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Association between virulence profile, biofilm formation and phylogenetic groups of *Escherichia coli* causing urinary tract infection and the commensal gut microbiota: A comparative analysis

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A B S T R A C T

Variety of virulence factors are involved in the pathogenicity of *Escherichia coli,* the common cause of the urinary tract infections (UTIs). The aim of this study was to determine some virulence factors involved in the pathogenicity and the phylogenetic grouping of *E. coli* from UTIs compared with the *E. coli* isolates from gut microbiota (fecal flora). The isolates were tested for biofilm formation, haemagglutination, cell surface hydrophobicity (CSH), hemolysin production, phylogenetic grouping and the distribution of 6 known virulence genes. Isolates from UTIs showed a significantly higher prevalence of haemagglutination and hemolysin production compared with fecal flora ($P \leq 0.05$), while biofilm formation and cell surface hydrophobicity (CSH) were not significantly different among the groups. Prevalence of virulence genes *fimH*, *kpsMT ll*, *iutA*, *sat*, *hlyA*, and *cnf1* among all isolates were: 94.5%, 66.95%, 67.8%, 39%, 23.07% and 21.08%, respectively. The genes for *hly*A, *cnf1*, *kpsMT ll* were found to be higher in UTI isolates compared to fecal flora ($P \leq 0.05$). The frequency of the isolates in the phylogenetic groups B2, D, A and B1 were 36.7%, 31.3%, 16.2% and 15.6%, respectively. All the virulence genes except *fimH* were found to be significantly higher in the isolates of groups B2 and D. The results suggests that certain factors are necessary for the host colonization and infection and they are common in both virulent and non-virulent strains, and that the strains in the groups A and B1 having the lower virulence factors must acquire these factors when the condition is in favor of their dissemination to the urinary tract. In contrast the isolates in the groups B2 and D appeared to be potentially virulent.

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1. Introduction

Escherichia coli is a common member of gut microbiota, both in human and various warm-blooded animals. It is also the most common cause of urinary tract infections (UTIs), and clinically results in complicated or non-complicated infections appearing as cystitis or pyelonephritis, depending on the host and bacterial factor as well as the route of bacterial dissemination [1]. Various virulence factors are involved in the bacterial pathogenic potential such as adhesive factors used by bacteria for primary colonization, iron acquisition system, and production of various toxic substances to invade the cells and overcome the host immune response [2]. Recent works showed a relation between *E. coli* phylogenetic characteristics and presence of virulence genes [3]. Phylogenetic analysis is based on the presence or absence of 3 markers: an outer membrane hemin receptor gene (*chuA*), the gene encodes for an uncharacterized protein (*yja*) and TSPE4.C2 (an unspecified DNA fragment). Accordingly different strains of *E. coli* can be categorized into four major phylogenetic groups (A, B1, B2, and D) which can be divided into seven subgroups A0, A1, B1, B2(2), B2(3), D1 and, D2 using a rapid polymerase chain reaction (PCR) [4,5]. Various works demonstrated that isolates on group B2 and D are usually more virulent, whereas majority of the isolates in group A and B1 are nonpathogenic and isolated from nonpathogenic gut microbiota [4].

In uropathogenic *E. coli* (UPEC) many factors have been reported to be responsible for the virulence such as fimbrial or non fimbrial

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adhesins, hemolytic factors and cytotoxic necrotizing factors (*cnf*1) [6]. The *cnf1* has been reported in 40% of UPEC and has a role in the dissemination and persistence of cells in the urinary tract [6]. Among other factors involved in the pathogenicity of UPEC are *kpsMT II* responsible for the capsular polysaccharide production, *fim* (type1 pili) and *fimH* mediates the invasion of the bacteria and iron scavenging properties mediated by *iutA* (aerobactin receptor) [7,8]. It has been suggested that the certain virulence genes are usually occur in the combinations [8]. Persistence of *E. coli* in the intestine has been linked to the presence of different virulence factors such as special adhesions, K1 capsular type, aerobactin and the iron −chelating compounds [9]. In many UTIs especially persisting infections the biofilm are involved and are usually associated with the catheters and prostheses. Haemagglutinating activities are involved in bacterial adhesion, with mannose resistance haemagglutination expressing mostly P fimbria and usually seen in isolates from UTIs and mannose sensitive haemagglutination expressing type 1 pili and found mostly in normal gut microbiota [10]. Adhesions had a crucial role in the *E. coli* biofilm formation which is an important cause of treatment failure in patients despite appropriate antimicrobial therapy [11]. Biofilm formation and interaction and reversible attachment with a biotic surfaces are highly dependent on the psychochemical and electrostatic interaction between the surfaces and the bacteria [12]. Cell surface hydrophobicity (CSH) may contribute to the bacterial adherence, although it is not clearly documented that CSH is a significant determinant of adherence among UPEC isolates [13]. Studying the virulence factor with respect to various phylogenetic groups can reveal an important aspect of pathogenicity of UPEC. The aim of this study was to determine the production of mannose resistance haemagglutination, cell surface hydrophobicity, biofilm formation, virulence genes which are most commonly found in *E. coli* isolates, determination of phylogenetic groups and compare them in isolates from UTIs with the commensal organisms resided in the normal fecal flora.

2. Material and methods

2.1. Specimen collection and bacterial isolation

From October 2014 to June 2015 a total of 251 isolates of *E. coli* were collected from the patients admitted to the three university hospitals affiliated with the Kerman University of Medical science in Kerman, Iran (Afzalipour hospital, Shafa hospital and Shahid-Bahonar hospital, Kerman, Iran), the hospitalized patients (n = 100), or the non-hospitalized (Out-patients, n = 151) and 100 bacteria isolated from the fecal flora of the healthy subjects with no sign or symptom of gastroenteritis. The identification of all the isolates were confirmed by biochemical tests [14]. The bacterial strains were stored in the trypticase soy broth (TSB) with 30% and 40% glycerol and were stored either in −20 °C for phenotypic or −70°C for genotypic characterization [15].

2.2. Haemagglutination

To determine mannose-resistant agglutination (MRHA), the method of Duguid et al., was used with some modification [12]. Briefly, bacteria was grown on nutrient broth in shaker incubator (120 RPM). The bacteria in this starter culture were transferred to the sheep blood agar plates. After the overnight incubation, a few colonies of each bacterium were suspended in the phosphates buffer saline to give a turbid suspension, equal to 8–10 McFarland standards. The citrated human O^+ blood group was used to test the haemagglutination. The red blood cells were prepared after 3 times washing and centrifugation of the fresh blood cells (2000 g, 4 °C, 10min). The test was performed in the presence of 0.5% (W/V) D-mannose, in 4 °C, after 2, 5, 10 and 15 min incubation. The weak reactions were considered positive if the reaction would enhanced during further incubation.

2.3. Determination of cell surface hydrophobicity (CSH)

Cell surface hydrophobicity (CSH) of the isolates was determined by the salt aggregation method according to Lindahl et al. [16]. The bacterial cells were grown on the nutrient agar overnight. The subculture was performed on the nutrient broth as the starter culture. The bacteria was sub cultured again, and was harvested at the logarithmic phase of growth (6–7 h with an OD of 1.2–1.5 at 600 nm). The cell suspension was experimentally adjusted to 0.9–1 at 600 nm, and one drop of bacteria was mixed with a drop of ammonium sulfate on a clean slide. The isolates aggregated in ammonium sulfate with the concentration of 0.2–0.4 M were regarded as hydrophobic, and those which have been that aggregated with 1.6–2 M were regarded as non-hydrophobic. Positive control of the experiment consists of the highest concentration of salt (molarity 2), and negative control was a mixture of bacterial suspension mixed with an equal volume of phosphate buffer.

2.4. Biofilm assay

To determine biofilm formation by the bacteria, the isolates were cultured in Luria Bertani (LB) broth in the static conditions. The overnight cultures of each bacterial isolate were diluted to an OD of 0.1 at 600 nm in fresh LB broth. In each of wells of the sterile 96-well of polystyrene micro titer plate (Falcon, USA) aliquots (100 μL) of the bacteria suspension were added and incubated for 24 h at 30 °C. After the incubation, the plates were washed with water (3 times), and the biofilm formation was visualized by staining with 0.1% crystal violet in the water fallowed by three times rinse with water. The Biofilm formation was visualized at OD 595 nm after addition of 200 μL of 95% ethanol [17]. The Positive control for the assay was *Pseudomonas aeruginosa* strain PAO1 and the culture medium without bacteria was used as the negative control.

2.5. Polymerase chain reaction (PCR) method for the detection of virulence genes

The DNA was extracted by boiling method [15]. Briefly, 2 to 3 colonies from an overnight culture of the test isolates in trpticase soy agar was suspended in 300 μl of HPLC grade distilled water. The suspension was boiled for 10 min at 100 C°, centrifuged, and the pellet was suspended to the initial volume with water, and was used in the PCR assay. The genes *fim*H, *iut*A, *kpsMT II*, *hly*A, *cnf1* and *sat* were amplified by PCR using the primers listed in Table 1 [18–20]. PCR amplification was performed in a total volume of 25 μL containing: 0.5 μL of each primer (10 pM), 25 μL of DNA Polymerase Master Mix RED (Ampliqon Co, Inc, Denmark), 1 μL of DNA and 10.5 μL of water (DNase and RNase free water) in Flex-Cycler PCR Thermal Cycler (Analytik Jena, Germany) under the following conditions: initial denaturation at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 58–63 °C for 1 min (Table 1), extension at 72 °C For 1 min. The final extension step was continued for another 5 min at 72 °C. PCR products were electrophoresis in 1.5% agarose gel in the 0.5 TBE buffer Tris, EDTA, Boric acid, containing Green viewer dye (Pars Tous, Co, Iran). The gels were visualized using Gel Doc TM XR image analysis station (Bio-Red, Hercules, USA).

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