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312. Molecular epidemiology of ampicillin-resistant uropathogenic *E.coli* strains

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INTRODUCTION AND PURPOSE

Resistance to aminopenicillins (ampi- and amoxicillin) becomes increasingly common among *E.coli* strains causing the urinary tract infections (UTI) in outpatients.
We have studied the ampicillin-resistant uropathogenic *E.coli* strains with the aim to determine whether the difference in geographic origin and antibiotic resistance patterns correlates with molecular types of these strains.

METHODS

Bacterial isolates. In total, 271 *E.coli* strains were collected from the urine samples of outpatients with UTI in four medical centers located in 3 different cities of Russia: Moscow (2 centers), Novosibirsk (1 center) and Smolensk (1 center) in 1998. Species identification was done using the API 20E system (BioMérieux, France).

Susceptibility testing. Susceptibility of bacterial isolates to 6 antimicrobial agents: ampicillin (AM), gentamicin, nitrofurantoin, trimethoprim/sulphamethoxazole, nalidixic acid and ciprofloxacin was determined by standard agar dilution method on Mueller-Hinton agar (Becton Dickinson, USA). The reference strains: *E.coli* ATCC25922 and *P.aeruginosa* ATCC27853 were used for the quality control. Interpretation of susceptibility testing results was performed using the breakpoints recommended by NCCLS (1998).

Molecular typing by ERIC-PCR. 44 ampicillin-resistant isolates equally representing each of the 4 participating medical centers were randomly selected for genetic analysis by ERIC-PCR. Prior to PCR bacterial strains were grown overnight on MacConkey agar at 35°C. The genomic DNA was extracted from single bacterial colonies using the InstaGene matrix (BioRad, USA) in accordance with manufacturer's recommendations. ERIC-PCR was performed by adding 2 pmol of primer ERIC1 (5'-GTGAATCCCCAGGAGCTTACAT-3'), 2 µl of InstaGene preparation which contained approximately 50 ng of template DNA, and autoclaved Milli-Q water to a final volume of 25 µl to one Ready-To-Go® PCR Bead (Amersham Pharmacia Biotech, USA). The amplification reactions were carried out in a PTC-200 thermal cycler (MJ Research, USA) under the following conditions: initial 3 min denaturation at 95°C followed by 35 cycles: 1 min annealing at 47°C, 1 min extension at 72°C and 30 sec denaturation at 94°C with a final 4-min extension step. After electrophoresis in 1.3% agarose gel the DNA fragments were stained with ethidium bromide and documented using the PhotoDoc-IT Link Gel Documentation System (UVP, USA). Cluster analysis of genomic fingerprints was done using the GelCompar software (Applied Maths BVBA, Belgium) by the unweighted pair group method using arithmetic averages (UPGMA).

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RESULTS

Analysis of susceptibility testing of 271 *E.coli* strains isolated from outpatients with UTI in Russia has demonstrated the highest rate of resistance to ampicillin (33.2%) among all antimicrobials tested. About 48% of AM-resistant strains were simultaneously resistant to trimethoprim/sulphamethoxazole; 16% - to gentamicin; 11% - to nalidixic acid and 7% - to nitrofurantoin or ciprofloxacin.

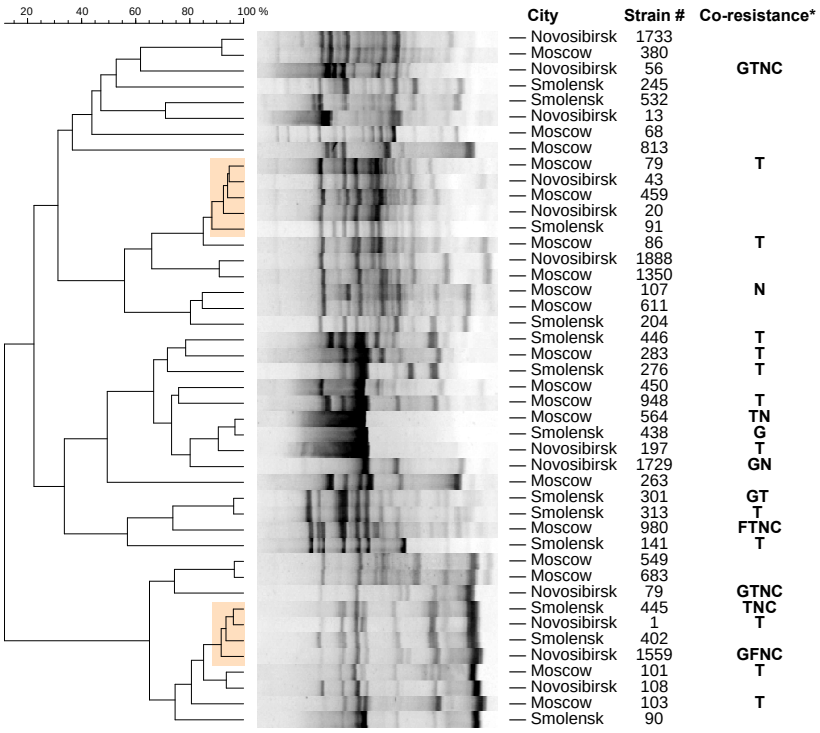

Figure 1. UPGMA clustering of ERIC1 banding patterns obtained with 44 ampicillin-resistant uropathogenic *E.coli* isolates and their phenotypic characters.

* - Antibiotic resistance in addition to ampicillin-resistance marker shared by all the strains:
G - gentamicin; **F** - nitrofurantoin; **T** - trimethoprim/sulphamethoxazole;
N - nalidixic acid; **C** - ciprofloxacin.

Genomic fingerprinting by ERIC-PCR revealed diversity of AM-resistant strains. Forty four isolates were allocated in 30 genotypes, of which 21 (47.7%) were represented by single strain. Two genotypes comprised 5 and 4 isolates respectively (shown by color-marked branches on Figure 1). These isolates although sharing very similar ERIC-PCR patterns were obtained from geographically distant areas and possessed different antibiogram patterns.
Among the isolates analysed by ERIC-PCR, 8 strains co-expressed resistance to several (2-4) non-β-lactam agents in addition to AM resistance. All multi-drug-resistant isolates appeared to be genetically unrelated according to ERIC-PCR typing.

CONCLUSIONS

We conclude that a high degree of genotypic variability is characteristic of uropathogenic *E.coli* strains within and between different geographic regions.

The lack of correlation between antimicrobial resistance patterns and molecular types of the strains suggests convergent acquisition of resistance determinants by genetically unrelated uropathogenic strains rather than epidemic spread of resistant isolates in community.