Features of uropathogenic *Escherichia coli* able to invade a prostate cell line

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SUMMARY

RWPE-1 normal prostate cells were tested as an experimental model for adhesion/invasion assays by genotypically and phenotypically characterized community uropathogenic strains of *Escherichia coli* (UPEC), a frequent cause of urinary tract infections (UTIs) and significant etiologic agent also in bacteriuria in males, resulting in ascending infections. Several studies have shown that UPEC, until recently considered extracellular bacteria, can invade epithelial cells of the urinary tract both in vitro and in vivo (Mulvey, 2002, Dhakal et al., 2008). The ability to invade the urothelium as well as the development of intracellular bacterial communities, large biofilm-like inclusions, correlate with prolonged persistence of UPEC in the host (Flores-Mireles et al., 2015). The majority of studies focused on bladder cells but the ability of UPEC strains to adhere, invade, replicate and persist in the normal prostate epithelial cell line RWPE-1 has been also demonstrated (Rudick et al., 2011). In order to extend the knowledge on the mechanisms linked to uropathogenic *E. coli* infection process, in the present study, 58 urinary *E. coli* strains were tested for adhesiveness to and invasion ability in the normal human prostate cell line, RWPE-1. Strains from a laboratory collection were isolated in 2006 from community patients diagnosed with UTI by general practitioners (Longhi et al., 2012). The cohort of patients included 25 men and 33 women, average age 58 (17-86 years), and 7 children aged between 3 months and 9 years. The possible relationship between antibiotic resistance, virulence factors and in vitro biofilm formation was also evaluated. Antibiotic susceptibility was evaluated by Kirby-Bauer disc diffusion method according to the CLSI guidelines (CLSI, 2005) and re-checked whenever necessary by broth dilution test. Bacterial biofilm production was determined by crystal violet assays after incubation for 48 h at room temperature and classified as reported by Stepnanovic et al. (2004). Non-neoplastic, immortalized human prostatic epithelial RWPE-1 cells (ATCC, Rockwell, MD), maintained in complete keratinocyte serum-free medium, were incubated in medium lacking antibiotics for at least 12 h prior to experiments. RWPE-1 cells, infected with bacteria
grown in Luria Broth (LB) at 37°C for 48 h under static conditions (multiplicity of infection of about 10 cfu/cell), were centrifuged twice at 500 x g for 2.5 min to promote bacterial adherence to the cell monolayer and incubated for 2 h at 37°C in 5% CO₂ as reported by Thumbikat et al. (2009) and Rudick et al. (2011). To measure bacterial adherence, after extensively washing with PBS, cells were lysed with 0.1% cold Triton X-100 and plated onto Tripticase Soy Agar (TSA). Bacterial adhesion was defined as the percentage of attached bacteria compared with the initial inoculum. Bacterial invasion was measured using a gentamicin protection assay: after 2 h of infection, RWPE-1 cells were incubated for 60 min with 100 µg/ml gentamicin (concentration at which all strains were susceptible), followed by washing, lysing, and bacterial plating as above described. Invasion was normalized to the number of adherent bacteria (number of cfu invasive/adherent x 100); the strains were considered invasive when the mean invasion level was equal or superior to 0.1% of the adhesion values. E.coli MG1655 and E.coli EIEC HN280 strains were utilized as negative and positive controls respectively. The phylogenetic group was determined by PCR assays (Clermont et al., 2000). Multiplex PCR for some genes associated with virulence in E. coli (PAI, papA, fimH, kpsMT III, papEF, ibeA, fyuA, sfa/focDE, istA, papG allele III, K1, hlyA, rfc, nfaE, papG allele I, kpsMTII, papC, gafD, cvaC, focG, traT, papG allele II, papG alleles II and III, afa/draBC, cnf1, sfaS, K5), was performed as described by Johnson and Stell, (2000). Random amplified polymorphic DNA (RAPD) reactions were performed using two arbitrary primers: 3 - [5'-d[GTAGACCCGT]-3’] and 4 - [5’-d[AAGAGCCCGT]-3’]. PCR was performed in triplicate as follows: 1 cycle at 95°C for 5 min, followed by 45 cycles at 95°C for 1 min, 36°C for 1 min, and 72°C for 2 min (Mastercycler pro, Eppendorf). Results were analyzed using Total Lab TL120 Trace version 2006 (Nonlinear Dynamics) setting position tolerance at 1.5%. The Dice coefficient of similarity was calculated, and the unweighted pair group method with arithmetic averages (UPGMA) was used to generate cluster XLstat 7.5 (Addinsoft). The similarity percentage cut-off to distinguish between clonally distinct groups was set at 95%. In order to compare VFs score between clus-
Table 1 - Age distribution of patients and characteristics of E. coli isolates.

<table>
<thead>
<tr>
<th>Age</th>
<th>Patients</th>
<th>E. coli invasiveness</th>
<th>Biofilm a</th>
<th>Antibiotic resistance b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N(M)</td>
<td>Negative</td>
<td>Positive</td>
<td>M F M F</td>
</tr>
<tr>
<td>&lt;17</td>
<td>7(4)</td>
<td>1 0 3 3</td>
<td></td>
<td>4 1 2 2 2 1 1 0 0 0 1 1 1 1 1 1 1 1 1</td>
</tr>
<tr>
<td>17-30</td>
<td>6(0)</td>
<td>0 3 0 3</td>
<td></td>
<td>3 1 2 2 1 1 1 1 1 1</td>
</tr>
<tr>
<td>31-60</td>
<td>18(6)</td>
<td>1 8 5 4</td>
<td></td>
<td>6 7 5 1 4 2 0 1 0 1 0 2 1 1 1 3 1 1 0 0 0 3 7</td>
</tr>
<tr>
<td>&gt;60</td>
<td>27(15)</td>
<td>7 11 8 1</td>
<td></td>
<td>10 8 9 11 5 4 3 1 1 1 0 1 2 1 1 2 1 0 1 2 1</td>
</tr>
<tr>
<td></td>
<td>58(25)</td>
<td>9 22 16 11</td>
<td></td>
<td>21 20 17 28 11 3 2 8 13 14 7 1 25</td>
</tr>
</tbody>
</table>

N: total number of patients; M: male; F: female.

The invasive group displayed stronger biofilm formation than non-invasive strains (33% vs 11%), with respect to non-invasive strains (48% vs 15%) (Table 1).

The biofilm producer strains were 70% (7/9) vs 12% (3/28) in invasive low/medium biofilm producers (p=0.002), although the sample size was low. Invasive strains had a high adhesive ability (p<0.01) (Figure 1). No substantial differences in the pattern of virulence factors were found between invasive and non-invasive strains. Genes such as fimH, papC, sfaS were present equally in both invasive and non-invasive strains, whereas the percentage papEF (33% vs 13%) and papG allele II (26% vs 10%) was slightly different but not statistically significant. Prevalence of serum resistance-associated traT gene and invasion of brain endothelium iba gene was 58% and 26% respectively in invasive strains vs 39% and 35% in non-invasive strains.

Multiple resistance was similarly present in invasive or non-invasive strains (22% vs 29%); resistance to three or more drug classes was present in invasive strains from male patients (6/16) and absent from women (0/11), although not significant because of the sample size (data not shown). In biofilm formation assays, 17 out 58 (29%) strains were classified as strong biofilm producers, and 21 out 58 isolates (36%) were weak or non-biofilm producers. The invasive group displayed stronger biofilm formation capability 41% (11/27) with respect to non-invasive strains 19% (6/31) (Table 1). In the elderly (27/58), invasive strong biofilm producer strains were 70% (7/9) vs 12% (3/18) invasive low/medium biofilm producers (p=0.002), although the sample size was low. Among adhesion factors, fimH adhesin was the prevalent VF detected, occurring in about 81% of strains (47/58). The presence of papC and kpsMTII genes was statistically correlated to different degree of adhesive ability: among UPEC with a low (≤10%) adhesive ability papC and kpsMTII were both present in 14% (3/22) of the strains, and in 69% (9/13) among isolates with a high adhesive ability (p<0.01) (Figure 1). No substantial differences in the pattern of virulence factors were found between invasive and non-invasive strains. Genes such as fimH, papC, sfaS were present equally in both invasive and non-invasive strains, whereas the percentage papE (33% vs 13%) and papG allele II (26% vs 10%) was slightly different but not statistically significant. Prevalence of serum resistance-associated traT gene and invasion of brain endothelium iba gene was 58% and 26% respectively in invasive strains vs 39% and 35% in non-invasive strains.
observed that the highest percentage of invasive strains with better invasive efficiency was found among *E. coli* isolated in male patients. Although the limitations of our study are the sample size and the lack of data due to the community origin of the patients, the results support the findings of Rudick et al. who described a different RWPE-1 cells invasion efficiency of a single *E. coli* isolated from a patient with chronic prostatitis with respect to a *E. coli* strain isolated from the urine of a woman with acute cystitis (Rudick et al., 2011).

Other studies regarding UTI pediatric patients reported that *E. coli* infections were more prevalent among males during the first year of life and among females thereafter. Although based on small sample, the invasive features of our strains were significantly associated with the pediatric cohort age group.

Moreover, in this study, it was not possible to associate a peculiar virulence spectrum to invasive ability, as also confirmed by RAPD analysis, supporting the hypothesis that multiple different factors, working in concert, may contribute to the invasive process (Lo et al., 2015).

Furthermore, it should be considered that not all bacterial genes putatively involved in the adhesion/invasion process were screened in this study and a number of unknown genes correlated to virulence undoubtedly remain uncharacterized. As regards susceptibility to antibiotics, we could not find any significant difference between invasive and non-invasive strains.

Although the resistance rate of *E. coli*, especially against amoxicillin-clavulanic, antimicrobials that are commonly prescribed in children, is currently a matter of concern, in our study the amoxicillin-clavulanic acid resistance rate was similar to that reported in other age groups. Jointly, our data suggest that some subtypes from the heterogeneous population of UPEC strains possess the ability to colonize and probably to persist in an intracellular milieu and that RWPE-1 cells could represent a useful cell model to study *E. coli*-epithelial cell interactions.

The identification of mechanisms that may facilitate UPEC adherence to and invasion of urinary cells may help to explain the susceptibility of some individuals to UTIs, potentially highlighting additional targets for therapeutic interventions. The determinants and strategies involved in the UPEC invasive step in prostate cells require further study and are now under our investigation.

**References**


