RESEARCH ARTICLE

Uropathogenic *Escherichia coli* (UPEC) strains may carry virulence properties of diarrhoeagenic *E. coli*

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Abstract

To analyze whether *Escherichia coli* strains that cause urinary tract infections (UPEC) share virulence characteristics with the diarrheagenic *E. coli* (DEC) pathotypes and to recognize their genetic diversity, 225 UPEC strains were examined for the presence of various properties of DEC and UPEC (type of interaction with HeLa cells, serogroups and presence of 30 virulence genes). No correlation between adherence patterns and serogroups was observed. Forty-five serogroups were found, but 64% of the strains belonged to one of the 12 serogroups (O1, O2, O4, O6, O7, O14, O15, O18, O21, O25, O75, and O175) and carried UPEC virulence genes (*pap*, *hly*, *aer*, *sfa*, *cnf*). The DEC genes found were: *aap*, *aatA*, *aggC*, *agg3C*, *aggR*, *astA*, *eae*, *ehly*, *iha*, *irp2*, $lpfA_{O113}$, *pet*, *pic*, *pilS*, and *shf*. Sixteen strains presented aggregative adherence and/or the *aatA* sequence, which are characteristics of enteroaggregative *E. coli* (EAEC), one of the DEC pathotypes. In summary, certain UPEC strains may carry DEC virulence properties, mostly associated to the EAEC pathotype. This finding raises the possibility that at least some faecal EAEC strains might represent potential uropathogens. Alternatively, certain UPEC strains may have acquired EAEC properties, becoming a potential cause of diarrhoea.

Introduction

According to their set of virulence factors and clinical properties, *Escherichia coli* strains can be classified as commensals, intestinal or extra-intestinal pathogens. *Escherichia coli* strains that cause intestinal infections [diarrhoeagenic *E. coli* (DEC)] have been classified into six different pathotypes: enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), Shiga toxin producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), diffusely adherent *E. coli* (DAEC), and enteroaggregative *E. coli* (EAEC) (Kaper *et al.*, 2004). ETEC expresses a variety of fimbrial adhesins and secrets heat-labile (LT) and/or heat-stable (ST) enterotoxins, while EIEC invades and multiplies within the enterocytes of the colon. STEC secrets one or more variants of Shiga toxin (Stx) and EPEC adheres to HeLa/HEp-2 cells by means of a type IV pilus, named the bundle-forming pilus (BFP), forming compact bacterial clusters that characterize the localized pattern of adherence (LA). In addition, EPEC strains and some STEC strains express the outer membrane adhesin intimin that is fundamental for the establishment of characteristic attaching and effacing lesions in the intestinal mucosa. DAEC and EAEC present characteristic adherence patterns in HeLa/HEp-2 cell lines called diffuse adherence (DA) and aggregative adherence (AA), respectively. DAEC and EAEC also produce various types of adhesins, and different toxins and cytotoxins (Kaper *et al.*, 2004).

Escherichia coli strains causing extra-intestinal infections, designated Extra-intestinal pathogenic *E. coli* (ExPEC) (Russo & Johnson, 2000), are frequently associated with urinary tract infections (UTI) and bacteremia in children and adults, as well as sepsis and meningitis in neonates (Kunin & Halmagyi, 1962; Russo & Johnson, 2000). In general, extra-intestinal infections are caused by strains that are transitorily present in the faecal microbiota and bear specific groups of genes encoding virulence factors (Donnenberg & Welch, 1996). If this type of strains colonizes the perineum, they may ascend the urethra and colonize the urinary tract, thus causing disease.

Virulence factors related to UTI causing *E. coli* strains, also designated uropathogenic *E. coli* (UPEC), include certain somatic antigens (especially O1, O2, O4, O6, O7, O15, O18, O25, O75 and O83), capsular antigens (K1 and K2), ability to adhere to the uroepithelial cells by fimbrial (P, S and Type I fimbriae) or afimbrial (Afa) adhesins, presence of OmpT (an outer-membrane protein), production of toxins (α-hemolysin, cytotoxic necrotizing factor and serine protease autotransporter toxin – Hly, CNF and Sat, respectively), and production of siderophores (aerobactin) (Donnenberg & Welch, 1996; Blanco *et al.*, 1997; Guyer *et al.*, 2000; Marrs *et al.*, 2005).

Recently, new putative genes have been described among the DEC strains (Paton *et al.*, 2001; Tatsuno *et al.*, 2001; Doughty *et al.*, 2002; Batisson *et al.*, 2003; Scaletsky *et al.*, 2005). Interestingly, one of these genes, IrgA homologue adhesin (Iha), has been shown to also contribute to the establishment of certain UTIs (Johnson *et al.*, 2000, 2005). Moreover, in a previous study, we have shown that some faecal EAEC strains carried DNA sequences associated with production of Hly (*hly*) and P fimbria (*pap*) (Suzart *et al.*, 1999), suggesting that some EAEC strains might be potential uropathogens. However, despite the large exchange of certain virulence genes found among *E. coli* strains, very few studies have described the presence of virulence characteristics of the DEC pathotypes among UPEC strains (Le Bouguénec *et al.*, 2001; Keller *et al.*, 2002; Matar *et al.*, 2005; Ogura *et al.*, 2007; Wallace-Gadsden *et al.*, 2007).

In this study, we have examined a collection of UPEC strains regarding the presence of virulence properties of the DEC and UPEC pathotypes. Our aim was to analyze whether UPEC strains share virulence characteristics with the DEC pathotypes and to recognize their genetic diversity.

Materials and methods

Bacterial strains

A total of 225 *E. coli* strains isolated from patients of both sex and different ages presenting symptomatic UTI were studied. Patients were hospitalized or visited the emergence room of Hospital São Paulo [Universidade Federal de São Paulo (UNIFESP)] in São Paulo city, Brazil. The strains were isolated in pure cultures and identified in the Microbiology Service of the Central Laboratory at Hospital São Paulo. Strains biochemically confirmed as *E. coli* were kept in Luria–Bertani (LB) broth with sterilized 15% glycerol at $-20\,^{\circ}C$.

HeLa cells adherence assay

Adherence assays were performed as described by Cravioto *et al.* (1979). Briefly, HeLa cells cultivated to 60% confluence, in minimal essential medium (MEM) supplemented with 2% foetal bovine serum and 2% D-mannose, were infected with 1 : 50 dilutions of bacteria grown overnight in LB broth. After 3 h of incubation at 37 °C, preparations were washed with phosphate-buffered saline (PBS), fixed with methanol, stained with May Grümwald and Giemsa, and examined under light microscope.

Serotyping

Identification of somatic (O) antigens was performed by standard methods employing all available O (O1–O181) antisera (Blanco *et al.*, 1992). All antisera were obtained and absorbed with the corresponding cross-reacting antigens to remove the nonspecific agglutinins. The O antisera were produced in the Laboratorio de Referencia de *E. coli* (LREC) (Lugo, Spain, http://www.lugo.usc.es/ecoli).

Search for genetic sequences related with virulence factors

Thirty genetic DNA probes related to putative and established virulence sequences of DEC and UPEC were used to search for homologous sequences in the 225 UPEC strains by colony hybridization assays conducted under high-stringent conditions (Maas, 1983). Probes were obtained from cloned fragments or by PCR amplifications (Tables 1 and 2) and labeled with [^{32}P] α-dCTP (Amersham, Biosciences UK Limited, Little Chalfont Buckinghamshire, England) using the Ready-To-Go DNA Labelling Beads (Amersham), and purified in Probe Quant G-50 Microcolumns (Amersham). The *aggA* primers were used only to test strains that were positive for the *aggC* sequence (Table 2).

Statistical methods

Results were compared by the χ^2 test with Yates' correction for continuity.

Table 1. Cloned DNA probes used in hybridization assays to search for putative and established virulence properties associated with pathogenic *Escherichia coli*

Probe	*E. coli* pathotype*	Associated property	Recombinant plasmid	Probe size (bp)	Restriction enzymes	References
aer	UPEC	Aerobactin	pKLS10	4800	HindIII–BglII	Lawlor & Payne (1984)
cdt	UPEC	Cytolethal distending toxin	pCVD488	1357	AccI	Albert *et al*. (1996)
cnf	UPEC	Cytotoxic necrotizing factor	pEOSW1	336	PstI–ClaI	Oswald *et al*. (1994)
hly	UPEC	Alpha-hemolysin	pSF4000	6000	AvaI	Welch *et al*. (1983)
kps	UPEC	Group II capsule	pSR366	1300	ClaI	Pavelka *et al*. (1991)
daaC	DAEC	Diffuse adherence	pSLM852	350	PstI	Bilge *et al*. (1989)
eae	EPEC	EPEC intimin	pCVD434	1000	SalI–KpnI	Jerse *et al*. (1990)
ehly	EHEC	EHEC hemolysin	pCVD419	3400	HindIII	Levine *et al*. (1987)
aatA†	EAEC	Dispersin transporter protein complex	pCVD432	1000	XbaI–SmaI	Baudry *et al*. (1990)
fim	All	Type I fimbriae	pIB254	9600	HindIII–SalI	Klemm & Christiansen (1987)

*UPEC, uropathogenic *Escherichia coli*; DAEC, diffusely adherent *E. coli*; EPEC, enteropathogenic *E. coli;* EHEC, enterohemorrhagic *E. coli*; EAEC, enteroaggregative *E. coli*.

†This probe has been used to detect strains presenting the AA phenotype, and was formerly known as the AA or CVD432 gene probe.

Results

Interaction with HeLa cells and serogroups of UPEC strains

Of all the 225 UPEC strains tested, 81 (36.0%) promoted cell detachment (CD) and their adherence patterns were not analyzed. Among the remaining 144 CD negative strains, 81 (56.3%) presented sporadic adherence (i.e. sparse bacteria in about 1% of the cells) or were nonadherent (NA). Thirty-three strains (22.9%) showed a noncharacteristic (NC) adherence pattern, i.e. distinct from those previously described (Kaper *et al*., 2004). The remaining strains presented DA (13.9%) or AA (6.9%) (Table 3). Strains presenting localized (LA) or localized adherence-like (LAL) patterns were not found.

The serogroups found among the 225 UPEC strains are shown in Table 3, according to their types of interaction with HeLa cells. Although the strains studied belonged to 45 distinct serogroups, only 12 of them accounted for 64.0% of strains. The most common serogroups in order of frequency were: O6 (44 strains), O2 (16 strains), O15 (13 strains), O14 (11 strains), O18 (10 strains), O25 (10 strains), O1 (8 strains), O175 (8 strains), O4 (7 strains), O21 (6 strains), O75 (6 strains), and O7 (5 strains). A total of 23 strains (10.2%) were O nontypable, and 23 (10.2%) were rough. No association between a particular adherence pattern and a specific serogroup was found. However, some of the strains expressing AA belonged to serogroups (e.g. O6 and O18) commonly found among UPEC strains (Ørskov *et al*., 1982).

Presence of DNA sequences related to virulence factors

Among the 30 DNA virulence sequences tested, the most frequent among the 225 UPEC strains were: *fim* (93.8%), *irp2* (68.9%), *kps* (51.5%), *pap* (45.8%), *hly* (44.0%), *iha* (40.4%), *aer* (39.6%), *sfa* (29.8%), and *cnf* (23.6%) (Table 4). Other sequences were found in $<15\%$ of the strains or were not found (*aafC*, *ldaA*, *paa* and *toxB*). Only two strains lacked all the sequences searched. Interestingly, one strain hybridized with the *eae* gene, which encodes an adhesive outer membrane protein responsible for the intestinal attaching and effacing lesions caused by strains of the EPEC and STEC (DEC) pathotypes (Kaper *et al*., 2004).

The 26 sequences found among the 225 UPEC strains studied were distributed in 160 distinct genetic profiles (combinations of virulence genes), which carried between one and 15 distinct genes. However, only three of these profiles (*fim*; *pap sfa fim hly kps cnf irp2*; and *pap sfa fim hly kps aer shf irp2 iha*) were found in five or more strains each. The *fim* sequence alone was the most frequent profile (13% or 5.7% of the strains).

Typical UPEC virulence genes (*pap*, *sfa*, *afa*, *hly*, and *cnf*) were concentrated in strains belonging to 11 of the 12 most frequent serogroups identified. Thus, 81.6% of strains belonging to serogroups O1, O2, O4, O6, O7, O14, O15, O18, O21, O25, and O75 possessed *pap*, *sfa*, *afa*, *hly* and/or *cnf* genes, vs. only 49.4% of strains belonging to other serogroups ($P < 0.001$).

Table 4 shows the frequencies of the virulence sequences detected in 225 UPEC strains according to their adherence pattern. All 81 CD strains hybridized with the *hly* probe. This group also presented *fim* (97.5%), *irp2* (76.5%), *pap* (74.1%), *kps* (67.9%), *sfa* (66.7%), *cnf* (53.1%), *aer* (44.4%), *iha* (43.2%), *pic* (24.7%), and *shf* (16.0%) sequences. Other sequences appeared in frequencies lower than 15% or were not found.

Among the strains presenting the NC adherence pattern, *fim* (97.0%), *irp2* (57.6%), *kps* (48.5%), *aer* (36.4%), *iha* (33.3%), *pap* (27.3%), and *hly* (27.3%) were the virulence sequences most frequently found, while in NA strains,

Table 2. DNA probes obtained by PCR used in hybridization assays to search for putative and established virulence properties associated with pathogenic *Escherichia coli*

Probe	*E. coli* pathotype*	Associated property	Primer sequence (5′–3′)	Probe size† (bp)	References
pap	UPEC	P pili	GACGGCTGTACTGCAGGGTGTGGCG ATATCCTTTCTGCAGGGATGCAATA	328	Le Bouguénec *et al*. (1992)
sfa	UPEC	S fimbriae	CTCCGGAGAACTGGGTGCATCTTAC CGGAGGAGTAATTACAAACCTGGCA	410	Le Bouguénec *et al*. (1992)
aafC	EAEC	AAF-II usher	ATGGTACCCGTTATAGCTCGCACATATTC ATGAGCTCTGCAGACTGATAATGCTC	1144	Elias *et al*. (1999)
aggA	EAEC	AAF-I fimbrial subunit	GCGTTAGAAAGACCTCCAATA GCCGGATCCTTAAAAATTAATTCCGGC	450	Nataro *et al*. (1992)
aggC	EAEC	AAF-I usher	TATTAAACCATGGTAGCG GCCAAGATCCGAGATTGA	538	Savarino *et al*. (1994)
agg3C	EAEC	AAF-III usher	GTTTGGAACCGGGAATTAACATTG ATACTTTAGATACCCCTCACGCAG	485	Bernier *et al*. (2002)
aggR	EAEC	Transcriptional activator of AAF-I and AAF-II	CTAATTGTACAATCGATGTA ATGAAGTAATTCTTG AAT	308	Czeczulin *et al*. (1999)
aap	EAEC	Dispersin protein coat	CTTTTCTGGCATCTTGGGT GTAACAACCCCTTTGGAAGT	232	Czeczulin *et al*. (1999)
astA	EAEC	EAEC heat-stable enterotoxin 1 (EAST1)	CCATCAACACAGTATATCCGA GGTCGCGAGTGACGGCTTTGT	111	Savarino *et al*. (1993)
irp2	EAEC	Yersiniabactin biosynthetic gene	AAGGATTCGCTGTTACCGGAC AAGGATTCGCTGTTACCGGAC	264	Czeczulin *et al*. (1999)
pet	EAEC	Plasmid encoded toxin (Pet)	GTGTTTCAACCAGGTTCAACA CCTTCACCAATTTTATGCAGT	1037	Gioppo *et al*. (2000)
pic	EAEC	Protein involved in intestinal colonization (Pic)	GGGTATTGTCCGTTCCGAT ACAACGATACCGTCTCCCG	1175	Czeczulin *et al*. (1999)
shf	EAEC	Cryptic ORF	ACTTTCTCCCGAGACATTC CTTTAGCGGGAGCATTCAT	613	Czeczulin *et al*. (1999)
pilS	EAEC	EAEC type IV pilus major subunit	ATGAGCGTCATAACCTGTTC CTGTTGGTTTCCAGTTTGAT	400	Dudley *et al*. (2006)
afa	DAEC, UPEC	Afimbrial adhesin	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	750	Le Bouguénec *et al*. (1992)
ldaG	EPEC	LDA afimbrial adhesin	AAAGATCTGTGATGAGGTTCAGGTGAAG AAATCTAGATGCAGACGCAACTACAGCCA	920	Scaletsky *et al*. (2005)
saa	STEC	STEC autoagglutinating adhesin	CGTGATGAACAGGCTATTGC ATGGACATGCCTGTGGCAAC	119	Paton & Paton (2002)
toxB	EHEC	ToxB protein	TAAAGCAGAAAAATGCGACAGAAGAT TAGTAAGTAGAGTAGAACTGGGGGATG	250	Badea *et al*. (2003)
iha	EHEC	IrgA homologue adhesin	CAAATGGCTCTCTTCCGTCAATGC CAGGTCGGGGTTACCAAGT	925	Szalo *et al*. (2002)
paa	EPEC	Porcine A/E-associated adhesin	CTCGAGAGTGCCTTTCCTGG GGATCCATGAGGAACATAA	760	Batisson *et al*. (2003)
lpfA	EPEC	Long polar fimbriae	ATGAAGCGTAATATTATAG TTATTTCTTATATTCGAC	573	Doughty *et al*. (2002)

*UPEC, uropathogenic *Escherichia coli*; EAEC, enteroaggregative *E. coli*; DAEC, diffusely adherent *E. coli*; EPEC, enteropathogenic *E. coli;* EHEC, enterohemorrhagic *E. coli*; STEC, Shiga toxin producing *E. coli*.

†Size of PCR-product used as DNA probes in hybridization assays.

fim (88.9%), *irp2* (65.4%), *kps* (40.7%), *iha* (37.0%), *aer* (37.0%), *pap* (35.8%), and $lpfA_{O113}$ (22.2%) prevailed. In both groups (NC and NA), other sequences appeared in frequencies lower than 15% or were not found.

Strains presenting DA frequently carried *fim* (90.0%), *irp2* (75.0%), *kps* (55.0%), *afa* (55.0%), *daaC* (50.0%), *iha* (50.0%), and *agg3C* (50.0%). Neither *pilS*, *aggR*, *aap*, *aatA*, *aggC*, *cdt*, *pet*, *saa*, *eae*, nor *ehly* sequences were found among these strains, and other sequences (*aer*, *pap*, *hly*, *cnf*, $lpfA_{O113}$, *astA*, *sfa*, *shf*, *pic*, in decreasing order of frequency) appeared in 35.0% of the strains or less.

All 10 AA strains presented *fim*, while *pap*, *hly*, *sfa*, $lpfA_{O113}$, *pilS*, *aggC, saa, cdt, eae* and *ehly* were not found among these strains. Other UPEC virulence sequences

Table 3. Types of interaction with HeLa cells and serogroups of 225 UPEC strains

Interaction with HeLa cells	Number (%) of strains	Serogroups (n° of strains)*
Cell detaching activity (CD)	81 (36.0)	ONT (4), O2 (10), O4 (6), O6 (31), O8, O11, O14 (3), O15 (2), O16, O18 (5), O21 (4), O25 (4), O54, O73, O75 (2), O78, O82, O92, O166, O175
No cell detaching activity:	144 (64.0)	
Nonadherent (NA)	81 (56.3)†	ONT (11), O1 (5), O2 (2), O5, O6 (6), O7 (5), O8, O9 (3), O10, O11, O12, O14 (6), O15 (7), O17, O18 (2), O21, O23 (3), O25 (3), O26, O32, O44, O54, O71, O75, O77, O81, O82, O92, O112, O117, O127, O153, O159 (2), O175 (5)
Noncharacteristic (NC)	33 (22.9)†	ONT (5), O1, O2 (2), O4, O6 (3), O8, O9, O14, O15 (2), O16, O17, O18 (2), O25 (3), O28, O32, O35, O75, O77, O83, O169, O173, O175
Diffuse adherence (DA)	20 (13.9)†	ONT (2), O1 (2), O2 (2), O6 (3), O11, O15 (2), O17, O19, O21, O55, O75 (2), O82, O175
Aggregative adherence (AA)	10 (6.9)†	ONT, O5 (2), O6, O14, O18, O92, O166, O169 (2)

*NT, nontypeable.

†Percentage related to the total of strains that did not present the cell detaching activity.

Table 4. Genetic sequences related to putative virulence factors associated with different *E. coli* pathotypes in 225 UPEC strains according to their phenotypes in the interaction with HeLa cells

Genetic sequences*	No. (%) of UPEC strains expressing different phenotypes in HeLa cells†					
	CD ($n=81$)	NA ($n=81$)	NC ($n=33$)	DA ($n=20$)	AA ($n=10$)	Total ($n=225$)
fim	79 (97.5)	72 (88.9)	32 (97)	18 (90.0)	10 (100)	211 (93.8)
irp2	62 (76.5)	53 (65.4)	19 (57.6)	15 (75.0)	6 (60)	155 (68.9)
kps	55 (67.9)	33 (40.7)	16 (48.5)	11 (55.0)	1 (10)	116 (51.5)
pap	60 (74.1)	29 (35.8)	9 (27.3)	5 (25.0)	–	103 (45.8)
hly	81 (100)	6 (7.4)	9 (27.3)	3 (15.0)	–	99 (44.0)
iha	35 (43.2)	30 (37)	11 (33.3)	10 (50.0)	5 (50)	91 (40.4)
aer	36 (44.4)	30 (37)	12 (36.4)	7 (35.0)	4 (40)	89 (39.6)
sfa	54 (66.7)	9 (11.1)	3 (9.1)	1 (5.0)	–	67 (29.8)
cnf	43 (53.1)	4 (4.9)	3 (9.1)	2 (10.0)	1 (10)	53 (23.6)
pic	20 (24.7)	6 (7.4)	3 (9.1)	1 (5.0)	1 (10)	31 (13.8)
shf	13 (16)	11 (13.6)	3 (9.1)	1 (5.0)	2 (20.0)	30 (13.3)
lpfA	5 (6.2)	18 (22.2)	3 (9.1)	2 (10.0)	–	28 (12.4)
daaC	5 (6.2)	8 (9.9)	3 (9.1)	10 (50.0)	1 (10)	27 (12.0)
agg3C	4 (4.9)	7 (8.6)	2 (6.1)	10 (50.0)	2 (20)	25 (11.1)
afa	5 (6.2)	4 (4.9)	2 (6.1)	11 (55.0)	1 (10)	23 (10.2)
astA	6 (7.4)	4 (4.9)	2 (6.1)	1 (5.0)	3 (30)	16 (7.1)
pilS	4 (4.9)	7 (8.6)	1 (3)	–	–	12 (5.3)
aatA	4 (4.9)	2 (2.5)	–	–	2 (20)	8 (3.6)
aggR	5 (6.2)	1 (1.2)	–	–	1 (10)	7 (3.1)
aap	4 (4.9)	1 (1.2)	–	–	2 (20)	7 (3.1)
aggC	4 (4.9)	2 (2.5)	–	–	–	6 (2.7)
saa	–	2 (2.5)	1 (3)	–	–	3 (1.3)
cdt	2 (2.5)	–	–	–	–	2 (0.9)
pet	–	–	–	–	1 (10)	1 (0.4)
eae	–	1 (1.2)	–	–	–	1 (0.4)
ehly	–	1 (1.2)	–	–	–	1 (0.4)

*DNA sequences related to putative *E. coli* virulence factors: *fim*, type I fimbriae; *irp*2, Yersiniabactin biosynthetic gene; *kpsMT*, group II capsule; *pap*, P pili; *hly*, α-hemolysin; *aer*, aerobactin; *sfa*, S fimbriae; *cnf*, cytotoxic necrotizing factor; *pic*, protein involved in intestinal colonization, Pic; *shf*, cryptic ORF; *daaC*, diffuse adherence; *agg3C*, AAF-III usher subunit; *afa*, afimbrial adhesin; *astA*, EAEC heat-stable enterotoxin 1; *pilS*, EAEC type IV pilus major subunit; *aggR*, transcriptional activator of AAF-I and AAF-II; *aap*, dispersin protein coat, previously named *aspU*; *aatA*, dispersin transporter protein complex; formerly used as an EAEC probe sequence; *aggC*, AAF-I usher subunit; *cdt*, cytolethal distending toxin; *pet*, plasmid encoded toxin, Pet; *eae*, EPEC intimin.

†AA, aggregative adherence; DA, diffuse adherence; NC, noncharacteristic; NA, nonadherent; CD, cell detaching activity.

Sequences searched for but not found: *aafC*, AAF-II fimbrial subunits; *paa*, Porcine A/E-associated adhesin; *toxB*, ToxB EHEC adhesin; *ldaG*, EPEC afimbrial adhesin.

Table 5. Type of interaction with HeLa cells, serogroups, and combinations of putative virulence genes of 16 UPEC strains presenting characteristics of the EAEC pathotype (aggregative adherence and/or the *aatA* sequence)

Interaction with HeLa cells* (No. of strains)	Serogroup	Combinations of putative virulence genes†
Aggregative adherence (*n* = 10)	O5	*fim irp2 iha*
	O5	*fim afa kps aer daaC agg3C shf irp2*
	O6	*fim aer cnf irp2*
	O14	*fim agg3C iha*
	O18	*fim astA irp2*
	O92	*fim aer aatA aggR aap pic irp2 iha*
	O166	*fim aatA aap astA pet irp2*
	O169	*fim aer shf iha*
	O169	*fim astA iha*
	ONT	*fim*
Cell detaching activity (*n* = 4)	O6	*aatA fim hly pap sfa kps cnf aggR aggC aggA aap irp2*
	O11	*aatA fim hly pap aer aggR aggC aggA aap pic irp2 iha lpfA*
	O15	*aatA fim hly pap sfa kps aer aggR aggC aggA aap pic irp2 iha pilS*
	ONT	*aatA fim hly pap kps cnf aggR aggC aggA aap*
Nonadherent (*n* = 2)	O15	*aatA fim pap kps aer aggC aggA irp2 iha lpfA*
	ONT	*aatA fim aap astA shf irp2 iha*

*Associations with HeLa cells were determined in 3 h.

†*aggA*, AAF-I fimbrial subunit. Other DNA sequences searched for in order to characterize the genetic profiles and the putative virulence factors related to these genes are described in Table 3.

found in this group included: *aer* (40.0%), *afa* (10.0%), *cnf* (10.0%), and *kps* (10.0%). Furthermore, the DEC virulence genes found among the AA-presenting strains include: *aggR*, *pic*, *pet*, *daaC* (10.0% each); *aap*, *aatA, agg3C, shf* (20.0% each), *astA* (30.0%) and *irp2* (60.0%). Except for *daaC*, all of these latter genes are associated with virulence properties of the EAEC pathotype.

Genetic profiles of UPEC strains presenting properties that characterize the EAEC pathotype (AA and/or *aatA*)

Among the 225 UPEC strains, 16 presented characteristics of the EAEC pathotype (AA and/or the *aatA*): two AA/*aatA*+, eight AA/*aatA* −, four CD/*aatA*+, and two NA/*aatA*+. The genetic profiles presented by each of these strains are shown in Table 5. The *aggR* sequence was found in one out of 10 AA, four CD and none of the NA strains, while *aggC* was found in all four CD strains and in one of two NA strains. All strains carrying *aggC* also carried the *aggA* sequence. The *agg3C* sequence was found in two out of 10 AA, and none of the CD or NA strains.

Discussion

In the present study, we have investigated a collection of 225 UPEC strains regarding the presence of different phenotypic and/or genotypic properties of the DEC pathotypes. The strains belonged to 45 serogroups. Of all strains tested, 64.0% belonged to one of 12 serogroups (O1, O2, O4, O6, O7, O14, O15, O18, O21, O25, O75, and O175) and frequently showed UPEC virulence genes (*pap* 45.8%, *hly* 44.0%; *aer* 39.6%; *sfa* 29.8%; *cnf* 23.6%), confirming that most UPEC strains belong to few seropathotypes with specialized virulence factors (Donnenberg & Welch, 1996; Blanco *et al.*, 1997; Marrs *et al.*, 2005). However, the analysis of the interaction with HeLa cells revealed that 6.9% of the adherent UPEC strains presented the AA pattern, which is used as a characteristic to classify faecal *E. coli* strains as EAEC (Kaper *et al.*, 2004). Apart from the present study which reports AA expression in UPEC strains, only Guth *et al.* (1995) described the occurrence of a few UPEC strains presenting this phenotype. It is possible that the AA frequency found among UPEC strains is even higher, because the expression of AA could not be evaluated with the CD strains that carried EAEC virulence genes.

AA expression is associated with the production of aggregative adherence fimbriae types I, II, and III (AAF/I, AAF/II, and AAF/III, respectively). These fimbriae are encoded by an apparently well conserved high molecular weight plasmid (pAA) (Vial *et al.*, 1988), which also carries *aggR*, *astA* and *pet* encoding a transcriptional activator, a heat-stable toxin (EAEC stable toxin, EAST1), and the plasmid encoded toxin (Pet), respectively. pAA also includes the anti aggregation protein (*aap*) and *shf* genes that codify the dispersin protein coat (Sheikh *et al.*, 2002) and a cryptic ORF (Czeczulin *et al.*, 1999), respectively. A segment of this plasmid, formerly known as the EAEC or CVD432 gene probe, and which has been used as a genetic probe to detect

EAEC strains, is presently named *aatA* and encodes the dispersin transporter protein complex (Nishi *et al*., 2003).

Sixteen UPEC strains studied presented characteristics used to classify faecal *E. coli* strains as EAEC (AA and/or *aatA*). A recent DNA microarray analysis of the distribution of plasmid and chromosomal genes among EAEC strains (Jenkins *et al*., 2005) has defined two groups of EAEC based on the presence of specific genes. Group 1 has been characterized by the presence of genes related to one of the three known AAF adhesin variants (*aggA*, *aafA*, and *agg3A*), their ushers (*aggC*, *aafC*, and *agg3C*) and their regulator *aggR*, while Group 2 would carry very small numbers of *pheU* island (highly conserved EAEC chromosomal pathogenicity island) and pAA related genes, particularly *aggR*, *aap*, and *aatA*. Jenkins *et al*. (2005) have also considered that the EAEC Group 2 strains may carry pAA and possibly lost segments of the *pheU* island or comprise non-EAEC strains that received pAA by horizontal transfer. Likewise, some of our UPEC strains carried pAA gene sequences (especially *aggR*, *aggC*, *aap*, and *astA*); these strains might have acquired pAA thus becoming a potential agent of diarrhoea. Furthermore, all six strains carrying *aggC* also carried *aggA* suggesting that these UPEC strains express AAF-I, because *aggA* and *aggC* encode the fimbrial subunit and usher of this fimbria, respectively. Further studies on the virulence property of the UPEC strains expressing AA should focus on a possible role of AAF/I in the colonization of the urinary tract.

Also, 13.9% of the adherent UPEC strains studied presented the DA pattern, a characteristic that identifies faecal *E. coli* strains as DAEC (Kaper *et al*., 2004). However, the DA pattern is also presented by UPEC strains expressing Afa, a group of adhesins belonging to Dr family (Servin, 2005). In fact, many of the UPEC strains showing DA in the present study carried UPEC virulence genes (*afa*, *pap*, *sfa*, and *hly*). Moreover, half of the DA strains also carried the *daaC* gene encoding the usher of F1845, another adhesin of the Dr family associated with DA expression in the DAEC pathotype (Bilge *et al*., 1993). As the usher sequences of the adhesins of the Dr family are highly homologous, cross reactivity between them may occur. However, the *daaC* sequence was detected in five UPEC strains devoid of *afa*; conversely, *afa* was found in one UPEC strain lacking *daaC*.

Another finding of the present study was the identification of the *eae* gene in a NA UPEC strain that belonged to serogroup O71. The *eae* gene encodes intimin, an adhesin promoting actin accumulation in eukaryotic cells; this property is associated with the ability of EPEC and STEC to cause attaching-effacing lesions (Kaper *et al*., 2004). In a study conducted by Matar *et al*. (2005), the *eaeA* gene was found in all 10 UPEC isolates analysed. Among these strains, 80% expressed it, suggesting that the *eae* gene product may play a certain role in UPEC pathogenesis. Differently from this finding, our data revealed that only one of 225 UPEC strains presented this gene, suggesting that it may not be important in UPEC pathogenesis. Preliminary characterization of the UPEC strain carrying *eae* revealed that it was able to accumulate actin in Mandin–Darby canine kidney (MDCK) cells (not shown), but the significance of this property in UTI remains to be studied.

We also sought to evaluate the prevalence of gene sequences related to the recently described adhesins of the DEC pathotypes (Iha, Saa, Paa, PilS, LPF, ToxB, and LDA) in our collection of UPEC strains. The most prevalent sequence of adhesin-encoding genes found was *iha* (40.4%), and the importance of Iha in strains causing UTI has been recently described (Léveillé *et al*., 2006). The second most common gene, $lpfA_{O113}$, was found in 12.4% of the strains. Although this sequence has been recently shown to be largely distributed in various DEC pathotypes (Toma *et al*., 2006), as far as we know, it has not been searched for in UPEC strains. LPF was originally described in *Salmonella*, where it plays an important role in adherence to murine Peyer's Patch *in vivo* (Bäumler *et al*., 1996). It has also been detected in two nonidentical loci in EHEC O157:H7 (Torres *et al*., 2002, 2004); one or both of these loci were shown to be involved in microcolony formation (Torres *et al*., 2002), increased adherence, and the colonization and persistence of *E. coli* O157:H7 in swine and sheep (Jordan *et al*., 2004). The *pilS* gene was found in 5.3% of the UPEC strains. This gene was described in one particular EAEC strain, where it encoded a functional type IV pilus related to AA expression and biofilm formation. However, none of the strains carrying *pil*S presented AA in our study. The occurrence of this gene in other *E. coli* pathotypes has yet to be determined. Saa has been described as the first agglutinating adhesin characterized in a LEE-negative STEC strain (Paton *et al*., 2001). In a search for the presence of *saa* genes in STEC strains, Ogura *et al*. (2007) also compared their genetic content with three UPEC strains, among which one (UTI89) was *saa*+. Our data revealed that only three (two NA and one NC) of 225 UPEC strains presented this gene suggesting that it may not play an important role in the adherence of UPEC strains to the host.

In summary, this study showed that some UPEC strains do carry DEC markers, mainly those associated with the EAEC pathotype. This observation raises the possibility that at least some faecal EAEC strains might represent potential uropathogens; thus, their presence in the faeces of asymptomatic individuals could explain the controversies among the epidemiological studies that seek to analyze the diarrhoeagenic potential of EAEC (Suzart *et al*., 1999). Alternatively, UPEC strains may have acquired EAEC markers becoming a potential cause of diarrhoea. Further studies focusing in EAEC/UPEC strains and other UPEC and/or EAEC properties will be of great interest to elucidate their role in the

pathogenicity of the different *E. coli* pathotypes. Although this study reports the occurrence of adhesin-encoding genes (*eae*, *lpfA*$_{O113}$, *pil*S, and *saa*) of the DEC pathotypes in UPEC strains, whether these genes are expressed *in vivo* and play any role in human UPEC infections are questions that still remain to be addressed.

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Authors' contribution

C.M.A. and F.A.S. contributed equally to this study.

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