

A subset of two adherence systems, acute pro-inflammatory *pap* genes and invasion coding *dra*, *fim*, or *sfa*, increases the risk of *Escherichia coli* translocation to the bloodstream

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Received: 24 April 2013 / Accepted: 9 June 2013 / Published online: 27 June 2013
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Abstract An analysis of the phylogenetic distribution and virulence genes of *Escherichia coli* isolates which predispose this bacteria to translocate from the urinary tract to the bloodstream is presented. One-dimensional analysis indicated that the occurrence of P fimbriae and α -hemolysin coding genes is more frequent among the *E. coli* which cause bacteremia. However, a two-dimensional analysis revealed that a combination of genes coding two adherence factors, namely, P + Dr, P + S, S + Dr, S + fim, and hemolysin + one adherence factor, were associated with bacteremia and, therefore, with the risk of translocation to the vascular system. The frequent and previously unrecognized co-existence of pro-inflammatory P fimbriae with the invasion promoting Dr adhesin in the same *E. coli* isolate may represent high-risk and potentially lethal pathogens.

Escherichia coli, the predominant facultative organism of the intestinal flora, can cause severe extra-intestinal infections, including infection of the kidney (pyelonephritis) or bloodstream (bacteremia). When it escapes from its usual habitat, *E. coli* can colonize the genital tract and, as a subsequent step, ascend to the bladder and kidneys. Ascending urinary tract infection (UTI) is well explained by tissue receptor–*E. coli* adhesins interactions. Several *E. coli* virulence factors, including toxins, are implicated in renal inflammatory injury and

bacteremia, but the mechanisms of translocation from the renal system to the bloodstream is poorly understood. It is thought that the strains which cause extra-intestinal infections harbor virulence factors that enhance the ability of *E. coli* to cause systemic infection. While an increase in the occurrence of *E. coli* bacteremia and urosepsis has been reported in recent years, relatively few studies have investigated the characteristics of the *E. coli* strains causing bacteremia. In fact, reports on the association between the risk of bacteremia/septicemia and occurrence of *E. coli* virulence factors are contradictory. While some studies propose that P fimbriae and α -hemolysin are associated with the risk of bacteremia [1], others indicate that there is no such association and that *E. coli* virulence factors do not differ between those isolated from bacteremia and those isolated from pyelonephritis [2].

UTIs affect from 5 to 36 % of renal transplant (RT) patients. In these patients, UTI is a frequent cause of bacteremia, septicemia, and acute graft failure [3]. *E. coli* is the most common etiologic agent of UTI in RT patients, as well as being the most frequent pathogen isolated from RT recipients with bacteremia [4]. To date, only a few studies have been available regarding the phylogenetic and virulence characteristics of *E. coli* isolates causing UTI and bacteremia in severely immunosuppressed patients, such as RT recipients [5–7].

Uropathogenic *E. coli* (UPEC) strains have particular phenotypic features which facilitate their persistence in urinary tracts and differentiate them from the other pathogenic and commensal *E. coli* strains. UPEC strains encode a number of virulence factors which enable the bacteria to colonize the urinary tract and persist in the face of a highly effective host defense. UPEC isolates exhibit a high degree of genetic diversity, owing to the possession of specialized virulence genes located on mobile genetic elements, called pathogenicity islands [8]. The virulence factors of *E. coli* which have been potentially implicated as important in establishing UTIs can be divided into two groups: (i) virulence factors associated with the surface of the bacterial cell and (ii) virulence factors which

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are secreted and exported to the site of action [9]. The surface virulence factors of UPEC include a number of different types of adhesive organelles (fimbriae), which promote bacterial attachment to, and/or invasion of, host tissues within the urinary tract, namely, type 1, P, S, and Dr adhesins [10]. For example, type 1 fimbriae mediate invasion of the bladder tissue, form intracellular bacterial communities, and are implicated in recurrent cystitis [10]. P fimbriae induce acute inflammatory signaling and are associated with acute pyelonephritis, while Dr adhesins promote cellular invasion and are associated with chronic pyelonephritis [11]. Interestingly, both adhesins are only carried by the same *E. coli* strain occasionally. The most important secreted virulence factors are a lipoprotein called α -hemolysin (HlyA), the cytotoxic necrotizing factor 1 (CNF1), and the uropathogenic specific protein (USP) [12]. In addition, phylogenetic analyses have revealed that UPEC strains differ substantially from other *E. coli* strains. Pathogenic *E. coli* strains, including UPEC strains, mainly belong to phylogenetic group B2 and, to a lesser extent, group D, the 'high-virulence' groups, whereas isolates of groups A or B1, the 'low-virulence' groups, contain few virulence determinants and are usually regarded as commensals [13].

The aim of this study was to gain insight into the phylogenetic distribution and virulence genes of *E. coli* isolates predisposing the translocation of *E. coli* from the urinary tract to the bloodstream in RT patients. We consider that the inconsistent findings on the association of individual virulence factors with the translocation to the bloodstream may result from the fact that *E. coli* require a battery of different adherence factors, in combination, in order to access the vascular bed. For example, we recently reported a case of maternal death owing to *E. coli* septicemia, where *E. coli* bearing both P and Dr adhesins spread from the kidney to the blood, causing lethal septic shock [14]. To investigate *E. coli* factors predisposing RT patients with UTIs to bacteremia, we performed an analysis of individual *E. coli* virulence factors in RT versus non-RT bacteremia and then conducted a two-dimensional analysis of all the virulence factors in all the groups tested.

The study population constituted 67 patients who had developed *E. coli* UTI and/or bacteremia and were hospitalized by the Department of Nephrology, Transplantology and Internal Diseases at the Gdańsk University of Medicine in Poland during the period from 2006 to 2009. The criteria for inclusion isolates in this study were based on the genotyping of isolates using the PCR MP and REA-PFGE methods [15]. A total of 215 isolates were genotyped, of which 103 strains came from blood and 112 were isolated from urine. The strains isolated from the same material among particular patients identified as the same genotype were rejected from the study. The genetic criteria for urinary tract and bloodstream transmission of the *E. coli* isolate were that of identical fingerprint profiles with at least one identical genotype isolated from the urine and the blood. A total of 77 *E. coli* isolates met the inclusion criteria and

they were divided into four groups: (I) 44 blood isolates from non-RT patients with positive urine culture and bacteremia, (II) 19 blood isolates from RT patients with positive urine culture and bacteremia, (III) six isolates from non-RT patients with positive urine culture but no bacteremia, and (IV) eight isolates from RT patients with positive urine culture but no bacteremia.

Multiplex PCR-based methods were used to determine the presence of six virulence factors genes, as previously described [16]. They included *fimG/fimH* (genes of type 1 fimbriae), *sfaD/sfaE* (genes of S fimbriae), *papC* (gene of P fimbriae), *hlyA* (gene encoding α -hemolysin), *cnf1* (gene encoding cytotoxic necrotizing factor 1), and *usp* (gene encoding uropathogenic specific protein). The isolates were also examined by PCR assay for the presence of the genes encoding fimbriae of the Dr family, *afa/dra(B-C)*, as described previously [17]. Statistical analysis of the results was carried out using a Chi-square test. The probability threshold *p*-value was assumed to be at the level of 0.05.

The one-dimensional analysis (Table 1) carried out on the pool of selected strains from all patients shows that bacteriocin Usp was the most frequently occurring virulence factor. Gene *usp* was carried by 90 % of the bacteremia strains which were able to translocate to the blood (groups I and II) and 71 % among strains with no bacteremia and no translocation (groups III and IV). Despite its higher frequency in blood, the presence of the *usp* gene was not statistically significant ($p=0.551$). The occurrence of only two individual factors, P fimbriae ($p=0.006$) and α -hemolysin ($p=0.007$), out of the seven virulence factors being investigated was statistically more frequent among *E. coli* isolates from bacteremia. The one-dimensional analysis thus suggests that P fimbriae and α -hemolysin are the virulence factors which may predispose *E. coli* strains to migrate from the urinary tract to the bloodstream. Surprisingly, the occurrence of Dr adhesin among all the bacteremic *E. coli* (80 %) and in the RT patients (53 %) was both very high and much higher than previously reported [2]. Despite the overall high frequency, the blood isolates from RT patients were characterized by a lower rate of individual virulence genes, such as, for example, Dr adhesins ($p=0.03$). A high frequency of genes coding S fimbriae (70 %) was also observed, but there was no difference between the groups being tested.

As pathogenic *E. coli* often carry multiple adherence factors, a two-dimensional data analysis was performed (Table 2). Consistent with our hypothesis, a combination of two adherence factors was associated with bacteremia/translocation to the blood. This included combinations of P + Dr and S + Dr, but not type 1 + Dr (p -values 0.022, 0.036, and 0.452, respectively). We observed that the strains which carry genes coding type 1 fimbriae and α -hemolysin simultaneously were also associated with bacteremia ($p=0.005$). This suggests that a combination of this kind could also be a predictive factor for the risk of translocation. The two-dimensional analysis demonstrated that the strain's blood transmission was not

Table 1 Unidirectional analysis of PCR-based detection $n =$ of genes encoding the examined *E. coli* virulence factors

Virulence factor ^a	Translocation groups I + II ($n = 63$) vs. no translocation groups III + IV ($n = 14$)		RT translocation group II ($n = 19$) vs. non-RT translocation group I ($n = 44$)		RT total groups II + IV ($n = 27$) vs. non-RT total groups I + III ($n = 50$)	
	%	<i>p</i> -value	%	<i>p</i> -value	%	<i>p</i> -value
P	62/21	0.006	58/64	0.667	44/60	0.191
S	71/50	0.121	74/70	0.795	70/66	0.696
cnf	46/36	0.482	37/50	0.336	37/48	0.355
usp	90/71	0.551	84/93	0.266	78/92	0.076
fim	76/57	0.148	68/80	0.341	59/80	0.051
hly	46/7	0.007	37/50	0.336	30/44	0.217
dr	71/64	0.597	53/80	0.030	52/80	0.010

Group I translocation non-RT [blood(+)/urine(+)] 44 *E. coli*

Group II translocation RT [blood(+)/urine(+)] 19 *E. coli*

Group III no translocation non-RT [blood(-)/urine(+)] 6 *E. coli*

Group IV no translocation RT [blood(-)/urine(+)] 8 *E. coli*

^a P P fimbriae (*papC*), S S fimbriae (*sfaD/sfaE*), *cnf* cytotoxic necrotizing factor (*cnfI*), *usp* bacteriocin *usp*, *fim* type 1 fimbriae (*fimG/fimH*), *hly* α -hemolysin (*hlyA*), *dr* Dr fimbriae (*afa/dra* B–C)

statistically associated with the presence of such virulence factors as a cytotoxic necrotizing factor coexisting with P, S, Dr, or type 1 fimbriae or with bacteriocin Usp (*p*-values 0.237, 0.276, 0.384, 0.199, and 0.481, respectively). The risk of

translocation was also not correlated with the coexistence of Dr fimbriae and bacteriocin Usp or type 1 fimbriae (*p*=0.384 and *p*=0.24, respectively). In contrast to bacteremia versus non-bacteremia, where significant differences were observed,

Table 2 Two-dimensional analysis of the coexistence of two genes encoding virulence factors among the investigated *E. coli* isolates

Virulence factor ^a	Translocation groups I + II ($n = 63$) vs. no translocation groups III + IV ($n = 14$)		RT translocation group II ($n = 19$) vs. non-RT translocation group I ($n = 44$)		RT total groups II + IV ($n = 27$) vs. non-RT total groups I + III ($n = 50$)	
	%	<i>p</i> -value	%	<i>p</i> value	%	<i>p</i> value
P + S	56/21	0.021	53/57	0.759	41/54	0.267
P + <i>cnfI</i>	38/21	0.237	26/43	0.206	22/42	0.083
P + <i>usp</i>	62/21	0.006	59/64	0.667	44/60	0.191
P + <i>fim</i>	48/7	0.005	42/50	0.565	30/46	0.162
P + <i>hly</i>	38/7	0.025	37/39	0.893	30/34	0.696
P + <i>dr</i>	48/14	0.022	32/55	0.094	26/50	0.041
S + <i>cnf</i>	44/29	0.276	37/48	0.425	33/46	0.282
S + <i>usp</i>	70/43	0.056	74/68	1.662	67/64	0.815
S + <i>fim</i>	59/21	0.011	58/59	0.929	48/54	0.624
S + <i>hly</i>	38/7	0.025	32/41	0.484	26/36	0.368
S + <i>dr</i>	52/21	0.036	37/59	0.105	33/54	0.083
<i>cnf</i> + <i>usp</i>	46/36	0.481	37/50	0.336	37/48	0.355
<i>cnf</i> + <i>fim</i>	40/21	0.199	32/43	0.388	30/40	0.367
<i>cnf</i> + <i>hly</i>	35/7	0.040	21/41	0.129	19/36	0.110
<i>cnf</i> + <i>dr</i>	33/21	0.384	16/41	0.052	19/38	0.078
<i>usp</i> + <i>fim</i>	76/50	0.050	68/80	0.341	59/78	0.082
<i>usp</i> + <i>hly</i>	46/7	0.007	37/50	0.336	30/44	0.217
<i>usp</i> + <i>dr</i>	67/50	0.240	47/75	0.033	44/74	0.010
<i>fim</i> + <i>hly</i>	38/0	0.005	26/43	0.206	19/38	0.078
<i>fim</i> + <i>dr</i>	54/43	0.452	37/61	0.073	30/64	0.004
<i>hly</i> + <i>dr</i>	38/7	0.025	21/45	0.067	19/40	0.055

^a P P fimbriae (*papC*), S S fimbriae (*sfaD/sfaE*), *cnf* cytotoxic necrotizing factor (*cnfI*), *usp* bacteriocin *usp*, *fim* type 1 fimbriae (*fimG/fimH*), *hly* α -hemolysin (*hlyA*), *dr* Dr fimbriae (*afa/dra* B–C)

we identified no significant differences between *E. coli* virulence factors from RT and non-RT patients. The reason for the lower frequency of Dr adhesins in RT patients is not clear and may be associated with immunosuppression or, perhaps, the stage of renal disease and/or function, which may require independent evaluation.

The phylogenetic group analysis was determined by a triplex PCR assay performed with a combination of three DNA markers (*chuA*, *yjaA*, and the DNA fragment *TSPE4.C2*), as previously described [18]. As expected, group B2 was associated with the presence of classic virulence factor genes, whereas group B1 isolates had the lowest virulence factor score. In our analysis, phylogenetic *E. coli* groups A, B1, B2, and D for the RT recipients' isolates being tested (groups II and IV) accounted for 0 %, 33 %, 57 %, and 10 % of the isolates, respectively. In the isolates from the non-RT patients (groups I and III), phylogenetic groups A, B1, B2, and D accounted for 0 %, 16 %, 84 %, and 0 %, respectively. The shift from group B2 toward group B1 observed could, thus, account for the lower frequency of virulence factor genes among RT isolates when compared with those from non-RT patients.

To summarize, in this paper, we have reported that *E. coli* translocation to the bloodstream was associated with a high frequency combination of two adherence factors, namely, P, and Dr, S, or Type 1 adhesins. P mediates acute inflammatory signaling, while Dr, S, and Type 1 may mediate tissue invasion. This observation implies that *E. coli* bearing P fimbriae with the capacity to cause severe inflammatory response and an adhesin which can mediate tissue invasion may establish an effective translocation strategy for passing all tissue barriers in order to access the bloodstream and cause bacteremia. An unrecognized combination of P and Dr adhesins may contribute to the establishment of a clonal group of a super-pathogenic *E. coli*, which, if combined with resistance to antibiotics, may represent a new trend in the evolution of uropathogens.

Acknowledgments This work was supported by the Polish Ministry of Science and Higher Education Grant No. N401331239, awarded to A.Ś and in part by U54 RR026140 (NCRR)/U54 MD007593 (NIMHD).

Conflict of interest The authors declare that they have no conflict of interest.

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