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Full Length Research Paper

Molecular phylogeny *of Escherichia coli* isolated from clinical samples in Hilla, Iraq

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***Escherichia coli* strains can be assigned to one of the main phylogenetic groups (A, B1, B2 and D). Strains of these groups differ in their phenotypic characteristics, including the ability to use certain sugars, antibiotic resistance profiles and growth rate-temperature relationships. A total of 45 *E. coli* isolates were obtained from different clinical samples by standard bacteriological methods. PCR was conducted to determine the phylogenetic grouping of these isolates by targeting two genes, chuA, yjaA and anonymous DNA fragment TspE4.C2. Three phylogeny genetic markers (chuA, yjaA and TspE4.C2) were used. Results found that the most isolates of *E. coli* belong to the phylogeny group A (44.4%) which includes: urine (3 samples), stool (8 samples), rectal (6 samples), and vagina (3 samples), followed by group B1 (22.2%) which included urine (4 samples), stool (2 samples), rectal (3 samples) and vagina (1 samples), group B2 (17.7%) which included urine (5 samples), and vagina (3 samples), while group D (15.5%) which included urine (3 samples), rectal (1 sample) and 3 vagina samples. Phylogeny pedigree was done according to the data recovered previously. This study explains that the distributions of *E. coli* isolates in phylogenetic groups (A, B1, B2 and D) varied depending on the characteristics of host population and also on the variation in bacterial sources.**

Key words: Phylogeny, *Escherichia coli*, polymerase chain reaction.

INTRODUCTION

Escherichia coli is a normal inhabitant of the intestine. Some *E. coli* strains can cause a wide variety of intestinal and extra-intestinal diseases such as diarrhea, urinary tract infection and septicemia (Orskov and Orskov, 1992). Phylogeny is the study of evolutionary relatedness among various groups of organism. *E. coli* strains can be assigned to one of the main phylogenetic groups (A, B1, B2 and D) (Herzer et al., 1990). According to Lecointre et al. (1998) groups A and B1 are sister groups whereas group B2 is included in an ancestral branch. Strains of these groups differ in their phenotypic characteristics including the ability to use certain sugars, antibiotic resistance profiles and growth rate-temperature relationships (Touchon et al., 2009). The distribution (presence/absence) of a range of virulence factors thought to be involve in the ability of a strain to cause diverse diseases also varies among strains of these phylogenetic groups (Escobar-Pàramo et al., 2004a) indicating a role of the genetic background in the expression of *E. coli* virulence. Consequently, these groups are differently associated with certain ecological niches, life history characteristics and propensity to cause disease. For example, groups B2 and D strains are less frequently isolated from environment (Walk et al., 2007) than A and B1 strains (Gordon and Cowling, 2003).

Furthermore, genome size differs among these phylogeny groups, with A and B1strain having smaller genomes than B2 and D (Bergthorsson and Ochman, 1998). Phylogenetic trees of housekeeping gene sequences from the *E. coli* reference collection indicated that group D diverged first and that groups A and B1 are sister groups that separated later (Wang et al., 1997). More recent analysis suggests that perhaps B2, rather than D is ancestral (Escobar-Pàramo et al., 2004b). The source of *E. coli* according to phylogeny groups may classify into intestinal or extraintestinal. The extraintestinal pathogenic strains usually belongs to groups B2 and D (Johnson and Stell, 2000), the

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Table 1. Primers of phylogenetic groups used in PCR.

Gene	Primer sequence (5' 3')	Size of product bp	Reference
chuA F	GACGAACCAACGGTCAGGAT	279	Clermont et al. (2000)
chuA R	TGCCGCCAGTACCAAAGACA		
yjaA F	TGAAGTGTCAGGAGACGCTG	211	
yjaA R	ATGGAGAATGCGTTCCTCAAC		
TspE4C2 F	GAGTAATGTCGGGGCATTCA	152	
TspE4C2 R	CGCGCCAACAAAGTATTACG		

commensal strains belong to groups A and B1 whilst the intestinal pathogenic strains belong to groups A, B1 and D (Pupo et al., 1997). It has developed a polymerase chain reaction (PCR) based method (Clermont et al., 2000) to characterize the phylo-groups using genetic markers: chuA (a gene required for hem transport in enterohemrrhagic O157:H7 *E. coli*) (Bonacorsi et al., 2000), yjaA (a gene initially identified in recent complete genome sequence of *E. coli* K-12) (Blattner et al., 1997) and TspE4.C2 (an anonymous DNA fragment) (Bonacorsi et al., 2000).

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

MATERIALS AND METHODS

Samples and bacterial culture

A total of 45 *E. coli* isolates recovered from various clinical samples by standard bacteriological methods, 15 isolates were isolated from urine samples from patients with urinary tract infection, high vaginal strains comprised 10 genital tract (cervix or vagina) isolated from pregnant and non-pregnant women suffering from vaginitis, 10 rectal strains were isolated from the rectum of women. Also, 10 samples of stool swabs concluded from patients complaining of diarrhea. 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) in addition to swabs taken from private clinics during the period from February 2010 to May 2010. The samples were processed on MacConkey and Eosin methylene blue agar and were incubated at 37°C overnight. The identification of Gram negative bacteria, purple color was performed by standard biochemical methods (catalase test, oxidase test, indol test, methyl red test, Vogues – Proskauer test, citrate test, urease test, motility test, triple sugar iron test, Ornithine Decarboxlase test, gelatin liquefaction and carbohydrate fermentation test) according to Bergy's manual for determinative bacteriology (Holt et al., 1994).

DNA extraction for Gram negative bacteria

This method was performed according to the genomic DNA purification kit supplemented by a manufacturing company (promega, USA).

Detection of phylogeny groups by PCR

PCR was conducted to determine the phylogenetic grouping of the isolates by targeting two genes, chuA, yjaA and anonymous DNA fragment TspE4.C2 (Clermont et al., 2000). Each 25 µl of PCR reaction mixture for PCR contained 2.5 µl of upstream primer, 2.5 µl of downstream primer, 2.5 µl of free nuclease water, 5 µl of DNA extraction and 12.5 µl of master mix. Thermal cycler conditions were as follows: 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 59°C for 30 s and 72°C for 30 s. A final extension of 72°C for 7 min was performed at the end of PCR. The primers used were chuA, yjaA and TspE4.C2 which generated 279, 211 and 152 bp fragment respectively. The data of the three amplification resulted in assignment of the isolates to phylogenetic groups as follows: $chuA^+$, $yjaA^+$, group B2; $chuA^+$, $yjaA^-$, group D; $chuA^-$, $TspE4.C2^+$, group B1 and $chuA^-$, $yjaA^+$, $TspE4.C2^-$, group A. The PCR amplification product was visualized by electrophoresis on 1% agarose gels for 45 min at 60 V. The size of the amplicons was determined by comparison to the 100 bp allelic ladder (promega, USA) (Table 1).

RESULTS

This study was conducted to show 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* isolated from clinical samples were detected by identifying the presence of specific PCR amplified fragments (chuA, yjaA, and TspE4.C2). chuA marker was found only in urine (5 samples) and vagina (3 samples) in group B2 and was also present in urine (3 samples), vagina (3 samples) and rectal (1 sample) which was positive after amplification (Figure 1). chuA represents the phylogenetic marker for extraintestinal *E. coli* isolates. In the same way, the yjaA marker found in *E. coli* isolated from urine (3 samples), stool (8 samples), rectal (6 samples), and vagina (3 samples) were positive after amplification (Figure 2), yjaA represents the phylogenetic marker for intestinal *E. coli* isolates. It was confirmed that *E. coli* isolates were distributed among phylogeny groups regardless of source of the isolates whether from urine, vagina, stool or from rectal swab. Clone TspE4.C2 was also found in *E. coli* isolated from urine (4 samples), stool (2 samples), rectal (3 samples) and vagina (1 sample) (Figure 3). TspE4.C2 represents a phylogenetic marker

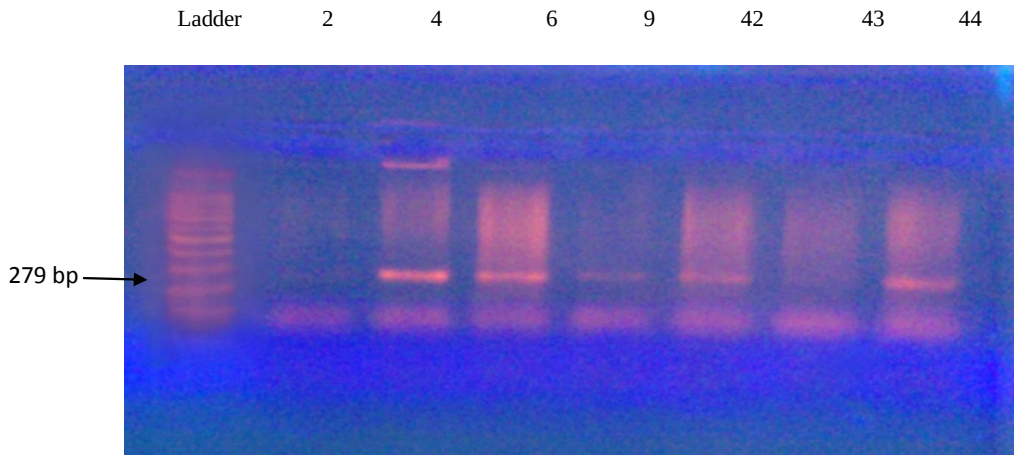

Figure 1. Gel electrophoresis of PCR of chuA amplicon obtained from urine samples; lane 1 (ladder), molecular weight marker of ladder (100 bp).

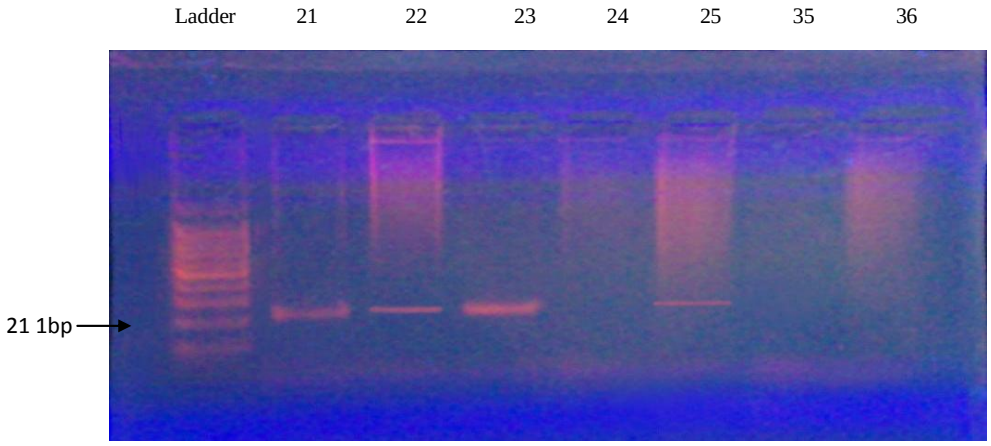

Figure 2. Gel electrophoresis of PCR of yjaA amplicon obtained from rectal samples; lane 1 (ladder), molecular weight marker of ladder (100 bp).

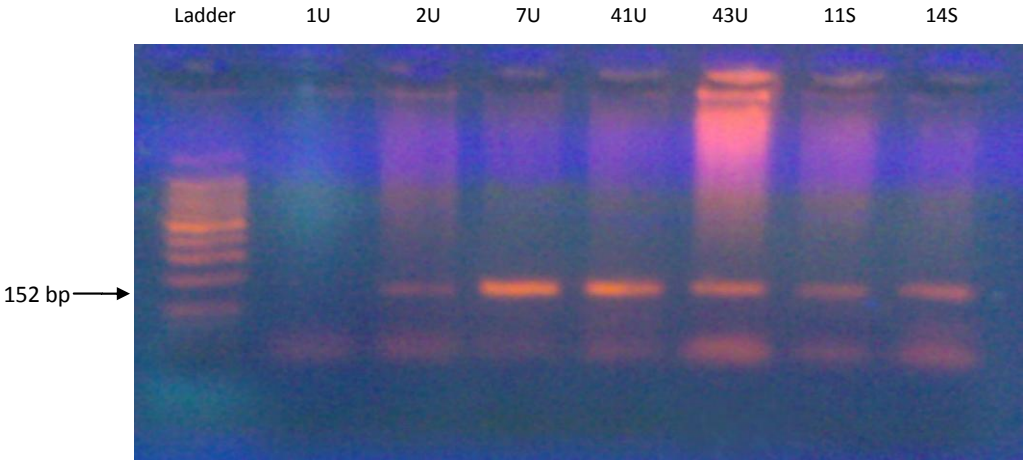

Figure 3. Gel electrophoresis of PCR of TspE4.C2 amplicon obtained from urine (U) and stool (S) samples; lane 1 (ladder), molecular weight marker of ladder (100 bp).

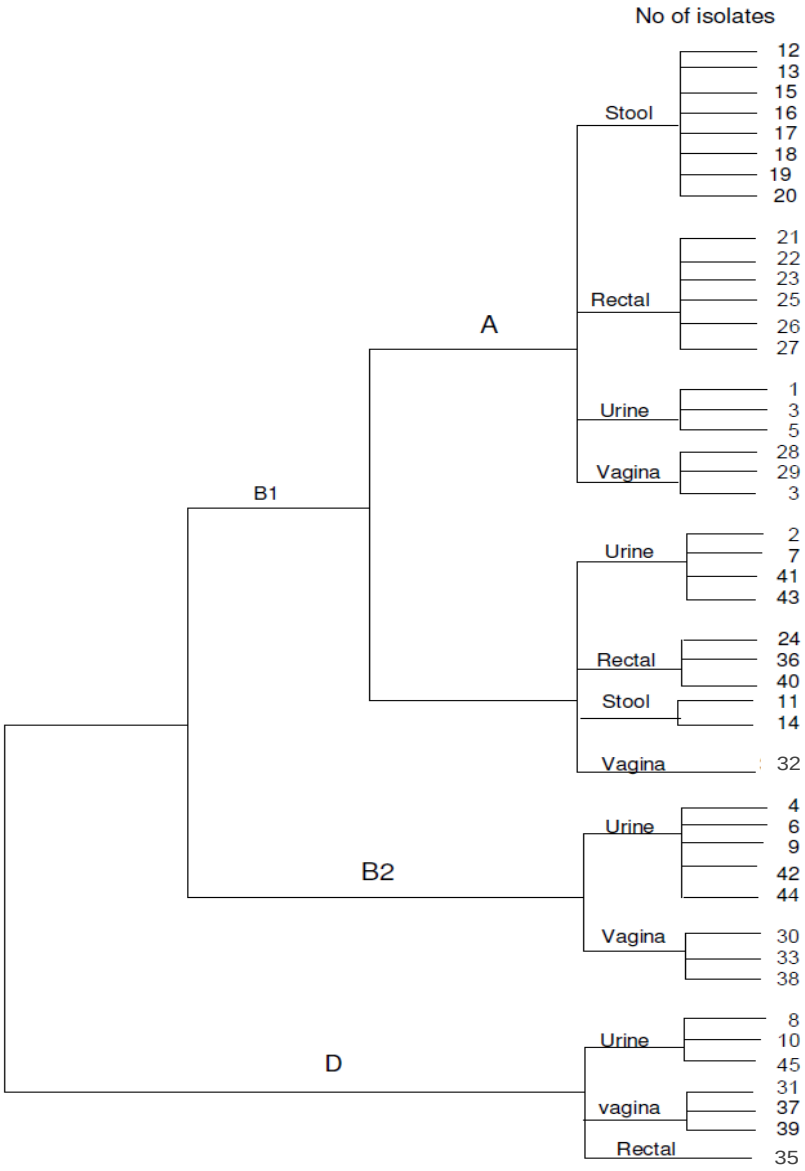

Figure 4. Phylogenic groups of *E. coli* isolated from clinical samples. A: yjaA+ marker, B1: TspE4.C2+ marker, B2: chuA+, yjaA+ marker, D: chuA+ marker

for intestinal *E. coli* isolates. Phylogenetic tree of 45 *E. coli* isolates is shown in Figure 4.

The phylogenetic tree was drawn according to Clermont et al. (2000) method. So, the result shows that 20 isolates belong to group A (44.4%) and 10 isolates which belong to group B1 (22.2%) were the most prevalent among intestinal flora. Also, 8 isolates belong to group B2 (17.7%) and 7 isolates belong to group D (15.5%).

DISCUSSION

Results of this study revealed that the chuA gene was present in all isolates belonging to groups B2 and D and was absent from all isolates belonging to groups A and B1. The yjaA gene allowed perfect discrimination between group B2 and group D and it was present in all isolates belonging to group A. Also, the TspE4.C2 is present in group B1 strains and absent from all group A isolates (Clermont et al., 2000). Too little information is available on yjaA and DNA fragment to speculate on their evolutionary history. In contrast, the study by Wyckoff et al. (1998) of heme transport genes suggested that chuA was acquired by sister groups B2 and D (Lecointre et al., 1998) soon after their emergence rather than being present in common ancestor and subsequently being lost by groups B1 and D. The distribution of phylogenetic groups differs considerably between intestinal and extraintestinal *E. coli* isolates (Duriez et al., 2001). The

results show that *E. coli* isolates belong to four phylogenetic groups (A, B1, B2 and D). Some isolates obtained from clinical samples such are urine, vagina, stool and rectal belongs to group A and less to group B1.

This indicates that the source of *E. coli* isolated is intestinal while other *E. coli* isolates belong to group B2 and group D, this indicate that the source of *E. coli* isolates are extraintestinal. Also, these finding are in agreement with those reported by Duriez et al. (2001) who observed that group B2 was rare among commensal isolates whereas groups A and B1 were the most common. Some reports indicate that strains belonging to groups A and B1 can also cause disease of extraintestinal site (Rijavec et al., 2008). In contrast, Zhang et al. (2002) found that this group was the most frequent among both commensal (48%) and extraintestinal (69%). Thus, it is not clear whether all *E. coli* in the intestinal tract of healthy individual at specific time should be considered commensal regardless of their phylogenetic background or only the isolates belonging to groups A and B1 (Zhang et al., 2002). Similarly, it is not clear whether *E. coli* isolates belonging to groups A and B1 from patient with urinary tract infection should be considered as true pathogens or as commensal *E. coli* that produce infection in a compromised host (Sabate et al., 2006).

Phylogenetic characterization of *E. coli* strains on the basis of a very phenotypic or genotypic feature initially appeared to be very difficult. Such genotypic trait must meet different criteria for use in phylogenetic characterization: First, the gene must have been acquired or deleted when the group that is characterized emerged. Secondly, the same gene must have been stabilized there by ruling out its subsequent deletion or horizontal transfer among bacteria belongs to other phylogeny groups. Finally, recombination event in the candidate gene must be very rare. In other words, the gene product must not be targeted by natural selection which favors new genetic recombination (Whittam, 1996).

REFERENCES

Bergthorsson U, Ochman H (1998). Distribution of chromosome length variation in natural isolates of *Escherichia coli*. Mol. Biol. Evol. 15: 6-16.

Blattner FR, Plunkett GI, Bloch CA , Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y(1997). The complete genome sequence of Escherichia coli K-12. Science 277: 1453-1461.

Bonacorsi SP, Clermont O, Tinsley C (2000). Identification of the *Escherichia coli* chromosome specific for neonatal meningitis associated strains. Infect. Immun. 68: 2096-2101.

Clermont O, Bonacorsi S, Bingen E (2000). Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. 66: 4555-4558.

Duriez P, Clermont O, Bonacorsi S, Bingen E, Chaventre´ A, Elion J, Picard B, Denamur E (2001). Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human population. Microbiol. 147: 1671-1676.

Escobar-Pàramo P, Clermont O, Blanc-Potard AB, Bui H, Le Bouguence C, Denamur E (2004a). A specific genetic background is required for acquisition and expression of virulence factors in *Escherichia coli*. Mol. Biol. Evol. 21:1085-1094.

Escobar-Pàramo P, Sabbagh A, Darlu P, Vaury O, Lecointre G, Denamur E (2004b). Decreasing the effects of horizontal gene transfer on bacterial phylogeny: the *Escherichia coli* case study. Mol. Phylogenet. Evol. 30: 243-250.

Gordon DM, Cowling A (2003). The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. Microbiol. 149: 3575-3586.

Herzer PJ, Inouye S, Inouye M, Whittam TS (1990). Phylogenetic distribution of branched RNA-linked multi-copy single-stranded DNA among natural isolates of *Escherichia coli*. J. Bacteriol. 172:6157-6181.

Holt JC, Krieg NR, Sneath A, Stachley JT, William ST (1994). Bergy's manual of determinative bacteriology, 9th ed. USA, pp.552.

Johnson JR, Stell AL (2000). Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J. Infect. Dis. 181: 261-272.

Lecointre G, Rachdi L, Darlu P, Denamur E (1998). *Escherichia coli* molecular phylogeny using the incongruence length difference test. Mol. Biol. Evol. 15: 1685-1695.

Orskov, Orskov I (1992). *Escherichia coli* serotyping and disease in man and animals. Can. J. Microbiol. 38: 699-704.

Pupo GM, Karaolis DKR, Reeves PR (1997). Evolutionary relationships among pathogenic and non-pathogenic *Escherichia coli* strains inferred from multilocus enzyme electrophoresis and *mdh* sequence studies. Infct. Immun. 65: 2685-2692.

Rijavec M, Muller-Premer M, Zakotnik B, Žgur-Bertok D (2008). Virulence factors and biofilm production among *Escherichia coli* strains causing bacteraemia of urinary tract origin. J. Med. Microbiol. 57: 1329-1334.

Sabate M, Moreno E, Perez T, Andreu A, Prats G (2006). Pathogenicity island markers in commensal and pathogenic *Escherichia coli* isolates. Clin. Microbiol. Infect. 12: 880-886.

Touchon M, Tenaillon O, Hoede C, Barbe V, Baeriswly S (2009). Organized Genome Dynamic in the *Escherichia coli* species Results in Highly Diverse Adaptive Paths. PLOS Genet. 5(1): e1000344.

Walk ST, Alm EW, Calhoun LM, Mladonicky JM, Whittam TS (2007). Genetic diversity and population structure of *Escherichia coli* isolated from freshwater beaches. Environ. Microbiol. 9: 2274-2288.

Wang FC, Whittam TS, Selander RK (1997). Evolutionary genetics of the isocitrate dehydrogenase gene (icd) in *Escherichia coli* and *Salmonella enterica.* J. Bactriol. 179: 6551-6559.

Whittam T (1996). Genetic variation and evolutionary processes in natural population of *Escherichia coli.* In: Neidhardt F C, editor; Neidhardt F C, editor. *Escherichia coli* and *Salmonella:* cellular and molecular biology. Washington, D.C.: American Society for Microbiology, pp. 2708-2720.

Wyckoff EE, Duncan D, Torres AG, Mills M, Maase K, Payne SM (1998). Structure of the *Shigella dysenteriae* haem transport locus and its phylogenetic distribution in enteric bacteria. Mol. Microbiol. 28: 1139-1152.

Zhang L, Foxman B, and Marrs C (2002). Both urinary and rectal *Escherichia coli* isolates are dominated by strains of phylogenetic group B2. J. Clin. Microbiol., 40: 3951-3955.