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Correlation Between *hlyA* and *cnf1* Virulent Genes with Antibiotic Resistance and non-ESBLs *Escherichia coli* Isolates Collected from Patient with Urinary Tract Infections in Kerman, Iran

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Abstract

Background: *Escherichia coli* is the most common cause of urinary tract infections. Different studies have been reported that acquisition of drug resistance mechanism may be related to loss of virulent factors. In this study, we investigated the correlation of antibiotic resistance and ESBLs production with presence of *hlyA* and *cnf1* virulence genes.
Methods: During June 2014 to March 2015, 250 *Escherichia coli* isolates were collected from different patients with urinary tract infections in Kerman, Iran. Disk diffusion method was used for determination of antibiotic susceptibility profile and combined disk method was used for detection of ESBLs producing isolates. The *hlyA* and *cnf1* genes were detected by PCR method.
Results: In total, 44.8% of isolates were considered as extended spectrum beta-lactamases (ESBLs) producing by combined disk method. *hlyA* and *cnf1* genes were detected in 28.8% and 29.2% of isolates, respectively. Statistical analysis revealed significant correlations between susceptibility to NA, CIP, CTX, CAZ, PIT antibiotics with *hlyA* and *cnf1* positive isolates and also significant correlation observed between ESBLs negative isolates with *hlyA* positive isolates (P value < 0.05).
Conclusions: Acquire of antibiotic resistance mechanism may lead to loss of virulence factor.

Keywords: Antibiotic Resistance, ESBLs, Virulence Genes, *Escherichia coli*

1. Background

Escherichia coli is a gram-negative bacilli, which is able to colonize in humans (1). This bacterium is the most common cause of urinary tract infections (UTIs) in all age groups (2). Ability of *E. coli* in the pathogenesis is dependent on the virulent factors (2, 3). Several virulent factors including hemolysin (*hlyA*) and cytotoxic necrotizing factor type 1 (*cnf1*) are responsible for pathogenic potential of uropathogenic *E. coli* isolates (UPEC) (3, 4). These virulence factors are involved in damage to the tissues as well as bacterial dissemination (3, 4). Antibiotic resistance is another important factor in ability of *E. coli* in the pathogenesis and increased of morbidity and mortality of patients (5). At the moment, multi-drug resistant (MDR) and ESBLs producing isolates of *E. coli* have been reported worldwide and treatment options for MDR isolates is limited (5, 6). Several studies have been reported that acquisition of drug resistance mechanism may be related to loss of virulent factors (4, 6). Therefore, the aim of this study was determinate of correlation between antibiotic resistance and ESBLs production with presence of virulent genes *hlyA* and *cnf1* in *E. coli* isolates collected from patients with a UTI in Kerman, Iran.

2. Methods

2.1. Sample Collection and Identification of Isolates

In a cross-sectional study, we collected 250 non-duplicate isolates of *E. coli* during June 2014 to March 2015 from patients with symptoms of a urinary tract infection who were admitted to the university-affiliated hospital in Kerman. Basic characteristics data such as gender and age were recorded. Bacterial isolates were identified by standard and biochemical methods including: oxidase test, lactose fermentation, growth on Triple Sugar Iron (TSI), Simmons' Citrate Agar Media, and MRVP test (7) (All the culture media were from Merck, Co, Germany.).

2.2. Antibiotics Susceptibility of Isolates

The disk diffusion method was used for determination of antimicrobial susceptibility profile of isolates according to the Clinical and Laboratory Standard (CLSI) (8). The following antibiotics (Mast, UK) were tested: imipenem (IMI:10 μg), ceftazidime (CAZ: 30 μg), cefotaxime (CTX:

30 μg), amoxicillin/clavulanic acid (AUG: 20/10 μg), cefepime (CPM: 30 μg), Piperacillin/tazobactam (PIZ: 100/10 μg), nalidixic acid (NA: 30 μg), and ciprofloxacin (CIP: 5 μg). *E. coli* ATCC 25922 was used as control strain in antimicrobial susceptibility test (8).

2.3. Detection of ESBLs Producing Isolates

ESBLs producing isolates was detected by combined disk method with clavulanic acid according to CLSI guideline (8). The following antibiotics (Mast, UK) were used for ESBLs detection: CAZ (30 μg), CTX (30 μg), CPD (30 μg) alone as well as with 10 μg clavulanic acid. Klebsiella pneumonia ATCC 700603 (ESBLs positive) and *E. coli* ATCC 25922 (ESBLs negative) were used as control strains (8).

2.4. Detection of hlyA and cnf1 Virulent Genes

The total DNA was extracted by the boiling method (9). Virulent genes included *hlyA* and *cnf1* detected by polymerase chain reaction (PCR). Specific oligonucleotide were: *hlyA-F* - 5'-AAC AAG GAT AAG CAC TGT TCT GGCT-3' and *hlyA-R* -5'-ACC ATA TAA GCG GTC ATT CCC GTCA for *hlyA-R* gene (Product size: 1177 bp) (10) and *cnf1-F* - 5'-AAG ATG GAG TTT CCT ATG CAG GAG -3' and *cnf1-R* -5'-CAT TCA GAG TCC TGC CCT CAT TAT T for *cnf1* gene (Product size: 543 bp) (11). The PCR amplification was carried out in Biometra PCR Thermal Cycler by using PCR Master mix (Ampliqon Inc, Denmark) according to manufacture guideline, under the following conditions: initial denaturation for 5 minutes at 95°C followed by 30 cycles of 60 seconds at 95°C, and 45 seconds annealing at the specific melting temperature of each primer (62°C for *hlyA* and 62°C for *cnf1*). PCR assay was performed in a total volume of 15 μL containing: 0.5 μL of each primer (10 pM), 7.5 μL of PCR Master mix, 1 μL of DNA (20 ng) and 5.5 μL of H_2O (DNase and RNase free water). PCR products were electrophoresis in 1% agarose gel.

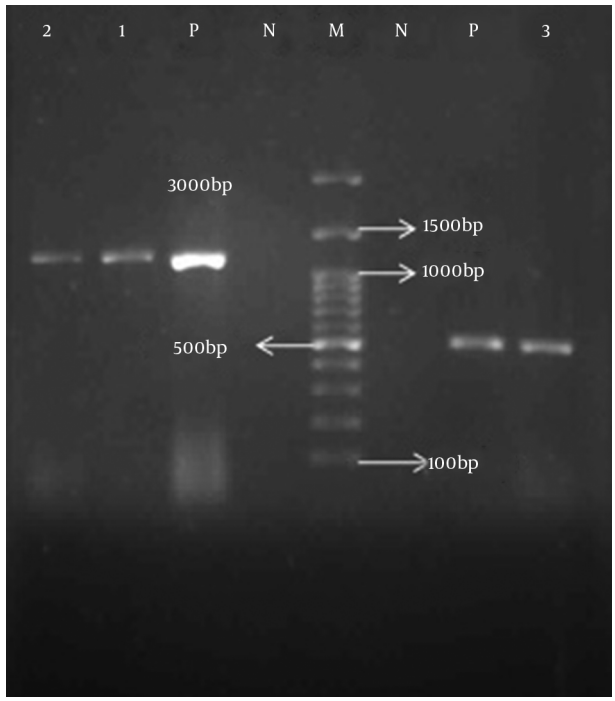
2.5. Statistical Analysis

SPSS v.22 (SPSS Inc., Chicago, IL, USA) was used for data analysis. We used the Chi-squared test to assess the variables correlation between antibiotic resistance and ESBLs producing with presence of *hlyA* and *cnf1* genes. P value < 0.05 was considered statistically significant.

3. Results

The age of the patients with a UTI ranged from 1 - 93 years. Among 250 UPEC isolates, 193 (77.2%) isolates were from females and 57 (22.8%) were from males. The rate of resistance to imipenem (IMI:10 μg), ceftazidime (CAZ: 30 μg), cefotaxime (CTX: 30 μg), amoxicillin/clavulanic acid (AUG: 20/10 μg), cefepime (CPM: 30 μg) and piperacillin/tazobactam (PIZ: 100/10 μg), nalidixic acid (NA: 30 μg) as well as ciprofloxacin (CIP: 5 μg) antibiotics were 4.8%, 46%, 47.2%, 61.6%, 46.8%, 24%, 68%, and 55.2%, respectively. In total, 44.8% of isolates were ESBLs positive. *hlyA* and *cnf1* genes detected in 28.8% and 29.2% of isolates, respectively (Figure 1). Co-exist of *hlyA* and *cnf1* genes observed in 23.2% of isolates. *hlyA* and *cnf1* genes observed in 8% and 10% among of ESBLs positive isolates, respectively. Prevalence of NA, CIP, CTX, CAZ, PIT-sensitive isolates were over NA, CIP, CTX, CAZ, PIT-resistant isolates among the UPEC isolates with the *cnf1* and *hlyA* (Table 1). Statistical analysis revealed significant correlation between susceptibility to NA, CIP, CTX, CAZ, PIT antibiotics with presence of *hlyA* and *cnf1* in isolates and also significant correlation observed between ESBLs negative (non ESBLs) isolates with *hlyA* positive isolates (P value < 0.05) (Table 1). In our study, there was no significant correlation with age and gender of patients with presence of *hlyA* and *cnf1* in isolates.

Figure 1. Agarose Gel Electrophoresis of PCR Amplified *hlyA* and *cnf1* Genes



N; Negative Control, P; Positive Control, 1 and 2; Positive Isolates for *hlyA* gene, 3; Positive Isolates for *cnf1* Gene, M; DNA Size Marker.

4. Discussion

Virulence factors such as *hlyA* and *cnf1* in UPEC isolates are involved in pathogenicity of this bacterium (2, 4, 6).

Table 1. Distribution of Antibiotic Susceptibility and ESBLs Producing with *hlyA* and *cnf1* Genes Positive Isolates

Virulent Genes (n)	Antibiotic Agents and Rate of Resistance to Deferent Antibiotic, No. (%)									
	NA	CIP	CTX	CAZ	CPM	IMI	PIZ	AUG	ESBLs	
	R, 170 (68)	R, 138 (55.2)	R, 118 (47.2)	R, 115 (46)	R, 117 (46.8)	R, 12 (4.8)	R, 60 (24)	R, 154 (61.6)	P, 112 (44.8)	N, 138 (55.2)
***hlyA*(72)**	28 (16.4)	25 (18.1)	24 (20.3)	22 (14.1)	28 (23.9)	5 (41.6)	11 (18.3)	27 (17.5)	20 (17.8)	52 (37.6)
***cnf1*(73)**	35 (20.5)	28 (20.2)	27 (22.8)	25 (21.7)	29 (24.7)	5 (41.6)	11 (18.3)	28 (18.1)	25 (22.3)	48 (34.7)

Abbreviations: AUG, Amoxicillin/Clavulanic; CAZ, Ceftazidime; CIP, Ciprofloxacin; CPM, Cefepime; CTX, Cefotaxime; IMI, Imipenem; N, Negative; NA, Nalidixic Acid; P, Positive; PIZ, Piperacillin/Tazobactam; R, Resistant.

The prevalence hemolysin (*hlyA* gene) and cytotoxic necrotizing factor type 1 (*cnf1* gene) among UPEC isolates were 28.8% and 29.2%, respectively. The prevalence of *hlyA* and *cnf1* in our study were similar to other studies in Iran. The prevalence of the *hlyA* gene was 23.3% in UPEC isolated from Iranian patient with UTI, in Jahrom Iran (12). In another study in Iran, prevalence of *hlyA* and *cnf1* genes has been reported as 13.5% and 22.9%, respectively (13).

In agreement to other studies, prevalence of resistance to NA, CIP, CTX, CAZ, CPM, and AUG is high in our city. The rate of resistance to NA, CIP, CTX, CAZ, and AUG were reported about 53%, 51%, 69%, 72%, 77%, respectively, in Tehran, Iran (14). Furthermore, the rate of ESBLs producing isolates in our study in similar to other studies in Iran (14). In several studies in Iran, ESBLs isolates were reported in 20% to 89.8% in different regions in Iran (14, 15).

Correlations between virulent genes with antibiotic resistance in UPEC isolates have been investigated in many studies in different country of worldwide (4, 6, 16). In the present study, we observed a statistically significance between antimicrobial susceptibilities and presence of *hlyA* and *cnf1* genes ($P < 0.05$).

In our study, we observed that the *hlyA* and *cnf1* positive isolates were significantly associated with the sensitivity to NA, CIP, CTX, CAZ, and PIT as well as with the non-ESBL isolates. Harwalkar et al. reported that prevalence of *hlyA* and *cnf1* in CIP-sensitive isolates is higher than in ciprofloxacin-resistant of UPEC isolates (4). Unlike Harwalkar et al., we observed an increased prevalence of the *cnf1* gene in the non-ESBL isolates compared to the ESBL positive isolates; however, the difference was not statistically significant and the *hlyA* gene showed a significant association with the ESBL negative isolates (4).

Also, our study, similar to Velasco et al., showed that the resistance to nalidixic acid caused loss of virulence genes; therefore, resistance to nalidixic acid may lead to the loss of the ability to pathogenicity (17). In a study done by Arisoy et al., they showed an association between susceptibility to antibiotics and virulence factors in the *E. coli* isolates recovered from UTI patients (18). Similar to the present study, they reported that virulence gene *hlyA* was increased in sensitive isolates. Furthermore, Asadi et al. showed stronger associations between virulence factors and antibiotic resistance in UPEC isolates (12).

Finally, our findings in agreement with other studies suggested that resistance to NA, CIP, and ESBLs production may be associated with marked reduction in pathogenicity of UPEC isolates (4, 6, 17).

4.1. Conclusion

The study showed that, there are a many statistically significances between non-resistance and non-ESBLs with presence of *hlyA* and *cnf1* genes. Our findings, similar to other studies, show that acquire of antibiotic resistance mechanism may be lead to loss of virulence factor.

Footnotes

Conflicts to Interests: None declared.

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