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Detection the Virulence Factor (Cytotoxic necrosis factor1) Produce from Uropathogenic *Escherichia coli* Isolates

Muthanna H. Hassan[1*], May T. Flayih[1], Nahi Y. Yaseen[2], Ahmed A.Suleiman[3]
[1] Departmentof Biology,College of Science,Baghdad University,Iraq
[2] Director General of Iraqi Center of Cancer & Medical Genetics Researches , Al-MustansiriyahUniversity,Iraq
[3]Desert research center, AL-AnbarUniversity, Iraq

Abstract
In this study, only four isolates produce CNF1 from 76 isolatesof uropathogenic *Escherichia coli.cnf1* gene was detected by using PCR technique, while cytotoxic necrotizing factor 1(CNF1) was determined by Immunoblotting assay.

Keywords: Uropathogenic *Escherichia coli*, CNF1.

الكشف عن عامل الضراوة (التنخر الخلوي) المنتج من عزلات الاشريشيه القولونية المسببة لالتهابات القناه البولية

مثنى حامد حسن[١]*، مي طالب فليح[١]، ناهي يوسف ياسين[٢]، احمد عبد الجبار سليمان[٣]
[١]قسم علوم الحياه ، كليه العلوم ، جامعة بغداد ، العراق
[٢]المركز العراقي لبحوث السرطان والوراثة الطبية، الجامعة المستنصرية، العراق
[٣]مركز ابحاث التصحر، جامعه الانبار، العراق

الخلاصة
في هذه الدراسة تم الحصول على اربعة عزلات منتجه لعامل التنخر الخلوي منبين ٧٦ عزله للاشريشيه القولونيه المسببه لالتهابات القناه البوليه. جين*cnf1* تم الكشف عنه باستخدام تقنيه PCR بينماعامل التنخر الخلويCNF1 قد كشف عنه باستخدام النسخ المناعي.

Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections of humans. More than 80% of UTIs are caused by Uropathogenic *E. coli*(UPEC) strains[1].The virulence factors in UPEC strains are P fimbriae (*pap*), a fimbrial adhesin I (*afaI*), hemolysin (*hly*), cytotoxic necrotizing factor 1(*cnf 1*), and S fimbriae (*sfa*) [2].CNF1 a protein toxin,first described in 1983 by Caprioli and coworkers as a toxin capable of causing multinucleation (cytotoxic) in cultured cells and necrosis in rabbit skin (necrotizing) [3], This toxin is an AB-type toxin which cause urinary tract infections and neonatal meningitis, it has a 115 kDa single-chain molecule comprising an N-terminal receptor binding domain and a C-terminal catalytic domain, which contains deamidase activity [4].The PCR studies showed that the gene for CNF1 is more common than that for CNF2, CNF1 and CNF3, which are chromosomally encoded while CNF2 is located on a transmissible plasmid [5].All CNFs are identical in length (1013/1014 aa) and comprise a modular structure with an *N*-terminal receptor binding domain ,in conjunction with the central translocation domain, it mediates cellular entry . The *C*-terminal part of CNF1 (720 to 1014aa) harbors the full catalytic activity [6] .The aim of

*Email:Muthanna_477_aliraqi@yahoo.com

studydetection the virulence factor (Cytotoxic necrosis factor1) produce from uropathogenic *Escherichia coli* isolates by different methods.

Materials and Methods

Collection of samples

Through the period extending from April 2012 till December 2012, 186 samples of urine were collected from patients with urinary tract infectionsand prostate cancer in sterilized containers from five hospitals in Baghdad and Anbar.

Identification of *E. coli*

The samples were processed on MacConkey agar, Eosin methylene blue agar and blood agar,and then incubated at 37°C for 24hrs. The identification of gram negative bacteria was performed by standard biochemical tests (catalase, oxidase, Urease , Indole production, Methyl red, Voges-Proskauer and Citrate utilization test) and confirmedbyvitek2 and Api-20 E according to Bergy's Manual for Determinative Bacteriology [7,8].

DNA extraction

DNA extraction from uropathogenic *E. coli* was carried out according to the genomic DNA purification kit supplied by manufactured company (promega, USA). A DNA concentration (µg/ml) was between 50-56, while DNA purity was between 1.7-1.9.

Detection of *cnf1*gene by PCR

To amplify *cnf1* gene, amixture of solution composed of 25µl of PCR reaction contained 2.5µl of each upstream and downstream primer, 2.5µl of free nuclease water, 5µl of DNA extraction and 12.5µl of master mix. The PCR amplification product were visualized by electrophoresis on 1.5% agarose gel for 45min at 60v. The size of the amplicons were determined by comparison to the 100 bp allelic ladder (promega, USA), the conditions for reaction are listed in table-1.The*cnf1*-F was (GAACTTATTAAGGATAGT)forward primer sequence from (5'to3') and*cnf1*-R was (CATTATTTATAACGCTG) reversed sequence from (5' to3').The size amplified product 543 base pairs(9).

Table 1-The cycling conditions of reaction

Loop's steps	*Temperature*	*Time*	*Number of cycles*
Number of cycles	**94°C**	**4 min**	**1**
Denaturation	**94°C**	**45 sec**	**35**
Annealing	**46°C**	**45 sec**	
Extension	**72°C**	**45 sec**	
Final extension	**72°C**	**min 7**	**1**
Hold	**4**	**indefinite**	**1**

Western and dot blot technique

Western blot analysis was carried out as previously described by Fabbri *et al*. (1999) with the modifications ;equal concentrations of CNF1 were subjected to SDS-PAGE(8% Tris-glycine gels),after electrophoretic separation, the proteins were transferred to 0.45µm nitrocellulose membranes with a Trans-Blot sodium dodocyl semidry electrophoretic transfer cell, and the membranes were then blocked overnight in phosphate buffered saline (PBS) that contained 5% skim milk and 0.1% Tween 20 (PBS-T) ,membranes were incubated with mouse polyclonal anti-CNF1 sera (1:500) washed in TBST, and then incubated with HRP-conjugated goat anti-mouse IgG (1:1,000) or HRP-conjugated goat antimouse. Reactive proteins were detected with enhanced visualized with diaminobenzidine (DAB) substrate. Dots blots were tested for CNF1 MAb reactivity as described above for Western blot analyses without electrophoresis.

Results and Discussion

Isolation and identification of *E. coli*

A total of one hundred and eighty six samples of urine were collected from UTI and cancer prostate patients from five hospitals in Baghdad and Anbar. All samples were cultured on MacConkey agar, EMB agar and blood agar plates,according to the growth characteristics about 76 (40.86%) samples

were identified as *E. coli*as shown figure -1 .Thenconfirmed by Api-20E system and vitek-2 system. [11,12].

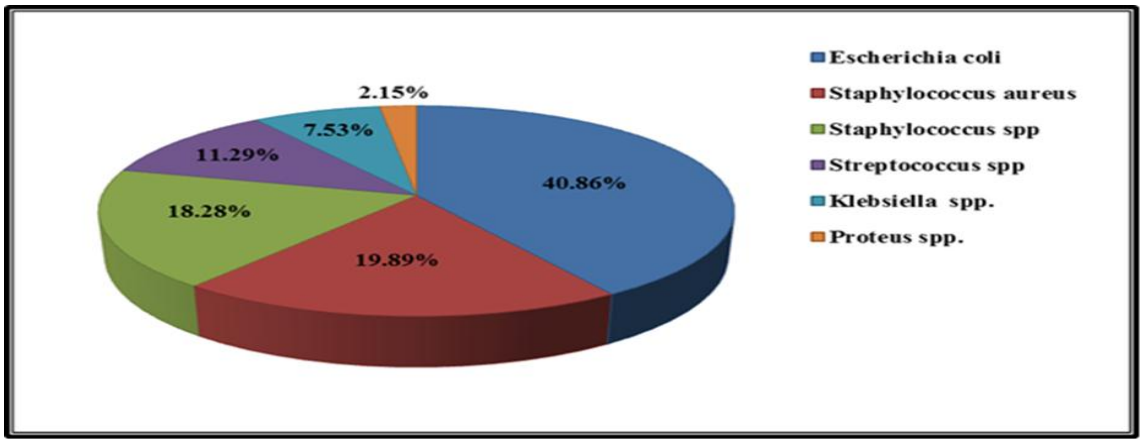

Figure 1- Percentage of Bacterial isolates from sample

By using PCR , *cnf1* gene with size 543bp ,as virulence factor,was detected in 4 isolates of *E. coli*as shown in figure 2. Moreover four isolates of *E. coli* that have *cnf1* gene produced CNF1protein that confirmed by using CNF1 monoclonal antibodies (Figure 3 and 4) [13]. The production of CNF1 will render such bacteria to grow and survive at the site of infection due to its ability to prevent the healing of wound through its effect on polymorphonuclear leukocytes, The action mechanisms of cytotoxic necrotizing factor-1(CNF-1) involve the Rho-dependent rearrangement of the cytoskeleton in eukaryotic cells with a complex of consequences [14].

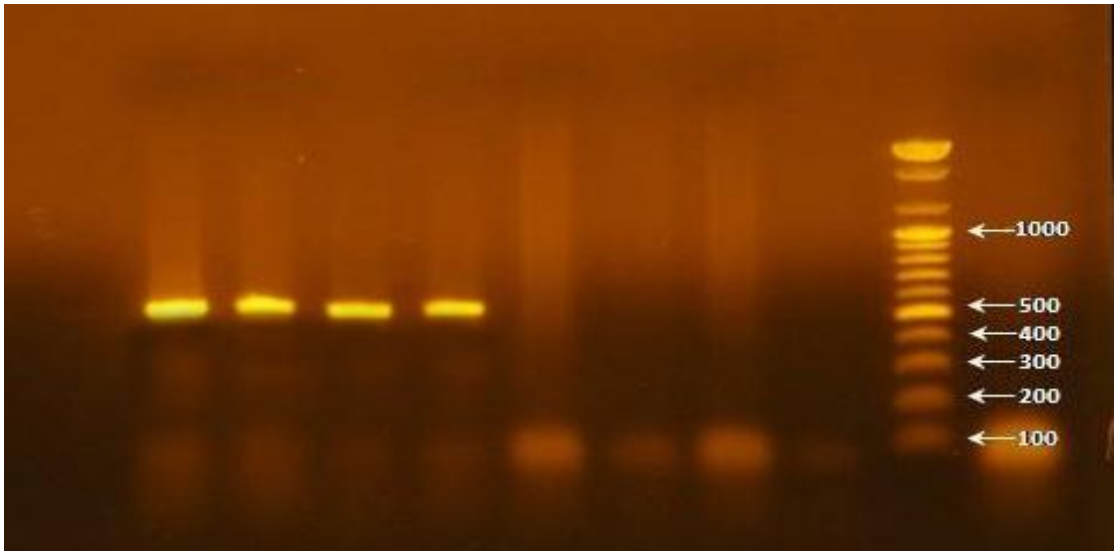

Figure 2-*cnf1* gene in whole DNA *E.coli* isolates by PCR on1.5% agarose gel at 5 V/cm for 1 hour, stained with ethidium bromide and visualized on a UV transiluminator documentation system. The size of amplified gene at 543pb

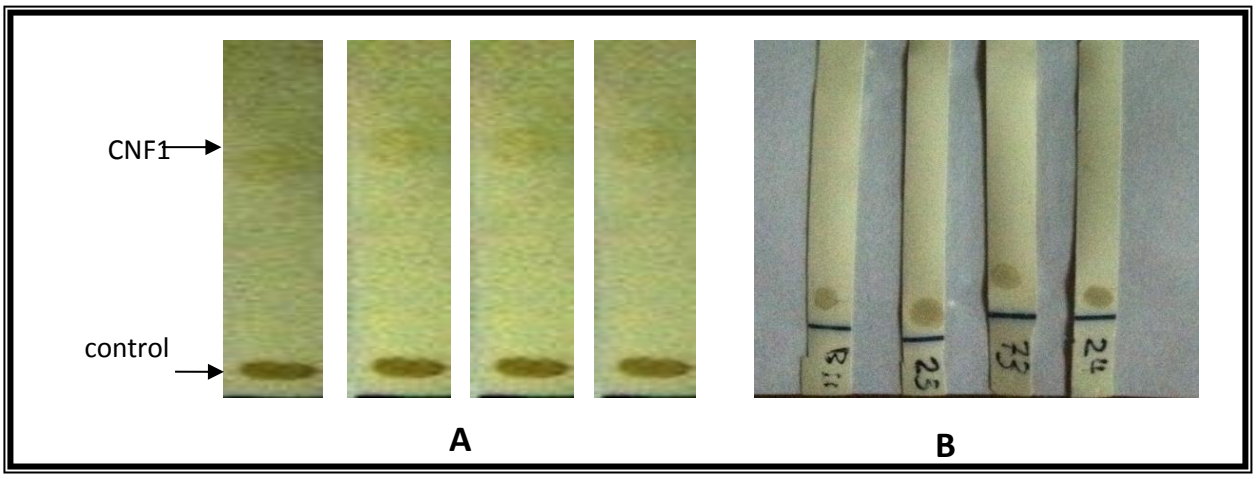

Figure 3-CNF1 in *E.coli* isolates by dot blot analysisA- positive result B-negative result

Figure 4-CNF1 in *E.coli* isolates by western blot showed positive result analysis

References

1. Ulet GC, Totsika M, Schaale K, Carey AJ, Sweet MJ andRahimi E. **2013**. Uropathogenic *Escherichia coli* virulence and innate immune responses during urinary tract infection. *Curr Opin Microbiol*. 16: S1369–5274
2. Momtaz H, Dehkordi FS, Rahimi E, and Asgarifar A. **2013**. Detection of *Escherichia coli*, *Salmonella* species, and *Vibrio cholerae* in tap water and bottled drinking water in Isfahan, Iran. *BMC Public Health.*13(1):556
3. Caprioli, A., Falbo, V., Roda, L.G., Ruggeri, F.M. and Zona, C.**1983.** Partial purification and characterization of a *Escherichia coli* toxic factor that induces morphological cell alterations. *Infection and Immunity* 39, 1300–1306
4. Hoffmann, C., and G. Schmidt. **2004.** CNF and DNT. Rev. *Physiol. Biochem. Pharmacol*. 152:49–63
5. Kadhum, H.J., Finlay, D., Rowe, M.T., Wilson, I.G.,and Ball, H.J. **2008**.Occurrence and characteristics of cytotoxic necrotizing factors, cytolethal distending toxins and other virulence factors in *Escherichia coli* from human blood and faecal samples. *Epidemiol. Infect*., 136, 752–760
6. Marianne P., Panagiotis P., Carsten S., Andreas S., Klaus A. and Gudula, S.**2014**. Lu/BCAM adhesion glycoprotein is a receptor for *Escherichia coli*cytotoxic necrotizing factor 1 (CNF1). *J. Plos Pathogens* 10 (1) e1003884.
7. Holt JC, Krieg NR, Sneath A, Stachley JT,and William ST **1994**. *Bergy,s manual of determinative bacteriology*, 9th ed. USA, pp.552.
8. Collins, C. H., Lyne, P. M., Grange, J. M. andFalkinham, J. O. **2007**.*Microbiological Method*. 8th ed., P.76-287. Arnold Amember of TheHodder Headline Group.London.
9. Blanco, M., Blanco, J., Blanco, J.E., Gonzalez, E.A., Gomes, A.T., Zerbini,L.F., yano,T., Pestana de castro, A.F.**1994**Genes coding for shiga-like toxins in bovine verotoxin production *Escherichia coli* VTEC strains belonging to different O:K:H serotype *vet. microbiol.*,42:105-110.

10. Fabbri, A., Gauthier, M. and Boquet, P. **1999.** The 5' region of *cnf1* harbours a translational regulatory mechanism for CNF1 synthesis and encodes the cell-binding domain of the toxin. *Mol. Microbiol., 33,* 108–118.

11. Setia, A., Bhandari, S. K. , House, J. D. , Nyachoti, M. C. and Krause, D. O.**2009**. Development and *in vitro* evaluation of an *E.coli* probiotic able to inhibit the growth of pathogenic K88 *E. coli* .*J. Anim. Sci*. 5 (4):1-24.

12. Reddy, K.R. **2010**. *Microbiology and Parasitology*.*Question & Answer review*.4thed., P.58-189.PARAS Medical Publisher. London.

13. Oswald,E.,Marc,t.,Roger,B., and Jean,D.R**1994**.Detection of *Escherichia coli* strains producing cytotoxic necrotizing factor type two(cnf2)by enzyme-linked immunosorbent assay. Infection and Immunity 29, 1101–1109.

14. Sare, T., Alessia, F. and Carla, F. **2008**. The Rho activating CNF-1 toxin from pathogenic *Escherichia coli*: A risk factor for human cancer development. *J. Bio. Med*. 3:4