Phylogenetic Study of *Escherichia coli* Isolated from Clinical Samples in Hilla City, Iraq

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***Escherichia coli* clones, designated as extraintestinal pathogenic *E. coli* (ExPEC), are responsible for extraintestinal infections. Phylogenetic analysis has shown that *E. coli* is composed of four main phylogenetic groups (A, B1, B2 and D) and six subgroups (i.e. A0, A1, $B2_2$, $B2_3$, D1 and D2). Group A and B1 are generally associated with commensals, whereas group B2,D is associated with extra-intestinal pathotypes. In the present study, a total of 53 *E. coli* isolates, isolated from human clinical samples, were used. Phylogenetic grouping was done based on the PCR method using primers targeted at three genetic markers, *chuA*, *yjaA* and *TspE4.C2*. According to PCR-based phylotyping, subgroup $B2_3$ contained the majority of the collected isolates (40 isolates, 75.47 %), followed by subgroups A1and $B2_2$ (4 isolates for each subgroup, 7.55 %), followed by subgroups D2(3 isolates, 5.66 %) and A0, D1 (1 isolate for each subgroup, 1.88 %). No isolates were found to belong to group B1 . Based on the results, the majority of isolates were extra-intestinal pathotypes. Therefore, the role of *E. coli* in human infections including urinary tract infections, septicemia, vaginitis and meningitis should be considered for further research.**

Keywords: *Escherichia coli, Clinical Samples, Iraq.*

Extraintestinal pathogenic *E. coli* (ExPEC) represents a distinct group of pathogenic *E. coli* that causes most of the extraintestinal *E.coli* infections (urinary tract infection, prostatitis, bacteremia, septicemia, and neonatal meningitis, vaginosis) human infections (Donnenberg *et al*., 2002; Gordon and Cowling, 2003 ; Al-Khaqani *et al*.,2016 ; Abdulla *et al*.,2016). ExPEC isolates are genetically distinct from commensal *E. coli* found in the intestinal flora. Phylogenetic analyses have revealed that *E. coli* isolates are composed of four main phylogenetic groups (A, B1, B2, and D) (Gordon , 2004 ; Al-Khaqani *et al*., 2016). Isolates of each of the four groups have different phenotypic features, causing their ability to exploit different sugars, antibiotic-resistance profiles and growth rate-temperature relationships . The distribution (presence/absence) of a variety of genes thought to enable a strain to cause extra-intestinal disease also varies among isolates of the four phylo-groups (Johnson *et al*., 2001). Several studies have shown the relation between phylogeny and pathogenicity of *E.coli* isolates (Bashir *et al*., 2012 ; Escobar-Paramo *et al.,* 2004). Bearing in mind that most commensal isolates belong to A and B1 groups (Duriez *et al*., 2001), Phylogenetically and epidemiologically ExPEC are potentially different from those of intestinal pathogenic and commensal isolates (Smith *et al*., 2007). Most of the ExPEC isolates phylogenetically belong to B2 and to a lesser extent D groups and are equipped with various virulence factors that help these isolates during different mode of infection mechanisms like adhesion, invasion of host tissues, escape host defence mechanisms, signaling and production of different toxins interfering host cellular functions

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thereby promoting extraintestinal infection in both normal and immune compromised hosts (Dobrindt and Hacker, 2008; Wiles *et al*., 2008; Al-Dahmoshi *et al*., 2016)

The aim of this study was to investigate the phylo-genetic groups of *E. coli* isolated from different clinical samples which includes urine, vaginal , seminal fluid and wound swap in Hilla, Iraq using a molecular primer.

MATERIAL AND METHODS

A total of 200 various clinical samples represent by 50 urine samples from patients with urinary tract infection,50 high vaginal swap from pregnant and non-pregnant women suffering from vaginosis, 50 seminal fluid samples of male suffering from bacteriospermia and 50 wound swabs samples . All samples were obtained from patients or individuals who were admitted to Babylon Hospital for Maternal and Pediatrics, and to Al-Hilla Surgical Teaching Hospital in Babylon city (Iraq) during the period from May to August 2016. In order to isolate *E. coli*, samples were directly inoculated on MacConkey agar (Himedia/India) plates. After overnight incubation at 37°C, lactose fermenting colonies, Gram-negative, oxidase negative bacilli transferred to UTI Chromogenic medium (Condalab/Spain) and Eosin methylene blue agar (Himedia/India) to confirm *E. coli* isolates.

DNA extraction for gram negative bacteria

Typical *E. coli* colonies (with metallic green color on Eosin methylene blue agar and pink color on UTI Chromogenic medium) were grown in LB broth(Condalab/Spain) at 37ºC for 18 h, and following the protocols of Favor Prep Genomic DNA Mini Kit (Blood/Cultured Cell) (Favorgen/Taiwan). The extracted DNA checked using Agarose gel electrophoresis (0.7% in TBE buffer) (Condalab/Spain) and then visualized using and gel documentation (Vilber/France).

Detection of phylogeny groups by PCR

PCR was conducted to determine the phylogenetic grouping of the isolates by targeting three genes, *chuA, yjaA* and *TspE4.C2* using 20ìL reaction mix (IntronBio/Korea) (Clermont *et al*., 2000). Thermal cycler conditions were as follows: 95°C for 4 min, 30 cycles of (denaturation at 94ºC for 30sec.), (annealing at 59 ºC for 30sec.), (extension at 72ºC for 30sec). and final extension at 72ºC for 5 min. Agarose gel electrophoresis (1.5% in TBE buffer) and gel documentation (Vilber/France) were used to visualized and document the PCR products. The amplicon sizes were 279 bp for *chuA*, 211 bp for *yjaA* and 152 bp for *TspE4C2* were recorder using 100bp ladder (IntronBio/Korea). After electrophoresis the gel was photographed under UV light. The results allowed the classification of isolates into either one of the four major phylogroups (A, B1, B2, or D) (Abdallah *et al.,* 2011; Gordon *et al.,* .2008).

RESULTS

This study was carried out to expose the phylogeny of *E. coli* isolated from different clinical samples to investigate the source of these isolates whether they are intestinal or extraintestinal. However, the phylogenetic groups of *E. coli*

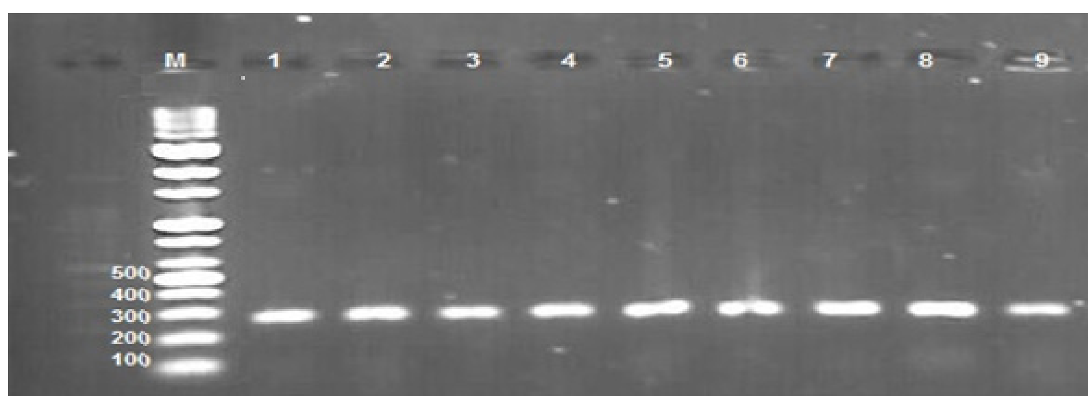

Fig. 1. 1.5% Agarose gel electrophoresis at 72 volt for 60 minutes of PCR to *chuA* amplicon (279bp); lane M represent DNA marker size(100bp). Lane(1-9) represent some of posetive *E.coli* isolates.

isolated from clinical samples were detected by identifying the presence of specific PCR amplified fragments (*chuA, yjaA, and TspE4.C2*) (Figures 1,2,3) respectively.

A total of 53 *E. coli* isolates represented by (12 vaginal, 17 UTI, 10 seminal fluid and 14 wound swap) samples and according to the presence or absence of these genes were assigned to one of four phylogenetic groups (i.e. A,B1, B2 and D) group A and B1 (intestinal groups); B2 and D (extraintestinal groups) and six subgroups (i.e. A0, A1, $B2_2$, $B2_3$, D1 and D2). According to PCR-based phylotyping the result showed that all vaginal samples ,14 urine samples, 8 of wound samples and 6 of seminal fluid samples were belong to $B2_3$ subgroup , 1 of urine samples and 3 of wound samples were belong to $B2_2$ subgroup , 2 of urine samples and 1 of seminal fluid samples were belong to D_2 subgroups , 2 of wound samples and 2 of seminal fluid samples were belong to A_1 subgroup , 1 of wound swap samples was belong to A_0 subgroup and 1 of seminal fluid samples was belong to D_1 subgroup. Also the results showed that the subgroup $B2_3$ contained the majority of the collected isolates (40 isolates, 75.47 %), followed by subgroups A1, $B2_2$ (4 isolates for each subgroup, 7.55 %) then subgroup D2 (3 isolates, 5.66%) and A_0, D_1 (1 isolate for each subgroup, 1.88 %). No isolates were found to belong to group B1 (Table 1and 2)

DISCUSSION

The niche of commensal *E. coli* is the mucous layer of the colon. However, there are *E. coli* clones that are distinct from the intestinal commensal *E. coli*, possessing specific fitness and virulence attributes which allow adaptation to other niches (e.g. urinary tract, central nervous system, blood) and confer enhanced ability to cause a broad

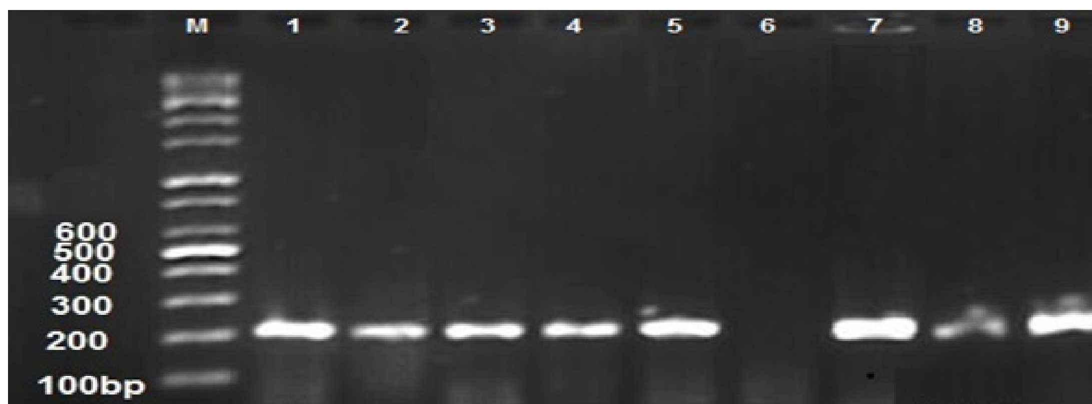

Fig. 2. 1.5% Agarose gel electrophoresis at 72 volt for 60 minutes of PCR to *yja A* amplicon (211bp); lane M represent DNA marker size(100bp). Lane(1-9) represent some of posetive *E.coli* isolates

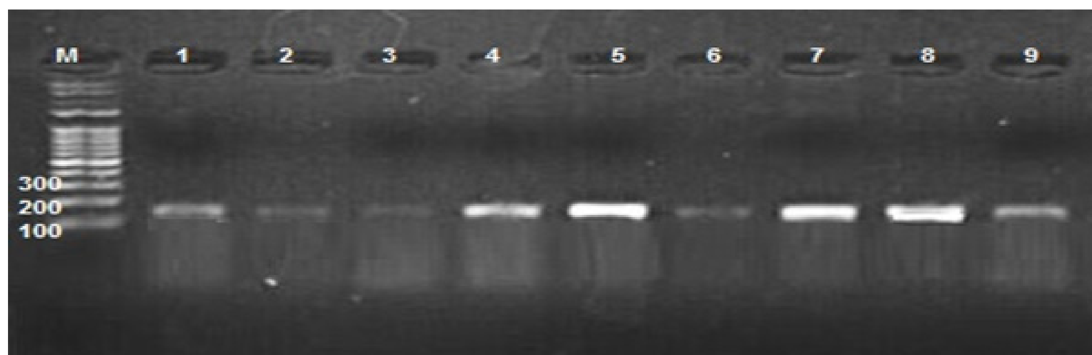

Fig. 3. 1.5% Agarose gel electrophoresis at 72 volt for 60 minutes of PCR to *TspE4.C2* amplicon (152bp); lane M represent DNA marker size(100bp). Lane(1-9) represent some of posetive *E.coli* isolatesl

Table 1. Percentage for isolation of extraintestinal *E. coli*

Sample	Urine	Vaginal Swab	Seminal fluid	Wound Swab	Total
No. of Samples	50	50	50	50	200
E. coli No.(%)	17(34%)	12(24%)	10(20%)	14(28%)	53(26.5%)
ExPEC, No.(%)	17(34%)	12(24%)	8(16%)	11(22%)	48 (24%)

Table 2. Percentage of *E. coli* isolates for each phylogenetic subgroups

Phylogenetic groups N(%)		Phylogenetic subgroups	N (%)	Total (%)
Intestinal Groups	Group A	Subgroup A0chuA - / yjaA - / TspE4.C2 –	1 (1.88)	5 (9.43)
		Subgroup A1chuA - / yjaA + / TspE4.C2 –	4 (7.55)	
	Group B1	chuA - / yjaA - / TspE4.C2 +	0 (0)	
Extraintestinal Groups	Group B2	Subgroup B22chuA + / yjaA + / TspE4.C2 –	4 (7.55)	48 (90.57)
		Subgroup B23chuA + / yjaA + / TspE4.C2 +	40 (75.47)	
	Group D	Subgroup D1chuA + / yjaA - / TspE4.C2 –	1 (1.88)	
		Subgroup D2chuA + / yjaA - / TspE4.C2 +	3 (5.66)	
	Total (%)		53 (100)	

spectrum of disease in extraintestinal sites (Johnson and Russo,2005) . ExPEC isolates usually belong to phylogenetic group B2 and to a lesser extent to group D, whilst commensal isolates are derived from groups A and B1 (Escobar-Páramo et al., 2004). Therefore, the rapid PCR-based phylogenetic typing developed by Clermont et al.(Clermont et al .,2000) has proven useful for rapidly screening ExPEC. According to this method, about more than two-thirds of the analyzed isolates in this study belonged to group B2 (83.01%) and this is larger than the studies of Obata-Yasuoka et al, 2002 (76%) ,Watt et al,2003 (68%) and Hilbert et al, 2008 (62%). A Compared to the data reported by these reserchers, group D isolates had a lower prevalence in this study (7.54% vs. 16%, 16%, and22%, respectively) , but the commensal phylogenetic group A exhibited nearest prevalence among the isolates investigated by this study (9.43% vs. 8%, 12% and 8% respectively). These differences in distribution of the phylogenetic groups among the isolates of geographically distinct populations in different studies may be due to the health status of the host, geographic climatic conditions, dietary factors, the use of antibiotics, or host genetic factors. (Duriez et al., 2001) . Based on the results of this study , the majority of isolates were extraintestinal pathotypes. Therefore, the role of *E. coli* in human infections including urinary tract infections, septicemia, vaginitis and meningitis should be considered for further researches.

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