Research Article

Al-Iraqia Medical College Journal (AIMCJ)
ISSN (Online): 3104-4565
ISSN (Print): 3104-4557

Molecular Detection of Cytotoxic Necrotizing Factor Type 1 Gene (CNF1) of Uropathogenic E. coli Isolated from Bladder and Prostate Cancer Patients

Estabraq Saad Rehan[*1], Nada H. Al-Mudallal[1]

[1] Department of Microbiology, Al-Iraqia University/ College of Medicine -Baghdad, Iraq

ARTICLE INFO
Received: 19/10 / 2025
Revised: 23/ 12/ 2025
Accepted: 26/ 12/ 2025
Publish online: 15 /4 / 2026

*Corresponding Author: Estabraq Saad Rehan
Email: estabraq.s.rehan@aliraqia.edu.iq

CITATION
Estabraq Saad Rehan, Nada .H.al-Mudallal. Molecular Detection of Cytotoxic Necrotizing Factor Type 1 gene (CNF1) of Uropathogenic E. coli isolated from Bladder and Prostate Cancers Patients. *AIMCJ*. 2026;3(1): 1-13.

DOI: https://doi.org/10.58564/AIMCJ3.1.2026.271

Abstract

Uropathogenic Escherichia coli (E. coli) produces cytotoxic necrotizing factor 1 (CNF-1). This factor influences epithelial cells and is a key virulence factor of uropathogenic E. coli, playing an essential role in bladder and prostate cancers.

Molecular identification of the cnf1 gene in uropathogenic E. coli isolated from patients with bladder and prostate cancer was performed using polymerase chain reaction (PCR).

This cross-sectional study included 150 urine samples obtained from patients with prostate and bladder cancer attending various hospitals in Baghdad, Iraq, from November 2024 to February 2025. All urine samples were analyzed both microscopically and macroscopically, cultured on blood agar and MacConkey agar, subjected to biochemical testing, and validated using the VITEK-2 system. Antibiotic susceptibility testing and molecular identification of isolated E. coli were performed by PCR targeting the cnf1 gene.

Of 150 urine samples, only 59.3% showed growth, with several bacterial species identified. Just 16% of samples were identified as E. coli and were obtained from prostate and bladder cancers. PCR analysis revealed that only 8 isolates contained the cnf1 gene. The antibiotic susceptibility test demonstrated varying levels of sensitivity and resistance. All 8 isolates containing the cnf1 gene showed elevated resistance to cefclidine (100%). The remaining 16 isolates lacking the cnf1 gene exhibited greater resistance to loracarbef (100%).

The research found that the cnf1 gene is genotoxic and can influence cellular differentiation, apoptosis, and proliferation. These findings underscore the importance of the cnf1 gene in urological infections and highlight the need for further studies on its association with antimicrobial resistance.

Keywords: Uropathogenic Escherichia coli, PCR, Prostate cancer, Bladder cancer, CNF1.

AIMCJ. (2026),3; (1):1-13
https://doi.org/10.58564/AIMCJ3.1.2026.271

Introduction

Uropathogenic Escherichia coli (UPEC)-triggered urinary tract infections (UTIs) typically result in cystitis, pyelonephritis, and prostatitis (1). These harmful strains possess various virulence factors that enable them to colonize, invade, and damage the host (2, 3). E. coli exhibits a variety of virulence factors that enhance its potential to cause disease, such as hemolytic activity, cytotoxicity, and hemagglutination, and is linked to particular virulence genes: hlyA, cnf-1, and papC (4). Cytotoxic necrotizing factor (CNF), encoded by the cnf1 gene, is genotoxic and can influence cellular differentiation, apoptosis, and proliferation (5).

UPEC releases CNF1 to aid the migration and invasion of prostate cancer cells, contributing to prostate cancer metastasis. In vitro research shows that it enhances pro-migratory and pro-invasive effects by infiltrating prostate cancer cells (6). Infections caused by E. coli and Enterococcus are associated with increased levels of pro-inflammatory cytokines linked to cancer development and progression (7).

Bacteria are isolated from only a small fraction of prostatitis cases, but when a bacterial species is cultured from prostatic secretions, most are Escherichia coli (8). Several investigations using non-culture-based methods have confirmed the presence of E. coli in more cases of prostatitis by detecting bacterial DNA in inflamed prostates (9). Moreover, UPEC was found to increase the risk of bladder cancer by enhancing CDKN2A methylation (10). Cytotoxic necrotizing factor type 1 (CNF1) is a toxin produced by several strains of uropathogenic Escherichia coli. (UPEC) continuously activates small GTPases from the Rho family by deamidating a single amino acid in these target proteins (11). By removing the amide group from a glutamine residue, the regulatory Rho, Rac, and Cdc42 GTPases in eukaryotic cells become irreversibly activated. This change enables new cellular processes, including gene transcription, cell proliferation, and cell survival (12).

CNF1 was initially characterized in 1983 by Caprioli and colleagues as a toxin that induces multinucleation ("cytotoxic") in cultured cells and necrosis ("necrotizing") in rabbit dermis. Caprioli et al. (1983) described a chromosomally encoded family of AB toxins (13). The protein toxin CNF1 enhances invasion of renal cells and, in vitro, promotes actin stress fiber and membrane ruffle formation in a Rho GTPase-dependent manner. It also induces apoptosis and epithelial damage in the bladder by triggering polymorphonuclear phagocytosis (14). Activation of Ras homolog family member C (RhoC) in bladder cancer cells induces the release of vascular endothelial growth factor (VEGF), leading to angiogenesis in the bladder cancer microenvironment (15). It also activates Cdc42, which, in turn, stimulates PAK1 phosphorylation and upregulates MMP-9 expression (6). In Iraq, prostate cancer ranks as the second most prevalent malignancy among Iraqi males. At the same time, bladder cancer (BC) is the 6th most prevalent cancer in Iraq. Bladder cancer affects males (79.4%) and females (20.6%), accounting for (4.7%) of all cancer cases in Iraq for both genders (16).

The aim of the study: Molecular detection of the cnf1 gene of UPEC isolated from bladder and prostate cancer patients.

AIMCJ. (2026),3; (1):1-13
https://doi.org/10.58564/AIMCJ3.1.2026.271

Material and Methods:

Sample collections and isolation of bacteria:

Between November 2024 and March 2025, one hundred fifty (150) midstream urine samples were collected from patients diagnosed with prostate and bladder cancer who attended Ghazi Al-Hariri Hospital and Al-Amal National Hospital for cancer care in Baghdad. Seventy-five samples from patients with prostate cancer and seventy-five from those with bladder cancer were placed in sterile wide-mouthed containers and promptly sent to the laboratory for further analysis.

Inclusion criteria: patients with early diagnosis of prostate and bladder cancer.

Exclusion criteria: patients treated with chemotherapy, radiotherapy, and antibiotics.

Microscopic examination (G.U.E):

Using a wide-mouth sterile cup, 150 samples were collected and assessed macroscopically for color and turbidity. Subsequently, 5 mL from each sample was transferred to a sterile plain tube for centrifugation, and the pellet was analyzed under a light microscope for pus cells, blood, and transitional epithelial cells. All samples displaying this characteristic feature were selected for the next step of identification (17).

Culturing of samples:

Every sample that tested positive in the microscopic analysis was cultured on selective and differential media (MacConkey agar, blood agar, and eosin methylene blue agar) and incubated at 37°C for 24 hours. Subsequently, the colonies were identified as E. coli based on their morphological characteristics (18). One colony was moved to brain heart infusion broth for storage and testing.

Biochemical tests:

Based on the growth of E. coli shown above, specific biochemical tests were performed to confirm the isolate as E. coli. The assays include TSI agar utilization, indole utilization, gas production, Voges-Proskauer test, methylene blue test, and citrate utilization, according to the classification of Bergey's Manual (19)—conformational diagnosis performed by the VITEK2 system.

Molecular detection and determination of the gene: DNA Template Preparation by the Boiling Method. The DNA template was prepared by the boiling method as described by (20). Briefly, 5 isolated colonies of overnight-grown bacteria were thoroughly suspended in 2 mL of distilled water and boiled in a water bath for 10 min. After centrifugation, the supernatant was used as the template DNA for PCR.

PCR Amplification:

The primers used to detect the cnf1 gene, provided by Macrogen (Seoul, South Korea), are 498 bp in length, as shown in Table 1.

The PCR amplification procedure for detecting local isolates at the genetic level follows these steps: The final PCR volume was 25 µl (12.5 µl of 2x Master Mix, 5 µl of template DNA, 1 µl of each forward and reverse primer, and 5.5 µl of nuclease-free water) in uniplex PCR Eppendorf tubes. The amounts changed in multiplex PCR. The mixture was vortexed briefly, then placed in a thermocycler for polymerase chain reaction. Annealing gradients were set, where appropriate, in increments from 52°C to 62°C; the annealing programs used for each PCR mixture are illustrated in Table 2.

AIMCJ. (2026),3; (1):1-13
https://doi.org/10.58564/AIMCJ3.1.2026.271

Table 1: The primer used for amplification of the cnf-1 gene and its sequence

Test gene	Primers used and their sequences	Extracted PCR product size (bp)	reference
Cytotoxic necrotizing factor-1	cnf-1 F-AAGATGGAGTTTCCTATGCAGGAG cnf-1 R-CATTCAGAGTCCTGCCCTCATTATT	498bp	(21)

Table 2: Thermocycling conditions.

Stage	No. of cycles	Time	Temperature
Initial denaturation	35	7 min	94ºC
Denaturation		1 min	94ºC
Annealing		45 sec	63º C
Elongation		1 min	72º C
Final extension		5 min	72º C

Determination of antibiotic susceptibility by using the VITEK-2 compact system

The Minimum inhibitory concentrations of antibiotics (MICs) were determined for 24 well-identified (UPEC) strains using the VITEK-2 compact system. 16 isolates were UPEC-negative for the cnf1 gene, while the other 8 were UPEC-positive. Results are shown in Table 3 according to Clinical and Laboratory Standards (22).

Statistical analysis:

Data are presented as mean ± SD, along with other descriptive statistics. Fisher's exact test and percentages were used in the current study to calculate p-values for some parameters. Values of $p>0.05$ were considered statistically non-significant, while $p\leq0.05$ was considered significant. Statistical analysis was performed using SPSS Inc., Chicago (v 20) (23).

Results:

A total of 75 urine samples were gathered from prostate cancer patients aged 55 to 80 years, and another 75 samples from bladder cancer patients aged 24 to 75 years.

Microscopic examination (Gram stain):

All isolates exhibited characteristics of Gram-negative, short bacilli and were non-spore-forming (24)—isolation of E. coli from the urine of patients with prostate and bladder cancer. Of 150 isolates, only 89 demonstrated bacterial growth; 31.9% of these were identified as E. coli originating from prostate and bladder cancer, as illustrated in Figures 1 and 2.

AIMCJ. (2026),3; (1):1-13
https://doi.org/10.58564/AIMCJ3.1.2026.271

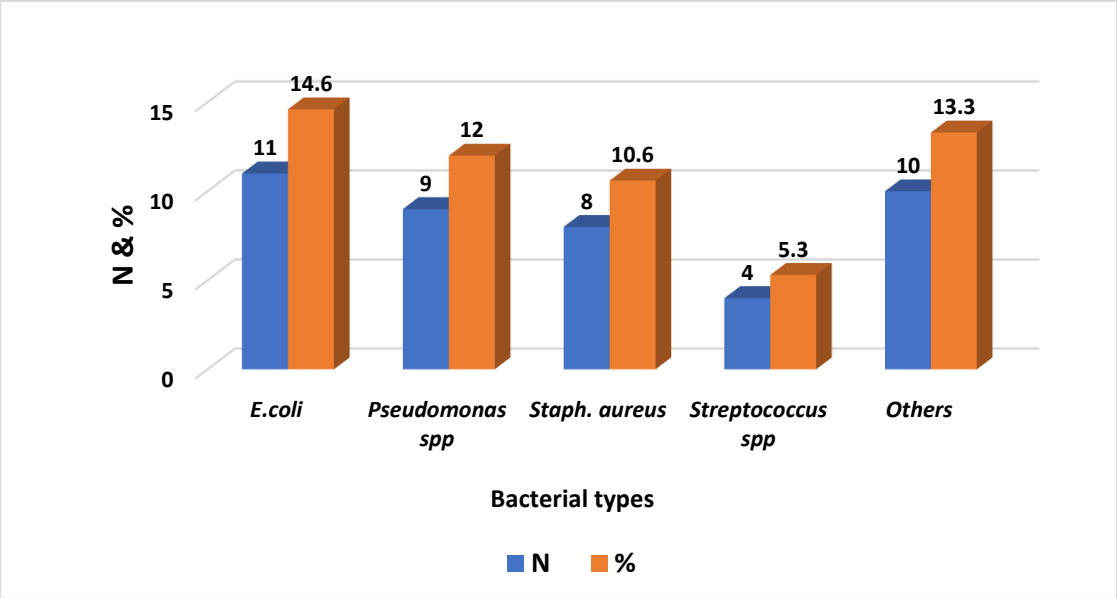

Figure 1: Number and percentage of bacterial growth from urine samples of prostate cancer patients.

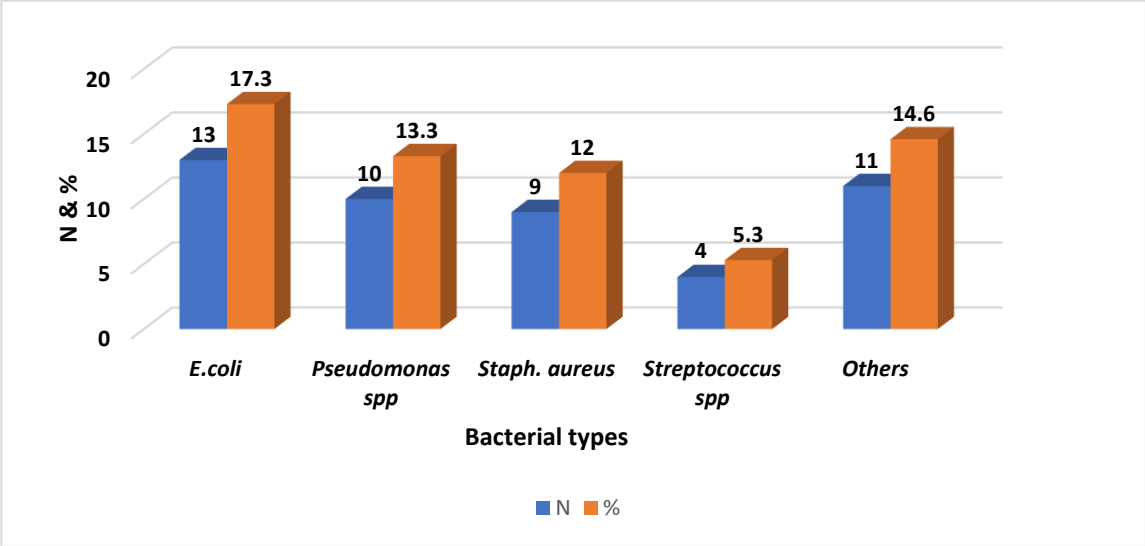

Figure 2: Number and percentage of bacterial growth from the urine samples of bladder cancer patients.

AIMCJ. (2026),3; (1):1-13
https://doi.org/10.58564/AIMCJ3.1.2026.271

Biochemical tests

All isolates were negative for oxidase, urease production, Voges-Proskauer, and citrate utilization tests. They were positive for Indole, Methyl red, Lactose fermentation, and motility tests (25).

All 24 E. coli isolates cultured on Kligler iron agar tested positive for this test, developing an acid slant and an acid bottom, producing gas, and being negative for H2S production (26).

Genomic DNA extraction from E. coli

Clear bands of genomic DNA from 24 E. coli isolates were used for further PCR detection of the cnf1 gene, as shown in Figure 3.

Identification of the *cnf1 gene* by PCR

Primers were used to amplify cnf1 from E. coli in this study. The PCR product showed a 498-bp band corresponding to the cnf1 gene, as shown in Figure 4 and Table 3. Source and number of E. coli isolates harboring the cnf1 gene.

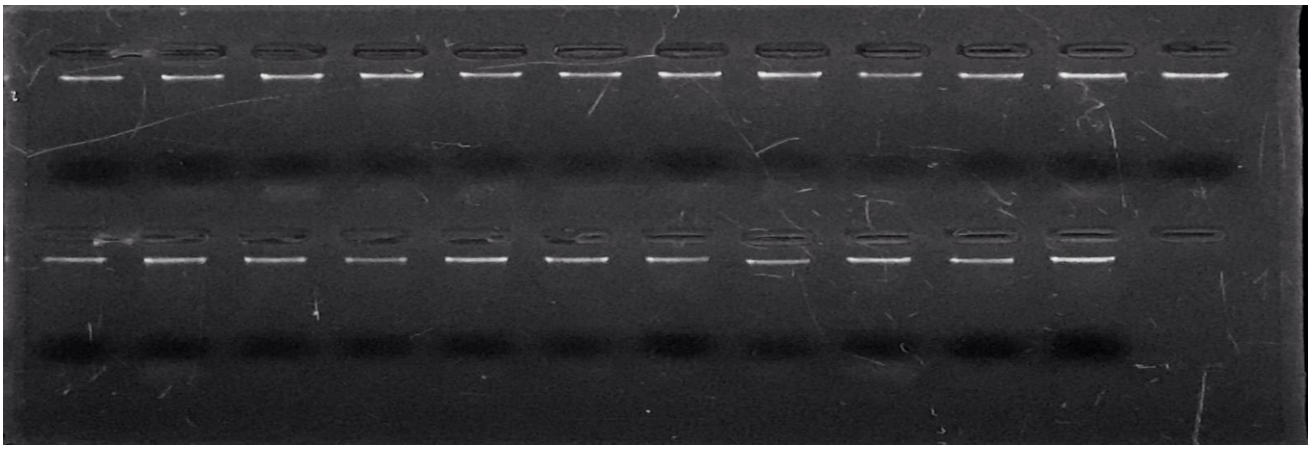

Figure 3: Gel profiles of the amplified products by PCR from the extracted DNA of subjects with prostate and bladder cancers.

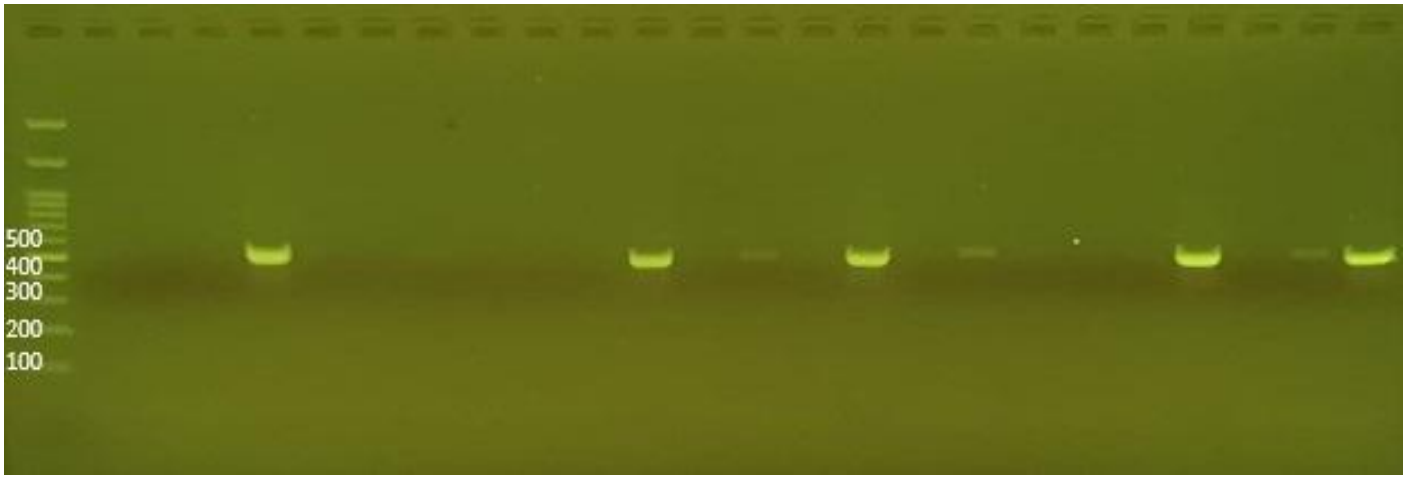

Figure 4: Gel electrophoresis of PCR products of the *cnf1 gene* (498bp) for *E. coli* isolates.

AIMCJ. (2026),3; (1):1-13
https://doi.org/10.58564/AIMCJ3.1.2026.271

Table 3: Number and percentage of isolates that harbor the cnf1 gene

Source of isolation	Total number of isolates	Number of isolated harbors gene	Percentage of *E. coli* isolate %	Percentage of *cnf1 gene* %
Prostate cancer	11	3	14.6	37.5
Bladder cancer	13	5	17.3	62.5
Total	24	8	31.9	100%

Antibiotic sensitivity of *E. coli* isolates

All 8 isolates harboring the cnf1 gene show higher resistance rates to cefclidine 8 (100%), cefoxitin, piperacillin, piperacillin/tazobactam, and doxycycline 6 (75%), and to ampicillin, ampicillin/sulbactam, piperacillin/sulbactam, cefditoren, imipenem, meropenem/avibactam, loracarbef, and tetracycline 5 (62.5%). They also show higher sensitivity rates to tigecycline and levofloxacin (8 [100%]), ceftolozane/tazobactam and tobramycin (7 [87.5%]), and ciprofloxacin and amikacin (6 [75%]), as shown in Table 4 and Figure 5.

The other 16 isolates that didn't harbor the cnf1 gene showed higher resistance to loracarbef (16/16, 100%), ampicillin, amoxicillin/clavulanic acid, piperacillin, cefclidine, and cefazolin (14/16, 87.5%), and Piperacillin/tazobactam (13/16, 81.25%). They were highly sensitive to colistin and tigecycline (16/16, 100%), Ciprofloxacin (15/16, 93.75%), and ceftolozane/tazobactam (13/16, 81.25%), as shown in Table 5 and Figure 6.

Table 4: antibiotic sensitivity test results for 8 isolate *cnf1 gene* positive *E. coli*

8 Isolates	AMP	AUG	A/S	P/PG	PIS	TZP	CFS	CFZ	LOR	CTN	FOX	CDN	CFE	CFT	CZA
R	5	2	5	6	5	6	8	1	0	2	6	5	4	5	3
R%	62.5	25	62.5	75	62.5	75	100	12.5	0	25	75	62.5	50	62.5	37.5
S	3	6	3	2	3	2	0	7	0	6	2	3	4	3	5
%	37.5	75	37.5	25	37.5	25	0	87.5	0	75	25	37.5	50	37.5	62.5
8isolates	C/T	CPE	AZT	DOR	IMI/IMP	MEV	AK	TO	LOR	CP	LEV	DO	CL	TE	TGC
R	1	3	3	0	5	5	2	1	5	2	0	6	0	5	0
R%	12.5	37.5	37.5	0	62.5	62.5	25	12.5	62.5	25	0	75	0	62.5	0
S	7	5	5	0	3	3	6	7	3	6	8	2	0	3	8
0	87.5	62.5	62.5	0	37.5	37.5	75	87.5	37.5	75	100	25	0	37.5	100

AIMCJ. (2026),3; (1):1-13
https://doi.org/10.58564/AIMCJ3.1.2026.271

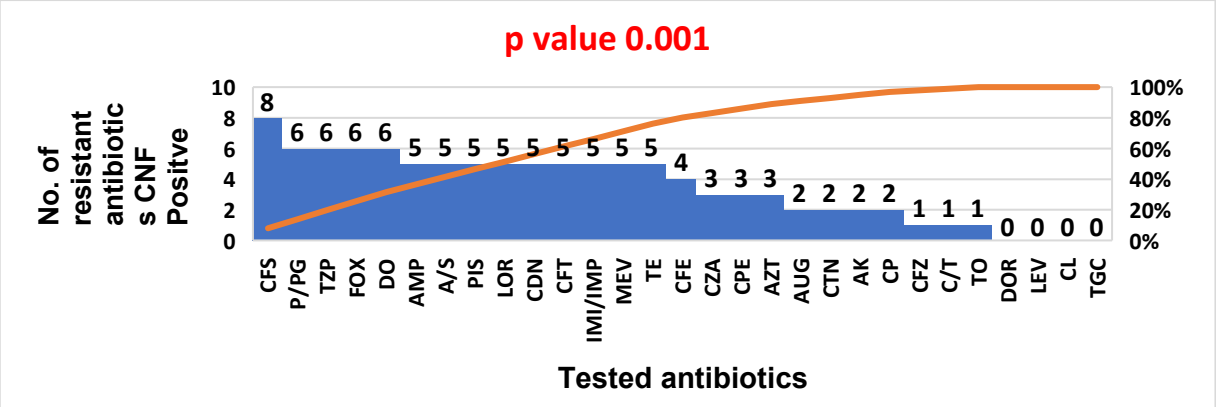

Figure 5: Number of resistant antibiotics for 8 *cnf1 gene-positive*

Table 5: antibiotic sensitivity test results of 16 isolates, *cnf1 gene-negative E. coli*

16 Isolates	AMP	AUG	A/S	P/PG	PIS	TZP	CFS	CFZ	LOR	CTN	FOX	CDN	CFE	CFT	CZA
R	14	14	12	14	12	13	14	14	16	11	12	11	10	11	7
R%	87.5	87.5	75	87.5	75	81.25	87.5	87.5	100	68.75	75	68.75	62.5	68.75	43.75
S	2	2	4	2	4	3	2	2	0	5	4	5	6	5	9
%	12.5	12.5	25	12.5	25	18.75	12.5	12.5	0	31.25	25	31.25	37.5	31.25	56.25
16 Isolates	**C/T**	**CPE**	**AZT**	**DOR**	**IMI/IMP**	**MEV**	**AK**	**TO**	**LOR**	**CP**	**LEV**	**DO**	**CL**	**TE**	**TGC**
R	3	11	10	0	9	7	4	5	14	1	2	8	0	8	0
R%	18.75	68.75	62.5	0	56.25	43.75	25	31.25	87.5	6.25	12.5	50	0	50	0
S	13	5	6	0	7	9	12	11	2	15	14	8	16	8	16
%	81.25	31.25	37.5	0	43.75	56.25	75	68.75	12.5	93.75	87.5	50	100	50	100

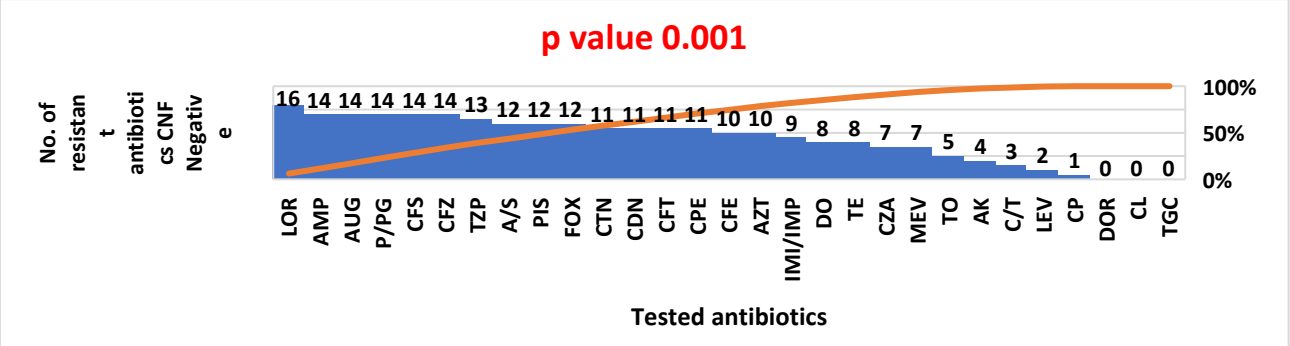

Figure 6: Number of resistant antibiotics for 16 isolates, cnf1 gene negative

AIMCJ. (2026),3; (1):1-13
https://doi.org/10.58564/AIMCJ3.1.2026.271

Discussion

The results of this study indicate that the cnf1 gene was detected in 33.33% of UPEC isolated from bladder and prostate cancer patients, surpassing the prevalence rates reported in other local studies in Iraq (27). In 2019, Al-Shubidi et al. found that 18% of UTI patients harbored the CNF1 gene (28). In 2022, Alhadidi et al. reported a cnf1 prevalence of 21.8% in UPEC (29). Khalaf et al. reported that only 10% of their isolates harbored cnf1 (30). This study has a lower CNF1 rate than other studies in Iraq; Nehmaa et al. reported a CNF1 rate of 35.5% among UPEC (31), while Odda et al. observed CNF1 in 41.57% of UPEC isolated from UTI patients (32). Globally, the present research indicated that the prevalence of cnf1 was higher than in Pakistan, Turkey, India, Iran, and France (33-36).

The present findings revealed that all samples positive for the cnf1 gene exhibited a high resistance rate to cefsulodin (8/100%); cefoxitin, piperacillin, piperacillin/tazobactam, and doxycycline (6/75%); and ampicillin, ampicillin/sulbactam, piperacillin/sulbactam, cefditoren, imipenem, meropenem/varbactam, loracarbef, and tetracycline (5/62.5%). By contrast, they demonstrated high sensitivity to tigecycline and levofloxacin (8/100%); ceftolozane/tazobactam and tobramycin (7/87.5%); and ciprofloxacin and amikacin (6/75%).

The remaining 16 isolates lacking the cnf1 gene exhibited increased resistance to loracarbef (16, 100%), ampicillin, amoxicillin/clavulanic acid, piperacillin, cefsulodin, and cefazolin (14, 87.5%), and to piperacillin/tazobactam (13, 81.25%). They also demonstrated significant sensitivity to colistin and tigecycline (16, 100%), ciprofloxacin (15, 93.75%), and ceftolozane/tazobactam (13, 81.25%).

A study conducted in Iraq by Al-Shubidi et al. found that UPEC isolates from UTI patients carrying the cnf1 gene showed significant resistance to amoxicillin (82.37%) and amikacin (92.35%), respectively (27). In contrast, Kalaf et al. reported that E. coli with the cnf1 gene exhibited high resistance to amoxicillin (92%) but variable resistance to other antibiotics, with tetracycline at 62% and amikacin at 4%. Nehmaa et al. determined that E. coli strains from bladder cancer patients showed complete resistance to ceftizoxime, cefsulodin, cefuroxime, and norfloxacin, and high resistance to piperacillin (94.73%), ticarcillin (94.63%), trimethoprim (91.13%), and tetracycline (90%). They also showed significant susceptibility (>85%) to ceftazidime/avibactam, amikacin, imipenem, meropenem, and gentamicin (37).

Research conducted in Iran by Derakhshandeh and associates on uncomplicated urinary tract infections caused by E. coli positive for the cnf1 gene showed that the isolates exhibited resistance to trimethoprim-sulfamethoxazole (SXT) (74.1%), cefotaxime (CTX) (68.2%), and amoxicillin-clavulanic acid (AMC) (94.1%) (38). Another study conducted in Egypt by El-Gendy et al. found that the microbiota isolated from cancer patients exhibited high resistance to β-lactams, cephalosporins, carbapenems, fluoroquinolones, β-lactamase inhibitors, folate synthesis pathway inhibitors, phosphonic acids, aminoglycosides, polymyxins, tetracyclines, macrolides, and chloramphenicol antibiotics (39). Other studies demonstrate varying degrees of antibiotic resistance (40,41).

AIMCJ. (2026),3; (1):1-13
https://doi.org/10.58564/AIMCJ3.1.2026.271

Conclusion

The findings of our study demonstrate the prevalence of the cnf1 gene in UPEC isolated from prostate and bladder cancer patients. All UPEC isolates carrying the cnf1 gene exhibited 100% resistance to cefoxitin, piperacillin, piperacillin/tazobactam, and doxycycline; 75% resistance was observed for ampicillin, ampicillin/sulbactam, piperacillin/sulbactam, cefditoren, imipenem, meropenem/varbactam, loracarbef, and tetracycline (62.5%). The other 16 isolates that did not harbor the cnf1 gene showed higher resistance to loracarbef (100%); ampicillin, amoxicillin/clavulanic acid, piperacillin, Cefsulodin, and cefazolin (87.5%); and piperacillin/tazobactam (81.25%).

Ethical Consideration:

Ethical approval was obtained from the College of Medicine, Al-Iraqia University, ethical review committee (FM.SA.157).

Funding: nil

Conflicts of interest: nil

Acknowledgment

We want to thank all the participants for their voluntary participation and for providing the data.

Author contributions

All authors contributed to sample collection and the preparation of the original draft, and read and approved the final version of the manuscript.

References:

1. Bien J, Sokolova O, Bozko P. Role of uropathogenic Escherichia coli virulence factors in development of urinary tract infection and kidney damage. *International journal of nephrology*. 2012;2012(1):681473.
2. Meng J, Feng P, Doyle MP. Pathogenic Escherichia coli. In: Compendium of Methods for The Microbiological Examination of Foods [Internet]. American Public Health Association; 2001http://ajph.apha-publications.org/doi/abs/10.2105/9780875531755ch35
3. Feng Y, Mannion A, Madden CM, Swennes AG, Townes C, Byrd C, Marini RP, Fox JG. Cytotoxic Escherichia coli strains encoding colibactin and cytotoxic necrotizing factor (CNF) colonize laboratory macaques. *Gut pathogens*. 2017 Dec 6;9(1):71.
4. Gohar NM, Aly HF, Ayoub MI. Important virulence factors and related genes in uro pathogenic E. coli and their relation to fluoroquinolone resistance. *J Pure Appl Microbiol*. 2018 Sep 1;12(3):1393–403.
5. Abbas AS, Manjunatha S, Rahmathulla SB, Prasad EM. Isolation, detection, and characterization of necrotoxic E. coli (cnf1 gene)
from patients with urinary tract infection. *World Journal of Pharmaceutical Research* SJIF Impact Factor 2015; 4(9): 2299-2311
6. Guo Y, Zhang Z, Wei H, Wang J, Lv J, Zhang K, et al. Cytotoxic necrotizing factor 1 promotes prostate cancer progression through activating the Cdc42–PAK1 axis. *Journal of Pathology*. 2017 Oct 1;243(2):208–19.

AIMCJ. (2026),3; (1):1-13
https://doi.org/10.58564/AIMCJ3.1.2026.271

7. Wei H, Dong L, Wang T, Zhang M, Hua W, Zhang C, Pang X, Chen M, Su M, Qiu Y, Zhou M. Structural shifts of gut microbiota as surrogate endpoints for monitoring host health changes induced by carcinogen exposure. *FEMS Microbiology Ecology*. 2010 Sep 1;73(3):577-86.
8. Yoon B Il, Kim S, Han DS, Ha US, Lee SJ, Kim HW, et al. Acute bacterial prostatitis: how to prevent and manage chronic infection? *Journal of Infection and Chemotherapy*. 2012;18(4):444–50.
9. Hochreiter WW, Duncan JL, Schaeffer AJ. Evaluation of the bacterial flora of the prostate using a 16S rRNA gene-based polymerase chain reaction. *The Journal of Urology*. 2000 Jan;163(1):127-30.
10. Tolg C, Sabha N, Cortese R, Panchal T, Ahsan A, Soliman A, Aitken KJ, Petronis A, Bägli DJ. Uropathogenic E. coli infection provokes epigenetic downregulation of CDKN2A (p16INK4A) in uroepithelial cells. *Laboratory investigation*. 2011 Jun 1;91(6):825-36.
11. Davis JM, Carvalho HM, Rasmussen SB, O'Brien AD. Cytotoxic necrotizing factor type 1 delivered by outer membrane vesicles of uropathogenic Escherichia coli attenuates polymorphonuclear leukocyte antimicrobial activity and chemotaxis. *Infection and immunity*. 2006 Aug;74(8):4401-8.
12. Fabbri A, Travaglione S, Rosadi F, Ballan G, Maroccia Z, Giambenedetti M, Guidotti M, Ødum N, Krejsgaard T, Fiorentini C. The Escherichia coli protein toxin cytotoxic necrotizing factor 1 induces epithelial-mesenchymal transition. *Cellular microbiology*. 2020 Feb;22(2):e13138.
13. Falbo V, Pace T, Picci L, Pizzi E, Caprioli A. Isolation and nucleotide sequence of the gene encoding cytotoxic necrotizing factor 1 of Escherichia coli. *Infection and immunity*. 1993 Nov;61(11):4909-14.
14. Amiri A, Nouriyeh P, Zibaei M, Zibaei S, Firoozeh F. Bacterial Protein Toxins with Emphasis on Bacterial Enterotoxins. *Infection Epidemiology and Microbiology*. 2025 Feb 10;11(1):77-93.
15. Guo Y, Wang J, Zhou K, Lv J, Wang L, Gao S, Keller ET, Zhang ZS, Wang Q, Yao Z. Cytotoxic necrotizing factor 1 promotes bladder cancer angiogenesis through activating RhoC. *The FASEB Journal*. 2020 Jun;34(6):7927-40.
16. CANCER REGISTRY OF IRAQ ANNUAL REPORT.
17. Sánchez-López E, Gomes D, Esteruelas G, Bonilla L, López-Machado AL, Galindo R, Cano A, Espina M, Ettcheto M, Camins A, Silva AM. Metal-based nanoparticles as antimicrobial agents: an overview. *Nanomaterials*. 2020 Feb 9;10(2):292.
18. Murugesan S, Samuel S, Rudrapathy P. Microbiological profile and antibiotic susceptibility pattern of uropathogens isolated among cancer patients at a tertiary care cancer center, South India. *Int. J. Pharm. Sci*. 2020;11:b56-63.
19. Systematic Bacteriology Second Edition Volume Two Introductory Essays.
20. Ali MR, Khudhair AM. Detection of colony adhesion factors and genetic background of adhesion genes among multidrug-resistant uropathogenic Escherichia coli isolated in Iraq. *J Pure Appl Microbiol*. 2018 Dec 1;12(4):2017–26.
21. Chapman TA, Wu XY, Barchia I, Bettel Heim KA, Driesen S, Trott D, Wilson M, Chin JJ. Comparison of virulence gene profiles of Escherichia coli strains isolated from healthy and diarrheic swine. *Applied and Environmental Microbiology*. 2006 Jul;72(7):4782-95.
22. CLSI CPHL 2025.
23. Arbuckle JL. IBM ® SPSS ® Amos TM 23 User's Guide. 2014.
24. Cabrera A, Mason E, Mullins LP, Sadarangani M. Antimicrobial resistance and vaccines in Enterobacteriaceae, including extraintestinal pathogenic Escherichia coli

AIMCJ. (2026),3; (1):1-13
https://doi.org/10.58564/AIMCJ3.1.2026.271

and Klebsiella pneumoniae. *npj Antimicrobials and Resistance*. 2025 Apr 28;3(1):1-4.

25. El-Alawi SH. Using culture-independent approaches to gain insights into the human urinary tract microbiome in healthy male and female individuals. MLA Citation [Internet]. 2021. Available from: https://fount.aucegypt.edu/retro_etds
26. Hasan RN, Jasim SA, Ali YH. Detection of fimH, kpsMTII, hlyA, and traT genes in Escherichia coli isolated from Iraqi patients with cystitis. *Gene Reports*. 2022 Mar 1;26:101468.
27. Hassan MH, Flayih MT, Yaseen NY, Suleiman AA. Detection of the Virulence Factor (Cytotoxic necrosis factor1) Produced from Uropathogenic Escherichia coli Isolates. *Iraqi Journal of Science*. 2015:1920-4.
28. Thaer Alshubidi Z, Neamati A, Homayoni M. Prevalence of Drug Resistance and Some Pathogenic Factors in Uropathogenic Escherichia coli (UPEC) Strains Isolated from Patients with Urinary Tract Infection. *Personalized Medicine Journal*. 2024 Jun 1;9(33):7-13.
29. Alhadidi HA, Al-Qaysi SA, Al-Halbosiy MM. Prevalence and cytotoxic effects of some colibactin and cnf genes among Escherichia coli isolated from urinary tract infections. *Microbiology and Biotechnology Letters*. 2022;50(2):283-92.
30. Khalaf ZZ, Flayyih MT. Detection of cnf1 and cnf2 Genes in Clinical Isolates of E. coli. *The Egyptian Journal of Hospital Medicine*. 2022 Oct 1;89(2):8088-94.
31. Nehmaa SA. Distribution of CNF1 among Escherichia coli isolates from urinary tract infections and bladder cancer in southern Iraq. *University of Thi-Qar Journal of Science*. 2023 Apr 29;10(1 (SI)):190-3.
32. Odaa NH, Rasheed HM. Distribution of cytotoxic necrotizing factor type 1 in clinical isolates of E. coli isolated from urinary tract infections in Iraq. *Microbial Biosystems Journal (International Scientific Journal of Microbial Biology)*. 2025 Mar 1;10(1):33–8.
33. Bashir S, Haque A, Sarwar Y, Ali A, Anwar MI. Virulence profile of different phylogenetic groups of locally isolated community-acquired uropathogenic E. coli from the Faisalabad region of Pakistan. *Annals of clinical microbiology and antimicrobials*. 2012 Aug 6;11(1):23.
34. Onlen Guneri C, Koksal F, Kizilyildirim S, Bedir B, Nagiyev T. The Distribution of Cytotoxic Necrotizing Factors (CNF-1, CNF-2, CNF-3) and Cytolethal Distending Toxins (CDT-1, CDT-2, CDT-3, CDT-4) in Escherichia coli Isolates Isolated from Extraintestinal Infections and the Determination of their Phylogenetic Relationship by PFGE. *International Journal of Clinical Practice*. 2022;2022(1):7200635.
35. Chakraborty A, Adhikari P, Shenoy S, Saralaya V. Molecular characterization of uropathogenic Escherichia coli isolates at a tertiary care hospital in South India. *Indian journal of medical microbiology*. 2017 Apr 1;35(2):305-10.
36. Landraud L, Gauthier M, Fosse T, Boquet P. Frequency of Escherichia coli strains producing the cytotoxic necrotizing factor (CNF1) in nosocomial urinary tract infections. *Letters in Applied Microbiology*. 2000 Mar 1;30(3):213-6.
37. Nehmaa SA, Chmagh AA, Mohammed KA. Prevalence of pks-positive Escherichia coli in Iraqi patients with urinary tract infections or bladder cancer. *Annals of Biology*. 2023. 39(2): 236-243
38. Derakhshandeh A, Firouzi R, Motamedifar M, Arabshahi S, Novinrooz A, Boroojeni AM, Bahadori M, Heidari S. Virulence characteristics and antibiotic resistance patterns among various phylogenetic groups of uropathogenic Escherichia coli isolates.

AIMCJ. (2026),3; (1):1-13
https://doi.org/10.58564/AIMCJ3.1.2026.271

Japanese journal of infectious diseases. 2015;68(5):428-31.

39. El-Gendy MMAA, Alghamdi HA, Abdel-Wahhab KG, Hassan NS, El-Bondkly AAM, Abdel-Wahab M, et al. Evaluation of multidrug resistance in the Gram-negative microbiome of cancer patients and the adverse effects of their metabolites on albino rats and epithelial or fibroblast cell lines. Infectious Agent Cancer. 2025 Dec 1;20(1).
40. Akinpelu SO, Olasehinde GI, Ikuerowo SO, Akinnola OO. Prevalence and antibiotic Susceptibility patterns of uropathogens in Men with prostate cancer and benign prostate hyperplasia from Southwestern Nigeria. *BMC Microbiology*. 2024 Sep 21;24(1):361.
41. Delcaru C, Podgoreanu P, Alexandru I, Popescu N, Măruţescu L, Bleotu C, Mogoşanu GD, Chifiriuc MC, Gluck M, Lazăr V. Antibiotic resistance and virulence phenotypes of recent bacterial strains isolated from urinary tract infections in elderly patients with prostatic disease. *Pathogens*. 2017 May 31;6(2):22.