Rev. Inst. Med. trop. S. Paulo
48(4):185-188, July-August, 2006

VIRULENCE FACTORS OF UROPATHOGENIC *Escherichia coli* FROM A UNIVERSITY HOSPITAL IN RIBEIRÃO PRETO, SÃO PAULO, BRAZIL

Edilene SANTO(1), Claudia MACEDO(2) & José Moacir MARIN(3)

SUMMARY

The aim of the study was to determine the occurrence of virulence genes expressing fimbriae, production of hemolysin, colicin and aerobactin among a hundred *Escherichia coli* isolates obtained from in-and outpatients of a tertiary-care teaching hospital, between July and August 2000, showing clinical and laboratory signs of urinary tract infection (UTI). The presence of genes (*pap*, *afa*, *sfa*) for fimbriae expression was assayed using specific primers in a polymerase chain reaction. Among the isolates studied, the prevalence of the virulence factors was 96.0%, 76.0%, 24.0%, for hemolysin, aerobactin and colicin, respectively; the prevalence of genes coding for fimbrial adhesive systems was 32.0%, 19.0% and 11.0% for *pap*, *sfa* and *afa* respectively. The strains isolated from the outpatients displayed a greater number of virulence factors compared to those from hospitalized subjects, emphasizing the difference between these two kinds of patients.

KEYWORDS: *Escherichia coli*; Urinary infection; Virulence factors.

INTRODUCTION

Escherichia coli is one of the major causes of human infectious diseases and is also the most common cause of urinary tract infection (UTI)[19]. At least 10 to 20% of women experience an acute symptomatic UTI at some point during their lives[7]. The severity of the infection depends both on the virulence of the infecting bacteria and on the susceptibility of the host. Urinary infections most often occur in patients with anatomically and functionally normal urinary tracts, and involve spontaneous ascent of bacteria from the urethra to the bladder and in a few patients to the kidney[8]. Adhesion of *E. coli* to the uroepithelium may protect the bacteria from urinary lavage, increasing their ability to multiply and invade renal tissue[12]. The uropathogenic *E. coli* (UPEC) possess adherence factors called pili or fimbriae, which allow them to successfully initiate infections. The two main pili (type 1 and P) found in patients with UTIs, are morphologically similar but differ in their ability to mediate hemagglutination in the presence of mannose[20]. Specific adhesion is mediated by bacterial proteins termed adhesins which may or may not be associated with fimbriae. *Pap* (pyelonephritis-associated pili), *sfa* (S fimbrial adhesin) and *afa* (afimbrial adhesin) operons are most commonly found encoding respectively, P, S and Afa (also designated Dr hemagglutinin) adhesins[3,15].

Besides bacterial adherence, several virulence factors may contribute to the pathogenicity of UPEC, including the production of α-hemolysin, colicin and aerobactin[4,5].

In the present study, we analyzed urinary tract *E. coli* isolates to search for possible evidence of a correlation between biological characteristics that could represent pathogenicity traits of these strains.

MATERIAL AND METHODS

Study design and patients: Virulence factors of nosocomial and community-acquired infections data according to obtained from the Clinical Hospital of the School of Medicine of Ribeirão Preto (HCFMRP), concerning *E. coli* isolated from UTI in-and outpatients, were monitored and analyzed. The study was carried out from July to August 2000. HCFMRP is a university teaching tertiary care hospital.

Bacterial isolates: A total of 100 strains of *E. coli* isolated from 100 patients (only one isolate from each) distributed as: 13 subjects aged between 0 and 15 years (11women and two men), 18 aged between 16 and 39 years (16 women and two men), and 69 aged > 40 years (40 women and 29 men), were analyzed. Thirty-three of the subjects were hospitalized (inpatients) and 67 were outpatients. UTI diagnoses were established by the hospital medical staff based on clinical symptoms and laboratory investigation. Urinary frequency, internal dysuria and suprapubic or pelvic pain are the characteristic symptoms of cystitis, in general acute pyelonephritis presents fever, flank pain, nausea and vomiting. The laboratory criterion for acute *E. coli* UTI was the presence of a positive culture response with at least 10^5 CFU of *E. coli* per mL of clean-voided urine. Among the subjects there were 15 cases of

(1) Departamento de Microbiologia, FCAV, Universidade Estadual Julio de Mesquita, UNESP, 14884-900 Jaboticabal, SP, Brasil.
(2) Departamento de Genética, FMRP/Universidade de São Paulo, 14040-900 Ribeirão Preto, SP, Brasil.
(3) Departamento de Morfologia, Estomatologia e Fisiologia, FORP/USP, 14040-904 Ribeirão Preto, SP, Brasil.
Correspondence to: José Moacir Marin: Phone: +55.16.3602-4101; email: jmmarin@forp.usp.br

pyelonephritis. *Escherichia coli* was identified with the use of standard methods[6]. The strains were stored and subcultured, for further analysis as previously described[1,15].

Hemagglutination and expression of type 1 and P fimbriae: The hemagglutinins were detected by agglutination of erythrocytes from humans blood group O and from guinea pig, in presence or in the absence of D-mannose[14].

Hemolysin production: Production of hemolysin was assayed by growing the isolates overnight (16 h), at 37 ºC in Luria-Bertani Broth (LB), 50 µL are spot inoculated onto sheep blood agar base. The culture was incubated at 37 ºC overnight (16 h) and hemolysin production was verified by the presence of a clear hemolytic halo around the spot.

Aerobactin production: Production of aerobactin was assayed by growing isolated strains in LB medium containing 200 µM of α-α dipyridyl at 37 ºC for 24 h, without shaking. The growth was spun for three min (12000 g), supernatants were filtered through a nitrocellulose membrane (0.22 µm) and aliquots of 50 µL were added to orifices made in LA medium previously seeded with strain LG 1522[2]. The plates were incubated at 37 ºC for 48 h and the production of aerobactin was visualized by the growth of strain LG 1522 around the orifices.

Colicin production: Cultures were examined for colicin production by the overlay method, described previously[22], with indicator strain MA335 of *E. coli*.

DNA extraction: *E. coli* strains were grown in LB broth at 37 ºC overnight (16 h). Bacteria were pelleted from 1.5 mL broth, suspended in 200 µL sterile distilled water, and boiled at 100 ºC for 15 min. Following centrifugation of the lysate, a 150 µL sample of the supernatant was stored at –20 ºC as a template DNA stock[11].

PCR: Specific primers were used to amplify sequences of the *pap*, *sfa* and *afa* genes as indicated in Table 1. Primer sequences, predicted sizes of the amplified products, and specific conditions were described by LE BOUGUENEC *et al.*[15].

Table 1
Primer sequences and predicted sizes of the amplified products of PCR

Primer	Oligonucleotide sequences (5'-3')	Size of amplicons
pap 1	GACGGCTGTACTGCAGGGTGTGGCG	328 bp
pap 2	ATATCCTTTCTGCAGGGATGCAATA	
sfa 1	CTCCGGAGAACTGGGTGCATCTTAC	410 bp
sfa 2	CGGAGGAGTAATTACAAACCTGGCA	
afa 1	GCTGGGCAGCAAACTGATAACTCTC	750 bp
afa 2	CATCAAGCTGTTTGTTCGTCCGCCG	

* *pap*, *sfa* and *afa* primers as reported by LE BOUGUENEC *et al.*[15].

RESULTS AND DISCUSSION

Non properly managed from their onset, urinary tract infection can in time, become a real threat, capable of leading to renal failure. A better knowledge of the virulence characteristics of the microorganism causing the infection allows the clinician to anticipate the evolution of infection in the host.

The occurrence of virulence factors in the present study ranged from 11.0% for *sfa* to 96.0% for hemolysin. Among the adhesins, the P fimbriae was the most prevalent (42 strains), followed by type 1 fimbriae (25 strains) (Table 2), these results not agree with published reports[10,18,21], which emphasize the predominance of fimbriae type 1 among the UPEC strains.

Table 2
Virulence factors associated genes in 100 *Escherichia coli* isolates from UTI patients

Factor	Outpatients	Hospitalized patients	Total of strains
Hemolysin	64	32	96
Colicin	17	7	24
Aerobactin	53	23	76
P fimbriae	28	14	42
Type 1 fimbriae	20	5	25
pap	23	9	32
sfa	14	5	19
afa	8	3	11

The presence of hemolysin and colicin, was related to tissue damage, and the prevalence of aerobactin which confers the capacity to bind iron[5] among our isolates, was higher than those reported by other investigators[10,21]. However, the high prevalence of these virulence factors among the isolates from pyelonephritis in the present report agrees with that reported by others[16,17]. In general the virulence factors from outpatient isolates were found in more than double amounts compared with those from the nosocomial isolates (Table 2).

Based on the distribution of the various target sequences, the strains studied exhibited 10 most common virulence patterns, referred to as Ec followed by an Arabic numeral (Table 3). The strains isolated from patients admitted in hospital exhibited a great diversity of gene patterns, showing the Ec 1, Ec 2, Ec 3 and Ec 7 patterns in common with outpatients patterns and 14 new patterns that appear once a time, it means 43.7% of diversity in virulence patterns (results not showed), in agreement with other report[21]. The presence of the *afa* operon together with aerobactin was detected in the same strain as the Ec 4 pattern (Table 3); this association had been previously reported[21]. A co-dependence of these virulence factors in a particular pathogenic pathway has been discussed[13] but needs to be confirmed.

Out of the 100 uropathogenic *E. coli* isolates tested by PCR, 49 carried sequences related to the three adhesion-encoding operon families investigated. Twenty, 10 and six of the isolates respectively, exhibited *pap*, *afa* and *sfa* genotypes alone (Table 4). The distribution of the S fimbriae-encoding operons found among the isolates studied was lower than previously reported[1,18,21] but agree with those reported by others[3,15]. Regarding P fimbriae, pooled results from other studies indicate that among *E. coli* isolates from patients with pyelonephritis

Table 3
Common virulence patterns identified in *E. coli* strains

Pattern	Virulence factor								No. of strains
	Hemolysin	Aerobactin	P fimbriae	Type 1 fimbriae	Colicin	pap	sfa	afa	
Ec 1	+	–	–	–	–	–	–	–	5
Ec 2	+	+	–	–	–	–	–	–	16
Ec 3	+	+	+	–	–	–	–	–	7
Ec 4	+	+	+	–	–	–	–	+	4
Ec 5	+	+	–	+	–	–	–	–	4
Ec 6	+	–	+	–	–	–	–	–	3
Ec 7	+	+	–	+	–	–	–	–	3
Ec 8	+	+	+	–	+	+	–	–	3
Ec 9	+	+	+	–	–	+	+	–	3
Ec 10	+	+	–	–	+	–	–	–	3

and cystitis, approximately 80% and 30% respectively, possess P fimbriae[4,10]. Although the frequency of *pap* in a cystitis strain agrees with literature data, we found that only 26.6% of the pyelonephritis strains contain *pap* sequences (Table 4). It is noteworthy that most of the pyelonephritis strains studied were isolated from patients with chronic infections and other diseases that complicate their clinical pictures of. Additional epidemiological studies have to be carried out to confirm this observation. However, these data agree with those reported by USEIN *et al.*[21] in Romania.

A total of 12 isolates carried both *pap* and *sfa* operons, and one isolate carried both *sfa* and *afa* operons what it is new from other reports[1,3,15,18,21], while the simultaneous presence of *pap-afa* operons was not detected, differently from others[3,15,18] . A small number of UTI strains possessing *afa*-afimbrial adhesions have been reported[1,3,15]. Growing importance is attributed to the *afa* operon, which has been implicated in the development of chronic interstitial nephritis[9]. We found a small increase in the percentage of *afa* PCR-positive strains in isolates from patients with pyelonephritis (13.3%), compared with those associated with cystitis (9.4%) (Table 4); however, this finding indicates the need for studies on the association.

Table 4
Distribution of the *pap*, *sfa*, *afa* adhesion-encoding operons in 85 *E. coli* isolates from UTI in-and outpatients and from 15 pyelonephritis isolates

Operon	*E. coli* isolates (n = 85)	Pyelonephritis isolates (n = 15)
pap	18 (21.1)*	2 (13.3)*
afa	8 (9.4)	2 (13.3)
sfa	6 (7.0)	0 (0)
pap-sfa	10 (11.8)	2 (13.3)
pap-afa	0 (0)	0 (0)
sfa-afa	1 (1.1)	0 (0)
None	42 (49.5)	9 (60.0)

* ( ) percent

In conclusion, the strains isolated from patients attended at the outpatient clinic exhibited a higher number of virulence factors per strain and seemed to be more aggressive than strains from hospitalized patients, emphasizing the importance of these infected patients that arrive at a tertiary care teaching hospital. This study of the *E. coli* strains isolated from UTI in this kind of hospital was meant as a step towards improving the knowledge regarding their virulence genetic determinants.

RESUMO

Fatores de virulência de *Escherichia coli* uropatogênicas provenientes de um Hospital Universitário em Ribeirão Preto, São Paulo, Brasil

O objetivo do trabalho foi determinar a ocorrência de fatores de virulência, tais como, a expressão de fímbrias, produção de hemolisina, colicina e aerobactina em 100 cepas de *Escherichia coli* isoladas de pacientes ambulatoriais e hospitalizados de um hospital universitário de nível de atendimento terciário, entre os meses de julho e agosto de 2000, que apresentavam sinais clínicos e laboratoriais de infecção do trato urinário (ITU). Foram pesquisados os genes *pap*, *afa* e *sfa* responsáveis pela expressão de fímbrias através da técnica de PCR. A freqüência dos fatores de virulência entre as cepas estudadas foi de 96,0%, 76,0% e 24,0% para hemolisina, aerobactina e colicina respectivamente, e a prevalência dos genes para os sistemas de adesinas fimbriais foi de 32,0%, 19,0% e 11,0% para os genes *pap*, *sfa* e *afa* respectivamente. As cepas isoladas dos pacientes ambulatoriais exibiram um número maior de fatores de virulência quando comparadas com aquelas provenientes de indivíduos hospitalizados.

ACKNOWLEDGMENTS

The authors thank Tania Marques for technical assistance. This work is part of a thesis submitted by Edilene Santo to UNESP University in partial fulfillment of the requirements for the Master's degree.

REFERENCES

1. BLANCO, M.; BLANCO, J.E.; ALONSO, M.P. *et al*. - Detection of *pap*, *sfa* and *afa* adhesin-encoding operons in uropathogenic *Escherichia coli* strains: relationship with expression of adhesins and production of toxins. **Res. Microbiol., 148**: 745-755, 1997.

2. CARBONETTI, N.H. & WILLIAMS, P.H. - Detection of synthesis of the hydroxamate siderophore aerobactin by pathogenic isolates of *Escherichia coli.* In: SUSSMAN, M. **The virulence of *Escherichia coli*. Reviews and methods**. Orlando, Academic Press, 1985. p. 419-424.

3. 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*. **Canad. J. Microbiol., 40:** 286-291, 1994.

4. DONNENBERG, M.S. & WECH, R.A. - Virulence determinants of uropathogenic *Escherichia coli*. In: MOBLEY, H.L.T. & WARREN, J.W., ed. **Urinary tract infections: molecular pathogenesis and clinical management**. Washington, ASM Press, 1996. p. 135-174.

5. EMODY, L.; KERÉNYI, M. & NAGY, G. - Virulence factors of uropathogenic *Escherichia coli*. **Int. J. Antimicrob. Agents, 22** (suppl.): S29-S33, 2003.

6. FARMER, J.J. - Enterobacteriaceae: introduction and identification. In: MURRAY, P.R.; BARON, E.J.; PHALER, M.A.; TENOVER, F.C. & YOLKEN, R.H. ed. **Manual of clinical microbiology. 7. ed.** Washington, ASM Press, 1999.

7. FOXMAN, B. - Epidemiology of urinary tract infection: incidence, morbidity and economic costs. **Amer. J. Med., 113** (suppl): 5S-13S, 2002.

8. FOWLER Jr., J.E. & STAMEY, T.A. - Studies of introital colonization in women with recurrent urinary infections. VII. The role of bacterial adherence. **J. Urol., 117:** 472-476, 1977.

9. GOLUSZKO, P.; MOSELEY, S.; TRUONG, L.D. *et al*. - Development of experimental model of chronic pyelonephritis with *Escherichia coli* O75: K5: H-bearing Dr fimbriae: mutation in the dra region prevented tubulointerstitial nephritis **J. clin. Invest., 99:** 1662-1672, 1997.

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

11. KESKIMAKI, M.; EKLUND, M.; PESONEN, H. *et al*. - EPC, EAEC, and STEC in stool specimens: prevalence and molecular epidemiology of isolates. **Diag. Microbiol. infect. Dis., 40:** 151-156, 2001.

12. KORHONEN, T.K.; VIRKOLA, R.; WESTERLUND, B. *et al*. - Tissue interactions of *Escherichia coli* adhesins. **Antonie van Leeuwenhoek, 54:** 411-420, 1988.

13. LALIOUI, L. & LE BOUGUENEC, C. - *afa*-8 gene cluster is carried by a pathogenicity island inserted into the $tRNA^{Phe}$ of human and bovine pathogenic *Escherichia coli* isolates. **Infect. Immun., 69:** 937-948, 2001.

14. LATHAM, R.H. & STAMM, W.E. - Role of fimbriated *Escherichia coli* in urinary tract infections in adult women: correlation with localization studies. **J. infect. Dis., 149:** 835-840, 1984.

15. LE BOUGUENEC, C.; ARCHAMBAUD, M. & LABIGNE, A. - Rapid and specific detection of the *pap*, *afa*, and *sfa* adhesion-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. **J. clin. Microbiol., 30:** 1189-1193, 1992.

16. O'HANLEY, P.; LOW, D.; ROMERO, I. *et al*. - Gal-Gal binding and hemolysin phenotypes and genotypes associated with uropathogenic *Escherichia coli*. **New Engl. J. Med., 313:** 414-420, 1985.

17. SANDBERG, T.; KAIJSER, B.; LIDIN-JANSON, G. *et al*. - Virulence of *Escherichia* coli in relation to host factors in women with symptomatic urinary tract infection. **J. clin. Microbiol., 26:** 1471-1476, 1988.

18. SILVEIRA, W.D.; BENETTI, F.; LANCELLOTTI, M. *et al*. - Biological and genetic characteristics of uropathogenic *Escherichia coli* strains. **Rev. Inst. Med. trop. S. Paulo, 43:** 303-310, 2001.

19. STAMM, W.E. - Scientific and clinical challenges in the management of urinary tract infections. **Amer. J. Med., 113** (suppl. 1 A): 1S-4S, 2002.

20. SVANBORG, C. & GODALY, G. - Bacterial virulence in urinary tract infection. **Infect. Dis. Clin. N. Amer., 11:** 513-529, 1997.

21. USEIN, C.R.; DAMIAN, M.; TATU-CHITOIU, D. *et al*. - Prevalence of virulence genes in *Escherichia coli* strains isolated from Romanian adult urinary tract infection cases. **J. cell. molec. Med., 5:** 303-310, 2001.

22. VIDOTTO, M.C.; MULLER, E.E.; FREITAS, J.C. *et al*. - Virulence factors of avian *Escherichia coli*. **Avian Dis., 34:** 531-538, 1990.

Received: 15 August 2005

Accepted: 26 April 2006