1. Introduction

*Escherichia coli* (E. coli) strains or pathotypes are genetically diverse groups which cause several types of infections. These strains encode a number of adhesions mediating their persistence and colonization to epithelial cells; thereby, they pass the host immune defense mechanisms and initiate extra-intestinal infections[1-3]. In addition to fimbrial adhesions, toxins, iron uptaking siderophores, and polysaccharide capsule also participate in the pathogenesis of isolates. Toxin production in parallel with colonization of *E. coli* may induce inflammatory reactions in the urinary tract[4-6]. Alpha-hemolysin (hlyA) and cytotoxic necrotizing factor 1 (cnf1) are two known toxins which have been demonstrated to cause direct cytotoxicity to the host tissues. Four different toxins from the serine protease autotransporters of Enterobacteriaceae (SPATE) were determined in strains of *E. coli* from pyelonephritis, the *sat* (secretory protein), *pic* (protease leading to colonization), *var* (vaculating autotransporters toxin) and *Tsh* (the temperature sensitive hemagglutinin), all of which are widely spread in UPEC but not in commensal strains[7-9].
Furthermore, *E. coli* adhesions such as type 1 fimbriae and pili are important for bacterial colonization. The non-fimbrial adhesins are expressed alongside the fimbrial adhesins in the host following stimulatory factors such as environmental and host signals[10].

Drug-resistant strains from Enterobacteriaceae, especially those producing ESBL, are typically treated with carbapenems. The increase of resistance to imipenem among these species will lead to the difficulties in eradication of infection[11-13]. Several carbapenemases encode carbapenemase enzymes such as metallo-beta-lactamases such as *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>NDM</sub>, *bla*<sub>SERV1</sub>, *bla*<sub>GES</sub>[14,15]. Several previous studies have detected some of these genes in Iran with various MICs of imipenem. However, the relation of carbapenemases and virulence factors among imipenem-resistant *E. coli* strains has not been fully elucidated. Therefore, this study was performed to detect the frequency of virulence genes and the genes responsible for phylogenetic groups, serogroups and antibiotic resistance among *E. coli* isolates of serogroup O1 from clinical infections.

### 2. Materials and methods

#### 2.1. Bacterial isolates

This cross-sectional study was performed in 2017. One hundred *E. coli* clinical isolates were collected from patients within an age range from < 1 to 89 years old. *E. coli* isolates were identified via common biochemical methods and then stored in trypticase soy broth (TSB) containing 30% glycerol for further study.

#### 2.2. Antibiotic susceptibility profile in *E. coli* strains

The antibiotic susceptibility of strains was tested according to CLSI guidelines. The antibiotic discs (Mast) used in the present study included 14 antibiotics, namely, phosphomycin (FO, 200 µg), ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), co-amoxiclav (AMC, 30 µg), cefazolin (CZ, 30 µg), nitrofurantoin (NI, 300 µg), piperacillin-tazobactam (PTZ, 110 µg), gentamycin (GM, 10 µg), tetracycline (TE, 30 µg), cotrimoxazole (TS, 25 µg), amoxicillin (AMX, 25 µg), imipenem (IMI, 10 µg), meropenem (MER, 10 µg), and ciprofloxacin (CIP, 5 µg). *E. coli ATCC 25922* was cultured for the quality assurance of the test.

#### 2.3. Virulence typing of *E. coli* strains

Specific primers in the PCR for detection of genes for drug resistance and virulence genes in this study have been shown in Table 1. Virulence genes including capsular polysaccharide synthesis K1 (*kpsMTII*), cytotoxic distending toxin (*cdt*), cytotoxic necrotizing factor 1 (*cnf1*), receptor toll/interleukin 1 (TcpC), secretion autoinducer toxin (sat), vaculating autoinducer toxin (vat), serum survival (*traT*), α-hemolysin (*hlyA*), invasion of brain endothelium (*ibeA*), ferric aerobactin receptor (iron uptake/transport) (*iuA*), curli fimbriae (*csgA*) and serine protease autoinducer (*pic*) were studied by PCR.

#### 2.4. Phylogenetic typing of strains by PCR method

Genomic DNA was prepared by boiling method and used as templates for PCR. Phylogenetic grouping for each of the strains was performed by PCR according to the *chuA* and *yjaA* genes and the DNA fragment *TspE4.C2*. The primer pairs used were *chuA* F-(5’-GACGAGACCAACAGCAGGGCA-3’) and R- (5’-TTGCGATACGGAGCACTGAC-3’), *yjaA* F-(5’-GAAGGGAATCGCTTATGATAC-3’) and R- (5’-CGTCCAGTACGGAGTATC-3’). The PCR was performed with Bio-Rad thermal cycler T100 under the following conditions: denaturation for 4 min at 94 °C; 30 cycles of 1 min at 94 °C, 1 min at 61 °C, and 1 min at 72 °C; and a final extension for 10 min at 72 °C.

#### 2.5. Amplification of *E. coli* strains of O-serogroup

The most common serogroups of the extraintestinal *E. coli* include antigens O1, O2, O4, O6, O7, O12, O15, O16, O18, O25, O75 and O157 which were analyzed using PCR. Primers used in this study are listed in Table 2. PCR was performed with a Bio-Rad thermal cycler T100 under the following conditions: denaturation for 5 min at 94 °C; 30 cycles of 1 min at 94 °C, 1 min at 61 °C, and 50 s at 72 °C; and a final extension for 10 min at 72 °C.

### Table 1

| Genes | Primers PCR product size (bp) |
|-------|-----------------------------|
| *cdt* | FP: 5’-AAATCACCAGAATCCATCAGTTA-3’; RP: 5’-AAATCCTCATGCAACATCAGTTA-3’ | 430 [16] |
| *kpsMTII* | FP: 5’-GGCCATTTTGCTGACTTGTG-3’; RP: 5’-CATCAGAGCTTATGAGTACGCA-3’ | 272 [16] |
| *TcpC* | FP: 5’-GAGTGAAGGAGGTGAAGCC-3’; RP: 5’-GCATGCGCTCATTATCCGC-3’ | 544 [17] |
| *tauA* | FP: 5’-GGTCTCGGACGCGAATAGC-3’; RP: 5’-GCATGCGCTCATTATCCGC-3’ | 302 [18] |
| *traT* | FP: 5’-GTTGCGTGGGCGTAAGCAG-3’; RP: 5’-GCAGTTCACACCCGTAGA-3’ | 290 [18] |
| *hlyA* | FP: 5’-GCATCACTAACGGTGCTCTCC-3’; RP: 5’-AATGACCAAACTGTTATGAGCT-3’ | 534 [19] |
| *cnf1* | FP: 5’-AAGATGGAGTTTCCTATGAGAG-3’; RP: 5’-CATTCAAGTGCTTACCTTAAA-3’ | 498 [7] |
| *ibeA* | FP: 5’-AGGCAGATGTGGGCGCGCTAC-3’; RP: 5’-TGGTGCTCCGGACCACATCGC-3’ | 171 [7] |
| *vat* | FP: 5’-AACCGTGTTGCGGCCAACATCC-3’; RP: 5’-AATGGCCCTTAGGACGGCGAT-3’ | 420 [7] |
| *sat* | FP: 5’-TCGAGAAGCTAGAAGACATG-3’; RP: 5’-CCATTATCACCAGAAAAACGGC-3’ | 930 [7] |
| *pic* | FP: 5’-ACTCGATCTTCAAGCTCAGATG-3’; RP: 5’-GACCTAAATGTCACTGTCCAGG-3’ | 572 [7] |
| *csgA* | FP: 5’-GGCCGAAATGGTGCTCAGATG-3’; RP: 5’-CGTATCCAGAATGCCTTCCG-3’ | 295 [20] |
The antibiotic resistance pattern of 37 isolates of E. coli was as follow: phosphomycin (0%), ceftazidime (57%), cefotaxime (60%), co-amoxiclav (84%), cefazolin (87%), nitrofurantoin (11%), piperacillin-tazobactam (8%), gentamycin (73%), tetracycline (100%), cotrimoxazole (78%), amoxicillin (100%), imipenem (3%), meropenem (3%), and ciprofloxacin (70%).

3.3. Phylogenetic typing of E. coli strains

Of the 37 strains belonging to O1 serogroup, 30% belonged to the A phylogroup. These strains belonged to this phylogroup containing the virulence genes of csgA, hlyA and traT. Interestingly, kpsMII and pic virulence genes were not detected in these strains.

Twenty-one percent of the strains belonged to the B1 phylogroup, which included 56.7% of the strains belonging to O1 serogroup containing the virulence genes iutA, kpsMII, csgA, traT, and hlyA, but the virulence genes of sat, vat and pic were not detected in these strains.

The B2 phylogroup had the highest prevalence in serogroup O1 (39%). All of these strains contained the iutA gene, while the virulence genes of ibeh and vat were not detected in these strains.

Table 2

| Primers     | Sequences (5'-3') | PCR product size (bp) |
|-------------|-------------------|-----------------------|
| gndbs.F     | 5'-ATACCGACGCAGCGATCTG-3' | -                     |
| rb01.R      | 5'-CAGAAATACACTTGGAAGAC-3' | 189                   |
| rb02.R      | 5'-GTGACTTTCGTACAAAGC-3' | 274                   |
| rb04.R      | 5'-AGGAGCCATTGGCCACACTC-3' | 193                   |
| rb06.R      | 5'-AAATGGGACCCACATTAC-3' | 584                   |
| rb07.R      | 5'-CCGAGATCCAGCATGCTCG-3' | 722                   |
| rb012.R     | 5'-GTGTCAAATGCTTGCGAAACC-3' | 313                   |
| rb015.R     | 5'-TGATAATGACCAACTCGACG-3' | 419                   |
| rb016.R     | 5'-GGATCATTTATGCTGGTACG-3' | 536                   |
| rb018.R     | 5'-GAAGATGGCTATAATGGTTG-3' | 360                   |
| rb025.R     | 5'-GAGATCCAAAAACAGTTGG-3' | 400                   |
| rb075.R     | 5'-GTAAATGCTTGGCAGAAAACC-3' | 672                   |

3.4. PCR detection of E. coli virulence genes

Twelve virulence genes were evaluated in strains. The prevalence rates of cnf, cdt, iutA, csgA, kpsMII, ibeh, vat, traT, TcpC, sat, hlyA and pic were 19%, 8%, 75%, 84%, 43%, 8%, 16%, 81%, 0%, 38%, 92% and 8%, respectively. Nearly 17% of the strains contained the vat gene. All of these strains contained csgA, traT, iutA and hlyA virulence genes and belonged to the A and D phylogroups. In addition, 38% of the isolates contained sat gene that belonged to A and B2 phylogroups. All of these strains had the hlyA and csgA virulence genes. Three strains also had the pic virulence gene; all of these strains belonged to the B2 phylogroup, which also had the csgA, traT and hlyA virulence genes. The results of PCR for virulence genes and phylogenetic typing are shown in Figure 1.
4. Discussion

*Escherichia coli* strains are the most common causes of intestinal, extraintestinal and urinary tract infections by encoding several virulence factors which mediate the adhesion, toxicity, and entry of strains into the blood[21-25]. Antibiotic resistance has been increased during recent years in Gram-negative bacteria, especially *E. coli* which is predominant species of Enterobacteriaceae in the hospital and even community-associated infections.

The results showed that all of the isolates were resistant to amoxicillin and tetracycline, and over 70% resistant to cotrimoxazole, cefazolin, co-amoxiclav, gentamicin, and ciprofloxacin, while no resistance was observed to phosphomycin. Resistance to imipenem, meropenem, and piperacillin-tazobactam was also very low (below 8%).

Altogether, *hlyA*, *csgA*, *iutA* and *traT* genes were mostly detected, but the frequency of *pic* and *vat* genes was low. The predominant secretory enzyme produced by *E. coli* isolates is a lipoprotein known as *hlyA* which is associated with the development of urinary tract infections, such as pyelonephritis[26]. Interestingly, 92% of isolates in this study were *hlyA* positive, suggesting a relation between sepsis and urinary tract infections. Despite this study, in Mexico, the prevalence of *hlyA* was low (7.4%) and was much lower than that in this present study[27].

The prevalence of *iutA* and *traT* was high in *E. coli* isolates in China (75.8% for *traT*)[28] and India (68% for *iutA*)[29], which is similar to this study, but 23% of isolates from India were *hlyA* and *cnf1* positive. The high rate of drug resistance and the presence of virulence factors in *E. coli* isolates increase the danger of fatal infections with no response to therapy. We detected *cdt* toxin gene in 8% of isolates. Similarly, in Mexico, 2 out of 20 stool samples were *cdt* positive, which was caused by *E. coli*[30].

The *vat* toxin was detected in 51% of septicemia and 12% of fecal samples in India[7], which was higher than our results, but the prevalence of the *pic* gene was 9% which is lower than the result of this study. Another study in Germany demonstrated that 10 (35.7%), 8 (28.6%) and 7 (25%) UPEC strains were positive for the *vat*, *hlyA*, and *cnf1* genes, respectively[31]. In another study in Zabul, Iran, the prevalence of *vat* toxin was 18% in *E. coli* isolates[32]. There is the difference in prevalence of virulence factors mainly due to differences in epidemiology, clinical samples, ages, clonal groups and various strains of *E. coli*.

All of *E. coli* isolates were sensitive to phosphomycin. Half of the isolates were multidrug resistant. The predominant virulence factors of *E. coli* isolates were the *hlyA*, *iutA*, *traT*, and *csgA* genes. O1 serogroup was the most common serogroup in *E. coli* in Iranian strains and B2 phylogroup was the most prevalent phylogroup. In terms of antibiotic resistance, the strains belonging to B1 phylogroup from O1 serogroup were the most resistant strains of *E. coli* from blood samples.

Conflict of interest statement

We declare that we have no conflict of interest.

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