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Original article

Distribution of virulence associated traits among urine *Escherichia coli* isolates from patients in onco-hematology

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ABSTRACT

Escherchia coli is the most common etiological agent of urinary tract infections. In this study we had two goals: First of all, to find out if urine stains isolated from our patients – having the particularity of being immunocompromised – would have a virulence genes distribution different from the one observed in strains isolated from ordinary patients. Second, we wanted to identify a common virulence profile associated to these particular strains. The prevalence of virulence factors (VF)-encoding genes was analyzed by PCR. Of the tested VF-encoding genes, *malX* (80%), *ompT* (79%), *fyuA* (74%), *usp* (67%), *chuA* (66%), *iroN* (59%), *iutA* (56%), *papC* (36%), *pap AH* (30%), *papEF* (28%), *hlyA* (28%), *papG allele II* (25%), *cnf1* (21%), *focG* (20%),*cvaC* (20%) and *papG allele III* (7%) were significantly associated to urinary strains. Virulence genes distribution of urinary strains isolated from onco-hematology patients and the one observed in strains isolated from ordinary patients are almost the same. The virulence profiles containing adhesins type 1, S and F1C fimbriae, siderophore genes and three individual genes *ompT, usp* and *malX* were present in half of the urinary strains and were significantly associated to them. Two virulence signatures occurred significantly in UTI-causing strains (12%). These findings provide first insight into the virulence of UTI-causing *E. coli* strains isolated in onco-hematology patients.

1. Introduction

Escherichia coli are natural inhabitants of the human intestinal tract; however, paradoxically it remains one of the most frequent causes of nosocomial and community-acquired bacterial infections. A group of *E. coli* is capable of causing enteric disease, and a different group can cause extra-intestinal disease, including urinary tract infection (UTI) [1].

In ordinary patients, urinary infections can be characterized by various clinical symptoms or it can develop into asymptomatic carriage in recurrent cases of urinary tract infection. However, neutropenic patients may constitute a special risk group for these infections and present additional therapeutic difficulties [2,3].

Although uropathogenic *E. coli* (UPEC) strains exist within the intestinal tract of humans, they are distinct from most diarrheagenic or commensal *E. coli* strains in that UPEC isolates possess specific factors that permit their successful transition from the intestinal tract to the urinary tract. These VFs are encoded by genes located at the selected regions of chromosomal DNA, plasmids and transposons. The DNA fragments forming a complex of virulence determinants (adhesins, toxins, secretion mechanisms, capsules and iron uptake systems) are called pathogenicity islands (PAIs). PAIs are flexible genetic elements, holding the mobility sequences, which are transferred horizontally between the bacterial cells [4,5].

Numerous VF associated with UPEC have been determined, including fimbrial organelles, fimbrial polyadhesins and adhesive pili (P and type 1 pili, Dr family of adhesins, S and F1C fimbriae) [6], multiple iron acquisition systems (aerobactin, enterobactin, enterobactin-like, including IroN, and yersiniabactin) [6–9], toxins

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(cytotoxic necrotizing factor 1 and hemolysin) [6], and other miscellaneous factors. However, to date, no core set of VFs has been identified that defines all UPEC isolates.

The aim of the present paper was: First of all, to find out if urine stains isolated from our patient – having the particularity of being immunocompromised – would have a virulence genes distribution different from the one observed in strains isolated from non-immunocompromised patients. Second, we wanted to identify a common virulence profile associated to these particular strains.

2. Material and methods

2.1. Patients and bacterial isolates

The *E. coli* strains were collected from patients undergoing stem cell transplantation or chemotherapy at the National Center for Bone Marrow Transplantation in Tunisia over 5 years between the 1st of January 2007 and the 31st of December 2011. Sixty one non redundant urinary *E. coli* strains were isolated from urine samples of 49 infected patients. UTI were diagnosed when clinical symptoms such as pollakiuria, fever, nausea, dysuria, low back and pubic pain were observed, white blood cells are less than 10^4 and bacteriuria is greater than 10^5. Cytobacteriological examination of urine was interpreted according to the *Référentiel en Microbiologie médicale* (Rémic) [10] The attending physicians diagnosed a single case of urosepsis (2%) three cases of pyelonephritis (5%), eight cases of cystitis (13%) and the remaining 80% are cases of bacteriuria. We considered patients who stayed for the clinical treatment in the hematology and transplantation unit to be inpatients (30 patients), and patients who were not hospitalized but who visited a day ward were considered to be outpatients (31 patients). Not all the patients were under prophylactic treatment. There were 39 women and 10 men, their age ranged between three and 61 years and none of these patients were associated with catheter use.

Table 1
Virulence genes.

Function	Gene		Product size (pb)	Reference
Adhesins	*papAH*	(P fimbriae)	720	[6]
	papC	(P fimbriae)	200	[6]
	papEF	(P fimbriae)	336	[6]
	papG II	(P fimbriae)	190	[6]
	papG III	(P fimbriae)	258	[6]
	sfaS	S fimbriae (sialic acid-specific)	240	[6]
	focG	f1C fimbriae	360	[6]
	fimH	D-mannose-specific adhesin type 1fimbriae	508	[6]
Toxins	*hlyA*	α-hemolysin	1177	[6]
	cnf1	cytotoxic necrotizing factor 1	498	[6]
Iron uptake system	*fyuA*	yersiniabactin receptor	880	[6]
	iutA	Aerobactin receptor	300	[6]
	chuA	Heme receptor	279	[7]
	iroN	Catecholate sidérophores receptor	665	[9]
	entF	Enterobactin synthesis	511	[8]
Miscellaneous	*ompT*	Outer membrane protein T (protease)	559	[9]
	usp	Uropathogenic-specific protein (bacteriocin)	615	[9]
	malX	Pathogenicity-associated island marker (from strain CFT073)	472	[11]
	cvaC	marker for ColV plasmids	680	[6]

During the same 5-year period, 122 commensal isolates were collected from the stools of 104 uninfected patients. These controls were matched with urinary strains, as closely as possible, by the date of collection of urine samples. Two arbitrarily chosen fecal *E. coli* strains per urinary strain were studied. Strains isolated from the same patient didn't belong to the same infectious episode and didn't have the same antibiotic susceptibility profiles.

2.2. Virulence genotyping

Nineteen VFs genes (Table 1) which are related the most to UTI have been explored by using simplex and multiplex PCR-based assay.

The primers and PCR conditions used to amplify these genes were the same as described in the references cited [6–9,11]. Virulence scores were the numbers of virulence genes detected for each isolate.

*Escherichia co*li J96, CFT073 and three clinical strains harboring *sfaS*, *entF* and *cvaC* were used as positive controls. Each sample was investigated for the presence or absence of each VF by visual inspection of ethidium bromide-stained agarose gels for PCR products of the appropriate sizes.

2.3. Virulence profiling

For a better understanding of the impact of the individual genes on the virulence, each isolate was assigned a virulence signature based on the presence or absence of each gene tested.

2.4. Statistical analysis

Comparisons of proportions were tested using the chi-square test or Fisher's exact test. P values less than 0.05 were considered to indicate significance.

3. Results

3.1. Prevalence of VF genes

Among the 61 urine isolates, the prevalence of individual VF genes ranged from 2% (*sfaS*) to 97% (*entF*) (Table 2). All 19 VF encoding genes were detected in at least one isolate. Four VF genes occured in more than 75% of strains: *entF*, *fimH*, *malX* and *ompT*. Five genes occurred in more than 50% of the strains: *fyuA*, *usp*, *chuA*, *iroN* and *iutA*. Five virulence genes were present in more than 25% of the strains: *papC*, *pap AH*, *papEF*, *papG alle II* and *hlyA*. The five remaining genes were present in less than 25% of strains: *cnf1*, *focG*, *cvaC*, *papG allele III and sfaS*.

All the VF genes occurred, significantly, more frequently among UTI strains than among fecal strains except for *fimH* and *entF* which were detected in almost all the strains and *sfaS* which was scarce in both groups. All urine strains had at least one gene significantly associated with UTIs (Table 2).

The P fimbriae *papC*, *papAH*, the F1C fimbriae *focG* and the toxin genes *cnf1* and *hlyA*, were significantly associated to pyelonephritis.

Virulence scores ranged from 2 to 16 in UTI strains while they ranged from 0 to 15 among stool isolates. High virulence scores (>7) were significantly associated with UPEC ($p < 0.001$).

3.2. Profiling of VF genes

Fifty two virulence signatures were found among urinary strains, of which two signatures were significantly associated with UTIs ($p < 0.05$). These virulence signatures were VS1 containing *papC, papAH, papEF, papG allele II, focG, fyuA, iutA, iroN, chuA hlyA,*