The virulence factors and antibiotic sensitivities of Escherichia coli isolated from recurrent urinary tract infections

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ABSTRACT

Objective: We determined the antibiotic sensitivities of uropathogenic *Escherichia coli* (UPEC) strains isolated from the urine of patients who have recurrent urinary tract infections (UTIs).

Methods: Our study was carried out between November 2000 and January 2002 at the Infectious Diseases Clinic, Istanbul Haydarpasa Numune Hospital, Istanbul, Turkey. We compared the virulence factors (fimbrial adhesion, hemolysin production, motility property) of 50 strains of *Escherichia coli (E. coli)* isolated from urine with the same properties of 25 strains of *E. coli* isolated from stool specimens of healthy individuals. In addition, we detected the virulence factors of UPEC strains using a microbiological and biochemical methods and by using disk diffusion method, we were able to investigate the sensitivity of the strains to the antimicrobials.

Results: We found the level of mannose-resistant (MR) fimbriae bearing in the UPEC strains to be significantly higher than that in the controls (odds ratio=10.27, $p<0.001$). The difference in mannose-resistant hemoagglutination (MRHA) and mannose sensitive hemoagglutination (MSHA) bearing levels in UPEC strains were rather high. This difference was regarded as significant in terms of showing the virulence of fimbriae bearing strains (odds ratio=29.03, $p<0.001$).

Conclusion: Our study demonstrates that strains with MR fimbriae have a rather high virulence ($p<0.001$), and that a combination of MR+MS fimbriae increased that virulence ($p<0.001$). As MR strains have a greater adhesive property, the determination of MR fimbriae bearing as high shows that fimbriae bearing plays an important role in widespread and resistant strains, especially in recurrent UTIs such as in our study. In addition, hemolysin capability was also a virulence factor in recurrent UTIs ($p<0.01$). In addition, the sensitivity of the strains to the antimicrobials appeared in the following order; imipenem 93%, norfloxacin 89%, ciprofloxacin 85%, netilmicin 80%, amikacin 78%, ceftriaxone 74%, gentamicin 72%, nitrofurantoin 71%, ampicillin-sulbactam 60%, amoxicillin-clavulanate 58%, Trimethoprim/sulfamethoxazole 45%, ampicillin 35%.

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In addition to the high morbidity of recurrent urinary tract infections (UTIs), the cost of diagnosis and treatment, loss of time, and the development of resistant strains impose a significant burden on society.[1] The *Escherichia coli* (*E. coli*) bacteria most frequently isolated from UTIs possesses different characteristics to the *E. coli* found in the normal intestinal flora. These strains adhere to the cells with the help of the fimbriae and increase pathogenicities by opposing urine flow. Such strains are known as uropathogen *E. coli*

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(UPEC).[2,3] The most important virulence factors of UPECs are fimbrial adhesins and hemolysins.[3,4] Hemoagglutination (HA) is the first observation based evidence of bacteria adhesin properties. The HA activity is related to the presence of fimbriae. Depending on their hemoagglutination properties, fimbriae are classified in 2 forms: mannose-sensitive (MS) and mannose-resistant (MR).[4] Mannose sensitive hemoagglutination (MSHA) is the form of agglutination inhibited with D-mannose. Pathogenic strains' host specific adhesins permit erythrocyte agglutination by a route unaffected by D-mannose and this form is known as mannose-resistant hemoagglutination (MRHA). Bacterial hemolysins are a group of extracellular cytotoxic polypeptides, which dissolves erythrocytes. The hemolysins in the bacterium provide bacterial persistence in the lower urinary system. Most UPEC strains are hemolytic.[4,5] In this study, we investigated these virulence factors and antibiotic sensitivities of *E. coli* isolated from urine of patients who have recurrent UTIs.

Methods. Recurrent UTI is defined as symptoms that returns 2 or more times in 6 months or symptoms of a single episode that last longer than 2 weeks or symptoms that last longer than 48 hours after treatment has begun.[1,4,6] Fifty *E. coli* strains isolated as a factor from polyclinic patients diagnosed with recurrent UTI were included in this study, carried out between November 2000 and January 2002 at the Infectious Diseases Clinic, Istanbul Haydarpasa Numune Hospital, Istanbul, Turkey. There were 135 patients who were given appointments and followed approximately for one year at specific intervals. The mean age of the patients was 32.2 ± 5 (20-60) years, 37 (74%) of the 50 patients were females, 13 (26%) of them were males. The virulence factors of the 50 *E. coli* strains isolated from these patients' urine in recurrent UTI were investigated in terms of fimbrial adhesion, hemolysin production and motility property; also their sensitivities to various antimicrobials were determined. Twenty-five *E. coli* strains isolated from fecal cultures from healthy individuals with no history of UTI were used as the control group.

The following pre-experimental preparations were made before determination of MR and MS fimbriae:

Tampons and solutions. Saline-phosphate buffer solution (PBS) was prepared by mixing 8.5 gr NaCl, 0.22 gr $NaH2PO42H_2O$, 1.20 gr KH_2 PO_4 and 1000 ml distiled water. For the the Alsever solution, a 2.05 gr glucose, 0.78 gr sodium citrate, 0.42 gr NaCl, 100 ml distilled water were mixed.

Escherichia coli strains. The sediment of the *E. coli* strains was suspended with PBS for 3 times and the surface liquid poured away. The bacterial suspensions beneath were used in the experiment.

Erythrocyte suspensions. An 8% human erythrocyte suspension in PBS was used in the determination of MRHA. While an 8% guinea pig erythrocyte suspension in PBS was used in the determination of MSHA.

D (+) mannose. Used with the addition of D (-) mannose in hemoagglutination inhibition experiments to give a final concentration of 4% on erythrocyte suspensions.

Strains giving HA with human erythrocytes with and without mannose were determined as MRHA positive. It was thought that strains giving no HA possessed no fimbriae. Strains giving HA without mannose and not giving HA with mannose were subjected to processing with test human erythrocyte suspension in terms of MSHA. As bacteria would leave the erythrocyte surface in the event of warming, the experiment was performed at 3-5^{o}C.

Hemoagglutination experiments for the determination of MRHA were carried out using human erythrocytes. Strains to be used in the experiment were obtained from fresh cultures in 5% sheep's blood agar. For the hemoagglutination experiment on micro slide 30 µL of PBS was dropped onto both sides of the slide. A 30 µL of 8% erythrocyte suspension was added to one of the colony suspensions with mannose and one without mannose. Then rotated on ice for 2 minutes hemoagglutination formation in erythrocytes was evaluated as macroscopically negative or positive.

The experiment for MSHA determination was performed using guinea pig erythrocytes and bacterial suspensions with the same method. For the broth culture experiment, of great importance with regard to confirming the MRHA and MSHA experiments described above, 30 µL of bacterial suspension was applied to both sides of the slide. A 30 µL of human erythrocyte suspension without mannose and with mannose rotated on ice for 2 minutes. Hemoagglutination formation in erythrocytes was evaluated as macroscopically negative or positive.[1,4,7,8]

Hemolysin production was evaluated by the presence of ß-hemolysis around colonies following 18-24 hours of incubation at 35^{o}C in blood agar culture medium.[4]

Investigation of motility. Strains that spread by reproducing and clouded the motility culture medium were regarded as motile.

Investigation of antimicrobial susceptibility. For antibiotic sensitivity experiments Mueller-Hinton culture medium was used following the Karby-Bauer disc diffusion method. The antimicrobials are selected according to the National Committee for Clinical Laboratory Standard (NCCLS). Antibiotic discs that were used are as follows; ampicillin (10 µg), ampicillin/sulbactam (20 µg), amoxicillin/clavulanic acid (30 µg), netilmicin (10 µg), amikacin (30 µg), gentamicin

(30 µg), ciprofloxacin (10 µg), norfloxacin (10 µg), ceftriaxone (30 µg), imipenem (10 µg), nitrofurantoin (50 µg) and trimethoprim/ sulfamethoxazole (TMP-SMX) (25 µg).[1,4,9]

For the antimicrobial susceptibility test's control, ATTC- 25922 *E. coli* variant were used.

Statistical analyses. The statistical analyses was conducted using the Fisher's exact test as well as SPSS statistical software.

Results. A total of 58% (29/50) of the *E. coli* strains isolated as a factor in recurrent UTIs were determined as MR fimbriae bearing, whereas 12% (3/25) of the fecal strains in the controls were MR fimbriae bearing. The difference between the level of fimbriae bearing in UPEC and controls was statistically significant. These values confirmed that MR fimbriae bearing strains are the most virulent strains (odds ratio=10.27, $p<0.001$).

In the hemoagglutination experiment using a guinea pig blood, 68% (34/50) of UPEC, 56% (14/ 25) of fecal strains were MS fimbriae bearing. No statistically significant difference between MS fimbriae levels was determined between the UPEC and control specimens (odds ratio=1.67, $p>0.05$). Although the level of both MR and MS fimbriae bearing was 36% (18/50) in UPEC strains, no co-presence of MR + MS fimbriae was determined in the fecal strains. This co-presence determined in the UPEC strains was statistically significant. It was confirmed by these results that fimbriae bearing strains are more virulent (odds ratio=29.03, $p<0.001$). Co-presence of not giving hemo-agglutination and without fimbriae was 10% (5/50) in UPEC and 32% (8/25) in fecal strains. The level of non-fimbriae bearing strains in the fecal strains was determined as significantly high compared with the UPEC strains (odds ratio=0.23, $p<0.05$). It was determined that hemolysis ratio was 32% (16/50) in UPEC and 16% (4/25) in fecal strains. The level of hemolysis in MR fimbriae bearing UPEC strains was significantly higher than that in the fecal strains. It was thus determined from these results that hemolysin making strains are more virulent (odds ratio=11.29, $p<0.01$).

In the UPEC strains the level of MR fimbriae bearing and hemolysin production was determined as 18% (9/50). This level is that for strains bearing and for both virulence factors. No co-presence of MR fimbriae bearing and hemolysin production was determined in any fecal strain. MS fimbriae bearing and hemolysin production was 14% (7/50) in UPEC strains, but 4% (1/25) in fecal strains. The difference in MS fimbriae bearing and hemolysin production between UPEC and fecal strains was not statistically significant (odds ratio=3.37, $p>0.05$).

When UPEC and fecal control strains' motilities were examined, it was determined that 70% (35/50) of the UPEC strains and 76% (19/25) of the fecal control strains were motile. As a result of the statistical analysis; motility was not evaluated as a significant virulence factor (odds ratio=0.73, $p>0.05$).

The sensitivity of the virulent strains, isolated from urine of patients who have recurrent urinary tract infections, to the antimicrobials appeared in the following order; imipenem 93%, norfloxacin 89%, ciprofloxacin 85%, netilmicin 80%, amikacin 78%, ceftriaxone 74%, gentamicin 72%, nitrofurantoin 71%, ampicillin/sulbactam 60%, amoxicillin/ clavulanate 58%, TMP-SMX 45%, ampicillin 35%.

Discussion. Urinary tract infection today is frequently encountered among polyclinic and clinic patients and therefore, heads the list of infections for which antimicrobial therapy is most administered. Some of the risk factors for recurrent UTI are a history of UTI, sexual activity at frequent intervals, recent antibiotic use in the last one year period, the low age for the first transmission of UTI, inappropriate hygienic measures, urinary incontinence, and in children vesicoureteral reflux (VUR).

According to our study, the difference in MR fimbriae bearing levels (odds ratio=10.27, $p<0.001$) and MS fimbriae bearing levels (odds ratio=1.67, $p>0.05$) between the UPEC and control strains demonstrated that MR fimbriae bearing strains are more virulent and plays an important role in resistant and recurrent infections. Also, it was determined that strains bearing both fimbriae (odds ratio=29.03, $p<0.001$) are more virulent and have a greater capacity to cause infections.

Wullt,[10] Amabile de Campos et al,[11] Srikanth et al,[12] Geerlings et al[13] and Johnson[14] determined the importance of microbial virulence factors, especially, mannose resistant fimbriae bearing and hemolysin capability, in the occurrence of urinary tract infections.

The difference between the 2 groups in the hemolysin production levels (odds ratio=11.29, $p<0.01$) showed that hemolytic strains are more virulent. Hemolysis support bacterial reproduction by emitting iron and are thought to increase bacterial virulence by directly harming the host tissues.[1,4,8]

Mandal et al[15] demonstrated that virulence factors associated with UPEC were hemolysin production, presence of mannose resistant and mannose sensitive fimbriae. They found resistance to amoxicillin, TMP-SMX, nalidixic acid, norfloxacin and ciprofloxacin among UPEC isolates, which ranged from 70-95%. Junquera et al[9] showed that penicillins, TMP-SMX and quinolones can no longer be considered to be the antimicrobials of choice for empirical treatment of *E. coli* in urinary

tract infections. Along the study period, they observed a reduction in the initial susceptibility differences among hospital and community isolates. Leon Gonzalez et al[16] determined that ciprofloxacin and norfloxacin resistance are starting to be significant. Barrett et al[17] determined the overestimate sensitivity to nitrofurantoin and underestimate sensitivity to quinolones and TMP-SMX and considerable overestimation of sensitivity to cephalosporins.

Resistant and recurrent UTIs lead to several complications and increase the cost of treatment. The development of resistance accelerates with the widespread, combined and generally unaware use of chemotherapeutics for therapeutic and protective purposes. This leads to the treatment of recurrent UTIs in which *E. coli* is a factor, to become more complicated with the occurrence of colonized strains. Particularly in the determination of antimicrobials to be selected in empirical therapy protocols on initiating antimicrobial treatment and in recurrent UTIs, knowledge of regional resistance levels and antibiotics being issued in the light of antibiogram results, plus a preference for the narrowest spectrum antibiotics, will allow these disadvantages to be overcome.

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