ORIGINAL PAPERS

Adv Clin Exp Med 2007, **16**, 5, 651–657
ISSN 1230-025X

DOROTA WOJNICZ

Virulence Factors of Uropathogenic *Escherichia Coli* Strains Isolated from Children with Chronic Pyelonephritis

Czynniki wirulencji uropatogennych szczepów *Escherichia coli* izolowanych od dzieci chorych na przewlekłe odmiedniczkowe zapalenie nerek

Department of Biology and Medical Parasitology, Silesian Piasts University of Medicine in Wrocław, Poland

Abstract

Background. Urinary tract infection (UTI) is one of the most common bacterial infections among children. Chronic pyelonephritis is a renal injury induced by recurrent or persistent renal infection. The severity of the UTI depends both on the virulence of the infecting bacteria and the susceptibility of the host. Uropathogenic *Escherichia coli* (UPEC) strains display a variety of virulence factors that help to colonize host mucosal surfaces and circumvent host defenses to allow invasion of the normally sterile upper urinary tract.
Objectives. The purpose of this study was to determine the occurrence of virulence factors among UPEC isolates.
Material and Methods. Sixty-six *E. coli* strains isolated from the urine of children with chronic pyelonephritis were tested for mannose-resistant hemagglutination in order to indicate P fimbriae. Cell-surface hydrophobicity was assayed by two methods: the bacterial adhesion to hydrocarbon-xylene test (BATH) and the salt aggregation test (SAT). The ability to adhesion was directly measured by using uroepithelial cells. The bacteriophage K1A was used to detect the presence of the capsular antigen K1. The production of α-hemolysin was assessed using blood agar.
Results. Among the isolates studied, the prevalence of the virulence factors α-hemolysin, hydrophobicity, P fimbriae, adhesion to uroepithelium, and K1 capsule were 77%, 74%, 42%, 42%, and 29%, respectively. Most of the *E. coli* strains (37.9%) expressed two virulence factors.
Conclusions. Hydrophobicity and the production of α-hemolysin in *E. coli* strains are important virulence factors in the pathogenesis and development of chronic pyelonephritis (**Adv Clin Exp Med 2007, 16, 5, 651–657**).

Key words: *Escherichia coli*, virulence factors, chronic pyelonephritis.

Streszczenie

Wprowadzenie. Zakażenia układu moczowego (z.u.m.) są jednymi z najczęściej występujących infekcji wśród dzieci. Przyczyną przewlekłego odmiedniczkowego zapalenia nerek są stale nawracające zakażenia układu moczowego, z powodu których dochodzi do uszkodzenia miąższu nerek. Przebieg z.u.m. zależy od zjadliwości bakterii oraz wrażliwości organizmu człowieka. Czynniki wirulencji uropatogennych szczepów *E. coli* (UPEC), umożliwiające adhezję do tkanek żywiciela, a także chroniące bakterie przed działaniem układu odpornościowego makroorganizmu, pozwalają na kolonizację górnych dróg moczowych.
Cel pracy. Określenie częstości występowania czynników wirulencji wśród UPEC.
Materiał i metody. W badaniach wykorzystano 66 szczepów *E. coli* wyizolowanych z moczu dzieci chorych na przewlekłe odmiedniczkowe zapalenie nerek. Obecność fimbrii P ustalono na podstawie testu hemaglutynacji. Hydrofobowość powierzchni komórek oznaczono dwiema metodami: bakteryjnej adhezji do ksylenu (BATH) i agregacji bakterii w siarczanie amonu (SAT). Określono przyleganie pałeczek *E. coli* do uroepitelium. W celu wykrycia otoczkowego antygenu K1 użyto bakteriofaga K1A. Zdolność do wytwarzania α-hemolizyny ustalono z użyciem agaru z krwią baranią.
Wyniki. Wśród badanych szczepów *E. coli* 77% wytwarzało α-hemolizynę, 74% miało hydrofobową powierzchnię. Szczepy, u których wykryto fimbrie P (42%) wykazywały jednocześnie zdolności adhezyjne do uroepitelium. Antygen K1 stwierdzono u 29% izolatów. Najwięcej pałeczek *E. coli* (37,9%) charakteryzowało się obecnością dwóch czynników wirulencji.

Wnioski. Jednoczesne występowanie w pałeczkach *E. coli* powierzchni hydrofobowej oraz zdolności do wytwarzania α-hemolizyny ma istotne znaczenie w patogenezie i rozwoju przewlekłego odmiedniczkowego zapalenia nerek (**Adv Clin Exp Med 2007, 16, 5, 651–657**).

Słowa kluczowe: *Escherichia coli*, czynniki wirulencji, odmiedniczkowe zapalenie nerek.

Escherichia coli are the most common microorganisms causing urinary tract infections (UTIs). Uropathogenic *E. coli* (UPEC) strains possess several virulence determinants that allow them to colonize the urinary tract, avoid host defenses, and cause damage to the uroepithelium [1]. Virulence factors of recognized importance in the pathogenesis of UTI include those produced on the surface of the cell: adhesins (P fimbriae, certain other mannose-resistant adhesins, and type 1 fimbriae), hydrophobic cell surface, the K capsule, and those factors produced within the cell and then exported to the site of action, e.g. α-hemolysin (Hly) [2, 3]. The adherence of *E. coli* to uroepithelial cells is perhaps the most important event in the pathogenesis of UTI. Adhesion to the uroepithelium may protect the bacteria from urinary lavage, increasing their ability to multiply and invade renal tissue. P-fimbriae is the most important adhesin expressed by uropathogenic *E. coli* [4].

E. coli is the most common infecting pathogen in children, accounting for up to 80% of UTIs. Other pathogens include *Enterococcus spp.*, a variety of enterobacteria (e.g. *Klebsiella*, *Proteus*, *Enterobacter*, and *Pseudomonas*) and, occasionally, *Staphylococcus*, *Streptococcus*, and *Candida albicans*. The virulence of the invading bacteria and the susceptibility of the host are of primary importance in the development of UTI [5]. Among children aged less than one year, the prevalence of UTI in girls is 6.5% compared with 3.3% in boys. The incidence of UTI in infants ranges from approximately 0.1% to 1.0% in all newborn infants to as high as 10% in low-birth-weight infants. After one year of age, the prevalence of UTI in boys decreases to 1.9%, whereas it increases slightly to 8.1% in girls. In preschool-aged children the prevalence of asymptomatic infections diagnosed by suprapubic aspiration is 0.8% in girls compared with 0.2% in boys. In the school-age group the incidence of bacteriuria among girls is 30 times higher than among boys (1.2% versus 0.04%) [6, 7]. The aim of this study was to analyze the prevalence of five virulence factors in *E. coli* strains isolated from the urine of children with chronic pyelonephritis.

Material and Methods

Bacterial isolates from 66 bacteriuric patients (more than 10^4 *E. coli* bacteria per milliliter of clean-voided urine) were obtained from the J. Korczak Lower Silesian Pediatric Center. The bacteria were characterized biochemically as *E. coli* using the API 20E System. All the strains were stored in deep-agar tubes at +4°C (1.5% nutrient agar) and cultured by passaging on Tryptic Soy agar (TSA) before use.

Hemagglutination and Expression of P Fimbriae

Hemagglutinins were detected by agglutination of erythrocytes from humans with blood group O in the presence or absence of D-mannose [8].

Hydrophobicity

The surface hydrophobicity of bacterial cells was assessed. Two methods were used: testing the bacterial adhesion to hydrocarbon-xylene (BATH) and the salt aggregation test (SAT). The BATH test was performed as originally proposed by Rosenberg et al. [9]. The bacterial suspensions were vortexed with xylene (1 ml) for 60 seconds and then left for 30 minutes. After the samples had separated into two layers, the aqueous layer was removed and the absorbance at 470 nm (A_{470nm}) was measured. The results were expressed as the percent decrease in optical density of the lower aqueous phase compared with the optical density of the cell suspension without xylene. A strain was considered hydrophilic if it expressed ≤ 35% adhesion to xylene. SAT was performed according to the method of Ljungh et al. [10]. The suspensions (50 µl) were mixed with a series of dilutions of ammonium sulfate (50 µl) ranging from 0.2 to 2.0 mol/l. The lowest concentration of ammonium sulfate at which bacterial aggregation was visible was determined. Autoaggregating strains were described as strongly hydrophobic. A strain was considered hydrophobic if it aggregated in ammonium sulfate concentrations of ≤ 1.4 mol/l.

Adherence Assay

Human periurethral epithelial cells were collected from fresh urine sediment from healthy females who were not being treated with antibiotics at the time. The cells were washed three times in PBS. The pellets were then resuspended in PBS to give 10^5 cells/ml (Bürker chamber

count). Equal volumes of epithelial cells (10^5 cells/ /ml) and antibiotic-treated bacterial suspensions (3×10^8 cells/ml) were mixed for 60 minutes at 37°C. Unattached bacteria were removed from the suspension by centrifugation. The resulting pellets were dried on glass slides in air and were Gram stained. The number of attached bacteria on 100 separate cells was calculated by direct light microscopy and adherence was determined as the mean number of bacteria attached per cell ± the standard deviation (*SD*) [11].

Detection of Capsular Antigen K1

Test strains of *E. coli* were inoculated into 4 ml of nutrient broth and incubated at 37°C for four hours. Every single streak of each culture was then placed on nutrient agar and allowed to dry (about 5 minutes). When the streaks were dry, 10 µl of a bacteriophage K1A suspension was placed in the center of each. After an 18-h incubation, the presence of Kl was indicated by a distinct reduction in the density of the bacterial growth where the bacteriophage suspension had been placed. K1 capsule production was verified by the presence of a clear zone of lysis [12]. The K1A phage and the *E. coli* B2095 (O2 : K1) standard strain used for proliferation of the phage were from the collection of Dr. G. Schmidt (Institut fűr Experimentalle Biologie und Medizin, Forschungsinstitut Borstel, Germany)

α-Hemolysin Production

The production of hemolysin was assayed by growing the different strains in Luria-Bertani Broth (LB) medium overnight (at 37°C) and dripping 50 µl of this culture on a Petri dish containing sheep blood agar. Then the culture was incubated at 37°C overnight and hemolysin production was verified by the presence of a clear hemolytic halo around the colony [13].

Results

It is essential for clinicians to know the virulence characteristics of microorganisms causing an infection in order to better anticipate the evolution of the disease in the host. Figure 1 shows the distribution of *E. coli* virulence factors in the diagnostic group. The results show that most of the *E. coli* strains (77%) produced α-hemolysin when grown on blood agar medium. Among the 66 investigated strains, 49 (74%) exhibited hydrophobic surface properties. P fimbriae were expressed in 28 (42%) of the *E. coli* strains isolated from the urine of patients. The adherence assays showed that 42% of the isolates presented adhesion to uroepithelial cells and the mean number of bacteria adhering to epithelium ranged from 18 ± 4 to 58 ± 8. Only 19 (29%) of the UPEC strains produced the K1 capsule.

The examined *E. coli* strains were divided into six groups according to their number of virulence factors (Table 1). Of all the *E. coli* strains, only two (3%) iso-

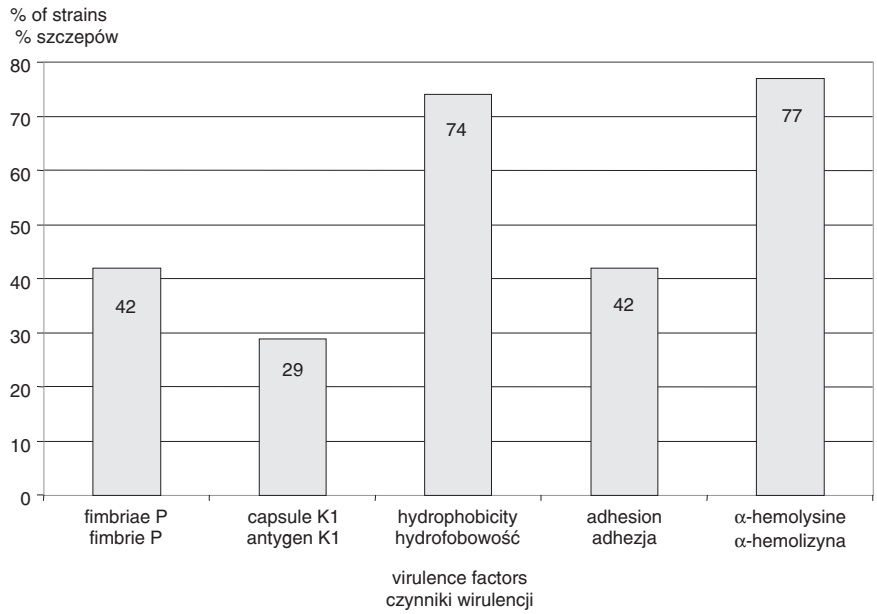

Fig. 1. Virulence factors in *E. coli* rods from urine of children with chronic *pyelonephritis*

Ryc. 1. Czynniki wirulencji pałeczek *E. coli* wyizolowanych z moczu dzieci chorych na przewlekłe odmiedniczkowe zapalenie nerek

Table 1. Virulence patterns identified in UPEC strains

Tabela 1. Wzory wirulencji określone u UPEC

Virulence patterns (Wzory wirulencji)	Number of virulence factors (Liczba czynników wirulencji)	Toxic phenotypes (Cechy zjadliwości)	Number of strains (Liczba szczepów)	%
VP 0	0	--------	2	3.0
VP 1	1	C H Hly^+	4 1 4	6.1 1.5 6.1
VP 2	2	H + Hly^+ C + H	24 1	36.4 1.5
VP 3	3	F + C + A C + H + Hly^+ F + A + Hly^+ F + H + A	4 2 1 1	6.1 3.0 1.5 1.5
VP 4	4	F + H + A + Hly^+ F + C + A + Hly^+ F + C + H + A	14 2 2	21.2 3.0 3.0
VP 5	5	F + C + H + A + Hly^+	4	6.1
		Total:	66	100.0

F – fimbriae P, C – K1 capsule, H – hydrophobicity, A- adhesion to epithelium, Hly+ – α-hemolysin.

F – fimbrie P, C – otoczka K1, H – hydrofobowość, A – adhezja do nabłonka, Hly+ – α-hemolizyna.

lates showed no virulence factors (VP 0). Nine (13.7%) *E. coli* strains possessed one virulence factor: 4 (6.1%) were Hly positive (Hly^+), 4 (6.1%) synthesized capsule K1, and 1 (1.5%) was hydrophobic (VP 1). Of the 66 *E. coli* strains, 25 (37.9%) possessed two virulence factors: 24 (36.4%) were Hly^+ and their surface was hydrophobic and 1 (1.5%) produced Hly and capsule K1 (VP 2). Eight strains (12.1%) had three virulence factors: 4 (6.1%) produced P fimbriae, the K1 capsule, and adhered to epithelium, 2 (3%) possessed capsular antigen, hydrophobic surface, and secreted α-hemolysin, and 2 (3%) P-fimbriated strains adhered to the uroepithelial cells, 1 of which was characterized by hydrophobicity and 1 was Hly^+ (VP 3). Eighteen strains (27.2%) possessed four virulence factors (VP 4): 14 of them (21.2%) were Hly^+, their cell surfaces were hydrophobic, produced P fimbriae, and were able to adhere to epithelium; 4 (6%) synthesized fimbriae P, the K1 capsule, were able to adhere, of which 2 of these strains of them were Hly^+ and 2 had hydrophobic surfaces (VP 4). Only 4 (6.1%) of the 66 UPEC strains expressed all the tested virulence factors (VP 5).

Discussion

Urinary tract infections which are not properly treated from their onset can become a real threat in time, finally leading to renal failure. This is partly due to the fact that urinary signs and symptoms are often not reliable in distinguishing upper and lower UTIs [14]. In general, the more virulence factors a strain expresses, the more severe an infection it is able to cause. Certain virulence factors specifically favor the development of pyelonephritis, others cystitis, and still others asymptomatic bacteriuria [15]. In this study the prevalence of five virulence factors (P fimbriae, hydrophobicity, the K1 capsule, adhesion to the epithelial cells, and α-hemolysin) in *E. coli* isolates causing pyelonephritis in children was determined.

Bacterial adhesion to tissue cells is an important step in the infection process. Specific surface attachments (adhesions, fimbriae), the production of extracellular polysaccharides, and hydrophobicity appear to be the most essential factors in the adherence of bacteria [16]. Epidemiological studies in adults and children over many years in diverse geographic locations have consistently demonstrated that P-fimbriated *E. coli* strains are present in nearly 100% of strains causing pyelonephritis [17, 18]. These *E. coli* strains are able to bind to the digalactoside expressed on the mucosal cells in the urethra and the ureters, thus facilitating establishment of bacteriuria and further transport to the kidneys. It stimulates mucosal cells to release cytokines such as interleukin-6 and interleukin-8, causing fever and an increase of C-reactive protein in the blood and chemotaxis of

neutrophils, initiating an inflammatory response. [2, 19]. In a monkey model, P fimbriae were required for the establishment of pyelonephritis and provided a competitive edge for organisms inoculated into the bladder. In human experiments, P fimbriae enhanced the early establishment of bacteriuria [20]. P-fimbriated *E. coli* strains are more frequently present in pyelonephritis than in lower UTI [21].

In this study the prevalence of P fimbriae was lower (42%) than those reported by other investigators [21, 22]. Among the pyelonephritogenic strains, expression of P fimbriae was found in 71% [21]. The results obtained by Kallenius et al. [22] indicate that 91% of *E. coli* strains causing pyelonephritis, 19% of UPEC causing acute cystitis, and 14% of *E. coli* causing asymptomatic bacteriuria possessed P fimbriae, compared with 7% of *E. coli* isolated from the feces of healthy controls. Plos et al. [23] confirmed that P fimbriae are important in the pathogenesis of ascending UTI and pyelonephritis in humans and have been identified in 80% of pyelonephritic *E coli* isolates. In experimental pyelonephritis, P fimbriae are as important as vesico-ureteric reflux in the development of ascending infection [24].

The hydrophobicity of the bacterial surface facilitates the colonization of tissues. This virulence factor is important for the adhesion of bacteria to water-insoluble substrates. It is assumed that bacterial adhesion decreases when the hydrophobic cell surface becomes hydrophilic. In the present report the high prevalence of hydrophobicity among the isolates from pyelonephritis agrees with that reported by others. Among the pyelonephritogenic strains, expression of cell-surface hydrophobicity was found in 92% [21].

Capsular polysaccharide may be involved in pyelonephritis virulence, perhaps by conferring resistance to the complement-dependent bactericidal effect of serum and phagocytosis [25, 26]. The presence of the K antigens is associated with upper urinary tract infections, and antibody to the K1 antigen has been shown to afford some degree of protection in experimental infections. Regardless of their chemistry, these capsules may be able to promote bacterial virulence by decreasing the ability of antibodies and/or complement to attach to the bacterial surface and the ability of phagocytes to recognize and engulf the bacterial cells [27]. *E. coli* strains expressing the K1 capsule comprise the majority of isolates from neonates with septicemia and acute pediatric pyelonephritis. K antigens account for 70% of the *E coli* isolates involved in acute pyelonephritis in children [28]. In the present study, capsulated strains were rarely detected (29%).

α-hemolysin**,** though not essential for the establishment of chronic pyelonephritis, might contribute to tissue injury and survival in renal parenchyma. Such injury would also facilitate bacterial entry into the blood stream [29]. A considerably high number of strains produced hemolysin, a characteristic normally described as being an important trait, although hemolysin production alone does not always equate with virulence, but may be a decisive factor in the virulence of many nephropathogenic strains, as demonstrated by Blanco et al. [29, 30]. Arisoy et al. [31] described a lower frequency of α-hemolysin-producing strains (1.24%) than that found in the present study (77%). This could be explained by differences in the pathogenic status of the strains because the cited studies analyzed UPEC isolated from symptomatic as well as asymptomatic infections. In this case, asymptomatic infections probably are caused by low-pathogenic *E. coli* strains. Early work performed with a pyelonephritis mouse model demonstrated that α-hemolysin enhances nephropathogenicity [32].

Based on the distribution these five virulence factors, the studied strains exhibited six most common patterns, referred to as VP followed by a number (Table 1). The production of α-hemolysin and hydrophobicity were detected the most frequently in the *E. coli* strains from pyelonephritis (VP 2). All the uropathogenic *E. coli* strains possessing P fimbriae were able to adhere to epithelial cells (VP 3, VP 4, and VP 5).

Some of the virulence patterns found in the studied strains could suggest the presence of pathogenicity islands (PAIs) described in uropathogenic *E. coli.* Several of the genes associated with the acquisition and development of UTIs are encoded on PAIs, e.g. hemolysin and P fimbriae [33]. PAIs encompass large segments of sometimes unstable chromosomal DNA (5 to 200 kb) containing virulence gene clusters that are often flanked by insertion sequence elements or tRNA genes. Four such islands have been identified in pathogenic *E. coli* isolates. PAIs I, II, IV, and V carry a number of virulence gene clusters, among them the P (*pap*) and P-related (*prf*) fimbriae gene clusters and two α-hemolysin loci, *hlyI* and *hlyII* . The virulence genes are often linked in one cluster and their expression may be independent [34].

References

[1] Zmysłowska A, Kozłowski J, Zielińska E, Bodalski J: Zakażenia układu moczowego u dzieci do 3. roku życia. Pol Merk Lek 2003, 14, 319–321.

[2] Wullt B: The role of P fimbriae for *Escherichia coli* establishment and mucosal inflammation in the human urinary tract. Int J Antimicrob Agents 2003, 21, 605–621.

[3] Emody, L, Kerenyi M, Nagy G: Virulence factors of uropathogenic *Escherichia coli*. Int J Antimicrob Agents 2003, 22**,** 29–33.

[4] Twaij M: Urinary tract infection in children: a review of its pathogenesis and risk factors. J R Soc Health 2000, 120, 220–226.

[5] Nowakowska M, Piechula B, Rudy M, Radosz-Komoniewska H, Zientara M: Bacterial etiologic agents in urinary tract infections in children. Med Sci Monit 2000, 6, 49.

[6] Chon CH, Lai FC, Shortliffe LM: Pediatric urinary tract infections. Pediatr Clin North Am 2001, 48, 1441-1459.

[7] Schlager TA: Urinary tract infection in children younger than 5 years of age: epidemiology, diagnosis, treatment, outcomes and prevention. Paediatr Drugs 2001, 3, 219–227.

[8] Latham RH, Stamm WE: Role of fimbriated *Escherichia coli* in urinary tract infections in adult women: correlation with localization studies. J Infect Dis 1984, 149, 835–840.

[9] Rosenberg M, Gutnick D, Rosenberg E: Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. FEMS Microbiol Lett 1980, 9, 29–33.

[10] Ljungh A, Hjerten S, Wadstrom T: High surface hydrophobicity of autoaggregating *Staphylococcus aureus* strains isolated from human infections studied with the salt aggregation test. Infect Immun 1985, 47, 522–526.

[11] Baskin H, Dogan Y, Bahar IH, Yulug N: Effect of subminimal inhibitory concentrations of three fluoroquinolones on adherence of uropathogenic strains of *Escherichia coli*. Int J Antimicrob Agents 2002, 19,79–82.

[12] Devine DA, Roberts AP, Rowe B: Simple technique for detecting K1 antigen of *Escherichia coli*. J Clin Pathol 1990, 43, 76–78.

[13] Santo E, Macedo C, Marin JM: Virulence factors of uropathogenic *Escherichia coli* from a university hospital in Ribeirao Preto, Sao Paulo, Brazil. Rev Inst Med Trop S Paulo 2006, 48, 185–188.

[14] Usein CR, Damian M, Tatu-Chitoiu D, Capusa C, Fagaras R, Tudorache D, Nica M, Le Bouguénec C: Prevalence of virulence genes in *Escherichia coli* strains isolated from Romanian adult urinary tract infection cases. J Cell Mol Med 2001, 5, 303–310.

[15] Vraneš J, Schönwald S, Šterk-Kuzmanović N, Ivančić B: Low virulence of *Escherichia coli* strains causing exacerbation of chronic pyelonephritis. Acta Clin Croat 2001, 40,165–170.

[16] Wolska K, Sówka A, Bukowski K, Jakubczak A: Adherence of *Pseudomonas aeruginosa* strains to solid surfaces. Acta Microbiol Pol 2001, 50, 311–314.

[17] Johnson J: Microbial virulence determinants and the pathogenesis of urinary tract infection. Infect Dis Clin North Am 2003, 17, 261–278.

[18] Johnson JR: papG alleles among *Escherichia coli* strains causing urosepsis: Associations with other bacterial characteristics and host compromise. Infect Immun 1998, 66, 4568– 4571.

[19] Kaper JB, Nataro JP, Mobley HL: Pathogenic *Escherichia coli.* Nat Rev Microbiol 2004, 2, 123–140.

[20] Wullt B, Bergsten G, Connell H, Rollano P, Gebretsadik N, Hull R, Svanborg C: P fimbriae enhance the early establishment of *Escherichia coli* in the human urinary tract. Mol Microbiol 2000, 38, 456–464.

[21] Siegfried L, Kmetova M, Puzova H, Molokacova M, Filka J: Virulence-associated factors in *Escherichia coli* strains isolated from children with urinary tract infections. J Med Microbiol 1994, 41, 127–132.

[22] Kallenius G, Mollby R, Svenson SB: Occurrence of P-fimbriated *Escherichia coli* in urinary tract infections. Lancet 1981, 2, 1369–1372.

[23] Plos K, Connell H, Jodal U: Intestinal carriage of P fimbriated *Escherichia coli* and the susceptibility to urinary tract infection in young children. J Infect Dis 1995, 171, 625–631.

[24] Roberts JA, Suarez GM, Kaack B, Kallenius G, Svenson SB: Experimental pyelonephritis in the monkey. VII. Ascending pyelonephritis in the absence of vesicouretral reflux. J Urol 1985, 133, 1068–1075.

[25] Horwitz MA, Silverstein SC: Influence of the *Escherichia coli* capsule on complement fixation and on phagocytosis and killing by human phagocytes. J Clin Investig 1980, 65, 82–94.

[26] Mushtaq N, Redpath MB, Luzio JP, Taylor PW: Prevention and cure of systemic *Escherichia coli* K1 infection by modification of the bacterial phenotype Antimicrob Agents Chemother 2004, 48, 1503–1508.

[27] Cisowska A: Polisacharydowa otoczka K1 pałeczek *Escherichia coli* i jej znaczenie w chorobotwórczości tych drobnoustrojów. Post Mikrobiol 2003, 42, 3–22.

[28] www.link.med.ed.ac.uk/ridu/UTI2.HTM

[29] Blanco J, Alonso MP, Gonzalez EA, Blanco M, Garabal JI: Virulence factors of bacteraemic *Escherichia coli* with particular reference to production of cytotoxic necrotizing factor (CNF) by P-fimbriated strains. J Med Microbiol 1990, 31, 175–183.

[30] Blanco J, Blanco M, Alonso MP, Blanko JE, Garabal JI, Gonzalez EA: Serogroups of *Escherichia coli* strains producing cytotoxic necrotizing factors CNF1 and CNF2. FEMS Microbiol Lett 1992, 96,155–159.

[31] Arisoy M, Aysev D, Ekim M, Ozel D, Köse SK, Ozsoy ED, Akar N: Detection of virulence factors of Escherichia coli from children by multiplex polymerase chain reaction. Int J Clin Pract 2006, 60, 170–173.

[32] Waalwijk C, MacLaren DM, de Graaff J: In vivo function of haemolysin in the nephropathogenicity of *Escherichia coli.* Infect Immun1983, 42, 245–249.

[33] Blum G, Falbo V, Caprioli A, Hacker J: Gene clusters encoding the cytotoxic necrotizing factor type 1, Prs-fimbriae and alpha-hemolysin form the pathogenicity island II of the uropathogenic *Escherichia coli* strain J96. FEMS Microbiol Lett 1995, 126,189–195.
[34] Yamamoto S: Molecular epidemiology of uropathogenic *Escherichia coli*. J Infect Chemother 2007, 13, 68–73.

Address for correspondence:

Dorota Wojnicz
Department of Biology and Medical Parasitology
Silesian Piasts University of Medicine
Mikulicza-Radeckiego 9
50-367 Wrocław
Poland
Tel.: +48 71 784 15 18
E-mail: wojnicz@biolog.am.wroc.pl

Conflict of interest: None declared

Received: 27.07.2007
Revised: 5.09.2007
Accepted: 18.10.2007