Persistence of *Escherichia coli* Clones and Phenotypic and Genotypic Antibiotic Resistance in Recurrent Urinary Tract Infections in Childhood

Siiri Köljalg,1* Kai Truusalu,1 Inga Vainumäe,2 Jelena Sstepetova,1 Epp Sepp,1 and Marika Mikelsaar1

Institute of Microbiology, University of Tartu, Ravila 19, 50411,1 and Department of Paediatrics, University of Tartu, Lunini 6, 51014,2 Tartu, Estonia

Received 24 July 2008/Returned for modification 14 September 2008/Accepted 23 October 2008

We assessed the clonality of consecutive *Escherichia coli* isolates during the course of recurrent urinary tract infections (RUTI) in childhood in order to compare clonality with phenotypic antibiotic resistance patterns, the presence of integrons, and the presence of the sul1, sul2, and sul3 genes. Altogether, 78 urinary *E. coli* isolates from 27 children, who experienced recurrences during a 1-year follow-up after the first attack of acute pyelonephritis, were investigated. The MICs of sulfamethoxazole, trimethoprim-sulfamethoxazole (SXT), ampicillin, cefuroxime, cefotaxime, and gentamicin and the presence or absence of the intI gene for class 1 integrons and the sulfamethoxazole resistance-encoding genes sul1, sul2, and sul3 were determined. All *E. coli* strains were genotyped by pulsed-field gel electrophoresis. There were no significant differences in the prevalences of resistance to beta-lactams and SXT between initial and consecutive *E. coli* isolates (41 versus 45% and 41 versus 29%, respectively). However, the *E. coli* strains obtained after SXT administration more frequently carried two or more sul genes than the nonexposed strains (9/21 [43%] versus 11/57 [19%], respectively; \( P = 0.044 \)). In 78% of the patients, the recurrence of unique clonal *E. coli* strains alone or combined with individual strains was detected. Phenotypic resistance and the occurrence of sul genes were more stable in clonal strains than in individual strains (odds ratios, 8.7 [95% confidence interval (95% CI), 1.8 to 40.8] and 4.4 [95% CI, 1.1 to 17.7], respectively). Thus, in children with RUTIs, the majority of *E. coli* strains from consecutive episodes are unique persisting clones, with rare increases in the initially high antimicrobial resistance, the presence of sul genes, and the presence of integrons.

Persistent urinary tract infections usually emerge in early childhood. Approximately 1 to 8% of children between the ages of 1 month and 11 years have experienced at least one urinary tract infection (23, 31, 41, 42). Recurrent urinary tract infection (RUTI) endangers renal function; even the first episode of acute pyelonephritis can lead to renal scarring in 9.5% to 57% of cases, according to Hoberman et al. (17) and Lin et al. (34), respectively. In childhood the most important risk factor for RUTI has been considered to be the presence of vesicoureinary reflux, alone or combined with dysfunctional voiding (3, 49, 58).

The second important risk factor is closely associated with antimicrobial therapy for RUTI. In children, as in adults, the most frequent urinary pathogen is *Escherichia coli*, and the prevailing treatment schemes include the beta-lactam antibiotics, trimethoprim-sulfamethoxazole (SXT), and aminoglycosides (1, 25). However, increased resistance among urinary *E. coli* strains to some beta-lactam antibiotics and SXT has been reported in different countries (18, 27). Furthermore, children with vesicoureinary reflux require long-term antimicrobial prophylaxis (20, 39), usually with SXT or nitrofurantoin. This, in turn, can select bacteria with increased resistance during the course of RUTI (8).

Until lately, RUTI has been considered mostly as reinfections of the urinary tract with consecutive new strains of *E. coli*. This concept accepts the antimicrobial pressure during therapy as a selective and driving factor for the appearance of new infecting antibiotic-resistant *E. coli* strains from the large reservoir of patients’ intestinal tracts (50) and suggests a long-term follow-up of phenotypic antibiotic resistance in emerging strains.

The availability of highly discriminative genotyping methods has changed the paradigm of RUTI as reinfections: a high incidence of persistent urinary strains both in adults (10, 48) and in children (22) has been documented. However, the role of the failure of antimicrobial therapy, due to initial or changed antimicrobial resistance after a horizontal transfer of genes or gene cassettes (26, 29), in causing the persistence of *E. coli* in the urinary tract is not well known. Also, no association between the presence of integrons and sul genes, in either clonal relapsing or recurrent individual *E. coli* strains, and antibiotic therapy has been established yet.

The aim of the pediatric study was to assess the clonality of consecutive *E. coli* isolates during the course of RUTI and to compare it with phenotypic antibiotic resistance patterns, the presence of integrons, and the presence of the sul1, sul2, and sul3 genes. Additionally, the association between the sul genes and SXT treatment was determined.

**MATERIALS AND METHODS**

*Patients and strains.* This study was part of a prospective 1-year follow-up study of 64 children for whom a first occurrence of acute pyelonephritis (International Classification of Diseases classification no. 10) was confirmed bacteriologically by the isolation of a single uropathogen before antibacterial treatment. The diagnostic criteria for acute pyelonephritis (modified from the work of Pylkkänen et al. [46]) were as follows: fever with an axillary temperature of \( \geq 38.5^\circ\text{C} \) or a rectal temperature of \( \geq 39.0^\circ\text{C} \), a serum C-reactive protein concentration of \( \geq 20 \) mg/liter, bacteriuria (a CFU count of \( \geq 10^5\) ml), and pyuria (a

* Corresponding author. Mailing address: Institute of Microbiology, University of Tartu, Ravila 19, 50411, Tartu, Estonia. Phone: 372 7 374170. Fax: 372 7 374172. E-mail: Siiri.Koljalg@klinikum.ee.

* Published ahead of print on 29 October 2008.
white blood cell count in urine of >5/µl high-power field). The recurrence of UTI was defined as an episode occurring at least 2 weeks after the end of the initial antibacterial treatment. The scheduled urine samples were collected within 2 weeks and 1, 2, 3, 6, and 9 months and 1 year after the initial (index) episode, and recurrences were found for half of the patients (32/64). The present study focused on RUTIs where E. coli was isolated in the index episode and also in a recurrent episode(s) and where the subsequent isolates were available for the study.

We studied a total of 78 urinary E. coli isolates recovered from 27 children (21 girls and 6 boys) with RUTI. The patients’ ages ranged from 2 to 180 months (median age, 38 months). The patients’ data regarding SXT administration were analyzed. E. coli strains were isolated after the urine samples had been plated on a cystine-lactose-electrolyte-deficient (CLED) medium, and the isolates were identified by standard laboratory methods (6). The bacterial isolates were stored at ~80°C for further studies.

**Antibacterial susceptibility testing.** The susceptibilities of E. coli strains to antibacterial agents were determined according to standard laboratory procedures (7) using Etest (AB Biodisk, Solna, Sweden). MICs of sulfamethoxazole, ampicillin, cefotaxime, cefuroxime, and gentamicin were determined according to the manufacturer’s instructions. Briefly, Mueller-Hinton agar plates were inoculated with a bacterial suspension corresponding to a 0.5 McFarland optical density standard. Etest strips were placed on the surfaces of the agar plates. The plates were incubated under an aerobic atmosphere at 37°C. MICs were registered after 24 h at the point of intersection between the elliptical zone of inhibition that developed on the test strip and the zone of microbial growth.

**PFGE.** Pulsed-field gel electrophoresis (PFGE) (9) was used to compare a patient’s E. coli isolates in order to distinguish between relapses due to persistence and those due to the acquisition of a new strain. Bacterial strains were prepared as described in the manufacturer’s (Bio-Rad, France) instructions. Briefly, one colony was picked up from blood agar and inoculated into 5 ml of Trypticase soy broth. Cells were grown overnight at 37°C and pelleted at 12,000 × g for 30 min. DNA was extracted by using a commercially available PFGE kit with NotI and XbaI enzymes (GenePath; Bio-Rad, France). Electrophoresis was performed in a CHEF-DR II apparatus (Bio-Rad, France). Gene Tools software (version 1.2; Syngene, United Kingdom) was used for processing the gel images. PFGE results were considered discriminatory if a pattern of at least 10 bands formed. Isolates with identical patterns or with a difference of 1 to 3 fragments were defined as clonal (52) and therefore indicated a relapse of UTI.

**Preparation of bacterial DNA and detection of class 1 integrons and sul resistance genes by PCR.** The total DNA of E. coli was extracted by using a QIAamp DNA minikit (Qiagen, Germany) according to the manufacturer’s protocol.

All strains were tested for the presence of class 1 integrons by PCR amplification of a class 1 integrase-specific fragment of the intI1 gene. The primer sequences used were 5’-AGCATCTGAAGAGCGAAG-3’ and 3’-CS (5’-AAGCAGACCTGACCTGA-3’) (33). The primers for the amplification of the sul1, sul2, and sul3 genes were sul1-F (5’-GTGACGGTGTTCGACATTCT-3’) and sul1-R (5’-TTTACGGAGGACCAAGCTT-3’), sul2-F (5’-TCAAGGACATGGCATTCC-3’) and sul2-R (5’-ATCGAAGGCAGCCGAAT-3’), and sul3-F (5’-GAGCAAGATTGGGAATC-3’) and sul3-R (5’-CATGTCAGCTAACTAGGTTCA-3’) (11, 32, 44).

**Statistical analysis.** Statistical analyses were performed using the SigmaStat (Jandel Scientific) and Excel (Microsoft Corp.) software programs, employing the Fisher exact test, the chi-square test, and the Mann-Whitney rank sum test. P values less than 0.05 were considered statistically significant.

### RESULTS

**Number of isolates from consecutive episodes.** During a 1-year follow-up period, a total of 2 to 6 (mean, 2.9 ± 1.2) E. coli isolates per patient were obtained from the index episode and recurrent episodes.

**Antimicrobial susceptibility profiles.** Of 78 isolates, 26 (33%) were resistant to SXT and 61 (78%) to sulfamethoxazole. Of these 61 sulfamethoxazole-resistant strains, 36 (59%) were sensitive to the SXT combination. One of 78 isolates (1%) was resistant to gentamicin, 31/78 (40%) to ampicillin, 13/78 (17%) to cefuroxime, and 2/78 (3%) to cefotaxime, whereas 34/78 (44%) were resistant to beta-lactams overall. No statistically significant shifts were found in the antimicrobial resistance pattern or MICs during particular RUTI courses when initial and recurrent E. coli strains were compared (Table 1).

**Genotypic characteristics of recurrences.** PFGE with the NotI enzyme gave discriminatory results for 63/78 isolates (81%). The remaining 15 E. coli strains were successfully typed using the XbaI enzyme. In addition, the molecular clonality of 16 strains restricted with the NotI enzyme was confirmed with the XbaI enzyme.

According to a PFGE profile pattern, unrelated (clones B to X) E. coli strains were detected in 21/27 patients (78%) (Fig. 1). For 11 of these patients, the persistence of clonal strains (n = 24) was found; for 10 patients, both clonal (n = 24) and individual (n = 16) strains were found; and for the remaining 6 patients, only individual strains (n = 14) were detected (Table 2). On the other hand, 16/27 (59%) patients had more than one strain during the RUTI course. Initial infecting strains reappeared in 16 out of 27 (59%) patients during the RUTI; in 11 of these patients, only clonal strains were detected, and in 5 patients, clonal and individual strains were consecutively detected. In the remaining 11 patients, the index strain was individual, and recurrences were caused by other individual strains (6 patients) or by clonal strains that appeared later (5 pa-

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**TABLE 1. Resistance patterns of initial and recurrent E. coli strains**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. (%) resistant</th>
<th>MIC (µg/ml)</th>
<th>Range</th>
<th>50%*</th>
<th>No. (%) resistant</th>
<th>MIC (µg/ml)</th>
<th>Range</th>
<th>50%*</th>
<th>No. (%) resistant</th>
<th>MIC (µg/ml)</th>
<th>Range</th>
<th>50%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>31 (40)</td>
<td>0.5–256</td>
<td>6</td>
<td></td>
<td>9 (33.3)</td>
<td>0.5–256</td>
<td>4</td>
<td></td>
<td>22 (43.1)</td>
<td>1–256</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>13 (17)</td>
<td>0.016–256</td>
<td>3</td>
<td></td>
<td>7 (25.9)</td>
<td>0.016–48</td>
<td>4</td>
<td></td>
<td>6 (11.8)</td>
<td>0.047–256</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>2 (3)</td>
<td>0.016–256</td>
<td>0.064</td>
<td></td>
<td>1 (3.7)</td>
<td>0.016–64</td>
<td>0.064</td>
<td></td>
<td>1 (2.0)</td>
<td>0.016–256</td>
<td>0.064</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1 (1)</td>
<td>0.19–256</td>
<td>0.5</td>
<td></td>
<td>0 (0)</td>
<td>0.19–12</td>
<td>0.5</td>
<td></td>
<td>1 (2.0)</td>
<td>0.25–256</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>26 (61)</td>
<td>0.016–32</td>
<td>0.064</td>
<td></td>
<td>11 (40.7)</td>
<td>0.023–32</td>
<td>0.064</td>
<td></td>
<td>15 (29.4)</td>
<td>0.016–32</td>
<td>0.064</td>
<td></td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>61 (78)</td>
<td>3–512</td>
<td>32</td>
<td></td>
<td>20 (71.4)</td>
<td>6–512</td>
<td>32</td>
<td></td>
<td>41 (80.4)</td>
<td>3–512</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

* 50%; MIC at which 50% of isolates were inhibited.
tients). The patients infected with individual strains only were younger than the others (median ages, 7.5 versus 48 months; P = 0.03).

**Phenotypic resistance of the index strain.** In the comparison of initial individual and clonal *E. coli* strains, a nonsignificant trend toward a higher prevalence of phenotypic resistance to the antibiotics investigated was found for the initial individual strains (7/11 [64%] versus 7/16 [44%), respectively; P = 0.440). In contrast, the gentamicin and cefotaxime MICs for the clonal strains were higher than those for the individual index strains (median gentamicin MICs, 0.75 and 0.38 μg/ml, respectively [P = 0.046]; median cefotaxime MICs, 0.094 and 0.032 μg/ml, respectively [P = 0.0079]).

**Prevalence of integrons.** The resistance-encoding class 1 integrons were found in 55 (71%) of the *E. coli* strains. The MICs of cefuroxime, cefotaxime, and gentamicin were higher for *intI*-positive than for *intI*-negative strains (median cefuroxime MICs, 4.0 and 2.0 μg/ml, respectively [P = 0.001]; median cefotaxime MICs, 0.064 and 0.032 μg/ml, respectively [P = 0.03]; median gentamicin MICs, 0.75 and 0.5 μg/ml, respectively [P = 0.009]). Initial *E. coli* strains had a nonsignificantly higher integron occurrence than recurrent strains (23/27 versus 32/51, respectively; P = 0.066). The presence of integrons was similar in clonal (35/48) and individual (20/30) *E. coli* strains.

**Distribution of the sulfamethoxazole resistance-encoding *sul* genes.** *sul* genes were found in 42 (54%) *E. coli* strains (Table 3). Overall the most prevalent *sul* gene was *sul2*, found in 31/78 (40%) strains, followed by *sul1* (25/78 [32%]) and *sul3* (9/78 [12%]). Particular strains possessed different *sul* gene combinations: *sul1* along with *sul2* and *sul2* alone were the most common patterns, each detected in 12 out of 78 *E. coli* strains. Phenotypic resistance to sulfamethoxazole was more prevalent among strains harboring any *sul* gene than among those harboring none (37/42 [88%] versus 24/36 [67%]; P = 0.029) (Table 3).

The initial infecting *E. coli* strains and the strains isolated in recurrences harbored *sul* genes with equal frequencies (7/27 versus 13/51, respectively; P = 1.000). The individual *E. coli* strains harbored more *sul1* genes and more of the combination of *sul1* with *sul2* than the clonal strains (15/30 versus 10/48 [P = 0.031] and 9/30 versus 3/48 [P = 0.008], respectively) (Table 3).

**Stability of resistance characteristics during RUTI.** During different infection episodes of a single patient, phenotypic antibiotic resistance and the occurrence of *sul* genes were more stable for patients harboring clonal strains than for those with individual strains (14/21 versus 3/16 patients [odds ratio, 8.7 {95% confidence interval, 1.8 to 40.8}], respectively, with antibiotic-resistant strains and 14/21 versus 5/16 patients [odds ratio, 4.4 {95% confidence interval, 1.1 to 17.7}], respectively, with *sul* gene-harboring strains). At the same time, the integron occurrence was stable irrespective of clonal or individual strain recurrences.

Among consecutive clonal strains, the phenotypic resistance, the presence of *sul* genes, and the occurrence of integrons showed the high stability of the markers (in 70, 67, and 74% of isolates, respectively) relative to the preceding *E. coli* strain.

**Influence of SXT treatment.** SXT was used for treatment of 10 out of 27 patients, and 21 out of 78 *E. coli* strains were isolated after SXT exposure. *E. coli* strains isolated after SXT treatment were more resistant than nonexposed strains to SXT (median MICs, 32 versus 0.064 μg/ml, respectively [P = 0.022]; 11/21 strains [53%] resistant versus 15/57 [19%], respectively [P = 0.056]), sulfamethoxazole (median MICs, 512 versus 32 μg/ml, respectively [P = 0.035]), and ampicillin (median MICs, 256 versus 4 μg/ml, respectively; 14/21 strains [67%] resistant versus 17/57 [30%], respectively [P = 0.004]).

The *E. coli* strains obtained after SXT treatment more frequently had two or more *sul* genes than nonexposed strains (9/21 [43%] versus 11/57 [19%] strains, respectively; P = 0.044). In two out of four patients who had the same clonal strains before and after SXT treatment, additional *sul* genes were inserted, while for the other two patients, no changes occurred after SXT treatment.

**DISCUSSION**

In 78% of the children in our study, unique clones of *E. coli* persisted during different RUTI episodes. At the same time, about half of the patients (59%) were infected with more than one *E. coli* strain during a RUTI course.

Among clonal isolates, the overall frequency of antibacterial resistance did not increase and the occurrence of resistance-encoding genes was unaltered during recurrences. These findings indicate the high prevalence of relapses due to persisting strains in children with RUTI, seemingly starting with difficulties in the eradication of the infectious agent during the first attack of acute pyelonephritis. On the other hand, SXT treatment enhanced the presence of *sul* genes, resulting in the higher resistance of *E. coli* strains to SXT and sulfamethoxazole.

Our findings with regard to the overall high resistance of urinary *E. coli* strains to antibiotics such as SXT (33%), ampicillin (40%), and any beta-lactams studied (44%) are in agreement with those of other recent studies (2, 13, 27, 45). This shows the limited possibility of using these antibiotics in the empirical treatment of UTIs. On the other hand, we found that persistent strains were less sensitive, with higher MICs of cefotaxime and gentamicin, the drugs of choice in UTI treatment. It is possible that insufficient concentrations of the antibiotics at the infection site could be responsible for the reemergence of infections caused by the same strain.
TABLE 2. Characteristics of index and consecutive *E. coli* strains (*n* = 78) classified into clonal and individual isolates during different RUTI episodes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Strain</th>
<th>Clonal isolates</th>
<th>Individual isolates</th>
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<tr>
<td></td>
<td></td>
<td>Clone^a</td>
<td>Resistance^b</td>
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<tr>
<td>1</td>
<td>B</td>
<td>AMP, CXM, SXT</td>
<td>1, 2</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>AMP, CXM, SXT</td>
<td>1, 2</td>
</tr>
<tr>
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<td>K</td>
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</tr>
<tr>
<td>5</td>
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<td>AMP, SXT</td>
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<tr>
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<tr>
<td>19</td>
<td>Ind</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>20</td>
<td>R</td>
<td>AMP, SXT</td>
<td>1, 2, 3</td>
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</table>

With both clonal and individual isolates

<table>
<thead>
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<th>Patient</th>
<th>Strain</th>
<th>Clonal isolates</th>
<th>Individual isolates</th>
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<td>None</td>
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<tr>
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</tr>
<tr>
<td>14</td>
<td>I</td>
<td>None</td>
<td>None</td>
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<tr>
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<td>U</td>
<td>AMP</td>
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</tr>
<tr>
<td>20</td>
<td>R</td>
<td>AMP, SXT</td>
<td>1, 2, 3</td>
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High incidences of relapses due to bacterial persistence during RUTI have also been shown in several other studies with adults (77% [Ejrnaes et al. (10)] and 68% [Russo et al. (48)]) and children (65% [Jantunen et al. (22)]). In contrast, in some papers, mainly new individual strains are associated with UTI recurrences (4, 12, 19, 28). This disagreement may result from methodological differences, since in the latter studies PFGE was not used to identify the strains. PFGE has now been used for more than 2 decades and is considered the "gold standard" in epidemiological typing. Still, several authors (22, 51) have reported E. coli strains that are nontypeable by use of PFGE. We have solved this problem by using the additional restriction enzyme XbaI.

A possible explanation of our finding of frequent relapses in childhood RUTI is that intracellular clones of E. coli may resist antibacterial treatment. Recent experimental studies have shown that the majority of urinary E. coli strains are able to form intracellular bacterial colonies in the bladder epithelia of mice (14, 37, 38) and thus to survive. The same kind of mechanism has been suggested for humans (47). Therefore, in order to treat the first attack of acute pyelonephritis efficiently, the intracellular location of bacteria should be considered and the proper antimicrobial that will penetrate human cells should be chosen (e.g., ciprofloxacin). The frequently used beta-lactams appear to be risk factors for RUTI (I. Vainumäe, K. Truusalu, K. Ulst, S. Köljalg, T. Talvik, and M. Mikelsaar, submitted for publication). Moreover, we have found frequent intermittence of clonal and individual E. coli strains in patients' urine samples during different RUTI episodes in a particular patient. It is possible that E. coli strains forming intracellular bacterial colonies can facilitate the invasion of the bladder epithelium by other strains, as shown by Garofalo et al. (14).

We found that infection with a new strain was more frequent among younger children. The intestinal tract has been suggested as the main reservoir for UTI-causing E. coli strains (21, 36, 56). Following birth, the intestinal tract of a newborn is gradually colonized with different E. coli strains, and therefore,
the variety of the E. coli population could be greater in older children (40). It is possible that younger infants have not yet acquired strains that are able to form intracellular bacterial colonies, and in the presence of predisposing factors, the recurrences could be caused by new E. coli strains derived from their intestinal tracts.

The high (71%) prevalence of class 1 integrons is in agreement with the prevalence found in a Swedish study (65% [Grape et al. (15)]) on urinary E. coli isolates in a general population but higher than those in similar Indian (36% [Mathai et al. (35)]) and Korean (55%) [Yu et al. (57)]) and Australian (46% [White et al. (55)]) studies. White et al. (55) found that ampCillin, gentamicin, and trimethoprim resistance among Enterobacteriaceae was associated with integrons. In our study, MICs of gentamicin and cephalosporins were higher for integron-positive strains than for integron-negative strains, a situation that may result in difficulties in achieving proper therapeutic serum drug concentrations in cases of generalization of UTI.

Hillier et al. (16) have shown that previous antibiotic exposure is a principal risk factor for resistant E. coli in UTIs. For RUTIs, by assessing the frequent clonality of consecutive E. coli strains, we succeeded in showing that increasing resistance is not the main goal of persisting bacteria. A negative correlation between resistance and the expression of virulence factors has been found in several previous studies (24, 53, 54). Possibly, some other virulence factors, such as tissue invasion, capsulation, or slime production, may be promoted in clonal strains.

Usually sulfamethoxazole resistance is encoded by the sul1, sul2, and sul3 genes. We found that more than half (54%) of the E. coli strains possessed one or more of these sul genes, and in 88% of these strains, phenotypic sulfamethoxazole resistance occurred. In results similar to ours, the sul2 gene has been found to be predominant in E. coli strains isolated in UTI episodes (5, 30). In our study, SXT treatment was associated with the occurrence of sul genes and with increased phenotypic resistance to SXT and sulfamethoxazole. Horizontal gene transfer has been associated with escalated SXT resistance among Enterobacteriaceae (5, 43). In our study, the presence of sul genes was more stable in clonal strains than in individual strains; changes in sul gene status were mostly associated with reinfection (e.g., acquiring a new E. coli strain during a course of RUTI). The remarkable stability of resistance markers, such as phenotypic resistance patterns and sul genes, among clonal strains may be a helpful tool for the preliminary differentiation between relapse and reinfection in a particular RUTI episode.

Thus, in children with RUTI, the majority of E. coli strains from consecutive episodes are unique persisting clones, with rare increases in the initially high antimicrobial resistance, the presence of sul genes, and the presence of integrons. It appears that the difficulty of eradicating the infectious agent during the initial episode is involved in the development of a complicated course of UTI.

ACKNOWLEDGMENTS

This work was supported by Targeted Financing (SF0182555s03 and SF0180132s08) and the Estonian Science Foundation (grant 7497).

We are grateful to Agnes Laszimer for excellent technical assistance. Special thanks to Irja Lutsar for critical reading of the manuscript.

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