The relation of phylogroups, serogroups, virulence factors and resistance pattern of *Escherichia coli* isolated from children with septicemia

F. Nojoomi and A. Ghasemian

1) Department of Microbiology, Faculty of Medicine, Aja University of Medical Sciences, Tehran, Iran

**Abstract**

The characterization of virulent and drug-resistant *Escherichia coli* strains helps to control and provide more accurate information regarding infection and eradication. The aim of this study was to determine the relationship between antibiotic susceptibility, phylogroups and virulence factors of *E. coli* isolates from children with septicemia. One hundred dereplicated *E. coli* isolates were collected from paediatric patients with septicemia in five hospitals in Tehran (May 2015 to May 2018). The antibiotic susceptibility of isolates was performed as per the 2016 guidelines of the Clinical and Laboratory Standards Institute. Extended-spectrum β-lactamases and carbapenemase genes, phylogroups, serogroups and virulence encoding genes were detected by PCR. Phylogroup B2 was dominant (40%) among strains, followed by phylogroups D (30%), A (8%) and B1 (7%). CTX-M1 was significantly higher in the B2 group (*n* = 21, *p* < 0.001). Furthermore, the virulence genes *iutA* (*n* = 27, *p* = 0.002), *csgA* (*n* = 39, *p* < 0.001), *kpsMII* (*n* = 39, *p* = 0.002), *ibeA* (*n* = 4, *p* = 0.004), *vat* (*n* = 5, *p* = 0.003), *traT* (*n* = 24, *p* < 0.001), *sat* (*n* = 12, *p* = 0.001) and *hlyA* (*n* = 33, *p* < 0.001) showed significantly higher rates in phylogroup B2. Three O25/CTXM1/OXA-48 and *cnf*, *iutA*, *csgA* and *traT* positive isolates belonged to phylogroup B2. Pulsed-field gel electrophoresis analysis showed 85% similarity among 25% of isolates. More than half of the isolates were multidrug-resistant *E. coli*. A significant relation was observed among *iutA*, *csgA*, *kpsMII*, *ibeA*, *vat*, *traT*, *sat* and *hlyA* genes and phylogroup B2. The characterization of virulent and drug-resistant strains helps control and properly eliminate infections. There was no genetic relation among strains in the pulsed-field gel electrophoresis pattern.

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**Corresponding author:** F. Nojoomi, Department of Microbiology, Faculty of medicine, Aja University of medical sciences, Tehran, Iran

**E-mail:** fnojoomi2@gmail.com

**Introduction**

*Escherichia coli* is among the dominant bacterial species causing septicemia, pyelonephritis, urinary tract infections (UTIs), cystitis and travellers’ diarrhoea, as well as meningitis in newborns [1]. *E. coli* is the most common organism which causes gastrointestinal, urinary tract and extraintestinal infections (ExPEC) [2]. Sepsis is a potentially life-threatening clinical infection associated with bacterial organisms, mainly due to Gram-negative species, and *E. coli* is the most common Gram-negative agent [3]. The body’s response to sepsis or bacteremia leads to septic shock due to the development of disease and to conditions that could lead to a high mortality rate among children [4–6]. Patients with sepsis must be promptly diagnosed because the disease is possibly preventable [7]. The most common infection before sepsis is UTI [8]. Septicaemic infections develop after UTI and genital infections in all age groups.

Phylogenetic analyses have determined that *E. coli* strains may be placed in four major phylogenetic groups, including A, B1, B2 and D types. The relationship between phylogenetic groups and virulence factors has not been fully proven [9,10]. ExPEC infections mainly belong to the B2 and D phylogroups [11]. Furthermore, antibiotic resistance has increased among *E. coli* strains, and the worldwide spread of some virulent clonal complexes such as ST151 have restricted chemotherapy options [12,13]. ExPEC strains contain a variety of virulence...
factors such as α-haemolysin (hly), cytotoxic necrotizing factor (cnf), invasion of endothelial brain protein (ibeA), fimbiae curli (csgA), toxin-secreted autotransporter (sat), serine protease autotransporter (pic), vacuolization of toxin autotransporter (vat), amyloid protein associated with serum resistance (traT) and capsule K1 (kpsMTII) [14,15]. The antigenic structures of strains can be detected with molecular methods such as PCR. ExPEC causing septicaemia mainly belong to one of 12 common O antigens including O1, O2, O4, O6, O7, O12, O15, O16, O18, O25, O75 and O157 [16,17].

The aim of this study was to determine the relationship between antibiotic resistance and serogroups and virulence factors of E. coli isolates among children with septicaemia.

Materials and Methods

Clinical isolates

We studied 456 dereplicated ExPEC E. coli isolates from various age groups (age range, 8 months to 89 years), identified using phenotypic and biochemical tests (blood agar, MacConkey agar, citrate, indole, motility) from blood samples among children with septicaemia in five hospitals of Tehran, Iran, during May 2015 to May 2018. One hundred of them were from patients aged 8 months to 11 years. No patient aged 12 to 15 years old with septicaemia in 2015 to May 2018. One hundred of them were from patients aged >12 years. We also excluded isolates from gastrointestinal infections. The isolates were identified by biochemical tests and were next preserved in trypticase soy broth and 30% glycerol at −20°C.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was implemented with the disc diffusion method (Kirby-Bauer) as per 2016 Clinical and Laboratory Standards Institute (CLSI) recommendations using 15 antibiotics, including fosfomycin (200 μg), ceftazidime (30 μg), co-amoxiclav (30 μg), cefazidime (30 μg), cefotaxime (30 μg), erythromycin (15 μg), nitrofurantoin (300 μg), gentamicin (10 μg), tetracycline (30 μg), piperacillin/tazobactam (110 μg), amoxicillin (25 μg), meropenem (10 μg), imipenem (10 μg), ticarcillin (25 μg) and ciprofloxacin (5 μg). E. coli ATCC 25922 and Staphylococcus aureus ATCC 25923 strains were used as the quality control of the discs in susceptibility testing.

Phenotypic extended-spectrum β-lactamase (ESBL) production was confirmed with synergy tests (using co-amoxiclav and cefazidime/cefotaxime discs) and Etest according to CLSI guidelines. Additionally, the combined discs including imipenem with ethylenediaminetetraacetic acid (EDTA) and the Carba-NP test were performed for determination of carbapenemase production.

DNA isolation and PCR technique

Isolation of bacterial DNA was implemented with the boiling method as described elsewhere [18,19]. The PCR reaction was used to amplify phylogenetic sequences, serogroups and virulence genes.

Phylogenetic typing

Phylogenetic grouping was conducted as C2 by PCR according to the chuA and yjaA genes and the DNA fragment TspE4 as described by Clermont et al [20]. The primer pairs were ChuA F-(5′-GACGAACCAACGGTCAGGAT-3′), R-(5′-TGCCGGCCAGTACCAAGACGAGACTGTG-3′), YjaA F-(5′-TGAGGTCAAGGAGACTGCTG-3′), R-(5′-ATGGAGAATGCGTTCCCTCAAC-3′) and TspE4C2 F-(5′-GAGTAATGCGGGGACATTCA-3′), R-(5′-CGCGCAACAAAG TATTACG-3′). The PCR conditions were as follows: 4 minutes at 94°C; 30 seconds, 33 cycles of 45 seconds at 94°C, 45 seconds at 55°C and 45 seconds at 72°C; and a final extension step of 10 minutes at 72°C.

O serogroup amplification

The specific primers and thermal profiles we used are presented in Table 1. PCR conditions included 5 minutes at 94°C; 30 cycles of 30 seconds at 94°C, 1 minute at 61°C and 50 seconds at 72°C; and 10 minutes at 72°C.

Virulence typing

Virulence genes including cytotoxic lethal distending toxin (cdt), capsular polysaccharide synthesis K1 (kpsMTII), receptor Toll/interleukin 1 (TcpC), cytotoxic necrotizing factor 1 (cnf1), ferric aerobactin receptor (iron uptake/transport) (ibeA), serum survival (traT), invasion of brain endothelium (ibeA), α-haemolysin (hlyA), surface exclusion, curli fimbiae (CsgA), secretion autoinducer toxin (sat), vaculating autoinducer toxin (vat) and serine protease autoinducer (pic) were amplified by PCR using the specific primers listed in Table 2.

Pulsed-field gel electrophoresis genotyping

Pulsed-field gel electrophoresis (PFGE) was performed as previously described [27]. In brief, equal volumes of the standardized bacterial suspensions (OD610 = 0.6) and 1% Seakem Gold agarose were mixed gently and allowed to solidify into agarose plugs. The plugs were lysed with cell lysis buffer (50 mM Tris, 50 mM EDTA (pH = 8), 1% sarcosine, 1 mg/mL proteinase K; Promega, Madison, WI, USA) and incubated for 4 hours at 54°C. Then lysed plugs were washed two times with ddH2O and six times with TE buffer. The plugs were digested with 10 U of XbaI (Promega) for 24 hours at 37°C. DNAs were separated by using a CHEF-Mapper with pulse times of 2.25 to 54.2 seconds at 200 V for 24 hours [28]. Analysis of the PFGE banding patterns based on the unweighted pair-group method

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was carried out by BioNumerics 6.0 software with arithmetic averages with 1.5 position tolerance.

Statistical data
Data were analysed by SPSS 21 (IBM, Armonk, NY, USA). The t test (chi-square analysis) and F test (one-way ANOVA) were done for this purpose. The 95% confidence interval was optimized, and p ≥ 0.05 was considered a significant result.

Ethical approval
This study was ethically approved by AJA University of Medical Sciences.

Results

Demographic data of patients
Paediatric patients included 32 boys (mean age of 6 years) and 68 girls (mean age of 7 years). Twenty per cent of children (12 boys and eight girls) were diagnosed with both sepsicaemia and UTI at the same time. Hospital wards included intensive care unit (24%), haematology (13%), urology and nephrology (21%), renal unit (12%), emergency clinic (11%), cardiac care unit (5%), gastroenterology (8%), cardiology and rheumatology (5%) and pulmonary/respiratory diseases (4%). Previous hospitalization during 2 months or more was observed among 87 of 100 children (p 0.0013).

Susceptibility pattern
The majority of isolates were resistant to erythromycin (89%), tetracycline (86%) and amoxicillin (80%). However, all were susceptible to fosfomycin and nitrofurantoin, and the majority was susceptible to imipenem, meropenem (85% for both) and piperacillin/tazobactam (60%). Furthermore, 57%, 14%, 33%, 71%, 48%, 56%, 24% and 49% were resistant to cefazidime, amikacin, gentamicin, ciprofloxacin, co-amoxiclav, cefotaxime, tigecycline and nalidixic acid respectively. Fifty-five per cent of isolates exhibited resistance to three classes of antibiotics (erythromycin, tetracycline and cefazidime), indicating multidrug-resistant (MDR) E. coli.

Of 57 ceftazidime-resistant E. coli, 46 were ESBL producers. In the combined discs including imipenem with ethylendiaminetetraacetic acid (EDTA) and Carba-NP test, among imipenem-resistant strains, 14 produced carbapenemases phenotypically.

PCR amplification of genes
Eight strains belonged to phylogroup A. All of them amplified csgA, traT and hlyA virulence genes. Six of these strains harboured the blaCTX-M-1 β-lactamase gene. Moreover, four of the strains carried both the sat and iutA genes (Table 3). In addition, seven strains were classified as phylogroup B1, and all were CTX-M1 positive. Five of them amplified the O15 and O75 serogroups. All of strains which were O75 positive also contained iutA, csgA and KpsMII virulence genes. Two children had the VET gene and had a history of antibiotic therapy in the past 2 months. Phylogroup B2 was dominant (40%) among strains from children. Sixty per cent of them belonged to serogroups O25, O18 and O75. All these strains amplified cnf, ibeA and sat virulence genes. Moreover, iutA, csgA, KpsMII and hlyA were dominant virulence genes among strains in phylogroup B2. Fourteen of the children had a history of antibiotics therapy in the past 2 months.

Thirty per cent of isolates classified in phylogroup D predominately belonged to the O1 (n = 24) and O2 (n = 3) serogroups. Sixteen patients showed liver and kidney disorders. In this phylogroup, iutA, traT, csgA, KpsMII and hlyA genes were predominant (Table 3). Two isolates could amplify the pic gene. Moreover, two strains belonged to serogroup O75 and were CTX-M1 positive. The virulence genes tcpC and cdt, and Klebsiella pneumoniae carbapenemase (KPC) and Verona

### Table 1. Primers used for amplification of O serogroups [20]

| Primer        | Sequence                                      | Product size (bp) |
|---------------|-----------------------------------------------|-------------------|
| gidbs F       | 5′-ATCCGACGACGCGATCGTGC-3′                    | 500               |
| nbO1 R        | 5′-CCCATATGCGGTAGATGTTG-3′                    | 480               |
| nbO2 R        | 5′-GGTGACTTTGCTTAAAG-3′                       | 450               |
| nbO12 R       | 5′-GGCTGAAATTCTGCGTACCGC-3′                   | 420               |
| nbO15 R       | 5′-GGCTGACTTTGCTTAAAG-3′                      | 400               |
| nbO16 R       | 5′-GGCTGACTTTGCTTAAAG-3′                      | 400               |
| nbO18 R       | 5′-GGCTGACTTTGCTTAAAG-3′                      | 400               |
| nbO25 R       | 5′-GGCTGACTTTGCTTAAAG-3′                      | 400               |
| nbO75 R       | 5′-GGCTGACTTTGCTTAAAG-3′                      | 400               |
| nbO157 R      | 5′-GGCTGACTTTGCTTAAAG-3′                      | 400               |

### Table 2. Primers and used for detection of virulence genes

| Primer | Primer direction | PCR product size (bp) | Reference |
|--------|------------------|-----------------------|-----------|
| cdt    | F: 5′-AAATCAGCGAGATCAGCTGAGTA-3′         | 470 [21]             |
| kpsMTI | F: 5′-GAGATCGCTTCAAGTGGGAGG-3′           | 450 [22]             |
| TopC   | F: 5′-GGCTGACGCTTCAAGTGGGAGG-3′          | 450 [23]             |
| iutA   | F: 5′-GAGATCGCTTCAAGTGGGAGG-3′           | 450 [24]             |
| traT   | F: 5′-GACATCGCTTCAAGTGGGAGG-3′           | 450 [25]             |
| hlyA   | F: 5′-GACATCGCTTCAAGTGGGAGG-3′           | 450 [26]             |
| cnf1   | F: 5′-GACATCGCTTCAAGTGGGAGG-3′           | 450 [27]             |
| ibeA   | F: 5′-GACATCGCTTCAAGTGGGAGG-3′           | 450 [28]             |
| vat    | F: 5′-GACATCGCTTCAAGTGGGAGG-3′           | 450 [29]             |
| sat    | F: 5′-GACATCGCTTCAAGTGGGAGG-3′           | 450 [30]             |
| pic    | F: 5′-GACATCGCTTCAAGTGGGAGG-3′           | 450 [31]             |
| CsgA   | F: 5′-GACATCGCTTCAAGTGGGAGG-3′           | 450 [32]             |
The prevalence of *cnf*, *cdt*, *iutA*, *csgA*, *kpsMII*, *ibeA*, *vat*, *traT*, *sat*, *pic* and *hlyA* were 11%, 0, 61%, 86%, 66%, 14%, 14%, 81%, 28%, 4% and 74% respectively. The prevalence of SHV, TEM1, CTX-M1, OXA-48 and IMP were 13%, 11%, 38%, 3% and 4% respectively. None of them carried the *KPC* or the *VIM* genes.

The O1 and O75 serogroups were predominant (each 20%), followed by O2, O18, O25, O4, O6, O7, O12, O15, O16 and O157, at 10%, 10%, 7%, 5%, 5%, 5%, 0, 5%, 3% and 0 respectively. Ten per cent of isolates were not typed with serogroup identification in this study. Three isolates were O25/CTXM1/OXA48 positive, which encoded *cnf*, *iutA*, *csgA* and *traT* and belonged to phylogroup B2.

A comparative study was performed between children (*n* = 100) and adults (*n* = 356) with regard to phylogroups, O1 and O75 serogroups, MDR strains and each of virulence genes. It is noteworthy that only MDR strains were significantly higher among adults, but none of the factors was significantly different. The existence of *blaCTX-M1* (72% vs. 38%, *p* 0.0111), *blaIMP* (27.80% vs. 4%, *p* <0.001) and *blaOXA-48-like* (21% vs. 3%, *p* <0.001) genes were significantly higher among adults. Therefore, a higher rate of drug resistance was observed among adults compared to children.

**PFGE analysis**

PFGE typing of 100 isolates from paediatric patients revealed that 95 various types were classified. Twenty-five isolates (25%) demonstrated ≥85% similarity as analysed by PFGE.

**Discussion**

In this study, most of the *E. coli* strains that caused sepsicaemia in paediatric patients genetically showed a high-level virulence and carried several virulence genes. We found that among paediatric patients with sepsicaemia, there was a higher prevalence of the *csgA*, *traT*, *iutA*, *hlyA* and *kpsMII* genes. We also showed that phylogroup B2 was predominant among the isolates we studied here. This reveals that *E. coli* virulence determinants such as curli fimbriae, siderophore, capsule, α-haemolysin and serum amyloid protein associated with resistance play crucial roles in the colonization and invasion of the blood by the isolates. One study of hospitalized patients found that the α-haemolysin gene was an important factor leading to the urosepsis by *E. coli*, and similar to our results, the B2 phylogroup was the predominant phylogroup determined among these patients [29]. In a study from India, the dominant virulence gene among hospitalized patients was *hly*, and phylogenetic group B2 was the predominant type, similar to our results [30]. The high prevalence of the *hlyA* gene in this study suggests a possible relation between sepsicaemia and urinary tract isolates. Curli pili mostly contribute to the infection processes by providing the bacterial attachment structure, invasion to the host cells, spread to other organs and promotion of biofilm formation [31]. In this study, it was demonstrated that 86% of *E. coli* causing sepsicaemia in paediatric patients amplified the curli fimbriae (*csgA*) gene. Two other important virulence genes in this study were *kpsMII* (66%) and *traT* (81%). The *traT* and *iutA* had high prevalence among *E. coli* isolates in China (75.8% for *traT*) [32] and India (68% for *iutA*) [33], similar to this study, but 23% of them amplified *hlyA* and *cnf1* in India. The role of *traT* in *E. coli* isolates from blood in paediatric patients with sepsicaemia and bacteraemia has been demonstrated. The prevalence of *traT* has been indicated to have a relation to the expression of the K1 capsule [34]. Therefore, it may be suggested that *traT* and *kpsMII* with the *csgA* genes are major causes of sepsis among *E. coli* isolates from paediatric patients in Tehran hospitals. There was a significant relation between these virulence genes and phylogroup B2.
It was found that 40% of strains isolated from paediatric patients possessed O1 and O75 antigens. In this study, among the 12 most common E. coli O-antigen serogroups, the O1 and O75 antigens were more commonly detected among paediatric patients with septicemia. The E. coli O1 serogroup mostly expresses the capsular K antigens and thus cause extraintestinal infections in humans [35]. In our study, the prevalence of O1/K antigenic serogroups was high; however, this serogroup is the main causative factor leading to UTIs and sepsis infection of paediatric patients.

Furthermore, among isolates with resistance to both cepazidime and imipenem, the prevalence of SHV, TEM1, CTX-M1, OXA-48 and IMP were 13%, 11%, 38%, 3% and 4% respectively. Similar to these results, CTX-M1 is the predominant gene among ESBL-producing E. coli isolates worldwide. None of them could amplify the KPC and VIM genes. There was a significant relation between the presence of CTX-M1 and phylgroup B2. Receipt of antibiotic therapy in 2 past months (p 0.032) and liver and kidney disorders (p 0.001) were significant risk factors for MDR E. coli acquisition in paediatric patients. Genetic analysis of the isolates showed no clonal spread of E. coli causing septicemia among children. We observed a higher rate of drug resistance and the presence of ESBL- and carbapenemase-encoding genes among isolates from adults compared to children. Additionally, none of the total 456 isolates carried the KPC or VIM genes.

In conclusion, it was found that approximately half of E. coli isolated from paediatric patients with septicemia belonged to phylgroup B2, and half also carried O1 and O75 antigens. The majority of the isolates carried csgA, kpsMII, iutA and traT virulence factors; moreover, a significant relation was found between these genes and phylgroup B2. More than half of the isolates were MDR E. coli. The characterization of virulent and drug-resistant strains contributes to the control and proper therapy of infections. Genetic analysis by PFGE showed no clonal spread of E. coli isolates among paediatric patients. However, previous hospitalization was a significant risk factor in children, which should be considered with regard to the acquisition of nosocomial infections. It is noteworthy that there was no significant difference regarding phylgroups, serogroups and virulence factors between ExPEC from children and adults, but drug resistance and the existence of ESBL and carbapenemase genes were significantly higher among adults, thereby highlighting a higher rate of drug resistance among adults.

Conflict of Interest

None declared.

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