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Antimicrobial resistance in *Escherichia coli* in urine samples from children and adults: a 12 year analysis

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Aim: To investigate the distribution and antimicrobial resistance in urinary tract pathogens, primarily *Escherichia coli*, in two age groups, children $\leq$2 y and adults 18–50 y, over a period of 12 y. *Methods:* From the database of the microbiological laboratory all urinary tract culture data were extracted and structured according to date, patient age, bacteriological findings, antimicrobial susceptibility results and sample type. Statistical longitudinal analysis of bacteriological findings and antimicrobial resistance trends in the two age groups were performed. *Results:* Statistical significance was obtained for the following results. *Escherichia coli* was the most common pathogen in both age groups and irrespective of sample type. In *E. coli* resistance to ampicillin and trimethoprim was higher in children than in adults and increased over time in both age groups. Resistance to fluoroquinolones was higher in adults than in children and increased over time in both groups. Resistance to pivmecillinam, cefadroxil and nitrofurantoin was below 2% in 2001 in both age groups.

Conclusion: The steadily increasing and now high *E. coli* resistance levels in children to ampicillin and trimethoprim render empirical therapy with these drugs doubtful. The stable and low levels of resistance to pivmecillinam, cefadroxil and nitrofurantoin (<2% in 2001) make these drugs reasonable alternatives in uncomplicated lower urinary tract infections.

Key words: *Antimicrobial resistance, children, urinary tract infections*

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Urinary tract infections (UTI) in children, both cystitis and pyelonephritis, are common infections in all age groups. Several population-based investigations studying the incidence of UTI have been performed in Sweden. In a national multicentre study from the 1990s the minimum cumulative incidence at the age of 2 y was 2.2% in boys and 2.1% in girls (1).

Pyelonephritis in children may cause irreversible kidney damage in the form of focal renal scarring and progressive renal disease (2). Delayed diagnosis and/or inadequate treatment increase the risk of sequelae. The development of antimicrobial resistance increases the risk of inadequate treatment.

It is difficult to obtain adequate urine samples in young children not yet in control of bladder function. "Bag urine" often yields false-positive cultures (3) and suprapubic aspiration is considered the gold standard for urine sampling, but is cumbersome (4). Midstream samples are only possible in older children.

Escherichia coli is the most common cause of UTI in both adults and children. In Sweden most children with fever and suspected UTI are initially treated with trimethoprim–sulfamethoxazole, children with suspected cystitis most often receive trimethoprim or nitrofurantoin, whereas adults with uncomplicated UTI in most cases receive pivmecillinam, trimethoprim or a fluoroquinolone.

The development of antimicrobial resistance in *E. coli* from UTIs is well documented (5–7). However, no detailed comparison of rates or trends in children and adults could be found. The aim of this study was to investigate the development of antimicrobial resistance in UTI pathogens in children and adults over a long period. A time-series analysis of the period 1990–2001 was conducted, comparing children aged 0–2 y and adults aged 18–50 y.

Material and methods

Study design

This study is a retrospective, longitudinal analysis of bacteria isolated from urinary tract cultures performed in Kronoberg county, Sweden, during the period 1990–2001. The study presents species distributions and antimicrobial resistance development during the study period. All patients, irrespective of clinical symptoms and signs or mode of urine collection, in the age groups

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0–2 and 18–50 y old on whom urinary tract cultures were performed in the period 1990–2001 were included to the analysis.

Within each year repeat positive cultures were excluded, i.e. the patient was not allowed into the analysis with the same species in a urinary tract culture more than once per year.

Urinary tract cultures

Urine samples were either freshly voided midstream urine samples (adults), or collected in adhesive bags (bag urine) from infants and small children or obtained as bladder punctures via suprapubic aspiration. Cultures were quantified as the number of colony-forming units (cfu) per litre: $<10^5$, $\geq 10^5$ to $<10^8$, or $\geq 10^8$ cfu/L. Urine samples were classified as positive or negative in accordance with the guidelines for testing issued by the Infectious Disease Society of America (8).

Identification

All bacteria occurring in urine samples at $\geq 10^5$ cfu/L were identified by their biochemical reaction profile. Bacteria occurring at $<10^5$ cfu/L were not speciated. The following bacteria were classified as primary uropathogens: *E. coli*, *Staphylococcus saprophyticus* and secondary uropathogens: *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp., *Enterococcus* spp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of bacteria was determined using the disc diffusion method as described by Swedish Reference Group on Antibiotics (SRGA) (9). The medium used was Isosensitest agar (ISA; Oxoid, Basingstoke, UK). Inhibition zone diameters were measured to the nearest millimetre with a slide gauge. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality-control strains. Test results were only accepted if the inhibition zone diameters of the control strains were within performance range.

Enterobacteriaceae were tested against the following antimicrobial agents: ampicillin, pivmecillinam, cefadroxil, trimethoprim, a fluoroquinolone and nitrofurantoin. Breakpoint values for qualitative interpretation were based on the recommendations of SRGA (9).

All zone diameters were measured, stored in the database and retrieved for analysis in the present study.

Data presentation

The distribution of species for two aggregated 4 y periods (1990–1993 and 1998–2001) in the age groups 0–2 and 18–50 y old are presented. A comparison of the distributions of species in urine between bladder puncture and bag urine is presented. Serial analysis and presentation of antimicrobial resistance frequencies was performed for *E. coli* for the age groups 0–2 y and 18–50 y. The number of isolates of other bacteria did not permit age-related analysis.

Statistics

The χ^2-test was used to test for statistical significance. Linear regression analysis was used for the analysis of year versus resistance rate and comparison of resistance development rate in the two age groups (statistical software SPSS version 11.0; SPSS, Sweden).

Results

The distribution of uropathogenic bacteria for the two age groups is shown in Table 1. *E. coli* was the most common species in both age groups during the two periods.

A comparison between the species distributions in urine obtained through bladder puncture and bag urine in the age group 0–2 y is shown in Table 2. *E. coli* was the most common species in both sample types, but was significantly more dominant in bladder puncture urine. Bag urine more often yielded bacteria of doubtful clinical significance (coagulase-negative staphylococci and enterococci).

Figure 1 shows the yearly antimicrobial resistance rates during 12 y from 1990–2001 in children aged 0–2 y and in adults aged 18–50 y. Resistance to ampicillin, trimethoprim and fluoroquinolones increased in both children and adults. The increase in resistance to ampicillin and trimethoprim in children preceded the increase in adults by 2 y ($p = 0.055$) and 1 y ($p = 0.001$), respectively. For fluoroquinolones the development of resistance in adults seemed to precede resistance in children.

Decreasing resistance was registered in children to nitrofurantoin ($p < 0.046$) and in adults to cefadroxil ($p < 0.007$). Resistance to mecillinam was low and stable over the 12 y in both adults and children. The

Table 1. Species distribution in children (0–2 y) and adults (18–50 y) during 1990–1993 and 1998–2001.

	Children 0–2 y		Adults 18–50 y	
Species	1990–1993 ($n = 1014$)	1998–2001 ($n = 584$)	1990–1993 ($n = 4671$)	1998–2001 ($n = 4110$)
Escherichia coli	53.5 (±4.2)	64.8 (±5.1)	65.5 (±1.7)	73.7 (±1.5)
Enterococcus faecalis	15.4 (±5.6)	12.2 (±7.6)	9.3 (±2.8)	5.7 (±3.0)
Proteus mirabilis	7.2 (±5.1)	6.7 (±7.8)	3.8 (±2.8)	2.7 (±3.0)
Proteus vulgaris	2.4 (±6.0)	0.3 (±8.1)	0.2 (±2.8)	0.1 (±3.1)
Klebsiella pneumoniae	5.2 (±6.0)	4.1 (±8.0)	3.3 (±2.8)	3.2 (±3.0)
Klebsiella oxytica	4.3 (±6.1)	2.4 (±8.0)	0.6 (±2.9)	0.7 (±3.1)
Staphylococcus aureus	3.5 (±6.0)	1.4 (±8.0)	2.8 (±2.9)	1.4 (±3.1)
Staphylococcus saprophyticus	0.2 (±6.1)	0	9.6 (±2.7)	8.9 (±2.9)

Data are shown as percentages (confidence interval in parentheses, given as $\pm 1.96 \times$ SE).

Table 2. Distribution of uropathogens in urinary tract cultures obtained through bladder puncture and bag urine from children 0–2 y old during 1990–2001.

	Bladder puncture (n = 222)	Bag urine (n = 610)	p
Primary and secondary uropathogens			
Escherichia coli	78.4	47.7	<0.001
Enterococcus faecalis	3.6	10.8	=0.002
Proteus mirabilis	3.6	4.8	=0.582
Klebsiella pneumoniae	3.2	4.1	=0.695
Klebsiella oxytica	2.7	2.6	=0.869
Pseudomonas aeruginosa	0.5	3.6	=0.030
Others	8.1	6.4	=0.482
Subtotal	94.6	80.0	
Doubtful uropathogens			
Coagulase-negative staphylococci	3.2	17.7	<0.001
Streptococcus agalactiae	1.4	0.5	=0.378
Others	0.9	1.8	=0.542
Subtotal	5.4	20.0	
Total	100	100	

Data are shown as percentages.

isolation frequency of other pathogens was too low to permit analysis of antimicrobial resistance in the two age groups. In children <2 y only 58 *Proteus mirabilis*, 57 *Klebsiella* spp. and 111 *Enterococcus faecalis* were isolated during 1998–2001.

Discussion

As expected, *E. coli* was the most common bacterium in urine samples from children and adults. *E. coli* was significantly more common in urine from adults than in urine from children, which can probably be explained by the difficulties in obtaining high-quality urine samples from children. In the recently published ECO-sens survey of uncomplicated UTIs in women in 16 European countries and Canada (6), *E. coli* was the most common pathogen in all countries and constituted 77% of all pathogens. In a recent Swedish quality-assurance project (10) concerning UTIs in children below the age of 2 y, *E. coli* was the most common pathogen in boys (79%) and girls (89%) (pers commun with the authors of Ref. 10). The present study comprised all urine cultures irrespective of clinical background, which may explain why *E. coli* was less dominant in this material than in dedicated prospective materials.

The significant difference in quality between urine obtained from small children through suprapubic bladder puncture (significantly more *E. coli* and less coagulase-negative staphylococci and enterococci) and bag urine speaks against the use of bag urine and emphasizes the caution needed in the interpretation of the bacteriological results of a procedure that is regularly contaminated by the periurethral flora (11). This is in accordance with previous results (3, 12). The suprapubic bladder puncture technique should be used more often (10), as it prevents false-positive cultures with ensuing unnecessary investigations and irrelevant antibiotic treatment.

As shown in Table 1, the proportion of *E. coli* increased between the earlier and later years of the period in both children and adults. This was primarily due to the change in the criteria for interpreting the results of urine cultures which was instituted between the early and late part of the study: *E. coli* 10^5 cfu/L was considered of no significance in the early part of the investigation but of significance in the latter period.

Antimicrobial resistance in *E. coli* is increasing in Sweden and internationally (5–7). In some European countries resistance to ampicillin and sulfamethoxazole in *E. coli* is close to 50% and to trimethoprim and trimethoprim–sulfamethoxazole 25%. Resistance to antimicrobial agents used only in UTI (pivmecillinam, nitrofurantoin, fosfomycin) seems to be less of a problem than resistance to other antimicrobials (aminopenicillins, trimethoprim, trimethoprim–sulfamethoxazole and fluoroquinolones) (6).

In 1990 the Swedish Medicines Agency published 6–8% trimethoprim resistance in *E. coli*, but made no distinction between children and adults (13). In another study from the same year trimethoprim resistance in *E. coli* from children with their first pyelonephritis was found to be 19% (14). In 1993–1995, in a quality-assurance project initiated by the Swedish Association of Paediatric Nephrology, *E. coli* in children below the age of 2 y exhibited trimethoprim resistance of 11% (pers commun with the authors of Ref. 10).

The Swedish Reference Group of Antibiotics and its subcommittee on methodology (SRGA and SRGA-M), together with the Swedish Institute for Infectious Disease Control, have performed nationwide antimicrobial resistance surveillance in *E. coli* since the early 1990s. Resistance to ampicillin has increased from 15 to 20%, to trimethoprim from 10 to 15% and to fluoroquinolones from <1 to 5%, whereas resistance to pivmecillinam, cefadroxil and nitrofurantoin have remained <3% for many years. These results are available on the Internet (9). The present study showed a gradual increase in the resistance to ampicillin, trimethoprim and fluoroquinolones from 1990 to 2001 in both adults and children. In 2001 trimethoprim resistance in young children in this study was 17%. Children with their first acute pyelonephritis are found in this age group. By mechanisms of cross-resistance and associated resistance, *E. coli* with resistance to trimethoprim are prone to be resistant to a large number of other drugs (15), including trimethoprim–sulfamethoxazole, ampicillin, ampicillin–clavulanic acid and fluoroquinolones, making the blind choice of effective therapy in complicated UTIs in children difficult. For hospitalized patients the use of third generation cefalosporins, carbapenems and aminoglycosides still carries a <1% risk of resistance in *E. coli*.

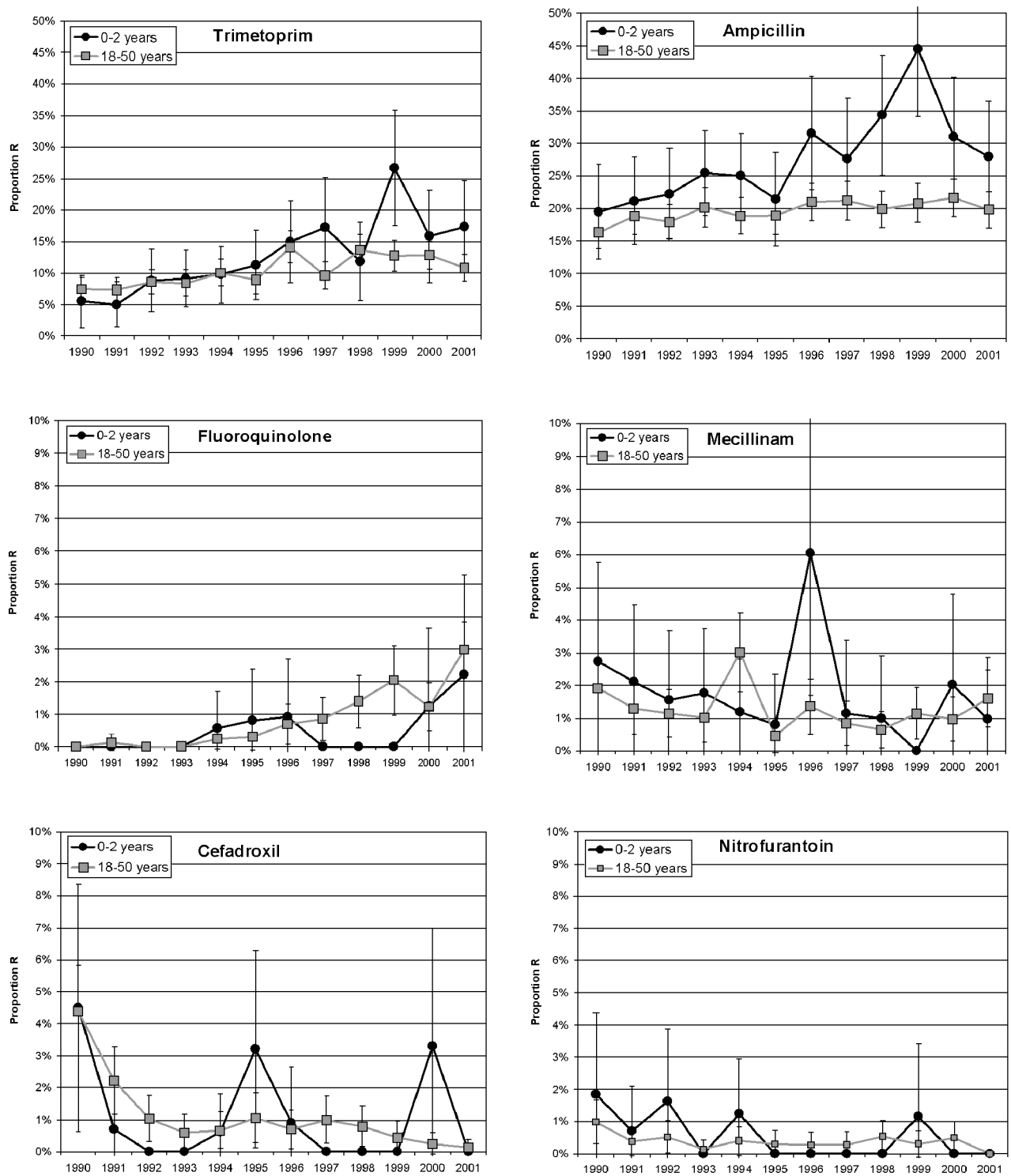

Fig. 1. Antimicrobial resistance development (%, 95% confidence interval) in *Escherichia coli* in children (0–2 y) and adults (18–50 y) during 1990–2001. For each of the curves the statistical probability for a significant positive and negative trend has been calculated. Trimethoprim for positive trend in children $p = 0.001$ and adults $p = 0.004$. Ampicillin for positive trend in children $p = 0.009$ and adults $p = 0.005$. Fluoroquinolone for positive trend in children $p = 0.038$ and adults $p < 0.001$. Mecillinam for negative trend in children $p = 0.439$ and adults $p = 0.069$. Cefadroxil for negative trend in children $p = 0.457$ and adults $p = 0.007$. Nitrofurantoin for negative trend in children $p = 0.046$ and adults $p = 0.088$.

For non-hospitalized patients the choice of an active oral cephalosporin or a fluoroquinolone are the only choices with <5% risk of resistance. For non-febrile patients with uncomplicated UTI the choice is between pivmecillinam, nitrofurantoin or an oral cefalosporin with <5% risk of resistance and trimethoprim with resistance of 17% and seemingly increasing.

The fact that resistance to pivmecillinam, cefadroxil and nitrofurantoin decreased during the 12 y study period is quite remarkable. There is no evidence of a clonal shift among *E. coli* in the early 1990s, but the trends shown in this study are suggestive.

Fluoroquinolones may be considered a reserve alternative in children with difficult-to-treat infections. With this in mind it is of concern that fluoroquinolone resistance in *E. coli* is increasing rapidly in Europe, in some areas to levels above 10% (6), and slowly but steadily in Sweden (5, 7, 16, 17). In this study, fluoroquinolone resistance increased in both adults and children, but most markedly in adults (from 0 to 3% over the study period).

E. coli with resistance to trimethoprim has been shown to spread within families (18). It is thus reasonable to assume that antimicrobial resistance in *E. coli* is to some extent dependent on cross-infection. The levels of resistance in *E. coli* in children and adults in the present study and the time sequence of the trends of resistance development indicate that trimethoprim and ampicillin resistance "leaks" from children to adults, whereas fluoroquinolone resistance leaks in the opposite direction. The significance of intrafamilial cross-infection of antibiotic-resistant *E. coli* remains to be elucidated.

In conclusion, the present study covering 12 y of *E. coli* from urinary samples in children aged 0–2 y and adults aged 18–50 y, has shown increasing resistance to ampicillin, trimethoprim and fluoroquinolone in adults and children, with higher levels of resistance to ampicillin and trimethoprim in children and higher levels of fluoroquinolone resistance in adults. Resistance to pivmecillinam, nitrofurantoin and cefadroxil remained low throughout the 12 y period. The study confirmed that suprapubic bladder puncture is superior to bag urine for urine culture and that the results of culturing bag urine may be directly misleading.

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