The *Escherichia coli* phylogenetic group B2 with integrons prevails in childhood recurrent urinary tract infections

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The aim of our study was to characterize the phylogenetic groups of *Escherichia coli*, antibiotic resistance, and containment of class 1 integrons in the first attack of pyelonephritis and in subsequent recurrences in young children. Altogether, 89 urine *E. coli* isolates from 41 children with urinary tract infection (UTI) were studied for prevalence and persistence of phylogenetic groups by pulsed-field gel electrophoresis (PFGE), antibacterial resistance by minimal inhibitory concentrations (MIC) and class 1 integrons by PCR. Phylogenetic group B2 was most common (57%), followed by D (20%), A (18%) and B1 (5%). Overall resistance to betalactams was 61%, trimethoprim-sulfamethoxazole 28%, and was not associated with phylogenetic groups. According to PFGE, the same clonal strain persisted in 77% of patients. The persistence was detected most often in phylogenetic group B2 (70%). Phylogenetic group B2 more often contained class 1 integrons than group A. Integron positive strains had higher MIC values of cefuroxime, cefotaxime, and gentamicin. In conclusion, phylogenetic group B2 was the most common cause of the first episode of pyelonephritis, as well as in case of the persistence of the same strain and contained frequently class 1 integrons in childhood recurrent UTI. An overall frequent betalactam resistance was equally distributed among phylogenetic groups.

Key words: Urinary tract infection; recurrence; phylogenetic group; integron; antibiotic resistance.

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Urinary tract infection (UTI) is one of the leading infection sites in young children. The most common bacterial species causing UTI in children is *Escherichia coli*. *E. coli* is considered a harmless facultative member of normal human colonic microbiota. Paradoxically, *E. coli* strains are also the most frequent cause of urinary tract and some other extraintestinal infections with high morbidity (1–3). *E. coli* strains differ in their ability to cause infection due to different phylogenetic groups in which they belong (4–6). The *E. coli* strains have been assigned to four main groups – A, B1, B2, and D (6, 7). Strains from phylogenetic group B2 and D are generally more virulent, but less resistant and contain resistance-encoding integrons to a lesser extent than strains from phylogenetic groups A and B1. The latter phylogenetic groups are considered to be commensal with low virulence, but have a higher resistance rate accompanied by the occurrence of integrons (8–10). Recently, contradicting data about the higher resistance of uropathogenic *E. coli* strains belonging to phylogenetic group B2 have been published (11).

Information about the clonality of recurrent UTI isolates is important in understanding the essence of infection. The persistence of the primary infecting strain or re-infection with a different strain challenges different treatment strategies, as has been clearly shown in *Helicobacter pylori* infection in
patients with ulcer perforation (12). Pulsed-field gel electrophoresis (PFGE) has been acknowledged as a gold standard for strain identity detection (13). At the same time, the role of phylogenetic groups in clonal and non-clonal recurrences of chronic childhood UTI is not yet clarified.

In our previous study of persistent UTI, nearly 80% of investigated children experienced the recurrence by a unique clonal E. coli alone or combined with individual strains. However, the phenotypic resistance to betalactams and trim ethoprim-sulfamethoxazole, and the occurrence of sul genes and integrons were characteristic of only about half of clonal E. coli strains (14). It is possible that in the other half of patients with recurrent urinary tract infection (RUTI), the failure of eradication of the pathogen and its clonal persistence are closely interrelated with the highly virulent phylogenetic grouping. Until now, the difference in the antibacterial susceptibility of E. coli strains of particular phylogenetic groups has been a matter of debate and can be connected with clonal spread of particular UTI-causing strains (15).

We aimed to characterize the phylogenetic groups of E. coli, antimicrobial resistance, and containment of class 1 integrons in the first attack of pyelonephritis and in recurrences in young children.

**MATERIAL AND METHODS**

**Patients and strains**

Altogether 89 urinary E. coli isolates from 41 children (35 girls and 6 boys; 2–123 months old with a median age of 48 months) with a first acute community-acquired pyelonephritis (APN) derived from the index (n = 41) and recurrent (n = 48) episodes were studied. All patients were followed up at Tartu University Children’s Clinic during 1999. The diagnostic criteria for acute pyelonephritis and recurrences have been described in our earlier study (14). The persistence of infection was defined as re-occurrence of a molecularly identical strain during 1999. The diagnostic criteria for acute pyelonephritis (APN) derived from the index (n = 41) and recurrent (n = 48) episodes were studied. All patients were followed up at Tartu University Children’s Clinic during 1999. The diagnostic criteria for acute pyelonephritis and recurrences have been described in our earlier study (14). The persistence of infection was defined as re-occurrence of a molecularly identical E. coli strain during the infection course. In 12 children, predisposing factors such as vesicoureteral reflux (VUR) and/or functional disorders of the bladder were diagnosed. The single E. coli strain of each patient detected from the index case and at each time point of scheduled urine samples (after 2 weeks, 1, 2, 3, 6, 9 months, and 1 year) was investigated.

Total of 26 patients with index (n = 26) and recurrent E. coli isolates (n = 48) described in our previous study (14) and 15 patients from the same follow-up with only the index strain available were included in the current study.

*Escherichia coli* strains were isolated after seeding the urine samples on a cysteine lactose electrolyte-deficient (CLED) medium and the isolates were identified using standard laboratory methods (16). The bacterial isolates were stored at –80 °C prior to further studies.

**Phylogenetic typing of E. coli**

The total DNA of the E. coli was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer’s protocol. E. coli phylogenetic groups were determined by triplex polymerase chain reaction (PCR) as described by Clermont et al. (17).

**Antibacterial susceptibility testing**

The antibacterial susceptibility of E. coli strains was determined using E-tests (AB Biodisk, Solna, Sweden). The minimum inhibitory concentration (MIC) of trimethoprim-sulfamethoxazole, ampicillin, cefuroxime, cefotaxime, gentamicin, and ciprofloxacin was tested according to the manufacturer’s instructions.

**Detection of the class 1 integrons**

The presence of class 1 integrons was studied by amplification of a class 1 integrase-specific fragment of the *intI* gene using PCR according to Levesque et al. (18) with slight modifications (14).

**Pulsed-field gel electrophoresis**

Pulsed-field gel electrophoresis with the enzyme NorI and/or XhoI was used to compare E. coli isolates (n = 74) from 26 patients with the index and recurrent strains to distinguish between persistence relapse and acquisition of a new strain reinfection. The preparation of bacterial strains was performed as described in the manufacturer’s (Bio-Rad, Paris, France) instructions. The clonal strains were characterized by up to a three-band difference in PFGE, e.g., one genetic event, indicating a relapse of UTI. The individual strains differed from other investigated strains by more than three-band in PFGE, indicating epidemiologically unrelated UTI episodes.

**Statistical analysis**

The statistical analyses were performed using the Sigma Stat (Jandel Scientific, San Rafael, CA, USA) and Excel (Microsoft Corp., Redmond, WA, USA) software programs, employing the Fisher exact test, chi-square, and Mann–Whitney rank sum test. p values less than 0.05 were considered statistically significant.

**RESULTS**

**Phylogenetic groups of E. coli**

Altogether, more than half (51/89; 57%) (Table 1) of E. coli isolates of children with UTI belonged to phylogenetic group B2. Other phylogenetic groups occurred less frequently: group D 18/89 (20%) of isolates, group A 16/89 (18%) of isolates, and group B1 4/89 (5%) of isolates. Differences in phylogenetic groups of E. coli strains between patients with or without predisposing factors were not detected.
Phylogenetic groups of *E. coli* at the first attack of pyelonephritis and in patients with studied recurrences

First attack of APN was most often caused by strains of phylogenetic group B2 (25/41; 61% of isolates). Groups D, A, and B1 occurred less frequently (11/41, 27%; 4/41; 10% and 1/41, 2%, respectively; Table 1).

Phylogenetic group B2 also prevailed as the cause of recurrent episodes with a frequency of 54% (26/48), followed by group A (12/48; 25%), group D (7/48; 15%), and group B1 (3/48; 6%). Among patients with studied recurrences, often one (18/26; 69%) or two (7/26; 27%) different *E. coli* phylogenetic groups were found in a particular patient during the RUTI course. However, one patient was even infected with three different phylogenetic groups of *E. coli* during different infection episodes.

Antimicrobial susceptibility profiles

More than a half of the investigated strains (56/89; 63%) were resistant at least to one of the studied antibiotics. Antibacterial resistance was found as follows: trimethoprim-sulfamethoxazole 25/89 (28%), ampicillin 39/89 (44%), cefuroxime 25/89 (28%), cefotaxime 3/89 (3%), gentamicin 2/89 (2%), and ciprofloxacin 0/89. Overall, betalactam resistance was 61% (54/89). A high prevalence of patients with UTI recurrences were infected with betalactam-resistant strains (17/26; 65%).

We did not find differences in antibiotic resistance between different phylogenetic groups, index and recurrent strains, or changes in antimicrobial resistance during the infection course. Similarly, no association between antibiotic susceptibility and phylogenetic groups was found among persisting strains.

Molecular typing results of RUTI isolates

Pulsed-field gel electrophoresis was applied to 74 of *E. coli* isolates from 26 patients, and it revealed 29 individual and 45 clonal strains belonging to 20 different genotypes. In 20/26 (77%) of patients, the single unique clonal strains were detected.

The index *E. coli* strain was found during recurrences in 16 of 26 (62%) patients and in 4 patients (15%), clonal strains appeared only during recurrences. Altogether, in 15/26 (58%) of patients, individual *E. coli* strains alone or together with clonal strains were detected.

In 35% (9/26) of patients, *E. coli* strains were isolated during more than 6 months of follow-up. The persistence of clonal strains was detected in 12/20 (60%) of patients for longer than 3 months and among them in 2/20 (10%), even for longer than 6 months (Table 2).

Among the UTI patients with molecularly characterized recurrences (n = 26; Table 3), the persistence occurs most often by phylogenetic group B2 (14/20; 70%). The index episodes were more common in strains of phylogenetic group B2 compared with non-B2 group (18/20 vs 8/15, respectively, p = 0.022).

When comparing clonal relapses caused by the same strain as the initial infectious episode (n = 16 patients), phylogenetic group B2 (13/16, 81%) was shown to be prevalent when compared with groups A (0/16, 0%), B1 (1/16, 6%), and D (2/16, 13%) (Table 3).

There were no differences in antibacterial resistance between clonal and individual strains. At the same time, the clonal strains compared with individual strains possessed lower sensitivity to cefotaxime (MIC median 0.079 vs 0.032, respectively; p = 0.004) and gentamicin (MIC median 0.75 vs 0.5, respectively; p = 0.011).

Prevalence of integrons

The resistance-encoding class 1 integrons were found from 67/89 (75%) of the *E. coli* strains.

<table>
<thead>
<tr>
<th>Phylogenetic group of <em>E. coli</em></th>
<th>No. of strains</th>
<th>Recurrent episodes</th>
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<td>n</td>
<td>First attack of APN</td>
<td>Recurrent episodes</td>
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<td>Total Betalactam resistance</td>
<td>SXT resistance</td>
<td>intI gene containment</td>
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<tr>
<td>B2</td>
<td>51</td>
<td>25 9 6 23</td>
<td>26 11 7 22,2,3,4</td>
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<tr>
<td>non-B2</td>
<td>38</td>
<td>16 6 5 14</td>
<td>22 11 6 81</td>
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<tr>
<td>A</td>
<td>16</td>
<td>4 1 1</td>
<td>12 5 5 62</td>
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<td>B1</td>
<td>4</td>
<td>1 0 0</td>
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<td>D</td>
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<td>11 5 4 11</td>
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SXT, trimethoprim-sulfamethoxazole. \(^1\) p < 0.001. \(^2\) p = 0.045. \(^3\) p = 0.010. \(^4\) p = 0.009.
E. coli strains from phylogenetic group B2 consisted more often resistance-encoding integrons compared with group A (45/51 vs 8/16, respectively; \( p = 0.003 \)) and B1 (45/51 vs 1/4, respectively; \( p = 0.012 \)) (Table 1). Nearly all (25/26; 96%) patients surveyed for recurrences were infected with at least one integron positive strain during the course of infection. Initial E. coli strains had a higher integron occurrence compared with recurrent ones (37/41 vs 30/48, respectively; \( p = 0.003 \)). Statistically significant differences in the integron containment of initial vs recurrent strains among phylogenetic group D were found (11/11 vs 3/7, respectively; \( p = 0.003 \)). There were no differences in antibiotic resistance according to sensitive-intermediate-resistant (SIR) designations among integron-positive and -negative strains. However, \( \text{IntI} \) positive compared with negative strains had higher MIC values of cefuroxime (MIC median 4.0 vs 2.0, respectively; \( p = 0.001 \)), cefotaxime (MIC median 0.079 vs 0.032, respectively; \( p = 0.014 \)), and gentamicin (MIC median 0.75 vs 0.44, respectively; \( p = 0.013 \)).

The presence of integrons was similar in clonal (34/45) and individual E. coli strains (18/29) in patients with RUTI.

### Table 2. Timetable of urinary tract infection episodes (index and recurrent) and molecular type pattern of E. coli isolates according to pulsed-field gel electrophoresis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>No. of episodes</th>
<th>No. of strains</th>
<th>Index 2 weeks to 3 months</th>
<th>3–6 months</th>
<th>6–9 months</th>
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\( \text{ind, individual strain; A–T, different E. coli clones.} \)

### Table 3. Patients (n = 26) with recurrent isolates

<table>
<thead>
<tr>
<th>Phylogenetic group of Escherichia coli</th>
<th>No. of patients</th>
<th>Persistence of index strain</th>
<th>Total at any UTI episode</th>
<th>Persistence of any strain</th>
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<tbody>
<tr>
<td>B2</td>
<td>18(^{3,4})</td>
<td>13(^{4})</td>
<td>20</td>
<td>14</td>
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<tr>
<td>non-B2</td>
<td>8(^{3})</td>
<td>3(^{a})</td>
<td>15</td>
<td>6</td>
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<tr>
<td>A</td>
<td>3(^{3})</td>
<td>0(^{4})</td>
<td>7</td>
<td>2</td>
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<tr>
<td>B1</td>
<td>1(^{a})</td>
<td>1(^{a})</td>
<td>3</td>
<td>1</td>
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<tr>
<td>D</td>
<td>4(^{3})</td>
<td>2(^{a})</td>
<td>5</td>
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\( ^{3}\text{Strains isolated from either index and/or recurrent episodes. UTI, urinary tract infection.} \)

**DISCUSSION**

We found a high prevalence of E. coli phylogenetic group B2 in initial attacks and recurrences of childhood UTI. At the same time, in the case of phylogenetic group A as the first episode of UTI, the following infectious episodes were caused by genetically different strains. During particular infectious episodes, besides different genotypes, different phylogenetic groups occurred in studied E. coli strains.
*Escherichia coli* phylogenetic group B2 has been shown to be highly uropathogenic in several studies (9, 19, 20). Similarly, in our study, phylogenetic group B2 was the leading cause of childhood RUTI and also caused the initial attack of pyelonephritis more often than the strains of phylogenetic group non-B2. This is by our understanding the first report on B2 group preference for clonality and relapses of childhood RUTI.

In the case of the index strain of phylogenetic group A, recurrences were caused by other phylogenetic groups. Concerning healthy children, a similar connection has been observed among fecal *E. coli* isolates where phylogenetic groups B2 and D were dominating and appeared clonal, unlike more genetically unique strains among groups A and B1 (4).

Predisposing factors like urinary tract abnormalities and individual genetic differences are believed to cause higher risk for developing UTI and recurrences leading to renal scarring (21–23). UTI and subsequent bacteremic complications caused by phylogenetic group A have been associated with predisposing factors like abnormalities in urinary tract and immunocompromised status (24, 25). The latter has been associated with the lower amount of virulence genes of phylogenetic group A as compared with B2 (5, 6, 9, 19). Nevertheless, in our study, predisposing factors like VUR and/or a functional disorder of the bladder did not influence the phylogenetic belonging of *E. coli* infecting children either in the initial attack or in the recurrences of UTI.

We found that altogether in 74% of children with RUTI, one particular strain reappeared in following infectious episodes. This observation is in accordance with studies of Ejrnaes et al. (13) and Jantunen et al. (26), who found that relapses of UTI are more frequent than reinfections by a new strain, although they did not associate this with the phylogenetic grouping of *E. coli* strains. The strength of our study appears in the long follow-up period (1 year), as, in almost half of patients, RUTI episodes continued over a 6-month period. We found that in 80% (16/20) of patients with clonal strains, the first infection episode was caused by a strain that remained persistent. It has been suggested that the occurrence of relapses is due to either higher virulence of the index strains of the B2 group, e.g., *papG* II, USP genes, or biofilm production, or increased antibiotic resistance markers (24, 25, 27). In our study, no differences in antibacterial resistance between phylogenetic groups in the index or recurrent strains were found.

The main cause of an absence of an antibacterial effect may be connected to predisposing factors like vesicoureteral reflux (21), forming extra- and intracellular bacterial biofilm-like communities within the bladder (28), too short courses of antibiotic therapy (29), or inherent heterogeneity of a bacterial population where persisters have reduced growth rate and attended to higher adaptation to unfavorable environmental conditions during antibiotic exposure (30).

On the other hand, the reappearing *E. coli* strain may derive from patients’ gut microbiota and therefore not be affected by antibiotics active in the urinary tract. Recurrent dislocation of particular *E. coli* strains into the urinary tract under the predisposing factors may lead to RUTI caused by the same strain.

The studies about the prevalence of main phylogenetic groups of *E. coli* in the human gut have revealed differences between countries indicating either group A or B2 being predominant in feces (31). Although the intestinal tract has been suggested as the main reservoir for UTI-causing *E. coli* strains (32, 33), the predominating phylogenetic group in UTI is B2 in different countries (34). This indicates the importance of virulence factors rather than the size of the infecting bacterial load in UTI pathogenesis.

Integrons as mobile genetic elements contain resistance genes, which can move from one bacterium to another and lead to the spread of antibiotic resistance. In our study, phylogenetic group B2 more often carried resistance-encoding class 1 integrons compared with non-B2 group; nevertheless, there were no differences in antibacterial susceptibility among phylogenetic groups. A possible linkage between virulence genes and class 1 integrons revealed differences between countries indicating either group A or B2 being predominant in feces (35). The coexistence of a higher load of virulence genes (27) and integrons of phylogenetic group B2 may possess an advantage in the infectious process. In previous studies of mostly non-UTI isolates, phylogenetic group B2 has been shown to be more sensitive and tended to have less integrons (8, 10). This indicates the possible different survival strategies of *E. coli* in different body sites.

Integron-containing strains have been shown to emerge phenotypic antibacterial resistance (10, 36). We found a high prevalence (67/89; 75%) of class 1 integrons in UTI *E. coli* isolates; nevertheless, the antibacterial resistance of integron-containing strains was not higher compared with integron negative ones. At the same time, higher doses according to increased MIC values of certain antibiotics (cefuroxime, cefotaxime and gentamicin) should be needed for bactericidal action. In our study, overall and especially in phylogenetic group D, integron containment was higher in the index compared with recurrent strains. The study of
Kärkkäinen et al. (37) revealed more virulence-associated factors among the index compared with recurrent isolates. Antibacterial resistance did not change during the RUTI episodes in our study. Possessing resistance genes may be important in the initial infection process for bacteria to survive in the host organism. In the prolonged infection course, different virulence factors might be emphasized and keeping resistance genes as energy-consuming process is abundant.

Thus, our study confirms the importance of phylogenetic group B2 in the first attack of APN and recurrent UTI episodes.

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