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## ACTUALITIES IN THE ANTIBIOTIC RESISTANCE PHENOMENON OF *E. COLI* ISOLATED FROM URINARY TRACT INFECTION

**Olga Burduniuc**

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### **Summary**

Research on the detection of enterobacteria of antibiotic resistance markers in the current context of rapid increase in the prevalence of resistant strains of *E. coli*, the need to use methods of molecular biology is becoming increasingly stringent, which is far more sensitive than phenotypic testing and surveillance and epidemiological monitoring of strains producing CTX-M are important in determining treatment tactics for review empirical treatment protocols. Our study is something new for our country by molecular analysis of plasmid genes with great potential for the communities.

### **Rezumat**

#### ***Actualități privind rezistența la antibiotice a E. coli izolată de la pacienții cu infecții ale tractului urinar***

În contextul actual de creștere rapidă a prevalenței rezistenței tulpinilor de *E. coli* necesitatea utilizării metodelor de biologie moleculară devine din ce în ce mai stringentă, acestea fiind metode mult mai sensibile decât testarea fenotipică, iar supravegherea și monitorizarea epidemiologică a tulpinilor producătoare de CTX-M sunt importante în stabilirea tacticilor terapeutice pentru revizuirea protoalelor de tratament empiric. Studiu efectuat de noi reprezintă ceva nou pentru republica noastră prin analiza moleculară a unor gene plasmidice cu mare potențial de diseminare în colectivități.

### **Introduction**

Antimicrobial resistance has become an important problem worldwide. Bacterial resistance to antimicrobial agents has been emerging and rapidly disseminating among many nosocomial and community-acquired pathogens [1,4].

Infections caused by resistant microorganisms determine a high morbidity and mortality level due to therapeutic failures and rising costs for medical care [1,14].

The main causes of increasing resistance to antibiotics are inappropriate use and invalid prescription of these preparations by misinterpretation of symptoms, uncertain diagnosis and perceived expectations of the patient, duration too long / too short or inappropriate dose administration, self-medication, use of antibiotics in the veterinary sector, poor arsenal of diagnostic measures, medicaments and vaccines, inadequate supervision and control over medicaments use [4, 8].

Difficulties in treatment of infectious diseases occur more often in infections caused by producing strains of ESBLs (Extended Spectrum Beta Lactamases) - enzymes that develop resistance to extended-spectrum antibiotics [15].

The emergence of strains of *Enterobacteriaceae* producer of ESBLs is at present days a strong threat, in terms of effectiveness of antibiotics use in therapy of infections [9].

*E. coli*, the most common member of the family *Enterobacteriaceae* accounts for 75-90 % of all urinary tract infections in both patients and out patients

Uropathogenic *E. coli* are the primary causes of community-acquired UTIs with an estimated 20% of women over the age of 18 years suffering from at least one UTI in their life time [1].

Characteristics and dimensions of the phenomenon of antibiotic resistance were identified by laboratory methods and demonstrated by unfavorable clinical evolution of patients [6, 11, 13].

Analyzing the literature data we highlight a number of specialized phenotypic and genotypic methods for to determine the secretors strains of ESBLs [15, 16].

The specific type of beta-lactamase is not possible to be detected by routine tests (disc - diffusion, chromogenic tests etc.) Combination of several types of beta-lactamases in the same microorganism makes it more difficult to detect correctly. Studies made by other researchers demonstrate the importance of molecular biology techniques in detecting of ESBLs [2, 7, 12].

Correct detection of ESBLs producing strains, CTX-M remains a challenge for the microbiology laboratory and is very important to prevent clinical failure due to inadequate antibiotic therapy.

The purpose of the proposed study was to identify phenotypes of resistance among circulating strains of *E. coli* in our geographical area and determining the molecular mechanism of resistance prove, by detecting the presence of resistance markers (beta-lactamase) using phenotypic and molecular methods.

### **Material and methods**

For the study were collected 118 urine and stool samples from people with a diagnosis of urinary tract infection (UTI). The study was organized under the Scientific Research Institute of Mother and Child Health Care, Diagnostic Center "Modus Vivendi". Researches were conducted in the laboratory of Center epidemiology of the highly contagious disease and Biosafety National Center for Public Health, in collaboration with the bacteriology laboratory of the hospital Bichat - Claude Bernard, Paris, France.

Urine samples were sown on media differential diagnoses: Endo, blood agar, hypersaline agar with egg, enterococci agar, Mueller Hinton, Sabouraud. For the research were selected only strains isolated from urine significantly positive ( $\geq 10^5$  CFU / ml). Strains of *E. coli* were identified by gender and / or species by conventional biochemical tests (Voges - Proskauer test, indole test, urea, citrate, fenilalanindezaminaze etc.). Confirmatory tests were performed using API 20E galleries (BioMérieux, France). Subsequent phase of the research included the susceptibility testing of *E. coli* strains to antimicrobial preparations using phenotypic methods (Kirby-Bauer disk diffusion test, synergy test - bidimensional distribution of the two discs with antibiotics) and molecular biology (polymerase chain reaction , sequencing, multiplex PCR, Rep - PCR).

Evaluation of the results was performed in accordance with recommendations of Clinical and Laboratory Standards Institute guidelines (CLSI M100-S20, 2010).

Strain of *E. coli* ATCC 25922 was used as a reference strain for antibiotic susceptibility testing on culture media used.

Susceptibility testing of *E. coli* strains to antimicrobial preparations included the following classes of antibiotics: beta-lactam antibiotics - amoxicillin (AMX), ticarcillin (TIC), amoxicillin / clavulanate (AMC), cefotaxime (CTX), ceftazidime (CFT), ceftoxitin ( FOX), cefepim (CFP), ertapenem (ETP), aminoglycosides - gentamicin (GM), amikacin (AN), kanamycin (K); fluorquinolone - nalidixic acid (NA), ofloxacin (OFX), ciprofloxacin (CIP), tetracycline - tetracycline (TE) and sulfonamides - cotrimoxazole (sulfamethoxazole trimetropim) (SXT).

## Results and discussions

Microbial resistance to antibiotics is a global public health problem is largely due to inappropriate use of antibiotics. Phenomenon of antibiotic resistance in Europe is in continuous growth. The urinary tract infections (UTI), refers to the infectious diseases commonly encountered in medical practice which record a high incidence and prevalence with medical and economic consequences. Strains involved in the etiology of UTI are part of the epidemiological and bacteriological studies in recent years [3, 14].

The results of studies show that *E. coli*, germs that represent a normal microflora of the gastrointestinal tract under certain conditions becomes a significant resistance to antibacterial preparations and can cause intestinal and extraintestinal infections (including urinary) [5, 15,].

According to studies concerning the prevalence and etiological structure of urinary infections in 95% of cases are caused by agents of the family *Enterobacteriaceae* (of which 80-95% of cases *E. coli*, rarer *Proteus* spp. or *Klebsiella* spp.) and the rest about 5% *Pseudomonas aeruginosa*, staphylococci, yeasts of the genus *Candida*, etc. [8, 10, 15, 16].

The results of biochemical screening tests and confirmation of *Enterobacteriaceae*, revealed that from all the strains isolated from patients with UTI, were predominant strains of *E. coli* (92.9%).

*E. coli* strains isolated from patients with UTI were tested to identify the degree of resistance to five classes of antibiotics. Resistance to antibiotics determined by the method of Kirby-Bauer disk diffusion revealed that studied *E. coli* strains, isolated from people with ITU has a high level of resistance, including antibacterial preparations beta - lactam. Also beta-diffusion method does not provide sufficient data to assess the level of resistance and correct monitoring of antibacterial therapy. Were used synergy tests which could elucidate one of the mechanisms of antibiotic resistance of *E. coli* strains - the presence of beta - lactamases. This test uses a beta-lactamase inhibitor, clavulanic acid usually in combination with an oximino-cephalosporin such as ceftazidime, cefotaxime, ceftriaxone. The antibiotic discs were placed in such a way, that you can view images of synergy between discs with amoxicillin / clavulanic acid and the cephalosporin III generation (CG3). Sequence location of the discs with appropriate antibiotics was following: TIC - FOX - CFP - AMX - GM-CFT - AMC - CTX.

As a result, we determined that of 118 *E.coli* strains isolated from urine cultures, 91 strains of *E. coli* have resistance to the ticarcillin, 49 strains – to the amoxicillin, 26 strains - to the cotrimoxazole, each 21 strains – to the nalidixic acid and ofloxacin, 18 strains - to the ciprofloxacin and 7 strains of *E. coli* to other antibiotics (Fig. 1).

*E. coli* strains producing of beta-lactamase were preserved in an environment containing ox-heart broth and 10% of glycerol and then were stored by freezing at - 80°C to be further tested by molecular biology techniques.

Conduct adequate treatment requires rapid and accurate identification of antibiotic resistant strains using molecular biology techniques.

Determination of the resistance profiling data showed that those strains have resistance to the following groups of antibiotics: aminoglycosides - gentamicin (72%), fluoroquinolone - nalidixic acid (89%) and sulphonamides - cotrimoxazole (72%). Strains isolated from the stool are also polyresistant. They are resistant to the same classes of antimicrobial preparations as *E. coli* ESBLs isolated from urine: 53% to the aminoglycosides (gentamicin), 56% to the fluoroquinolone (ciprofloxacin), 44% to the sulphonamides (co-trimoxazole).

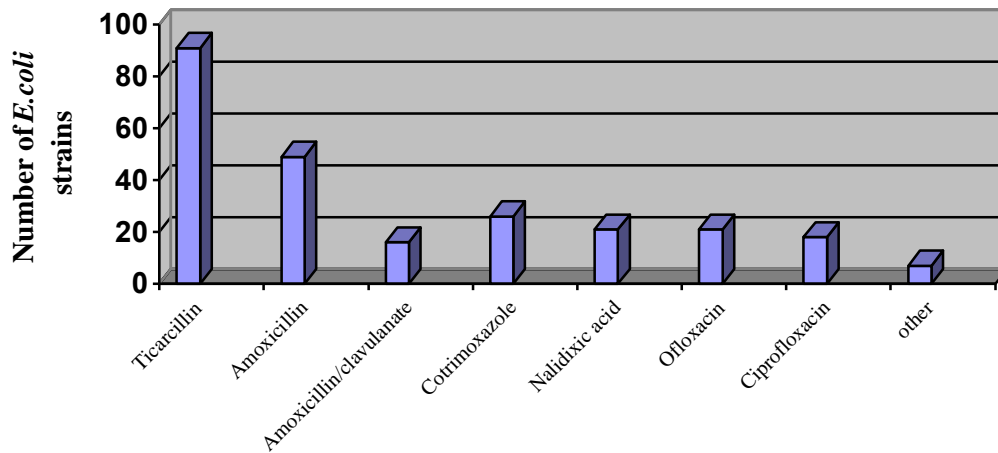


Figure 1. Profile of resistance of *E. coli* strains

*E. coli* ESBLs strains isolated from urine samples were of CTX-M type, namely: a strain of type CTX-M-1, 3 strains of type CTX-M-14, other 3 strains are of CTX -M-15 type. *E. coli* ESBLs strains found in stool samples were predominantly of CTX type and only one enzyme of SHV type. In contradistinction to urinary strains those were relatively more varied and namely a strain - CTX-M-1, 2 strains - CTX-M-3, 8 strains - CTX-M-14 and 3 strains - CTX-M-15.

The ability to clone of *E. coli* ESBLs strains detected in urine and stool was investigated by Rep-PCR method, only four strains of *E. coli* contain ESBLs in both urine and feces (U + / F +). At three of four of *E. coli* strains ESBLs U + / F + tested, was present Rep-PCR similar profile.

Determining phylogenetic group of urinary strains was found that 58,5% of *E. coli* ESBLs strains are from group B<sub>2</sub>, 27,9% - group A, 12,7% - group D and 0,9 from other groups. The strains detected in the faeces have following phylogenetic diversity: 53,4% - group A, 23,1% - group B<sub>2</sub> and group D, and 0,4 - other groups (Figure 2).

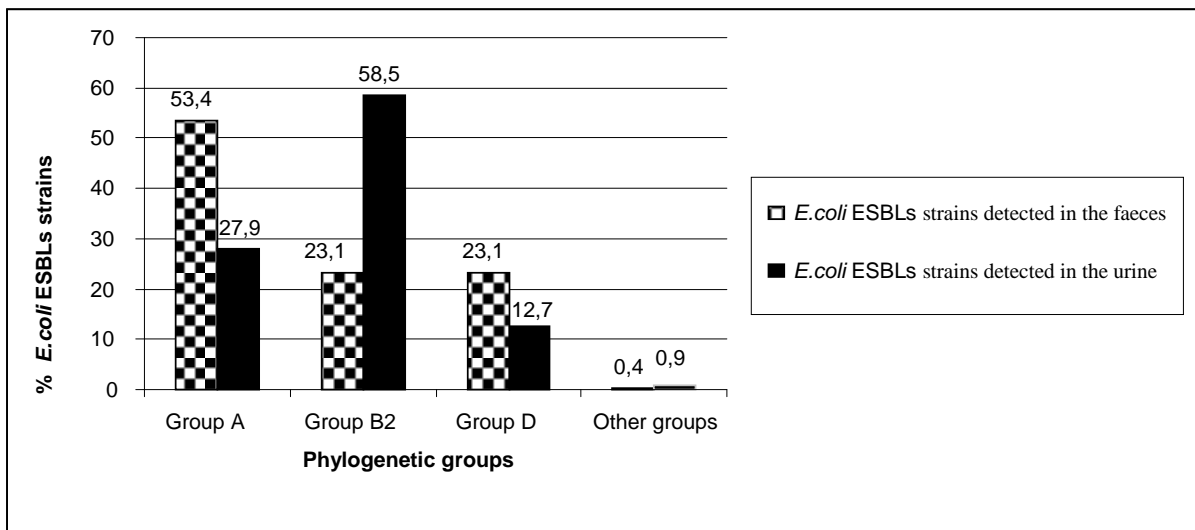


Figure 2. Phylogenetic groups of *E. coli* ESBLs strains

Phenotypic and genotypic monitoring of antibiotic resistance markers in the human population is a constitutive key in the national surveillance system for antimicrobial resistance phenomenon, following to be developed in Moldova as a part of state public health surveillance.

## Conclusions

1. Molecular biology methods involved relatively high costs, but provided reproducible results in a very short time, detecting in *E. coli* strains isolated from urine and coprocultures ESBLs – beta-lactam antibiotics resistance marker.
2. The movement of *E. coli* strains secretors of ESBLs of the CTX-M type within Republic of Moldova demonstrates the complexity of mechanisms of resistance.
3. It is hereby recommended that in view of the resistance rates to antimicrobial agents used in UTI therapy, antimicrobial agent usage policies, especially empirical therapies, should be based on antimicrobial resistance surveillance studies.

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