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Characterization of *Escherichia coli* Isolates from Hospital Inpatients or Outpatients with Urinary Tract Infection

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Uropathogenic *Escherichia coli* (UPEC) is the most common cause of community- and hospital-acquired urinary tract infections (UTIs). Isolates from uncomplicated community-acquired UTIs express a variety of virulence traits that promote the efficient colonization of the urinary tract. In contrast, nosocomial UTIs can be caused by *E. coli* strains that differ in their virulence traits from the community-acquired UTI isolates. UPEC virulence markers are used to distinguish these facultative extraintestinal pathogens, which belong to the intestinal flora of many healthy individuals, from intestinal pathogenic *E. coli* (IPEC). IPEC is a diarrheagenic pathogen with a characteristic virulence gene set that is absent in UPEC. Here, we characterized 265 isolates from patients with UTIs during inpatient or outpatient treatment at a hospital regarding their phylogenies and IPEC or UPEC virulence traits. Interestingly, 28 of these isolates (10.6%) carried typical IPEC virulence genes that are characteristic of enteroaggregative *E. coli* (EAEC), Shiga toxin-producing *E. coli* (STEC), and atypical enteropathogenic *E. coli* (aEPEC), although IPEC is not considered a uropathogen. Twenty-three isolates harbored the *astA* gene coding for the EAEC heat-stable enterotoxin 1 (EAST1), and most of them carried virulence genes that are characteristic of UPEC and/or EAEC. Our results indicate that UPEC isolates from hospital patients differ from archetypal community-acquired isolates from uncomplicated UTIs by their spectrum of virulence traits. They represent a diverse group, including EAEC, as well as other IPEC pathotypes, which in addition contain typical UPEC virulence genes. The combination of typical extraintestinal pathogenic *E. coli* (ExPEC) and IPEC virulence determinants in some isolates demonstrates the marked genome plasticity of *E. coli* and calls for a reevaluation of the strict pathotype classification of EAEC.

Escherichia coli is one of the most extensively studied Gram-negative bacteria in microbiology. This species has been associated with intestinal and extraintestinal infections in humans and many animals. Currently, six major groups of intestinal pathogenic *E. coli* (IPEC) have been recognized: enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) (1).

In addition, three types of extraintestinal pathogenic *E. coli* (ExPEC), including neonatal meningitis-causing *E. coli* (NMEC), sepsis-causing *E. coli* (SEPEC), and uropathogenic *E. coli* (UPEC), have been associated with meningitis in newborns, systemic infections, and urinary tract infections (UTIs), respectively. UPEC causes around 90% of community-acquired UTIs and up to 50% of nosocomial UTIs (2). It has been estimated that catheter-associated UTIs represent one of the most common causes of nosocomial infection and that treatment costs are as high as $400 million annually in the United States (3–5). Detailed molecular epidemiological studies assessing the population structure of UPEC isolates from nosocomial UTIs or from hospital inpatients and outpatients have not been performed to date. Most UTI cases have been reported for women, children, elderly people, and immunocompromised patients (6, 7). A combination of various risk factors represent the most plausible cause of such a phenomenon, including physiological and anatomical changes, an active sex life, age, and the close proximity of the urethra, vagina, and rectum in women (8). It is broadly accepted that the fecal flora of the host is the primary source of UPEC isolates (9, 10). Despite the fact that UPEC and ExPEC in general belong to the normal intestinal flora of many healthy individuals, where they coexist with commensal *E. coli*, they are often distinguished based on their virulence gene contents and their allocation to certain phylogenetic lineages (11, 12). However, in hospital settings, many patients are immunocompromised, have indwelling urinary catheters, and are exposed to a plethora of antimicrobial compounds that might promote UTIs caused by *E. coli* strains that are not considered typical uropathogens. In this context, we hypothesize that nosocomial UTIs result not only from infections by typical UPEC but can be also caused by various *E. coli* strains with an unusual virulence gene repertoire. Furthermore, a marked genome plasticity promotes the spread and exchange of various virulence gene sets among the *E. coli* population. If this occurs, intermediate *E. coli* variants can arise that cannot be unambiguously discriminated from particular IPEC isolates or from commensal *E. coli* isolates based on their virulence gene pool, as they display a mosaic of different virulence-associated genes (13).

The goal of this study was to describe the molecular and phe-

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notypic characteristics of the UPEC isolates collected from patients suffering from UTIs during inpatient or outpatient treatment at two hospitals. These isolates were screened by multiplex PCR (MPCR) in order to detect archetypal virulence determinants of the ExPEC and IPEC pathotypes. Additionally, the isolates were subjected to molecular epidemiological analysis using multilocus sequence typing (MLST). Selected strains were phenotypically compared with regard to biofilm formation, motility, adhesion to eukaryotic cells, and expression of virulence factors (VFs).

MATERIALS AND METHODS

Bacterial isolates. A total of 265 *E. coli* isolates were collected from the urine samples of patients with UTIs during inpatient and outpatient treatment at the University Hospital in Würzburg, Germany (hospital A), during the periods of July to August 2005 and September to December 2006, and at the Department of Urology, Hospital St. Elisabeth, Straubing, Germany (hospital B), during 2004 to 2005. We considered patients who stayed for the clinical treatment in a given ward of the hospital to be inpatients, and patients who were not hospitalized but who visited an acute day ward or polyclinic were considered to be outpatients. One hundred forty-six patients treated at hospital A were females (age range, 1 to 90 years; median, 59.5 years) and 73 patients were males (age range, 1 to 89 years; median, 68 years). Twenty-one patients treated at hospital B were female (age range, 36 to 89 years; median, 72 years) and 25 patients were male (age range, 23 to 89 years; median, 72 years). The isolates originated from patients with significant *E. coli* bacteriuria (defined as $\geq 10^5$ CFU ml^{-1} in clean-voided urine). One hundred six isolates (40%) were associated with catheter use, with 58 strains (22%) isolated from patients with long-term (>48 h) catheterization. None of the patients from whom atypical UPEC isolates with characteristics of IPEC were isolated had been admitted to the hospital because of diarrhea.

PCR assays. Bacterial DNA was isolated using the InstaGene matrix kit (Bio-Rad, Munich, Germany) according to the manufacturer's recommendations; 2 μl of the DNA was used as a template for PCR analyses.

We performed multiplex PCR (MPCR) assays for the detection of IPEC and ExPEC virulence-associated genes, as previously described by Müller et al. (13) and Johnson et al. (14), respectively. MPCR of IPEC-specific genes included the genes *escV* for the detection of locus of enterocyte effacement (LEE)-positive strains (typical EPEC, atypical EPEC, and STEC), *bfpB* for typical EPEC, stx_1 and stx_2 for STEC, *elt*, *estIa*, and *estIb* for ETEC, *invE* for EIEC, and *astA*, *aggR*, pCVD432, and *pic* for EAEC. The isolates that were positive for one or more IPEC marker genes were further screened by MPCR for the detection of ExPEC virulence determinants, such as the fimbrial adhesin-coding genes, including *papA*, *papC*, *papEF*, *papG* alleles I, II, and III, *sfa-focDE*, *sfaS*, *focG*, *gafD*, *bmaE*, and *fimH*. Furthermore, the toxin-coding genes *hlyA*, *cnf1*, *cdtB*, *vat*, and *sat*, the group II and group III capsule determinants (*kpsMT* II and *kpsMT* III), as well as K1 and K5 capsule genes (*kpsMT* K1 and *kpsMT* K5), were included in the screening. We also detected genes that are representative of important siderophore systems, such as *iutA*, *fyuA*, *iroN*, and *chuA*. In addition, the miscellaneous ExPEC virulence genes *cvaC*, *ibeA*, and *traT* were included in our virulence marker survey. The EAEC isolates were further tested for the presence of aggregative adherence fimbriae I (AAF/I) (*aggA* and *aggC*), AAF/II (*aafA*), AAF/III (*agg3A*), Hda (*hdaA*), AAF/V (*aaf5A*), dispersin (*aap*), and plasmid-encoded toxin (*pet*). The primers used for the detection of AAF/I, AAF/II, AAF/III, and Hda have been described (15–18). Since the nucleotide sequence of the AAF/V fimbria-coding gene cluster is already publicly available (GenBank accession no. AB571097), we designed the primers aafVA-f (5′-TTGCGAGTCTGGTA TTCAGC-3′) and aafVA-r (5′-CGGTATATTGTCCGGGTTTG-3′) for its detection. A PCR yielded a fragment of 420 bp.

LEE pathogenicity island-harboring isolates were investigated for the presence of *eae* (outer membrane adhesin intimin) and the translocated intimin receptor-encoding gene *tir*, as well as for the effector protein-encoding genes *espF*, *espG*, and *map*. The intimin alleles were typed as described in reference 19.

All PCRs were carried out with the REDTaq ReadyMix PCR mix (Sigma-Aldrich, Germany) in a total volume of 25 μl containing 2 μl of template DNA. The PCR products were visualized in 2% agarose gel in Tris-acetate-EDTA buffer by staining with RedSafe nucleic acid staining solution. The MPCRs were validated by using a subset of reference strains as described previously (13–18).

Multilocus sequence typing. The 265 urine isolates from hospital patients were allocated to different clonal lineages according to Wirth et al. (20). New sequence types (STs) were submitted to the MLST database (see http://mlst.ucc.ie/mlst/dbs/Ecoli). The phylogenetic relationships between distinct sequence types were determined based on a recently improved phylotyping PCR approach (21). The minimum spanning tree based on the allelic numbers of the MLST loci was calculated using SeqSphere software version 0.9.38 β (Ridom GmbH).

Serotyping. All *astA*- and/or *aggR*-positive strains were serotyped at the Robert Koch Institute, Wernigerode, Germany, using a microtiter method and antisera to *E. coli* O antigens 1 to 187 and H antigens 1 to 56, as described previously (22).

Phenotypic assays. The ability of bacterial strains to produce colicins and aerobactin was evaluated according to Zdziarski et al. (23). The hemolytic phenotype was determined on Columbia blood agar (Heipha, Heidelberg, Germany).

Adherence assays. Adherence patterns to both human laryngeal carcinoma (HEp-2) and human bladder carcinoma (T24) cell lines were assayed as described previously by Bielaszewska et al. (24), with slight modifications. Briefly, HEp-2 cells and T24 cells were grown as semiconfluent monolayers in Eagle's minimum essential medium (EMEM) and McCoy's 5A modified medium, respectively. Both media were supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% nonessential amino acids. The bacteria were incubated on the cultured cell lines for 3 h at 37°C and 5% CO_2 in the presence of 0.5% D-mannose. Afterwards, the cells were washed three times with phosphate-buffered saline (PBS), fixed with methanol for 1 min, and stained for 30 min with Giemsa stain freshly diluted 1:10 in water. The adherence patterns were evaluated under oil immersion with a phase contrast microscope (Axio Imager A1; Zeiss, Jena, Germany). The reference strains EAEC 042, EPEC 2348/69, and K-12 C600 (references 81, 82, and 83, respectively) were included in this assay to validate our results. When necessary, eukaryotic cells prefixed with 2% glutaraldehyde were used to carry out this assay with bacterial isolates that displayed strong hemolytic activities.

ESBL expression. Bacterial growth was tested on extended-spectrum beta-lactamase (ESBL) agar plates (chromID ESBL; bioMérieux, Nürtingen, Germany).

Motility assays. Swarming motility assays were performed on lysogeny broth (LB) agar plates containing 0.3% agar to evaluate the motility of unconventional UPEC strains. The plates were inoculated with a colony of *E. coli*, and the swarming distance was measured (in mm) after 12 h of incubation at 37°C.

Bacterial growth in LB or pooled human urine samples. The bacterial growth rates were determined using an optical density measurement of selected typical UPEC isolates and asymptomatic colonizers of the bladder (UPEC strains 536 and CFT073 and ABU strain 83972) grown in both LB and sterile pooled human urine samples, as previously described by Alteri and Mobley (25), with slight modifications. Briefly, the bacteria were grown statically at 37°C, and the optical density at 600 nm was measured every 20 min.

Quantitative biofilm test. Biofilm formation was analyzed in a microtiter plate assay upon bacterial growth at 37°C for 48 h in artificial urine medium (AUM) (26) or M9 minimal medium (27), as described before (28). An interpretation of the results and grouping into no or weak biofilm producers, moderate biofilm producers, or strong biofilm producers was done as previously published (29).

RESULTS

Phylogenetic characterization of *E. coli* isolates recovered from hospital inpatients and outpatients with UTIs. IPEC strains are generally represented in the phylogenetic groups B1, E, and A, while ExPEC strains are principally distributed in groups B2 and D (11, 21, 30–32). In order to better characterize the 265 *E. coli* UTI isolates from our hospital patients, we evaluated their phylogenetic relationships by MLST and allocated the isolates to the main *E. coli* phylogenetic lineages as well as to sequence types (STs) and clonal complexes (CCs) (Fig. 1). Interestingly, 55.8% of our isolates belonged to phylogroups B2 and D, 31.7% of the urine isolates were grouped into lineages A, B1, and E, and 6.8% and 4.1% of the isolates represented lineages C and F, respectively. One isolate was allocated to clade V (Table 1 and Fig. 1). The two collections of isolates from hospitals A and B markedly differed in their fractions of phylogroup A, B2, and F strains. Whereas only one isolate (2.2%) from hospital B belonged to phylogenetic group A and 67.4% and 0% of the isolates were allocated to lineages B2 and F, respectively, the strain collection from hospital A included 19.2% of the phylogroup A strains. The lineage B2 and F strains represented 45.7% and 5%, respectively, of the collection from hospital A. The phylogroup B2 strains were less frequently found but were still predominant in this collection (Fig. 1 and Table 1).

We identified 89 different STs by MLST. The most prevalent STs were ST73, ST10, ST131, ST141 and ST88 (Table 2 and Fig. 1). One hundred seventy-one of the 265 isolates (64.5%) were allocated to characteristic ExPEC STs and CCs, such as CC73, CC95, and CC14 (Fig. 1), which were defined based on their composition according to the *E. coli* MLST database (http://mlst.ucc.ie/mlst/dbs/Ecoli). Interestingly, a large proportion of the urine isolates ($n = 94$ [35.5%]) were grouped into typical STs and CCs of IPEC and commensal *E. coli*, including CC10 and CC155 (Fig. 1 and Table 2). Thirteen isolates belonged to ST131, which includes a recently and globally emerging multidrug-resistant clone causing UTIs and septicemia in hospitals and the community. ST88 is correlated with extended-spectrum beta-lactamase (ESBL) production in nosocomial isolates (33, 34). Three (23%) of the ST131 isolates and two (18%) of the ST88 strains were phenotypically ESBL positive. Although the two strain collections from the hospitals differ in their overall ST compositions, the major clones determined in this study include isolates from both collections, and except for phylogroup F and clade V, all the other phylogenetic lineages are represented by *E. coli* strains from both collections. This indicates that our general findings regarding the population structure of UTI isolates from hospital inpatients and outpatients are independent of the sampling site.

Our molecular epidemiological analyses thus demonstrated that UTIs in hospital inpatients and outpatients can be caused by a heterogenous group of *E. coli* strains that belong to several major *E. coli* phylogenetic groups and that the ability to colonize the urinary tract and cause UTIs does not require a typical ExPEC genetic background.

Detection of IPEC virulence determinants in *E. coli* isolates from hospital inpatients and outpatients with UTIs. The high prevalence of characteristic clonal lineages of IPEC among the UTI isolates from hospitals led us to screen these isolates for typical IPEC virulence markers using a multiplex PCR. Interestingly, 28 of the 265 isolates (10.6%) harbored at least one IPEC virulence gene (Table 3). Twenty-five of the 28 aUPEC strains (89.3%) (all but aUPEC strains UR5779/201, UR0589/201, and UR05521/201) were isolated from inpatients, thus most likely representing nosocomial UPEC isolates, and 10 of them are associated with catheter use (Table 3). Of the 28 aUPEC strains, three isolates (10.7%) were classified as STEC because they contained genes coding for Shiga toxin and enterohemorrhagic *E. coli* (EHEC) hemolysin. Two of these strains harbored the locus of enterocyte effacement (LEE) pathogenicity island, as evidenced by the presence of the *escV*, *eae*, *tir*, *espF*, *espG*, and *map* genes. The third STEC isolate carried the STEC autoagglutinating adhesin (*saa*) gene, which often occurs in STEC strains that lack the LEE pathogenicity island (35) (Table 3).

We also found one EPEC isolate (positive for *escV*, ι-*eae*, *tir*-EPEC, *espF*, *espG*, and *map*) that did not contain the bundle-forming pilus (*bfp*) determinant and was thus classified as an atypical EPEC (aEPEC). Additionally, one UTI isolate was categorized as EAEC due to the presence of pCVD432 sequences, as well as the *aaf*5*A*, *aggR*, and *aap* genes encoding the major fimbrial subunit of aggregative adherence fimbria V, the transcriptional activator AggR, and dispersin Aap, respectively, which are usually present in EAEC. However, this isolate did not carry the *astA* gene, which codes for the EAEC heat-stable toxin 1 (EAST1) (36). On the other hand, 23 other UTI isolates contained *astA* but were pCVD432 negative and did not carry the *aggR* and *aap* genes. None of these 23 *astA*-positive isolates carried *sepA*, which encodes a serine protease autotransporter protein (SPATE) frequently found in *Shigella* spp. and EAEC strains (37).

Ten (35.7%) of the 28 atypical UPEC isolates belonged to phylogroup B2 and six (21.4%) belonged to group A. Groups E and F were each represented by three isolates (10.7% each), and lineages B1 and C were each represented by two strains (7.1% each). One isolate each belonged to phylogenetic lineage D and to clade V (3.6% each) (Table 3). Of the 28 strains, five (17.9%) belonged to ST141, three (10.7%) belonged to ST10, two (7.1%) belonged to ST354, and two (7.1%) belonged to ST617. The remaining 16 isolates belonged to different STs, i.e., ST32, ST88, ST95, ST117, ST133, ST330, ST429, ST675, ST783, ST997, ST1643, ST1858, ST2016, ST2017, ST2018, and ST2019. All these isolates were further analyzed for the presence of ExPEC virulence factors (Table 3).

Detection of ExPEC virulence determinants in atypical UPEC isolates. Some of the most relevant fimbrial adhesins expressed in ExPEC belong to the chaperone-usher pathway subclass and include type 1, P, and S/F1C fimbriae (38). Thus, we investigated the presence of those fimbrial operons and of 29 additional ExPEC virulence-associated determinants in the 28 aUPEC isolates harboring IPEC virulence genes (Table 3).

Type 1, P, and S/F1C fimbrial genes were found in 25 (89.3%), eight (28.6%), and seven (25%) of the aUPEC isolates, respectively. The toxin-coding genes *vat*, *hlyA*, *cnf1*, *cdtB*, and *vat* were also found in 10 (35.7%), eight (28.6%), seven (25%), three (10.7%), and one (3.6%) of those strains, respectively.

The most frequently found siderophore system-associated genes *chuA*, *fyuA*, *iroN*, and *iutA* were found in 20 (71.4%), 17 (60.7%), 13 (46.5%), and 12 (42.9%) of the 28 aUPEC strains, respectively.

Nineteen (67.9%) of the 28 strains tested positive for *traT* (a gene associated with survival in blood serum). Eleven strains (39.3%) also tested positive for group II capsule (*kpsMT* KII).

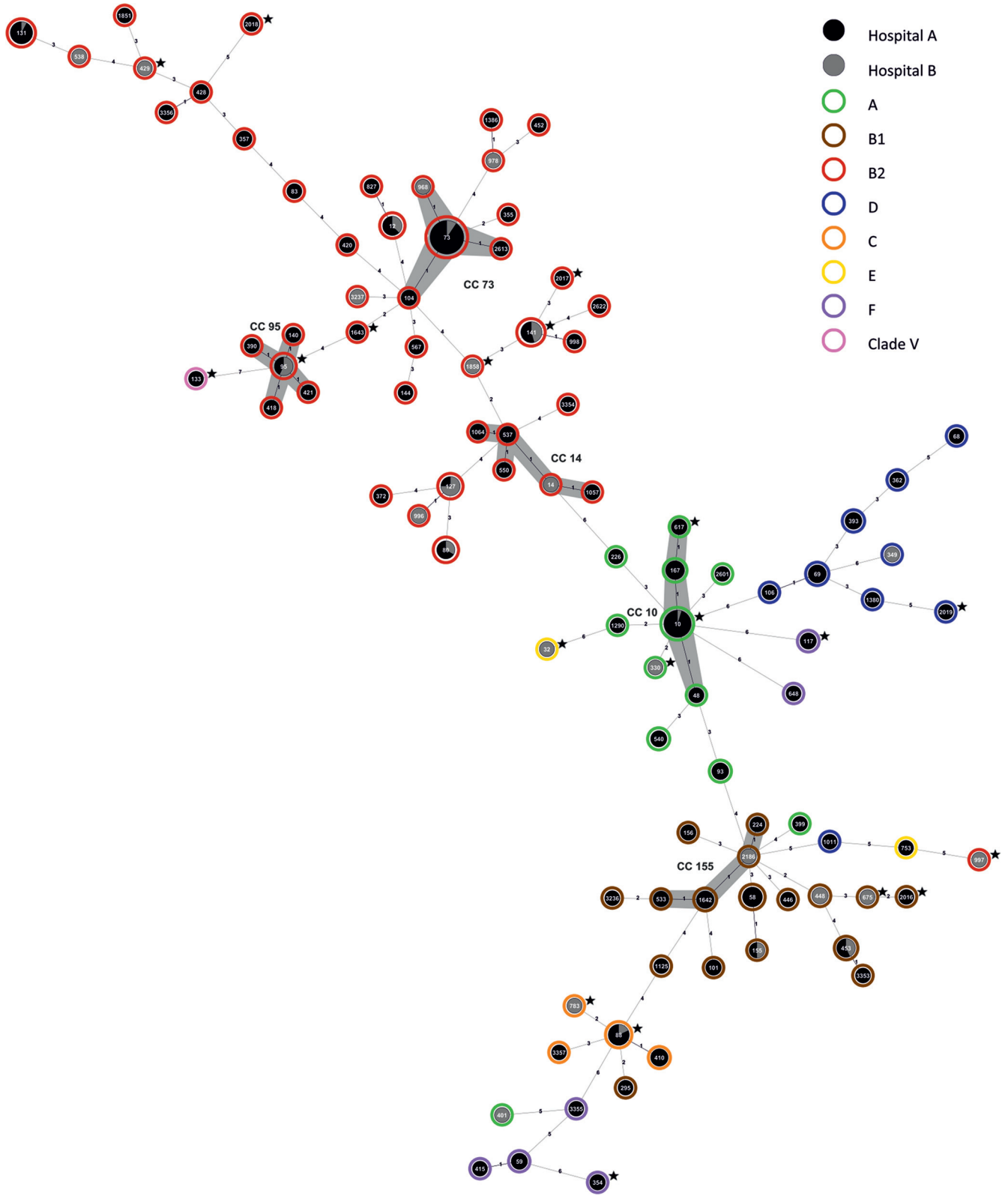

FIG 1 Phylogenetic background of *E. coli* isolates from hospital inpatients and outpatients with UTIs. The molecular phylogeny of the urine isolates from hospital A (black) or from hospital B (gray) as indicated by the pie charts is based on the MLST allelic profiles. The minimum spanning tree illustrating the clonal relationship of the isolates has been generated with SeqSphere (Ridom GmbH). The size of each pie chart mirrors the number of strains allocated to an individual ST. Phylogenetic groups (A, B1, B2, C, D, E, F, and clade V) as determined by an improved quadruplex phylotyping PCR approach (21) are indicated by the outermost colored rings on the tree. Sequence types marked with an asterisk comprise isolates with intestinal pathogenic *E. coli* (IPEC) virulence markers.

TABLE 1 Allocation of UPEC isolates from hospital inpatients and outpatients with suspected urinary tract infection to main phylogenetic lineages

Phylogroup	Total ($n = 265$)		Hospital A ($n = 219$)		Hospital B ($n = 46$)	
	No. of isolates	%	No. of isolates	%	No. of isolates	%
A	43	16.2	42	19.2	1	2.2
B1	41	15.5	32	14.6	9	19.6
B2	131	49.4	100	45.7	31	67.4
C	18	6.8	15	6.8	3	6.5
D	17	6.4	16	7.3	1	2.2
E	3	1.1	2	0.9	1	2.2
F	11	4.1	11	5.0	0	0
Clade V	1	0.4	1	0.5	0	0

Further analysis revealed that five of the group II capsule-positive strains harbored the K5 capsule, and the other six group II capsule-positive strains carried the K1 capsule determinant. Additionally, three strains (10.7%) gave positive results for *cvaC* (microcin ColV), but none of the 28 strains carried the *rfc* gene (for O4 lipopolysaccharide synthesis) or the group III capsular gene cluster.

Altogether, our analysis demonstrates that UTI isolates from hospital inpatients and outpatients are a heterogeneous group of isolates that differ in their virulence gene repertoires, serotypes, phylogenetic backgrounds, and virulence- or fitness-associated traits. About 10% of these urine isolates represent IPEC pathotypes or at least combine typical ExPEC and IPEC marker genes and thus differ from typical UPEC isolates.

Further characterization of atypical UPEC strains: analysis of aggregative adherence patterns. Twenty-three isolates carried the *astA* gene that is characteristic of EAEC. However, because this locus can also occur in other IPEC and even in ExPEC strains (13, 39, 40) and because the bacterial adhesion phenotype of eukaryotic cells still remains the gold standard for the identification of EAEC, we screened all *astA*-positive isolates for their ability to adhere to HEp-2 cells.

Of the 23 *astA*-positive strains, four (17.4%) exhibited the aggregative adherence pattern that is typical of EAEC strains, despite lacking the pAA plasmid (Fig. 2C to F and Table 3). One strain showed a diffuse adherence phenotype, whereas the remaining 19 strains exhibited only weak adherence to HEp-2 cells (Table 3).

Curiously, EAEC strain 1352 (negative for *astA* and positive for *aggR*, *aap*, and *aaf*5A) did not show the "stacked-brick" adherence pattern on HEp-2 cells that is considered to be characteristic of EAEC (Fig. 2G and Table 3). Of the three STEC strains we investigated, one exhibited a typical localized adherence pattern on HEp-2 cells, whereas the other two strains adhered in an undefined pattern. The aEPEC isolate did not show the typical localized adherence pattern (Table 3).

As these strains were recovered from patients suffering from UTIs, we also evaluated their adherence patterns on T24 bladder epithelial cells. The bacterial aggregative adherence (AA) and the diffuse adherence (DA) phenotypes observed with HEp-2 cells were confirmed with T24 cells. Interestingly, the AAF/V fimbria-positive isolate 1352 showed an aggregative pattern only on T24 cells but not on HEp-2 cells (Fig. 3G and Table 3).

TABLE 2 Allocation of UPEC isolates from hospital inpatients and outpatients with urinary tract infection to characteristic sequence types of ExPEC or IPEC

Characteristic STs of ExPEC and/or commensal *E. coli*[a]		Characteristic STs of IPEC and/or commensal *E. coli*[a]	
ST	No. of isolates	ST	No. of isolates
73	32	10	21
131	13	58	9
88	11	167	7
141	11	453	7
12	8	410	5
127	8	448	3
95	7	540	3
69	6	101	2
80	6	155	2
1642	5	156	2
93	4	1643	2
393	4	32	1
59	3	48	1
117	3	68	1
537	3	83	1
104	2	106	1
354	2	133	1
357	2	224	1
420	2	226	1
428	2	295	1
533	2	349	1
617	2	362	1
14	1	399	1
140	1	401	1
144	1	429	1
355	1	452	1
372	1	550	1
390	1	675	1
415	1	753	1
418	1	783	1
421	1	1011	1
446	1	1064	1
538	1	1064	1
567	1	1125	1
648	1	1380	1
827	1	1851	1
968	1	1858	1
978	1	2018	1
996	1	2186	1
997	1	2601	1
998	1	2613	1
1057	1	2622	1
1290	1		
1386	1		
2016	1		
2017	1		
2019	1		
3236	1		
3237	1		
3353	1		
3354	1		
3355	1		
3356	1		
3357	1		

[a] ST, sequence type. Characteristic STs of ExPEC or IPEC have been defined based on the prevalence of corresponding isolates according to the *E. coli* MLST database (http://mlst.ucc.ie/mlst/dbs/Ecoli).

TABLE 3 Characteristics of aUPEC isolates from hospital in- and outpatients

Isolate	Phylogroup	Serotype	Diagnosis/presence of catheter[a]	ST/CC	IPEC marker(s)	ExPEC marker(s)	Phenotype				
							Colicin	Aerobactin	Hemolysis	Adherence to[b]: HEp-2 cells	Adherence to[b]: T24 cells
1352	A	O15:H-	Cystitis	ST10, CC10	*pic*, *aggR*, *aap*, pCVD432, *aaf5*	*fyuA*, *iutA*, *kpsMT* K5	−	−	−	NA	AA
UR5703/202	E	O145:H-	Cystitis	ST32, CC32	*escV*, *eae*, Tir EHEC, *espF*, *espG*, *espP*, *map*, *cif*, stx_2, EHEC-*hly*		−	−	−	NA	LA
131	B1	O76:H19	Hemorrhagic cystitis, catheter <48 h	ST675	stx_1, stx_2, *saa*, EHEC-*hly*	*cdiA*, *traT*	+	−	−	NA	NA
UR3457	B2	O78:H-	Catheter >48 h	ST2018	*escV*, *eae*, Tir EPEC, *espF*, *espG*, *map*	*chuA*, *ibeA*, *traT*	−	+	−	NA	NA
UR5779/201	A	Ont:H-	Cystitis	ST330, CC10	*escV*, *eae*, stx_2, *espF*, *espP*, *map*, EHEC-*hly*, *estI*	*chuA*, *fyuA*, *traT*	−	−	−	LA	NA
UR05826/201	A	O8:H-	Cystitis	ST10, CC10	*astA*	*fyuA*, *iutA*, *cdiA*, *gafD*, *traT*	−	+	−	AA	AA
UR05791/202	A	Ont:H-	Catheter >48 h	ST617, CC10	*astA*	*iutA*, *malX*, *traT*	−	+	−	NA	NA
UR05890/201	A	Ont:H-	UTI	ST617, CC10	*astA*	*iutA*, *malX*, *traT*	−	+	−	NA	NA
UR766	A	O40:H-	Cystitis	ST10, CC10	*astA*	*iroN*, *iutA*, *cvaC*, *traT*	+	+	−	NA	NA
UR3930	B1	Ont:H16	Cystitis	ST2016	*astA*	*cdiA*, *iutA*, *traT*	−	−	−	NA	NA
5269	B2	O6:H1	Ureteral stones, catheter >48 h	ST1858	*astA*	*pap*, *sfa-foc*, *chuA*, *fyuA*, *iroN*, *cdiA*, *vat*, *cnf1*, *hlyA*, *malX*, *ibeA*, *kspMT* K5	−	−	+	DA	NA
UR923	B2	Ont:H14	Cystitis	ST2017	*pic*, *astA*	*chuA*, *fyuA*, *cdiA*, *vat*, *malX*, *ibeA*	−	−	−	NA	NA
4403	B2	O2:H6	Ureteral calculi, catheter <48 h	ST141	*astA*	*pap*, *sfa-foc*, *chuA*, *fyuA*, *iroN*, *cdiA*, *vat*, *cnf1*, *hlyA*, *malX*, *kpsMT* K1	−	−	+	NA	NA
UR04550/201	B2	Orough:H-	UTI, catheter >48 h	ST141	*astA*	*pap*, *sfa-foc*, *chuA*, *iroN*, *fyuA*, *cdiA*, *vat*, *cnf1*, *hlyA*, *malX*, *traT*, *kpsMT* K1	−	−	+	NA	NA
UR04550/202	B2	Orough:H6	UTI, catheter >48 h	ST141	*astA*	*pap*, *sfa-foc*, *chuA*, *iroN*, *fyuA*, *cdiA*, *vat*, *cnf1*, *hlyA*, *malX*, *traT*, *kpsMT* K1	+	−	+	NA	NA
UR5889/201	B2	O2:H6	UTI	ST141	*astA*	*pap*, *sfa-foc*, *chuA*, *iroN*, *fyuA*, *cdiA*, *vat*, *cnf1*, *hlyA*, *malX*, *kpsMT* K1	−	−	+	NA	NA
UR1232	B2	O18:H-	Cystitis	ST95, CC95	*astA*	*sfa*, *chuA*, *fyuA*, *iroN*, *iutA*, *cdiA*, *vat*, *cdtB*, *ibeA*, *malX*, *cvaC*, *traT*, *kpsMT* K1	+	−	−	NA	NA
4402	B2	O83:H4	Ischuria, catheter <48 h	ST429	*astA*	*chuA*, *fyuA*, *iroN*, *cdtB*, *vat*, PAI, *ibeA*, *traT*, *kpsMT* K1	+	−	−	NA	NA
UR1318	B2	Ont:H45	Cystitis	ST1643	*astA*	*chuA*, *fyuA*, *iroN*, *vat*, *malX*, *traT*	−	−	−	NA	NA
2383	B2	Ont:H-	Bladder carcinoma	ST141	*astA*	*chuA*, *iroN*, *cdiA*, *traT*	−	−	−	AA	AA
5226	C	Ont:Hnt	Carcinoma of the renal pelvis, catheter >48 h	ST783	*astA*	*pap*, *cnf1*, *hlyA*, *fyuA*, *traT*	−	+	+	AA	AA
BK3196	C	Ont:H-	UTI	ST88, CC23	*astA*	*pap*, *sfa*, *fyuA*, *iroN*, *iutA*, *cvaC*, *traT*	+	−	−	NA	NA
UR1811	D	O92:H23	Cystitis	ST2019	*astA*	*chuA*, *cdtB*	−	−	−	NA	NA
315	E	Ont:H-	Bacteriuria	ST997	*astA*	*chuA*, *iroN*, *cdiA*, *traT*	−	−	−	NA	NA
764/4174	F	Orough:H-	Bacteruria	ST354, CC354	*astA*	*pap*, *sfa-foc*, *chuA*, *fyuA*, *iutA*, *cnf1*, *hlyA*, *ibeA*, *kpsMT* K5	−	−	−	DA	DA
UR3962	F	Ont:H4	Cystitis	ST117	*astA*	*chuA*, *fyuA*, *iroN*, *vat*, *traT*	−	−	−	NA	NA
UR05521/201	F	O153:H34	UTI	ST354, CC354	*astA*	*chuA*, *ibeA*, *kpsMT* K5	−	+	−	NA	NA
UR3427	Clade V	Ont:H-	UTI, catheter <48 h	ST133	*astA*	*chuA*	−	−	−	AA	AA

[a] UTI, urinary tract infection.

[b] NA, no adherence; LA, localized adherence; AA, aggregative adherence; DA, diffuse adherence.

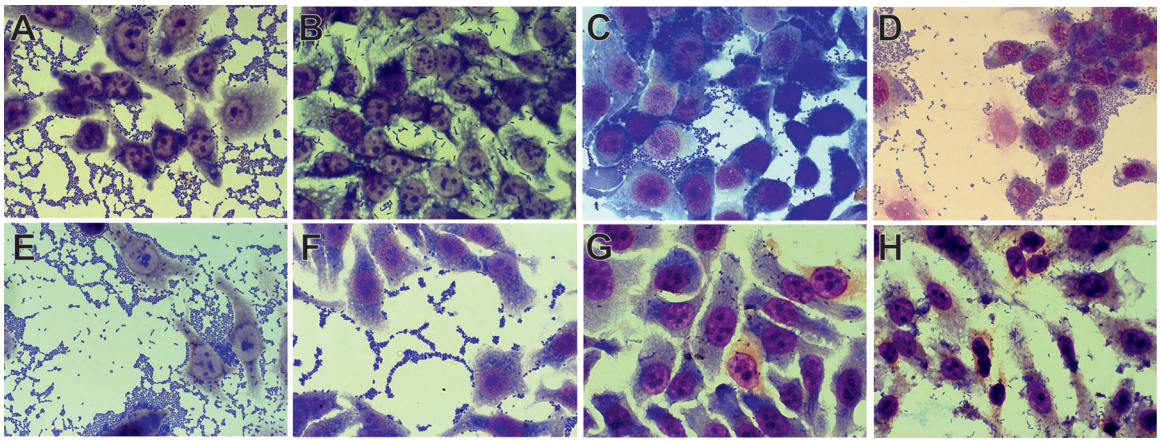

FIG 2 Adherence pattern of *astA*-positive atypical UPEC isolates from hospital inpatients and outpatients identified on HEp-2 cells. Shown are EAEC strain 042 (positive control) (A), *E. coli* K-12 strain C600 (negative control) (B), aUPEC 2383 (Ont:H-, ST141) (C), aUPEC 5226 (Ont:Hnt, ST782) (D), aUPEC UR3427 (Ont:H-, ST133) (E), aUPEC UR05826/201 (O8:H-, ST10) (F), aUPEC 1352 (O15:H-, ST10) (G), and aUPEC UR5889/201 (O2:H6, ST141) (H).

Aggregative adherence pattern in *astA*-harboring isolates was not associated with the presence of known AAF fimbrial determinants. Due to the fact that adherence to HEp-2 cells in an AA pattern has been attributed to EAEC and the expression of AAF fimbriae (15, 16, 41, 42), we investigated the presence of AAF(-like) fimbrial determinants (AAF/I, AAF/II, AAF/III, Hda, and AAF/V fimbriae) in all 24 isolates with EAEC virulence markers, in particular those harboring the *astA* and/or the *aggR* genes (Table 3). Altogether, six aUPEC isolates exhibited an AA pattern on HEp-2 and/or T24 cells (Fig. 2 and 3 and Table 3). Unexpectedly, in the four *astA*-positive strains with an AA phenotype on HEp-2 and T24 cells (aUPEC isolates UR05826/201, 5226, UR3427, and 2383), none of the known *aaf* determinants were detected by PCR. In contrast, the aUPEC isolate 1352 with an AA phenotype that is restricted to T24 cells tested positive for the recently described AAF/V fimbrial gene cluster. This strain tested positive for pCVD432 and the *aggR* and *aap* genes.

Growth of atypical UPEC isolates in pooled human urine samples. To compare the growth abilities of atypical UPEC isolates and of typical symptomatic or asymptomatic bladder-colonizing *E. coli* isolates (536, CFT073, UTI89, and 83972), we measured bacterial growth in LB and sterile-filtered pooled human urine samples, respectively, at 37°C (Fig. 4). No significant differences in the growth rates of selected aUPEC 4403 (positive for *astA* and negative for *aggR*), 1352 (positive for *astA*, *pic*, *aggR*, and pCVD432), UR05889/201 (positive for *astA* and *hlyA*), 131 (STEC), UR5779/201 (STEC), and UR3427 (aEPEC) isolates and of typical UPEC isolates, as well as of the asymptomatic bacteriuria isolate 83972, were observed. This result indicates that aUPEC isolates can grow as efficiently as archetypal UPEC isolates in pooled human urine samples.

Biofilm formation of aUPEC strains. Biofilm formation may promote urinary tract infection, especially in the case of catheter-associated UTIs. To assess whether the ability to form a biofilm may support UTIs by the aUPEC isolates, we screened all 28 aUPEC isolates for their ability to form biofilms in AUM and M9 minimal medium (Fig. 5). Generally, biofilm formation was higher in AUM than in M9 medium. Based on the detected biofilm formation, 18 (64.3%) isolates were classified as at least moderate biofilm producers in M9, and 10 isolates (35.7%) were classified as

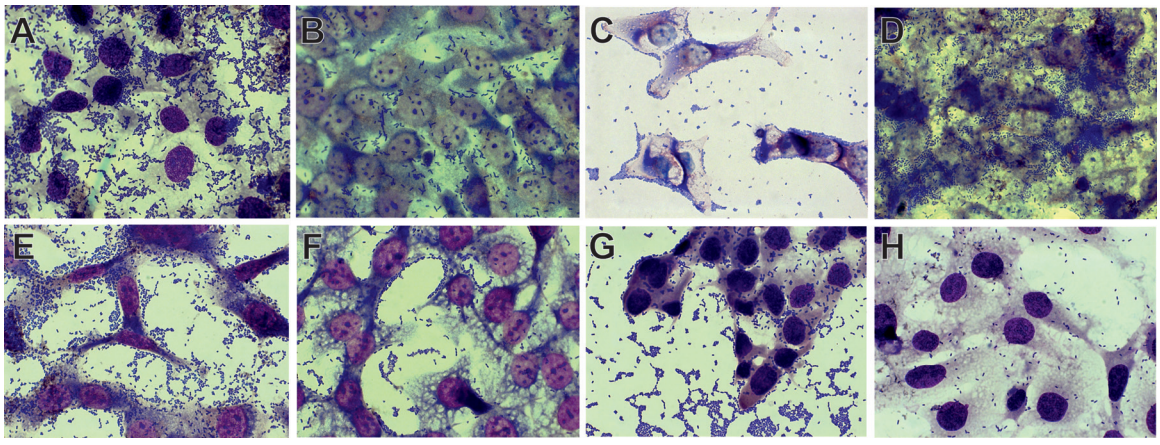

FIG 3 Adherence pattern of *astA*-positive atypical UPEC isolates from hospital inpatients and outpatients on T24 bladder epithelial cells. Shown are EAEC strain 042 (positive control) (A), *E. coli* K-12 strain C600 (negative control) (B), aUPEC 2383 (Ont:H-, ST141) (C), aUPEC 5226 (Ont:Hnt, ST782) (D), aUPEC UR3427 (Ont:H-, ST133) (E), aUPEC UR05826/201 (O8:H-, ST10) (F), aUPEC 1352 (O15:H-, ST10) (G), and aUPEC UR5889/201 (O2:H6, ST141) (H).

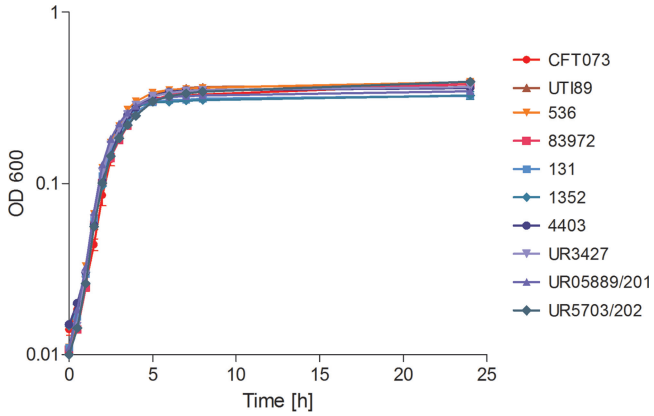

FIG 4 Growth characteristics of atypical UPEC isolates in pooled human urine samples. Error bars represent the standard deviations of the results from at least three independent experiments. The growth curves of typical UPEC and asymptomatic bacteriuria *E. coli* isolates at 37°C are indicated in red and orange, and growth curves of atypical UPEC isolates are colored in blue and violet.

weak or non-biofilm producers. Five *astA*-positive isolates produced marked biofilms in M9 medium, whereas all three STEC isolates failed to form biofilms. Only three of the five *astA*-positive biofilm producers (in M9 medium) formed moderate biofilms in AUM.

Strains with moderate and strong biofilm production in M9 medium were more frequently associated with the presence of determinants coding for type 1, P and S/F1C fimbriae. All of the 18 moderate and strong biofilm producers strains tested positive for type 1 fimbriae, eight (44.4%) tested positive for P fimbriae, six (33.3%) tested positive for F1C fimbriae, five (27.7%) tested positive for S fimbriae, and one (5.5%) tested positive for G fimbrial adhesins; of the 11 non- or weak biofilm-producing bacteria, only seven (66.6%) strains were positive for type 1 fimbriae, and only one (9.1%) strain was positive for P and S/F1C fimbriae. None of the 29 strains was positive for either afimbrial adhesins or M-specific adhesin.

DISCUSSION

UTIs in hospital patients can be caused by a broad variety of *E. coli* variants with typical ExPEC or IPEC phylogenetic backgrounds. Our results demonstrate that *E. coli*-associated UTIs in hospital patients are caused by a diverse group of strains. Our molecular epidemiological data indicate that the patients' isolates comprised typical ExPEC lineages, e.g., ST73/CC73, ST131, and ST141. In addition, we identified many dominant clones of phylogenetic groups A, C, and B1, e.g., CC10, CC23, and CC155, which have not been typically associated with ExPEC virulence potential. Similarly, *E. coli* urine isolates from elderly patients have been shown to be epidemiologically diverse (43): 150 community- or hospital-derived *E. coli* urine isolates from the Nottingham area (United Kingdom) were allocated to 52 STs. CC131 (22% of the isolates), ST73 (11% of the isolates), and ST69 (9% of the isolates) were identified as the most highly represented clones in this collection. Interestingly, only 3% of the urine isolates belonged to ST95 (43). A very similar picture regarding the most prevalent phylogenetic lineages can be painted for the strain collection used in this study. Also, in our collection of urine isolates, only a relatively small proportion of ST95 isolates was identified. The ST88 (CC23) phylogroup C clone was recently associated with the spread of different beta-lactamases in nosocomial *E. coli* isolates (33, 34). Also, *E. coli* of the phylogenetic lineages A and C that belong to CC10 and CC23 frequently caused extraintestinal infections in humans, and together with CC155 isolates, they are often multiresistant (44–46). These results show that extraintestinal infections, especially in hospital patients, are frequently caused by *E. coli* variants with genetic backgrounds, such as phylogroups A, C, and B1, which are rather uncommon in ExPEC from community-acquired infections and which are often associated with multiple antibiotic resistances.

Certain EAEC strains have the potential to cause UTIs. Some of the urine isolates from hospital patients represent IPEC or at least they carry virulence-associated genes of diarrheagenic *E. coli*. Twenty-eight (10.9%) of the 265 urine isolates carried one or more known IPEC virulence genes, sometimes in combination with classical UPEC virulence genes. From these isolates, 23 carried the EAEC heat-stable enterotoxin 1-coding gene *astA*, and four of them clearly exhibited the typical EAEC aggregative adherence phenotype on both HEp-2 and T24 bladder epithelial cells. Although the function of this enterotoxin is not completely understood, it has been associated with diarrhea in children (47). Despite the fact that *astA* and, less frequently, the *aap* gene have been reported in *E. coli* pathotypes other than EAEC (39, 40, 48–52), their detection remains useful for the diagnosis of EAEC in-

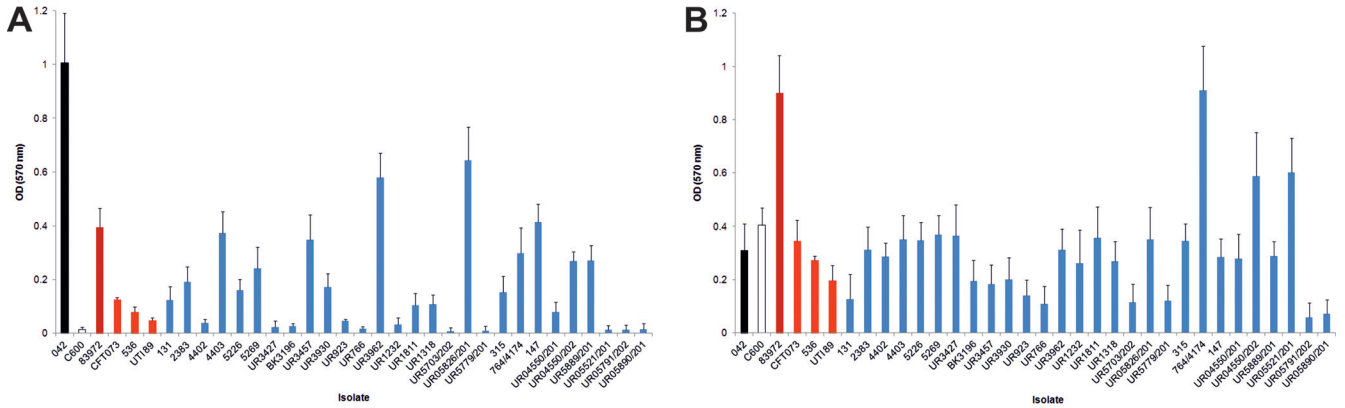

FIG 5 Biofilm formation of atypical UPEC isolates upon growth at 37°C in M9 minimal medium (A) and artificial urine medium (B). Error bars represent the standard deviations of the results from at least three independent experiments.

fection if they are screened in combination with other EAEC genes. However, the widespread presence of the *astA* gene in urine isolates investigated in our study warrants further efforts to better understand its role in UPEC pathogenesis. Contrary to *astA*, the *aggR* gene appears to be mostly restricted to some EAEC strains, and thus it has been suggested to be a reliable marker for the detection of typical EAEC (53). In this study, we detected one isolate (1352) carrying both *aggR* and *aap* genes that was also positive by PCR for the *aaf*5*A* gene and pCVD432. This gene combination was also found by Cerna and coworkers (54) in patients suffering from diarrhea, and these genes were therefore recommended as target genes for the rapid diagnosis of EAEC infection. Boll and coworkers reported that an EAEC strain of serotype O78 caused an UTI outbreak and that AAF fimbriae as specific EAEC virulence factors can promote urovirulence (55). Interestingly, isolate 1352 carried the *aaf*5 determinant (GenBank accession no. AB255435), which codes for an AAF fimbrial variant related to AAF/III, and it showed a strong AA phenotype on T24 but not on Hep-2 cells. The other four isolates tested positive for aggregative adherence on HEp-2 cells and *astA* and were negative for *aggR*, *aap*, *pet*, pCVD432, and all known AAF fimbrial determinants. The fact that the pAA-like plasmids have not been detected in these isolates suggests that *astA* might be located on another plasmid or on the chromosome as it has already been described (40, 56). The absence of known AAF fimbrial determinants supports the idea that other adhesins or so far unknown AAF fimbrial variants might be involved in the AA phenotype that we observed on both HEp-2 and T24 cells. The prevalence of EAEC marker genes in urine isolates has also been reported in Brazil (39, 57), and *E. coli* strains with a typical EAEC virulence marker content have been recently isolated from patients with prostatitis (58). Together with the recent documentation of an UTI outbreak caused by an EAEC O78:H10 strain (55, 59), this demonstrates the high heterogeneity of this pathotype and underlines that some EAEC strains have the potential to be uropathogenic. Against the background that EAEC represents a highly heterogeneous pathotype (42, 60, 61), clinically relevant EAEC subtyping should be improved, and the uropathogenicity of EAEC isolates from UTI cases requires further analysis.

Intestinal pathogenic *E. coli* causes UTIs. In addition to EAEC and EAEC-like strains, we identified three STEC isolates and one aEPEC isolate as causative agents of UTIs in hospital patients. aEPEC strain UR3457 belongs to phylogroup B2 (ST2018) and serotype O78:H-. Although this serotype is not frequently found in humans, it has also been reported in an STEC isolate recovered from a patient suffering from neonatal bacteremia, bloody diarrhea, and hemolytic-uremic syndrome (HUS) (62). The interconversion between some STEC and aEPEC isolates may arise via the loss and gain of Shiga toxin-coding phages (63, 64). Therefore, it is plausible to assume that a similar event might have occurred with aEPEC UR3457 either *in vivo* during infection or *in vitro* during laboratory storage or consecutive passages. Thus, this strain may represent either an LEE-positive STEC O78:H- isolate that lost its *stx*-harboring bacteriophage, or a typical EPEC that lost the EAF plasmid. Additionally, we found an intermediate isolate carrying the typical *estIa* gene of enteroinvasive *E. coli* (EIEC) and the marker genes that are most characteristic of STEC, such as *stx*, *eae*, and EHEC-*hly*. Although this is an unusual finding, similar isolates were described by Müller et al. (13), which reflects the genomic plasticity in *E. coli*. A recent phylogenomic analysis based on comparative whole-genome sequence analysis of 114 attaching and effacing *E. coli* (AEEC) isolates further corroborates this observation and demonstrates that the virulence gene and phylogenetic marker content in individual isolates of the same phylogenetic lineage can be inconsistent (65).

The finding that approximately 10% of the isolates from our study exhibited genotypic and phenotypic features of IPEC indicates that an improved risk assessment of UPEC from hospital patients should also consider IPEC virulence markers. Otherwise, a relatively low but considerable number of isolates will be mistyped.

Despite the fact that the frequency of UTIs caused by IPEC appears to be quite low (58, 66–69), our results indicate that *E. coli*-associated UTIs may be caused by a diverse group of *E. coli* variants, some of which represent STEC, aEPEC, and EAEC, or heteropathogenic strains that combine IPEC and ExPEC virulence traits. The ability of such organisms to colonize the host and cause infection is not only dependent on their virulence-associated traits but also on risk factors that are highly relevant to infections in hospital patients, such as immunosuppression, underlying diseases, age, and long-term indwelling catheterization (23, 70–72).

Genome plasticity promotes heteropathogenic *E. coli* that combine IPEC and ExPEC virulence traits. The majority of ExPEC and IPEC virulence-associated genes are located on mobile and genetic elements, such as plasmids, bacteriophages, and pathogenicity islands (73–75). The combination of typical ExPEC and IPEC virulence determinants in the isolates characterized in this study further supports the finding that *E. coli* exhibits a marked genome plasticity and that gene transfer may result in "intermediary strains" with characteristics of both IPEC and ExPEC. Our results also suggest that horizontal gene transfer and genomic plasticity may promote an evolutionary transition from IPEC to ExPEC, or vice versa. Some studies suggest that ExPEC virulence determinants might have evolved as a product of commensalism, because some known virulence-associated genes are also required to persist in the gut (76–78). The emergence of novel *E. coli* variants as a result of a combination of traits of already known pathotypes represents a serious problem, as was recently clearly demonstrated by the latest *E. coli* O104:H4 outbreak in Germany (24, 79, 80). The emergence of such strains demonstrates that such a heteropathogenic potential is of considerable public health concern and further underlines that a stepwise transition from a nonpathogen to a single type of pathogen is not the only route of evolution.

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