Determination of Phylogenetic Typing of *E.coli* isolated from UTIs in many hospitals of Baghdad city

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Abstract

Background: *Escherichia coli is* the major of causative agent in most of Urinary tract infections (UTIs), Phylogenetic analyses of *E.coli* isolated from urinary tract infections fall into four phylogenetic groups (A,B1, B2 and D), All Strains of these four groups differ in the characteristics of phenotype as antibiotic resistance , growth rate-temperature relationships and ability to use certain sugars.

Ami of study: To investigate the phylogenetic typing *E.coli* isolated from 660 urine samples from patients with UTIs in many hospitals of Baghdad city,as well as study the relationship between phylogenetic of *E.coli* with many important factors as gender; age and hospital wards.

Materials and Methods: From a total of six hundred - sixty urine samples were collected from UTIs patients in many hospitals (Baghdad/Iraq), through the period from March 2015 to September 2015 and inoculated on (MaCconckey and blood) agar, then incubated at 37 °C for 24 h. ,all isolates were identified by VITEK 2-GN system and Antimicrobial susceptibility pattern was determined by diffusion method (Kirby-Bauer disk) as well as the Phylogenetic grouping of the *E. coli* isolates was determined by a multiplex polymerase chain reaction (PCR)technique.

Results: In this study, *E.coli* was isolated from 660 urine sample from UTIs patients; (400) isolated of *E.coli* strains , high percentage of *E. coli* (41%) was isolated from AL-Yermouk hospital and (18.25%) form AL-Iskan hospital, whilst low percentage as (11.25%) , (13.75%), (14.75%) respectively from (Ghazi AL-Hariri , Iben Beledi and AL-Nooman) hospitals respectively.

Most of strains were isolated from female patients of AL-Yermouk hospital (31%) followed by (15%) from AL-Iskan hospital , as well as AL-Yermouk hospital have high percentage in males (11%) but close ratios in both hospitals (AL-Iskan and Ghazi AL-Hariri) hospitals respectively as (3.25 and 3)% ,whilst in (Iben Beledi hospital and AL-Nooman) hospitals were low ratio as (1 %) and (0.5), so greater percentage (43%) of isolates in this study belonged to phylogroup B2 and (28.25, 24) % for both phylogroup D and A respectively, whilst low percentage (3.5%) for B1, so in this study show 1.25% was untypable as well as the isolation rate of *E.coli* were higher among age (11-20) years (31.75 %), but low percentage (19.25 &19) % in both age groups (≤10 and 21-32) years, so high Prevalence of most phylogenic type (A, B2 and D) as (7.25, 12.5 and 10.75) % respectively in age group (21-30) years, whilst (6%) of phylogenic B1in age group (41-50)years **.**

Key words: *Escherichia coli(E.coli)*, phylogenetic typing groups, urinary tract infections (UTIs),

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الخلاصة

تعتبر بكتريا الاشيرشيا القولونية احدى العوامل الرئيسية المسببة لمعظم حالات التهابات المسالك البولية، وان تطور النمطي لبكتريا الاشيرشيا القولونية المعزولة من التهابات المسالك البولية يمكن تقسيمها الى أربع مجموعات للتنميط الوراثي هي A ، B1، B2 و D , حيث ان جميع سلالات هذه المجموعات الأربع تختلف في خصائص النمط الظاهري كالمقاومة للمضادات الحيوية، معدل نمو و علاقاتها بدرجة الحرارة والقدرة على استخدام بعض السكريات.

هدف الدراسة: دراسة التنميط الوراثي لبكتريا الاشيرشيا القولونية المعزولة من 660 عينة بول لمرضى يعانون من التهاب المسالك البولية في العديد من المستشفيات في مدينة بغداد، وكذلك دراسة العلاقة التنميط الوراثي لبكتريا الاشيرشيا القولونية مع العديد من العوامل المهمة كالجنس العمر و نوع المستشفى.

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المواد وطرق العمل : من مجموع 660 عينة بول جمعت من مرضى التهابات المسالك البولية في العديد من المستشفيات (بغداد/ العراق)، خلال الفترة من مارس 2015 إلى سبتمبر عام 2015 ولقح البول على وسطي الدم و المكونكي اكار، ثم حضنت في درجة 37 ° م لمدة 24 ساعة. وقد تم تشخيص جميع العزلات بجهاز الفايتك اما حساسيتها للمضادات الحيوية فقد درست باستخدام طريقة الانتشار (طريقة كيربي باور) وحددت الانواع التنميط الوراثي الاربع للعزلات باستعمال تقنية P.C.R .

النتائج: في هذه الدراسة تم عزل بكتريا الاشيرشيا القولونية و بنسبة عالية (41٪) من مستشفى اليرموك و (18.25٪) من مستشفى الاسكان، بينما كانت بنسبة منخفضة (11.25٪)، (13.75٪)، (14.75٪) على التوالي من مستشفى (غازي الحريري، ابن البلدي و النعمان)على التوالي.

وقد عزلت معظم السلالات من الاناث المريضات لمستشفى اليرموك التعليمي وبنسبه31) ٪ (تليها (15٪) لمستشفى الاسكان، اما نسبة الذكور فقد كانت عالية في مستشفى اليرموك (11٪) لكنها قريبة نسبيا في كل من مستشفى الاسكان وغازي الحريري (3.25 و 3)٪على التوالي , بينما منخفضة في كل من مستشفى ابن البلدي و النعمان وبنسبة (1 و 0.5)% .

وفي هذه الدراسة وجد نسبة عالية من العزلات (43٪) تنتمي الى النمط الوراثي phylogroup B2 و(28.25, 24)٪ لكلا نوعي النمط الوراثي D و A على التوالي، في حين نسبة منخفضة (3.5٪) الى النمط الوراثي B1، وايضا اظهرت النتائج ان 1.25٪ غير منمط اضافة لذلك فقد كان معدل عزل البكتريا عاليا في الفئة العمرية (11– 20) سنة وبنسبة (31.75٪)، ولكنها كانت منخفضة (19.25 و 19)٪ في كل من المجموعتين العمريتين (≤10 و32–21) سنة،وايضا سجلت الدراسة انتشار عالية لمعظم أنواع النمط الوراثي A، B2وD بنسب (7.25، 12.5 و 10.75)٪ على التوالي في الفئة العمرية (21–30) سنة، بينما (6٪) من النمط الوراثي B1 في الفئة العمرية (41–50) سنة.

الكلمات المفتاحية: لإشريكية القولونية (E.coli)، مجموعات الكتابة النشوء والتطور، والتهابات المسالك البولية (التهاب المسالك البولية)

Introduction

Urinary tract infection (UTI$_s$) is one of the most common bacterial infections (Selvarangan *et al.*,2004), one of the most common of gram negative bacterial isolated from (UTIs) were *Escherichia coli* (Sivick and Mobley, 2010), Although these bacteria (*E.coli*) is conceder as an extracellular pathogen but it has ability to invade several stratified layers of the bladder cell, Invasion to the host cell simplifies both the persistence and establishment *E.coli* in the urinary tract (Johnson *et al.*,2005).

Consideved *E.coli* is important bacteria , because it was considered as one of the most sequenced organisms in GenBank in terms of the number of sequenced genomes available .

Sequencing- based the genotyping methods were becoming increasingly popular in many epidemiological studies of infections as urinary tract infection, but so far Korea only the classical phylogrouping method is used for analysing clinical *E.coli* isolates from patients with UTI$_s$ (Choi *et al.*,2012).

Enterobacterial repetitive intergenic consensus (ERIC) sequences are a genomic fingerprinting technique that generates specific strain patterns , which also known as intergenic repetitive units, that's located in intergenic regions as the palindromes of (127 bps),but differ the copy numbers in the different bacteria such as 30 copies reported in strains of *E. coli* K-12 (Duchaud *et al.*,2003) so ERIC founded in most bacterial species such as *Enterobacteriaceae* family (Hulton,1991).

E. coli strains fall into four main phylogenic groups; A, B1, B2 and D (Herzer *et al.*,1990), the phylogenetic groups A and B1 were considered as sister groups whilst phylogenetic group B2 was included in the ancestral branch (Lecointre *et al.*,1989).

Materials and Methods

Study patients: All patients who attendants to many Hospitals (Ghazi AL-Hariri ; Iben Beledi; AL-Iskan ; AL-Nooman and AL-Yermouk) in Baghdad city,the study conducted in the period from March 2015 to September 2015.

Specimen collection:

Growth conditions :

All samples were derived from the fresh midstream urine, inoculated (0.01 mL) on blood agar and MacConkey agar, then incubated at 37 °C for 24 h.

Identification of *E.coli* isolates : the isolates of *E.coli* were identified by VITEK 2-GN cards (bioMérieux, USA).

DNA extraction and Phylogenetic typing group of E.coli isolates

The DNA of the selected isolates was extracted by kit, So Phylogenetic groups of the *E coli* isolates were determined by multiplex PCR-based technique was performed by used three primers (ChuA.1, YjaA.1 and TspE4C2.1 which designed according to the basis of published sequencing and synthesized in Alpha DNA Company (Canada) as the following:

1- **ChuA.1**: (5′-GACGAACCAACGGTCAGGAT-3′)
2- **YjaA.1** : (5′-TGAAGTGTCAGGAGACGCTG-3′)
3- **TspE4C2.1**:(5′-GAGTAATGTCGGGGCATTCA-3′), which generate 279 b.p, 211b.p , and 152 bp fragments, respectively.

Statistical analysis

For the analysis of study data, Microsoft Office Excel 2010 and SPSS (Statistical Package for Social Sciences) were used as software program.

Results

Table-1: Distribution of *E.coli* according to gender and the hospitals ward isolated from.

Gender	Hospitals ward *E.coli* No.(%)					Total(%)
	G.H hospital No.(%)	I.B hospital No.(%)	A.I hospital No.(%)	A.N hospital No.(%)	A.Y hospital No.(%)	
Female	33 (8.25)	51(12.75)	60(15)	57(14.25)	124(31)	325(81.25)
Male	12(3)	4(1)	13(3.25)	2(0.5)	44(11)	75(18.75)
Total	45(11.25)	55(13.75)	73(18.25)	59(14.75)	168(41)	400(100)

NOTE: G.H: Ghazi AL-Hariri hospital I.B: Iben Beledi hospital , A.I: AL-Iskan hospital, A.N: AL-Nooman hospital.; A.Y: AL-Yermouk hospital

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he total isolated of (400) *E.coli* strains from urine sample of patients severing from Urinary tract infections (UTIs) , according to hospital wards, *E.coli* isolated were classified into five categories , these categories were outlined in the table -1 as the following :one hundred sixty –eight strains (41%) were isolated from AL-Yermouk hospital and 73(18.25%) form Al-Iskan hospital, whilst low numbers as 45 (11.25%) , 55(13.75%) and 59(14.75%) respectively isolated from (Ghazi AL-Hariri, Iben Beledi and AL-Nooman)hospitals respectively.

On the other hand; the proportion of positive cultures is very high among females (No.= 325(81.25%) as compared to males (No.=75(18.75%). Most of the strains were isolated from female patients of AL-Yermouk hospital (No.=124(31%)

followed by 60(15%) from Al-Iskan hospital, whilst the isolate from (AL-Nooman, Iben Beledi and Ghazi AL-Hariri) hospitals respectively as 57(14.25), 51(12.75) and 33(8.25) respectively, as well as AL-Yermouk hospital have high number of males 44(11%), but close ratios in both hospitals (Al-Iskan and Ghazi AL-Hariri) hospital as13(3.25%), 12(3%) but in (Iben Beledi and Nooman) hospital as low ratios as 4(1 %) and 2(0.5%) in (Table 1).
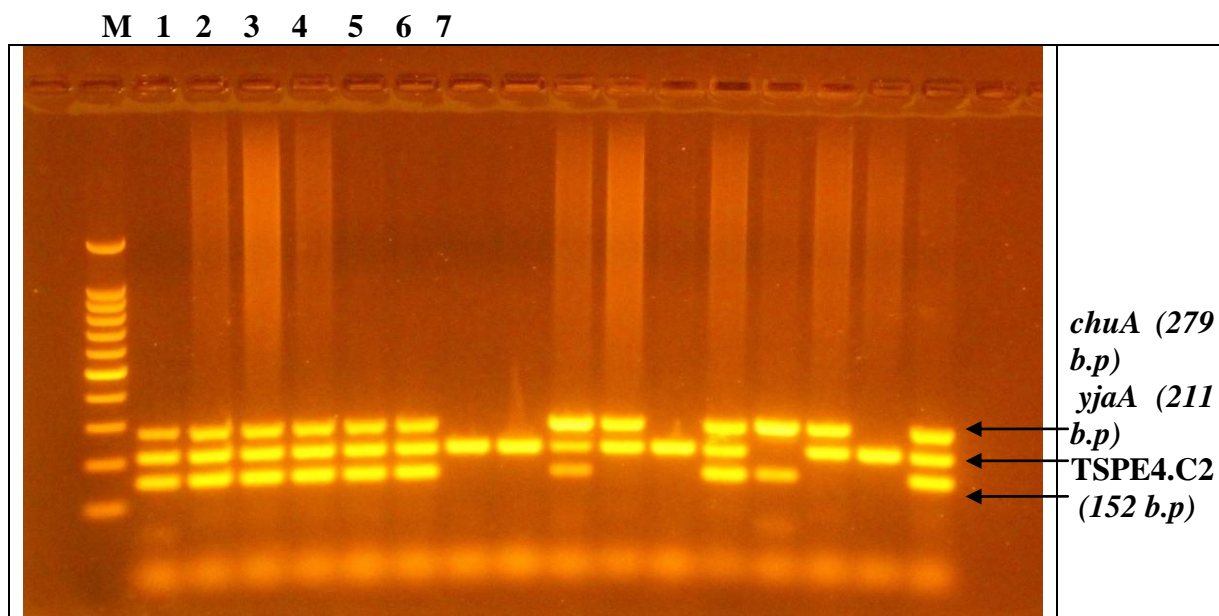

Figure (1): Triplex PCR profiles specifically for *E. coli* phylogenetic groups(Each combination of *chuA* and *yjaA* gene and TSPE4.C2) group A (Lanes 1 and 2); group B1 (lane 3); group D(lanes 4 and 5) ; group B2 (lanes 6 and 7) markers (in Lane M so Lane L contains a 100-bp marker).

Table- 2: Distribution the classes of Phylogenic of *E.coli* according to the Hospital ward.

Hospitals ward	Phylogenic classes (Number, %)				Untypable
	A	**B1**	**B2**	**D**	
G . H	15(3.75)	1(0.25)	14(3.5)	13(3.25)	2(0.5)
I. B	8(2)	3(0.75)	21(5.26)	22(5.5)	1(.25)
A . I	21(5.25)	3(0.75)	20(5.25)	29(7.25)	0(0)
A. N	7(1.75)	0(0)	34(8.5)	18(4.5)	0(0)
A.Y	45(11.25)	7(1.75)	83(20.75)	31(7.75)	2(0.5)
Total	96(24)	14(3.5)	172(43)	113(28.25)	5(1.25)

NOTE : G.H : Ghazi AL-Hariri hospital I.B: Iben Beledi hospital , A.I**:** AL-Iskan hospital, A.N: AL-Nooman hospital.; A.Y: AL-Yermouk hospital

Table -2 showed a greater percentage (43%) of isolates in the study belonged to phylogroup B2 and (28.25 and 24) % for both phylogroup D and A respectively, but a low percentage (3.5%) for B1,so in this study show 1.25% was untypable (see figure 1).

Table - 3: Distribution the classes of Phylogenic of *E.coli* according to Hospital ware and gender.

| Hospitals ward | Phylogenic types (Number , %) | | | | | | | | Untypable | | Total | |
| | A | | B1 | | B2 | | D | | | | | |
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
G.H	12 (3)	3 (0.75)	1 (0.25)	0 (0)	8 (2)	6 (1.5)	9 (2.25)	3 (0.75)	1 (0.25)	1 (0.25)	33 (8.20)	12 (31)
Iben-Beledi	8 (2)	0 (0)	3 (0.75)	0 (0)	18 (4.5)	3 (0.7)	21 (5.25)	1 (0.25)	1 (0.25)	0(0)	51 (12.75)	4 (1)
A.I	15 (3.75)	6 (1.5)	2 (0.5)	1 (0.25)	17 (4.25)	3 (0.75)	26 (6.5)	3 (0.75)	0(0)	0 (0)	60 (15)	13 (3.25)
A.N	7 (1.75)	0 (0)	0 (0)	0 (0)	33 (8.25)	1 (0.25)	17 (4.25)	1 (0.25)	0 (0)	0 (0)	57 (14.75)	2 (0.5)
A.Y	34 (8.5)	1 (2.75)	5 (1.25)	2 (0.5)	56 (14)	27 (6.75)	0(0)	0(0)	2(0.5)	0(0)	124(31)	44 (11)
Total	76 (19)	20 (5)	12 (3)	2 (0.5)	132 (33)	40 (10)	57 (14.8)	2 (0.5)	4 (1)	1 (0.25)	325(81.25)	75 (18.75)

NOTE : G.H : Ghazi AL-Hariri hospital I.B: Iben Beledi hospital , A.I**:** AL-Iskan hospital, A.N: AL-Nooman hospital.; A.Y: AL-Yermouk hospital

Results in table (3) showed the phylogenic group B1 was prevalent among Male patients of AL-Yermouk hospital (14%), followed AL-Iskan (3.75)% whilst Lower proportion of males were in Ghazi AL-Hariri Hospital(3%) , as well as males were more than females in all other phylogenic (A,B1,B2,D) in the five hospital (Ghazi AL-Hariri; Iben- Beledi; AL-Iskan; AL-Nooman and AL-Yermouk)as percentage (19,3,33,14.8)% respectively when compare with female in the same phylogenic and hospital (5,0.5 ,10,0.5) % respectively, also all phylogenic groups A, B1 and B2 have high percentage in male patients in AL-Yermouk hospital as (8.5, 1.25 , 14, 0.75) excepted phylogenic D that great percentage in AL-Iskan hospital .

Table -4: Distribution the classes of Phylogenic of *E.coli* according to age groups

| Phylogenetic type | Age groups(years) | | | | | | | Total |
	≤ 10	11-20	21-30	31-40	41-50	51-60	>60	
A	19	5	29	18	11	10	4	96
%	4.75	1.25	7.25	4.5	2.75	2.5	1	24
B1	3	2	4	1	4	0	0	14
%	0.75	0.5	1	0.25	1	0.25	0.25	3.5
B2	22	16	50	39	24	17	4	172
%	5.5	4	12.5	9.75	6	4.25	1	43
D	26	2	43	16	20	4	2	113
%	6.5	0.5	10.75	4	5	1	0.5	28.25
Un	0	0	1	2	0	2	0	5
%	0	0	0.25	0.5	0	0.5	0	1.25
Total	70	70	25	127	76	59	33	10
%	19.25	19.25	6.25	31.75	19	14.75	8.25	2.5

In this study the other important factor is age, the isolation rate of *E.coli* were higher percentage 31.75 % among age (11-20) years (and nearly same percentage (19.25 &19)% of the isolates from both age groups (≤10 and 21-32)years, so different prevalence of most phylogenic type (A, B2 and D) as (7.25, 12.5 and 10.75) % respectively in age group (21-30) years, whilst increased prevalence of phylogenic B1 in age group (41-50)years.

Discussion

Results of current study showed a high percentage of *E. coli* strains belonged to Phylogenetic B2 (42%) in five hospitals of Baghdad city , This is fully compatible with the findings of Choi *et. al.,*2012, who explain the B2 phylogroup was the most common (84%) type, so B2 and D phylogroup comprised the majority (95%) of the *E. coli* isolates, as well as in many studies, phylogroup B2 has been shown most prevalent *E.coli* isolate in UTIs as well as results of Micenková *etal.,*2016 showed phylogroup B2 were the most common (38.3%), followed by phylogroups A (28.3%), D (26.3%) and B1 (7.2%) (Micenková *etal.,*2016), this phylogrouping strategy has been used extensively worldwide.

Phylogenetic features of *E. coli* strains on the basis of Phenotypic and genetic traits that initially difficult appeared such as Phylogenetic characterization trait must meet different criteria for use in Phylogenetic character: as the following when the group that is characterized emerged, gene must deleted or acquired as well as in the candidate gene the recombination event must be very rare, that's meaning natural selection must not be targeted the gene product, which favours new genetic recombination so, the same gene must stabilized by ruling out its subsequent deletion or horizontal transfer among the bacteria belong to the others Phylogenetic groups (Whittam, 1996).

The *E. coli* isolates from urine sample of patients with urinary tract infections (UTIs) belonging to groups A and B1 that considered as commensal or true pathogens of *E. coli* which producing the infection in the host (Sabate *et. al.*, 2006), so results of this study showed male infected UTIs by *E.coli* more than females ,This results were fully compatible with the results of Montini *et. al.,*2011 who showed that nearly 7% of females and 2% of males have UTI during the first eight years of the life (Montini *etal.,*2011), as well as study of Johnson *etal.,* 2003 indicate (12%) males and (10-20%) females experience an acute symptomatic UTI,and even highly numbers develop asymptomatic bacteriuria (Johnson *et. al.,* 2003).

Females more than male because many causing such as in female urodynamic disturbances occurring with the age and changing in sex hormone as well as anatomy factor, the opening of the urethra in female is closer to the anus and external genital area so urethra is shorter , also bacteria are more likely to move up it, and these Bacteria are present around external genital area as normally , so after defecation, the direction of wiping (wiping back to front) can lead to fecal contamination of the urogenital orifices (Al-Dahhan *et. al.,*2016).

As well as the isolate of *E.coli* rate were higher among patients of age group (11-20) years, but this results was contrary to the results of Al-Dahhan *et. al.,*2016, who showed high percentage *E.coli* isolate (25.2%) in (41-50) age group, at the same time high percentage of isolate in females than male during the two years ,Whilst in al-qadysia and Babylon high *E.coli* isolated in age group (21-30) in percentage (20.8 and 20.9)% respectively as well as (12.8 and 20.5) % in both age groups (1-10) and (21-30) (Al-Dahhan *et. al.,*2016).

The researchers Kuhnert *et. al.,* explained in their study that all isolates belonging to groups B2 and D are potentially pathogenic (Kuhnert *etal.,* 1997),In the

Worldwide phylogenetic analyses have demonstrated that virulent *E. coli* strains belong mainly to group B2 and, to a lesser extent, to group D. In contrast, most of the commensal strains are associated with group A or group B1 (Basu *et. al.*, 2013; Ejrnaes,2011), whilst study of Yanping *et. al.* (2012) ,who reported that the predominant group *E.coli* recovered from UTI patients in her study belonged to phylogroup D.

Conclusion

The study conclude that *E.coli* was isolated from females more than males and most of the strains were isolated from patients of AL-Yermouk hospital followed by AL-Iskan hospital, whilst low numbers from (AL-Nooman, Iben Beledi and Ghazi AL-Hariri) hospitals, so AL-Yermouk hospital have high number of isolate in males, as well as the greater isolates of *E.coli* belonged to phylogroup B2 followed both phylogroup D and A, So the rate of *E.coli* isolated were higher among age (11-20) years whilst high Prevalence of most phylogenic type (A, B2 and D) in age group (21-30) years, but phylogenic B1 in age group (41-50) years.

As well as high isolated of *E. coli* from (AL-Yermouk and AL-Iskan) hospitals, whilst low numbers of (Ghazi AL-Hariri , Iben Beledi and AL-Nooman) hospitals respectively, so most of strains were isolated from female patients of AL-Yermouk , AL-Iskan hospital

Acknowledgments

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