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Phylogenetic Grouping of Dominant Fecal *Escherichia coli* Isolates from Healthy Males and Females in Al-Kut/Wasit Province/Iraq

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Abstract

Fecal isolates of *Escherichia coli* are divided into four main phylogenetic groups designated A, B1, B2 and D. Most commensal strains belong to group A and have fewer virulence factors than the extra intestinal pathogenic *E. coli* strains (ExPEC). This study was mainly designed for phylogenetic grouping of commensal fecal *E. coli* isolates from healthy males and females in Al-Kut City/Wasit Province/Iraq using PCR-based protocols. Also ExPEC's virulence genes were detected among these isolates. Among this study included isolates (n=205), group A was the most common among both females' and males' isolates (68.5% vs. 63.0%, respectively), followed by groups: B1 (15.7% vs. 22.6%, respectively), D (10.7% vs. 9.5%, respectively), and B2 (4.1% vs. 4.7%, respectively). Gender distribution of phylogenetic groups showed insignificant differences between females and males. Females' and males' isolates did not differ significantly for all of the detected ExPEC's virulence genes, except for *papC* which was significantly ($P \leq 0.05$) more prevalent among males' isolates (16.6%) than among females' isolates (6.6%). In both females' and males' isolates the most prevalent virulence genes were *fimH* (97.5% vs. 100%, respectively) and *iucC* (52.0% vs. 55.9%, respectively), whereas the least prevalent were *sfa/foc* (0% each) and *hly* (0.82% vs. 0%, respectively). Furthermore, virulence genes were concentrated in isolates clustered in group B2.

In both females and males, high percent of dominant commensal fecal *E. coli* isolates from Iraqi people in Al-Kut City clustered in phylogroup A followed by groups B1 and D whereas group B2 was rare. Also, dominant fecal strains with ExPEC characteristics were much less prevalent.

Keywords: Fecal *E. coli*; Phylogroups; Virulence genes; Males and females

Introduction

Escherichia coli is a member of the normal intestinal microflora of humans and animals. It is also a common cause of extraintestinal infections both in adults [1,2] and neonates [3,4]. The reservoir for these extraintestinal pathogenic *E. coli* (ExPEC) is the human bowel flora [5-7].

Fecal isolates of *E. coli* are divided into four main phylogenetic groups (ECOR groups) designated A, B1, B2 and D [8]. These groups differ in their phenotypic and genotypic characteristics [9]. The virulent extraintestinal *E. coli* strains belong mainly to group B2 and, to a lesser extent, to group D, whereas most commensal strains belong to group A [10]. Numerically, group A is the dominant group among normal fecal *E. coli* but relative percent distribution of these groups differs from one country to another [11,12]. In comparison with ExPEC, the human commensal fecal strains had fewer virulence factors [11,13]. Such VFs induce disease through their ability to help the organisms to avoid or subvert host defenses, colonize key anatomical sites, perturb host physiology, invade host tissues, and/or incite a noxious host inflammatory response [9,13-15].

Worldwide, many studies were carried out for fecal *E. coli* phylogenetic grouping, while here in Iraq little if any are available regarding this subject, therefore; this study was mainly designed for phylogenetic grouping of dominant commensal fecal *E. coli* isolates from healthy males and females in Al-Kut City/Wasit Province/Iraq. The distributions of several known extraintestinal virulence factors (*fimH, papC, sfa/foc* adhesin-encoding operons, and *hly* and *iucC* operons) were also surveyed among this study included isolates.

Methods

Bacterial isolates

A total of 205 fecal *E. coli* isolates from adult healthy females (n=75) and males (n=45) aged 18-45 years, who were not aware of any illness at the time of sampling, were included in this study. The volunteers were college students, college staff, and college staff's family members. From each volunteer an oral consent was obtained to publish this work. This work was approved by the Scientific Committee of the College of Science/ University of Wasit/ Wasit Province/Iraq.

Specimen collection, processing and identification of the isolates

The specimens were collected during the period December 2008 through June 2010. A single fecal specimen was collected per person and processed according to Plos et al. [16] to obtain dominant fecal.

Bacteria were isolated from freshly deposited feces. Fecal collection was carried out using a swab/transport tube system containing phosphate buffered saline. Bacteria were isolated by dilution-streaking

the sample (within 1-2 hours after collection) onto an eosine methylene blue (EMB, Himedia, India) agar plate. After incubation, from each plate the last three colonies (with the appropriate color and morphology that is characteristics of *E. coli*) at the end of the streak area were selected and then subcultured onto a tryptic soy agar (TSA) (Himedia, India) plate. Lactose positive isolates were tested for citrate utilization, and urease and indole production [17,18]. All incubations were carried out at 35°C. For most individuals 1-2 isolates were included in this study because some isolates were lost during the study period before performing PCR experiments.

Phylogenetic grouping of the isolates

The isolates were classified by use of the rapid phylogenetic grouping technique described by Clermont et al. [19]. This method is based on a triplex PCR involving the amplification of two genes (chuA and yjaA) and of an anonymous fragment of DNA from *E. coli*. Briefly, PCR was performed in a total volume of 25 µl containing 12.5 µl of KapaTaq 2x Ready Mix (KAPA Biosystems, USA), 20 pmol concentrations of each primer, and 5 µl of DNA template. The PCR conditions were as follows: denaturation for 4 min at 94°C, 30 cycles of 5 s at 94°C and 10 s at 59°C, and a final extension step of 5 min at 72°C. The results were interpreted as follows, according to Clermont et al. [19]: group B2 (chuA+, yjaA+, TspE.C2±), group D (chuA+, yjaA-, TspE.C2±), group B1 (chuA-, yjaA±, TspEC2+) and group A (chuA-, yjaA±, TspE.C2-).

Genotypic virulence characterization of the isolates

Multiplex PCR was used to detect five genes encoding virulence determinants usually associated with ExPEC: *fimH* (type 1 pili), *papC* (type P pili), *sfa/foc* (type S pili and type 1C fimbriae), *hly* (alpha-hemolysin), and *iucC* (aerobactin) [13,20]. Virulence factor genes were amplified according to Johnson and Stell (10) and Yamamoto et al. [21] with the primers described elsewhere [10,21-23] in a total volume of 50 µl containing 25 µl of KapaTaq 2x Ready Mix (KAPA Biosystems, USA), 20 pmol concentrations of each primer except *hly* (30 pmol), and 5 µl of DNA template. The reaction conditions were as follows: initial denaturation at 94°C for 5 min followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 63°C for 30 s, and extension at 68°C for 3 min, followed by a final 10 min extension period at 72°C. The amplification products were separated by electrophoresis in a 2% agarose gel containing ethidium bromide. A 100-bp DNA ladder (Kappa Universal, USA) was used in each gel as a molecular size marker.

Statistical analysis

Differences in the distributions of the studied determinants were tested by Chi square [24]. A P value of ≤ 0.05 was considered to indicate statistical significance.

Results

Among all this study included isolates (n=205), the most commonly detected phylogenetic group was group A (66.3%) (Table 1), followed by groups: B1 (18.5%), D (10.2%), and B2 (4.3%). Gender distribution of phylogenetic groups showed insignificant differences between females and males.

Females' and males' isolates did not differ significantly for all of the ExPEC's virulence genes detected here in this study, except for *papC* which was significantly (P ≤ 0.05) more prevalent among males' isolates (16.6%) than among females' isolates (6.6%) (Table 2). Among both females' and males' isolates the most prevalent genes were *fimH* (97.5% vs. 100%, respectively) and *iucC* (52.0% vs. 55.9%, respectively), whereas the least prevalent genes were *sfa/foc* (0% each) and *hly* (0.82% vs. 0%, respectively).

Study individuals		No. (%) of isolates positive for the indicated phylogroup			
Gender	No. of *E. coli* isolates	A	B1	B2	D
Females	121	83 (68.5)	19 (15.7)	5 (4.1)	13 (10.7)
Males	84	53 (63.0)	19 (22.6)	4 (4.7)	8 (9.5)
Total	205	136 (66.3)	38 (18.5)	9 (4.3)	21 (10.2)

Table 1: Distribution of phylogenetic groups among fecal E. coli isolates from healthy males and females.

ExPEC VFs' gene	No. (%) of isolates positive for the indicated trait		
	Females' isolates (n=121)	Males' isolates (n=84)	Total (n=205)
fimH	118 (97.5)	84 (100)	202 (98.5)
papC	8 (6.6)	14 (16.6)	22 (10.7)
sfa/foc	0	0	0
hly	1 (0.82)	0	1 (0.48)
iucC	63 (52.0)	47 (55.9)	110 (53.6)

Table 2: Prevalence of ExPEC virulence genes among fecal *E. coli* isolates from healthy males and females.

Phylogenetic distribution of virulence genes was summarized in Table 3. In both females and males virulence genes were concentrated in isolates clustered in group B2, among which the highest rates of multiple virulence factors possession (three or more virulence factors/isolate) were demonstrated in both females' (3/5:60.0%) and males' isolates (2/4:50.0%), whereas only 15.3% vs. 12.5%, respectively, 2.4% vs. 16.9% and 0% each of isolates belonged to groups D, A, and B1, respectively, had multiple virulence factors.

Discussion

The vast majority of both females' and males' isolates included in this study clustered in group A (68.5% vs. 63.0%, respectively) (Table 1), whereas isolates clustered in group B2 were the least prevalent (4.1% vs. 4.7%, respectively). Gender distribution of phylogroups showed insignificant differences between females and males. Similar results were obtained by others [25,26] who found that sex and age factors had no effect on distribution of phylogroups. Whereas Gordon et al. [27] found that in males the probability of isolating A or D strains increased with host age, whilst the probability of detecting a group B2 strain declined, while in females the probability of recovering A or B2 strains increased with increasing host age and there was a concomitant decline in the likelihood of isolating B1 or D strains. The reason for these differences may lie in that Gordon et al. [27] had used

fecal isolates from patients suffering from gastroenteritis, whereas, our isolates were from healthy volunteers who were not aware of any symptoms at the time of sampling. Also these researchers' study included a wider age range (0-80 years), while our study included only young ages (18-45 years).

Isolates	Phylogroups (No. of isolates)	No. (%) of isolates positive for the indicated virulence gene				
		fimH	*papC*	*sfa/foc*	*hly*	*iucC*
Females' isolates (n=121)	A (83)	80 (96.3)	2 (2.4)	0	0	35 (42.1)
	B1 (19)	19 (100)	0	0	0	12 (63.1)
	B2 (5)	5 (100)	3 (60.0)	0	1 (20.0)	5 (100)
	D (14)	14 (100)	3 (21.4)	0	0	11 (78.5)
Males' isolates (n=84)	A (53)	53 (100)	10 (18.8)	0	0	35 (66.0)
	B1 (19)	19 (100)	0	0	0	6 (31.5)
	B2 (4)	4 (100)	3 (75.0)	0	0	3 (75.0)
	D (7)	7 (100)	1 (14.2)	0	0	3 (42.8)
Total (n=205)	A (136)	133 (97.7)	12 (8.8)	0	0	70 (51.4)
	B1 (38)	38 (100)	0	0	0	18 (47.3)
	B2 (9)	9 (100)	6 (66.6)	0	1 (11.1)	8 (88.8)
	D (21)	21 (100)	4 (19.0)	0	0	14 (66.6)

Table 3: Phylogenetic distribution of virulence genes among fecal *E. coli* isolates from healthy males and females.

As a whole, this study results were consistent with those obtained by Duriez et al. [11] and Li et al. [26] who found that strains from phylogenetic groups A and B1 were the most common, followed by phylogenetic group D strains, while strains of the phylogenetic group B2 were rare. This study isolates were dominated by group A (66.3%), as it was shown in previous studies [28,29] that the *E. coli* clonal community of a person is numerically dominated by one strain, or at most a few strains. Other researchers [13,27,30] found much more prevalence of phylogroup B2 among fecal isolates in comparison with this study results. This confirmed the fact that human *E. coli* commensal microbiota varied in a population-specific manner as the geographic locations of the human populations seem to play an important role in structuring the *E. coli* populations [25]. So that, it seems likely that geographic and climatic factors play an important role in structuring the *E. coli* population, worldwide. In addition to the effects of diet and food processing differences between nations. Also, these differences may reflect the influence of host genetic on the commensal flora [11,25,26,31,32]. Therefore, the distribution of phylogenetic groups, subgroups and genetic markers is non-random in the hosts [33] and the four *E. coli* groups may differ in their ecological niches and life-history characteristics [34].

The most commonly detected virulence genes among this study included isolates were *fimH* (98.5%) and *iucC* (53.6%), whereas the least detected genes were *sfa/foc* (0%) and *hly* (0.48%). Females' and males' isolates did not differ significantly for all of the virulence genes detected here in this study, except for *papC* which was significantly ($P \leq 0.05$) more prevalent among males' isolates (16.6%) than among females' isolates (6.6%) (Table 2). The exact reason for this difference was not clear, but it may reflect the morphological, physiological, and dietary differences in the intestinal tract of males and females which influence the distribution of *E. coli* genotypes as the intestinal tract of males and females appear to represent different environments for *E. coli* [27].

Phylogenetic distribution of virulence genes (Table 3) revealed that in both females and males virulence genes were concentrated in isolates clustered in group B2, while isolates clustered in groups D, A, and B1 had the least prevalence of virulence genes. Our results were in agreement with what is known that the human commensal strains had fewer virulence factors than the extraintestinal pathogenic strains [11,13]. In addition, the concentration of virulence factors (two or three virulence factors per strain) was in isolates belonged to group B2 which indicated the influence of phylogenetic origin of an isolate in determining its virulence [11,13].

Conclusions

In both males and females, commensal fecal *E. coli* isolates from Iraqi people in Al-Kut city were dominated by phylogroup A followed by groups B1 and D, whereas group B2 was rare. Also, dominant fecal strains with ExPEC characteristics were much less prevalent.

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