A COMPARATIVE STUDY OF SOME VIRULENCE FACTORS AND PHYLOGENETIC CHARACTRIZATION OF *Escherichia.coli* ISOLATES CAUSING URINARY TRACT INFECTION AND THE COMMENSAL GUT MICROBIOTA

N.A. Ahmed[1*] **S. S. Mahmood[1]** **A. H. Abbas[2]**
Researcher **Assist. Prof.** **Assist. Prof.**
1-Dept. of Biot. Coll. of Sci. University of Baghdad
2-Tropical-Biological Research Unit, College of Science, University of Baghdad-Iraq
Corresponding author: noor02103@gmail.com

ABSTRACT

Variety of virulence factors are involved in the pathogenicity of *Escherichia coli* isolates, the common cause of the urinary tract infections (UTIs). This study was aimed to determine some virulence factors involved in the pathogenicity and the phylogenetic grouping of uropathogenic *E. coli* isolates from UTIs in compare with isolates from gut microbiota (fecal flora) In this study ,E coli isolates were collected from samples urine (n = 25), fecal samples (n = 25) samples of the same patients with UTI, and from fecal samples (n= 5 as control)of patients without UTI. The detection of phylogenetic grouping and some virulence genes among the all isolates were confirmed by PCR technique. The results showed that phylogenetic groups B2,B1(36%) and D (28%) were predominated among uropathogenic *E.coli* in comparison with group A (8%) ,whereas in commensal isolates groups B1(36%), B2(32%) ,D (28%) were more prevalent in compare with group A (4%).The prevalence of *cnf1* and *fimH* genes were higher in UPEC in comparsion with commensal isolates. However, the prevalence of *kpsMT II* gene was similar among both groups, while *hlyA* gene was higher in fecal isolates. According to this results , microbiota may considered the main source of UPEC bacteria.

Keywords: urinary tract infection ,gut microbiota, virulence genes, phylogenetic groups.
***Part of M.Sc. thesis of the 1st author**

مجلة العلوم الزراعية العراقية -2019 :50(4):1193-1198 **احمد وآخرون**

دراسة مقارنة انتشار بعض عوامل الضراوة وتوصيف النشوء الجيني لمسببات القولون الممرضة المسبب لعدوى المسالك البولية ومكروبات الأمعاء المتعايشة

نور عبد الاله احمد[1] **سهاد سعد محمود[1]** **علي حافظ عباس[2]**
باحث **أستاذ مساعد** **أستاذ مساعد**
[1] قسم التقنيات الاحيائية–كلية العلوم/جامعة بغداد /العراق
[2] وحدة الأبحاث البايولوجية للمناطق الحارة–كلية العلوم/جامعة بغداد/ العراق

المستخلص

تشارك مجموعة متنوعة من عوامل الضراوة في القدرة الإمراضية للإشريكية القولونية، والذي يعتبرالسبب الشائع للالتهابات المسالك البولية (UTI). تهدف هذه الدراسة إلى تحديد بعض عوامل الضراوة المتضمنة في الإمراضية والتجمع النشوئي لعزلات الإشريكية القولونية المسببة لالتهاب المسالك البوليه من عدوى المسالك البولية مقارنة مع العزلات من الميكروبات المعوية (النبيت الطبيعي للبراز) في هذه الدراسة، تم جمع عزلة *E. coli* من الادرار (n = 25)، البراز (n = 25) عينات من نفس المرضى الذين يعانون من عدوى المسالك البولية، ومن عينات البراز (n = 5)للسيطرة من المرضى الذين لا يعانون من عدوى المسالك البولية. تم التأكيد على الكشف عن التجمع النشوئي و بعض مورثات الضراوة بين جميع العزلات بواسطة تقنية PCR. أوضحت النتائج أن مجموعات التوالد B2 و B1(36%) وD (28%) كانت سائدة بين الإشريكية القولونية المسببة لالتهاب المسالك البوليه بالمقارنة مع المجموعة A (8%)، بينما في مجموعات العزلات المتعايشة(32%) B2, (36%) B1 ، وكانت D (28%)أكثر انتشارا مقارنة مع المجموعةA (4 %)، وكان انتشار جينات cnf1 و fimH أعلى في الإشريكية القولونية المسببة لالتهاب المسالك البوليه مقارنة مع العزلات المتعايشة. ومع ذلك ، كان انتشار المورث kpsMT II متشابهًا بين المجموعتين ، في حين كان المورث hlyA أعلى في العزلات البرازية. واعتمادا على هذه النتائج، قد تعتبر مايكروبات الامعاء المتعايشه المصدر الرئيسي للبكتيريا الإشريكية القولونية المسببة لالتهاب المسالك البوليه.

كلمات مفتاحية: التهاب المجاري البولية، مايكروبات الامعاء المتعايشة، مورثات الضراوه، المجموعات التطوريه.
***جزء من رسالة ماجستير للباحث الأول**

***Received:18/12/2018, Accepted:3/4/2019**

INTRODUCTION

The *Escherichia coli* is a normal inhabitant of the intestines, both in human and various warm blood animals. It is responsible for 80% of community-acquired UTIs and 30% of nosocomial infections (13) Despite the occurrence of commensal strains in the intestinal microbiota, E. coli can cause to various infections not only in the intestinal system but also in the bloodstream. E. coli that can cause infections outside the intestinal system are designated as extra intestinal pathogenic E. coli (ExPEC) (5). Extraintestinal pathogenic *E. coli* (ExPEC),is divided to three types including neonatal meningitis-causing *E. coli* (NMEC), sepsis-causing *E. coli* (SEPEC), and uropathogenic *E.coli* (UPEC), have been associated with meningitis in newborns, systemic infections, and urinary tract infections (UTIs), respectively. UPEC causes around 90% of community-acquired UTIs and up to 50% of nosocomial UTIs(16).Phylogenetic analyses have shown that E. coli strains fall into four main phylogenetic groups (A, B1, B2, and D) (9,10,1). Based on the presence or absence of 3 markers: an outer membrane hemin receptor gene (*chuA*), the gene encodes for an uncharacterized protein (*yja*) and *TSPE4.C2* unspecified DNA fragment.According to the phylogenetic classification, Pathogenic groups of ExPEC strains generally belong to groups B2 and D and commensal strains that survive in the intestinal system, i.e., non-pathogenic strains, are generally included in groups A or B1(7) involved in the bacterial pathogenic potential, included adherence factors (*e.g.*, type 1 and P fimbriae), toxins (*e.g.*, hemolysin and cytotoxic necrotizing factor), secretion systems, and siderophores (*e.g.*, aerobactin and yersinabactin) (11)., Theses virulence factors (VFs) encoded by virulence associated genes (VAGs) which enables them to effectively colonize and invade host cells and evade host defenses. Most VFs are encoded by mobile genetic elements called pathogenicity islands (PAIs) have previously been investigated in pathogenic bacteria. The aim of this study is to determine virulence genes which are most commonly found in *E. coli* isolates, determination of phylogenetic groups and compare them in isolates from UTIs with the commensal organisms resided in the normal fecal flora.

MATERIALS AND METHODS

Bacterial isolates

A total of 80 sample were collected including 40 urine sample,40 stool sample of the same patients suffering from (UTI) and 5 control samples of healthy people . 20(80%) were isolated from female and 5(20%) isolates were from Male their ages were between (20-50) years how admitted from four hospitals in Baghdad city during 4 month period started from December 2017 to April 2018. Identification of these isolates was performed biochemically with the VITEK 2 Compact System. isolates were stored at -20°C in brain heart with glycerol until they were used.

Detection of phylogenetic and virulence genes by PCR technique

Phylogenetic group and virulence genes were determined by PCR technique. The primers used in this study are listed in Table (1). For the grouping, simultaneous presence, or absence of the *chuA*, *yjaA* gene and a DNA fragment *tspE4.C2* combination in each isolate were determined. The isolates with: *chuA*–, *tspE4.C2* – were placed in the group A, *chuA*–, *yjaA*–, *tspE4.C2*+, group *B1; chuA*+, *yjaA*+, group B2, and *chuA*+, *yjaA*–, group D (11) . Briefly, the total DNA from *E. coli* isolates was extracted by boiling(12), PCR was done in a 25 µl reaction mixture containing 1 µl of template DNA, 1 µM of Each primer, 12.5 master mix that contain (Taq polymerase 2.5µl,2 dNTP(dATP, dCTP, dGTP, dTTP) 250µM, 3 Tris – BASE (pH 9.0) 10 mM , 4 KCl 30mM and 5 MgCl2 1.5mM), then the volum completed to 25 mm by deionized water. The PCR steps were: initiation (94°C, 15 min), 30 cycles of denaturation (94°C, 30 sec), annealing (59-63°C, 30 sec-1min) and extension (72°C, 30sec-1 min), and a final extension interval (72°C, 10 min).

Table 1. Primer sequences used in this study

Gene	Primer sequences (5′- 3′)	Annealing temperature (◦C)	Amplicon size (bp)	Reference
hlyA	F-AACAAGGATAAGCACTGTTCTGGCT R-ACCATATAAGCGGTCATTCCCGTCA	62	1177	17
kpsMT II	F-GCGCATTTGCTGATACTGTTG R-CATCCAGACGATAAGCATGAGCA	60	272	17
fimH	F-ATGAAACGAGTTATTACC R-TTGATAAACAAAAGTCAC	63	508	17
cnf 1	F-GAACTTATTAAGGATAGT R-CATTATTTATAACGCTG	62	543	2
tspE4C2	F-GAGTAATGTCGGGGCATTCA R-CGCGCCAACAAAGTATTACG	59	152	8
yjaA	F-TGAAGTGTCAGGAGACGCTG R-ATGGAGAATGCGTTCCTCAAC	59	211	8
chuA	F-GACGAACCAACGGTCAGGAT R-TGCCGCCAGTACCAAAGACA	59	279	8

RESULTS AND DISCUSSON

The results of identification showed that among 80 samples, 50 samples(including 25 urine and 25 stool) and all 5 control isolates were given a typical morphological characteristics and biochemical tests related to *Escherichia coli*, while the rest. Of isolates belonged to other pathogenic bacteria from different genera like.*Staphylococcus spp*., followed by *Klebsiella spp., Proteus spp., Pseudomonas spp. and Enterobacter spp.* other studies by.(17) reported that E.coli considered the main causeative agent of UT infection. The results of distribution of phylogenetic groups in uropathogenic *E. coli* isolates revealed that Group B2 was the most common (36%), followed by Groups B1 and D in same. Percentage (28%) .while Group A being the least frequent (8%) only, and that agreed with (11)study that the group B2 was the most prevalent group in both fecal and UTIs isolates, followed by group D. Among the commensal isolates, B1(36%) ,B2 (32%) .and D(28%) groups were more in compare with group A (4%) that was very rare group in fecal as in existing in pathogenic *E.coli* ,this result agreed with (11) On the other hand the results of virulence factors screening by PCR technique revealed that *fim*H, *kps*MT ll, *hly*A and *cnf*1 gene among all isolates of urine and stool were (62, 40, 12 and 12)% respectively. with highly attend to increase in pathogenic strains.The majority of the isolates have the *fim*H gene.and *kps*MT ll in(62, 40) % respectively. The *hly*A, and *cnf*1.genes were produced by the same percentage (12%) but it is more common in UPEC (table2,3).

Table 2. Comparison of virulence encoding genes depending on phylogenetic groups in urine sample

VF gene	Phylogenetic group			
	A(n=4)	B1(n=14)	B2(n=18)	D(n=14)
kpsMT II	2	3	4	1
HlyA	1	3	-	-
FimH	1	-	8	8
cnf1	-	-	2	2

Table 3. Comparison of virulence encoding genes depending on phylogenetic groups in fecal samples

VF gene	Phylogenetic group			
	A(n=2)	B1(n=18)	B2(n=16)	D(n=14)
kpsMT II	1	3	5	1
HlyA	1	1	-	-
FimH	-	4	8	2
cnf1	-	-	2	-

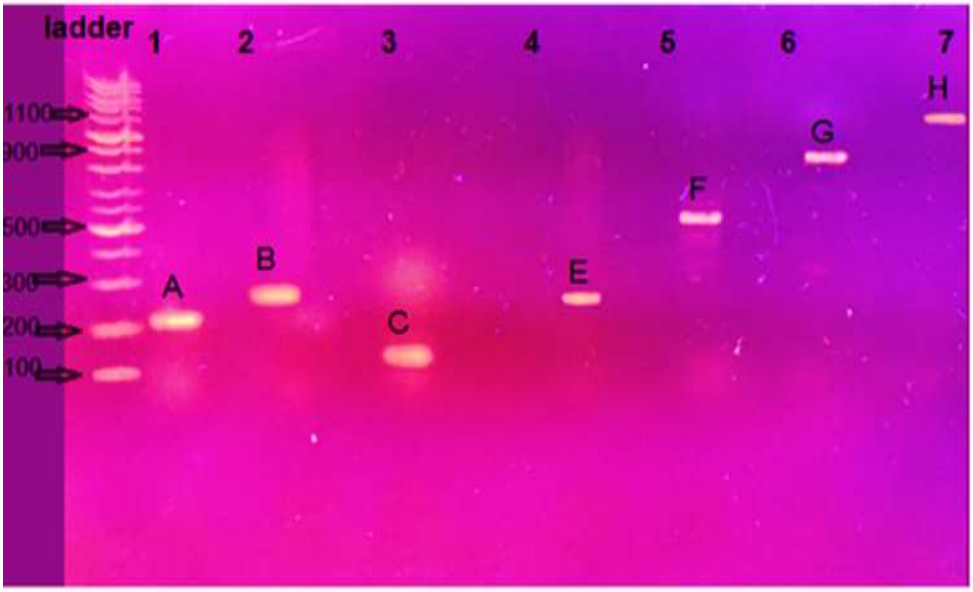

Figure 1. Agarose Gel electrophoresis for Phylogenetic and virulence factors genes:(A)-1. yjaA (211BP), (B)-2. *chuA*(279BP), (C) -3.*tspE4C2*(152BP) genes:(E*) -4. KpsMT II*(272BP),(F)-5. *cnf 1*(543BP), (G) -6.*fimH* (900BP),(H*)-7. hlyA* (1177BP. (Agarose 0.8%, 70min. at 70 voltage). Visualized under U.V light after staining with ethidium bromide. Line1 ladder : DNA marker (100-1500 bp).

Although, *E coli* presence as commensal in nature, pathogenic *E. coli* are the increasing cause of extra intestinal infections such as UTIs, meningitis, sepsis, and various type of intestinal infections (14). Hence , To develop a new strategies for the treatment of MDR isolates, it is essential to understand and characterize their factors essential for pathogenicity or virulence.it is also important to find the distinction between the commensal that are not harmful to their hosts and the pathogens that are aggressive and harmful to their hosts. In this study, the various virulence factors were detected in UTI isolates in compare with the commensal isolates of the fecal flora by molecular methods in order to find a unique characteristics for both. According to this study ,The results of phylogentic groups classification revelaed that group B2 more distributed in UPEC isolates while group B1 is more prevalent among commensal isolates, it was (11) found that the most common phylogroup was group B2 , followed by group D and B1 group was found to be higher in the isolates of the fecal flora , while in the UPEC the group B2 was prevalent. Whereas, In the fecal flora, the group D was less common. In addition (13) found that B2 phylogenetic group was the most prevalent in UTI isolates while group A and B1 were more prevalent in the fecal isolates. Different distribution of various phylotype in *E. coli* strains indicates that the environmental conditions such as climate, host genetic, nutritional, or using antimicrobial agents especially on the commensals in the fecal flora, may affect the distribution of the various phylogenetic groups in a geographical region. Also may this difference can probably be attributed to the bacterial characteristics in different geographic regions under the influence of antibiotics usage or host genetic factors(19). Moreover ,all the virulence factors were found to be more prevalent in pathogenic strains than in commensal strains, as reported previously (19) the *fim H* gene ,which responsible for fimbrea 1, is more prevalence among UPEC than commensal isolates and more prevalent in group B2 and D (18) results mentioned that *fimH* gene was the most prevalent virulence gene and was found in 68% in UPEC and it is highly distributed in group D (57.1%) and B2 **(**44.4%**)**, while it is less common in in group A 25% .but in B1group *FimH* gene was not present. The present results found that *KpsMT II* gene, which responsible for capsule production , is distributed in the same presentage among UPEC and commensal isolates,with highly significtly prevalent in in group B2 . while *hlyA* gene which also present

in the group A and B1 but was more prevelant in UPEC. finally, cytotoxic necrotizing factor 1 (CNF1) gene is more present in UPEC among group B2 and D while in commensal isolates this gene were present in **B2.** In general ,Preveouis studies(6) mentioned that commensal isolates usually belong to groups A and B1, whereas the extraintestinal pathogenic strains belong to groupsB2 and D(6), while in the present study mentioned the distributed of UPEC and commensal isolates within different groups ,this may related to the fact that intestinal *E. coli* are mixture of all the phylogenetic groups and may act as a reservoir for the pathogenic isolates. however, The reason for a commensal strain becoming virulent may be attributed to the multiple strategies of genome plasticity wherein the random point mutations were incorporated for adaptive pathogenic environments (8). In summary UTI isolates were found to have more virulence factors compared to fecal flora. The groups B2 and D were more prevalent in all isolates, they are insinuating and most of them are well equipped with various virulence factors. The virulence factors may be the mportant factors enabling the bacteria to attach, colonize and survive in the host tissue and to start infection whenever the host conditions are in favor of the bacterial infection.

REFERENCES

1.Abdallah ,K.S., Y. Cao and D.J. Wei 2011. Epidemiologic investigation of erxtraintestinal pathogenic *E. coli* (ExPEC) based on PCR phylogenetic group and fimH single nucleotide polymorphisms (SNPs) in China.Int. J Mol.Epidemiol. Genet.353:339-353

2.Ayub, M. J., S. Amir and K. Firdous. 2016. *E. coli* the most prevalent causative agent urinary tract infection in pregnancy: comparative analysis of susceptibility and resistance pattern of antimicrobials. Arch. Clin. 7(4): 25.

3.Blanco, M., J.E. Blanco, J. Blanco, M.P. Alonsob, C. Balsalobrec, M. Mouriñoc, C. Madridc, and A. Juárez. 1996. Polymerase chain reaction for detection of *Escherichia coli* strains producing cytotoxic necrotizing factor type 1 and type 2 (CNFl and CNF2). J Microbiol Methods. 26(1-2): 95-101.

4.Boisen, N., F. Ruiz-Perez, F. Scheutz, K.A. Krogfelt and J.P. Nataro. 2009. High prevalence of serine protease autotransporter cytotoxins among strains of enteroaggregative *Escherichia coli*. Am. J. Tro. Med. Hyg. 80(2):294-301

5.Bozcal, E., V. Eldem, S. Aydemir, M. Skurnik. 2018 . The relationship between phylogenetic classification, virulence and antibiotic resistance of extraintestinal pathogenic *Escherichia coli* in İzmir province, Turkey. Peer J. 6, e5470.

6.Carlos, C., M.M. Pires, N.C. Stoppe, E.M. Hachich, M.I. Sato, and T.A. Gomes. 2010. *Escherichia coli* phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. BMC Microbiol. 10:161.

7.Chakraborty, A., V. Saralaya, P. Adhikari, S. Shenoy, S. Baliga, and A. Hegde. 2015. Characterization of *Escherichia coli* phylogenetic groups associated with extraintestinal infections in South Indian population. Ann. Med. Health Sci. Res. 5(4): 241–246.

8.Chattopadhyay, S., S.J. Weissman, V.N. Minin, T.A. Russo, D.E. Dykhuizen and E.V. Sokurenko. 2009. High frequency of hotspot mutations in core genes of *Escherichia coli* due to short-term positive selection, Proc. Natl. Acad. Sci. 106(30): 12412–7.

9.Clermont, O., S. Bonacorsi, and E. Bingen. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environmen Microbiol. 66(10):4555-8.

10.Foxman, B and P. Brown. 2003. Epidemiology of urinary tract infections: transmission and risk factors, incidence, and costs. Infect Dis Clin North Am .17(2) : 227-41.

11.Hashemizadeh, Z., K. Davood and M. Davood. 2017. Association between virulence profile, biofilm formation and phylogenetic groups of *Escherichia coli* causing urinary tract infection and the commensal gut microbiota: A comparative analysis. Microbial Pathogenesis infect. 110: 545-550.

12.Islam,M.A.,A.Nabi,M.Rahman,M.Islam,D. Ahmed,A.G.S Faruque and P. H. Endtz. 2014.prevalence of faecal carriage of NDM-1-producing bacteria among patients with

diarrhea in Bangladesh. Journal of Medical Microbiology .63(4).620-622.

13.Lee, S., J.K. Yu, K. Park, E-J. Oh, S-Y. Kim and Y-J. Park. 2010. Phylogenetic groups and virulence factors in pathogenic and commensal strains of *Escherichia coli* and their association with blaCTX-M. Ann. Clin. Lab. Sci. 40:361 – 367.

14.Nagarjuna, D., G. Mittal., R. S. Dhanda., P. K. Verma and R. Gaind .2015. Faecal *Escherichia coli* isolates show potential to cause endogenous infection in patients admitted to the ICU in a tertiary care hospital. New Microbes and New Infections. 7: 57-66.

15.Picard. B, J.S. Garcia, S. Gouriou, P. Duriez, N. Brahimi, E. Bingen. 1999. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. Infect Immun.67(2):546–53.

16.Pootong, A., N. Mungkornkeaw, B. Norrapong, and S. Cowawintaweewat. 2018.Phylogenetic background, drug susceptibility and virulence factors of uropathogenic *E. coli* isolate in a tertiary university hospital in central Thailand. Tropical Biomedicine. 35(1): 195–204.

17. Seifu, W. D., and A.D. Gebissa. 2018. Prevalence and antibiotic susceptibility of uropathogens from cases of urinary tract infections (UTI) in shashemene referral hospital, Ethiopia. BMC Infectious diseases. 18(1): 30.

18.Tarchouna M., F. Asma, B. Walid, and B. Jalel. 2013. Distribution of uropathogenic virulence genes in *Escherichia coli* isolated from patients with urinary tract infection. Int J Infect Dis. 17(6): 450–453.

19.Vargova .R., M. Kmetova, K. Curova and L. Siegfried. 2017. Virulence genes in *Escherichia coli* strains isolated from urine of elderly patients. Biologia.72(3): 259-266.

20.Yun K.W., H.Y. Kim, H.K. Park, W. Kim and I.S. Lim. 2014. Virulence factors of uropathogenic *Escherichia coli* of urinary tract infections and asymptomatic bacteriuria in children. J. Microbiol. Immunol. Infect.47(6):455-61.