

**CHARACTERISTICS OF ANTIBIOTIC SENSITIVITY OF MUSEUM STRAINS OF ESCHERICHIA COLI ISOLATED IN 1946-1959**

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In the middle of XX century, from the moment of the widespread introduction of penicillin into the medical practice, doctors were hoping for the era of infectious diseases to end. But in two years after the mass application of the medicine it has become clear that some microorganisms can resist the antibiotic. As the duration and the scale of antibiotic use increased - the quantity of resistant microorganism strains increases as well. According to the WHO data, today the antibiotic resistance in infectious agents has reached such a scale that there is a risk that humanity can return to the pre-antibiotic era [1-3].

In order to successfully fight against antibiotic resistance it is necessary to understand the conditions of its development. According to the accepted theory, the reason of resistance development and speedy dissemination is the selective pressure on microorganisms because of the active application of antimicrobial agents in husbandry and unjustified use in clinical practice [4-7]. But in the latter years, the reports stating that antibiotic resistance is a phenomenon that precedes antibiotic therapy, and is rather related to the general mechanisms of resistance to the unfavorable conditions of the environment in microorganisms, and not connected to the widespread use of antimicrobial agents, are becoming increasingly frequent [8-10]. Taking the above mentioned data into account, the studies devoted to the antibiotic sensitivity of microorganisms isolated before the era of widespread antibiotic use are relevant. The thorough study of the collection strains isolated in the pre-antibiotic period and in different periods of antibiotic therapy introduction, in our view, could allow us to realize the mechanisms of development and evolution of antibiotic resistance in bacteria.

**The aim** of this study was to analyze the sensitivity to antibiotics in museum strains of *E. Coli* isolated in 1946-1959 years.

**Materials and methods.** The objects of the study were the 20 strains of *E. coli*, obtained from Microorganisms Museum of SE «IMI NAMS», from which 4 were isolated in the territory of Ukraine (I. I. Mechnikov Institute of Microbiology and Immunology, Kharkiv (Kh); 4 –in the territory of Russia: Institute of epidemiology and microbiology, Yekaterinburg (IEM (Ye), Institute of Vaccines and Serums, St. Petersburg (IVS (S-P), Institute of Microbiology, epidemiology and hygiene, Moscow (IMEH) (M); 6 strains were obtained from the Kaufmann collection (Denmark, Copenhagen), 3 strains were isolated in Hungary, one strain each – from Bulgaria and Sweden. The place of isolation of the strain that was received from the collection of L. A. Tarasevich Institute of standardization and control, Moscow (SISC (M)), is unknown.

The determination of sensitivity of microbial cultures to antibiotics was carried out with the help of disc diffusion method with the use of standard commercial disks («Farmactive», Inc., Ukraine; «Aspect», Inc., Ukraine; Himedia, India); method of serial dilutions in agar and broth with the use of Muller-Hinton nutritive Muller-Hinton medium (Himedia, India) [11]. The sensitivity of *E. coli* to ampicillin, ampicillie-sulbactam, karbenicillin, tikarcillin, piperacilin, cefazolin, cefuroxime, ceftazidime, cefepim, norfloxacin, ciprofloxacin, levofloxacin, gatifloxacin, gentamycin, amykacin, tetracycline, chloramphenicol, ko-trimoxazol. The preparation of microorganisms' suspensions with the determined concentration of microbial cells was carried out based in the McFarland scale with the use of electronic device Densi-La-Meter (PLIVA-Lachema Diagnostika, Czech Republic). Statistical evaluation of the obtained data was carried out with the help of computer programs Microsoft Excel 2007, STATISTICA 6.0.

**Results and discussion.** During the analysis of the results of the sensitivity of the studied strains to ampicillin it was established there were 4 strains that were not sensitive to the antibiotic (20,0±5,2) % (Table. 1).

**Table 1. Antibiotic sensitivity of the studied strains of *E. coli***

Antibiotics	Distribution of strains according to sensitivity, %					
	resistant		Mildly resistant		Sensitive	
	Abs.	M±m, %	Abs	M±m, %	Abs	M±m, %
<b>Penicillines</b>						
Ampicillin	4	20,0±5,2	1	5,0±2,8	15	75,0±6,0
Ampicillin-sulbactam	1	5,0±2,8	2	10,0±3,9	17	85,0±4,6
Carbenicillin	0	0	0	0	20	100,0
Tykarcillin	0	0	0	0	20	100,0
Piperacillin	0	0	0	0	20	100,0
<b>Cefalosporines</b>						
Cefazolin	4	20,0±5,2	1	5,0±2,8	15	75,0±6,0
Cefuroxime	1	5,0±2,8	3	15,0±4,6	17	75,0±6,0
Ceftazidime	0	0	1	5,0±2,8	19	95,0±2,8

Cefepime	0	0	0	0	20	100,0
<b>Aminoglycosides</b>						
Gentamycin	0	0	0	0	20	100,0
Amikacin	0	0	0	0	20	100,0
<b>Tetracyclines</b>						
Tetracycline	2	10,0±3,9	2	10,0±3,9	16	80,0±5,2
Doxycycline	0	0	0	0	20	100,0
<b>Nitrobenzenes</b>						
Chloramphenicol	2	10,0±3,9	0	0	18	90,0±3,9
<b>Fluoroquinolone</b>						
Norfloxacin	0	0	0	0	20	100,0
Ciprofloxacin	0	0	0	0	20	100,0
Levofloxacin	0	0	0	0	20	100,0
Gatifloxacin	0	0	0	0	20	100,0
<b>Sulfanilamides</b>						
Co-trimoxazole	0	0	0	0	20	100,0

There were 2 resistant strains isolated in 1957 in IMI (Kh): *E. coli* 561 (01011) – MIC 64 µg/ml and *E. coli* 168 (01012) – MIC 128 µg/ml; strain *E. coli* 4932-53 (01016) from Kaufmann collection (Copenhagen, Denmark), 1958 year of isolation – MIC 32 µg/ml and the *E. coli* strain 145 (01026), isolated in the IMEH (M) in the year 1958 – MIC 64 µg/ml. Moreover, one of the researched strains was found to be mildly sensitive to this antibiotic: *E. coli* F 103 (01023) – Kaufmann collection (Copenhagen, Denmark), isolation year 1958 – MIC 16 µg/ml. It should be noted that the *E. coli* strain 145 (01026) also was resistant to the inhibitor-protected agent – ampicillin-sulbactam. There was mild sensitivity to ampicillin-sulbactam in *E. coli* strains 561 (01011) and *E. coli* 4932-53 (01016) – MIC was 16 µg/ml. All strains were sensitive to carboxypenicillins (carbenicillin, ticarcillin) and to the ureidopenicillins (piperacillin).

The evaluation of the cephalosporines efficacy towards the studied strains of *E. coli* has established that (25,0±6,0) % of the strains were not sensitive to cefazolin (I generation), and (20,0±5,2) % of the strains were not sensitive to cefuroxime (II generation) in the analyzed group. Ceftazidime (III generation) was found to be more effective, as there was 1 (5,0±2,8) % resistant strain; no resistance was found to cefepime (IV generation). Among those strains, *E. coli* 4932-53 (01016) (Kaufmann collection, Denmark, 1958) was resistant to cefazolin (MIC 16 µg/ml) and cefuroxime (MIC 16 µg/ml); *E. coli* strains 561 (01011) (IMI (Kh), 1957) and *E. coli* F 103 (01023) (Kaufmann collection, Denmark, 1958) were resistant to cefazolin - MIC 64 and 32 µg/ml correspondingly, and mildly resistant to cefuroxime – MIC 16 µg/ml; *E. coli* strain 408 «Novgorodskaya» (01022) (IVS (S-P), 1947) was mildly resistant to the both above mentioned antibiotics (MIC 16 µg/ml), and the *E. coli* strain 145 (01026) (IMEH (M), 1958 p.) – resistant to cefazolin, MIC 64 µg/ml. The most widespread mechanism of the cephalosporine resistance in microorganisms is their fermentative inactivation as the result of hydrolysis by β-lactamases [12]. It should be noted that the studied strains were isolated significantly earlier than cephalosporines were discovered and introduced into the clinical practice. Cefazolin was synthesized in the year 1971, cefuroxime–

in the year 1977, ceftazidime – in the year 1983 [12]. The mentioned above confirms the theory that antibiotic resistance has developed long before antibiotic discovery itself, as the mechanism of adaptation to the environment.

Despite the fact that resistance to nitrobenzenes develops relatively slowly, (10,0±3,9) % of the strains were resistant to chloramphenicol: (*E. coli* 561 (01011) (IMI (Kh), 1957) and *E. coli* 145 (01026) (IMEH (M), 1958), MIC for this antibiotic was 32 and 64 µg/ml correspondingly. In most cases, resistance to chloramphenicol was caused by the bacterial enzyme chloramphenicol-acetyltransferase that inactivates the agent [13].

As for the sensitivity of *E. coli* to tetracycline antibiotics: doxycycline had 100,0 % efficacy, two strains were found to be resistant to tetracycline – *E. coli* F 103 (01023) (Kaufmann collection, Denmark, 1958) – MIC 32 µg/ml, *E. coli* 145 (01026) (IMEH (M), 1958) – MIC 16 µg/ml and two mildly resistant strains (MIC 8 µg/ml): *E. coli* 168 (01012), (IMI, 1957); *E. coli* 408 «Novgorodskaya» (01022) (IVS (S-P), 1947). The main mechanism of protection against tetracyclins in bacteria is its elimination from the bacterial cells.

It should be noted that among *E. coli* strains of the studied group no strains resistant to aminoglycosides, fluoroquinolones and co-trimoxazole were found.

Therefore, as a result of the study antibiotic resistant strains of *E. coli* were found: *E. coli* 561 (01011) – resistant to ampicillin, cefazolin, chloramphenicol; *E. coli* 4932-53 (01016) – resistant to ampicillin, cefazolin, cefuroxime; *E. coli* F 103 (01023) – resistant to cefazolin and tetracycline; *E. coli* 145 (01026) – resistant to ampicillin, cefazolin, chloramphenicol, tetracycline.

**Conclusion.** Among the museum cultures of *E. coli* isolated in 1946-1959 yy., strains non-sensitive to ampicillin, ampicillin-sulbactam, cephalosporines of I, II, III generations, tetracycline and chloramphenicol were found.

**Perspectives of further studies.** Antibiotic resistant museum strains of *E. coli*, isolated in 1946-1959 yy. will be used in further studies in order to elucidate the mechanisms of antibiotic resistance development in bacteria.

**CHARACTERISTICS OF ANTIBIOTIC SENSITIVITY OF MUSEUM STRAINS OF ESCHERICHIA COLI ISOLATED IN 1946-1959 YY. Popov M. M., Peretyatko O. G., Yagnyuk Yu. A., Bolshakova G. M., Kholodna T. V.**

In the middle of XX century, since the moment of the widespread introduction of penicillin into the medical practice, the doctors used to hope that the era of infectious diseases is over. But in two years after the mass application of the agent it has become clear that certain microorganisms can form resistance to antibiotics. For the successful fight against antibiotic resistance in microorganisms it is necessary to understand the conditions of its development. Therefore, the studies of antibiotic resistance in microorganisms isolated before the era of widespread use of antibiotics in clinical practice are relevant to modern practice. **The aim of the study** was to analyze the antibiotic sensitivity in museum strains of *E. Coli* isolated in the 1946-1959 yy. **Materials and methods.** The objects of the study were 20 strains of *E. coli*, obtained from the Museum of Microorganisms of the SE «IMI NAMS». The determination of the sensitivity of microbial cultures to antibiotics was carried out with the help of disc diffusion method with the use of standard commercial disks, with the help of serial dilutions in agar and broth with the use of Muller-Hinton medium. The sensitivity of *E. coli* to the following groups of antibiotics was studied: penicillins, cephalosporines of I-IV generations, fluoroquinolones, aminoglycosides, tetracyclines, chloramphenicol and co-trimoxazole. The preparation of microorganisms' suspensions with the determined concentration of microbial cells was carried out based in the McFarland scale with the use of electronic device Densi-La-Meter (PLIVA-Lachema Diagnostika, Czech Republic). Statistical evaluation of the obtained data was carried out with the help of computer programs Microsoft Excel 2007, STATISTICA 6.0. **Results and discussion.** 4 (20,0±5,2) % of the strains resistant to ampicillin were found, one of which was also resistant to the inhibitor-protected agent – ampicillin sulbactam. All strains were found to be sensitive to carboxypenicillins (carbenicillin, tikarcillin) and to ureidopenicillins (piperacilin). During the evaluation of efficacy of cephalosporins to the studied *E. coli* strains it was established that (25,0±6,0) % of the strains were not sensitive to cefazolin (I generation), (20,0±5,2) % were not sensitive to cefuroxime (II generation), and (5,0±2,8) % of the strains of the analyzed group were not sensitive to ceftazidime (III generation), while no resistance was found to cefepime (IV generation). Among the analyzed strains (10,0±3,9) % were found to be resistant to chloramphenicol and tetracyclin. Among the analyzed group of *E. coli* strains none were resistant to aminoglycoside antibiotics, as well as to fluoroquinolones and co-trimoxazole. **Conclusion.** Among the museum cultures of *E. coli* isolated in 1946-1959 yy., strains non-sensitive to ampicilline, ampicilline-sulbactam, cephalosporines of I, II, III generations, tetracycline and chloramphenicol were found. **Perspectives of further studies.** Antibiotic resistant museum strains of *E. coli*, isolated in 1946-1959 yy. will be used in our further

studies in order to elucidate the mechanisms of antibiotic resistance development in bacteria.

**Keywords:** *E. coli* museum strains, antibiotics, antibiotic resistance.

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