

ORIGINAL ARTICLE

Quadruplex PCR for Phylogenetic Analysis of Uropathogenic *Escherichia Coli* in Iraqi Population

Meraim A. Kazaal¹,

¹Department of Nursing Techniques, Technical Institute of Al-Diwaniyah, Al-Furat Al-Awsat Technical University, Iraq

Corresponding author:

meraim.kazaal@atu.edu.iq

Department of Nursing Techniques
Technical Institute of Al-Diwaniyah
Al-Furat Al-Awsat Technical University
Al-Diwaniyah, Iraq.

Received: Sep 07, 2023,
Revised: Oct 23, 2023,
Accepted: Oct 26, 2023,

DOI: 10.57238/jbb.2023.7109.1049



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article online

Abstract

Background *Escherichia coli* (*E. coli*) are the most common cause of urinary tract infection (UTI). Clermont and his coworkers recently classified *E. coli* strains into eight phylogenetic groups using a new quadruplex PCR method. This study aimed to identify the phylogenetic groups of *E. coli* based on Quadruplex PCR method to detect any of the phylogenetic groups that mainly cause UTIs in the Iraqi population.

Method Isolates of Uropathogenic *E. coli* (UPEC) and other bacteria are identified by culture media and biochemical test, and then UPEC is subjected to phylogenetic analysis by a quadruplex PCR method.

Results Out of 210 urine samples, only 114 (54%) samples have a positive culture for *E. coli* with a count of 10⁵ CFU/mL. The frequency of other bacteria are *Enterobacter* (24%), *Proteus* (10%), *Pseudomonas* (6%), *Klebsiella* (3%), *Citrobacter* (2%), and *Acinetobacter* (1%). In the present study, only seven groups of UPEC are detected and PCR profiles showed most UPEC isolate are related to B2 (42(36.8%)) followed by D (24(21.1%)), A 12((10.5%)), C(13(11.4%)), F (9(8%)), clade1 (8 (7%)), E (3(2.6%)) and unknown (3(2.6%)) but not detected any isolate below to group B1 (0(0%)). In the existing study, most phylogenetic groups were isolated from females and from patients in age groups 2, 3, and 4 (young and adults).

Conclusion Only seven phylogenetic groups of UPEC are detected, including A, B2, C, D, E, F, and clade 1. Phylogenetic group B2 was the most common cause of UTI in the Iraqi population. Moreover, most UPEC phylogenetic groups are isolated from young and adult females.

Keywords: UPEC; Phylogenetic group; Quadruplex PCR; UTI

1 Introduction

Urinary tract contaminations are among the most continuous bacterial irresistible illnesses, influencing the two inpatients and short-term patients about the world [1]. A urinary lot disease is a contamination in any piece of the urinary framework: kidney, ureters, bladder, and urethra [1,2].

Most UTIs are achieved by organisms that colonize the gastrointestinal part, which go on to escape from the butt and assault the urethra; starting there, they can make an excursion up to the bladder and, if the infection is not treated, will continue to corrupt the kidney [3–5]. Women are forever inclined to UTIs on the grounds that they have more limited urethras, which permit microorganisms speedy admittance to the bladder. Additionally, sex can bring microbes into

the urinary plot. *E. coli* is liable for over 81% of all UTIs, as per a report in a diary of arising irresistible sickness [2,6].

Clermont and his partners fostered a trio PCR measure to distinguish the qualities *chuA*, *yjaA*, and *TspE4.C2* in 2000. Respect the presence/nonappearance of these qualities, an *E. coli* strain could be characterized into one of the vitally phylogenetic gatherings, A, B1, B2, or D [7]. The growing body of multilocus sequence type (MLST) information for *E. coli* reports from various hosts and natural surroundings showed over 80% of the phylogenetic gatherings groupings are right [8].

Overall phylogenetic investigations have shown that harmful extra-digestive *E. coli* strains have a place for the most part with bunch B2 and, to a lesser degree, to bunch D. while most of the commensal strains are related with bunch B1 and A [9,10]. In 2013, Clermont developed triplex PCR to Quadruplex by adding *trpA* and *arpA* genes to previous processes. By Quadruplex PCR, four new phylogenetic groups are identified, which include E, F, C, and clade 1 [8]. To our insight, this is the main review using the new Quadruplex PCR technique to order phylogenetic gatherings of UPEC isolated from UTIs in Iraq. The present study aimed to determine phylogenetic groups of UPEC according to the quadruplex PCR of Clermont and his coworkers in 2013 [8] and detect any group that was mainly associated with UTI to find if the patient's age or gender affected prevalence, distribution, and typing of these groups.

2 Patients and samples test

2.1 Study design and sample collection

This exploration is a cross-sectional review that comes in concurrence with the morals of Al-Diwaniyah Teach-

ing Hospital. Showing Clinic and verbal informed assent was gotten from all members. 210 pee tests were gathered in the sterile holder from patients with UTI (age range from 1 to long term) in the period from April 2022 to Jun 2023.

2.2 Isolation and identification of bacteria

Urine samples were directly transported to the microbiology lab in Al-Diwaniyah Teaching Hospital. The distinguishing proof of *E. coli* from other bacterial isolates was finished relying upon morphological highlights and the rose pink shade of the territories on MacConkey agar plats that avowed by subculture on Eosin Methylene Blue agar and hatched for 24 hours at 37°C, the typical greenish metallic sheen tone show of *E. coli* [11,12] then the result asserted by biochemical tests and minutely evaluation with Gram's stain, after fundamental distinctive verification of *E. coli* bacterial cell refined in Supplement stock for DNA extraction for molecular survey.

2.3 Molecular study

DNA was extricated from bacterial stock according to the creators' rules of Genomic DNA tiny Pack (Geneaid). The eliminated DNA was electrophoresed on agarose gel (0.5% agarose stained with 5µL of ethidium bromide) to certify that DNA was present in every model. Phylogenetics is not completely settled by multiplex PCR. Improvement reactions for all preparations (Table 1) were driven in a 0.2 ml holder of Accu Power PCR Premix tube according to the bioneer association's direction.

Table 1: Genes, primers, PCR reaction, and products used in phylogenetic analysis [8,14].

PCR reaction	Target	Primer ID	Primer sequence	PCR product (bp)
Quadruplex	<i>chuA</i>	ChuA.1b	5-ATGGTACCGGACGAACCAAC-3	288
		chuA.2	5-TGCCGCCAGTACCAAAGACA-3	
	<i>yjaA</i>	yjaA.1b	5-CAAACGTGAAGTGTTCAGGAG-3	211
		YjaA.2b	5-AATGCGTTCCTCAACCTGTG-3	
	<i>TspE4C2</i>	TspE4C2.1b	5-CACTATTCGTAAGGTCATCC-3	152
		TspE4C2.2b	5-AGTTTATCGCTGCGGGTTCGC-3	
<i>arpA</i>	Acek.f	5-AACGCTATTCGCCAGCTTGC-3	400	
	ArpA1.r	5-TCTCCCATAACCGTACGCTA-3		
Group E	<i>arpA</i>	ArpAgpE.f	5-GATTCCATCTTGTCAAAATATGCC-3	301
		ArpAgpE.r	5-GAAAAGAAAAAGAATTCCCAAGAG-3	
Group C	<i>trpA</i>	ArpAgpC.1	5-AGTTTTATGCCAGTGCAG-3	219
		ArpAgpC.2	5-TCTGCGCCGGTCCACGCC-3	
Internal control	<i>trpA</i>	TrpBA.f	5-CGGCGATAAAGACATCTTAC-3	489
		TrpBA.r	5-GCAACGCGGCCTGGCGGAAG-3	

The PCR tube vortexed until the lyophilized pellet deteriorated and all mixes were mixed; then, at that point, the PCR tube was set into Capable TR/O Thermocycler under the going with conditions: beginning denaturation at 94°C for 4min and 30 cycles for each denaturation at 94°C for 5sec, hardening at 57°C for 20sec (bunch E) or 59 °C for 20sec (quadruplex and bunch C), heightening at 72°C for 1min, and last increase at 72 °C for 5min. PCR things were poor somewhere near electrophoresis with a 2% agarose gel stained with DNA-safe stain and imagined using Gel-Doc 2000 transilluminator [3,8,13].

2.4 Statistics

Measurable investigation was done utilizing SPSS20 with Microsoft Excel 2010, and we considered the probability value less than 0.05 to be statistically useful.

3 Result

3.1 Isolated bacteria from UTI

Out of 210 urine samples, only 114 (54%) samples have a positive culture for *E. coli* with enumeration equal to 10⁵ CFU/mL. The frequency of remaining bacteria are *Enterobacter* (24%), *Proteus* (10%), *Pseudomonas* (6%), *Klebsiella* (3%), *Citrobacter* (2%), and *Acinetobacter* (1%), as in Figure 1.

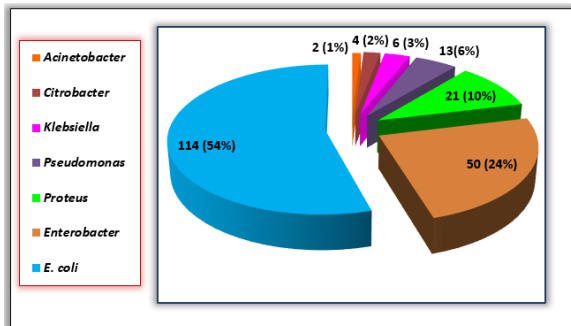


Figure 1: Percent of bacterial isolates in the urine sample of a patient with UTI (P<0.05).

4 Phylogenetic analysis of UPEC

Analysis and detection of UPEC Phylogenetic groups were performed according to quadruplex PCR of Clermont and his coworkers in 2013 [8] as in Figures 2 (i and ii). In the present study, only seven groups of UPEC are detected and PCR profiles showed most UPEC isolates are related to B2 (42(36.8%)) followed by D (24(21.1%)), A (12(10.5%)), C (13(11.4%)), F

(9(8%)), clade1 (8 (7%)), E (3(2.6%)) and unknown isolates (3(2.6%)) but not detected any isolate below to group B1(0(0%)) as in Figure 3.

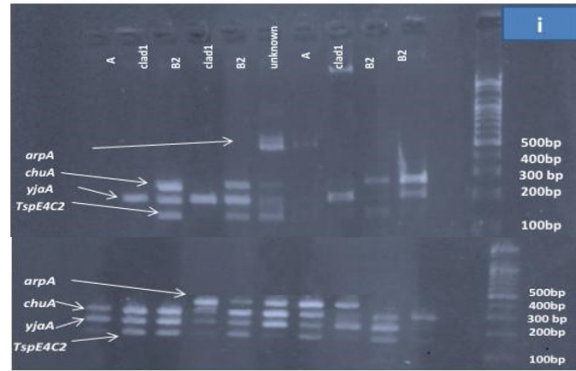


Figure 2: Analysis and detection of UPEC Phylogenetic groups by using Clermont quadruplex PCR.

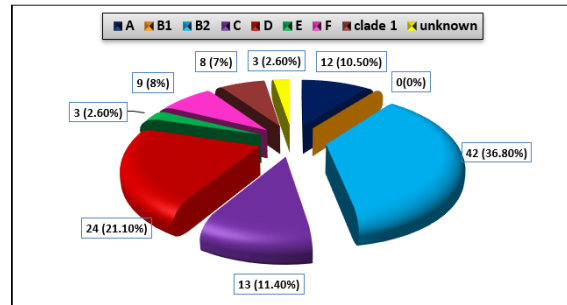


Figure 3: Distribution of UPEC Phylogenetic Groups Among Studied Population.

5 Distribution of UPEC Phylogenetic Groups according to age groups and gender

In the present study, patients who are infected by UPEC are grouped according to age range and gender as in Table 2. Most UPEC detected in patients with age range from 36 to 45 year 33(28.9%) followed by 26-35 year 30 (26.4%), 46-55 year 21 (18.4%), 16-25 year 20 (17.5%) and 1-15 year 10 (8.8%). Moreover, most UPECs were isolated from females 95 (83%), and only 19(17%) were isolated from males.

Most phylogenetic group A detected in age group 1 (50%) also appeared in 16.7% in each one of age groups 2 and 3 but not detected in age group 5 (0%) as in Table 3. phylogenetic group B2 was seen in all age groups, but the highest percent of B2 was recorded in age groups 4 (31%) and groups 5 (23.8%). Group C was detected in age groups 4 (46%), 3 (31%), and age group 2 (23%) but not identified in age groups 1

(0%) and 5 (0%). In the same line, group F was also observed in age groups 4 followed by age groups 3 and 2 (55.6%, 33.3%, and 11.1%, respectively). Group D mainly appeared in age group 5 (42%) and was also detected in age groups 2 and 3 (21% for each one) and appeared in 17% of patients in the age group but was not detected in group 1 (0%) whereas group E present only in age groups 1, 2 and 3 (33.3% for each one). On the other hand, 75% of clade 1 are detected in age group 3, and 12.5% isolated from each one of age groups 2 and

4. Moreover, Unknown isolates were detected in only age groups 4 (66.7%) and 2(33.3%). Table 4 displays there are massive contrasts in dispersion of UPEC phylogenetic according to gender ($p < 0.05$) when the highest percentage of phylogenetic groups A, B2, C, D, E and Clade 1 present in females (75%, 83.3%, 84.6%, 79%, 66.7% and 87.5% respectively) compared with males (25%, 16.7%, 15.4%, 21%, 33.3% and 12.5% respectively). Additionally, phylogenetic group F and unknown isolates were detected in females only (100

Table 2: Distribution of UPEC infection among age groups and gender.

Age groups	Age range (year)	N*	%
Group1	1-15	10	8.8
Group2	16-25	20	17.5
Group3	26-35	30	26.4
Group4	36-45	33	28.9
Group5	46-55	21	18.4
Total	1-55	114	100
Gender	N		%
Males	19		17
Females	95		83

*N = Number

Table 3: Dissemination of UPEC phylogenetic groups among age-groups

UPEC Phylogenetic		Patients age groups					P value
Groups	Number	Group1	Group 2	Group3	Group4	Group5	
		N (%)	N (%)	N (%)	N (%)	N (%)	
A	12	6 (50)	2 (16.7)	2 (16.7)	2 (16.7)	0 (0)	0.022*
B2	42	3 (7.1)	7(16.7)	9(21.4)	13(31)	10 (23.8)	0.0157*
C	13	0 (0)	3(23)	4(31)	6(46)	0 (0)	0.008*
D	24	0 (0)	5(21)	5(21)	4(17)	10(42)	0.0106*
E	3	1(33.3)	1(33.3)	1(33.3)	0 (0)	0 (0)	0.042*
F	9	0 (0)	1(11.1)	3(33.3)	5(55.6)	0 (0)	0.0131*
Clade 1	8	0 (0)	1(12.5)	6(75)	1(12.5)	0 (0)	0.0077*
Unknown	3	0 (0)	0 (0)	0(0)	2 (66.7)	1 (33.3)	0.038*

*N = Number, * = Different according to statistical measures ($p < 0.05$)

Table 4: Distribution of UPEC phylogenetic groups according to gender

UPEC Phylogenetic		Males	Females	P value
Groups	Number	N (%)	N (%)	
A	12	3 (25)	9 (75)	0.0011*
B2	42	7 (16.7)	35 (83.3)	0.0003*
C	13	2 (15.4)	11 (84.6)	0.00022*
D	24	5 (21)	19 (79)	0.0065*
E	3	1(33.3)	2 (66.7)	0.0074*
F	9	0 (0)	9 (100)	<0.0001*
Clade 1	8	1 (12.5)	7 (87.5)	0.00019*
Unknown	3	0(0)	3(100)	<0.0001*

*N = Number, * = Statistically significant ($p < 0.05$)

6 Discussion

This study found that *E. coli* is the primary etiological agent of UTIs, particularly in women in comparison to men and at different ages [15,16]. *E. coli* is a bacteria with dual functions, including being both a fundamental and beneficial microbiota in the digestive tract and an opportunistic pathogen that causes intestinal and extraintestinal diseases. At present, the clinical administration of UTIs is a significant worldwide issue due to the expansion in diseases brought about by *E. coli* strains that have obtained protection from ordinarily utilized antimicrobial specialists [17,18]. The present review is devoted to the Identification of phylogenetic gatherings of UPEC as per quadruplex PCR of Clermont and his collaborators in 2013 and the discovery of any gathering that basically connected with UTI so location in the event that the patient's age or orientation affected predominance, dispersion and composing of these gatherings [8].

In the present review, just six phylogenetic gatherings of UPEC are recognized by quadruplex PCR of Clermont, and three secluded excess unclassified. In current outcomes, most UPEC phylogenetic gatherings connected with bunch B2 and D have yet not identified any segregations connected with bunch B1. UPEC phylogenetic gatherings B2 and D, which are more normal in UIT, might have harmfulness elements and anti-toxin opposition qualities or might have other unique systems that make them more normal in UTIs contrasted with other UPEC phylogenetic gatherings [10]. Ochoa et al. (2016), Molina-Lopez et al. (2011), and Nuesch-Inderbinen et al. (Phylogenetic group B2 has been linked to numerous drug-resistant strains and increased expression of virulence factors such as fimbriae, which mediated their attachment to the urinary tract [19–21].

Different examinations found tenacious and repetitive UTIs have additionally been related to phylogenetic gathering B2, and this has been embroiled in the pathogenesis of pyelonephritis [22,23]. The present review concurs with an exploration of El-Shaer et al. (2018), who identified clinical confines were more predominant in phylogenetic gatherings B2 (22.2%) and D (23.6%), while ecological segregates were in bunches A (24.2%) and B1 (60.6%). El-Shaer and his companions found that a greater part of harmfulness qualities were higher in clinical *E. coli* segregates (bunch B2) [13]. Our discoveries in accordance with concentrated on Iran, which showed that bunch B2 was the most overwhelming phylogenetic gathering and the safest strain to normally utilize anti-toxins among patients with UTI [11,24]. Katongole et al.'s study, 2019) in Uganda on UTIs showed that Phylogenetic gathering B2 was the most transcendent (40%), trailed by A (6.23%), clade I (5%), D and E (every one 2.14%), B1 (1.43%)

and F and C (each 0.71%) [12]. The research carried out by Ramos et al. on pregnant moms with UTIs at Mulago Public Reference Emergency Clinic viewed B1 as the most overwhelming phylogenetic gathering [25]. Different investigations have shown phylogenetic gathering B1 to be related to commensal and less harmful kinds of *E. coli*. 8,22 The triplex PCR method was unable to identify the phylogenetic groups A, C, E, F, and clade I in the current study, which was consistent with previous findings [8,11,26,27].

The current study also recorded 2.6% of isolates belonged to the unknown group; this could be due to new strains or the un-typable strains by PCR, as explained by Clermont et al. (2013) [8], whereas the study of Katongole et al., (2019) [12] in Uganda found there are 41.3% of UPEC not typed. Clermont et al. (2013) and El-Shaer et al. (2018) showed that just 1% and 1.9% of *E. coli* strains couldn't be appointed to one of the eight perceived phylogroups utilizing the drawn-out quadruplex technique [8,13]. Furthermore, in Iranpour et al.'s research (2015), in excess of a quarter (27.1%) of *E. coli* separates of patients with UTI stayed unclassified [11]. This finding is hard to make sense of; however, these unassignable strains are likely very intriguing phylogroups or they come from recombination between two different phylogroups [8]. Along these lines, we prescribed to future review renamed this gathering rely upon consequences of quadruplex PCR of Clermont and other accessible investigations. Likewise, we prescribe different scientists to add images of PCR items that incorporate untyped phylogenetic gatherings to recognize these untyped gatherings.

In the present study, most phylogenetic groups were isolated from females and patients in age groups 2, 3, and 4 (young and adults). Children in age group 1 were infected by phylogenetic groups B2, A, and E, while older patients in age group 5 were infected by B2, D, and unknown phylogenetic groups. This dissemination of phylogenetic gatherings might be affected by invulnerable frameworks, chemicals, sexual action, and different territories of patients. In extra to the design of the female urinary lot, chemicals and sexual action might expand the opportunity of contamination in females, particularly in the age range from 16 to the long term [10]. Tragically, every one of the examinations that we acquired didn't address the relationship of the dissemination of phylogenetic gatherings as per the ages and sexual orientations of the patients, so the purposes for these outcomes need more clarification.

7 Conclusions

In the current study, only seven phylogenetic groups of UPEC are detected, including A, B2, C, D, E, F, and clade 1. Phylogenetic group B2 of UPEC was the most common cause of UTI in the Iraqi popula-

tion. Moreover, phylogenetic groups are mainly isolated from young and adult females.

Acknowledgments

I want to extend my thanks and gratitude to the medical and nursing staff in health centers and clinics for providing tremendous assistance in collecting samples. I also extend my thanks to the participants for their cooperation in giving us samples and information, which was the main reason for completing this work and praying to God to grant them health and safety.

Conflict of Interest: No conflicts of interest exist between the authors and the publication of this work.

Ethical consideration: The ethical committee approved the study at Al-Furat Al-Awsat Technical University.

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How to cite this article

Kazaal M. A.; Quadruplex PCR for Phylogenetic Analysis of Uropathogenic *Escherichia Coli* in Iraqi Population. Journal of Biomedicine and Biochemistry. 2023;2(4):1-8. doi: 10.57238/jbb.2023.7109.1049