Pediatrik ve Yetişkin Hastalardan İzole Edilen E. coli İzolatlarının Antimikrobiyal Duyarlılıklarının ve Virülans Faktörlerinin İrdelenmesi

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Öz

Bu çalışmada komplike olmayan idrar yolu enfeksiyonu tanısı almış pediatrik ve yetişkin hastalardan izole edilen E. coli izolatlarında antimikrobiyal direnç paternlerini ve virülans genlerinin prevalansını saptamayı hedefledik. Akut komplike olmayan idrar yolu enfeksiyonu tanısı almış toplam 83 pediatrik ve yetişkin ayaktan hastanın orta akım idrarından izole edilmiş kökenlerin, VITEK® 2 (bioMerieux, Marcy l'Etoile, France) otomatize sistemi ile identifikasyonları ve antimikrobiyal direnç paternleri belirlendi. Bakterilerin direnç durumları dışında virülans genlerinin (pap, aer, sfa, hly ve cnf-1) prevalansı araştırıldı. Antimikrobiyal duyarlılık testleri sonucunda; sıklıkla idrar yolu enfeksiyonları için ampirik tedavide kullanılan siprofloksasin, trimetoprim-sülfametoksazol, gentamisin, ampisilin ve sefolatinin saptanan E. coli izolatlaında araştırılan diğer ajanlardan daha az duyarlı olduğu saptandı. Her iki yaş grubunda da en yüksek oranda saptnan virülans genler pap ve aer olarak bulundu. Pediatrik yaş grubunda en az oranda saptanan genler; sfa ve hly iken yetişkin yaş grubunda en az oranda saptanan gen hly olarak saptandı. Üropatojenik E. coli izolatlarında virülans faktörleri ile antimikrobiyal direnç paternleri arasında bir ikişki olduğunu gösteren yayın sayısı literatürde yok denecek kadar azdır. Antimikrobiyallere direnç ile virülans faktörlerinin ilişkisinin değerlendirilebilmesi için daha büyük çalışma grupları gerekmektedir.

Anahtar Kelimeler: E.coli,Virülans,PCR,Antimikrobiyal Duyarlılık

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Sorumlu Yazar

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Evaluation of Antimicrobial Resistance and Virulence Genes in Uropathogenic Escherichia coli in Pediatric and Adult Patients

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Abstract

We aimed to evaluate the antimicrobial resistance patterns and the prevalence of certain virulence genes in uropathogenic E. coli isolated from pediatric and adult patients with uncomplicated urinary tract infection. We examined nonduplicate 83 uropathogenic E. coli isolated from mid-stream clean-catch urine samples of the pediatric and adult outpatients with the diagnosis of acute uncomplicated urinary tract infection. VITEK® 2 automated system (bioMerieux, Marcy l'Etoile, France) was used for identification and determination of antimicrobial resistance. We examined the isolates in respect to their antimicrobial resistance patterns and the presence of virulence genes (pap, aer, sfa, hly and cnf-1). Antimicrobial susceptibility testing results of the E. coli isolates revealed that commonly used empiric antimicrobials (ciprofloxacin, trimethoprim–sulfamethoxazole, gentamicin, ampicillin and cephalothin) for urinary tract infections were less effective than others. Most frequently detected virulence genes were pap and aer in both age groups. Sfa and hly genes were the least frequently detected genes in the pediatric age group; hly gene was the also the least common in the adult age group. There was no association with virulence factors and antimicrobial resistance patterns of the uropathogenic E. coli isolates in contrary to literature. More comprehensive studies with larger sample groups are needed to demonstrate the relation between virulence factors with antimicrobial drugs in different age groups.

Keywords: E. coli,Virulence,Antimicrobial Resistance,PCR

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INTRODUCTION

Urinary tract infection (UTI) is the infection of the urethra, bladder, ureter, and kidneys with any microbial pathogen and it is the most common bacterial infection in all ages1.The incidence of UTIs are estimated to be about 150-250 million cases worldwide and UTIs are associated with considerable morbidity and health care cost2-4.

The most common bacterial pathogen causing UTI is the uropathogenic *Escherichia coli* (UPEC)[1]. UPEC isolates harbor many genes encoding diverse virulence factors. These virulence factors are mainly adhesions, toxins, iron acquisition systems, biofilms and other virulence factors[5,6]. They contribute in persistence of the isolate to enhanced pathogenicity resulting infection by colonization, invasion, reduction of the host immunity[6,7]. The functions and genetic properties of the virulence factors of UPEC isolates are well characterized[8-10]. Host factors and bacterial virulence factors are important in urinary tract infection pathogenesis. Features such as capsule forming, ability to attach to uroepithelial cells, reproduction rate in urine, serum resistance, P and S fimbria existence, siderophore (aerobactin, enterobactin) production, cytotoxic necrotizing factor-1 and hemolysin existence, belonging to a specific O and K serogroup, colistin V production and resistance to antimicrobials can be specified within common virulence factors of UPEC[11].

Major virulence factors of the UPEC, such as p fimbriae (*pap*), s fimbriae (*sfa*), haemolysin (*hly*), cytotoxic necrotising factor (*cnf-1*) and aerobactin (*aer*) play important role in the pathogenesis of *E. coli* strains by overcoming host defense mechanisms and causing disease[12].

We aimed to evaluate the antimicrobial resistance patterns and the prevalence of certain virulence genes in uropathogenic *E. coli* isolated from pediatric and adult patients with uncomplicated urinary tract infection.

MATERIALS AND METHODS

Patients and Bacterial isolates

Uropathogenic *E. coli* isolates were collected from Celal Bayar University Hospital, Department of Medical Microbiology, during six-month period in 2013. We examined nonduplicate 83 uropathogenic *E. coli* isolated from mid-stream clean-catch urine samples of the pediatric and adult outpatients with the diagnosis of acute uncomplicated urinary tract infection. Organisms were included in our study when they occurred as a pure culture and at a concentration more than 10^5 CFU/ml. Isolation and identification were performed by conventional biochemical tests as well as by *VITEK® 2* automated system (*bioMerieux, Marcy l'Etoile, France*). Antimicrobial susceptibility testing of the isolates was performed by modified Kirby-Bauer disk diffusion method according to the recommendations of the CLSI[13]. The isolates

were stored in brain heart infusion broth (Merck, Darmstadt, Germany) with 10% glycerol at -80°C.

Extraction of bacterial DNA

The stored *E. coli* isolates were subcultured overnight at 37 °C in Luria-Bertani Broth (Merck, Germany) and genomic DNA was extracted by using commercial DNA extraction kit (PureLink Genomic DNA Mini Kit, Invitrogen, USA).

Multiplex Polymerase Chain Reaction

We used the primers previously reported by Yamamoto et al.[14] for detection of *pap*, *sfa*, *hly*, *cnf-1* and *aer* genes in this study (Table 1). Reference primer sequences were determined by the information obtained from Genbank. The primers used in this study were listed in Table 1. The multiplex PCR was performed in a total 50 µl containing the following components: 10 µl of bacterial DNA sample, x10 buffer solution (ThermoFisher Scientific, USA), 25 mM $MgCl_2$, deoxyribonucleotide triphosphate mixture (containing 5 mM of dATP, dTTP, dCTP and dGTP each), 5U of Taq DNA polymerase (ThermoFisher Scientific, USA), 20 pM each of *pap*, *cnf-1*, *sfa* primers and 40 pM each of *hly*, *aer* primers. We performed the PCR amplification in three steps: initial denaturation at 94 °C for 1 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 63 °C for 30 s and extension at 72 °C for 1.5 min and the final extension for 5 min at 72 °C. Ten microlitres of the reaction mixture was analyzed on a 1% agarose gel by electrophoresis. Bands were visualized under UV after staining (Safe-View G108, Applied Biological Materials, Canada).

Quality Control Strains

We used *E. coli* ATCC 25922 for *aer* and *E. coli* C7 mutant strain for *pap*, *cnf-1*, *sfa* and *hly* as positive control.

Statistical analyses:

Statistical analyses were performed by commercial statistical software *SPSS* version 21.0 (*SPSS Inc., Chicago, IL, USA*). Comparisons between patient groups and virulence genes were made with x^2 or *Fisher's exact test* for categorical variables and *Student t* test for continuous variables. A *p* value <0,05 was considered as significant.

RESULTS

Eighty-three *E. coli* isolates from pediatric and adult patients diagnosed with urinary tract infection in Celal Bayar University Hospital in Manisa were included in this study. The mean ages of the pediatric and adult patients was 6,2±4,7 and 38,4±5,1 years, respectively. Male to female ratio was 21/24 in pediatric age group and 21/17 in the adult age group.

Antimicrobial susceptibility testing results of the *E. coli* isolates revealed that commonly used empiric antimicrobials for UTI (e.g. ciprofloxacin, trimethoprim–sulfamethoxazole, gentamicin, ampicillin and cephalothin) were

less effective than others (Table 2). The most of the susceptible antimicrobial was carbapenem in both of the age groups. We determined higher resistance rates in the adult age group.

Most frequently detected virulence genes were *pap* and *aer* in both age groups (Table 3, Figure 1). *sfa* and *hly* genes were the least frequently detected genes in the pediatric age group; *hly* gene was the also the least common in the adult age group, however we did not determine statistically significant difference between the patient groups in respect to the virulence genes.

DISCUSSION

Host defence factors and bacterial virulence factors are important in pathogenesis of urinary tract infections. Main virulence factors can be specified as cellular structures, enzymes, toxins and adhesion structures. Most UPEC isolates possess specific virulence genes that encode various virulence factors. These factors are mostly associated with colonization and survival of the UPEC in the urinary tract.

Adhesion proteins such as P fimbriae (type-2) encoded by *pap* genes and S fimbriae (type-2) encoded by *sfa* genes are important for colonization. We detected *pap* genes as the most common virulence genes in both pediatric (64%) and adult patient groups (63%). *sfa* genes were higher in adult group than pediatric group. This was an expected condition since P fimbriae had higher affinity to urogenital epithelial cells than that of S fimbriae[15,16].

Iron-acquisition systems of the UPEC isolates are well characterised[17]. Aerobactin functions in iron acquisition, which enables pathogenic bacteria to survive in very low iron concentrations[17,18]. We determined *aer* genes in pediatric and adult age groups with the rates of 55% and 44%, respectively.

Haemolysin (*encoded by hly gene*) decreases the functions of white blood cells and causes severe damage to renal tubular cells leading severe complications[1,17]. Haemolysin is often associated with clinical severity of the UTIs caused by UPEC isolates[19]. Cytotoxic necrotizing factor-1 (*encoded by cnf-1 genes*) is another toxin that alters the phagocytic abilities of the immune cells. We determined *hly and cnf-1 genes* less than *pap* and *aer* genes in both patient groups. This data was logical since we evaluated isolates from uncomplicated UTIs in both groups.

Virulence factors related with adhesive systems are most commonly determined detected genes in UPEC[20-22]. In a recent study from Tunisia, frequency of virulence genes in 90 UPEC isolates was determined as 52 %, 41 %, 34 %, 19 %, 3 % and, for *aer, pap, sfa, hly,* and *cnf-1* genes, respectively[23]. Researcher from Korea figured out that most common virulence genes associated with UPEC isolated from children were related to production of fimbriae and iron- acquisition systems[1]. Farshad et al. determined the

prevalence rates of *sfa*, *pap C*, and *hly* VFs in children with UTI as 13.5%, 22.9%, and 14.6%, respectively[24].

UTIs are very common in both pediatric and adult ages. UTIs are accepted as quite important infections not only for their high frequency but also their possibility to lead severe complications. Treatment of UTIs requires use of antimicrobial drugs, which eventually leads another problematic condition referred as antimicrobial drug resistance. In our study, resistance rates of UPEC isolates from pediatric patients to cefuroxime, cephalothin, trimethoprim-sulfamethoxazole and ampicilin and resistance rates of isolates from adult patients to amoxicillin-cluvanic acid, cefuroxim, cephalothin, ciprofloxacin, gentamicin, trimethoprim-sulfamethoxazole, cefotaxim and ampicilin to be higher than 50%. However, we did not determine any association with resistance patterns and virulence factors. In the literature there are several studies linking an association with virulence factors and antimicrobial resistance, especially with adhesive proteins and antimicrobial drugs such as ciprofloxacin, nalidixic acid and trimethoprim-sulfametaxasole[25-28].

As a conclusion, we determined *pap* and *aer* genes most frequently in both pediatric and adult age groups. There was no association with virulence factors and antimicrobial resistance patterns of the UPEC isolates in contrary to literature.

More comprehensive studies with larger sample groups are needed to demonstrate the relation between virulence factors with antimicrobial drugs in different age groups.

REFERENCES

1. Yun KW, Kim HK, Park HK, Kim W, Lim IS. Virulence factors of uropathogenic Escherichia coli of urinary tract infections and asymptomatic bacteriuria in children. J Microbiol Immunol Infect. 2014; 47(6): 455-61.
2. Santo E, Salvador MM, Marin JM. Multidrug-resistant urinary tract isolates of Escherichia coli from Ribeirao Preto, Sao Paulo, Brazil. Braz J Infect Dis. 2007; 11(6): 575–8.
3. Hassan SA, Jamal SA, Kamal M. Occurrence of multidrug resistant and ESBL producing E. coli causing urinary tract infections. J of Basic and Applied Sci. 2011; 7(1): 39–43.
4. Agarwal J, Srivastava S, Singh M. Pathogenomics of uropathogenic Escherichia coli. Indian J Med Microbiol. 2012; 30(2): 141–9.
5. Johnson JR, Porter SB, Zhanel G, Kuskowski MA, Denamurd E. Virulence of Escherichia coli clinical isolates in a murine sepsis model in relation to sequence type ST131 status, fluoroquinolone resistance, and virulence genotype. Infect Immun. 2012; 80(4): 1554-62. article

6. Agarwal J, Mishra B, Srivastava S, Srivastava R. Genotypic characteristics and biofilm formation among Escherichia coli isolates from Indian women with acute cystitis. Royal Society of Tropical Medicine and Hygiene 2013; 107(3): 165-9. article
7. Anvarinejad M, Farshad S, Ranjbar R, et al. Genotypic analysis of E. coli strains isolated from patients with cystitis and pyelonephritis. Iranian Red Crescent Medical Journal 2012; 14(7): 408-16.
8. Chiou YY, Chen MJ, Chiu NT, Lin CY, Tseng CC. Bacterial virulence factors are associated with occurrence of acute pyelonephritis but not renal scarring. J Urol 2010; 184: 2098-102.
9. Cusumano CK, Hung CS, Chen SL, Hultgren SJ. Virulence plasmid harbored by uropathogenic Escherichia coli functions in acute stages of pathogenesis. Infect Immun 2010; 78: 1457-67.
10. Crepin S, Houle S, Charbonneau ME, et al. Decreased expression of type 1 fimbriae by a pst mutant of uropathogenic Escherichia coli reduces urinary tract infection. Infect Immun 2012; 80: 2802-15.
11. Yamamoto S. Molecular epidemiology of Escherichia coli. J Infect Chemother 2007; 13; 68-73.
12. Bean DC, Krahe D, Wareham DW. Antimicrobial resistance in community and nosocomial Escherichia coli urinary tract isolates, London 2005 – 2006, Ann Clin Microbiol Antimicrob. 2008; 7: 13.
13. Yamamoto S, Terai A, Yuri K, Kurazono H, Takeda Y, Yoshida O. Detection of urovirulence factors in Escherichia coli by multiplex polymerase chain reaction. FEMS Immunol Med Microbiol. 1995; 12(2): 85-90.
14. Clinical and Laboratory Standards Institute (Formerly NCCLS) (2011). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement CLSI document M100-S21, Clinical and Laboratory Standards Institute, Wayne, Pa.
15. Rahdar M, Rashki A, Miri HR, Ghalehnoo MR. Detection of pap, sfa, afa, foc, and fim adhesin-encoding operons in uropathogenic Escherichia coli isolates collected from patients with urinary tract infection. Jundishapur Journal of Microbiology. 2015; 8: e22647.
16. Adib N, Ghanbarpour R, Slotzadeh H, Alizade H. Antibiotic resistance profile and virulence genes of uropathogenic Escherichia coli isolates in relation to phylogeny. Trop Biomed 31.1 (2014): 17-25.
17. Zgur-Bertok D, Modric E, Grabnar M. Aerobactin uptake system, Col V

production, and drug resistance encoded by a plasmid from an urinary tract infection Escherichia coli strain of human origin. Can J Microbiol 1990; 36: 297-9.

18. Svanborg C, Godaly G. Bacterial virulence in urinary tract infection. Infect Dis Clin North Am, 1997; 11(3): 513-29.
19. Garcia TA, Ventura CL, Smith MA, Merrell DS, O'Brien AD. Cytotoxic necrotizing factor 1 and hemolysin from uropathogenic Escherichia coli elicit different host responses in the murine bladder. Infect Immun 2013; 81: 99-109.
20. Bogyiova E, Kmetova M, Biros E, Siegfried L. Detection of pap-sfa- and afa-specific DNA sequences in Escherichia coli strains isolated from extraintestinal material. Folia Microbiol (Praha). 2002; 47(6): 723–6.
21. Saidenberg AB, Robaldo Guedes NM, Fernandes Seixas GH, et al. A survey for Escherichia coli virulence factors in asymptomatic free-ranging parrots. ISRN Vet Sci. 2012; 2012: 984813.
22. Mohajeri P, Khademi H, Ebrahimi R, Farahani A, Rezaei M. Frequency distribution of virulence factors in uropathogenic Escherichia coli isolated from Kermanshah in 2011-2012. Int J Appl Basic Med Res. 2014; 4(2): 111–6.
23. Tarchouna M, Ferjani A, Ben-Selma W, Boukadida J. Distribution of uropathogenic virulence genes in Escherichia coli isolated from patients with urinary tract infection. Int J Infect Dis. 2013; 17(6): 450-3.
24. Farshad S, Emamghorashi F. The prevalence of virulence genes of E. coli strains isolated from children with urinary tract infection. Saudi J Kidney Dis Transpl. 2009; 20(4): 613–7.
25. Asadi S, Kargar M, Solhjoo K, Najafi A, Ghorbani-Dalini S. The association of virulence determinants of uropathogenic Escherichia coli with antibiotic resistance. Jundishapur Journal of Microbiology. 2014
26. Horcajada JP, Soto S, Gajewski A, et al. Quinolone-resistant uropathogenic Escherichia coli strains from phylogenetic group B2 have fewer virulence factors than their susceptible counterparts. J Clin Microbiol. 2005; 43(6): 2962–4.
27. Arisoy M, Rad AY, Akin A, Akar N. Relationship between susceptibility to antimicrobials and virulence factors in pediatric Escherichia coli isolates. Int J Antimicrob Agents. 2008; 31(1): 4–8.
28. Zhao L, Chen X, Zhu X, et al. Prevalence of virulence factors and antimicrobial resistance of uropathogenic Escherichia coli in Jiangsu province (China). Urology. 2009; 74(3): 702–7.

Table 1. PCR primers for detection of virulence genes in uropathogenic *E. coli.*

Gene		Primer Sequence (5' to 3')	Size of PCR product (bp)
hly	F	AACAAGGATAAGCACTGTTCTGGCT	1177
	R	ACCATATAAGCGGTCATTCCCGTCA	
cnf-1	F	AAGATGGAGTTTCCTATGCAGGAG	498
	R	CATTCAGAGTCCTGCCCTCATTATT	
pap	F	GACGGCTGTACTGCAGGGTGTGGCG	328
	R	ATATCCTTTCTGCAGGGATGCAATA	
sfa	F	CTCCGGAGAACTGGGTGCATCTTAC	410
	R	CATCAAGCTGTTTGTTCGTCCGCCG	
aer	F	TACCGGATTGTCATATGCAGACCG	602

Table 2. The antibiotic resistance rates of Uropathogenic *E. coli* isolates from pediatric and adult age groups.

Antibiotics	**Pediatric age group (*n= 45)***		**Adult age group (*n=38)***	
	n	**%**	**n**	**%**
AMC	19	42	20	52
CXM	25	55	30	78
KF	23	51	28	73
CIP	11	24	24	63
GN	16	35	21	55
SXT	30	66	24	63
CTX	18	40	24	63
CAZ	7	15	16	42
IPM	1	2	2	5
ATM	14	31	16	42
AMP	29	64	26	68

AMC: Amoxicilline-clavulonate, CXM: Cefuroxim, KF: Cephalothin, CIP: Ciprofloxacin, GN: Gentamicin, SXT: Trimethoprim-sulfamethoxazole, CTX: Cefotaxime, CAZ: Ceftazidime, IMP: Imipenem, ATM: Aztreonam, , AMP: Ampicillin

Table 3: PCR results of virulence genes in uropathogenic *E. coli* isolates.

Genes	Pediatric age group (*n= 45)*		Adult age group (*n=38)*		*p value*
	n	**%**	**n**	**%**	
pap	29	64	24	63	> 0.05
sfa	7	15	11	28	
cnf-1	9	20	12	31	
aer	25	55	17	44	
hly	8	17	8	21	

Figure 1. Agarose gel image of amplicons generated in multiplex PCR protocols with positive control strains (Line 1: *E. coli* C7 strain, Line 2: *E. coli* ATCC 25922), negative control (Line 3) and 13 out of 83 uropathogenic *E. coli* isolates.