Folia Microbiol. 47 (1), 73–77 (2002) http://www.biomed.cas.cz/mbu/folia/

Occurrence and Genetic Association of Selected Virulence Factors in Clinical *Escherichia coli* Isolates

E. BOGYIOVÁ[a], L. SIEGFRIED[a], M. KMEŤOVÁ[a], Z. ŠÁNDORČÍNOVÁ[a], A. LIPTÁKOVÁ[a], E. BIROŠ[b]

[a]*Institute of Medical Microbiology, School of Medicine, P.J. Šafárik University, 040 66 Košice, Slovakia*
[b]*Laboratory of Genetic Ecotoxicology, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, 142 20 Prague, Czechia*

e-mail bogyie@central.medic.upjs.sk

Received 7 May 2001
Revised version 6 August 2001

ABSTRACT. Occurrence of *cnf1*+ *E. coli* pathogenic strains among extraintestinal *E. coli* isolates was evaluated to explain an impact of cytotoxic necrotizing factor type 1 (CNF1) in human infections. A total of 120 *E. coli* isolates were characterized for presence of virulence factors *cnf1*– and *pap*– specific sequences by PCR, and the production of α-hemolysin using blood agar-plate test. Different association patterns among the detected virulence factors were obtained by comparison of various groups of clinical *E. coli* isolates. These differences probably reflect a potential impact of CNF1 in the colonization of vaginal environment.

The naturally thin anatomical association between gastrointestinal and genitourinary tracts gives an opportunity for infections caused by *E. coli* strains. Some *E. coli* strains are able to produce several virulence factors involved in pathogenesis of extraintestinal infections. These include α-hemolysin, various fimbrial and afimbrial adhesins, aerobactin, and others (Johnson 1991; Kmeťová and Siegfried 1999). It is suggested that cytotoxic necrotizing factor type 1 (CNF1) is also involved in pathogenicity of *E. coli* strains (Caprioli *et al.* 1987; De Ricke *et al.* 1990; Siegfried *et al.* 1993; Fiorentini *et al.* 1997).

Cytotoxic necrotizing factor (CNF1) is chromosomaly encoded 110-kDa protein toxin produced by necrotoxigenic *E. coli* (NTEC1) strains (De Rycke *et al.* 1990); CNF1 is genetically and immunologically related to a plasmid-encoded CNF2 (86 % DNA sequence homology) which is often produced by *E. coli* strains isolated from diarrheic stools of calves (Blanco *et al.* 1996*a*).

Several CNF1-dependent pathological effects were found in laboratory conditions; CNF1 is able to induce necrosis of rabbit skin, enlargement, and multinucleation of Vero or HeLa cells, and cytoplasmic membrane ruffling (Caprioli *et al.* 1983; Blanco *et al.* 1996*a*; Capo *et al.* 1998); it can also affect bactericidal activity of human polymorphonuclear leukocytes (Siegfried *et al.* 1993). Reorganization of F-actin cytoskeleton and formation of stress fibers are the most important CNF1 pathological effects detected *in vitro* (Fiorentini *et al.* 1995). Moreover, CNF1 can prevent apoptosis *via* Rho activation and is able to influence the expression of proteins belonging to the Bcl-2 family (Aktories 1997; Fiorentini *et al.* 1998). NTEC1 strains often cause various types of human extraintestinal infections, including urinary tract infections (UTI), prostatitis, children diarrhea, skin, and respiratory infections (Brauner *et al.* 1990; Andreu *et al.* 1997; Boquet 1998). The role of CNF1 in pathogenesis is still discussed. Yamamoto *et al.* (1996) found increased virulence of CNF1-producing strains in mouse model of UTI. In contrast, Johnson *et al.* (2000*a*) found no evidence that a *cnf1*-negative mutant strain was attenuated in murine model of ascending UTI; also Landraud *et al.* (2000) did not find significant participation of CNF1 in nosocomial infections.

NTEC1 strains usually possess other important virulence factors. CNF1 is found in about 70 % of α-hemolytic but rarely in nonhemolytic isolates (Blanco *et al.* 1996*b*; Island *et al.* 1998). It is also associated with P-fimbriae, which are considered to be the most important fimbrial adhesins in pathogenesis of upper urinary tract infections, mainly in pyelonephritis (Johnson 1991). These and other virulence factors enable the NTEC1 strains to colonize, invade, and persist in host organism. A strong genetic association among α-hemolysin, CNF1, P-fimbriae, and S-fimbriae was established (Daigle *et al.* 1994; Yamamoto *et al.* 1995; Blanco *et al.* 1997). Blum *et al.* (1995) described the genetic blocks of these virulence factors, which are present in the flanked chromosomal regions, and termed them 'pathogenicity islands' (PAI).

Here we focus on the presence of several *E. coli* virulence factors (CNF1, α-hemolysin, and *pap*-fimbriae) in extraintestinal *E. coli* isolates. We aimed to explain the incidence of necrotoxigenic strains and its association with incidence of α-hemolysin and P-fimbriae in *E. coli* strains isolated from gynecological samples of Slovak female population.

MATERIALS AND METHODS

Clinical isolates of E. coli. A total of 120 isolates were recruited during the period 1993–99 by standard bacteriological methods. Of the 120 strains, 60 were isolated from urine specimens (30 pyelonephritic strains were isolated from children up to 10 years of age, 30 cystitic strains from adult males and females). Thirty strains were isolated from gynecological material (cervix, vagina, labia, and embryonic fluid) collected from adult and healthy middle-aged females. Thirty fecal strains were isolated from stool specimens of up to 1-year-old healthy children. *E. coli* strains EB28, EB34, B47, and B48 were used as positive and negative controls for PCR detection. All strains were stored at –20 °C in Luria broth (LB, in g/L: tryptone 10, yeast extract 5, NaCl 0.5) supplemented with 20 % glycerol; strains were revitalized before testing.

Detection of α-hemolysin. Strains were tested for the production of α-hemolysin on blood-agar plates. Defibrinated sheep erythrocytes were added to blood-agar base (no. 2, *Oxoid*) in a 5 % final concentration; hemolysis was evaluated after overnight incubation at 37 °C (Brauner *et al.* 1990).

PCR detection of specific gene sequences. Bacteria were harvested from 1 mL of an overnight broth culture, suspended in 200 μL sterile water, and incubated (100 °C, 10 min). After subsequent centrifugation of the bacterial lysate, 150 μL sample supernatant was stored at –20 °C as a template DNA stock. Amplification of *cnf1*-specific sequences was performed with 50 μL volumes containing 10 μL prepared bacterial lysate; 900 ng *cnf1-A* (GAA CTT ATT AAG GAT AGT) and *cnf1-B* (CAT TAT TTA TAA CGC TG) probes; 0.2 mmol/L each dATP, dGTP, dCTP and dTTP; 1.5 mmol/L $MgCl_2$; 20 mmol/L Tris-HCl (pH 8.4); 50 mmol/L KCl; and 1 U of platinum polymerase (*Gibco BRL*). The PCR was performed with a thermal cycler Mini Cycler™ (*MJ Research*) with heated lid at 94 °C for 2 min for 1 cycle followed by 30 cycles (94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min); final extension 2 min at 72 °C (Blanco *et al.* 1996*a*). PCR detection of *pap*-specific (*papC*) gene sequences was performed with 50 μL volumes containing 1 μL prepared bacterial lysate; 0.45 μmol/L each of *pap1* (GAC GGC TGT ACT GCA GGG TGT GGC G) and *pap2* (ATA TCC TTT CTG CAG GGA TGC AAT A) primers in the same medium as above. The PCR (thermal cycler Mini Cycler™, *MJ Research*) was performed at 94 °C for 2 min for 1 cycle followed by 25 cycles (94 °C, 1 min, 65 °C, 1 min, 72 °C for 2 min); final extension 2 min at 72 °C (Bougenec *et al.* 1992). An aliquot (10 μL) of the final reaction mixture was electrophoresed through 2 % agarose gel for 45 min at 80 V. Amplified DNA fragments of *cnf1* and *pap* (543 bp and 328 bp) were visualized by UV fluorescence after ethidium bromide staining (*see* Fig. 1) (Blanco *et al.* 1996*a*).

Statistical analysis was performed by Pearson χ^2-test; significant differences were at $p < 0.05$.

RESULTS AND DISCUSSION

Occurrence of virulence factors. All three virulence factors were significantly more frequent in 90 extraintestinal isolates (Table I) compared to fecal isolates ($p < 0.01$). These data are in good agreement with the results of Brauner *et al.* (1990), Foxman *et al.* (1995) and Blanco *et al.* (1997), and confirm the participation of tested virulence factors in the development of extraintestinal infections.

Table I. Frequency[a] of triple combinations of virulence factors (Hly, *cnf1*, *pap*) detected in cystitic (C), pyelonephritic (P), gynecological (G) and fecal (F) strains

Hly	*cnf1*	*pap*	C		P		G		F	
+	+	+	10	33	16	53	12	40	3	10
+	+	–	1	3	0	–	1	3	2	6
+	–	+	6	20	4	13	0	–	4	13
+	–	–	1	3	1	3	1	3	0	–
–	+	+	0	–	1	3	2	6	0	–
–	+	–	1	3	0	–	0	–	0	–
–	–	+	3	10	2	6	5	15	3	10
–	–	–	8	27	6	20	9	30	18	60

[a]*First columns* – number of strains of the total (30 in each individual group); *second columns* – percentage.

As α-hemolysin-positive were detected 60 % (18/30) cystitic, 70 % (21/30) pyelonephritogenic (for all uropathogenic isolates it was 65 %), 46 % (14/30) gynecological, and 30 % (9/30) fecal isolates (Table II). A significantly increased frequency of α-hemolysin production was found in pyelonephritogenic ($p < 0.01$)

and in cystitic ($p \leq 0.02$) isolates compared to fecal ones. Gynecological isolates showed also a similar increase in frequency of α-hemolysin but this difference did not reach statistical significance ($p \leq 0.2$).

Table II. Occurrence of individual virulence factors among clinical isolates[a]

Virulence factors	C	P	G	F
Hly	60	70	46	30
cnf1	43	60	50	17
pap	63	77	63	33

[a]*See* footnote to Table I.

We found a higher frequency of α-hemolysin production in cystitic *E. coli* strains (60 %) than previously published, *viz.* 37.5 % by Foxman *et al.* (1995), 41 % by Blanco *et al.* (1997), and 40.5 % by Johnson *et al.* (2000*b*). Production of α-hemolysin in our pyelonephritogenic strains isolated from children (70 %) was similar as determined by Brauner *et al.* (1990) (72 %). Similarly, genotype-based detection (Jusková and Čižnár 1994) showed 72 % of α-hemolytic strains in UTI *E. coli* strains isolated from children.

The *cnf1* genotype analysis showed a significantly higher proportion of *cnf1*+ *E. coli* strains both in cystitic (13/30, 43 %; $p < 0.03$) and pyelonephritogenic (16/31, 52 %; $p < 0.01$) isolates than fecal ones (5/30, 17 %). Statistical analysis also showed a significantly higher frequency of NTEC1 strains (50 %) in gynecological isolates than fecal isolates ($p < 0.01$). Our data indicate the ability of NTEC1 strains to colonize vaginal environment and persist there without clinical manifestation.

We found an increased occurrence of NTEC1 strains in gynecological isolates, which even exceeded α-hemolysin production. Our findings are in good agreement with those of Siegfried *et al.* (1996) who scored the production of CNF1 in extraintestinal strains isolated from various sources (urine, stool, skin, respiratory tract, vagina). Cook *et al.* (1975) also reported a higher frequency of *E. coli* strains exhibiting necrotoxigenic activity in vaginal isolates derived from women with subsequently developing UTI but not in isolates from women without later UTI; however, they did not confirm the responsibility of CNF1 for necrotoxigenic activity.

The highest frequency of *pap*+ strains was detected in pyelonephritogenic isolates (77 %, $p < 0.001$) compared to fecal isolates (33 %). This frequency was identical in cystitic and gynecological isolates (63 %); statistical significance in fecal isolates was $p \leq 0.02$.

Associations between virulence factors. Statistical analysis of extraintestinal isolates confirmed the genetic association among the evaluated virulence factors. The Hly+/*cnf1*+/*pap*+ pattern was found to be the most frequent combination in extraintestinal isolates (53 % pyelonephritogenic, 40 % gynecological, 33 % cystitic isolates; 42 % for all extraintestinal isolates). Fecal isolates showed only 10 % of Hly+/*cnf1*+/*pap*+ *E. coli* strains; this decrease was statistically significant at $p < 0.01$.

In 21 isolates from the total number of 120 (17.5 %) showed the presence of two inspected virulence factors. The most frequent was the Hly+/*cnf1*–/*pap*+ pattern (14/21, 67 %). This combination appeared in the uropathogenic isolates (10/60) as well as in the fecal ones (4/30). According to occurrence frequency, the Hly+/*cnf*+/*pap*– (4/21) is the next; the third combination is Hly–/*cnf*+/*pap*+ (3/21). Fourteen strains (11 %) revealed only a single detected virulence factor (10 strains were *pap*+, 3 strains were α-hemolytic and 1 strain was *cnf1*+).

The occurrence of nonhemolytic necrotoxigenic *E. coli* strains is considered as null (Brauner *et al.* 1990; Foxman *et al.* 1995). In our clinical isolates, α-hemolysin was not expressed by 4 strains of 49 *cnf1*+ *E. coli* strains. Kuhar *et al.* (1998) reported the occurrence of *Hly–/cnf1*+ *E. coli* strains selected from O6 and O18 serotypes derived from intestinal and extraintestinal infections; the occurrence of *Hly–/cnf1*+ strains in our population could be confirmed by genotypic detection (Fig. 1).

Association between Hly and *cnf1*. Of the 62 α-hemolysin producing strains (Hly+), 45 (70 %) were positive for *cnf1* specific sequences (*cnf1*+) (Table III) which is in good agreement with the results of

Table III. Occurrence of toxins and their genetic linkage in clinical groups[a]

Hly	*cnf1*	C		P		G		F	
+	+	11	37	16	53	13	43	5	17
+	–	7	23	5	17	1	3	4	13
–	+	1	3	1	3	2	7	0	–
–	–	11	37	8	27	14	47	21	70

[a]*See* footnote to Table I.

Blanco *et al.* (1997). Moreover, the percentage of *cnf1*+ strains among the α-hemolytic ones was 55 % (5/9) in fecal isolates, 68 % (27/40) in uropathogenic isolates, and 93 % (13/14) in gynecological isolates. Strains isolated from female sexual genitals displayed increased association between α-hemolysin production and presence of *cnf1* specific DNA sequence than isolates from urinary tract ($p \leq 0.06$).

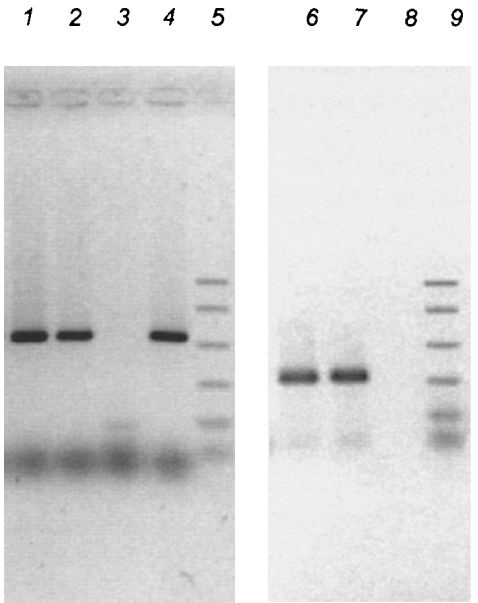

Fig. 1. Agarose gel electrophoresis of *cnf1* (*1–4*) and *papC* (*6–8*) PCR products. Presence of *cnf1* specific sequence leads to 543 bp PCR product, presence of *papC* to 328 bp PCR product; lane *1*: *cnf1*+ (*E. coli* EB28), *2*: *cnf1*+ (*E. coli* EB34), *3*: *cnf1*– (*E. coli* B47), *4*: *cnf1*+ (*E. coli* Ax33, clinical strain); *6*: *pap*+ (*E. coli* EB28), *7*: *pap*+ (*E. coli* Ax33, clinical strain), *8*: *pap*– (*E. coli* B47); lanes *5* and *9*: DNA ladder.

Of 120 isolates, four (3.3 %) were nonhemolytic with *cnf1*+ (Hly–/*cnf1*+ pattern) which indicates a higher frequency of these strains (2 %) found by Blanco *et al.* (1997). Generally, the Hly–/*cnf1*+ arrangement is rare – 3.3 % (2/60) in our uropathogenic and 6.6 % (2/30) in gynecological isolates. This supports the view that the NTEC1 strains are more frequently isolated from gynecological material than other extraintestinal infections.

P-fimbriae are known to be the important factor rendering an advantage for upper urinary tract settlement and for vaginal colonization by *E. coli*. These properties of Pap-fimbriae are ascribed to *pap*-specific Gal–Gal receptors present on epithelial cell surfaces (for review *see* Johnson 1991). We showed a similar occurrence of Hly–/*cnf*–/*pap*+ pattern in both uropathogenic and gynecological *E. coli* isolates, although the combination Hly+/*cnf*–/*pap*+ was absent in the gynecological ones. The Hly+/*cnf*+/*pap*+ arrangement thus seems to be a successful virulence-factor combination for gynecological tract colonization. CNF1 can be considered to be advantageous for colonization and persistence of α-hemolytic *E. coli* strains in vaginal environment.

Finally, it is important to note that vaginal persistence of the NTEC1 strains should be of permanent epidemiological and clinical importance. These strains are frequently found in the urine of patients with diverse types of UTI, and are often found in causal relation to bacterial prostatitis (Andreu *et al.* 1997). From this point of view, the female genital tract is a potential reservoir of necrotoxigenic *E. coli* strains. We can assume that the NTEC1 strains are able to persist there without clinical symptoms as vaginal flora; this should be recognized as an important epidemiological and clinical factor of the increased risk of urinary tract infections in the female population.

The authors wish to thank A. Caprioli, PhD (*Laboratorio di Medicina Veterinaria, Istituto Superiore di Sanità*, Roma) for kindly providing of the control *E. coli* strains. This work was supported by the *Scientific Grant Agency of Ministry of Education of Slovak Republic* and *Slovak Academy of Sciences*, grant no. 1/6042/99.

REFERENCES

AKTORIES K.: Rho proteins: targets for bacterial toxins. *Trends Microbiol.* **5**, 282–288 (1997).

ANDREU A., STAPLETON A.E., FENNEL C., LOCKMAN H.A., XERCAVINS M., FERNANDEZ F., STAMM W.E.: Urovirulence determinants in *Escherichia coli* strains causing prostatitis. *J.Infect.Dis.* **176**, 464–469 (1997).

BLANCO M., BLANCO J.E., BLANCO J., ALONSO M.P., BALSALBORE C., MOURINO M., MADRID C., JUÁREZ A.: Polymerase chain reaction for detection of *Escherichia coli* strains producing cytotoxic necrotizing factor type 1 and type 2 (CNF1 and CNF2). *J.Microbiol.Meth.* **26**, 95–100 (1996*a*).

BLANCO M., BLANCO J.E., ALONSO M.P., BLANCO J.: Virulence factors and O groups of *Escherichia coli* isolates from patients with acute pyelonephritis, cystitis and asymptomatic bacteriuria. *Eur.J.Epidemiol.* **12**, 191–198 (1996*b*).

BLANCO M., BLANCO J.E., RODRÍGUEZ E., ABALIA I., ALONSO M.P., BLANCO J.: Detection of virulence genes in uropathogenic *Escherichia coli* by polymerase chain reaction (PCR): comparison with results obtained using phenotypic methods. *J.Microbiol. Meth.* **31**, 37–43 (1997).

BLUM G., FALBO V., CAPRIOLI A., HACKER J.: Gene clusters encoding the cytotoxic necrotizing factor type 1, Prs-fimbriae and α-hemolysin from the pathogenicity island II of the uropathogenic *Escherichia coli* strain J96. *FEMS Microbiol.Lett.* **126**, 189–196 (1995).

BOQUET P.: Cytotoxic necrotizing factor 1 from *Escherichia coli*: a toxin with a new intracellular activity for eukaryotic cells. *Folia Microbiol.* **43**, 285–289 (1998).

BOUGENEC C.L., ARCHAMBAUD M., LABIGENE A.: Rapid and specific detection of the *pap*, *afa* and *sfa* adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. *J.Clin.Microbiol.* **30**, 1189–1193 (1992).

BRAUNER A., KATOULI M., TULLUS K., JACOBSON S.H.: Production of cytotoxic necrotizing factor, verocytotoxin and hemolysin by pyelonephritogenic *Escherichia coli*. *Eur.J.Clin.Microbiol.Infect.Dis.* **6**, 762–767 (1990).

CAPO C., MECONI S., SANGUEDOLCE M.V., BARDIN N., FLATAU G., BOQUET P., MEGE J.L.: Effect of cytotoxic necrotizing factor 1 on actin cytoskeleton in human monocytes: role in regulation of integrin-dependent phagocytosis. *J.Immunol.* **161**, 4301–4308 (1998).

CAPRIOLI A., FALBO V., RUGGERI F.M., BALDASSARI L., BISICCHIA R., IPPOLITO G., ROMOLI E., DONELI G.: Cytotoxic necrotizing factor production by hemolytic strains of *Escherichia coli* causing extraintestinal infections. *J.Clin.Microbiol.* **25**, 146–149 (1987).

CAPRIOLI A., FALBO V., RODA L.G., RUGGERI F.M., ZONA C.: Partial purification and characterization of an *Escherichia coli* toxic factor that induces morphological cell alterations. *Infect.Immun.* **39**, 1300–1306 (1983).

COOKE E.M., EWISN S.P.: Properties of strains of *Escherichia coli* isolated from a variety sources. *J.Med.Microbiol.* **8**, 107–111 (1975).

DAIGLE F., HAREL J., FAIRBROTHER J.M., LEBEL P.: Expression and detection of *pap*-, *sfa*- and *afa*-encoded fimbrial adhesin systems among uropathogenic *Escherichia coli*. *Can.J.Microbiol.* **40**, 286–291 (1994).

DE RYCKE J., GONZÁLES E.A., BLANCO J., OSWALD E., BLANCO M., BOVIN R.: Evidence for two types of cytotoxic necrotizing factor in human and animal clinical isolates of *Escherichia coli*. *J.Clin.Microbiol.* **28**, 694–699 (1990).

FIORENTINI C., DONELLI G., MATARRESE P., FABBRI A., PARADISI S., BOQUET P.: *Escherichia coli* cytotoxic necrotizing factor 1: evidence for induction of actin assembly by constitutive activation of the p21 Rho GTPase. *Infect.Immun.* **63**, 3936–3944 (1995).

FIORENTINI C., FABBRI A., MATARRESE P., FALZANO L., BOQUET P., MALORNI W.: Hindrance of apoptosis and phagocytic behavior induced by *Escherichia coli* cytotoxic necrotizing factor 1: two related activities in epithelial cells. *Biochem.Biophys.Res. Commun.* **241**, 341–346 (1997).

FIORENTINI C., MATARRESE P., STRAFACE E., FALZANO L., FABBRI A., DONELLI G., GOSSARIZZA A., BOQUET P., MALORNI W.: Toxin-induced activation of Rho GTP-binding protein increases Bcl-2 expression and influences mitochondrial homeostasis. *Exp.Cell Res.* **242**, 341–350 (1998).

FOXMAN B., ZHANG L., PALIN K., TALLMAN P., MARRS C.F.: Bacterial virulence characteristics of *Escherichia coli* isolates from first-time urinary tract infection. *J.Infect.Dis.* **171**, 1514–1521 (1995).

ISLAND M.D., CUI X., FOXMAN B., MARRS C.F., STAMM W.E., STAPLETON A.E., WARREN J.W.: Cytotoxicity of hemolytic, cytotoxic necrotizing factor 1-positive *Escherichia coli* to human T24 bladder cells. *Infect.Immun.* **66**, 3384–3389 (1998).

JOHNSON J.R.: Virulence factors in *Escherichia coli* urinary tract infection. *Clin.Microbiol.Rev.* **4**, 80–128 (1991).

JOHNSON D.E., DRACHENBERG C., LOCKATELL C.V., ISLAND M.D., WARREN J.W., DONNEN M.S.: The role of cytotoxic necrotizing factor type-1 and tissue injury in a murine model of urinary tract infection. *FEMS Immunol.Med.Microbiol.* **28**, 37–41 (2000*a*).

JOHNSON J.R., STEL A.L.: Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *J.Infect.Dis.* **181**, 261–272 (2000*b*).

JUSKOVÁ E., ČIŽNÁR I.: Occurrence of genes for P and S fimbriae and hemolysin in urinary *Escherichia coli*. *Folia Microbiol.* **39**, 159–161 (1994).

KMEŤOVÁ M., SIEGFRIED L.: Pathogenicity of *E. coli* — new results. Part 2. Strains of *E. coli* producing extraintestinal human diseases. (In Slovak) *Slov.Lek.* **8–9**, 328–330 (1999).

KUHAR I., GRABNAR M., ŽGUR-BERTOK D.: Virulence determinants of uropathogenic *Escherichia coli* in fecal strains from intestinal infections and healthy individuals. *FEMS Microbiol.Lett.* **164**, 243–248 (1998).

LANDRAUD L., GAUTHIER M., FOSSE T., BOQUET P.: Frequency of *Escherichia coli* strains producing the cytotoxic necrotizing factor (CNF1) in nosocomial urinary tract infections. *Lett.Appl.Microbiol.* **30**, 213–216 (2000).

SIEGFRIED L., JANIGOVÁ V., MARŠALA J., PUZOVÁ H.: Role of *Escherichia coli* cytotoxic necrotizing factor in morphological and functional changes of human polymorphonuclear leukocytes. *Med.Microbiol.Immunol.* **182**, 214 (1993).

SIEGFRIED L., JANIGOVÁ V., PUZOVÁ H., KMEŤOVÁ M.: Incidence of cytotoxic necrotizing factor and hemolysin production in *Escherichia coli* strains. Abstr. Book p. 108, 6th Internat. Congr. Infectious Diseases, Prague 1994.

YAMAMOTO S., TSUKAMOTO T., TERAI H., KURAZONO Y., TAKEDA Y., YOSHIDA O.: Distribution of virulence factors in *Escherichia coli* isolated from urine of cystitis patients. *Microbiol.Immunol.* **39**, 401–404 (1995).

YAMAMOTO S., NAKATA K., KAZUYO Y., KATAE H., TERAI A., KURAZONO H., TAKEDA Y., YOSHIDA O.: Assessment of the significance of virulence factors of uropathogenic *Escherichia coli* in experimental urinary tract infection in mice. *Microbiol. Immunol.* **40**, 607–610 (1996).