

Evaluation of clonal relatedness among different sources of *E. coli* isolates in Iranian children with urinary tract infection (UTI) and age-matched healthy people.

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Abstract

Urinary tract infections (UTIs) currently rank among the most prevalent infectious diseases worldwide, with chronic and recurrent infections being especially problematic. Uropathogenic *E. coli* (UPEC), which is a causative agent in most cases of UTIs, expresses a multitude of virulence factors. Some virulence factors and specific genes were examined by PCR method. Genetic diversity was evaluated by phylogenetic typing groups. To know the genetic linkages among various *E. coli*, we evaluated clonal relatedness among different sources of *E. coli* isolates in Iranian children with UTI and age-matched healthy people by PFGE. Some pathogenicity determinants were more prevalent in urinary strains rather than fecal *E. coli* strains, significantly. There were PAI IICFT073, PAI II J96, PAI I536, PAI ICFT073, PAI I536, PAI IV, *galD*, *focG*, *vat*, *usp*, *hlyD*, *sat*, *cnf1*, *picU*, *fliC* (H7), *kpsMTII*, *kpsMTIII*. UPEC were mainly found in phylogenetic typing groups B2 and D, while in fecal isolates, phylogenetic groups A and D were the most common. One hundred and fifty *E. coli* genomic DNA were digested by XbaI restriction enzyme and subjected to PFGE. A high level genetic diversity of DNA restriction pattern was reported. So, different clonalities were observed among different sources of *E. coli* isolates in Iranian children with UTI and age-matched healthy people. With attention to these results, genetic patterns showed that the strains had different clonalities. The differences in the PFGE pattern are a result of various genetic events caused by consistent changes with some independent genetic events. Lack of genetic similarity among *E. coli* isolates with different sources can be explained by the acquisition of different gene factors.

Keywords: Clonal relatedness, Fecal *E. coli* isolates, Healthy people, Iranian children, Urinary tract infection (UTI), Uropathogenic *E. coli* (UPEC).

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Introduction

UTI with chronic and recurrent infection is common in childhood around the world. It can have wide range complications, including kidney damage, kidney failure, or blood poisoning (sepsis) [1,2]. UTI occurs in about 3-5% of girls and 1% of boys less than 5 years. After the first incidence of UTI, 60%-80% girls get a second infection within 18 months old [3]. In adults, there are some evidences that about 40% women (in their early 20s) and 12% men (above 85) will experience UTI symptomatically with increasing incidences. During 6 to 12 months, about 25% of these women will get infected again. Uropathogenic *E. coli* (UPEC) is the primary

etiologic agent associated with UTIs [2]. UPEC can invade various types of bladder cells, such as the stratified layers of the bladder urothelium. Both the establishment and persistence of UPEC in the urinary tract is facilitated by invasion of the host cell [4]. UPEC persists, with interior urothelial cells lying in a dormant state within late endosomal vesicles. In addition, UPEC isolates chiefly carry individual virulence factors that help to colonize successfully in the mammalian urinary tract and can cause intestinal and extra-intestinal infection [2,4,5]. Furthermore, UPEC isolates possess several virulence factors which are uncommon in fecal *E. coli* isolates. The mechanisms underlying the evolution and emergence of such new bacterial pathogens are not well understood, and these atypical strains

now are attracting more attention. The variety of virulence determinants due to horizontal gene transfers of mobile elements, such as pathogenicity islands (PAIs), phages and plasmids contributing to a strain's pathogenic potential [6-10].

By applying PFGE, detection of alteration in great pieces of DNA is possible. It is also a very effective tool in epidemiological studies and gene mapping of microbes. In a monotonous magnetic field, segments more than 50 kb length transmit a direction across the gel and move in a zigzag pattern. DNAs separations as bands carry out better across the gel. In PFGE method, closely related bacterial species display patterns equivalent to DNA separation, and those which do not possess similarity or are less similar manifest various separation patterns. Therefore, these attributes can be used to identify the common bacterial agent of a disease. PFGE can also be employed to monitor and evaluate various microorganisms in clinical specimens and existent ones in soil and water. In the recent years, PFGE has proven to be an extremely remarkable and powerful tool for control, prevention, and surveillance of infections in several populations [11]. At first, PFGE illustrated by Schwartz in 1983 with the intermittent change of electricity current brings DNA migration. Recently, several techniques have been expanded to recognize and classify bacteria: however, all these methods are not appropriate. Otherwise, PFGE is the applicable method for detection, identification, and classification of genome of microorganisms accurately and sensitively. Kindergartens, schools, hospitals, prisons, and even homeless refugees are exposed to a greater danger of infection outbreak due to bacteria. Moreover, in some places, such as hospitals, there are patients with weakened immune systems: diseases are can spread quickly there. Hospitals are places where the possibility of introducing bacteria into surgery wounds, *in vitro* fertilization (IVF), and catheterization is possible. Determination of the exact time in suspicious cases of an outbreak is important because the outbreak source can be a hospital worker or hospital food. PFGE method gives researchers the ability of linear DNAs separation longer than 10 mega base pair (Mbp) [12]. Also, PFGE is a useful instrument to determine the relationship between several strains of a single species; to approximate the size and chromosomal mechanism; to illustrate and clarify the genome of eukaryotes and prokaryotes; to assist physical gene mapping; to permit large DNA fragment analysis; and to allow for primary origin detection of genes in fungi, protozoa, bacteria, and even mammals [13]. Albeit, PFGE discriminatory power has evaluated approval in compare with other subtyping methods [14-16]. So, the aim of this study is the evaluation of clonal relatedness among different sources of *E. coli* isolates in Iranian children with UTI and age-matched healthy people by PFGE method.

Materials and Methods

Bacterial isolates

This was a retrospective study wherein we considered 150 *E. coli* strains. We selected two groups. First of all, 100 *E. coli* strains were isolated from urine (50 cases) and stool (50 cases) samples collected from 2-12 year old children with UTI: these were all patients referred to the nephrology ward of the Mofid Children Hospital. From all children with UTI, we isolated *E. coli* from their stool specimen. We also isolated 50 *E. coli* from stool samples of age-matched healthy people. Urine samples were collected in the sterile containers and were cultured onto MacConkey agar, sheep blood agar, and Muller-Hinton agar for colony counting. After incubating at 37°C overnight, we examined microorganisms' growth. Each plate with growth more than 10⁵ CFU/ml was processed subsequently. Besides, 100 *E. coli* which were isolated from stool samples of UTI patients (50 strains) and healthy children as a control group (50 strains) were cultured on MacConkey agar plates and incubated at 37 °C overnight. In total, all *E. coli* strains were approved biochemically and stored in Luria-Bertani (LB) broth supplemented with sterilized 15% glycerol at -20°C for further examination (all media was purchased from Sigma Aldrich Company, Germany) [7]. Some virulence factors and specific genes were examined by PCR method. Genetic diversity was evaluated by phylogenetic typing groups [17].

PFGE

We used a standard operating protocol for PFGE [16]. Plugs were made ready from bacterial suspensions accompanied by proteinase K (10 µl at 20 mg/ml) treatment. The plugs were lysed in ES buffer, including 0.5 M EDTA, pH 9.0, and 1% sodium-lauryl-sarcosine. After treatment with proteinase K at 54°C for 1 to 2 hours, plugs were washed two times (for 15 min each time at 50°C) in sterile distilled water and four times (for 15 minutes each time) in TE buffer (Tris-EDTA). Slices of plug with 2 mm thickness were digested with XbaI endonuclease. CHEF-DRII PFGE apparatus (Bio-Rad) was performed for PFGE by separation on a 1% agarose gel (Seakem) into 0.5% tris-borate-EDTA buffer at 14°C and 6 V/cm. PFGE was done by Bio-Rad CHEF-DRIII system and condition was reported as: digestion temp (°C): 37; digestion time (h): 4-16; units of enzyme per plug: 30; run time (h): 20; initial switch time (s): 2.2; and final switch time (s): 54.2.

Salmonella choleraesuis serotype Branderup H9812 was used as the molecular size marker. The gels were stained with ethidium bromide and photographed under ultraviolet light. Calculation of phylogeny and drawing of phylogenetic tree (phenograms) unweighted paired group (UPGMA) method by BioNumerics Gelcompar II software version 4.0 (Applied Maths, Sint-Martens-Latem, Belgium) and band-based Dice Similarity Coefficient (DSC) was used Interpretation was done by using the guidelines set out previously [16-19].

Data analysis

Continuous variables were expressed as mean \pm Standard Deviation (SD). Discrete variables were reported as frequency and percentage. Chi square test and Fisher's exact test were used to access the relation between quantities' variables. For all statistical analyses, a p-value of <0.05 was considered to be significant. Statistical analysis was conducted using the SPSS version 24. χ^2 test was used for ratio comparison of the same groups (fecal isolates derived from healthy people and UTI patients). McNamara test was used for ratio comparison of the different groups (fecal isolates and urine isolates derived from UTI patients).

Results

Of the 50 fecal *E. coli* isolates, 44% fell into group A, 16% into B2, 14% in B1, and 32% into D, and urinary isolates 34% fell into group D, 54% into B2, 4% in B1, and 8% into A. Of the 50 fecal *E. coli* isolates (control), 48% fell into group A, 6% into B2, 26% in B1, and 22% into D. According to data presented in Table 1, the distribution of fecal *E. coli* isolates and urinary isolates among the four phylogenetic groups is as following: Distribution PAI genes, such as PAI ICFT073 (74 vs. 26%), PAI IICFT073 (38 vs. 14%), PAI I536 (36 vs. 6%), PAI IV536 (86 vs. 42%), PAI II J96 (30 vs. 10%) were more frequent virulence markers in urinary isolates than fecal *E. coli* and PAIII536 (22 vs. 4%), PAI III536 (6 vs. 0%), PAI I J96 (4 vs. 0%) markers was almost similar in urinary isolates and fecal *E. coli*. Distribution of adhesion genes, such as *bmaE* (16 vs. 6%), *gafD* (20 vs. 2%), *focG* (22 vs. 6%) were more frequent virulence markers in urinary than fecal *E. coli* and *afa* (6 vs. 10%), *fimH* (92 vs. 98%) markers were almost similar in both isolates. Distribution of toxin genes, such as *vat* (96 vs. 4%), *usp* (54 vs. 6%), *hlyD* (26 vs. 2%), *cdtB* (18 vs. 10%), *sat* (44 vs. 8%), *cnf1* (26 vs. 0%), *picU* (42 vs. 2%) were more frequent virulence markers in urinary isolates than fecal *E. coli*. *cvaC* (20 vs. 66%) was most frequent marker in fecal *E. coli*. Distribution of miscellaneous genes, such as *fliC* (H7 (26 vs. 10%)), *ompT* (62 vs. 58%) were more frequent virulence factors in urinary than fecal *E. coli*, and *ibeA* (14 vs. 26%) was almost similar in both of them. Distribution of protectins genes, such as *kpsMTIII* (70 vs. 46%) was more frequent virulence gene in urinary rather than fecal *E. coli*. The prevalence of *kpsMTI* (K1) (46 vs. 54%), *kpsMTIII* (14 vs. 2%), *rfc* (O4 LPS) (6 vs. 2%), were almost similar in both of them.

PFGE was performed in order to study the genetic linkage analysis among different sources of *E. coli* isolates. Genome sequence data showed that fragments obtaining from complete XbaI digestion would be anticipated only as 19 visible distinguishable fragments.

Of 150 strains examined in this study by pulsed field gel electrophoresis, 28 strains (18.6%) were identified as non-typeable. The numbers of bands obtained were varied considerably, and were overall out of 15 to 24 (mean 19 bands). The sizes of the bands were Kb 20-1135. Since some

were colorless bands, Pierson's similarity coefficient was used. The PFGE patterns of 150 distinct strains of *E. coli* were visually classified into pulsotypes with the assistance of BioNumerics software.

98% identity level and a similarity cut-off index of 76% were used to define PFGE profiles. Dendrogram drawing in the menu curve was conducted based on the peak bands. The high genetic diversity of DNA restriction pattern by means of PFGE was reported as following: 122 pulsotypes, including 14 clusters in all studied *E. coli* isolates; 84 pulsotypes with 14 clusters in fecal and urinary *E. coli* isolates derived from UTI patients; and 80 pulsotypes containing 8 clusters in fecal *E. coli* isolates derived from healthy people and UTI patients (Figure 1). Isolates with unrelated patterns were considered unrelated epidemiologically. The number of pulsotypes, clusters, and Dice coefficient based on studied isolates is shown in Table 2.

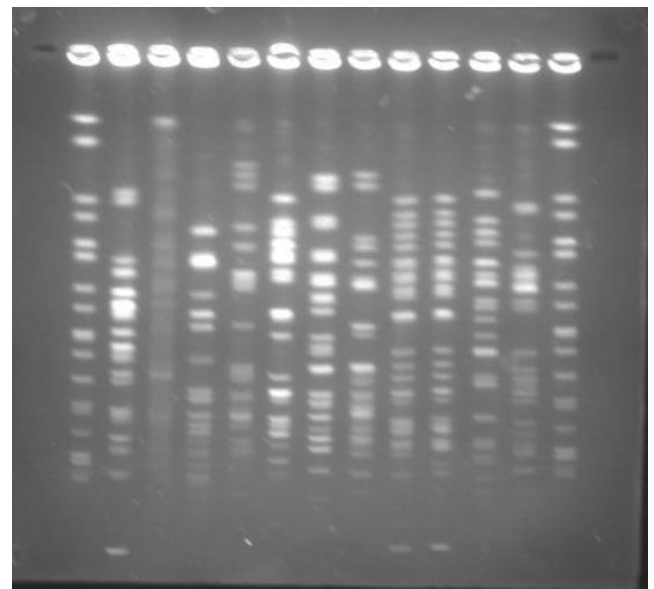


Figure 1. Pulsed-field gel electrophoresis image showing the DNA band patterns of studied strains. Fragments obtaining from complete XbaI digestion. (*Salmonella choleraesuis* serotype Branderup H9812 was used as the molecular size marker in first and the last lane).

As shown in Figure 2, clonal spreading was not observed in the studied population due to high diversity profiles of PFGE. ($D_i=0.98$) Dendrogram of all studied population isolates is provided below.

14 clusters were seen in fecal and urinary *E. coli* strains. Due to the high diversity profiles of PFGE, clonal spreading was not observed in fecal and urinary strains isolated from UTI patients. Dice coefficient of studied isolates was 0.912. Dendrogram of fecal and urinary strains isolated from UTI patients is shown in Figure 3.

8 clusters were identified. Due to the high diversity profiles of PFGE, clonal spreading was not observed in healthy people's fecal isolates and UTI patients' fecal isolates respectively. Dice coefficient of studied isolates was 0.908. Dendrogram of

healthy people's fecal isolates and UTI patients' fecal isolates is shown in Figure 4.

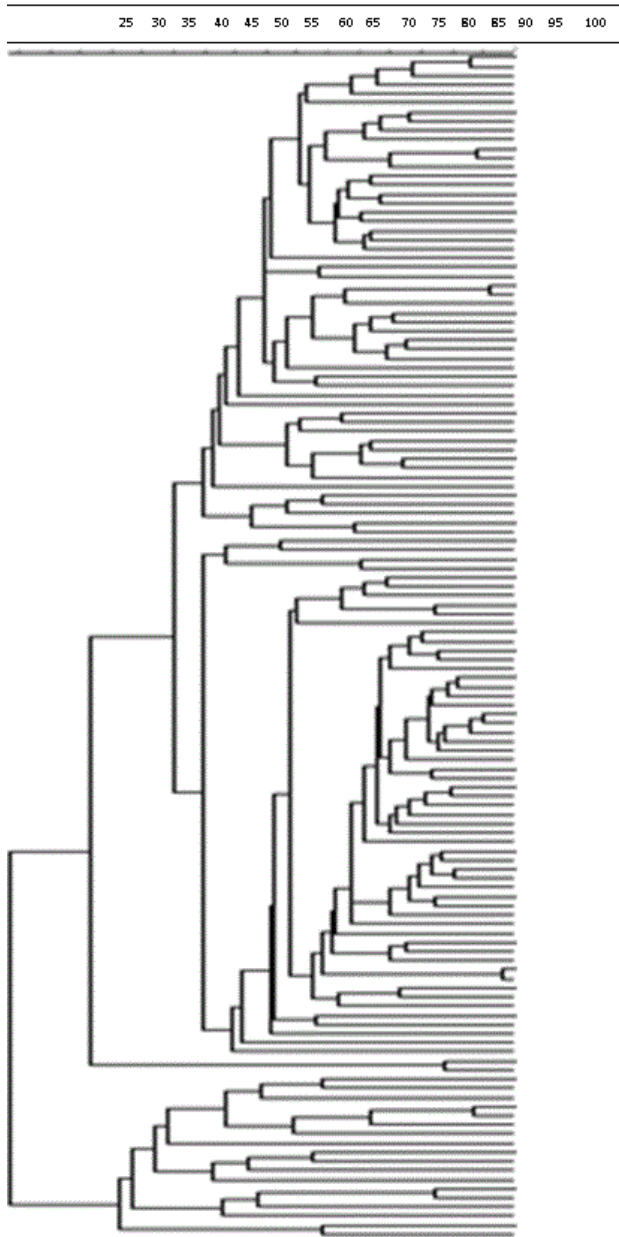


Figure 2. Dendrogram of all studied population isolates.

Table 1. Distribution of virulence factors and specific genes in fecal *E. coli* and urinary *E. coli*.

Genes	Fecal <i>E. coli</i> control N=50	Fecal <i>E. coli</i> patients N=50	Urinary <i>E. coli</i> N=50	p-value
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PAI genes				
PAI III 536	1 (2%)	0 (0%)	3 (6%)	0.324
PAI IV 536	9 (18%)	21 (42%)	43 (86%)	<0.001
PAI II CFT073	3 (6%)	7 (14%)	19 (38%)	<0.001
PAI I 536	1 (2%)	3 (6%)	18 (36%)	<0.001
PAI II 536	0 (0%)	2 (4%)	11 (22%)	<0.001
PAI I J96	0 (0%)	0 (0%)	2 (4%)	0.329
PAI II J96	2 (4%)	5 (10%)	15 (30%)	0.001
PAI I CFT073	7 (14%)	13 (26%)	37 (74%)	<0.001
Adhesion genes				
<i>afa</i>	1 (2%)	5 (10%)	3 (6%)	0.298
<i>bmaE</i>	0 (0%)	3 (6%)	8 (16%)	0.007
<i>fimH</i>	47 (94%)	49 (98%)	46 (92%)	0.534
<i>gafD</i>	0 (0%)	1 (2%)	10 (20%)	<0.001
<i>focG</i>	2 (4%)	3 (6%)	11 (22.0%)	0.006
Toxin genes				
<i>cdtB</i>	3 (6%)	5 (10%)	9 (18%)	0.194
<i>hlyD</i>	4 (8%)	1 (2%)	13 (26%)	0.001
<i>cnf1</i>	2 (4%)	0 (0%)	13 (26%)	<0.001
<i>cvaC</i>	0 (0%)	33 (66%)	10 (20%)	<0.001
<i>usp</i>	4 (8%)	3 (6%)	27 (54%)	<0.001
<i>vat</i>	2 (4%)	2 (4%)	48 (96%)	<0.001
<i>sat</i>	1 (2%)	4 (8%)	22 (44%)	<0.001
<i>picU</i>	1 (2%)	1 (2%)	21 (42%)	<0.001
Miscellaneous genes				
<i>fliC_H</i>	4 (8%)	5 (10%)	13 (26%)	0.020
<i>ibeA</i>	10 (20%)	13 (26%)	7 (14%)	0.325
<i>ompT</i>	29 (58%)	29 (58%)	31 (62%)	0.895
Protectins genes				
KPS MT I	24 (48%)	27 (54%)	23 (46%)	0.707
KPS MT II	15 (30%)	23 (46%)	35 (70%)	<0.001
KPS MT III	3 (6%)	1 (2%)	7 (14%)	0.083
<i>rfc</i>	3 (6%)	1 (2%)	3 (6%)	0.700

Table 2. Number of pulsotypes, number of clusters and Dice coefficient according to studied isolates.

Isolates	Pulsotypes (No.)	Clusters (No.)	Cluster (No.)	Dice coefficient
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All studied isolates	122	14	Cluster1 (4), Cluster 2 (3), Cluster 3 (3), Cluster 4 (2), Cluster 5 (2), Cluster 6 (2), Cluster 7 (6), Cluster 8 (4), Cluster 9 (2), Cluster 10 (2), Cluster 11 (24), Cluster 12 (8), Cluster 13 (3), Cluster 14 (2)	0.98
Fecal and urinary isolates from UTI patients	84	14	Cluster 1 (6), Cluster 2 (15), Cluster 3 (2), Cluster 4 (2), Cluster 5 (2), Cluster 6 (2), Cluster 7 (2), Cluster 8 (2), Cluster 9 (5), Cluster 10 (2), Cluster 11 (3), Cluster 12 (3), Cluster 13 (2), Cluster 14 (1)	0.912
Fecal isolates from healthy people and UTI patient	80	8	Cluster 1 (7), Cluster 2 (2), Cluster 3 (4), Cluster 4 (3), Cluster 5 (27), Cluster 6 (2), Cluster 7 (7), Cluster 8 (22)	0.908

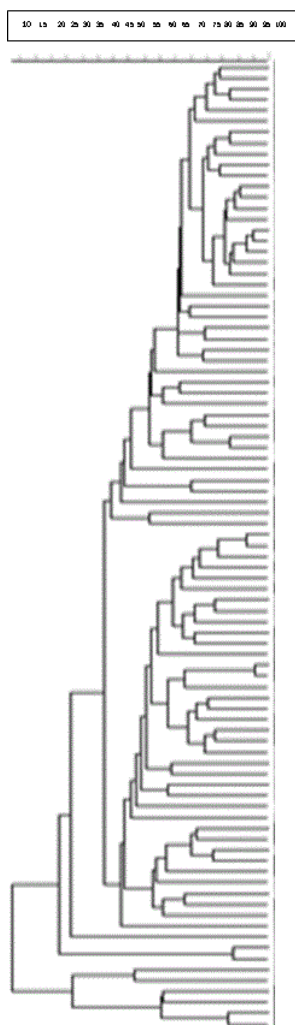


Figure 3. Dendrogram of fecal and urinary strains isolated from UTI patients.

Discussion

E. coli is an intestinal flora whose diversity creates large uncertainties in the identification of new genomes [20,21]. Most strains of *E. coli* are not harmful as long as they are living in the intestinal tract, but they may cause bacterial infections if they enter other parts of the body, such as

conjunctiva, gallbladder, genital tract, eyes, appendix, and respiratory tract [22].

The development of PFGE typing methods based on fingerprinting of bacterial genome has given valuable tools to confirm the relationship among outbreak strains [23-27].

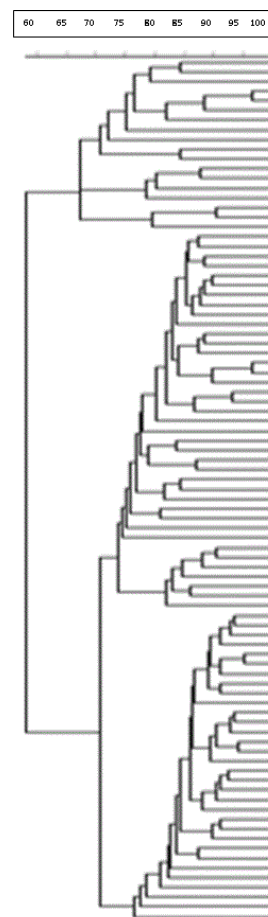


Figure 4. Dendrogram of healthy people's fecal isolates and UTI patients' fecal isolates.

In the current study, we applied PFGE followed by XbaI restriction digestion of chromosomal DNA to determine the genetic relatedness among *E. coli* isolates derived from different sources. This enzyme is the most frequently used restriction enzyme for UPEC outbreaks [28,29]. The number of bands obtained was varied, and they were overall out of 15 to

24 (mean 19 bands). The sizes of the bands were 20 to 1135 kb. Since the samples were collected in a year and from different sources of population, these results were predictable. Only, 4 identical clones detected in all urinary and fecal isolated from patients with UTI. Two urinary and fecal strains isolated from 1 patient with UTI had more than 98% similarity. Also, four identical strains were reported in fecal isolates of healthy individuals (one strain belonged to a sister and her brother, and the other strain belonged to a twin). The best description of this variation was that the isolates from the subjects were not of a common clone or origin, and they did not have the same genotypes. Other factors that caused this difference including the number and size of the bands obtained from the fingerprinting. Also, these variations have been observed in different part of the world. Some reasons are being diverse of strains isolated from patients in distinct parts of the world, being various laboratory conditions, and a wide variety of *E. coli* strains in several human societies, and being genetic differences of strains because of different types of samples and their location. *E. coli* isolates belonging to the clonal group had different virulence characteristics. The probable reasons for the presence of the same virulence factors in pathogens and a large variety in the pulsotypes were being variety of *E. coli* in the colon and as a consequence of the diversity of the uropathogens. Other reason was possibility of genetic DNA exchange through horizontal gene transfer between strains.

Other studies have shown different bands with high diversity of molecular weights. In molecular typing of Japanese *E. coli* isolates, Kawamori et al. reported 19 to 24 DNA bands with molecular sizes of 30 to 500 kb. With PFGE typing of UPEC strains, Ejrnaes et al. reported 15 to 20 distinct bands with 50 to 1200 kb molecular weights [30,31]. In an epidemiologic study of *E. coli* isolates in Northern Ireland, Watabe et al. observed 15 DNA fragments using PFGE [27].

In this study, the Dice similarity coefficient has been explained approximately as equal genetic relatedness [13,30,31], though there was little correlation among similarity evaluation obtained from single enzymes. Poor similarity and little correlation between Dice similarities can be explained by one enzyme digestion and by an enhanced pathogenic factor: the latter occurs due to the acquisition of virulence genes from the environment in bacterial genome. This hypothesis is supported by our finding. Based on other studies, single-enzyme digestion PFGE is presumably correlated deficiently with randomly amplified polymorphic DNA [13,32,33]. According to our study, there are some reasons for the weak correlations among Dice coefficients obtained from single fragment size resolution: these could explain the discrepancy between the 18-25 fragments identified by superlative published protocols for *E. coli* PFGE. On the other hand, concealed bands would not be considered and would influence the Dice index underneath. Two bands with simultaneous movement in a non-same position could be considered as only one non-matching band and would influence the Dice coefficient upgrade [13,34,35].

To summarize, we can conclude that our results suggest that the presence of certain features such as *PAI ICFT073*, *IICFT073*, *PAI I536*, *PAI IV536*, *PAI II J96*, *PAI II536*, *gafD*, *focG*, *vat*, *usp*, *hlyD*, *sat*, *cnf1*, *picU*, *fliC(H7)*, *kpsMTII*, *kpsMTIII* in *E. coli* has made the bacterium compatible with its extra-intestinal environment and may cause extra-intestinal infections in humans. On the other hand, the presence of some virulence genes such as *PAI IV 536*, *fimH*, *cva C*, *ompT*, *KSP MT I*, *KSP MT II* in the fecal bacteria can make it a reservoir for the emergence of virulence factors for ExPEC.

“Indeed, when an outbreak research is under way, time and resources may not incorporate more attempts. Without prior presumption permitted by epidemiologic data, however, a relatedness valid measure, rather than a method that simply recognizes matches, is required. Inferences about genetic linkages based on only one-enzyme PFGE should be mentioned with caution. The computer software programs analyse Dice coefficients and generate dendrograms relatively easily. In addition, failure to recognize relatedness concepts and similarity coefficients may have facilitated acceptance of dendrograms from single enzyme PFGE as precise relatedness measures. BioNumerics software produces banding patterns analysis from large sets of isolates, but it cannot be trusted to consider the presence or lack of bands without visual perception of the gel picture” [14,21,36]. The present study findings allow us to conclude epidemiologic relationships among isolates from PFGE data: however, more restriction enzymes would be required to make a reasonable approximation of genetic relatedness. Thus, poor resolution of fragment size could account for the discrepancy between the 18 to 25 fragments detected by most published PFGE protocols for O157 and the 40 fragments expected based on sequence data. There are other possible explanations for the discrepancy: (i) the sequence data may be in error or (ii) some of the XbaI recognition sites in the O157 genome may not be available to the enzyme if, for example, they happen to be adjacent to a methylated site. The impact of occult or comigrating bands is potentially great [37].

The observation of some virulence factors in urinary tract isolates, including: *PAI ICFT073*, *IICFT073*, *PAI I536*, *PAI IV536*, *PAI II J96*, *PAI II536*, *gafD*, *focG*, *wat*, *usp*, *hlyD*, *sat*, *cnf1*, *picU*, *fliC (H7)*, *kpsMTII*, *kpsMTIII* compared to fecal isolates was statistically significant. The frequency of pathogenic factors in urine strains was significantly higher than fecal strains of patients and healthy individuals. Phylogenetic typing showed that urinary strains belong to the phylogenetic groups of typing B2 and D, while the fecal strains of the patients belonged to phylogenetic typing groups A and D and fecal isolates of healthy individuals belonged to phylogenetic typing groups A and B1. According to the findings of other studies, phylogenetic typing of B2 and D is most commonly seen in extracellular and pathogenic *E. coli*. The simultaneous presence of some virulence factors with high frequency in urinary strains compared to fecal isolates indicated that the pathogens producing UTI have a potential pathogenic compared to other isolates of this study. The differences between the distribution of virulence factors and genomic

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typing between urinary and fecal strains of patients with healthy individuals' fecal isolates suggest that colonization of the virulent strain in the intestines can increase the risk of UTI.

Extra-intestinal pathogenic *Escherichia coli* (ExPEC) have a complex phylogeny, broad virulence factor (VF) and significant genomic plasticity, and are associated with a spectrum of host infective syndromes ranging from simple urinary tract infection to life-threatening bacteremia. Their importance as pathogens has come to the fore in recent years, particularly in the context of the global emergence of hyper-virulent and antibiotic resistant strains. Despite this, the mechanisms underlying ExPEC transmission dynamics and clonal selection remain poorly understood. Large-scale epidemiological and clinical studies are urgently required to ascertain the mechanisms underlying these processes to enable the development of novel evidence-based preventative and therapeutic strategies [38].

Isolates derived from UTI patients did not have a common origin and are not identical genotypes. Differences in size and number of bands obtained from strains fingerprinting in different parts of the world, diverse experimental conditions in other parts of the world, a wide variety of *E. coli* in communities, and various human genetic variations due to several types of strains isolated from gathered samples cause these varieties.

Study Limitation

There are established methods such as DNA-seq (Illumina), Pacbio or Oxford Nanopore sequencing which they can easily assess the genetic diversity across the strains and probably discover some additional, new features. Due to the high cost of mentioned methods and limitations in our budget, we could not use them.

Recommendations

The present study provides molecular and epidemiological information about virulence factor genes found in two groups of *E. coli*. It is necessary to have a simple pathotypes screening test which can be beneficial to facilitate along with other experiments in establishing an UTI assessment. Unfortunately, due to the high variation in pathogenicity determinants of UPEC strains, pathotypes could not be determined using pathogenicity determinants. Knowledge of the molecular details of UPEC is mainstay of successful strategies development for treatment of UTI and prevention of its subsequent complications. Globally, PFGE method is capable of identifying 90% of the bacterial genome, and of monitoring genetic changes in antibiotic-resistant genes in hospital-infection producing bacteria. Despite the high cost of materials and instruments, or the time-consuming procedure, PFGE is suggested as a practical and applicable typing method. Yet, it should be noted that although PFGE is taken as the gold-standard technique in epidemiological studies, using this method alone does not completely confirm the similarity of the two isolates. Thus, the utilization of the second strains typing

method or additional epidemiological analysis is useful to understand the dominant gene and how genetic differences cause diseases.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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