligns with the carbapenem resistance pattern of the present study(9.09%)(24) The antimicrobial susceptibility testing revealed that *E. coli* exhibited high susceptibility to Nitrofurantoin (75.5%, 108/143), Fosfomycin (91%, 130/143). These two agents demonstrated the most effective antimicrobial activity against *E. coli* isolates.

Antibiotic resistance and virulence genes
In this study, iutA showed a statistically significant association with ESBL-producing *E. coli* isolates (p = 0.0359), indicating a possible association between iron acquisition mechanisms and antibiotic resistance. This is in contrary with the previous studies.In this study, among the 143 study isolates, 74.1% were ESBL producers and is found to be a little higher while comparing with other recent studies where the ESBL production was around 51.2% in a study by **Maryam et al.** [24]

Carbapenem resistance and its association with virulence genes was also studied which did not reveal any association with the virulence genes looked up in this study
In a recent study conducted by **Fonseca -Martinez et al** *IutA* gene is commonly associated with Fluroquinolone, penicillin and Multi drug resistance (MDR) which is similar to the present study.[23]

Univariate analysis in this study revealed that only ***sat gene*** (secreted autotransporter toxin) was significantly associated with mortality (**p = 0.010**), with an odds ratio of 6.15.

In the present study *FimH* gene encoding type 1 fimbriae was present in 50% (33/66) of the study isolates. The type 1 fimbrial adhesin contributes to the pathogenesis of urinary tract infections. In *K.pneumoniae*, siderophore *entB* has higher affinity towards iron (20). A total of 37.8%(25/66) of the isolates in this study also harboured the gene *entB*.
In the present study, the *uge* (UDP galacturonate 4-epimerase) gene was detected in 3.02% (2/66) of *Klebsiella pneumoniae* isolates. *uge* did not occur independently but coexisted with other virulence genes like *fimH* and *entB* The study by **Nazari et al**. found *fimH* in 87% and *entB* in 100% of their clinical K. pneumoniae isolates. **Ballén et al**. similarly observed that nearly all isolates carried *entB* (100%) and *fimH* (98%), with *uge* present in 73%. These findings are in contrary with the present study.(25,26)

Virulence gene combinations:
Of the 66 isolates that carried the virulence genes, 39(59%) carried more than one gene in various combinations. Among the various gene combinations, *Fim H+ent b* is the most common one followed by *Fim H+uge, entb+uge and FimH+ entb+ uge*

In this study 28.7% (19/66) of isolates harbored the combination *fimH + entB*, making it the most prevalent virulence gene combination. Additionally, the combinations *fimH + uge*, *entB + uge*, and *fimH + entB + uge* were each observed in 3.3% (2/66) of isolates. A similar combination of ***fimH + entB*** predominance was reported by **Remya et al**.(27) The co-occurrence patterns of virulence genes *(fimH+entB)* are generally consistent with previous findings.

Antibiotic resistance profile:
The highest resistance was observed to cefotaxime with 37/66(56%) classified as resistant. Fluoroquinolone resistance was also elevated about 60.6%(40/66) of isolates were ciprofloxacin-resistant. Co-trimoxazole (trimethoprim-sulfamethoxazole) resistance was noted in 56%(37/66), nitrofurantoin shows 65%(43/66) of resistance, whereas resistance to aminoglycosides was lower (gentamicin-42%, amikacin -43%). These are consistent with the resistance patterns reported by **Soltani et al**., who found ~69% cefotaxime resistance and ~69% resistance to ciprofloxacin, nitrofurantoin and cotrimoxazole in clinical K. pneumoniae isolates. **Ballen et al** also noted the similar findings in uropathogenic K. pneumoniae.(26,28)

Resistance to multiple drugs was evident, particularly to cefotaxime (56%), ciprofloxacin (60.6%), and nitrofurantoin (65.1%), reflecting the current global trends in rising multidrug resistance (MDR) among Uropathogenic K. pneumoniae.

Antibiotic resistance and virulence genes:
Notably, *fimH* was more frequently found in nitrofurantoin (53.5%) and ciprofloxacin-resistant (57.5%) strains, while *entB* was present in 60.7% of ciprofloxacin-resistant isolates, suggesting a possible genetic linkage between resistance and virulence determinants The only statistically significant association we found was between *entB* and carbapenem resistance: *entB* was present in 60.7% of carbapenem-resistant isolates versus 21.1% of susceptible ones ($p \approx 0.001$). Neither *fimH* nor *uge* showed significant differences between resistant and susceptible groups ($p > 0.05$).Univariate analysis showed *uge* as the only gene significantly associated with mortality (odds ratio =20.3, $p=0.008$). Although the *uge* gene was less frequent (3.02%), its significant association with mortality (p=0.008) underlines its potential role in severe disease outcomes.
Carbapenem resistance was observed in 19.6%

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(41/209) of the total isolates. Among the 41 carbapenem-resistant isolates, only 26 were positive for the genes included in the study protocol. The most frequently detected carbapenemase gene was NDM.

Among 13 Carbapenem resistant *Escherichia coli* isolates 8 (61.5%) carried *blaNDM* gene, 2 (15.4%) isolates carried *blaOXA-48* gene and 2 (15.4%) had both *blaNDM* and OXA- 48. Among 28 Carbapenem resistant *Klebsiella pneumoniae* isolates 5 carried both *blaNDM* and *blaOXA-48* (17.85%), 9(32.1%) carried *blaNDM* gene, and 7 (25%) isolate carried *blaOXA-48* gene.

In 7 of the carbapenem-resistant E.coli and klebsiella pneumoniae isolates both *blaNDM* and *blaOXA-48* were co-harbored. In 8 of the carbapenem-resistant isolates, neither *blaNDM* nor *blaOXA-48* was detected, suggesting the presence of other genes, such as *bla*-VIM or *bla*-IMP genes. Additionally, carbapenem resistance may also be associated with the hyperproduction of extended-spectrum beta-lactamases (ESBLs) or AmpC beta-lactamases, upregulation of efflux pumps or the loss of porin channels.

CONCLUSION:

The study highlights a high prevalence of antimicrobial resistance in *E. coli* and *K. pneumoniae* urinary isolates, especially to commonly used antibiotics such as ampicillin, cefotaxime, and ciprofloxacin.Molecular analysis revealed that a significant proportion of isolates harbored virulence genes, with *fimH* and *iutA* being most common in *E. coli*, and *fimH* and *entB* in *K. pneumoniae*.

Co-occurrence of virulence genes was frequent, This co-expression likely provides pathogenic potential, enabling the bacteria to better adhere to host tissues (e.g., via *fimH*), acquire essential nutrients like iron (e.g., via *iutA* or *entB*), and cause tissue damage (e.g., via *sat* or *uge*).

The presence of multiple virulence traits contribute to more severe or persistent infections.Carbapenem resistance was observed in 19.6% of isolates, with *blaNDM* being the most frequently detected carbapenemase gene, followed by *blaOXA-48*. Eight carbapenem-resistant isolates lacked both *blaNDM* and *blaOXA-48* genes, suggesting the involvement of alternative resistance mechanisms such as other carbapenemase genes, ESBL overproduction, or porin loss.

A significant association was found between *iutA* and ESBL production in *E. coli*, and between *entB* and carbapenem resistance in *K. pneumoniae* .The presence of *sat* gene in *E. coli* and *uge* gene in *K. pneumoniae* was associated with higher mortality, indicating their potential role in disease severity.The combined analysis of resistance and virulence profiles provides a foundation for better therapeutic decision-making and highlights potential targets for future vaccine development against uropathogens.

LIMITATIONS:

Only a few virulence genes (3 in *E. coli* and 3 in *K. pneumoniae*) and two carbapenem resistance genes (*blaNDM*, *blaOXA-48*,) were analyzed, which may not reflect the full genetic diversity of these uropathogens. The study did not include whole genome sequencing, which would have allowed for a more comprehensive detection of resistance determinants and novel virulence markers. The isolates were obtained from a single tertiary care hospital, which may limit the generalizability of the findings to other regions or healthcare settings. As a point-prevalence study, temporal trends in antimicrobial resistance and virulence gene acquisition could not be assessed. Functional expression of detected genes (e.g., virulence gene expression or protein activity) was not evaluated, so their actual contribution to pathogenicity remains inferred.

FUTURE DIRECTIONS:

Future research should expand the panel of virulence and resistance genes studied to capture a more comprehensive genomic profile of *E. coli* and *K. pneumoniae* isolates. Whole genome sequencing (WGS) can be employed to identify novel or less common resistance mechanisms and virulence traits. Longitudinal surveillance across multiple healthcare settings would help track evolving resistance trends. Additionally, studying host-pathogen interactions and gene expression under clinical conditions may provide insights into pathogenicity. Importantly, understanding virulence gene profiles can support the development of targeted vaccines and innovative therapies for urinary tract infections caused by multidrug-resistant organisms.

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