Incidence of Resistance Phenotypes of *Escherichia coli* Strains Isolated from an Obstetrics and Gynecology Unit

Unicentric Prospective Transversal Study

OVIDIU BORUGA[1], HORIA TUDOR STANCA[2]*, IULIA CRISTINA BAGIU[3]*, IOANA DELIA HORHAT[4], MIHAELA CRACIUNESCU[3], ANDREI COSNITA[1], DELIA BERCEANU VADUVA[3], VLAD CHERCOTA[1], ADRIANA IULIANA MILCU[1], CIPRIAN IOVAN[5]

[1]Victor Babes University of Medicine and Pharmacy, Timisoara, Department 9 Surgery, Discipline of Ophtalmology, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[2]Carol Davila Bucharest University of Medicine and Pharmacy, 37 Dionisie Lupu Str., 030167, Bucharest, Romania

[3]Victor Babes University of Medicine and Pharmacy, Timisoara, Department 14 Microbiology, Discipline of Microbiology, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[4]Victor Babes University of Medicine and Pharmacy, Department 9 Surgery, Discipline of Otorhinolaryngology, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[5]Univerity of Oradea 1 Universitatii Str., 410087, Oradea, Romania

Multiple microbial resistance is a global alarming phenomenon in modern medicine, which is experiencing a decrease in the therapeutic options and in the rate of discovery of new antimicrobials. The aim of this study was to analyze the incidence of Escherichia coli resistance phenotypes to suitable antimicrobial chemotherapies in strains isolated from urine samples. The study was conducted between 01.04.2016 and 31.03.2017 in the Department of Microbiology of the Municipal Emergency Clinical Hospital of Timisoara - Obstetrics and Gynecology unit. We used 1931 urine samples collected from the patients in this department. Identification of pathogens was performed on the API system, and chemotherapy sensitivity testing was based on the disk diffusion method. Of the 1931 urine samples, 210 were non-sterile (94 samples from the obstetrics department, 116 from the gynaecology department). The identified pathogens were Staphylococcus aureus (7 samples), coagulase-negative staphylococci (7 samples), Streptococcus spp. (37 samples), Escherichia coli (138 samples), Klebsiella spp. (9 samples), Proteus spp. (6 samples), Serratia spp. (6 samples). The identified resistance phenotypes for Escherichia coli strains were: wild-type strains (36) 25.92%, penicillinase-secreting strains (50) 35.84%, penicillinase-hypersecreting strains (26) 18.91% cephalosporinases (11) 7.98%, ESBL-producing strains (15) 11.17%. The prevention and spread of multidrug-resistant bacteria requires knowledge of bacterial resistance mechanisms and the development and application of appropriate in-hospital protocols.

Keywords: resistance phenotypes, Escherichia coli, urinary tract infections

Due to the high number of urinary tract infections, with considerable medical, social changes and economic consequences [1], the bacterial strains involved in their aetiology remain in the attention of bacteriology and epidemiology studies. Presently, one of the priority research themes in clinical microbiology is the acquired resistance of bacteria to antibiotics. Resistance to beta-lactam antibiotics is included in the genetic code of bacteria, the acquisition of which is produced by mutation or by the acquisition of genetic material. The inactivation of β-lactam antibiotics by -lactamases is currently the most commonly encountered mechanism. These enzymes cleave the beta-lactam nucleus, producing antibiotic inactivation [2-4]. They can be inducible, only secreted in the presence of beta-lactams, or permanently produced constituents, even in the absence of the substrate. Beta-lactamases have been demonstrated in both Gram-negative and Gram-positive bacteria and can be inhibited in varying degrees by beta-lactamase inhibitors such as clavulanic acid and sulbactam [2,5-7]. Gram-negative bacteria produce a much higher number of beta-lactamases than Gram-positive ones. All Gram-negative bacteria produce chromosomally encoded beta-lactamases, most of which preferentially hydrolyze cephalosporins, third and fourth generation included, which are resistant to the action of plasmid-encoded beta-lactamases [2,3,5].

Experimental part

Material and method

The study was conducted between 01.04.2016 and 31.03.2017 in the Department of Microbiology of the Municipal Emergency Clinical Hospital of Timisoara - Obstetrics and Gynecology unit. It is a prospective unicentric transversal study that used a representative sample of 1931 urine specimens collected from patients admitted in this section. The sampling was done in accordance with the recommended aseptic rules. Antibiotic testing was performed by the disk diffusion method (Kirby-Bauer) which included the double-disc method for ESBL presence, according to the Clinical and Laboratory Standard Institute (CLSI) standard.

Results and discussions

Of the 1931 urine samples, 210 were non-sterile (94 samples from the Obstetrics Department, 116 from the

* email: hstanca@yahoo.com; bagiuiulia@yahoo.com;

# Authors with equal contributions

Gynecology Department). The identified pathogens were *Staphylococcus aureus* (7 samples), coagulase-negative staphylococci (7 samples), *Streptococcus spp.* (37 samples), *Escherichia coli* (138 samples), *Klebsiella spp.* (9 samples), *Proteus spp.* (6 samples), *Serratia spp.* (6 samples) (table 1).

Table 1
IDENTIFIED PATHOGENS

Pathogen	Number	Percent
Escherichia coli	138	65.71
Klebsiella spp.	9	4.28
Proteus spp.	6	2.85
Staphylococcus aureus	7	3.33
Coagulase-negative staphylococci	7	3.33
Serratia spp.	6	2.85
Streptococcus spp.	37	17.61

In order to highlight the behavior of *Escherichia coli* strains versus beta-lactam, we tested the following classes of antibiotics: aminopenicillins (ampicillin), amino-penicillins with beta-lactamase inhibitors (amoxicillin + clavulanic acid), ureidopenicillins (piperacillin), second generation cephalosporins (cefuroxime), third generation cephalosporins (ceftazidime, cefotaxime, cefpodoxime), cephamycin (cefoxitin), fourth-generation cephalosporins (cefepime) and carbapenems (imipenem) (table 2).

Tabel 2
EXPANDED SPECTRUM BETA-LACTAMASE PHENOTYPE (ESBL)

ANTIBIOTIC	CLASS
Aminopenicillins	R
Aminopenicillin+BIL	S/I/R
Carboxipenicillin	R
Ureidopenicillin	R
I-st geenration cephalosporins	R
II-nd generation cephalosporins	R
III-rd generation cvephalosporins	R
IV-th generation cvephalosporins	R
Cephamycin	S/I/R
Carbapenems	S

Testing for the production of extended spectrum beta-lactamases in enterobacteria was done by the diffusion method: the inhibition effect is made simple by placing a disk impregnated with a beta-lactamase inhibitor or a combination of a beta-lactamase inhibitor and a beta-lactamase (eg amoxicillin-clavulanic acid) in the vicinity of a third-generation cephalosporin disc. In the presence of ESBL, a synergy image is obtained, refered to as *champagne-cork*, between the third generation cephalosporin disc and the beta-lactamase inhibitor disc (alone or in association with another antibiotic) [4,8]. The synergy image may be discreet, atypical or absent, leading to misinterpretation of the characters of the microorganism (false sensitivity). In the absence of a synergistic picture, ESBL production will be suspected in any reduction observed in the inhibition diameter around third generation cephalosporins: CTX (cefotaxime) 27 mm, CAZ (ceftazidime) 22 mm, CRO (cefuroxime) 25 mm, or to monobactam: aztreonam 27 mm [3.5]. The resistance phenotypes determined for *Escherichia coli* strains were as follows: wild-type strains (36) 25.92%, penicillinase secreting strains (50) 35.84%, penicillinase strains (26) 18.91% cephalosporinases (11) 7.98%, ESBL producing strains (15) 11.17% (table 3).

Table 3
RESISTANCE PHENOTYPES FOR *E.coli* STRAINS

STRAIN	NUMBER	PERCENT
Wild-type strains	36	25.92
Pernicillinase-secreting strains	50	35.84
Penicillinase hypersecreting strains	26	18.91
Cephalosporinase-secreting strains	11	7.98
ESBL-producing strains	15	11.17

Conclusions

At this point it is important to reduce the social impact of multiple resistance phenomena to anti-infectious chemotherapies through detailed knowledge of bacterial resistance mechanisms, optimizing the use of existing antibiotics, formulating strategies to reduce the emergence of bacterial strains with multiple resistance to antibiotics. Their elaboration and application would lead, over time, to the modification of nosocomial and community bacterial biocenosis, with the reduction of multiresistant strains [9].

The antibacterial activity of essential oils from *S.officinalis* [10] or *Allium ursinum* extracts which can provide long-term antifungal activity without remission [11] recommends them as a new accessible natural sources of antiseptics with potential applications against pathogens. The development and application of adequate hospitalization protocols meant to reduce the number of nosocomial infections thus become priority concerns for the Romanian health system. Similarly, the complications, as well as the high costs associated with the management of patients with sepsis, can be significantly reduced by early initiation of intensive care [12,13].

References

1.BAGIU, I.C., VLAICU, B., ONISEI, D., ONISEI, D., BAGIU, R.B., Types of Alcohol Associated to Binge Drinking Behaviour Among Students from Timis County , Rev.Chim.(Bucharest), **67**, no. 9, 2016, p.1684

2.PATERSON DL, BONOMO RA., Extended-Spectrum â-Lactamases: a Clinical Update. Clinical Microbiology Reviews, 2005, 1894:657-686

3.EINHORN AE., Extended-Spectrum Beta-lactamases: Frequency, Risk Factors and Outcomes. Pharmacotherapy Publications, 2002, 22(1): 14-20

4.BUIUC D, NEGUT M., Tratat de Microbiologie Clinica. Editura Medicala, Bucuresti, 1999

5.LORIAN V., Antibiotics in laboratory medicine. Lippincott Williams &Wilkins, 2005

6.LICKER, M., ANGHEL, A., MOLDOVAN, R.,HOGEA, E., MUNTEAN, D., HORHAT, F., SECLAMAN, E., TAMAS, L., ANGHEL, M., BADITOIU, L., Microarray Technology for the Detection of Antimicrobial Resistance Genes Present in Hospital and Community Acquired Methicillin Resistant S.aureus Strains, Rev.Chim.(Bucharest), **68**, no.11,2017, p. 2546

7.LICKER, M., MOLDOVAN, R., HOGEA, E., ET AL., Microbial biofilm in human health - an updated theoretical and practical insight, Revista

Romana de Medicina de Laborator, Volume: 25 Issue: 1 Pages: 9-26
8.CODITA I., Ghid naional pentru aplicarea procedurilor de testare a sensibilitãii la antibiotice conform standardului CLSI/NCCLS, Ed. Universitara Carol Davila, Bucuresti, 2007
9.MIRELA PETRASCU ET. AL, Resistance phenotypes for Escherichia Coli and Klebsiella pneumoniae strains producing expanded beta-lactamases (BLSE) isolated from urinary tract infections, Clujul Medical, 2011. Vol. 84 - nr. 3,371-377
10.ILIE, C., GOLET, I., CRACIUNESCU, M, HOGEA, E., POPESCU, R., HORHAT, F.G., Chemical Composition and Antimicrobial Activity of Essential Oil of Western Romanian Salvia officinalis, Rev. Chim..(Bucharest), **67**, no.1, 2016, p. 131
11.RADU VASILE BAGIU, BRIGITHA VLAICU AND MONICA BUTNARIU, Chemical Composition and in Vitro Antifungal Activity Screening of the Allium ursinum L. (Liliaceae), Int J Mol Sci. 2012; 13(2): 1426-1436.
12.DAVID VL (DAVID, VLAD LAURENTIU) ET. AL., Early Prediction of Sepsis Incidence in Critically Ill Patients Using Specific Genetic Polymorphisms , Biochemical genetics,Volume: 55, Issue: 3,Pages: 193-203
13.BEDREAG, OVIDIU HOREA; ROGOBETE, ALEXANDRU FLORIN; CRADIGATI, CARMEN ALINA; ET AL., A novel evaluation of microvascular damage in critically ill polytrauma patients by using circulating microRNAs, Revista Romana de Medicina de Laborator Volume: 24 Issue: 1 Pages: 21-30

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