University of Thi-Qar Journal of Science (UTJsci)
E-ISSN:2709-0256, P-ISSN: 1991-8690, Vol. (10), No.1(Special Issue: ISCAMET), April 2023

Distribution of CNF1among Escherichia coli isolates from urinary tract infection and bladder cancer in southern of Iraq

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***Abstract*—_One of the most important virulence factors produced by uropathogenic*Escherichia coli* (UPEC) is cytotoxic necrotizing factor 1 (CNF1). It plays a vital role in regulation of Ras homolog family member A (Rho), Ras-related C3 botulinum toxin substrate (Rac), and Cell division control protein 42 homolog (Cdc42)guanosine triphosphatase (GTPases) proteins, which involved in the organization of the actin cytoskeleton in eukaryotic cells. Also, CNF1may play a role in cell proliferation, surviving and gene transcription. Therefore, detection of the gene encoding CNF1 in UPEC would be imperative in characterization of these organisms and establishing a proper management regime to prevent the poor prognosis of the associated diseases. The aim of the present study was to investigatethe prevalence of the gene encoding cnf1 in UPEC strainsthat have been isolated from urinary tract infection and bladder cancer patients.Out of three hundred and fifty midstream urine samples, 136*E. coli*strains were isolated (98 urinary tract infection, 18 bladder cancer, and 20 healthy people). Detection and determination of the gene encoding CNF1 based on PCR technique and sequencing of PCR amplified target DNA. The present results showed that thirty five percent (35.34%) of the tested isolates have *cnf1*gene. All *cnf1*+strains were isolated from urinary tract infection patients. This finding should bring attention to predict the prognosis of the infections caused by these organisms and follow up patients to prevent any further complications.**

***Keywords*—CNF1; Escherichia coli,PCR,Virulence factor.**

I. INTRODUCTION

The prognosis of the urinary tract infection subjects to both the host susceptibility and the bacterial virulence. *Escherichia coli* isconsidered as a normal florain human intestine. However,uropathogenic *E.coli* showscharacters that make it virulent., specifically those related to specific serotypes such as O4, O6, O14, O22, O75 and O83 (Garcia *et al.* 2013) which express certain virulence factors includingP-related fimbriae and haemolysinwhich are encoded by specific genes located in pathogenicity islands (PAIs) (Hochhut*et al*. 2006). Gene encoding CNF 1 also had correlation with other virulence genes in PKs-island. CNF 1 is a toxic protein made by many uropathogenic*E. coli*.The glutamine residue deamidation that stimulate by CNF1forGTP-binding protein RhoAwhich plays an essential role in the actin cytoskeleton organization in cells (Knust*et al*. 2010). This role represents inRhoA protein activity that reorganize the stress fibers ofactin network accompanying start membrane disrupting and multinucleation in culture cells(Fabbri*et al*. 2002).

Many Studies showed that the adhesins P-related fimbriae and haemolysin play essential roles in *Escherichia coli*pathogenicity causing the furthor sever clinical conditions of urinary tract infections (Garcia *et al.* 2013), but the role of CNF 1 has been less understood. Previous studies showed considerable roles for CNF1in initiation of an aggressive pro-inflammatory response to UPEC in the bladder (Garcia *et al.* 2013). Recent study demonstrated the role of CNF 1 in bladder cancer (Guo *et al*. 2017). They found that CNF1 stimulate bladder cancer cells by secreting vascular endothelial growth factor (VEGF) through the activation of Ras homolog family member C (RhoC), resulting in bladder cancer angiogenesis microenvironment. Further study found thaturopathogenic *Escherichia coli* (UPEC) that CNF1-producing provokes significantly more submucosal edema andinterstitial and neutrophil infiltration in the bladder than those other strain lack CNF1(Yang *et al*. 2018).

The aim of this study was to investigate the CNF1-producing uropathogenic *E. coli* prevalence inurinary tract infection and bladder cancer.

II. MATERIALS AND METHODS

A. *Samples collection*

Threehundred and fiftymidstream urine samples were collected from patients with urinary tract infections (250) and bladder cancer (50) attended different hospitals in Basra province and 70 samples were collected from healthy people.

B. *Isolation of Bacteria*

The collected samples were inoculated on MacConkey agar and eosin methyl blue agarand

incubated at 37ºC for 24 hours. The culturewas examined for their shape, size, color, and Gram stain reaction, for identification of *E.coli*. Then all plates were incubated at 37ºC for 24 hours, after that a single pure isolated colony was transferred to Brain-Heart infusion agar medium for the preservation and to carry out biochemical tests that confirmed the identification of isolates.

C. Identification of Isolates

Escherichia coli isolates was identified depending on morphological features on culture medium (MacConkey agar and eosinmethyle blue agar), biochemical testsincluding indole, methyl redVoges Proskauer, and citrate test (IMVC tests) and Gran stain according to the classification ofBergey'smanual (Whitman *et al*. 2015).

D. Molecular detection and determination of cnf1 gene.

DNA Extraction

In order to extract the genomic DNA, the bacterial isolates were cultured overnight in 10ml of broth at 37°C. Then the extraction was carried out by using (Promega kit).

PCR Protocol

The (PCR) reaction mix use a final volume (25 µl) containing (2 µl) of DNA, (1 µl) of each primer, (12.5 µl) master mix and (8.5 µl) nuclease free water.

The (PCR) amplification were done under thethese situation conditions:initial denaturation at 94c for 4 minutes (1 cycle),then 30 cycles were performed: denaturation 94c for 30 second, annealing temperature 65c for 30 second, followed extension of 72c for 1 minutes, then final extension of 72c for 4 minutes.

The primer used to detect *cnf 1* were as follow
F-AAGATGGAGTTTCCTATGCAGGAG
R-CATTCAGAGTCCTGCCCTCATTATT
PCR productsize498Pb.(Chapman *et al*. 2006).

DNA sequencing

For DNA sequencing, 20 µl of PCR products of selected *E. coli* isolates were sent to Macrogen Company (Seoul, South Korea). The above forward primer was used for DNA sequencing (Chapman et al. 2006). All the obtained sequences were analyzed and aligned to each other and to reference gene recorded in National Center for Biotechnology using the Bio Edit program (Hall 1999).

III. RESULTS AND DISCUSSION

The present results showed that out of 350 urine samples, 136 (38.7%) *E.coli* isolateswere identifiedof which, 98/250 (39.2%) were found in patients infected with urinary tract infection, 18/50 (36%) were found in bladder cancer and 20/70 (28.6%) were isolated from healthy people. *E coli* grew on eosin methylene blue which was used as a differential and selective medium for genus *E. coli* (Leininger *et al*. 2001). The colonies showed green sheen. This confirmed in principle that isolates belonged to genus *E. coli*. Isolates has the ability to ferment of lactose & production of strong acids and form large green colonies on the EMB agar by quick reduction in the pH of the EMB was observed on the medium.

MacConkey agar also was used as a selective medium for genus *E. coli*.All *E. coli*isolate showedpinkcolonies (Cundell*et al*. 2019).

The isolates and the cells appeared as Gram-negative bacilli rod shape after staining by Gram stain under microscope.

However for further identification, the oxidase test was performed and all isolates gave negative results. IMVICtests were performed to differentiates and identify *E. coli* from other *Enterobacterbactericea.*

E.coli has been considered as one of the most common pathogens associated with UTI in many other countries (Gajdács*et al* 2019). The results showed that the prevalence of *E. coli* among UTI patients was slightly low compared with other international studies which reported that the prevalence of *E.coli* was 50 to 57.5% (Kabugo*et al*. 2016, Mwaka*et al*. 2011) and much higher than the prevalencerate found inKurdistan Region in Iraq (21.1%) (Mahde*et al*. 2022). The variation of the prevalence rate could be due the differences in the sample size, different population, or the improvement in management of urinary tract infections.

Detection of *cnf1* gene was carried out by using PCR technique using specific primers. CNF1 encoding gene was detected in 41 (35.3%) strains (table 1), all of them were isolated from patients with urinary tract infections (table 1, figure 1). None of the bladder cancer samples showed positive results for *cnf1* gene.

Alignment of *cnf1*gene sequence with reference gene recorded in National Center for Biotechnology Information showed 98 to 100% similarity (figure 2).

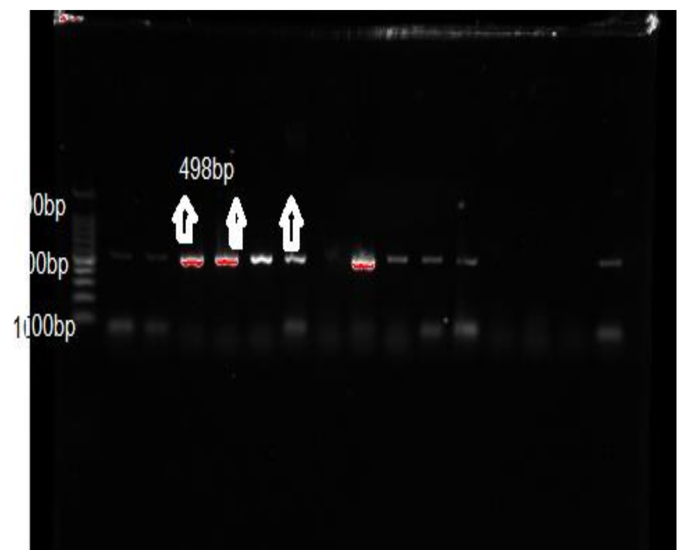

Figure 1. Gel electrophoresis of PCR products of Cytotoxic Necrotizing Factor 1 (*cnf1*) gene (498bp) for E. coli strains.

TABLE 1. DISTRIBUTION OF CYTOTOXIC NECROTIZING FACTOR 1(*CNF1)* GENE AMONG *E. COLI* ISOLATES

Sample source	Number of isolates	cnf1 geneN (%)

Healthy people	20	0
Urinary tract infection	98	41 (41.83%)
Bladder cancer	18	0
Total	136	41 (35.3%)

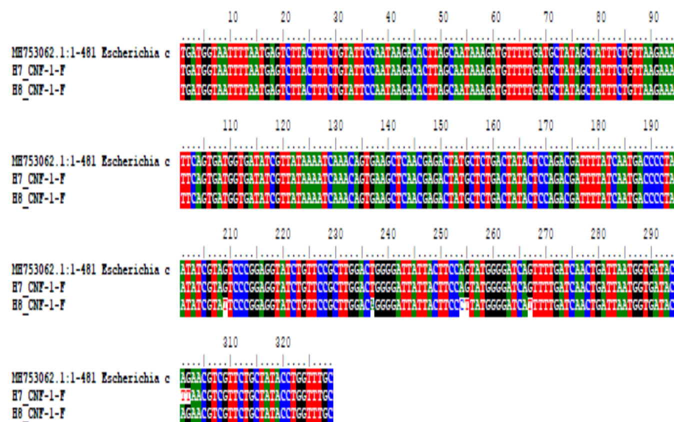

Figure 2. Alignment of the sequence of *cnf1* gene of representative tested isolates with reference*Escherichia coli* strain W010 cytotoxic necrotizing factor CNF-1 (CNF-1) gene, Sequence ID: MH753062.1.

The roles that cytotoxic necrotizing factor 1 (CNF1) play in the pathogenicity of UPEC has been broadly studied. It is one of most important UPEC toxins. Several studies reported that about 30% of the UPEC isolated from pyelonephritis cases have the *cnf1* gene, suggesting its effect in kidney infection (Bien *et al*. 2012).Other studies reported that CNF1 positive strains induce other inflammation in prostate, kidney and bladder (Guo *et al*. 2020, Smith *et al* 2008). It has been reported that CNF1 causes bladder cells apoptosis that may lead to bladder cell exfoliation providing bacterial access to underlying tissues (Mills *et al*. 2000). Other recent study found that CNF1 persuades the migration and invasion of prostate cancer cells encouraging prostate cancer progression through activation of the Cdc42-PAK1 axis (Guo *et al*. 2017). Furthermore, many reports showed that CNF1 markedly involves in activating Rho GTPases inducing cell motility and invasion by uropathogenic*E. coli* (Carlini*et al*. 2021, Doye*et al*. 2002, Diabate*et al*. 2015).
The present study showed that the prevalence rate of CNF1-positive strains (41.83%)was higher than that reported in Europe (34%),Iran (28%) and India (29%)(Landraud*et al*. 2000, Shabani*et al*. 2018, Arindam *et al*. 2017).The present results showed that all the *cnf1* positive strains were associated with urinary tract infection and all *E. coli* strains isolated from urine collected from patients with bladder cancer and heathy people were *cnf1* negative.
In conclusion findings indicated that *cnf1* positive strains were markedly associated with UTI (41.83%).This virulence gene (*cnf1*) may help these isolates to persist even with proper treatment and could be responsible for frequent persist infections. Despite ofthe importance of findings, there are certain limitations such as limited patients' information regarding their disease prognosis and responding to chemotherapy. Further study is required for longer time to follow up patients and to determine more phenotypical and genotypical characters of the infecting *E. coli* strains.

IV REFERENCES

1- ArindamChakraborty, PrabhaAdhikari, ShaliniShenoy, VishwasSaralaya. (2017). Molecular Characterisation of Uropathogenic Escherichia coli Isolates at a Tertiary Care Hospital in South India.*IJMM*, 35 (2), 305-310.

2- Bien J, Sokolova O, Bozko P. (2012). Role of uropathogenic *Escherichia coli* virulence factors in development of urinary tract infection and kidney damage. *Int J Nephrol.* :681473

3- Carlini, Francesca, ZairaMaroccia, Carla Fiorentini, Sara Travaglione, and AlessiaFabbri (2021). Effects of the *Escherichia coli* Bacterial Toxin Cytotoxic Necrotizing Factor 1 on Different Human and Animal Cells: A Systematic Review. *Int. J. Mol. Sci* 22, no. 22: 12610.

4- Chapman TA, Wu XY, Barchia I, Bettelheim KA, Driesen S, Trott D, Wilson M, Chin JJ. (2006). Comparison of virulence gene profiles of Escherichia coli strains isolated from healthy and diarrheic swine. *Appl Environ Microbiol*. 72(7):4782-95.

5- Cundell, A., & Sheepy, E. (2018). Student perceptions of the most effective and engaging online learning activities in a blended graduate seminar. *Online Learning*, 22(3), 87–102.

6- Diabate M, Munro P, Garcia E, Jacquel A, Michel G, Obba S. (2015). *Escherichia coli* alpha-hemolysin counteracts the anti-virulence innate immune response triggered by the Rho GTPase activating toxin CNF1 during bacteremia. *PLoSPathog.* 11:e1004732.

7- Doye A, Mettouchi A, Bossis G, Clement R, Buisson-Touati C, Flatau G. (2002). CNF1 exploits the ubiquitin-proteasome machinery to restrict Rho GTPase activation for bacterial host cell invasion. *Cell* 111, 553–64.

8- Fabbri A, Falzano L, Travaglione S, Stringaro A, Malorni W, Fais S, Fiorentini C.(2002). Rho-activating Escherichia coli cytotoxic necrotizing factor 1: macropinocytosis of apoptotic bodies in

human epithelial cells. *Int J Med Microbiol*. 2002 Feb;291(6-7):551-4.

9- Gajdács. M, M. Ábrók, A. Lázár, and K. Burián. (2019). Comparative epidemiology and resistance trends of common urinary pathogens in a tertiary-care hospital: a 10-year surveillance study. *Medicina*, 55(7).

10- Garcia TA, Ventura CL, Smith MA, Merrell DS, O'Brien AD (2013). Cytotoxic necrotizing factor 1 and hemolysin from uropathogenic Escherichia coli elicit different host responses in the murine bladder. *Infect Immun*. Jan;81(1):99-109.

11- Guo Y, Wang J, Zhou K, Lv J, Wang L, Gao S, Keller ET, Zhang ZS, Wang Q, Yao Z. (2020). Cytotoxic necrotizing factor 1 promotes bladder cancer angiogenesis through activating RhoC. *FASEB J*.34(6):7927-7940.

12- Guo Y, Zhang Z, Wei H, Wang J, Lv J, Zhang K, (2017). Cytotoxic necrotizing factor 1 promotes prostate cancer progression through activating the Cdc42-PAK1 axis. *J Pathol.* 243:208–19.

13- Hall TA. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser*. 41:95–98.

14- Hochhut B, Wilde C, Balling G, Middendorf B, Dobrindt U, Brzuszkiewicz E, Gottschalk G, Carniel E, Hacker J. (2006). Role of pathogenicity island-associated integrases in the genome plasticity of uropathogenic*Escherichia coli* strain. *MolMicrobiol*. Aug;61(3):584-95.

15- Kabugo. D, S. Kizito, D. D. Ashok. (2016). Factors associated with community-acquired urinary tract infections among adults attending assessment centre, Mulago Hospital Uganda. *African Health Sciences*, 16(4), 1131–1142.

16- Knust, Zeynep, and Gudula Schmidt (2010). Cytotoxic Necrotizing Factors (CNFs)−A Growing Toxin Family. *Toxins* 2, no. 1: 116-127.

17- Landraud L, Gauthier M, Fosse T, Boquet P. (2000). Frequency of Escherichia coli strains producing the cytotoxic necrotizing factor (CNF1) in nosocomial urinary tract infections. *LettApplMicrobiol*. Mar;30(3):213-6.

18- Leininger DJ, Roberson JR, Elvinger F. (2001). Use of eosin methylene blue agar to differentiate Escherichia coli from other gram-negative mastitis pathogens. *J Vet Diagn Invest*. May;13(3):273-5.

19- Mahde S. Assafi, Fawwaz F. Ali, ReemFouad Polis, NisreenJawadSabaly, SozanMuhsinQaraniBi en J, Sokolova O, Bozko P.(2012).Role of uropathogenic *Escherichia coli* virulence factors in development of urinary tract infection and kidney damage. *Int J Nephrol*:681473.

20- Mills M, Meysick KC, O'Brien AD. (2000). Cytotoxic necrotizing factor type 1 of uropathogenic *Escherichia coli* kills cultured human uroepithelial 5637 cells by an apoptotic mechanism. *Infect Immun.* 68:5869–80.

21- Mwaka A. D., Mayanja-Kizza H., E. Kigonya, and D. Kaddu-Mulindwa (2011). Bacteriuria among adult non-pregnant women attending Mulago hospital assessment centre in Uganda. *African Health Sciences*, 11(2), 182–189.

22- Shabani A, Amini A, EbrahimzadehNamvar A. (2017). Frequency of Necrotizing Factor Type 1 and Hemolysin Genes Among Escherichia coli Strains Isolated from Hospitalized Patients of Rouhani Hospital in Babol, IRAN in: A Short Report. *JRUMS*; 17 (7) :681-688.

23- Smith YC, Rasmussen SB, Grande KK, Conran RM, O'Brien AD. (2008) Hemolysin of uropathogenic *Escherichia coli* evokes extensive shedding of the uroepithelium and hemorrhage in bladder tissue within the first 24 hours after intraurethral inoculation of mice. *Infect Immun.* 76:2978–90.

24- Whitman, W. B.; DeVos, P.; Dedysh, S.; Hedlund, B.; Kämpfer, P.; Rainey, F.; Trujillo, M. E.; Bowman, J. P.; Brown, D. R.; Glöckner, F. O.; Oren, A.; Paster, B. J.; Wade, W.; Ward, N.; Busse, H-J.; Reysenbach, A-L., eds. (2015). *Bergey's manual of systematics of archaea and bacteria*. Hoboken, New Jersey: John Wiley & Sons.

25- Yang H, Li Q, Wang C, Wang J, Lv J, Wang L, Zhang ZS, Yao Z, Wang Q. (2018). Cytotoxic Necrotizing Factor 1 Downregulates CD36 Transcription in Macrophages to Induce Inflammation During Acute Urinary Tract Infections. *Front Immunol.* ;9:1–15.