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Phylogenetic analysis of *Escherichia coli* strains isolated from human samples

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A B S T R A C T

Escherichia coli (E. coli) is a normal inhabitant of the gastrointestinal tract of vertebrates, including humans. Phylogenetic analysis has shown that *E. coli* is composed of four main phylogenetic groups (A, B1, B2 and D). Group A and B1 are generally associated with commensals, whereas group B2 is associated with extra-intestinal pathotypes. Most enteropathogenic isolates, however, are assigned to group D. In the present study, a total of 102 *E. coli* strains, isolated from human samples, were used. Phylogenetic grouping was done based on the Clermont triplex PCR method using primers targeted at three genetic markers, *chuA*, *yjaA* and TspE4.C2. Group A contained the majority of the collected isolates (69 isolates, 67.64%), followed by group B2 (18 isolates, 17.64%) and D (15 isolates, 14.7%) and no strains were found to belong to group B1. The distribution of phylogenetic groups in our study suggests that although the majority of strains were commensals, the prevalence of enteropathogenic and extra-intestinal pathotypes was noteworthy. Therefore, the role of *E. coli* in human infections including diarrhea, urinary tract infections and meningitis should be considered.

Key words: *Escherichia coli, Phylogenetic grouping, Triplex PCR, Human*

INTRODUCTION

Escherichia coli, a normal inhabitant of the gastrointestinal tract, is the most common enteric organism causing various intestinal (diarrhea) and extra-intestinal (urinary tract infection, prostatitis, bacteremia, septicemia, and neonatal meningitis) human infections [1-4]. Phylogenetic analyses have revealed that *E. coli* strains are composed of four main phylogenetic groups (A, B1, B2, and D) [5, 6]. Strains of each of the four groups have different phenotypic features, causing their ability to exploit different sugars, antibiotic-

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resistance profiles and growth rate-temperature relationships [7]. The distribution (presence/absence) of a variety of genes thought to enable a strain to cause extra-intestinal disease also varies among strains of the four phylo-groups [8]. Several studies have shown the relation between phylogeny and pathogenicity of *E.coli* strains [9-13]. Bearing in mind that most commensal strains belong to A and B1 groups [14], and that the virulent extra-intestinal strain belongs mainly to group B2 and, to a lesser extent to group D [13], a great deal can be obtained by phylogenetic group determination of unknown *E.coli* strains.

Several techniques can be performed to determine phylogenetic group, such as multilocus enzyme electrophoresis (MLEE) [15], ribotyping, random amplified polymorphic DNA analysis [5], fluorescent amplified-fragment length polymorphism (FAFLP) analysis [16], PCR phylotyping using the presence/absence of three genomic DNA fragments [17], analysis of variation at mononucleotide repeats in intergenic sequences [18], and multilocus sequence typing (MLST) [19, 20]. MLST is now clearly the "gold standard" technique [21], but it is costly and time-consuming. PCR phylotyping technique was described by Clermont et al [17] and is based on a triplex PCR using a combination of two genes (*chuA*and *yjaA*) and an anonymous DNA fragment designated TSPE4.C2 [17]. This method, whose results strongly correlate with those obtained by other standard methods, is an excellent technique for rapid and inexpensive assigning of *E. coli* strains in different phylogenetic groups [17, 22].

Up to now, there have been very few published studies on phylogenetic group determination of human *E.coli* in Iran. Therefore, the objective of this study was to determine the distribution of phylogenetic groups of *E. coli* isolated from 102 patients of Shiraz hospitals using the Clermont triplex PCR method.

MATERIALS AND METHODS

Collection, identification and storage of *E. coli* strains: A total of 102 *E. coli* strains were isolated from patients (males/females with different ages) referred to various hospitals of Shiraz. Samples were collected with informed consent from different sites and fluids of the body including urine, meninge, wound and internal organs. In order to isolate *E. coli*, samples were directly inoculated on MacConkey agar (Merck-Germany) plates. After overnight incubation at 37°C, lactose fermenting colonies were streaked on EMB agar (Merck- Germany). Typical *E. coli* colonies (with metallic green color) were tested for oxidase presence, citrate utilization, L-lysine decarboxylase, motility, glucose and sucrose fermentation, indole production, tryptophan deamination, hydrogen sulfide production and urea hydrolysis. Isolated strains which exhibited a biochemical profile for *E. coli* were grown in LB broth (Merck- Germany), and kept as stock in a 25% glycerol solution at -70°C for long-term storage.

DNA extraction: DNA template preparation was performed by the boiling method as follows. First, a few colonies were resuspended in 250 µl sterile distilled water. The cells were lysed by heating at 95^{o}C for 10 min. After heating, they were immediately put on ice for 5 min. The supernatant was then harvested by centrifugation at 12,000 rpm for 5 min,

transferred to fresh centrifuge tubes and kept at -20°C. The supernatant was used as a source of template for amplification [23].

Triplex PCR: Phylogenetic classification of *E. coli* isolates was done by using a previously reported triplex PCR-based phylotyping technique [17, 22]. Briefly, the genomic DNA of bacterial strains was amplified by triplex PCR using primers targeted at three markers, *chuA, yjaA*and TspE4.C2. The primer pairs used for PCR amplification were: ChuA.1 (5`-GAC GAA CCA ACG GTC AGG AT-3`) and ChuA.2 (5`-TGC CGC CAG TAC CAA AGA CA-3`), YjaA.1 (5`-TGAAGTGTCAGGAGACGCTG-3`) and YjaA.2 (5`-ATG GAG AAT GCG TTC CTC AAC-3`), and TspE4.C2.1 (5`-GAG TAA TGT CGG GGC ATT CA-3`) and TspE4.C2.2 (5`-CGC GCC AAC AAA GTA TTA CG-3`), which generate 279-, 211-, and 152-bp fragments, respectively. The amplifications were performed in a total volume of 25 µl, each reaction mixture containing 11.25 µl distilled H_2O, 2.5 µl 10X buffer (supplied with *Taq*polymerase) (CinnaGen Co., Iran), 0.75 µl $MgCl_2$ (CinnaGen Co., Iran), 1 µl dNTPs (each deoxynucleoside triphosphate at a 200 mM concentration) (CinnaGen Co., Iran), 1 µl of each primer (20 pmol) (CinnaGen Co., Iran), 2.5 U of *Taq* polymerase (CinnaGen Co., Iran) and 3 µl of DNA template. A negative control (reaction lacking the template DNA) was included in all performed amplifications. Thermal cycler (MJ Mini, BIO-RAD-USA) conditions were as follows: initial denaturation at 94°C for 4 min, 30 cycles of 5 s at 94°C and 10 s at 57°C, and a final extension of 5 min at 72°C. Amplification products were separated in 2% agarose gels containing ethidium bromide [24]. 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) [23]. All amplification procedures were repeated at least three times. Phylogenetic grouping was done on the basis of the presence or absence of the 3 DNA fragments as follows: *chuA*–, TspE4.C2–, group A; *chuA*–, *yjaA*–, TspE4.C2+, group B1; *chuA*+, *yjaA*+, group B2; *chuA*+, *yjaA*–, group D. Because two possible profiles can be obtained for the groups A, B2, and D, each was subdivided as follows: *chuA*–, *yjaA*–, TspE4.C2–, group A subgroup A_0; *chuA*–, *yjaA*+, TspE4.C2–, group A subgroup A_1; *chuA*+, *yjaA*+, TspE4.C2–, group B2 subgroup $B2_2$; *chuA*+, *yjaA*+, TspE4.C2+, group B2 subgroup $B2_3$; *chuA*+, *yjaA*–, TspE4.C2–, group D subgroup D_1; *chuA*+, *yjaA*–, TspE4.C2+, group D subgroup D_2 [22].

RESULTS

A total of 102 *E. coli* strains isolated from patients were assigned to three phylogenetic groups (i.e. A, B2 and D) and six subgroups (i.e. A_0, A_1, $B2_2$, $B2_3$, D_1 and D_2). According to multiplex PCR-based phylotyping, group A contained the majority of the collected isolates (69 isolates, 67.64%), followed by group B2 (18 isolates, 17.64%) and D (15 isolates, 14.7%). No strains were found to belong to group B1. Most strains of group A (64 isolates, 92.75%) belonged to the subgroup A_0 and the others (5 isolates, 17.24%) were assigned to subgroup A_1. Ten isolates (55.55%) of group B2 belonged to subgroup $B2_3$, and eight isolates (44.44%) to subgroup $B2_2$. Also, most strains of group D (13 isolates,

86.66%) fitted in subgroup D_1 and the others (2 isolates, 13.33%) in the D_2 subgroup (Fig. 1).

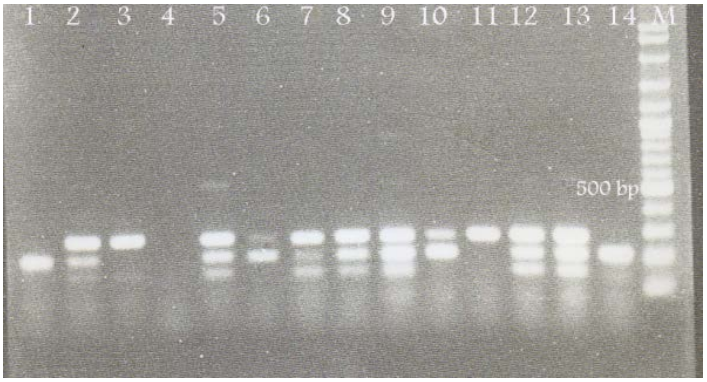

Figure 1: Triplex PCR based phylogenetic profile of *E.coli* isolates. Lane M contained 100 bp marker; Lane 5,7,8,9,12 and 13, subgroup $B2_3$; Lane 2 and 10, subgroup $B2_2$; Lane 1,6 and 14, subgroup D_1; Lane 3 and 11, subgroup A_1; Lane 4, subgroup A_0.

DISCUSSION

E.coli strains belong to four main phylogenetic groups [7], each of which exhibit ecological specialization and differ in their propensity to cause disease [25]. Hence, knowledge of the structure of bacterial populations is a prerequisite to understanding the epidemiology of infectious diseases. It is of clinical interest too, as group A and B1 are generally associated with commensals, most enteropathogenic isolates are assigned to group D, and extra-intestinal pathotypes are associated with group B2 [26]. Most *E.coli* strains that cause neonatal meningitis and septicemia belong to the B2 and D groups [4]. Duriez et al [14] determined the phylogenetic group of 168 commensal *E.coli* strains isolated from the stool of three geographically distinct human populations and showed that strains from phylogenetic groups A and B1 were the most common, followed by phylogenetic group D strains. Bingen-Bidois et al. [27] showed that groups B2 and D were the most prevalent phylogenetic groups in urosepsis strains of human *E. coli.* Obata-Yasuoka et al [28] assessed the prevalence of phylogenetic groups in 88 vaginal *E. coli* isolates and revealed that the majority of strains belonged to group B2. Moreno et al [29] compared urine and fecal *E.coli* isolates from the same hosts and showed that urine clones exhibited a significantly higher prevalence of group B2 than fecal-only clones. Bukh et al. [30] showed that two-thirds of 1533 *E. coli* isolates in Danish patients with community-acquired bacteraemia (CAB) were classified into phylogenetic group B2. Groups A and D were comparable in size, whereas B1 was the least abundant. Bashir *et al*. [9] analyzed phylogenetic groups in 59 uropathogenic *E.coli* isolates and revealed that group B2 was the most dominant followed by groups A, B1 and D. Smati et al. [31] assessed the relative proportions of *E. coli* phylogroups from the stool of 100 healthy individuals and observed that most isolated phylogroups belong to group A and B2.These differences in distribution

of the phylogenetic groups among the strains 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, in addition to the differences arising from different sampling areas. Some *E. coli* strains may also be primarily adapted to the gut conditions of certain populations [14]. Based on the results, the majority of strains were commensals, although the prevalence of enteropathogenic and extra-intestinal pathotypes was noticeable. Therefore, the role of *E. coli* in human infections including diarrhea, urinary tract infections, septicemia and meningitis should be considered for further research.

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REFERENCES

1. Donnenberg M. *Escherichia coli* virulence mechanisms of versatile pathogen. Elsevier Science, San Diego Calif. 2002.
2. Eisenstein BI, Joes GW**.** The spectrum of infections and pathogenic mechanisms of *Escherichia coli.* Adv Intern Med 1998;33:231-252.
3. Gordon D, Cowling A. The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. Microbiology 2003;149:3575-3586.
4. Orskov F, Orskov I. *Escherichia coli* serotyping and disease in man and animals. Can J Microbiol 1992;38:699-704.
5. Desjardins P, Picard B, Kaltenbock B, Elion J, Denamur E. Sex in *Escherichia coli* does not disrupt the clonal structure of the population: evidence from random amplified polymorphic DNA and restriction-fragment-length polymorphism. J Mol Evol 1995; 40:440-448.
6. Gordon DM. The influence of ecological factors on the distribution and genetic structure of *Escherichia coli*. In *Escherichia coli* and *Salmonellatyphimurium*. American Society for Microbiology 2004 [http:// www.ecosal.org/ecosal/index.jsp].
7. Herzer PJ, Inouye S, Inouye M, Whittam TS. Phylogenetic distribution of branched RNA-linked multicopy single-stranded DNA among natural isolates of *Escherichia coli*. J Bacteriol 1990;172:6175-6181.
8. Johnson JR, Delavari P, Kuskowski M, Stell AL. Phylogenetic distribution of extraintestinal virulence-associated traits in *Escherichia coli*. J Infect Dis 2001;183:78-88.

9. Bashir S, Haque A, Sarwar Y, Anwar A, Anwar M. Virulence profile of different phylogenetic groups of locally isolated community acquired uropathogenic *E. coli* from Faisalabad region of Pakistan. Ann Clin Microbiol Antimicrob 2012;11:23.
10. Bingen E, Picard B, Brahimi N, Mathy S, Desjardins P, Elion J, Denamur E**.** Phylogenetic analysis of *Escherichia coli* strains causing neonatal meningitis suggests horizontal gene transfer from a predominant pool of highly virulent B2 group strains. J Infect Dis 1998;177:642-650.
11. Boyd E, Hartl D**.** Chromosomal regions specific to pathogenic isolates of *Escherichia coli* have a phylogenetically clustered distribution. J Bacteriol 1998;180:1159-1165.
12. Escobar-Paramo P, Clermont O, Blanc-Potard A, Bui H, Le Bouguenec C, Denamur E**.** A specific genetic background is required for acquisition and expression of virulence factors in *Escherichia coli.* Mol Biol Evol 2004;21:1085-1094.
13. Picard B, Garcia JS, Gouriou S, Duriez P, Brahimi N, Bingen E, Elion J, Denamur E. The link between phylogeny and virulence in *Escherichia coli*extraintestinal infection. Infect Immun 1999;67:546-553.
14. Duriez P, Clermont O, Bonacorsi S, Bingen E, Chaventre A, Elion J, Picard B, Denamur E. Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. Microbiology 2001;147:1671-1676.
15. Ochman H, Selander RK. Standard reference strains of *Escherichia coli* from natural populations. J Bacteriol 1984;157:690-693.
16. Arnold C, Metherell L, Willshaw G, Maggs A, Stanley J**.** Predictive fluorescent amplified-fragment length polymorphism analysis of *Escherichia coli*: high-resolution typing method with phylogenetic significance. J Clin Microbiol 1999;37:1274-1279.
17. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 2000;66:4555-4558.
18. Diamant E, Palti Y, Gur-Arie R, Cohen H, Hallerman E, Kashi Y. Phylogeny and strain typing of *Escherichia coli,* inferred from variation at mononucleotide repeat loci. Appl Environ Microbiol 2004;70:2464-2473.
19. Lecointre G, Rachdi L, Darlu P, Denamur E. *Escherichia coli* molecular phylogeny using the incongruence length difference test. Mol Biol Evol 1998;15:1685-1695.
20. Reid S, Herbelin C, Bumbaugh A, Selander R, Whittam T**.** Parallel evolution of virulence in pathogenic *Escherichia coli.* Nature 2000;406:64-67.
21. Urwin R, M Maiden. Multi-locus sequence typing: a tool for global epidemiology. *Trends Microbiol* 2003;11:479-487.
22. Gordon DM, Clermont O, Tolleyand H, Denamur E. Assigning *Escherichia coli* strains to phylogenetic groups: multi-locus sequence typing versus the PCR triplex method. Environ Microbiol 2008;10:2484-2496.
23. Abdallah KS, Cao Y, Wei DJ. Epidemiologic Investigation of Extra-intestinal pathogenic *E. coli* (ExPEC) based on PCR phylogenetic group and *fim*H single nucleotide polymorphisms (SNPs) in China. Int J Mol Epidemiol Genet 2011;2:339-353.

24. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: a laboratory manual.1998. 2nd edition. N.Y., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press.
25. Walk ST, Alm EW, Calhoun LM, Mladonicky JM, Whittam TS. Genetic diversity and population structure of *Escherichia coli* isolated from freshwater beaches. Environ Microbiol 2007;9:2274-2288.
26. Sabarinath A,Tiwari KP,Deallie C,Belot G,Vanpee G, Matthew V, Sharma R, Hariharan H. Antimicrobial resistance and phylogenetic groups of commensal *Escherichia Coli* isolates from healthy pigs in Grenada. Webmed Central Vet Med 2011;25,WMC001942.
27. Bingen-Bidois M, Clermont O, Bonacorsi S, Terki M, Brahimi N, LoukilCh, Barraud D, Bingen E. Phylogenetic analysis and prevalence of Urosepsis strains of *Escherichia coli* bearing pathogenicity island-like domains. Infect Immun 2002;70:3216-3226.
28. Obata-Yasuoka M, Ba-Thein W, Tsukamoto T, Yoshikawa H, Hayashi H. Vaginal *Escherichia coli* share common virulence factor profiles, serotypes and phylogeny with other extraintestinal *E. coli*. Microbiology 2002;148:2745-2752.
29. Moreno E, Andreu A, Pigrau C, A Kuskowski M, R Johnson J, Prats G. Relationship between *Escherichia coli* Strains Causing Acute Cystitis in Women and the Fecal *E. coli* Population of the Host. J Clin Microbiol 2008;46:2529-2534.
30. Bukh AS, Schonheyder HC, Emmersen JMG, Sogaard M, Bastholm S, Roslev P. *Escherichia coli* phylogenetic groups are associated with site of infection and level of antibiotic resistance in community-acquired bacteraemia: a 10 year population-based study in Denmark. J Antimicrob Chemother 2009;64:163-168.
31. Smati M, Clermont O, Gal FL, Schichmanoff O, Jauréguy F, Eddi A, Denamur A, Picard B. Real-time PCR for quantitative analysis of human commensal *Escherichia coli* populations reveals a high frequency of sub-dominant phylogroups. Appl Environ Microbiol 2013;79:5005-5012.