Asian Pacific Journal of Tropical Disease

journal homepage: http://www.apjtcm.com

Original article https://doi.org/10.12980/apjtd.7.2017D7-155 ©2017 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

The antibiotic resistance and virulence factors of *Escherichia coli* of serogroup O1 isolated from Tehran, Iran

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ARTICLE INFO

Article history:
Received 30 Jun 2017
Received in revised form 14 Jul, 2nd revised form 4 Aug 2017
Accepted 25 Aug 2017
Available online 25 Sep 2017

Keywords:
Escherichia coli
Phylogroup typing
O1 serogroup
Virulence factors

ABSTRACT

Objective: To detect several virulence genes among *Escherichia coli* (*E. coli*) isolates of serogroup O1 collected in Tehran, Iran.
Methods: In this study, 100 clinical isolates of *E. coli* were collected in Tehran, Iran. The antibiotic susceptibility test was done for 14 antibiotics according to CLSI guidelines. Phylogenetic grouping was performed by PCR according to the *chuA*, *yjaA* and *TspE4.C2* genes. Serogroup typing was performed using the 12 O-antigens. The virulence genes were detected by PCR.
Results: A total of 37 isolates belonged to serogroup O1. All of *E. coli* isolates were sensitive to phosphomycin. Phylogroups A (30%), B1 (21%), B2 (39%), and D (10%) were detected. The prevalence rates of *cnf, cdt, iutA, csgA, kpsMII, ibeA, vat, traT, TcpC, sat, hlyA* and *pic* were 19%, 8%, 78%, 84%, 43%, 8%, 16%, 81%, 0%, 38%, 92% and 8%, respectively. Also, the *vat* gene was the most dominant gene in A phylogroup, and the genes of *sat* and *pic* were the most dominant virulence genes in the B2 phylogroup.
Conclusions: The phosphomycin, imipenem, and meropenem were the most effective antibiotics. Half of the isolates were multidrug resistant. The predominant virulence factors of *E. coli* isolates were the genes *hlyA, iutA, traT,* and *csgA*. Detection of virulent strains which exhibit wide antibiotic resistance can help the control programs in order to prevent the spread of infections. According to the results, the B2 phylogroup was the most common phylogroup and strains belonging to B1 phylogroup were the most resistant strains in O1 serogroup *E. coli* strains.

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), *vat* (vaculating autotransporters toxin) and *Tsh* (the temperature sensitive hemagglutinin), all of which are widely spread in UPEC but not in commensal strains[7-9].

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Foundation Project: Supported by Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran (Grant No. 52D/8164, 2017).
The journal implements double-blind peer review practiced by specially invited international editorial board members.

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 adhesions 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 carbapenemase genes encode carbapenemase enzymes such as metallo-beta-lactamases such as bla_{IMP}, bla_{VIM}, bla_{OXA-48}, bla_{NDM1}, bla_{GES}[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 (MEN, 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*), cytolethal 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) (*iutA*), curli fimbriae (*csgA*) and serine protease autoinducer (*pic*) were studied by PCR.

Table 1
Primers sequences used for amplification of virulence genes.

Genes	Primers	PCR product size (bp)	Reference
cdt	FP: 5'-AAATCACCAAGAATCATCCAGTTA-3' RP: 5'-AAATCTCCTGCAATCATCCAGTTTA-3'	430	[16]
kpsMTII	FP: 5'-GCGCATTTGCTGATACTGTTG-3' RP: 5'-CATCCAGACGATAAGCATGAGCA-3'	272	[16]
TcpC	FP: 5'-GAGTGGAAGGAGGTTGAGGC-3' RP: 5'-GCAGTGCCATTTTATCCGCC-3'	544	[17]
iutA	FP: 5'-GGCTGGACATCATGGGAACTGG-3' RP: 5'-CGTCGGGAACGGGTAGAATCG-3'	302	[18]
traT	FP: 5'-GGTGTGGTGCGATGAGCACAG-3' RP: 5'-CACGGTTCAGCCATCCCTGAG-3'	290	[18]
hlyA	FP: 5'- GCATCATCAAGCGTACGTTCC-3' RP: 5'- AATGAGCCAAGCTGGTTAAGCT-3'	534	[19]
cnf1	FP: 5'- AAGATGGAGTTTCCTATGCAGGAG-3' RP: 5'- CATTCAGAGTCCTGCCCTCATTAT-3'	498	[7]
ibeA	FP: 5'- AGGCAGGTGTGCGCCGCGTAC-3' RP: 5'-TGGTGCTCCGGCAAACCATGC-3'	171	[7]
vat	FP: 5'- AACGGTTGGTGGCAACAATCC-3' RP: 5'- AGCCCTGTAGAATGGCGAGTA-3'	420	[7]
sat	FP: 5'- TCAGAAGCTCAGCGAATCATTG-3' RP: 5'- CCATTATCACCAGTAAAACGCACC-3'	930	[7]
pic	FP: 5'- ACTGGATCTTAAGGCTCAGGAT-3' RP: 5'- GACTTAATGTCACTGTTCAGCG-3'	572	[7]
csgA	FP: 5'- GGCGGAAATGGTTCAGATGTTG-3' RP: 5'- CGTATTCATAAGCTTCTCCCGA-3'	295	[20]

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'-GACGAACCAACGGTCAGGAT-3'), R-(5'-TGCCGCCAGTACCAAAGACA-3'), *yjaA* F-(5'-TGAAGGTCAGGAGA CGCT G-3'), R-(5'-ATGGAGAATGCGTTCCTCAAC-3') and *TspE4C2* F-(5'-GAGTAATGTCGGGGCATTCA-3'), R-(5'-CGCGCCAACAAAGTATTACG -3'). The PCR was performed with Bio-Rad thermal cycler T100 under the following conditions: denaturation for 4 min at 94 °C; 33 cycles of 45 s at 94 °C, 45 s at 55 °C, and 45 s 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 2
Primers for detection of O-serogroups.

Primers	Sequences (5'-3')	PCR product size (bp)
gndbis.F	5'-ATACCGACGACGCCGATCTG-3'	-
rfbO1.R	5'-CCAGAAATACACTTGGAGAC-3'	189
rfbO2.R	5'-GTGACTATTTCGTTACAAGC-3'	274
rfbO4.R	5'-AGGGGCCATTTGACCCACTC-3'	193
rfbO6.R	5'-AAATGAGCGCCCACCATTAC-3'	584
rfbO7.R	5'-CGAAGATCATCCACGATCCG-3'	722
rfbO12.R	5'-GTGTCAAATGCCTGTCACCG-3'	239
rfbO15.R	5'-TGATAATGACCAACTCGACG-3'	536
rfbO16.R	5'-GGATCATTTATGCTGGTACG-3'	450
rfbO18.R	5'-GAAGATGGCTATAATGGTTG-3'	360
rfbO25.R	5'-GAGATCCAAAAACAGTTTGTG-3'	313
rfbO75.R	5'-GTAATAATGCTTGCGAAACC-3'	419
rfbO157.R	5'-TACGACAGAGAGTGTCTGAG-3'	672

2.6. Data analysis

The data of the study were analyzed by SPSS version 21, and the *Chi*-square test and *t*-test where any result with P value < 0.05 was considered as significant and 95% confidence interval was assigned.

2.7. Ethical approval

This study was approved by Ethical Committee of Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran.

3. Results

3.1. O serogroup typing in E. coli strains

Of the 100 isolates of *E. coli*, 37% of isolates belonged to O1 serogroup, which was the most prevalent serogroup in our specimens.

3.2. Drug resistance pattern in O1 serogroup

The antibiotic resistance pattern of 37 isolates of *E. coli* is 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 *ibeA* and *vat* were not detected in these strains.

Only 10% of the strains were identified as D phylogroup, all of which contained virulence genes of *iutA*, *csgA*, and *hlyA*. According to the results, the B2 phylogroup was the most common phylogroup and strains belonging to B1 phylogroup were the most resistant *E. coli* strains of O1 serogroup. The antibiotic resistance of O1 serogroup *E. coli* strains belonging to four phylogroups is shown in Table 3.

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*, *ibeA*, *vat*, *traT*, *TcpC*, *sat*, *hlyA* and *pic* were 19%, 8%, 78%, 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.

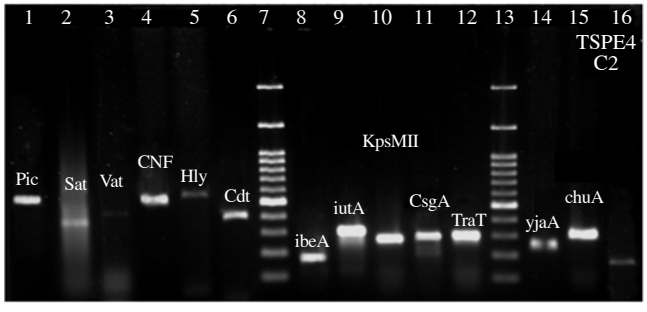

Figure 1. PCR for virulence typing and phylogenetic typing of *E. coli* strains.
Lane 1: 572 bp; Lane 2: 330 bp; Lane 3: 420 bp; Lane 4: 498 bp; Lane 5: 534 bp; Lane 6: 430 bp; Lanes 7 and 13: Ladder 100 bp; Lane 8: 171 bp; Lane 9: 302 bp; Lane 10: 272 bp; Lane 11: 295 bp; Lane 12: 290 bp; Lane 14: 211 bp; Lane 15: 279 bp; Lane 16: 152 bp.

Table 3
The antibiotic resistance of O1 serogroup *E. coli* strains belonging to four phylogroups (%).

Phylogroups	TE	TS	IMI	MEN	GM	CIP	FM	PTZ	AMC	CTX	CAZ	CZ	AMX	FO
Phylogroup A (N = 11)	100	73	9	9	64	55	18	9	82	46	54	91	100	0
Phylogroup B1 (N = 8)	100	88	0	0	88	88	0	0	88	75	75	100	100	0
Phylogroup B2 (N = 14)	100	86	0	0	79	79	14	7	86	65	50	72	100	0
Phylogroup D (N = 4)	100	75	0	0	50	50	0	25	75	50	50	100	100	0

4. Discussion

E. 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.

Acknowledgment

This study was supported by Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran (Grant No. 52D/8164, 2017).

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