Virulence characteristics and antimicrobial susceptibility of uropathogenic *Escherichia coli* strains

F.A. Oliveira[1], K.S. Paludo[2], L.N.V.S. Arend[3], S.M.S.S. Farah[3], F.O. Pedrosa[4], E.M. Souza[4], M. Surek[1], G. Picheth[1] and C.M.T. Fadel-Picheth[1]

[1]Departamento de Patologia Médica, Universidade Federal do Paraná, Curitiba, PR, Brasil
[2]Departamento de Biologia Estrutural Molecular e Genética, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brasil
[3]Laboratório Central do Estado do Paraná, Curitiba, PR, Brasil
[4]Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, Curitiba, PR, Brasil

Corresponding author: C.M.T. Fadel-Picheth
E-mail: fpicheth@ufpr.br

ABSTRACT. Eight virulence factors associated with uropathogenic *Escherichia coli* (UPEC) were investigated in 204 clinical isolates of *E. coli* recovered from urine cultures at counts ≥10^5. The bacteria were classified into two groups according to the number of leukocytes in urine samples from which they were isolated: group I ≤8 leukocytes/hpf, 104 strains; group II >8 leukocytes/hpf, 100 strains. Two multiplex PCR systems were used to detect genes encoding adhesin P (*pap*), adhesin S (*sfa*), afimbrial adhesin I (*afa*), siderophore aerobactin (*aer*), alpha-hemolysin (*hly*), cytotoxic necrotizing factor type 1 (*cnf1*), and *traT* associated with serum resistance. The PAI marker for the virulence island identified in strains CFT072 and CVD432, a marker of enteroaggregative *E. coli*, was also investigated using

PCR. The susceptibility profile of *E. coli* strains was determined by disk diffusion method. Ninety percent UPEC showed at least one of the virulence genes, the prevalence being *traT* (76%), *aer* (41%), PAI (32%), *sfa* (26%), *pap* (25%), *cnf1* (18%), *afa* (6%), and *hly* (5%). There was no significant difference in the distribution of virulence genes between groups I and II. A significantly higher degree of virulence was detected in UPEC group II. The CVD432 gene was not detected in any of the UPECs. Fifty-nine percent of the strains were resistant to at least one of the antimicrobials that we tested; the most common being resistance to ampicillin (51%) and trimethoprim-sulfamethoxazole (44%).

Key words: *Escherichia coli*; UPEC; Virulence genes; Uropathogen; Urinary tract infection; Antimicrobial resistance

INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infections (Zhang and Foxman, 2003; Stamm, 2006) and is defined as a significant number of pathogenic organisms in the urinary system (Zhang and Foxman, 2003; Marrs et al., 2005). Microscopy and culture of midstream urine is the gold standard diagnostic test for UTI. Quantitative bacteriuria of 10^5 colony forming units per mL (CFU/mL) is sufficient for a diagnosis of UTI (Chung et al., 2010). Clinical manifestations of UTI are very variable in extent and severity, while cystitis generally resolves without sequelae, pyelonephritis can cause serious morbidity and can be fatal.

Escherichia coli is a commensal of the human intestine, although several strains have acquired specific virulence attributes, which confer the ability to adapt to new niches and cause a broad spectrum of disease (Kaper et al., 2004). *E. coli* is by far the most common cause of UTI accounting for 80 - 90% of all UTIs seen among ambulatory populations (Zhang and Foxman, 2003; Stamm, 2006; Talan et al., 2008). These *E. coli* are named uropathogenic *E. coli* (UPEC) and have virulence properties that are associated with infection in the normal urinary tract including the expression of specific adhesins and toxins (Blanco et al., 1997a; Johnson and Stell, 2000; Kaper et al., 2004; Antão et al., 2009).

Bacterial adherence to uroepithelial cells is an essential stage for the initiation and development of UTI. This process allows bacteria to resist the flushing action of the urine flow and bladder emptying, promoting bacterial persistence and activation of the host signaling pathways. UPEC strains are able to produce various types of adhesins necessary for the recognition and attaching to receptors along the urinary tract including type 1 fimbriae, coded by the *fim* gene cluster; P fimbriae, coded by *pap* (pyelonephritis-associated pili) genes (Roberts et al., 1994; Antão et al., 2009); S fimbriae, coded by *sfa* genes (Korhonen et al., 1984; Antão et al., 2009); and Afa adhesins, coded by *afa* genes, for afimbrial adhesins (Blanco et al., 1997a; Johnson and Stell 2000; Kaper et al., 2004). It was shown that *afa*, *pap* and *sfa* are predictors of cystitis and/or pyelonephritis (Johnson et al., 2005).

Production of toxins such as hemolysin and cytotoxic necrotizing factor 1 (CNF1) causes tissue damage facilitating bacterial dissemination, releasing of host nutrients, and may

also modulate host signaling pathways affecting several processes, including inflammatory responses, host cell survival, and cytoskeletal dynamics (Wiles et al., 2008b). It was shown that hemolysin provokes sloughing of the uroepithelium and bladder hemorrhage while CNF1 induces bladder inflammation and submucosal edema in mice (Smith et al., 2008).

Other characteristics are also important for the UPEC ability to cause UTI. Among them, the expression of iron acquisition systems that utilize siderophores to scavenge iron from the environment and subsequently concentrate it in the bacterial cytosol is fundamental for the ability to colonize and persist in host iron-poor niches such as the urinary tract since limiting iron availability is an important host defense against invading bacterial pathogens (Russo et al., 1999; Wiles et al., 2008b). Its importance is reflected by the presence of several iron acquisition systems in UPEC strains which are up regulated *in vivo* (Snyder et at., 2004). Also, the serum resistance, the ability to avoid the bactericidal activity of serum, is a characteristic important for pathogenesis enabling bacteria to persist in body fluids and internal organs. The outer membrane protein TraT play a role in the defense of bacteria against serum killing (Binns et al., 1982; Mellata et al., 2003).

Some recent studies have found markers of enteroaggregative *E. coli* (EAEC), a diarreagenic pathotype of *E. coli*, among UPEC strains suggesting that some EAEC are potentially uropathogens (Abe et al., 2008; Park et al., 2009).

In this study *E. coli* strains isolated from urine with bacterial counts $\geq 10^5$ CFU/mL were analyzed for presence of virulence factors and susceptibility to antimicrobials. The study was approved by the University's Research Ethics Committee.

MATERIAL AND METHODS

Bacterial strains

A total of 204 *E. coli* strains were isolated from urine samples of in- and out-patients in the Paraná Clínicas Laboratory in Curitiba, PR during the period of April to September 2010. UTI was confirmed by urine culture with bacterial counts $\geq 10^5$ CFU/mL. Bacteria were identified using conventional microbiological methods and were divided into two groups according to the numbers of leukocytes present in the urine sample: discrete to moderate counts, ≤ 8/hpf (group I, 104 strains) or high, >8/hpf (group II, 100 strains). The bacteria were maintained in TSB/glycerol at -70°C.

Detection of virulence genes

The *E. coli* strains were grown overnight in MacConkey agar and isolated colonies were used for DNA extraction by the boiling method. Two distinct multiplex PCR assays were used to detect the presence of the virulence genes. Multiplex 1 included primers designed by Yamamoto et al. (1995) for the target genes *pap* (pilus associated with pyelonephritis), *afa* (afimbrial adhesin), *sfa* (S fimbriae), which code for adhesins; *hly* (hemolysin) and *cnf1* (cytotoxic necrotizing factor 1) coding for toxins, and *aer* for siderophore aerobactin. Multiplex 2 contained primers PAI, a marker of the pathogenicity island of the pyelonephritogenic UPEC *E. coli* CFT073, and the serum resistance associated gene *traT* (Johnson and Stell, 2000). The primers sequences are in Table 1.

Table 1. Sequence of primers used in PCR for virulence genes.

Primer	Sequence (5'-3')	Product size	Reference
pap3	GCAACAGCAACGCTGGTTGCATCAT	336 bp	Yamamoto et al., 1995
pap4	AGAGAGAGCCACTCTTATACGGACA		
sfa1	CTCCGGAGAACTGGGTGCATCTTAC	410 bp	Yamamoto et al., 1995
sfa2	CGGAGGAGTAATTACAACCTGGCA		
afa1	GCTGGGCAGCAAACTGATAACTCTC	750 bp	Yamamoto et al., 1995
afa2	CATCAAGCTGTTTGTTCGTCCGCCG		
hly1	AACAAGGATAAGCACTGTTCTGGCT	1177 bp	Yamamoto et al., 1995
hly2	ACCATATAAGCGGTCATTCCCGTCA		
aer1	TACCGGATTGTCATATGCAGACCGT	602 bp	Yamamoto et al., 1995
aer2	AATATCTTCCTCCAGTCCGGAGAAG		
cnf1	AAGATGGAGTTTCCTATGCAGGAG	498 bp	Yamamoto et al., 1995
cnf2	CATTCAGAGTCCTGCCCTCATTATT		
PAI F	GGACATCCTGGTACAGCGCGCA	930 bp	Johnson and Stell, 2000
PAI R	TCGCCACCAATCACAGCCGAAC		
traT F	GGTGTGGTGCGATGAGCACAG	290 bp	Johnson and Stell, 2000
traT R	CACGGTTCAGCCATCCCTGAG		

All the 204 UPEC strains were also tested for presence of CVD432, a plasmid sequence associated with enteroaggregative *E. coli*, using primers EAEC1 5'-CTGGCGAAAGACTGTATCAT-3' and EAEC2 5'-CAATGTATAGAAATCCGCTGTT-3' (Aranda et al., 2004).

The Multiplex PCR assays were performed in a final volume of 25 µL, containing DNA (2 µL boiling lysate), 1.5 mM MgCl$_2$, 0.2 mM dNTP, 1U Taq DNA Polymerase (Fermentas), 1X Taq DNA Polymerase Buffer, and 0.4 µM primers. For both multiplex systems the following program was used: 1 cycle of 94°C for 60 s; 30 cycles of 94°C for 60 s, 63°C for 30 s, 72°C for 90 s followed by a final cycle of 72°C for 300 s.

Strains EC 2 (*traT pap aer hly*), EC 3 (PAI *sfa cnf1*) and EC 71 (*afa*), were used as positive controls. The detection of PCR products was performed on 2% agarose gel electrophoresis in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3), stained with ethidium bromide 0.5µg/mL and visualized under UV light.

DNA sequencing

The amplicons were sequenced using the DYEnamic ET Dye Terminator Cycle Sequencing kit (GE Healthcare Life Sciences) according to the manufacturer's instructions and an automatic DNA sequencer ABI Prism 377 (Applied Biosystems). Sequences were analyzed using the BioEdit, BLASTn and ClustalW softwares.

Antimicrobial susceptibility testing

The susceptibility test was carried out using the disk diffusion method according to the CLSI, 2010. The following antimicrobials were tested: amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefoxitin, ceftriaxone, cephalothin, ciprofloxacin, gentamicin, nalidixic acid, trimethoprim/sulfamethoxazole and imipenem.

Hemolysis assay

The lytic capability of UPEC strains positive for *hly* gene was assessed by the

method of Scheffer et al. (1988) with some modifications. Briefly, brain heart infusion (BHI) broth (10 mL) was inoculated with the *E. coli* strains and incubated at 37°C in an orbital shaker at 150 rpm. Aliquots of cultures were harvest at hourly intervals beginning after 4 h of incubation up to the 8th hour. The cell suspensions or culture supernatant (0.2 mL) were incubated for 2 h at 37°C with 0.2 mL suspension containing 10^8/mL human erythrocytes in buffer (20 mM $CaCl_2$, 10 mM Tris, and 140 mM NaCl, pH 7.4). The hemoglobin released in the supernatant was determined at 540 nm. The results are expressed as percent of lysis compared with that of erythrocytes lysed in distilled water and Triton X-100 0.1%.

Statistical methods

Comparisons for hemolytic assays were performed with one-way ANOVA and the Tukey *post hoc* test for average comparisons. Differences between non-continuous variables were tested by chi-square test or two-tailed Fisher exact test, as appropriate. The Pearson linear correlation test was used for correlation analysis. Statistical analyses were performed with Statistica for Windows, version 8.0 (StatSoft Inc., Tulsa, OK, USA). All results were considered as statistically significant at $P < 0.05$.

RESULTS

Virulence-associated characteristics

The marker CVD432 of plasmid pAA, associated with EAEC, was not found among the 204 UPEC strains analyzed. One hundred and eighty four strains (90%), presented at least one of the eight UPEC virulence associated genes tested (Table 2), however in 20 strains (10%) none of these virulence genes were found. Fifty-seven strains (28%) had only one virulence gene; 45 strains (22%) two genes; combinations of three virulence genes were observed in 40 (20%) strains, and four to seven virulence genes in 42 (20%). None of the strains contained all the eight UPEC virulence associated genes tested.

The two groups of strains are significantly different considering the number of virulence genes (0, 1, 2, 3, or 4 - 7) present in each UPEC (Table 2). In group I, 39 strains contained only one virulence gene compared with 18 in group II ($P = 0.003$). In contrast, group II contained 29 strains with 4 or more virulence genes compared with only 13 in group I ($P = 0.005$). These results suggest that group I contains strains less virulent than those included in group II.

The prevalence of the UPEC virulence genes was *traT* (76%), *aer* (41%), PAI (32%), *sfa* (26%), *pap* (25%), *cnf1* (18%), *afa* (6%) and *hly* (5%). However, except for *hly*, found only in strains of group II, no significant difference was found in the distribution of these genes between the two groups of strains. The virulence associated genes were distributed in 50 distinct patterns; of these 20 were common to strains belonging to both groups of UPEC. Seven patterns were observed only in strains of group I, and 23 only among strains belonging to group II, suggesting that the last group present greater variability (Table 2). Significant correlations were observed between the presence of some of the virulence genes and leukocytes counts (Figure 1).

Table 2. Distribution and frequency of virulence associated genes in UPEC strains.

Genotype	Group 1	Group 2	Total
traT	34	17	51
aer	4	1	5
pap	1	0	1
traT aer	11	10	21
traT PAI	4	4	8
traT sfa	1	1	2
traT afa	1	0	1
traT pap	0	2	2
PAI sfa	3	2	5
PAI aer	2	2	4
PAI pap	0	1	1
afa aer	1	0	1
traT pap aer	2	8	10
traT PAI aer	4	2	6
traT afa aer	0	4	4
traT PAI pap	2	2	4
traT PAI sfa	1	2	3
traT sfa cnf1	1	1	2
traT sfa aer	1	1	2
traT pap cnf1	0	1	1
traT aer cnf1	0	1	1
PAI pap aer	1	0	1
PAI sfa cnf1	0	1	1
PAI sfa aer	0	1	1
sfa aer cnf1	0	1	1
pap sfa cnf1	2	0	2
pap afa aer	1	0	1
traT pap sfa cnf1	2	3	5
traT PAI sfa cnf1	3	1	4
traT PAI pap aer	0	2	2
PAI pap sfa cnf1	1	1	2
traT PAI pap cnf1	0	1	1
traT pap sfa aer	0	1	1
traT PAI afa aer	1	1	2
traT sfa aer cnf1	0	1	1
traT PAI sfa aer cnf1	2	1	3
traT PAI pap aer hly	0	3	3
traT pap sfa aer cnf1	2	1	3
traT PAI pap sfa cnf1	1	1	2
traT PAI sfa cnf1 hly	0	2	2
traT PAI pap sfa aer	0	1	1
traT PAI sfa afa aer	0	1	1
PAI pap sfa aer hly	0	1	1
PAI pap sfa cnf1 aer	0	1	1
traT PAI pap sfa aer cnf1	1	0	1
traT PAI pap sfa aer hly	0	2	2
traT PAI sfa afa aer cnf1	0	1	1
traT PAI pap afa aer hly	0	1	1
PAI pap sfa aer cnf1 hly	0	1	1
traT PAI pap sfa aer cnf1 hly	0	1	1

The number of leukocytes was positively correlated with the marker for pathogenicity island PAI (p = 0.022), *pap* (P = 0.002), *aer* (P = 0.005) and *hly* (P < 0.001), indicating that the greater the leukocyte number the more frequent these virulence genes are found. Also a positive correlation of 0.702 (P < 0.001) was observed between *cnf1* (found in 36 strains) and *sfa* (present in 53 strains) which were found together in 32 strains.

	traT	PAI	pap	sfa	afa	aer	cnfl	hlv	Leu
traT	NA								
PAI	-	NA							
pap	-	0.212 P=0.002	NA						
sfa	-	0.386 P<0.001	0.266 P<0.001	NA					
afa	-	-	-	-	NA				
aer	-	-	0.214 P=0.002	-	0.255 P<0.001	NA			
cnfl	-	0.227 P=0.001	0.332 P<0.001	0.702 P<0.001	-	-	NA		
hlv	-	0.344 P<0.001	0.317 P<0.001	0.208 P=0.003	-	0.196 P=0.005	-	NA	
Leu	-	0.162 P=0.022	0.218 P=0.002	-	-	0.198 P=0.005	-	0.245 P<0.001	NA

Figure 1. Correlation analysis between virulence genes. The correlation and results of the Fischer exact test indicating only the significant P values are indicated. Leu = leukocytes.

The strains positive for *hly* were tested for the ability to cause lysis of human erythrocytes. Eight of the ten strains presented hemolytic activity (Table 3). The hemolytic activity of bacterial cultures was determined at different times during cell growth. *E. coli* strains induced hemolysis mainly after 3-4 h of bacterial growth. Then, the hemolysis induction rapidly decreased, as previously described (Scheffer et al, 1988; Johnson, 1991). Two strains failed to produce significant hemolysis, however, since HlyA is a pore-forming toxin, at low concentration its binding not always lead to lysis (Boehm et al, 1990; Ostolaza and Goni,1995).

Table 3. Lytic capability of UPEC strains.

Strain	Hemolysis (%
Negative control	3,46 ± 0,14
UPEC *hly* negative	4,10 ± 0,80
UPEC 39	11,6 ± 0,67
UPEC 40	12,63 ± 0,76
UPEC 44	50,4 ± 5,51***
UPEC 74	34,49 ± 1,69***
UPEC 90	21,87 ± 2,4***
UPEC 92	24,7 ± 3,21***
UPEC 105	64,2 ± 13,24***
UPEC 134	16,97 ± 2,4**
UPEC 145	41,6 ± 2,99***
UPEC 162	41,6 ± 2,24***

**P < 0.01 and ***P < 0.001, compared with negative control.

Antimicrobial susceptibility test

One hundred and twenty strains (59%) showed resistance to one or more antimicrobials (Table 4). Resistance was observed for all the antimicrobials tested: ampicillin (51%), trimethoprim / sulfamethoxazole (44%), nalidixic acid (21%), gentamicin (16%), ciprofloxacin (13%), cephalothin (4%), cefoxitin (1%), amoxicillin-clavulanic acid (1%), ceftriaxone, (1%),

aztreonam, cefepime and imipenem (0.5% each). Resistance to ampicillin and sulfametoxazol/ trimetoprim (30%), ampicillin (13%), and ampicillin, ciprofloxacin, nalidixic acid and sulfametoxazol/trimetoprim (9%) were the most common (Table 4). Fifty-one (42%) of these strains were multi-resistant, and a possible ESBL-producing strain was found.

Table 4. Antimicrobial resistance patterns found in UPEC strains.

Antimicrobial	Strains (%)
Ampicillin, Trimethoprim/sulfamethoxazole	36 (30)
Ampicillin	16 (13)
Ampicillin, Nalidixic acid, Trimethoprim/sulfamethoxazole	11 (9)
Ampicillin, Ciprofloxacin, Gentamicin, Nalidixic acid, Trimethoprim/sulfamethoxazole	6 (5)
Gentamicin	6 (5)
Trimethoprim/sulfamethoxazole	6 (5)
Ampicillin, Gentamicin, Trimethoprim/sulfamethoxazole	6 (5)
Ampicillin, Ciprofloxacin, Nalidixic acid, Trimethoprim/sulfamethoxazole	5 (4)
Ampicillin, Cephalothin, Trimethoprim/sulfamethoxazole	5 (4)
Ampicillin, Gentamicin, Nalidixic acid, Trimethoprim/sulfamethoxazole	4 (3)
Cephalothin	2 (2)
Ampicillin, Ciprofloxacin, Nalidixic acid	2 (2)
Ampicillin, Cephalothin, Ciprofloxacin, Nalidixic acid, Trimethoprim∕sulfamethoxazole	2 (2)
Nalidixic acid	1 (1)
Ampicillin, Ciprofloxacin	1 (1)
Ampicillin, Nalidixic acid	1 (1)
Gentamicin, Trimethoprim/sulfamethoxazole	1 (1)
Ampicillin, Gentamicin, Nalidixic acid	1 (1)
Ampicillin, Gentamicin, Nalidixic acid, Trimethoprim/sulfamethoxazole	1 (1)
Ampicillin, Cephalothin, Gentamicin, Nalidixic acid, Trimethoprim/sulfamethoxazole	1 (1)
Ampicillin, Ceftriaxone, Ciprofloxacin, Gentamicin, Nalidixic acid	1 (1)
Ampicillin, Amoxicillin-clavulanic acid, Cefoxitin, Gentamicin, Nalidixic acid	1 (1)
Ampicillin, Cefoxitin, Ciprofloxacin, Nalidixic acid, Trimethoprim/sulfamethoxazole	1 (1)
Ampicillin, Amoxicillin-clavulanic acid, Ciprofloxacin, Gentamicin, Nalidixic acid, Trimethoprim/sulfamethoxazole	1 (1)
Ampicillin, Ciprofloxacin, Gentamicin, Imipenem, Nalidixic acid, Trimethoprim/sulfamethoxazole	1 (1)
Ampicillin, Aztreonam, Cefepime, Ceftriaxone, Cephalotin, Ciprofloxacin, Gentamicin, Nalidixic acid, Trimethoprim/sulfamethoxazole	1 (1)

DISCUSSION

Recent studies have shown that some UPEC strains share some characteristics of EAEC (Abe et al., 2008; Park et al., 2009), a pathotype of diarrheagenic *E. coli* defined by characteristic aggregative adherence pattern to HEp-2 cells. Although no single virulence factor has been irrefutably associated with EAEC virulence, several of them are regulated by a single transcriptional factor called AggR coded by the *aggR* gene (Kaper et al., 2004). The pattern of aggregative adherence was observed in 4% of UPEC strains, while the *aggR* gene was found in 3.1% (Abe et al., 2008) or 41% (Park et al., 2009) of isolates. Also the *aatA* gene, which code for the dispersin transporter protein complex, formerly known as EAEC or CVD432 gene, was detected in 3.6% of UPEC strains (Abe et al., 2008). These results are in contrast with those found in the present work where no positive results for CVD 432 were found among the 204 UPEC strains tested. These discrepancies might be explained by the loss or absence of the plasmid that contains the EAEC sequences in the UPEC strains analyzed in this study, and also to heterogeneity in gene composition of the plasmid pAA associated with EAEC.

In 10% of strains analyzed in this work none of the eight UPEC associated virulence genes tested were found. Lack of virulence markers was also observed in other studies i.e.

41 and 51% of UPEC strains when tested respectively for presence of *hly*, *cnf1*, *pap*, *sfa* and *afa* (Blanco et al., 1997b) or *pap*, *sfa* and *afa* (Santo et al., 2006). These findings reflect the heterogeneity in the distribution of virulence genes among UPEC strains and the complex and multifactorial characteristic of UPEC virulence (Johnson and Stell, 2000; Snyder et al., 2004).

Several distinct patterns were found among the UPEC strains regarding the number of virulence genes and their distribution among the strains (Table 2), a result also found in other studies (Blanco et al., 1997b; Johnson and Stell, 2000; Marrs et al., 2002). High heterogeneity of the composition of virulence associated genes was found, and except for strains carrying only *traT* (51 strains, 25%), or *traT aer* (21 strains, 10%), most strains have a unique virulence gene pattern (Table 2). Similar finding was also observed by Marrs et al. (2002), who found that only 5 to 10% of UPEC strains shared virulence gene patterns.

A significant difference was found among strains belonging to group I or II. A higher degree of virulence was found for UPEC of group II, associated with intense leukocyturia. This group contained a higher number of strains carrying multiple virulence genes (P = 0.005). Strains with only one virulence gene (P = 0.003), predominate in group I (milder leukocyturia). However, no statistically significant difference was found in the distribution of virulence genes between the two groups, except for *hly* (P = 0.033), which was found only in strains of group II. Eighty percent of the strains positive for *hly* induced significant lysis of human erythrocytes. α-hemolysin (HlyA), is associated with higher clinical severity in UTI patients (Johnson, 1991; Marrs et al, 2005). Even sublytic concentrations of HlyA can produce inactivation of the serine/threonine kinase Akt, which plays a central role in host cell cycle progression, metabolism, vesicular trafficking, survival, and inflammatory signaling pathways (Wiles et al., 2008a).

The *traT* gene, associated with serum resistance, was the most prevalent, present in 77% of strains. Johnson and Stell (2000) found *traT* frequencies ranging from 59 to 83% according to the carboxylesterase B type and presence of PAI, whose frequency ranged from 26 to 96%. In the present work the PAI frequency was 33%, and no correlation was found between PAI and *traT* gene, which may be associated to differences in the distribution of *traT* bearing plasmids and variability of pathogenicity islands described in UPEC strains (Binns et al., 1892; Lloyd et al., 2007).

The adhesins genes *sfa* and *pap* presented frequencies of 26 and 25%, respectively. Frequencies of *sfa* and *pap* varying from 0 to 64% and from 0 to 54%, respectively, have been reported in strains isolated from distinct groups of patients and belonging to different phylogenetic groups of UPEC (Blanco et al., 1997a; Santo et al., 2006; Takahashi et al., 2006, 2009). A significant positive correlation was found for simultaneous presence of *sfa* and *pap* (P < 0.001), which were found together in 11% of the strains. Since the binding to the host cells is a key factor for development of infection, the expression of different types of adhesins, with affinity to distinct specific receptors, confers advantages to pathogens (Antão et al., 2009).

The *afa* gene presented the lowest frequency among the adhesins, being found in 6% of the strains. Frequencies of *afa* ranging from 2 to 11% have been reported (Blanco et al., 1997a; Santo et al., 2006; Takahashi et al., 2006; Abe et al., 2008) indicating a lower prevalence of this gene among UPEC.

Among the toxin genes, *cnf1* was the most prevalent, present in 18% of strains, while *hly* was detected in only 5% of the strains, all belonging to group 2. Prevalence of these genes vary according to the phylogenetic group, clinical conditions of host and geographical localization. Frequencies ranging from 0 to 32% were found for *cnf1*; and from 0 to 44% for *hly*

(Blanco et al., 1997b; Johnson and Stell, 2000; Takahashi et al., 2006, 2009; Abe et al., 2008).

Prevalence of *aer* was 41%, which is similar to those found in other studies, although a large variation in gene frequencies has also been been observed (Abe et al., 2008; Takahashi et al., 2006, 2009).

Several pathogenicity islands, with distinct genetic compositions, have been described in UPEC strains. The combination of the virulence genes *pap aer hly* (Table 2), found in 9 strains, suggest the presence of the pathogenicity island I described in strain CFT073 (Guyer et al., 1998; Lloyd et al., 2007). The best studied UPEC strains such as CFT073, J96, 536 and UTI89 have one or more pathogenicity island. Associations of the genes *pap*, *hly* and *cnf1* and also *sfa* are present in some of them (Guyer et al., 1998; Oelschlaeger et al., 2002; Lloyd et al., 2007; Wiles et al., 2008b). Associations of two or more of these genes were observed in some of the strains analyzed in this study, which is suggestive of the presence of pathogenicity island in these strains. For example, the strong positive correlation (P < 0.001; Figure 1) found between *cnf1* and *sfa* may be a result of physical linkage between them, thus suggesting the presence of a pathogenicity island

UTIs are common infections and increased antimicrobial resistance hinder their treatment. The most commonly used antibacterial drugs in the treatment of UTI are trimethoprim-sulfamethoxazole, cephalosporins, semi-synthetic penicillins with or without beta-lactamase inhibitors and quinolones (Arslan et al., 2005; Chung et al., 2010). A high frequency (59%) of UPEC strains resistant to one or more antimicrobials was found in the present work (Table 4). The most frequent antimicrobial resistance found was against ampicillin (51%), in agreement with other studies that found ampicillin resistance ranging from 30 to 58% (McIsaac et al., 2004; Arslan et al., 2005; Houdouin et al., 2006; Talan et al., 2008). The second most frequent resistance observed in this work was to trimethoprim/sulfamethoxazole (44%), again a common finding, with frequencies varying from 10.9 to 36% world-wide (McIsaac et al., 2004; Arslan et al., 2005; Houdouin et al., 2006; Talan et al., 2008). Resistance to ciprofloxacin, also frequently used in the treatment of UTI, was found in 13% of strains; other studies found frequencies of 3% (Talan et al., 2008), 9% (Houdouin et al., 2006) and 17% (Arslan et al., 2005). These results show that resistance to the most frequently used antimicrobials agents are found in a high percentage of UPEC isolates, a cause for concern since this reduces the first-line alternatives for therapy.

A relationship between UPEC reduced virulence and antimicrobial resistance to quinolone and fluoroquinolone was observed in several studies. Nalidixic acid resistance was associated with a lower prevalence of *sfa*, pathogenicity island II$_{J96}$-like domains, strains of the phylogenetics subgroup B2/I, and group A (Houdouin et al., 2006). Piatti et al. (2008), found a higher prevalence of fluoroquinolone susceptible strains in the phylogenetic group B2. Also *hly* and *cnf1* were found to be associated with susceptibility to fluoquinolone and with group B2. Moreover, within group B2, fluoroquinolone resistant strains showed lower prevalences of *pap*, *hly* and *cnf1* than their susceptible counterparts. Similar results were also found by Takahashi et al. (2009). In our work no statistical significant difference was found between quinolone and fluoroquinolone resistant strains and prevalence of virulence genes, indicating that the relationship may be influenced by the genetic background.

In summary, a high variability was found among UPEC strains regarding the distribution of virulence genes and more virulent strains were isolated from urine samples with stronger inflammatory response. A high level of resistance was found due to antimicrobials frequently used for UTI treatment.

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