Genetic analysis of virulence and pathogenicity features of uropathogenic *Escherichia coli* isolated from patients with neurogenic bladder

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Abstract

The severity of urinary tract infections (UTI) produced by *Escherichia* (E.) *coli* is due to the expression of a wide spectrum of virulence factors conferring their uropathogenicity and amplified by specific clinical conditions, such that of the patients with motor deficit. In these patients the symptomatology specific to neurogenic bladder could lead to the installation of major complications of UTI such as renal failure. The purpose of this work was to investigate the virulence factors of 66 *E. coli* strains isolated from patients with neurological disorders and neurogenic bladder. PCR multiplex was used for the phylogenetic analysis and for the assessment of virulence factors in the respective strains. Our study demonstrated that the uropathogenic *E. coli* strains isolated from patients with neurogenic bladder are harbouring different virulence genes implicated in the initiation and the development of the infectious process, represented by adhesins (*fimH*), haemolysins (*hly, cnf*) and iron chelating agents (*aer*). The studied strains were equally distributed between the extraintestinal phylogenetic groups (B2 and D), known for their higher virulence potential and the intestinal ones (A and B1). The most virulent strains belonged to the extraintestinal phylogenetic group B2, harboring all tested virulence genes.

Keywords: *Escherichia coli*, virulence factors, phylogenetic groups, PCR

Introduction

Urinary tract infections (UTI) are inflammatory disorders caused by abnormal proliferation of pathogens in the urinary device, while causing changes in the normal functioning of the kidneys and urinary tract (1). UTI is a major public health problem, being one of the most common infectious diseases encountered in all ages, with a large share for the infectious pathology of neurological patient. The severity of UTI produced by *Escherichia* (E.) *coli* is amplified by the existence of a wide range of virulence factors conferring the uropathogenicity of these strains (2). The generally accepted hypothesis today is that uropathogenic *E. coli* strains (UPEC) evolved from non-pathogenic strains by acquiring new virulence factors by accessory DNA horizontal transfer often organized into "clusters" (pathogenicity islands) located at chromosomal or plasmidial level (3). Most pathogenic *E. coli* strains do not have a single evolutionary origin, being the result of various events of genetic transfer, so they do not belong to a monofiletic group (4).
Under the conditions of urinary stasis consecutive to the difficult urinary act, these infections are triggering local, regional or systemic complications because the association of infectious process with the urinary stasis is favoring conditions for the kidney damage. These will enhance and exacerbate each other, and the vicious circle that threatens the patients’ life can be stopped only by concomitant suppression of both agents aggressors. In the absence of proper therapy since the onset of disease, UTI in patients with motor deficit and with all the symptomatology specific to neurogenic bladder could lead to the installation of major complications such as renal failure (5).

The purpose of this work was to investigate the virulence factors of E. coli strains isolated from patients with neurological disorders and neurogenic bladder.

**Material and methods**

**Microbial strains**

There were investigated 60 E. coli isolated from positive urine cultures in patients with neurological diseases admitted to the Neuropsychiatry Clinical Hospital of Craiova, during December 2006 – November 2007. All patients included in the study were not carrying any urinary device.

**PCR multiplex for the inclusion of E. coli strains in phylogenetic groups**

The E. coli strains were included into the four major phylogenetic groups (A, B1, B2 and D) following the protocol proposed by Clermont et al., 2000 (6) using two virulence genes (chu A, encoding a hem transportor protein in E. coli O157: H7 and yjaA, initially identified in the genome of E. coli K-12) and one DNA fragment TspE4.C2.

The DNA amplification ADN was performed in a final volume of 50 µl using 6 µl bacterial and a reaction mix containing 2 U Taq polimerase (ROCHE BOEHRINGER), Buffer 1X, 1.5 mM Mg Cl2 with 0.2 mM of each dNTP (dATP, dCTP, dTTP, dGTP) (ROCHE BOEHRINGER) and 30 pmol of each primer (Chu A1- GAC GAA CCA ACG GTC AGG AT, Chu A2- TGC CGC CAG TAC CAA AGA CA, Yja A1-TGA GAG AAT GCG TTC CTCAAC, TspE4C2.1- GAG TAA TGT CGG GGC ATT CA, TspE4C2.2- CGC GCCAAC AAA GTA TTA CG). The amplification program was ruled out on a My Cycler BioRad apparatus consisting in an initial denaturation at 95°C for 4min; followed by 30 cycles : (1) denaturation at 94°C for 30 sec; (2) alignment at 59°C for 30 sec; (3) elongation at 72°C for 30 sec; final extension at 72°C for 5 min.

**PCR multiplex for virulence genes**

The assed virulence factors were represented by: fimH: encoding for type 1 fimbriae; region hlyCA: encoding for haemolysin production; cnf: encoding for cytotoxic necrotizing factor; aer: encoding for aerobactin.

The DNA amplification ADN was performed in a final volume of 50 µl using 10 µl bacterial and a reaction mix containing 1.25 U Taq polimerase (ROCHE BOEHRINGER), Buffer 10X with 1.5 mM Mg Cl2, 0.2 mM of each dNTP (dATP, dCTP, dTTP, dGTP) (ROCHE BOEHRINGER) and 30 pmol of each primer (fimH-1- AACAGCGATGATTTCAGTTGTGTG, fimH-2- TTTGCGTACCAGCATGGAATGCC, hly-1-AGATTCTTGCCATGTATCCT, hly-2- TCTTTGCGACTGTAGTGT, cnf-1-TTTATATGCTGCAAGTAGTA, cnf-2- CACTAGCTTTTAAATATTGA, aer-1- AACACCTGGCTTACGCAACTGT, aer-2- ACCCGTCTGCAAAATCATGGGAT).

The amplification program was ruled out on a My Cycler BioRad apparatus consisting in an initial denaturation at 94°C for 4min; followed by 30 cycles: (1) denaturation at 94°C for 30 sec; (2) alignment at specific temperature for each primer (fim H- 60°C, hly-55°C, cnf-50°C, aer-55°C) for 30 sec; (3) elongation at 72°C for 1 min with 2 sec. prolongation of each cycle.

The PCR products were evidenced after migration in gel electrophoresis in an agarose gel 2% prepared in TBE 0,5X, stained with ethidium bromure 0,5µg/ml, visualized in UV and photographed with a Polaroid camera (6).

The amplicon size (chu A -279 bp, yja A- 211 bp, TspE4C2- 152 bp, fim H- 465 bp, hlyA-565 bp, cnf-693 bp, aer- 269 bp) was appreciated by comparison with a molecular size marker (Promega 100pb), and the E. coli J 96 was used as positive control, while the distilled sterile water as negative one.

Results and discussion

In many uropathogenic E. coli strains, their virulence is due to their to the extraintestinal B2 or D phylogenetic group and to different virulence factors, such as haemolysins or adhesins (7, 8).

**Phylogenetic analysis of E. coli strains**

The four phylogenetic group are dividing the E. coli strains in extraintestinal (B2 and D) and intestinal ones (A and B1).

The uropathogenic Escherichia coli strains isolated from patients with neurogenic bladder were equally distributed between the extraintestinal, B2 and D phylogenetic groups and the intestinal ones (A and B1). The four phylogenetic groups were defined by different combinations of these genes, i.e. group A: yja A, group B1: TspE4C2, group B2: chuA + yjaA; chuA + yjaA + TspE4C2, group D: chu A + TspE4C2 (Fig. 1).

**Figure 1.** Gel electrophoresis of PCR multiplex products for phylogenetic analysis of E. coli strains. Agars well contained: agar wells no. 1, 2, 6, 8, 13 şi 14: amplicon yjaA, representing the A group; agar wells no.: 4, 7, 10 şi 12: chu A + TspE4C2, representing the D group; agar well no. 5: amplicon chuA, representing the B1 group; agar well no. 3: amplicon TspE4C2 representing the D group; agar wells no.: 9 and 11: amplicons chuA + yjaA; chuA + yjaA + TspE4C2 representing B2 group.
Distribution of virulence genes among the studied uropathogenic E. coli

The urine is representing the most important barrier against the microbial colonization of the urinary tract by uropathogenic E. coli strains, which are carrying different fimbrial and afimbrial adhesins facilitating the adherence to the uroepithelial cells, as well as other virulence factors contributing to the development of the infectious process, such as toxins and siderophores (9, 10).

The prevalence of different virulence genes among the studied strains varied from 10% for cnf gene to 80% for fimH gene (table no. 1).

Table no. 1. The prevalence of virulence genes among uropathogenic Escherichia coli strains

<table>
<thead>
<tr>
<th>Virulence gene</th>
<th>Group B2</th>
<th>Group D</th>
<th>Group B1</th>
<th>Group A</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fimH</td>
<td>15</td>
<td>13</td>
<td>5</td>
<td>15</td>
<td>48 (80%)</td>
</tr>
<tr>
<td>Aer</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>14</td>
<td>32 (53,3%)</td>
</tr>
<tr>
<td>Hly</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8 (13,3%)</td>
</tr>
<tr>
<td>Cnf</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6 (10%)</td>
</tr>
</tbody>
</table>

As that could be observed (table no. 1) the microbial strains belonging to the extraintestinal phylogenetic groups B2 and D possess the most virulence genes (59 genes), as compared to those belonging to the intestinal groups B1 and with only 6 virulence genes. The majority of strains belonging to the phylogenetic B2 group harbor the complete set of four virulence factors (38 strains). For B2 and A groups an equal distribution of fimH was observed (15 strains), while aer is predominating in group A strains (14 strains), followed by B2 (9 strains) and D (8 strains), in the B1 group existing only one strain harboring this gene. The hly and cnf have not been identified in the strains belonging to D, B1 and A groups. Concerning the total prevalence of different virulence strains among the uropathogenic strains the fimH gene was present in the majority of the tested strains (80%) (Fig. 2), aer in 53,3% (Fig. 3), hly in 13,3% and cnf in 10% of the studied strains (Fig. 4 and 5).

Figure 2. Gel electrophoresis of fimH amplicons. Each well contains the amplicons obtained from different E. coli strains positive for fimH, well no. 11- molecular weight marker of 100 bp and well no. 15- molecular weight marker of 50 bp.
The analysis of the association between the presence of different combinations of virulence genes, allowed us to include the tested strains into seven virulence patterns noted Ec 1 to 7 (table no. 2). The four strains without any virulence marker were included in Ec 1, 22 strains harbouring fimH gene in Ec 2 and 6 strains harboring aer gene in Ec3. The pattern Ec4 included the strains simultaneously positive for fim + aer (15 strains). The two patterns Ec 5 and Ec 6 were represented by the strains possessing a combination of three virulence genes, Ec 5 with fim + aer + hly (2 strains) and Ec 6 with fim + hly + cnf (3 strains). In the Ec 7 pattern there were included the 3 strains harbouring simultaneously the four tested virulence
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Our distribution was similar to that reported in similar studies performed on *E. coli* strains isolated from UTI in different Romanian population groups (11).

**Table no. 2.** The representation of the prevalence of different virulence patterns identified for the uropathogenic *E. coli* tested strains.

<table>
<thead>
<tr>
<th>Patterns</th>
<th>Fim</th>
<th>Hly</th>
<th>Aer</th>
<th>cnf</th>
<th>No. strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ec 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Ec 2</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Ec 3</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Ec 4</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Ec 5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Ec 6</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>Ec 7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
</tbody>
</table>

**Conclusion**

In conclusion, our study demonstrated that the uropathogenic *E. coli* strains isolated from patients with neurogenic bladder are harbouring genes for different virulence factors implicated in the initiation and the development of the infectious process, represented by adhesins (*fimH*), haemolysins (*hly, cnf*) and iron chelating agents (*aer*). The studied strains were equally distributed between the extraintestinal phylogenetic groups (B2 and D) known for their higher virulence potential and the intestinal ones (A and B1). The most virulent strains belonged to the extraintestinal phylogenetic group B2 and harbored all tested virulence genes.

**Selective references**

1. NEGUȚ M, BUIUC D. Tratat de Microbiologie Clinică, Ediția a II-a, Ed. Medicală, 2008, București
7. PITOUT JD, LAUPLAND KB, CHURCH DL et al. Virulence factors of *Escherichia coli* isolates that produce CTX-M-type extended-spectrum beta-lactamases. Antimicrobial Agents Chemother. 2005, 43 (6), 2844-2849