Acta clin Croat 2001; 40:165-170 Original Scientific Paper

LOW VIRULENCE OF *ESCHERICHIA COLI* STRAINS CAUSING EXACERBATION OF CHRONIC PYELONEPHRITIS

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SUMMARY – *Escherichia (E.) coli,* which causes over 80% of uncomplicated urinary tract infections, may simultaneously express a number of virulence factors of relevance for urinary tract infections. Some of the recognized *E. coli* virulence factors are adherence to uroepithelial cells, certain O and K serotypes, hemolysin production, and aerobactin production. One of *E. coli* adhesins, P-fimbriae, are known as a major virulence factor in the development of acute uncomplicated pyelonephritis. The aim of this study was to determine the virulence properties of *E. coli* strains isolated from the urine of patients with chronic pyelonephritis, and to compare them to the properties of strains isolated from patients with acute pyelonephritis, acute cystitis, and asymptomatic bacteriuria. For each strain, O-serogroup, adhesin type, motility, production of hemolysin, and the amount of capsular polysaccharide were examined. The strains isolated from patients with acute pyelonephritis were found to mostly express all five or four virulence markers tested, while the less virulent strains were detected in the group of patients with chronic pyelonephritis, where most strains expressed up to three virulence markers. The lowest virulence was observed among the strains isolated in the group of patients with asymptomatic bacteriuria. Expression of P-fimbriae and production of hemolysin were found to be the most important virulence factors with the highest power for discrimination between chronic and acute upper urinary tract infection ($p<0.01$).

Key words: *Escherichia coli, infections, microbiology; Pyelonephritis, microbiology; Urinary tract infections, microbiology*

Introduction

Urinary tract infections (UTIs) pose a major health problem in terms of the proportion of the population affected, and the sequels and cost of bacteriuric episodes. *Escherichia (E.) coli* is the microorganism that is most commonly isolated from individuals with diagnosed endogenous infection of the urinary tract. The probability of UTI depends on the virulence of the infecting bacteria and susceptibility of the host. Most factors predisposing to UTI can be anatomic, increased uroepithelial cell adherence, and nonsecretion of P blood group or of the Lewis blood group antigens[1,2]. Factors described as virulence factors for uropathogenic *E. coli* (UPEC) are adherence ability, hemolysin production, resistance to the bactericidal action of normal human serum and to phagocytosis, aerobactin production, and certain O and K antigens[3,4]. A variety of adhesins with different molecular binding properties have been identified on uropathogenic strains of *E. coli,* such as type 1, P, G, S fimbriae, M-adhesin and Dr group of adhesins[3]. Almost all *E. coli* isolates associated with acute pyelonephritis in uncompromised hosts have expressed P-fimbriae[5-7]. The ability of pyelonephritogenic *E. coli* strains to adhere to uroepithelial cells correlates with their ability to cause a mannose-resistant, specific hemagglutination of human P_1, P_2 or P^k_1 erythrocytes[8]. The minimum receptor-active struc-

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Received March 7, 2001, accepted June 15, 2001

ture for P-fimbriated *E. coli* strains is the disaccharide sequence α-D-Gal-(1→4)-β-D-Gal (Gal, galactose)[9].

The aim of the study was to determine the virulence properties of *E. coli* strains isolated from the urine of patients with chronic pyelonephritis and to compare them to the properties of strains isolated from patients with acute pyelonephritis, acute cystitis, and asymptomatic bacteriuria. For each strain, O-serogroup, adhesin type, motility, production of hemolysin, and the amount of capsular polysaccharide were examined.

Material and Methods

Bacteria

Bacterial isolates from 160 bacteriuric patients (greater than 10^4 organisms per ml for voided clean-catch specimens, any number for suprapubic puncture specimens) were obtained from the Department of Microbiology, Dr. Fran Mihaljević University Hospital for Infectious Diseases, Zagreb. Bacteria were characterized biochemically as *E. coli* using the API 20E System (API, Analytab Products, Plainview, NY). All strains were stored in deep-agar tubes at +4 °C (1.5% nutrient agar, Difco Lab., Detroit, MI) and subcultured by passaging on Tryptic Soy agar (TSA, Difco Lab., Detroit, MI) before use.

Diagnostic criteria and patients

The male and female patients ranged in age from infancy to 98 years (median age 28 years). Four clinical categories were established from charts reviewed for 160 patients with significant *E. coli* bacteriuria (>10^4 bacteria/ml). The level of urinary tract infection was determined indirectly as follows: acute pyelonephritis was defined by body temperature of at least 38.5 °C, increase in the microsedimentation rate (≥25 mm/h), and a level of C-reactive protein (>20 mg/l), which indicated renal inflammation[10]. If parenchymal renal scarring was detected by intravenous urography using a standardized method, the diagnosis of chronic pyelonephritis was made. Acute cystitis was defined by burning and frequency of urination, body temperature of <38 °C, normal sedimentation rate, and level of C-reactive protein. Asymptomatic bacteriuria was defined as significant bacteriuria (≥10^5/ml) with the same strain of bacteria present in at least two consecutive cultures in patients without symptoms or abnormal laboratory findings, in whom significant bacteriuria had been detected during screening or at follow-up after symptomatic infections.

Determination of bacterial adhesins

The expression of adhesins was defined by hemagglutination and inhibition of hemagglutination in microtiter plates, as previously described[11]. Hemagglutination was performed using human erythrocytes of blood groups A_1P_1, $0P_1MM$, $0P_1NN$, $0P_2MM$, and $0P_2NN$ (Croatian Institute of Transfusion Medicine, Zagreb), sheep, ox and guinea pig erythrocytes. The erythrocytes were washed three times in phosphate-buffered saline (PBS; pH 7.2, 0.9 mM) and suspended to a concentration of 2% (vol/vol); they were used on the same day.

Serotyping

All *E. coli* isolates were serotyped using 17 different O-antisera (Institute of Immunology, Zagreb). These O types (O1, O2, O4, O5, O6, O7, O8, O9, O11, O15, O17, O18, O20, O25, O50, O62, and O75) were selected because of their frequent occurrence as urinary pathogens[7]. Serotyping was performed on glass slides and confirmed using a mechanized microtechnique[12,13]. Strains that did not show agglutination with any of the 17 O-antisera used were defined as O non-typeable (NT), and strains that agglutinated in saline were defined as rough strains (RF).

Hemolytic activity

The production of hemolysin was tested on ox blood agar plates. The bacteria growing on TSA were stabbed with a sterile straight wire into 5% ox blood agar. After 18 to 24 h of incubation at 37 °C, the clearing zone was observed.

Motility

With a tubed motility agar, the strains were stabbed into the medium and observed macroscopically for motility after 18 to 24 and 48 h of incubation.

Estimation of capsular polysaccharide amount

The amount of capsular polysaccharide was estimated semiquantitatively by using the Hiss staining method[14].

Statistical analysis

Proportions were compared by the χ^2-test or by Fisher's exact test when the number in any cell of the 2x2 table was ≤5. A p value of <0.05 was considered statistically significant.

Results

The distribution of *E. coli* serogroups among 160 strains studied is summarized in Table 1. Altogether, 76.63% of the 160 *E. coli* strains could be grouped with the O-antisera used, and 7.50% of the strains were rough. Serogroup O6 was most frequently identified, i.e. in 37 (23.13%) strains, followed by O2 serogroup (12.50%). These two predominant serogroups together comprised 35% (n=57) of all *E. coli* strains. Other O-serogroups accounted for less than 10.0% each, and 27 (16.87%) strains were non-agglutinable with the available antisera.

Table 1 shows the frequency of *E. coli* serogroups in the four patient groups. Serogroups O6 and O2 predominated among the strains isolated from the urine of patients with acute pyelonephritis, and acute cystitis. The serogroup distribution was different in strains of the chronic pyelonephritis group. In this category of patients, the most frequently detected serogroup was O15, followed by O1, O4, O6, and O2 serogroups. Only small differences were observed in the distribution of these serogroups, and two rough strains were detected. Five (out of 31) strains were non-typeable.

Table 2 shows distribution of *E. coli* virulence factors in the four diagnostic groups. P-fimbriae were expressed in 25 E. coli strains isolated from the urine of patients with acute pyelonephritis, and in four strains isolated from patients with acute cystitis, whereas in other diagnostic groups they were not detected (χ^2=80.33, p<0.01). Also, there was a statistically significant difference in hemolysin production between the strains (χ^2=35.86, p<0.01). Only five (16%) strains isolated from patients with chronic pyelonephritis produced hemolysin, similarly to the strains isolated from patients with asymptomatic bacteriuria. There were no statistically significant differences in the motility of strains detected in different diagnostic groups (χ^2=0.27, p>0.05). On the contrary, differences in the distribution of capsulated strains were statistically significant (χ^2=9.13, p<0.05). Noncapsulated strains were mostly observed in the asymptomatic bacteriuria and acute cystitis groups. There were no statistically significant differences in the amount of capsular polysaccharide between the strains isolated in acute pyelonephritis and chronic

Table 1. Frequency of E. coli serogroups in the four patient groups

	AP	CP	AC	ABU		Total
	n	n	n	n	n	%
NT	1	5	10	11	27	16.87
RF	0	2	5	5	12	7.50
O1	1	4	3	0	8	5.00
O2	9	3	7	1	20	12.50
O4	4	4	3	1	12	7.50
O5	1	0	1	0	1	0.62
O6	15	4	16	2	37	23.13
O7	1	1	0	1	3	1.88
O8	1	0	2	3	6	3.75
O9	0	0	0	2	2	1.25
O11	0	0	1	2	3	1.88
O15	0	6	1	1	8	5.00
O17	0	0	0	1	1	0.62
O18	3	2	4	2	11	6.88
O20	0	0	0	0	0	0.00
O25	0	0	1	0	1	0.62
O50	0	0	0	2	2	1.25
O62	0	0	0	0	0	0.00
O75	2	0	2	2	6	3.75
Total	37	31	56	36	160	100.00

ABU=asymptomatic bacteriuria; AC=acute cystitis; AP=acute pyelonephritis; CP=chronic pyelonephritis; NT=non-typeable strain; RF=rough strain

Table 2. Distribution of E. coli virulence factors according to diagnostic groups

	Virulence factors							
Diagnosis	P-fimbriae		Hemolysin		Motility		Capsule	
	Yes	No	Yes	No	Yes	No	Yes	No
Chronic pyelonephritis	25	12	30	7	22	15	29	8
Acute pyelonephritis	0	31	5	26	20	11	23	8
Acute cystitis	4	52	26	30	34	22	37	19
Asymptomatic bacteriuria	0	36	9	27	23	13	17	19
Total	29	131	70	90	99	61	106	54

pyelonephritis groups. Abundant capsular polysaccharide was detected in 11 out of 29 capsulated strains in the acute pyelonephritis group, and in nine out of 23 capsulated strains in the chronic pyelonephritis group (χ^2=0.56, p>0.05).

Comparing the number of virulence markers expressed, the acute pyelonephritis group strains were most virulent, followed by the strains of the acute cystitis group. Less virulent strains were detected in the group of patients with chronic pyelonephritis. In this group, only three strains expressed four or all five virulence markers tested, similar to those in the asymptomatic bacteriuria group (Table 3).

Table 3. Combination of virulence determinants in E. coli strains isolated in different diagnostic groups

	Number of virulence markers*						
Diagnosis	0	1	2	3	4	5	Total
Chronic pyelonephritis	0	1	5	7	7	17	37
Acute pyelonephritis	1	6	9	12	3	0	31
Acute cystitis	1	10	15	21	9	0	56
Asymptomatic bacteriuria	3	15	10	6	2	0	36
Total	5	32	39	46	21	17	160

*Virulence markers: P-fimbriae, hemolysin production, motility, expression of capsule, and one of the 10 pyelonephritogenic O-antigens (O1, O2, O4, O6, O7, O8, O18, O25, O50 and O75).

Discussion

The results of this study showed that the strains isolated from the urine of patients with exacerbation of chronic pyelonephritis were less virulent than the strains isolated from the urine of patients with acute pyelonephritis and acute cystitis. None of these strains were P-fimbriated, and they produced hemolysin only rarely. The observed predominance of O6 and O2 serotypes in acute pyelonephritis was not seen among the strains isolated from patients with chronic pyelonephritis. Several virulent clones or virulence properties of *E. coli* have been reported to be dominant in the urinary strains from children and adults[15,16], suggesting that in healthy population only highly virulent bacteria may cause ascending infection. Nimmich *et al.* observed that uropathogenic strains of *E. coli* in which O1, O2, O4 or O6 serogroups were detected, had mostly produced hemolysin and expressed P-fimbriae[17], whereas O'Hanley *et al.* report that O4, O6, O25, O50 and O75 serogroups were often detected among the strains isolated from patients with acute pyelonephritis, and that these strains were significantly often P-fimbriated and hemolytic[18]. The data presented in this study indicate that less virulent strains may be able to cause UTI in patients with renal disease. Although numerous studies on UTI have been published, limited data are available on the properties of uropathogenic *E. coli* strains in specific patient groups. In male patients with acute pyelonephritis or febrile UTI, and in patients with nosocomial UTI, urinary *E. coli* strains were shown to be more often hemolytic and less often P-fimbriated than the strains causing pyelonephritis in women or children with normal urinary tracts[19,20]. In contrast, diabetic patients with nephropathy or other complications were shown to be more prone to infections caused by *E. coli* strains not expressing P-fimbriae or producing hemolysin[21].

Siitonen and Nurminen have suggested the importance of bacterial motility in colonization of urinary mucous membrane, based on the results of the experimental mouse model of UTI[22]. We were not able to detect any correlation between motility and pathogenicity of the strains. There were no statistically significant differences between the strains from different diagnostic groups.

Acidic capsular polysaccharides have been suggested to shield the bacterial cell against the bactericidal action of the complement and phagocytes[23]. In the present study, the capsulated strains were more often detected among the strains isolated from the urine of patients with upper UTI than among the strains from patients with acute cystitis and asymptomatic bacteriuria. Nevertheless, 23% of pyelonephritic strains in this study were

noncapsulated, corresponding to the observations of Ikäheimo *et al.*[24]

Conclusions

1. Strains isolated from patients with acute pyelonephritis mostly expressed all five or four virulence markers tested, whereas less virulent strains were detectetd in the group of patients with chronic pyelonephritis, where most strains expressed up to three virulence markers.

2. Expression of P-fimbriae and production of hemolysin were found to be the most important virulence factors with the highest power for discrimination between chronic and acute upper UTI.

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Sažetak

NISKA VIRULENCIJA SOJEVA *ESCHERICHIA COLI* – UZROČNIKA EGZARCEBACIJE KRONIČNOG PIJELONEFRITISA

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Escherichia (E.) coli, koja je uzročnik više od 80% svih nekompliciranih infekcija mokraćnog sustava, može istodobno iskazivati brojne čimbenike virulencije značajne u patogenezi infekcija mokraćnog sustava. Neki od poznatih čimbenika virulencije *E. coli* su sposobnost prianjanja na uroepitel, iskazivanje određenih antigena O i K, te proizvodnja hemolizina i aerobaktina. P-fimbrije, vrst adhezina *E. coli,* poznati su glavni čimbenik virulencije u nastanku akutnog nekompliciranog pijelonefritisa. Cilj ovoga istraživanja bio je određivanje virulentnih svojstava sojeva *E. coli* izoliranih iz mokraće bolesnika u kojih je dijagnosticiran kronični pijelonefritis, te usporedba ovih sojeva sa sojevima izoliranim iz mokraće bolesnika u kojih je dijagnosticiran akutni pijelonefritis, akutni cistitis i asimptomatska bakteriurija. Svakom od 160 istraživanih sojeva određena je serogrupa O, tip iskazanog adhezina, pokretnost, sposobnost stvaranja hemolizina, te postojanje kapsule i količina kapsularnog polisaharida. Utvrđeno je da sojevi izolirani iz mokraće bolesnika oboljelih od akutnog pijelonefritisa većinom iskazuju svih pet ili četiri istraživana biljega virulencije, dok su sojevi izolirani iz mokraće bolesnika s kroničnim pijelonefritisom bili manje virulentni, te su većinom iskazivali do tri čimbenika virulencije. Najniža virulencija opažena je u skupini sojeva izoliranih iz mokraće bolesnika u kojih je utvrđena asimptomatska bakteriurija. Najznačajniji biljezi virulencije s najvišom moći razlučivanja između kronične i akutne upale gornjeg mokraćnog sustava bili su iskazivanje P-fimbrija i proizvodnja hemolizina ($p<0,01$).

Ključne riječi: *Escherichia coli, infekcije, mikrobiologija; Pijelonefritis, mikrobiologija; Infekcije mokraćnog sustava, mikrobiologija*