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Relationship Between O Serotype and Virulent Genes in *Escherichia Coli* Causing Urinary Tract Infections

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Original Paper

Introduction. *Escherichia coli* are the most frequent pathogens in acute urinary infections. They are classified based on various types of O antigen. *Escherichia coli* strains that cause urinary tract infections possess several genes encoding urovirulent factors. To assay the relation of virulent factors of *E coli* in acute urinary infections, the serotypes and virulence factor genotypes were determined.
Materials and Methods. We studied 96 *E coli* isolates from children with acute urinary infections. Four urovirulence determinants were analyzed by DNA colony hybridization, including the genes for type 1 fimbriae (*pil*), P fimbriae (*pap*), S fimbriae (*sfa*), hemolysin (*hly*), and cytotoxic necrotizing factor 1 (*cnf1*). O serotypes were also determined.
Results. The most frequently found virulence factor-encoding gene in the *E coli* strains studied was the gene for type 1 fimbriae (27.4%). The prevalence of *pap*, *sfa*, *hly*, and *cnf1* were higher in serotypes causing pyelonephritis than cystitis. The most common type of O antigen was O1 (12.2%). There was a significant correlation between serotype and genotype in uropathogenic *E coli*.
Conclusions. The high prevalence of O6 serotypes in children urinary tract infections and the high percentage of virulent genes in serotype O6 suggested a close relation between serotype and genotypes of uropathogen *E coli*.

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INTRODUCTION

Escherichia coli are the most frequent causes of acute urinary infections (UTIs). The *O antigen* is a liposaccharide composed of bacterial cell wall. For the first time, *E coli* were classified based on various types of O antigen by Kauffmann.[1,2] *Escherichia coli* strains that cause UTI possess virulence properties that facilitate their colonization and persistence in the bladder. Several genes encode urovirulent factors such as hemolysin (*hly* gene), cytotoxic nercotizing factor type 1 (*cnf1* gene), pyelonephritis associated pili (*pap* genes), and S-family adhesions (*sfa* gene).[3] Some of these genes, *hly, pap* or p fimbriae, and *cnf1,* play an important role in the pathogenesis of *E coli* strains.[4-6] To assess the virulence profiles of *E coli* in acute UTIs, the relation between O serotypes and virulence factor genotypes were determined.

MATERIALS AND METHODS

Sample

Escherichia coli strains were isolated from urine samples of children aged from l month to 14 years with UTI who presented at Motahary Hospital, in Jahrom, Iran. *E coli* isolates were identified by standard methods. Their clinical presentations were cystitis or pyelonephritis. Cystitis was clinically defined as a syndrome involving dysuria, frequency, and urgency, whereas acute pyelonephritis was defined as a syndrome characterized by fever (temperature > 38°C), flank pain, and/or lumbar tenderness, often associated with dysuria, urgency, and frequency.

DNA Extraction

Escherichia coli isolates were grown in Luria Bertani broth at 37°C overnight. Bacteria were

then pelleted from broth, resuspended in sterile distilled water, and boiled at 95°C for 10 minutes. After centrifugation, the supernatants were stored as DNA template at -20°C until they were used in the polymerase chain reaction (PCR).

Detection of Genes

Detection of *pap*, *sfa*, *cnf1*-1, and *hly* genes was done by amplifying the genes by PCR. The primers sequences were previously reported in the literature,[7] and obtained from the TIB MOLBIOL Syntheselabor GmbH (Berlin, Germany). Other enzymes and chemicals were provided by the Cinnagen Chemical Company (Tehran, Iran). Amplification was performed in a thermal cycler (Eppendorf, Hamburg, Germany) according to the methods described by Yamamoto and colleagues.[8] All PCR mixtures contained 1x PCR buffer, 200 µM each deoxynucleoside triphosphate, 25 pmol of each primer, 1.5 mM $MgCl_2$, 1.25 U of Taq polymerase, and 10 µL of DNA extracts. The PCR amplification included an initial denaturation step at 94°C for 5 minutes, followed by 25 cycles with the following profiles: 94°C for 30 seconds and 65°C for 30 seconds for *sfa*, *pap*, and *hly*; and 50°C for 30 seconds, 72°C for 1 minute, and a final extension in 72°C for 10 minutes for *cnf*. Amplifications were carried out in a gradient thermal cycler (Eppendorf, Hamburg, Germany). Expected sizes of the amplicons were ascertained by electrophoresis in 1.5% agarose gel with an appropriate molecular size marker (100-bp DNA ladder, MBI, Fermentas, Lithuania).

Serotyping

Slide agglutination was done according to manufacturers' guidelines of the kit (Mast Assure Bacterial Agglutinating Antisera, Mast Group, Merseyside, UK). After preparing heat-treated organism suspension as antigen suspension, 2 loopfuls of that were placed on microscopic slide. One drop of antiserum was placed on 1 drop of the suspension and 1 drop of normal saline on the other drop of the suspension as control. These reagents were mixed by titling back and forth for 60 seconds while viewing it under indirect light against a dark background. Distinct clumping or agglutination within this period without clumping in the saline control was regarded as a positive result.

We had limitations for testing all types of O antigen; therefore, based on a literature review, 4 more common O types of uropathogen *E coli* were selected and tested. The O types included O1, O6, O15, and O18.

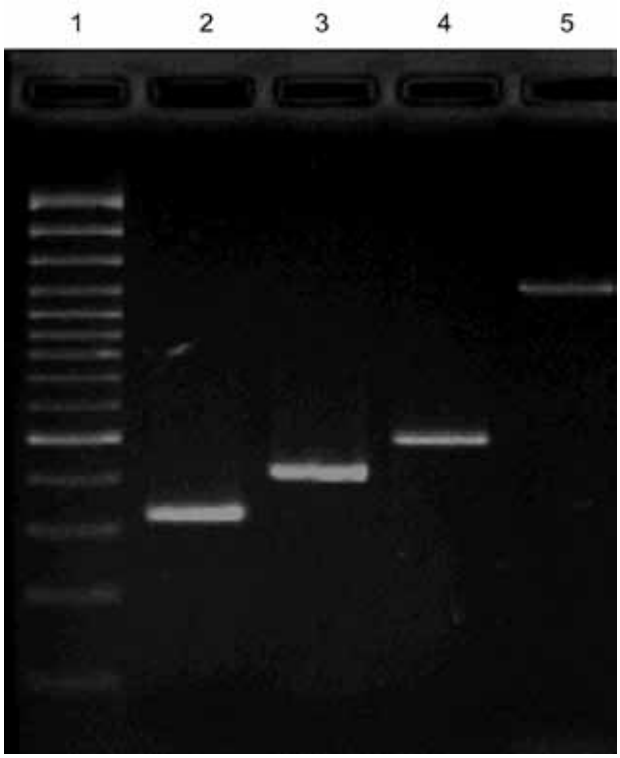
Statistical Analyses

To establish the significance of the results, the Fisher exact test and the chi-square test were used. The level of significance was set at a *P* value of less than .05.

RESULTS

A total of 96 strains of *E coli* were isolated from urine samples of children with UTI, aged 1 month to 14 years (mean, 21.8 ± 26.9 months). Cystitis was diagnosed in 49.2% of the children and pyelonephritis, in 50.8%.

The most frequently found virulence factor-encoding gene in the *E coli* strains studied, independently of serotype, was the gene for type 1 fimbriae (27.4%), which was followed by *cnf* (22.9%), *sfa* (14.6%), and *hly* (13.5%; Figure). Pyelonephritis was more prevalent in cases positive for virulent



Representative polymerase chain reaction results for virulence genes in *Escherichia coli* strains isolated from children with urinary tract infection. Lane 1 is molecular-size marker (100-bp plus ladder); Lane 2, *pap* (328 bp); Lane 3, *sfa* (419 bp); Lane 4, *cnf-1* (498 bp); and Lane 5, *hly* (1177 bp).

Frequency of genes in various serotypes O

Gene, %	O Serotype				*P*
	O1	O6	O15	Nontypable	
pap	33.3	90.0	50.0	15.7	< .001
sfa	25.0	50.0	25.0	7.1	.002
cnf	25.0	80.0	25.0	14.3	< .001
hly	0	70.0	25.0	7.1	< .001

genes. The most common types of O antigen were O1 (12.2%), O6 (10.2%), and O15 (4.1%). No case of O18 antigen was detected.

There was a significant association between serotypes and presence of virulence genes. Ninety percent of serotype O6 and 33.3% of serotype O1 were *pap*-positive ($P < .001$). A similar relation was observed for other genes (Table).

DISCUSSION

The high prevalence of type 1 fimbriae is in accordance with previous results from studies conducted by other investigators,[9] who have found a high prevalence of type 1 fimbriae among uropathogenic *E coli* strains. Some studies indicate that type 1 fimbriae are more important for colonization of the bladder than for colonization of the kidney.[9] Our results showed that the proportions of type 1 fimbriae among isolates producing pyelonephritis were more than those producing cystitis.[10]

The present study showed a strong correlation between the production of virulent genes and serotypes. Study of Terai and coworkers showed 9 O serotypes (O1, O2, O4, O6, O16, O18, O22, O25, and O75) accounted for 79.4%, 73.7%, and 78.4% of the prostatitis, pyelonephritis, and cystitis strains, respectively. The authors also demonstrated the correlation between serotype and genotype in uropathogenic *E coli*.[11] Blanco and colleagues showed an apparent correlation between the *pap* and *sfa* operons and the O serogroups of the strains. Thus, 93% of strains belonging to O1, O2, O4, O6, O7, O14, O15, O18, O22, O75, and O83 possessed *pap* and/or *sfa* operons, versus only 32% of strains belonging to other serogroups.[12] In another study, Blanco and colleagues showed the majority of bacteremic O2, O4, O6, and O83 *E coli* strains were *hly+cnf1+* and expressed P fimbriae or mannose-resistant hemagglutination type III, whereas the strains of serogroup O18 were *hly+cnf1-* and had P fimbriae or mannose-resistant hemagglutination type III, whereas the strains of serogroup O18 were *hly+cnf1-* and had P fimbriae.[13]

There is a similar relation in the serotype causing intestinal and extraintestinal infection. Martinez-Medina and colleagues showed intestinal and extraintestinal *E coli* O25:H4 serotype shared similar virulence gene sets, and certain strains were phylogenetically related.[14]

CONCLUSIONS

The high prevalence of O6 serotypes in children with UTI and the high percentage of virulent genes in serotype O6 suggested a close relation between serotype and genotypes of uropathogen *E coli*. We had a limitation for serotyping of more types of *E coli*; as a result, some of samples were negative by using these limited serotypes. Extended study for evaluation of relation between other serotypes and virulent genes is needed for better clinical and practical use.

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CONFLICT OF INTEREST

None declared.

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