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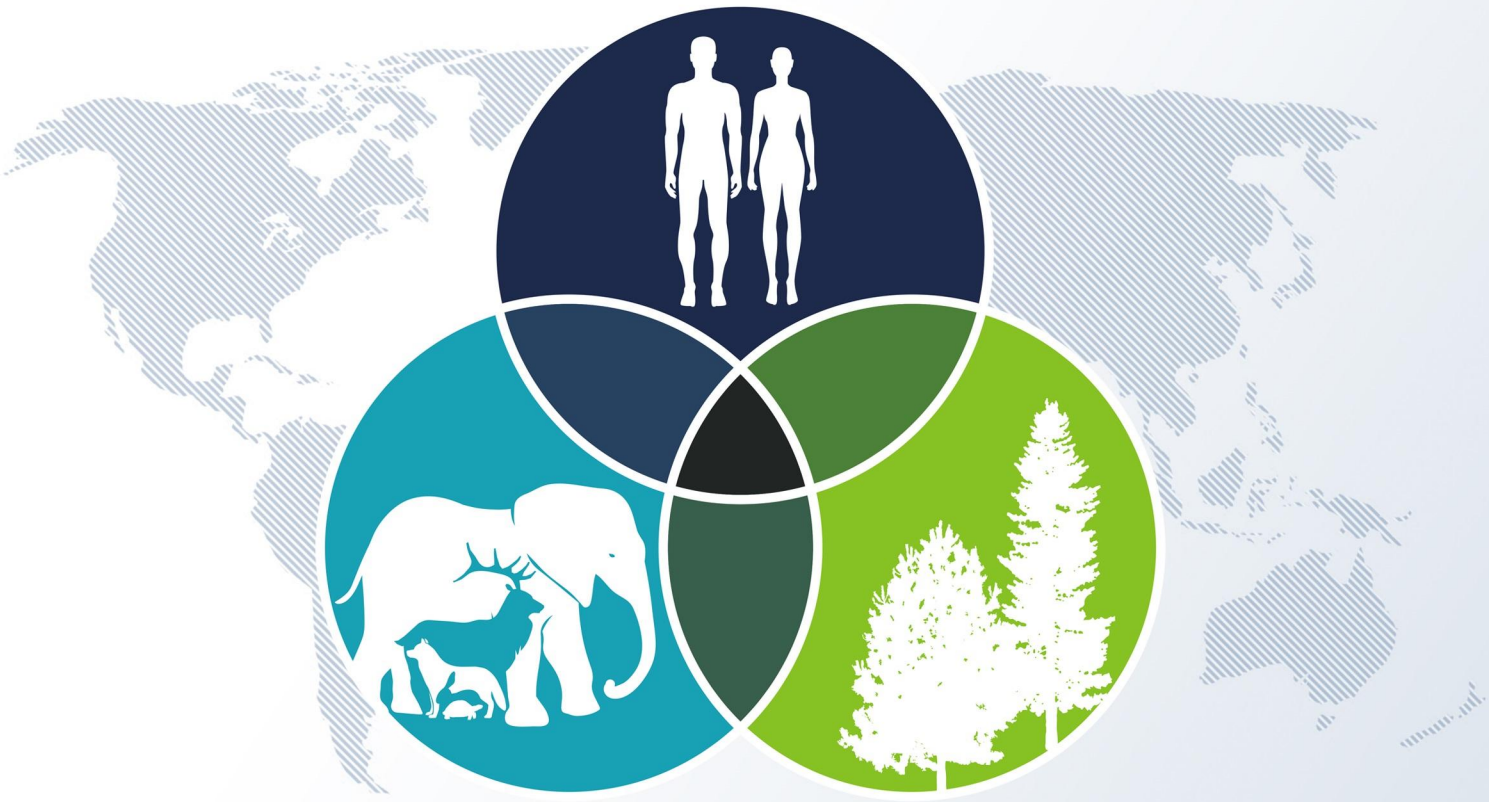

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*Asociația de Biosiguranță și Biosecuritate din Republica Moldova* (ABBRM) este o organizație profesională cu caracter științifico-practic și instructiv-educativ, neguvernamentală, apolitică și nonprofit, creată în 2017.

Obiectivul principal al asociației este dezvoltarea bunelor practici și culturii în domeniul biosiguranței și biosecurității și promovarea cunoștințelor în cadrul grupurilor profesionale și de cercetare-inovare.

***Biosiguranța*** – include principii de securizare, tehnologii şi reguli ce trebuie urmate pentru a preveni expunerea neintenţionată la agenţi patogeni şi toxine sau eliberarea/scurgerea lor accidentală.

„*Prote*jarea personalului, populației de expunerea neintenţionată la patogeni/material cu biohazard".

***Biosecuritatea*** – include un spectru larg de măsuri (politici de biosecuritate, regim de reglementări, măsuri ştiinţifice şi tehnice) aplicate într-un cadru organizat, necesar minimalizării riscurilor (prevenirea acţiunilor, atentatelor teroriste de eliberarea intenţionată de patogeni sau toxine precum şi a pierderii, furtului sau folosirii greşite a acestora).

*„Protejarea și prevenirea furtului, abuzului intenționat a patogenilor/materialului cu biohazard".*

***Managementul riscului*** – este un proces de luare a deciziilor în urma căruia rezultatele din evaluarea riscului (procesul de estimare a pericolelor la locul de muncă) sunt integrate cu principii economice, tehnice, sociale şi politice pentru generarea unor strategii de reducere a riscului.

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# One Health - Important Component of the Global Health Security

**Thomas BINZ, PhD,**
**President of the European Biosafety Association,**
**Federal Office of Public Health, Switzerland**

*As demonstrated by Covid-19, infectious disease can emerge any time in the world. Therefore, we must repay attention and use the One Health approach in order to protect humans and animals from a possible next infectious disease that has pandemic potential. The major aim of One Health is to improve health and well-being through the prevention of risks and the mitigation of effects of crises that originate at the interface between humans, animals and their various environments. Today's and future global challenges demand much greater, multidisciplinary team solutions.*

*One Health is a new interdisciplinary trend that has to be adressed when national and international plans and strategies related to zoonotic diseases, food safety, antimicrobial resistance, and climate change are established.*

*We need to promote the One Health approach, as humans cannot be healthy if the animals and the environment are not healthy. Therefore, it is necessary to design and implement programs, policies, legislation and research in which multiple sectors communicate and work together to achieve better human and animal public health outcomes.*

*As a key element to One Health, Biosafety and Biosecurity issues have been found on the agenda of many international organizations and professionals active in different sectors, such as public health, animal health, plant health. In order to get a new young generation of ONE HEALTH, in 2019, the Moldavian Biosafety and Biosecurity Association founded the One Health & Risk Management Journal. The Association brings together a diverse community of individuals who share their scientific results for biosafety and biosecurity issues.*

*Reading the "One Health & Risk Management» journal is not only a valuable method to inform about a healthy future based on the One Health approach, but also a fine intellectual pleasure.*

Thomas Binz

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RESEARCH ARTICLES – ARTICOLE DE CERCETARE – ARTICLES DE RECHERCHE – НАУЧНЫЕ СТАТЬИ

BIOLOGICAL SCIENCES



# VIABILITY AND PHENOTYPIC HETEROGENEITY OF *RHODOCOCCUS RHODOCHROUS* CNMN-Ac-05 IN THE PRESENCE OF FULLERENE $C_{60}$

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***Keywords:*** *fullerene $C_{60}$, rhodococci, viability, phenotypic heterogeneity.*

***Introduction***. *In recent years, due to wide applications of nanotechnologies in various fields, the safety of nanomaterials has become a pressing issue. Fullerene $C_{60}$ is not an exception. Research on the activity of microorganisms and their interaction with nanoparticles is of major importance, both for microorganisms and for the ecosystem as a whole.*

***Material and methods.*** *Fullerene $C_{60}$ powder was purchased from Sigma-Aldrich. The object of study was R. rhodochrous CNMN-Ac-05 strain. The number of viable bacterial cells was estimated by colony-forming units (CFU). The morphological features of the rhodococci colonies have been described according to the usual microbiological method.*

***Results***. *It was established that fullerene $C_{60}$ in concentrations of 1-25 mg/L fullerene $C_{60}$ stimulated the growth of R. rhodochrous by 2.4-2.8 times. As the concentration of fullerene $C_{60}$ increased up to 50-100 mg/L, the multiplication and growth of rhodococci decreased by 29.5% and 38% respectively. In the presence of 1-10 mg/L fullerene $C_{60}$ the rhodococci population remained homogeneous, being composed of 100% S type colonies. The increase of fullerene $C_{60}$ concentration led both to the decrease in the CFU number and to the appearance of R type colonies, up to 1.3% of population.*

***Conclusions***. *Fullerene $C_{60}$ in concentrations 1-100 mg/L had no obvious toxic effect on the rhodococci strain. The optimum concentration is 10 mg/L. The concentrations higher than 25 mg/L led to the dissociation of rhodococcal population and diminution in the CFU counts, but not to the total inhibition.*

***Cuvinte cheie:*** *fulerena $C_{60}$, rodococi, viabilitate, heterogenitate fenotipică.*

**VIABILITATEA ŞI HETEROGENITATEA FENOTIPICĂ A TULPINII RHODOCOCCUS RHODOCHROUS CNMN-Ac-05 ÎN PREZENŢA FULERENEI $C_{60}$**

***Introducere.*** *Siguranţa nanomaterialelor devine din ce în ce mai actuală, având în vedere utilizarea tot mai intensă a nanotehnologiilor în diferite domenii. Fullerena $C_{60}$ nu este o excepţie. Cercetările privind activitatea vitală a microorganismelor şi interacţiunea lor cu nanoparticulele are importanţă majoră atât pentru fiecare microorganism în parte, cât şi pentru ecosistem în totalitate.*

***Material şi metode***. *Fulerena $C_{60}$ a fost achiziţionată de la Sigma-Aldrich. Obiectul cercetării a servit tulpina R. rhodochrous CNMN-Ac-05. Numărul de celule bacteriene viabile a fost estimat prin unităţi formatoare de colonii (UFC). Caracterele morfologice ale coloniilor de rodococi au fost descrise conform metodei microbiologice uzuale.*

***Rezultate***. *S-a stabilit că fulerena $C_{60}$, în concentraţii de 1-25 mg/L, stimulează creşterea tulpinii R. rhodochrous de 2,4-2,8 ori. Odată cu creşterea concentraţiei până la 50-100 mg/L, multiplicarea şi creşterea rodococilor a scăzut cu 29,5% şi, respectiv, 38%. Tulpina de rodococi crescută în prezenţa a 1-10 mg/L fulerena $C_{60}$ a rămas omogenă, populaţia fiind alcătuită din 100% colonii de tip S. Mărirea concentraţiei de fulerenă $C_{60}$ a dus nu doar la scăderea numărului de celule, dar şi la apariţia coloniilor de tip R, până la 1,3% din populaţie.*

***Concluzii***. *Fulerena $C_{60}$ în concentraţiile 1-100 mg/L nu are efect toxic evident asupra tulpinii de rodococi. Concentraţia optimală este de 10 mg/L. Concentraţiile mai mari de 25 mg/L duc la disocierea populaţiei şi la scăderea semnificativă a numarului CFU, dar nu şi la inhibare totală.*

## INTRODUCTION

Fullerenes are an allotropic form of carbon with many synthesized modifications. Due to its structure, composed of 60 carbon atoms, fullerene $C_{60}$ are good heat and electricity conductor that possess an excellent tensile strength. These properties make it to be a unique functional material for electronics and optics, energy, biochemistry, and molecular medicine. More recently, fullerenes have been used for bioremediation of environment contaminated with polyethylene (1), pesticides (2, 3), or other xenobiotics of different nature (4).

Wide applications use of nanotechnologies in various fields, including the field of remediation and reduction of environmental contamination, the safety of nanomaterials has become a current issue. Regarding the action of fullerene $C_{60}$ on living cells, scientists observed some effects: on the one hand, the antioxidant action of fullerene was shown (5, 6, 7), on the other hand the antimicrobial activity was demonstrated (6, 8-11). For example, a nano-composite containing fullerene was effective in degrading pesticides Imidacloprid, Isoproturon and Malathion, but at the same time, it was used for inactivating *Pseudomonas aureus*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Escherichia* coli bacteria (2).

Numerous studies of fullerene $C_{60}$ have revealed that its biological activity is rather complex and multilateral, due to several factors, such as tropism for cell membranes, due to its lipophilicity, interaction with free radicals, and fullerene's ability to transfer energy to the oxygen molecule and convert it to single oxygen (7, 12, 13). It has been shown that the toxicity of fullerene $C_{60}$ is determined by a number of factors, such as particle size, organic matter and ionic strength of the medium, fullerene $C_{60}$ concentration and time of exposure, bacterial growth conditions, the age of $C_{60}$ used, and the bacterial species tested (5, 8, 14).

Over time, it has been shown that the advantages of immobilized microorganisms, compared to free cells, are the enhanced stability of the biocatalyst and posibility of recovering and reusing microorganisms. Moreover, immobilization could also protect the cells and thus increase the tolerance to high concentration of pollutants (15, 16). Voznyakovskii, et al. (2020) (17) demonstrated the possibility of using microorganisms immobilized on carbon structures for eliminating the consequences of contamination with petroleum products.

The *Rhodococcus rhodochrous* CNMN-Ac-05 strain was retrieved from the stock of our laboratory, which is a destructor of benzothiazole and its metabolites (18). The purpose of our research was to evaluate the effects of fullerene on the viability and phenotypic heterogeneity of *R. rhodochrous* CNMN-Ac-05 in order to estimate the possibility of using fullerene $C_{60}$ along with the rhodococcal strain in bioremediation procedures.

## MATERIAL AND METHODS

**Chemicals.** Fullerene $C_{60}$ powder (purity 98%) was purchased from Sigma-Aldrich. The particle size is approximately 0.7 nm in diameter.

**Bacterial strain and culture conditions.** *Rhodococcus rhodochrous* CNMN-Ac-05 was deposited within the National Collection of Non-Pathogenic Microorganisms of the Republic of Moldova, being able to degrade benzothiazoles and persistent organic pollutants (18). *R. rhodochrous* was grown in 100 mL portions of Tryptic soy (TS) broth (Sigma-Aldrich) in 300 mL Erlenmeyer flasks incubated at 28°C and 200 rpm. The cells were harvested over 36 h of culture and centrifuged at 6.000 rpm for 20 min. The bacterial pellet was washed first with a NaCl solution (0.8%) and then with distillated water.

**Determination of the effects of fullerene $C_{60}$.** Bacterial biomass was resuspended in distillated water (pH 7.2) to prepare cell suspension 6 mg/mL (1.4 mg cell dry weight/mL). The concentration of the cell biomass was determined spectrophotometrically by measuring the optical density of culture at λ=540 nm, with subsequent recalculation to cell dry weight according to the calibration curve. Colloidal aqueous suspension of fullerene $C_{60}$, 2 mg/mL was prepared on ultrasonic cleaner at 50 kHz for 5 min. For experiments, 5 mL of bacterial cells suspension was added in 250 mL Erlenmeyer flasks containing 95 mL of medium PAS and fullerene $C_{60}$ in following concentrations (mg/L): 1, 10, 25, 50, and 100. The PAS medium contained (g/L): 4.35 $K_2HPO_4$, 1.7 $KH_2PO_4$, 2.1 $NH_4Cl$, 0.2 $MgSO_4$, 0.05 $MnSO_4$, 0.01 $FeSO_4$ $7H_2O$, and 0.03 $CaCl_2$ $2H_2O$. pH adjusted at 7.2. Inoculated flasks were

incubated in a rotary shaker (180 rpm) at 28°C for 24 h. After a serial dilution, the 50 µL of suspension was spread on agar plates with TS medium; afterwards the plates were incubated at 28°C for 96 h until the bacterial colonies appeared. The number of viable bacterial cells was estimated by colony-forming units (CFU) inoculated on agar plates.

The morphological features of the rhodococci colonies were described according to Egorov method (19), by using a magnifying glass (8-fold magnification).

Statistical analysis was performed via MS Excel. All results were expressed as means of three individual replicates ±CI (confidence intervals). All the differences were considered statistically significant at $P<0.05$.

## RESULTS

The results of the action of fullerene $C_{60}$ on the viability of *R. rhodochrous* CNMN-Ac-05 are shown in Figure 1. These results indicated that the concentration of the test substance was of major importance. The optimal concentrations for rhodococcal cell multiplication were 1-25 mg/L. At these concentrations, the growth of the strain was stimulated by 2.4-2.8 times compared to the control samples. The highest CFU count value was obtained at a concentration of 10 mg/L. However, an increase in the fullerene $C_{60}$ concentration up to 50 and 100 mg/L, obviously decreased the rhodococci capacity of multiplication and growth. The values of the CFU number were lower by 29.5% and 38.0% compared to the control samples.

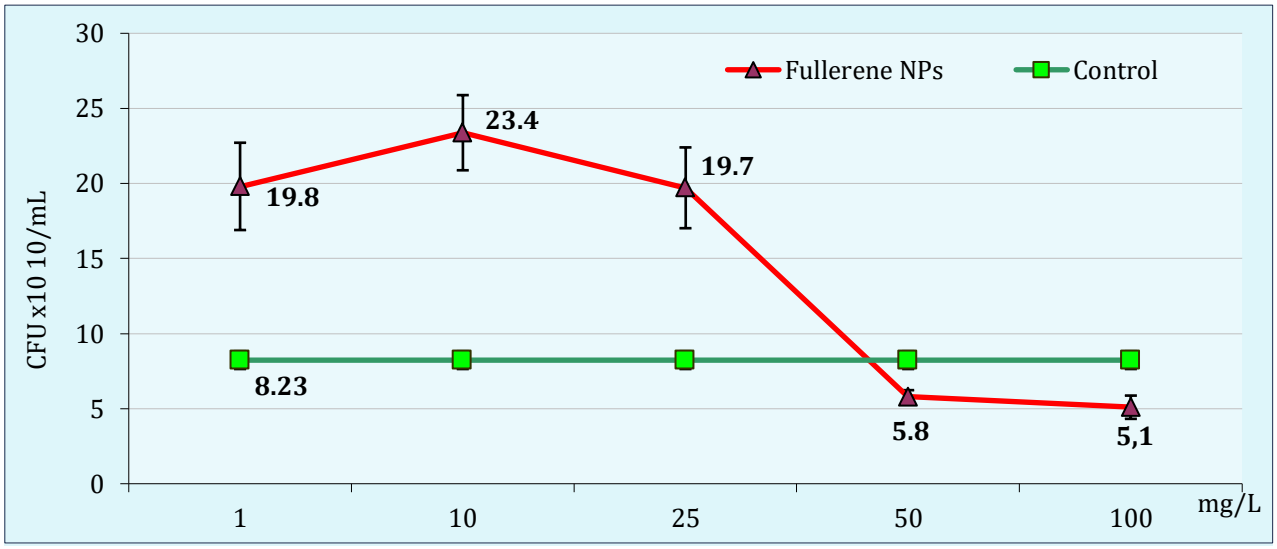



Figure 1. Influence of fullerene $C_{60}$ on the viability *R. rhodochrous* CNMN-Ac-05.

In addition to cell multiplication, the phenotypic modification of *Rhodococcus* colonies in the presence of fullerene $C_{60}$ was described. Macroscopically observable features and colony types of *R. rhodochrous* CNMN-Ac-05 are presented in Table 1 and Figure 2.

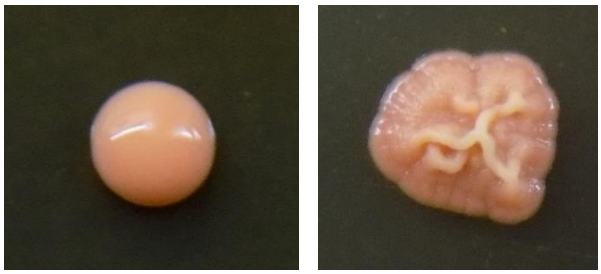
Basic morphological features of *R. rhodochrous* CNMN-Ac-05 colonies were similar to the S type (smooth). Thus, cultivation on TS medium without fullerene (control), resulted in the formation of 100% S type colonies. The colonies of S type were dominant in all the experimental variants, regardless of fullerene $C_{60}$ concentration, ranging between 98.7-100% (fig. 3).

The population of *R. rhodochrous* cultivated in the presence of 1 and 10 mg/L of fullerene $C_{60}$ remained homogeneous, being composed of 100% S type colonies, similar to the control sample. Moreover, cultivation in the presence of 25 mg/L fullerene $C_{60}$, under conditions of active multiplication, resulted in phenotypic dissociation of strain into two types of colonies, S (99.6%) and R (0.4%) types.

The further increase of fullerene $C_{60}$ concentration (50-100 mg/L) caused both a substantial decrease in the cell number and an increase in the phenotypic heterogeneity of the rhodococci. The highest percentage of R type cells – 1.3% was observed at the concentration of 100 mg/L.

Table 1. Types of colonies formed by *R. rhodochrous* CNMN-Ac-05.

| Morphological features | Colony type | |
|---|---|---|
| | S | R |
| **Form** | Round | Irregular |
| **Size, mm** | 1.0-4.0 | 1.0-4.0 |
| **Margin** | Entire | Undulate |
| **Elevation** | Convex | Convex |
| **Surface** | Smooth and glistening | Rough and dull |
| **Color** | Pink | Pink |
| **Opacity** | Opaque | Opaque |



**Type S** **Type R**

Figure 2. Types of colonies formed by *R. rhodochrous* CNMN-Ac-05 cultivated in the presence of fullerene $C_{60}$.

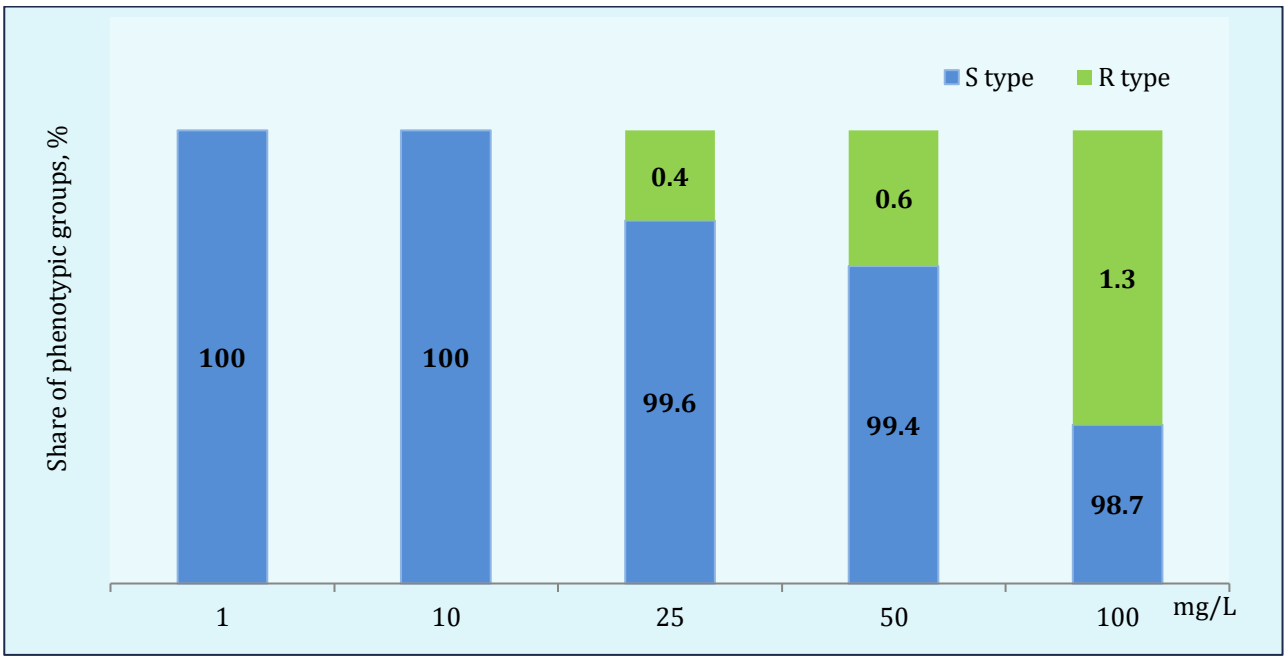



Figure 3. Dissociation of *R. rhodochrous* CNMN-Ac-05 grown in the presence of fullerene $C_{60}$.

## DISCUSSIONS

The genus *Rhodococcus* belongs to the phylum and class *Actinobacteria*, the order *Actinomycetales*, and the family *Nocardiaceae*. Rhodococci possess a broad catabolic diversity and numerous enzymatic capabilities. They are able to use a wide range of organic compounds as sole sources of carbon and energy for growth, and that makes them well-equipped for biotransformation and biodegradation of xenobiotic compounds (15, 20, 21).

Since rhodococci are nocardioform bacteria, the phenotypic heterogeneity is quite common among them. According to Goodfellow et al. (22), rhodococci colonies may be mucoid, rough or smooth, as well as a pigmented, yellow, orange, red, or colorless buff and cream. The splitting of a homogeneous rhodococci population into variants with different morphological, physiological, biochemical, and genotypic properties was observed by many researchers (23-26).

Phenotypic heterogeneity refers to the phenomenon when individual cells within an isogenic population, that have a uniform genetic background, can nevertheless display differences in phenotype (27, 28). Non-genetic variations, that exist within an isogenic population, benefit the population through division of labor and improving the ability to exhibit a high level of metabolic activity. Therefore, phenotypic heterogeneity allows bacterial populations to

improve their ability to adapt to changing environments (29, 30).

In the present study, the concentration-toxicity ratio of fullerene $C_{60}$ to rhodococcal cells was clearly established. In case of concentrations of 1 and 10 mg/L, the population of rhodococci was homogeneous and grew very actively, whereas starting with 25 mg/L, R type colonies appeared. Since typical S colonies are produced only under optimal cultural conditions, the appearance of R type colonies indicates a stressful condition, caused by the presence of increased concentrations of fullerene $C_{60}$. This phenomenon was confirmed by the experimental variants, when rhodococci were grown in the presence of 50 and 100 mg/L of fullerene $C_{60}$. The number of R type colonies increased in these variants, additionally to the decrease in the CFU count. Similar results were obtained by Sah et al. (2010) (1) that reported a moderately decrease in the CFU counts of bacterial strains at the concentration of 100 mg/L fullerene $C_{60}$.

## CONCLUSIONS

1. The fullerene $C_{60}$ in tested concentrations did not display any obvious toxic effects on *R. rhodochrous* CNMN-Ac-05 cells. The addition of fullerene $C_{60}$ in concentrations up to 25 mg/L, stimulates the growth and multiplication of rhodococcal cells. The optimum concentration was 10 mg/L.
2. Concentration increases beyond 25 mg/L caused a dissociation of the rhodococcal population, expressed by the appearance of R type colonies, as well as a significant decrease of the CFU counts (29.5-38.0%), no total inhibition occurs. Higher fullerene $C_{60}$ concentrations resulted in lower growth activity and higher phenotypic heterogeneity of the strain.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

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# ACTIVITÉ PHYSIQUE CHEZ LES ÉTUDIANTS : UNE ETUDE EPIDEMIOLOGIQUE TRANSVERSALE DANS LA REPUBLIQUE DE MOLDAVIE ET EN FRANCE

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***Keywords:*** *students, physical activity, somatometric indices.*

***Introduction****. Physical activity is the key component of a student's healthy lifestyle, which is a fundamental factor contributing to academic success. The importance of physical activities during the study period is determined particularly by the student's working regime, which is often characterized by sedentary lifestyle, reduced mobility and constant working position.*

***Material and methods.*** *A cross-sectional epidemiological study (2017-2018) was carried out. The study sample consisted of 783 students, including 430 students from the Republic of Moldova and 353 students from France. The data collection was performed by using a questionnaire that was completed by each study participant online, in electronic form.*

***Results.*** *The mean body mass index (kg/m²) of students from Chisinau was 21.5±0.10 (ranging between 16.16 and 34.72) and of students from Rouen - 22.65±0.22 (15.75-43.76) (p<0.001). 43.9% students from Chisinau and 56.4% students from Rouen practice sport (p<0.001). Both men (98.5%) and women (98.4%) from Chisinau practice same-intensity physical activities, until sweating. A slightly more different situation was reported in students from Rouen, where only 88.8% of women exercise until sweating, compared to men – 93.1%.*

***Conclusions.*** *The results of the research showed a slightly significant difference among students from both countries, as well as in their gender distribution.*

***Cuvinte cheie:*** *studenţi, activitate fizică, indici somatometrici.*

**ACTIVITATEA FIZICĂ LA STUDENŢI: UN STUDIU EPIDEMIOLOGIC TRANSVERSAL ÎN REPUBLICA MOLDOVA ŞI FRANŢA**

***Introducere.*** *Activitatea fizică reprezintă una din componentele de bază ale unui stil de viaţă sănătos al unui student – un factor primordial, care contribuie la succesul academic. Importanţa activităţilor fizice în perioada studiilor este determinată, în special, de faptul că regimul de lucru al studentului se caracterizează adesea prin sedentarism, mobilitate redusă sau poziţie de lucru invariabilă.*

***Material şi metode.*** *A fost proiectat un studiu epidemiologic transversal (2017-2018), eşantionul de studiu fiind constituit din 783 studenţi, dintre care 430 de respondenţi din Republica Moldova şi 353 - din Franţa. Datele au fost colectate în baza unui formular cu întrebări, care a fost completat de către fiecare participant la studiu, în format electronic, în regim online.*

***Rezultate.*** *Valoarea medie a indicelui masei corporale (kg/m²) pentru studenţii din Chişinău a constituit 21,5±0,10 (limitele cuprinse între 16,16 şi 34,72), iar pentru studenţii din Rouen - 22,65±0,22 (15,75-43,76) (p<0,001). Astfel, practică sportul: 43,9% studenţi din Chişinău şi 56,4% studenţi din Rouen (p<0,001). Atât bărbaţii (98,5%), cât şi femeile (98,4%) din Chişinău practică activităţile fizice cu aceeaşi intensitate, până la transpiraţie. O situaţie puţin mai deosebită atestându-se la studenţii din Rouen, unde femeile fac exerciţii fizice până la transpiraţie doar în 88,8% cazuri, în comparaţie cu bărbaţii – 93,1%.*

***Concluzii.*** *Rezultatele cercetării au arătat că diferenţele au fost puţin semnificative atât după criteriul de gen, cât şi la nivel de ţări.*

## INTRODUCTION

L'activité physique fait partie intégrante de la vie quotidienne des jeunes. Le rôle des activités physiques et d'autres formes d'exercice dans la vie sociale est multilatéral. Parallèlement à une alimentation équilibrée, l'activité physique régulière est l'un des moyens les plus simples et les plus efficaces de prévention de la survenue de maladies chroniques, des maladies cardiovasculaires et des maladies mentales. Le progrès technico-scientifique, le développement vertigi-neux de la science et le nombre en permanence croissance de nouvelles informations, nécessaires au spécialiste contemporain, rendent l'activité quotidienne des jeunes, principalement des étudiants, plus intense, plus tendue (1). De manière appropriée, le rôle de l'activité physique comme moyen d'optimiser le style de vie, le travail, le repos actif augmente, en maintenant et en perfectionnant la capacité intellectuelle des étudiants tout au long de la période d'étude (2).

L'activité physique est l'une des composantes fondamentales du mode de vie sain d'un étudiant – un facteur fondamental contribuant à la réussite académique. En même temps, un statut social spécial, un mode de vie et des conditions de travail spécifiques, l'exposition à divers facteurs de risque distinguent les étudiants de toutes les autres catégories de population et déterminent aussi leur vulnérabilité. Ainsi, en raison de l'horaire chargé, de la grande quantité d'informa-tion à assimiler, les étudiants négligent trop souvent l'activité physique, ce qui génère des états d'anxiété, de fatigue, de stress dans le processus d'interaction dans l'environnement universitaire ou avec les membres de la famille (3, 4).

L'importance de l'activité physique pendant la période universitaire est déterminée en particulier par le fait que le travail de l'étudiant est souvent caractérisé par un mode de vie sédentaire, une mobilité réduite, une posture de travail invariable pour une période de 10 à 12 heures. Dans ces conditions, l'exercice est le facteur fondamental dans la réduction des conséquences négatives pour la santé, ainsi que de l'effort intellectuel et psycho-émotionnel. Ainsi, le temps utilisé pour pratiquer l'exercice physique est compensé par la capacité générale élevé de travail, y compris l'activité intellectuelle. Ce fait est démontré, notamment, par les résultats d'études, souvent supérieures, des étudiants pratiquant régulièrement un exercice physique (3). *L'objectif* de l'étude était d'évaluer le niveau d'activité physiques chez les étudiants de l'Université d'Etat de Médecine et de Pharmacie « Nicolae Testemiţanu » (Chisinau, République de Moldavie) et de la Faculté de Médecine de l'Université de Rouen (Rouen, France) et d'identifier les facteurs influençant la durée et la diversité. *L'hypothèse* de l'étude : les étudiants des deux pays ont le même niveau d'activité physique, conditionné par la présence de facteurs limitant l'activité.

## MÉTHODES

### *Conception de l'étude*

Une étude épidémiologique transversale a été conçue entre le mois de janvier 2017 et décembre 2018. L'étude a impliqué des étudiants de l'Université de Médecine et de Pharmacie « Nicolae Testemiţanu », Chisinau, la République de Moldavie (ci-après – Ch) et la Faculté de Médecine de l'Université de Rouen, Rouen, France (ci-après – Ro).

### *Validation de l'étude*

L'étude fait partie du projet international EurECAS (European Evaluation of Comportment and Addiction among Students). Le protocole d'étude a été approuvé par le Comité d'éthique de l'Université de Rouen (27.01.2016) et le Comité d'éthique de l'université de la République de Moldavie (no 19 du 21.11.2017).

### *Collecte des données*

La sélection des sujets pour la constitution de l'échantillon s'est faite à titre volontaire. Le critère de sélection pour la participation à l'étude a été le statut d'un étudiant, ce dernier devait être étudiant à la faculté de médecine des universités mentionnées ci-dessus. L'acceptation et la participation volontaire ont été faites en remplissant le questionnaire anonyme en ligne. Les étudiants qui ont déjà obtenu leur diplôme de médecine et ceux qui ont refusé de participer à l'étude n'ont pas été inclus dans l'échantillon de l'étude.

Les données ont été recueillies à l'aide d'un questionnaire en ligne que chaque participant à l'étude a rempli. Le questionnaire a été complété une seule fois au cours de chaque année académique II et IV, pendant la période de préparation universitaire de chaque étudiant. Le contenu des questions a été adapté selon le questionnaire utilisé à l'Université de Rouen (France) et a été repris aussi de la version traduite en langue roumaine de l'Université de Médecine et de Pharma-

cie « Iuliu Hatieganu », Cluj Napoca (Roumanie).

Il a été recueilli des données sur l'age, le sexe, le poids et la taille pour calculer l'indice de masse corporelle (poids/taille$^2$), cursus, statut (en couple ou célibataire), la pratique d'un sport et le nombre d'heure de pratique par semaine, et la présence de facteurs empêchant l'activité physique.

***Analyse statistique***

Les résultats reçus ont été évalués statis-tiquement à l'aide du test t-Student, pour les variables quantitatives et par le test du Chi 2 pour les variables qualitatives. Les données sont présentées par moyenne arithmétique (M) ± écart type. En tant qur différences statistiquement significatives ont été considérées celles ayant un $p<0,05$. L'analyse statistique des résultats obtenus a été réalisée à l'aide du logiciel SPSS-20.

## RÉSULTATS

***Population***

L'échantillon de l'étude était composé par 783 étudiants, dont 430 étudiants de la République de Moldavie et 353 étudiants de la France. La répartition des étudiants par sexe était la suivante: 25,4% (109) hommes et 74,6% (321) femmes (Ch) et 14,7% (52) hommes et 85,3% (301) femmes (Ro). L'âge moyen était de 21,2 ans (±0,03) (Ch) et de 20,9 (±0,1) (Ro).

***Caractéristique générale de l'échantillon d'étude***

L'étude a port sur 430 étudiants de l'UEMPh « Nicolae Testemitanu » (R. Moldavie) dont 32 (7,44%) de la Faculté de Pharmacie, 372 (86,51%) des étudiants de la Faculté de Médecine et 26 (6,04%) des étudiants de la Faculté de Médecine dentaire. La répartition par années d'études était la suivante : 320 (74,41%) étudiants – en deuxième année d'études et 110 (25,58%) étudiants – en quatrième année d'études. De l'Université de Rouen (France) à l'étude ont participé 353 étudiants de la Faculté de médecine. Sur l'ensemble des sujets de la République de Moldavie – 337 (78,37%) ont déclaré être célibataires et 93 (21,62%) sont en couple, et de France – 259 (73,37%) sont célibataires et 94 (26,62 %) sont en couple.

***Caractéristique des indices somatométriques***

Une analyse comparative des indices somatométriques des étudiants de Chisinau et Rouen a été réalisée (tab. 1).

Tableau 1. Analyse comparative des indices somatométriques des étudiants de Chisinau et Rouen.

| No. | Indices somatométriques | Étudiants de Chisinau n=430 | Étudiants de Rouen n=353 | P |
|---|---|---|---|---|
| 1 | Taille (cm) | 169,6±0,03 | 167,27±0,40 | ** |
| 2 | Masse corporelle (kg) | 62,3±0,23 | 63,46±0,68 | * |
| 3 | Indice de masse corporelle | 21,5±0,10 | 22,65±0,22 | *** |

*Note:* *p>0,05; **p<0.05; ***p<0.001.

La taille moyenne des sujets de Chisinau était de 169,6±0,03 cm (extrêmes : 147 et 196 cm), et des étudiants de Rouen 167,27±0,40 cm (148-190 cm) ($p<0,05$). Les étudiants hommes de Chisinau avaient une taille moyenne – 180,5±0,02 cm (extrêmes: 164-196 cm) et ceux de Rouen 177,58±0,87 cm (163-190) ($p<0,001$), respectivement femmes – 165,9±0,04 cm (147-183 cm) et 165,49±0,35 (148-181 cm) ($p>0,05$).

La masse corporelle moyenne des étudiants de Chisinau était de 62,3±0,23 kg (extrêmes : 40 et 115 kg), et de Rouen – 63,46±0,68 kg (42-125 kg) ($p>0,05$). La moyenne pour les étudiants de sexe masculin de Chisinau était de 75,2±0,22 kg (49-115 kg) et celle des étudiants de Rouen – 72,08±1,6 (51-115 kg) ($p>0, 05$), respectivement féminin – 58,0±0,26 kg (40-98 kg) et 61,98±0,72 (42-125 kg) ($p<0,001$).

La valeur moyenne de l'indice de masse corporelle (kg/m$^2$) pour les étudiants de Chisinau était de 21,5±0,10 (extrêmes : entre 16,16 et 34,72), et pour les étudiants de Rouen – 22,65±0,22 (15,75-43,76) ($p<0,001$). La moyenne pour les sujets de sexe masculin de Chisinau était 23,0±0,29 (16,37 à 33,97), et de Rouen – 22,86±0,48 (17,24 à 34,34) ($p<0,05$), respectivement féminin - 21,05±0,16 (16,16-34,72) et 22,62±0,25 (15,76-43,77) ($p<0,001$).

***Caractéristique de l'activité physique***

Le questionnaire comportait la question visant la pratique d'un sport par les étudiants. A cette question, ont offert une réponse affirmative un numéro de 43,9% d'étudiants de Chisinau et 56,4% d'étudiants de Rouen (p<0,001), dont 34,4% d'hommes (Ch) et 14,6% (Ro) (p<0,001), respectivement femmes 65,6% (Ch) et 85,4% (Ro) (p<0,001). Ainsi, les hommes de Chisinau et les femmes de Rouen sont plus actifs. Pour un sport d'équipe, ont opté 71 personnes (37,6%) de Chisinau et 37 personnes (18,6%) de Rouen, et pour un sport individuel – 118 personnes (62,4%) de Chisinau et 179 personnes (81,4%) de Rouen.

La durée du sport a également été analysée dans l'étude (tab. 2). La répartition hebdomadaire du temps alloué aux sports est différente selon le sexe biologique.

Tableau 2. Répartition des étudiants par sexe selon la durée du sport (heures/semaine).

| Durée du sport (heures/semaine) | Hommes | | | | Femmes | | | |
|---|---|---|---|---|---|---|---|---|
| | Chisinau (65) | | Rouen (29) | | Chisinau (124) | | Rouen (170) | |
| | *no. abs.* | *P±m1(%)* | *no. abs.* | *P±m2(%)* | *no. abs.* | *P±m1(%)* | *no. abs.* | *P±m2(%)* |
| **< 1 heure** | 6 | 9,23±3,0 | 6 | 20,68±6,9 | 12 | 9,67±2,6 | 39 | 22,94±3,2 |
| | p>0,05 | | | | p<0,01 | | | |
| **1 à 2 heures** | 7 | 10,76±3,3 | 7 | 24,13±8,0 | 32 | 25,8±3,9 | 51 | 30,0±3,5 |
| | p>0,05 | | | | p>0,05 | | | |
| **2 à 3 heures** | 15 | 23,07±5,2 | 5 | 17,24±5,7 | 45 | 36,29±4.3 | 33 | 19,41±3,0 |
| | p>0,05 | | | | p<0,01 | | | |
| **3 à 4 heures** | 8 | 12,3±4,0 | 3 | 10,34±3,7 | 7 | 5,64±2,0 | 23 | 13,52±2,6 |
| | p>0,05 | | | | p<0,05 | | | |
| **4 à 5 heures** | 7 | 10,76±3,5 | 3 | 10,34±3,7 | 5 | 4,03±1,3 | 9 | 5,29±1,7 |
| | p>0,05 | | | | p>0,05 | | | |
| **5 à 6 heures** | 7 | 10,76±3,8 | 1 | 3,44 | 8 | 6,45±2.1 | 4 | 2,35±0,6 |
| | p>0,05 | | | | p>0,05 | | | |
| **6 à 7 heures** | 6 | 9,23±3.1 | 1 | 3,44 | 5 | 4,03±1,3 | 2 | 1,17±0,4 |
| | p>0,05 | | | | p>0,05 | | | |
| **> 7 heures** | 9 | 13,84±4,2 | 3 | 10,34±3,7 | 10 | 8,06±2,4 | 9 | 5,29±1,7 |
| | p>0,05 | | | | p>0,05 | | | |

Un autre aspect qui a été étudié consiste dans l'intensité de la pratique d'activités physiques/ d'exercices jusqu'à la transpiration (fig. 1). Ainsi, on peut observer que les hommes et les femmes de Chisinau pratiquent des activités physiques avec la même intensité. Une situation un peu plus particulière est retrouvée chez les étudiants de Rouen, où seulement 88,8% des femmes font de l'exercice jusqu'à ce qu'elles transpirent.

À l'étape suivante, les répondants devaient répondre à une série de questions sur l'activité physique (effort physique) comme les sports, la marche, les travaux ménagers ou d'autres activités nécessitant une consommation d'énergie. Les hommes pratiquaient l'activité physique pour éviter la tension dans 80,7±3,7% des cas (Ch) et 78,8±5,6% de cas (Ro) (p>0,05) et les femmes – 83,2±2,1% de cas (Ch) et 83,4±2,1% cas (Ro) (p>0,05). Bien que les étudiants puissent avoir des problèmes de santé de toute nature, 59,6±4,7% (Ch) et 36,5±6,6% (Ro) hommes (p<0,01), respectivement 63,9±2,6% (Ch) et 50,8±2,8% (Ro) des femmes (p<0,001) pratiquaient une activité physique malgré des problèmes de santé persistants. Dans les cas où les étudiants ont pratiqué une activité physique, ils ont été tentés d'augmenter la durée de l'effort physique pour obtenir les effets ou les avantages attendus depuis longtemps : hommes – 77,1±4,0% (Ch) et 63,5±6,6% (Ro) (p>0,05), tandis que les femmes – 75,7±2,3% (Ch) et 63,8±2,7% (Ro) (p<0,01). Une autre situation assez inquiétante est l'incapacité des étudiants de réduire l'intensité de leur effort physique : hommes – 54,1±4,7% (Ch) et 38,5±6,7% (Ro) (p>0,05) et femmes – 50,1±2,7% (Ch) et 34,2±2,7% (Ro) (p<0,001). À cet égard, les hommes ont mentionné qu'ils consacraient une grande partie de leur temps libre à l'activité physique dans 73,4±4,2% des cas (Ch) et 44,2±6,8%

(Ro) (p<0,001), et les femmes – 64,8±2,6% (Ch) et 42,2±2,8% (Ro) (p<0,001). Ainsi, certains d'entre eux ont pratiqué des activités physiques plus longtemps que prévu : hommes – 68,8±4,4% (Ch) et 44,2±6,8 (Ro) (p<0,01), femmes – 63,9±9,6% (Ch) et 43,8±2,8% (Ro) (p<0,001). On a également demandé aux étudiants de positionner le rôle des activités physiques par rapport à la famille. Les hommes ont mentionné qu'ils faisaient de l'exercice plutôt que de passer du temps avec des amis ou de la famille dans 66,9±4,6% des cas (Ch) et 40,4±6,8% (Ro) (p<0,01), tandis que les femmes – 50,5±2,7% des cas (Ch) et 37,9±2,7% (Ro) (p<0,01).

Au cours du dernier mois d'activité, 53,3% des étudiants ont signalé la présence de facteurs limitant leur activité physique (tab. 3).

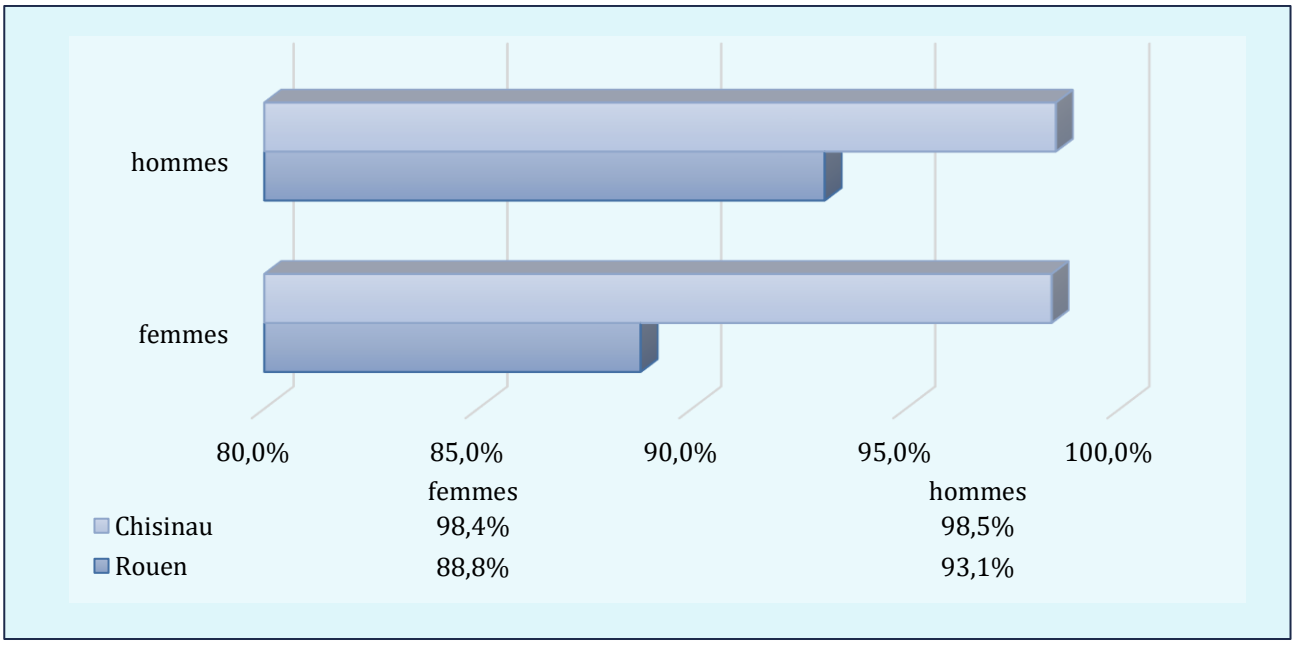


Figure 1. L'intensité de l'activité physique par les étudiants.

Tableau 3. Répartition des étudiants qui ont signalé la présence de facteurs limitant l'activité physique.

| | | Chisinau n=430 | | Rouen n=353 | |
|---|---|---|---|---|---|
| | | ***oui*** | ***non*** | ***oui*** | ***non*** |
| **Total** | no. abs. | 229 | 201 | 200 | 153 |
| | % | 53,3 | 46,7 | 56,6 | 43,4 |
| **Hommes** | no. abs. | 44 | 65 | 25 | 27 |
| | % | 19,2 | 32,3 | 12,5 | 17,6 |
| **Femmes** | no. abs. | 185 | 136 | 175 | 126 |
| | % | 80,8 | 67,7 | 87,5 | 82,4 |

L'analyse des données par sexe a révélé la présence de facteurs limitant l'activité physique à un plus grand nombre d'hommes de Chisinau – 19,2%, que chez les hommes de Rouen – 12,5%. Cependant, plusieurs femmes de Rouen (87,5%) ont signalé la présence de facteurs qui limitaient l'activité physique, par rapport aux femmes de Chisinau (80,8%).

En raison de la présence de facteurs limitant l'activité physique, 211 (49,1%) étudiants de Chisinau et 209 (59,2%) de Rouen n'ont pas pratiqué d'activité physique pendant 7 jours – 82 étudiants (Ch) et 51 (Ro) ; 7-13 jours – 32 (Ch) et 34 (Ro) ; 14-20 jours – 29 (Ch) et 27 (Ro) ; 21-27 jours – 7 (Ch) et 16 (Ro) et plus de 28 jours – 61 (Ch) et 81 (Ro) (fig.2).

## DISCUSSIONS

Le but de cette étude était de comparer les activités physiques effectuées par les étudiants des

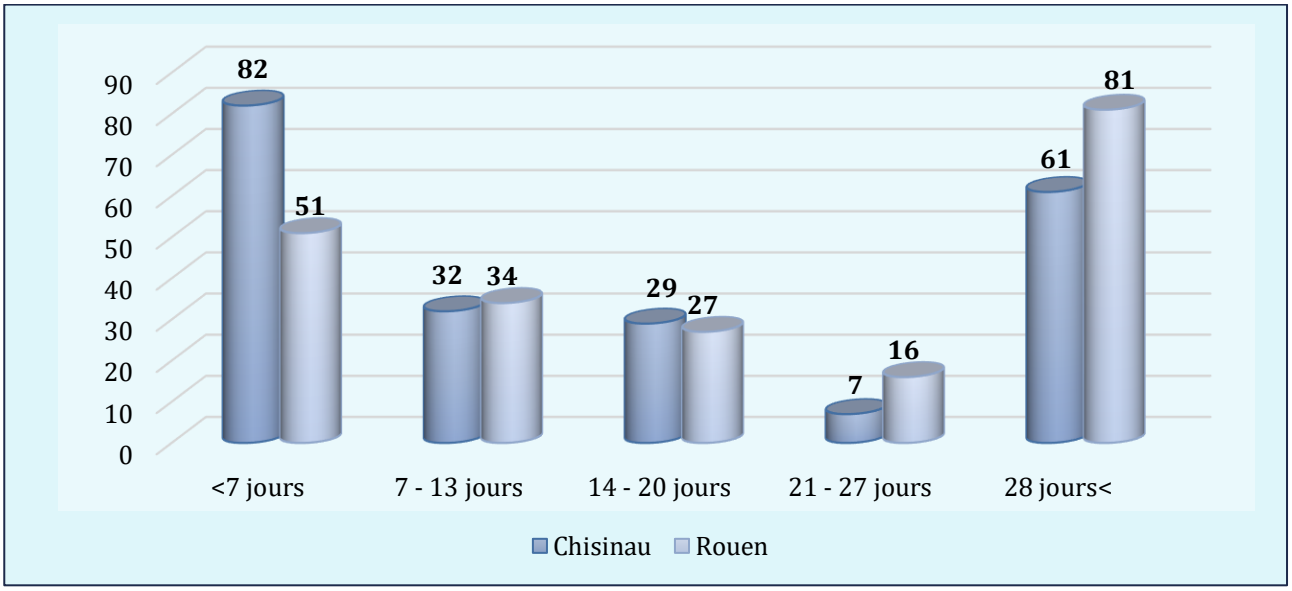


Figure 2. Manque d'activité physique (jours).

facultés de médecine dans deux pays eu ropéens: la République de Moldova et la France. A cette fin, une analyse comparative par pays et par critère de genre, y compris les indices somatométriques, a été utilisée. Les résultats ont établi que la plupart des différences étaient statistiquement significatives dans les scores majeurs et moyens des aspects étudiés : indices somatométriques, indice de masse corporelle, manque ou présence d'activités physiques, fréquence des facteurs limitant l'activité physique, le sport, durée des activités sportives, l'intensité de l'activité physique et diverses caractéristiques des activités physiques. Les recherches indiquent que les hommes et les femmes diffèrent tant à l'échelle mondiale qu'en termes spécifiques, en ce que concerne la confiance en soi dans le sport (5, 6). En général, les chercheurs ont noté que les femmes, habituellement, succombent aux hommes dans diverses activités physiques (7, 8, 9), un fait noté dans notre étude aussi.

L'activité physique apporte des avantages évidents pour la santé, le mouvement faisant partie intégrante du bon fonctionnement du corps humain. Ainsi, le manque d'activité physique et un mode de vie sédentaire constituent des facteurs de risque pour la santé. Cependant, les étudiants sont dans l'une des périodes de vie stressantes et exigeantes, ils font partie du processus d'étude, nécessitant du temps et d'énergie, même pendant le week-end. L'exercice est bénéfique à la fois en ce qu'il abaisse le niveau d'anxiété et conduit directement à l'amélioration de la qualité du sommeil. Mais aujourd'hui, les jeunes sont coincés dans leur routine quotidienne en négligeant le sport, ignorant qu'à mesure qu'ils vieillissent, il est extrêmement important pour les gens de rester actifs, car le sport aide à ralentir le processus de vieillissement physique et mental, en augmentant l'oxygénation des tissus et en renforçant le système immunitaire.

À l'heure actuelle, la sédentarité, le stress et le surpoids semblent gagner du terrain au détriment du sport (10, 11). C'est inquiétant pour les étudiants parce qu'ils doivent faire du sport, pas nécessairement des sports de performance, mais ils doivent garder leur corps fort et en bonne santé. Le sport développe à la fois les capacités physiques du corps et celles mentales et intellectuelles (6, 12).

Les activités physiques et intellectuelles quotidiennes, ainsi qu'une alimentation saine, peuvent renforcer le bouclier protecteur du cerveau et prévenir la maladie (13). Il y a aussi un besoin croissant de sport, car l'obésité et les maladies cardiaques s'installent même chez les jeunes (6). Par conséquent, les étudiants devraient être conscients de l'importance de l'activité physique pour le corps et la santé mentale et développer de certaines compétences qu'ils peuvent également prendre en compte plus tard dans la vie. Et notamment les étudiants en médecine devraient en être conscients, car ce sont eux qui promeuvent la santé et les principes d'un mode de vie sain dans la société.

L'effet du mouvement physique sur la santé dépend beaucoup de l'intensité, de la durée et de la fréquence. Une partie importante des avantages de l'effort physique sont obtenues quand ils dépassent une certaine durée (10 minutes) d'effort physique constant, les effets étant optimaux quand il atteint une durée de 30 minutes par jour. Pour rester en forme, il est idéal de faire de l'exercice au moins cinq fois par semaine, en introduisant le mouvement dans quotidiennement (14, 15).

La santé physique pendant les études universitaires ne cesse de se détériorer et le nombre d'étudiants atteints de pathologies chroniques augmente d'une année à l'autre. La détérioration de la santé physique et mentale est la conséquence du non-respect du régime d'activité intellectuelle par l'activité physique, la nutrition et le manque de sommeil adéquat et qualitatif. Certains chercheurs attestent un taux plus élevé de pathologies chroniques chez les futurs médecins que chez les étudiants des autres spécialités (16).

En Slovénie, le gouvernement a adopté en 2007 un plan national de santé publique sur l'activité physique pour améliorer la santé (HEPA Slovénie 2007-2012). Les trois piliers principaux de ce plan sont l'activité physique, l'activité physique dans les écoles et sur le lieu de travail et l'activité physique associée au transport. L'objectif fondamental du programme national HEPA est d'encourager toutes les formes d'activité physique régulière tout au long de la vie. Le programme a une large portée, avec des domaines et des groupes cibles, y compris les enfants et les adolescents, les familles, le travail, les personnes âgées, les personnes ayant des besoins spéciaux, le secteur social/santé, le secteur des transports et les organisations sportives (17).

Afin d'accroître l'importance de l'activité physique et de garantir son succès auprès les jeunes (18, 19), il est nécessaire d'analyser, d'évaluer et de mettre en œuvre des théories d'apprentissage innovantes et de nouvelles perceptions de l'éducation physique. Des recommandations ont été élaborées par les acteurs européens dans l'étude de l'UE sur « Le mode de vie des jeunes et sédentarisme » (17).

En France, l'ICAPS (intervention centrée sur l'activité physique et le comportement sédentaire des adolescents) est un programme à plusieurs niveaux avec de multiples acteurs, etc. Le programme vise à encourager les jeunes à poursuivre davantage d'activités physiques et à offrir des possibilités d'activité physique tant à l'intérieur qu'à l'extérieur des établissements d'enseignement. Les résultats des quatre premières années ont été positifs et indiquent que les mesures visant à réduire les niveaux d'obésité peuvent être couronnées de succès (17). A Rouen, le programme « Ta santé en un clic » (www.tasanteenunclic.org), programme novateur de promotion de la santé, spécifiquement adressé aux étudiants, propose des actions pour les étudiants pour lutter contre la sédentarité (20, 21).

Au Royaume-Uni, le gouvernement a alloué 100 millions de livres sterling pour un programme sportif non éducatif « Sport Unlimited ». Le programme vise à accroître les possibilités pour les jeunes de participer à des activités sportives en dehors des heures de classe, augmentant ainsi le niveau de participation à cinq heures par semaine. Les partenariats régionaux dans le domaine du sport consultent les jeunes pour s'assurer que les activités proposées sont celles qui sont préférées des jeunes. Le programme est une approche basée sur le partenariat et un certain nombre d'agents locaux en dehors des écoles peuvent fournir, également, des installations et des services pour les clubs de jeunes, les clubs sportifs, le secteur commercial privé et les centres de loisirs (17).

**CONCLUSIONS**

1. Les résultats de la recherche ont montré que les différences étaient légèrement significatives tant selon le sexe qu'au niveau des pays. Ainsi, l'hypothèse de l'étude a été confirmée.
2. L'activité physique (dans cette étude : sports, promenades, entretien ménager ou autres activités nécessitant une consommation d'énergie) a été mentionnée par plus de 75% des étudiants de Chisinau et de Rouen. Il parle du fait que l'activité physique n'est pas négligée, mais le nombre d'étudiants qui font de l'activité physique doit être augmenté.
3. De nombreux pays européens ont mis en œuvre avec succès des programmes visant à pro

mouvoir le sport chez les jeunes. À cet égard, dans la République de Moldova, il est nécessaire d'élaborer et de mettre en œuvre des programmes d'État qui capitaliserait sur l'importance de l'activité physique chez les étudiants.

## CONFLIT D'INTÉRÊTS

Les auteurs déclarent qu'il n'y a pas de conflit d'intérêts dans cet article.

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# EVALUATION OF THE COMPLIANCE OF PEOPLE WITH THE CONTAINMENT MEASURES AND WEARING-MASK BEHAVIOURS IN DIFFERENT STAGES OF COVID-19 PANDEMIC: AN OBSERVATIONAL STUDY FROM TURKEY

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***Keywords:*** *mask, containment measures, Covid-19, Turkey, lockdown.*

***Introduction.*** *Protection measurements should be paid attention so that the regions affected to a great extent gain time for medical care and medical facilities can cope with increasing intensive care cases. The purpose of this study was to investigate the change in the rate of behaviours of people related to going out and wearing a mask during the pandemic in Turkey.* ***Material and methods.*** *This observational study investigated people's behaviours of going out and mask- wearing in the province of Kahramanmaras in Turkey during 4 different periods with 14-day intervals before and after Covid-19 pandemic. A total of 48 hours camera record made in 4 different periods at 12 pedestrian crossings used intensively by people was examined. Two researchers recorded and examined the number and gender of the people using these pedestrian crossings and their wearing-mask behaviours on a data collection form. The obtained data were presented as tables and graphics, showing numbers and percentages. Appropriate mask wearing according to gender was analysed by Chi-Square test.* ***Results.*** *The number of people using pedestrian crossings decreased by 70.19% for men and 87.07% for women compared to before the pandemic. When comparing the appropriate mask-wearing according to gender, it was concluded that women had a higher statistically significant rate on the appropriate mask-wearing compared to men ($p<0.05$).* ***Conclusions.*** *Compliance to mask-wearing and control measures was high at the beginning of the pandemic. A high percentage of women wore masks correctly. About 40 days after the pandemic started, people wore the mask less correctly.*

***Cuvinte cheie:*** *mască, măsuri de izolare, Covid-19, Turcia, lockdown.*

**EVALUAREA COMPLIANȚEI PERSOANELOR CU MĂSURILE DE IZOLARE ȘI COMPORTAMENTUL DE A PURTA MASCA LA DIFERITE ETAPE ALE PANDEMIEI COVID-19: UN STUDIU OBSERVAȚIONAL ÎN TURCIA**

***Introducere.*** *Măsurile de protecție necesită o atenție sporită, astfel încât regiunile afectate să beneficieze de suficient timp pentru îngrijiri medicale, iar instituțiile medicale să poată gestiona numărul crescut de pacienți, internați la terapie intensivă. Scopul acestui studiu a fost de a investiga comportamentul persoanelor, ca răspuns la purtarea măștilor, în perioada pandemiei în Turcia.* ***Material si metode.*** *În acest studiu observațional a fost investigat comportamentul persoanelor, din provincia Kahramanmaras, Turcia, la purtarea măștii în aer liber, în 4 perioade diferite, cu intervale de 14 zile, în perioada pre-pandemică și pandemică Covid-19. Ca dovadă au fost examinate cca 48 de ore video, înregistrate de camerele de supraveghere a traficului, efectuate în 4 perioade diferite, la 12 treceri pentru pietoni, utilizate frecvent de pietoni. Aceste înregistrări au fost examinate de doi cercetători, iar numărul, sexul persoanelor, care utilizează aceste treceri de pietoni și comportamentul lor de a purta masca, precum și de a o purta corect, au fost înregistrate într-un formular de colectare a datelor. Datele obținute au fost prezentate sub formă de tabele și grafice în cifre și procente. Purtarea corectă a măștii, în funcție de sex, a fost analizată prin testul Chi-Square.* ***Rezultate.*** *Numărul persoanelor care folosesc trecerile pentru pietoni s-a diminuat cu 70,19%, în cazul bărbaților și cu 87,07%, în cazul femeilor, comparativ cu perioada pre-pandemică. La fel, femeile au avut o rată semnificativă și în ceea ce privește purtarea corectă a măștii, față de bărbați ($p<0,05$).* ***Concluzii.*** *Respectarea normelor de purtare a măștii și a măsurilor de control au fost stricte la început de pandemie, iar comparativ cu bărbații, femeile purtau și foloseau corect masca, într-un procent mai ridicat. Ulterior, aproximativ la 40 de zile de la declanșarea pandemiei, populația deja purta masca mai puțin corect.*

## INTRODUCTION

COVID-19 disease caused by SARS-CoV2 has turned into a pandemic with unknown transmission ways (1). The first COVID-19 case was identified on 11 March 2020 in Turkey, and the WHO declared its pandemic announcement on this date (2, 3).

The WHO suggested using barrier measures such as keeping a distance between other people at least one meter and wearing a medical mask (4). It is likely that the COVID-19 may slow down by taking people in risky regions under control and wearing a mask. Protection measurements should be paid attention so that the regions affected to a great extent gain time for medical care and medical facilities can cope with increasing intensive care cases (5, 6). Many European countries and the countries like USA, Canada and Australia imposed restrictions from forbidding school, entertainment activities and big events to a whole lockdown (7). On the other hand, South Korea and Hong Kong could prevent the COVID-19 outbreak to a certain extent. Mass masking, which plays an important role in barrier measures, may lead to a control on infectors and infected individuals in the outbreak by reducing the spread of infected droplets, especially from individuals with asymptomatic COVID-19 but the efficiency of mass masking is still disputable. Although the WHO and ECDC (European Centre for Disease Prevention and Control) have prepared conflicting reports on the fact that mass masking to be applied by healthy people may prevent being infected with the COVID-19, it is considered that mass masking would help the decrease of infection spreading (8-12).

*The purpose of this study* was to investigate the change in the rate of behaviours of people related to going out and wearing a mask during the pandemic in Turkey.

## MATERIAL AND METHODS

This observational study investigated the people's behaviours of going out and wearing a mask in the province of Kahramanmaras in Turkey during 4 different periods with 14-day intervals, starting with the date of 10 March and then 24 March 2020, 7 April 2020 and 21 April 2020. This study was administrated on Tuesdays during 12.00-13.00, the most crowded times of the day. As there were curfews at some weekends in Turkey, a weekday was considered to be more appropriate for the study. The observations on the above-mentioned dates were made through the camera records of the City Security Management Systems (KGYS-MOBESE) taken from the Provincial Security Directorate of Kahramanmaras by an official letter. A total of 48 hours camera record made in 4 different periods at 12 pedestrian crossings used intensively by people was examined.

Two researchers recorded and examined the number and gender of the people using these pedestrian crossings and their wearing-mask behaviours on a data collection form. Wearing a mask by covering mouth and nose was considered as the appropriate mask-wearing. The assessment made by two researchers independently at one randomly selected pedestrian crossing from the above mentioned 12 ones, exhibited four reliability coefficients in the test which were above 0.95. While a total of 12.625 people were determined by crossing, 154 people with clothes covering their faces were not evaluated in terms of wearing mask behaviour. The obtained data were presented as tables and graphics with numbers and percentages. Wearing appropriate mask according to gender was analysed by Chi-Square test. An ethics committee approval was not needed for this observational study since no personal data were used, thus the study was carried out in accordance with the Helsinki declaration.

## RESULTS

In this study, the passers-by at 12 different pedestrian crossings within 4 different periods were investigated. It was determined that a total of 6013 people used these pedestrian crossings on 10 March 2020, while this number was found to be 2301 on 24 March 2020, 1441 on 07 April 2020 and 2870 on 21 April 2020. While a 76,04% decrease was observed in the number of people using these pedestrian crossings on 07 April 2020 compared to the date of 10 March 2020 (70.19% for male, 87.07% for female), an increase was found in the number of passers-by. It was determined that women respected the curfew more than men. The number and gender of people passing through the above-mentioned pedestrian crossings and their wearing mask status are provided in Table 1.

Table 1. Distribution of the number of people in the crosswalk and their mask wearing rates.

| | 10.03.2020 (0*) | | | | 24.03.2020 (1.872*) | | | | 07.04.2020 (34.109*) | | | | 21.04.2020 (95.591*) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | %[a] | %[b] | %[c] | n | %[a] | %[b] | %[c] | n | %[a] | %[b] | %[c] | n | %[a] | %[b] | %[c] |
| **Region 1** | | | | | | | | | | | | | | | | |
| Male | 1460 | 66.8 | 0.14 | 0.14 | 877 | 89.9 | 8.6 | 6.7 | 530 | 81.4 | 73.0 | 59.4 | 979 | 71.7 | 85.6 | 59.6 |
| Female | 724 | 33.2 | 0 | 0 | 99 | 10.1 | 29.3 | 26.8 | 121 | 18.6 | 82.0 | 72.1 | 386 | 28.3 | 95.7 | 92.3 |
| Total | 2184 | | 0.09 | 0.09 | 976 | | 10.3 | 8.4 | 651 | | 74.6 | 61.6 | 1365 | | 88.3 | 68.3 |
| **Region 2** | | | | | | | | | | | | | | | | |
| Male | 42 | 72.4 | 0 | 0 | 17 | 70.8 | 11.8 | 11.8 | 7 | 63.6 | 42.9 | 42.9 | 11 | 64.7 | 54.5 | 27.3 |
| Female | 16 | 27.6 | 0 | 0 | 7 | 29.2 | 0 | 0 | 4 | 36.4 | 50.0 | 50.0 | 6 | 35.3 | 83.3 | 66.7 |
| Total | 58 | | | | 24 | | 8.3 | 8.3 | 11 | | 45.5 | 45.5 | 17 | | 64.7 | 41.2 |
| **Region 3** | | | | | | | | | | | | | | | | |
| Male | 158 | 68.7 | 0 | 0 | 84 | 82.4 | 7.1 | 4.8 | 51 | 82.3 | 64.7 | 33.3 | 87 | 67.4 | 82.8 | 50.6 |
| Female | 72 | 31.3 | 0 | 0 | 18 | 17.6 | 11.8 | 11.8 | 11 | 17.7 | 100 | 81.8 | 42 | 32.6 | 89.7 | 76.9 |
| Total | 230 | | 0 | 0 | 102 | | 7.9 | 5.9 | 62 | | 71.0 | 41.9 | 129 | | 84.9 | 58.7 |
| **Region 4** | | | | | | | | | | | | | | | | |
| Male | 671 | 67.2 | 0.15 | 0.15 | 277 | 83.7 | 5.7 | 5.7 | 159 | 83.7 | 77.4 | 57.9 | 346 | 82.0 | 76.0 | 52.9 |
| Female | 327 | 32.8 | 0.30 | 0.30 | 54 | 16.3 | 17.6 | 17.6 | 31 | 16.3 | 93.3 | 83.3 | 76 | 18.0 | 93.2 | 87.7 |
| Total | 998 | | 0.20 | 0.20 | 331 | | 7.6 | 7.6 | 190 | | 79.9 | 61.9 | 422 | | 79.0 | 58.9 |
| **Region 5** | | | | | | | | | | | | | | | | |
| Male | 217 | 65.6 | 0 | 0 | 93 | 76.2 | 3.2 | 3.2 | 62 | 80.5 | 71.0 | 59.7 | 90 | 75.0 | 75.6 | 46.7 |
| Female | 114 | 34.4 | 0 | 0 | 29 | 23.8 | 25.9 | 25.9 | 15 | 19.5 | 100 | 100 | 30 | 25.0 | 84.6 | 76.9 |
| Total | 331 | | 0 | 0 | 122 | | 8.3 | 8.3 | 77 | | 76.6 | 67.5 | 120 | | 77.6 | 53.4 |
| **Region 6** | | | | | | | | | | | | | | | | |
| Male | 203 | 75.2 | 0 | 0 | 98 | 88.3 | 11.2 | 10.2 | 67 | 84.8 | 88.0 | 76.1 | 88 | 79.3 | 86.4 | 62.5 |
| Female | 67 | 24.8 | 0 | 0 | 13 | 11.7 | 23.1 | 23.1 | 12 | 15.2 | 90.9 | 63.6 | 23 | 20.7 | 95.7 | 91.3 |
| Total | 270 | | 0 | 0 | 111 | | 12.6 | 11.7 | 79 | | 88.5 | 74.4 | 111 | | 88.3 | 68.5 |
| **Region 7** | | | | | | | | | | | | | | | | |
| Male | 277 | 69.1 | 0 | 0 | 133 | 79.6 | 6.0 | 6.0 | 69 | 78.4 | 73.9 | 68.1 | 143 | 83.1 | 72.0 | 58.7 |
| Female | 124 | 30.9 | 0 | 0 | 34 | 20.4 | 8.8 | 8.8 | 19 | 21.6 | 84.2 | 73.7 | 29 | 16.9 | 92.6 | 85.2 |
| Total | 401 | | 0 | 0 | 167 | | 6.6 | 6.6 | 88 | | 76.1 | 69.3 | 172 | | 87.6 | 62.9 |
| **Region 8** | | | | | | | | | | | | | | | | |
| Male | 480 | 67.6 | 0.20 | 0.20 | 182 | 78.4 | 6.6 | 5.5 | 125 | 82.2 | 70.4 | 60.8 | 192 | 71.4 | 84.9 | 64.1 |
| Female | 230 | 32.4 | 0.44 | 0.44 | 50 | 21.6 | 12.5 | 8.3 | 27 | 17.8 | 88.9 | 88.9 | 77 | 28.6 | 100 | 92.2 |
| Total | 710 | | 0.28 | 0.28 | 232 | | 7.8 | 6.1 | 152 | | 73.7 | 65.8 | 269 | | 88.7 | 71.1 |
| **Region 9** | | | | | | | | | | | | | | | | |
| Male | 10 | 41.7 | 10.0 | 0 | 4 | 66.7 | 0 | 0 | 2 | 40.0 | 50.0 | 50.0 | 8 | 66.7 | 75.0 | 50.0 |
| Female | 14 | 58.3 | 0 | 0 | 2 | 33.3 | 0 | 0 | 3 | 60.0 | 66.7 | 66.7 | 4 | 33.3 | 100 | 100 |
| Total | 24 | | 4.2 | 0 | 6 | | 0 | 0 | 5 | | 60.0 | 60.0 | 12 | | 83.3 | 66.7 |
| **Region 10** | | | | | | | | | | | | | | | | |
| Male | 29 | 41.4 | 0 | 0 | 20 | 71.4 | 15.0 | 10.0 | 19 | 65.5 | 52.6 | 52.6 | 32 | 69.6 | 87.5 | 62.5 |
| Female | 41 | 58.6 | 0 | 0 | 8 | 28.6 | 0 | 0 | 10 | 34.5 | 80.0 | 80.0 | 14 | 30.4 | 78.6 | 64.3 |
| Total | 70 | | 0 | 0 | 28 | | 10.7 | 7.1 | 29 | | 62.1 | 62.1 | 46 | | 84.8 | 63.0 |
| **Region 11** | | | | | | | | | | | | | | | | |
| Male | 119 | 41.0 | 0.84 | 0.84 | 75 | 72.8 | 8.0 | 6.7 | 39 | 83.0 | 79.5 | 74.4 | 82 | 74.5 | 72.0 | 50.0 |
| Female | 171 | 59.0 | 0 | 0 | 28 | 27.2 | 21.4 | 21.4 | 8 | 17.0 | 100 | 100 | 28 | 25.5 | 92.3 | 92.3 |
| Total | 290 | | 0.34 | 0.34 | 103 | | 11.6 | 10.7 | 47 | | 83.0 | 78.7 | 110 | | 76.9 | 60.2 |
| **Region 12** | | | | | | | | | | | | | | | | |
| Male | 267 | 59.7 | 0 | 0 | 75 | 75.8 | 2.6 | 2.6 | 42 | 84.0 | 47.6 | 33.4 | 68 | 70.1 | 72.1 | 58.8 |
| Female | 180 | 40.3 | 0 | 0 | 24 | 24.2 | 4.5 | 4.5 | 8 | 16.0 | 100 | 87.5 | 29 | 29.9 | 93.1 | 93.1 |
| Total | 447 | | 0 | 0 | 99 | | 3.1 | 3.1 | 50 | | 56.0 | 42.0 | 97 | | 78.4 | 69.1 |
| **All region** | | | | | | | | | | | | | | | | |
| Male | 3933 | 65.4 | 0.15 | 0.13 | 1935 | 84.1 | 7.4 | 6.3 | 1172 | 81.3 | 72.5 | 59.0 | 2126 | 74.1 | 82.3 | 57.5 |
| Female | 2080 | 34.6 | 0.10 | 0.10 | 366 | 19.9 | 18.0 | 16.8 | 269 | 18.7 | 86.8 | 78.2 | 744 | 25.9 | 94.3 | 89.3 |
| Total | 6013 | | 0.13 | 0.12 | 2301 | | 9.0 | 7.8 | 1441 | | 75.1 | 62.5 | 2870 | | 85.2 | 65.2 |

*Note:* %[a]: Percent column. gender percentage
%[b]: Percent row. having mask
%[c]: Percent row. wearing correct mask
*Covid-19 number of cases in Turkey (22)

On 10 March 2020, when there was no Covid-19 case in Turkey, 65.4% of 6013 people, who passed the pedestrian crossings were males and this rate was found to be as follows in the other dates: 84.1%, 81.3% and 74.1% (fig. 1).

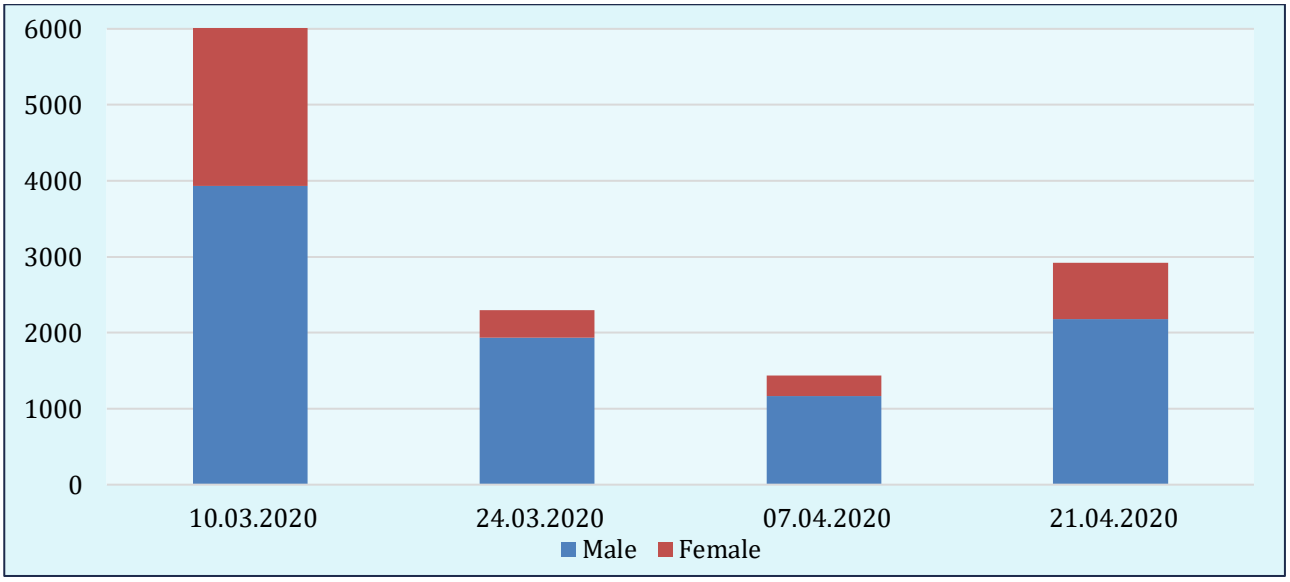


Figure 1. Distribution of the number of people in the crosswalk by gender.

When comparing the appropriate mask-wearing according to gender, it was concluded that women had a statistically significant rate on the appropriate mask-wearing compared to men on 24 March 2020, 07 April 2020 and 21 April 2020 ($p<0.001$) (respectively, $\chi^2$=45,140 and 34,091 and 246,876).

It was also found out that the rate of women's appropriate mask-wearing behaviour increased over time during the COVID 19 pandemic period, whereas men complied with appropriate mask-wearing at a lesser extent despite the increase in the behaviour of wearing a mask on 21 April 2020, this case is shown in Figure 2.

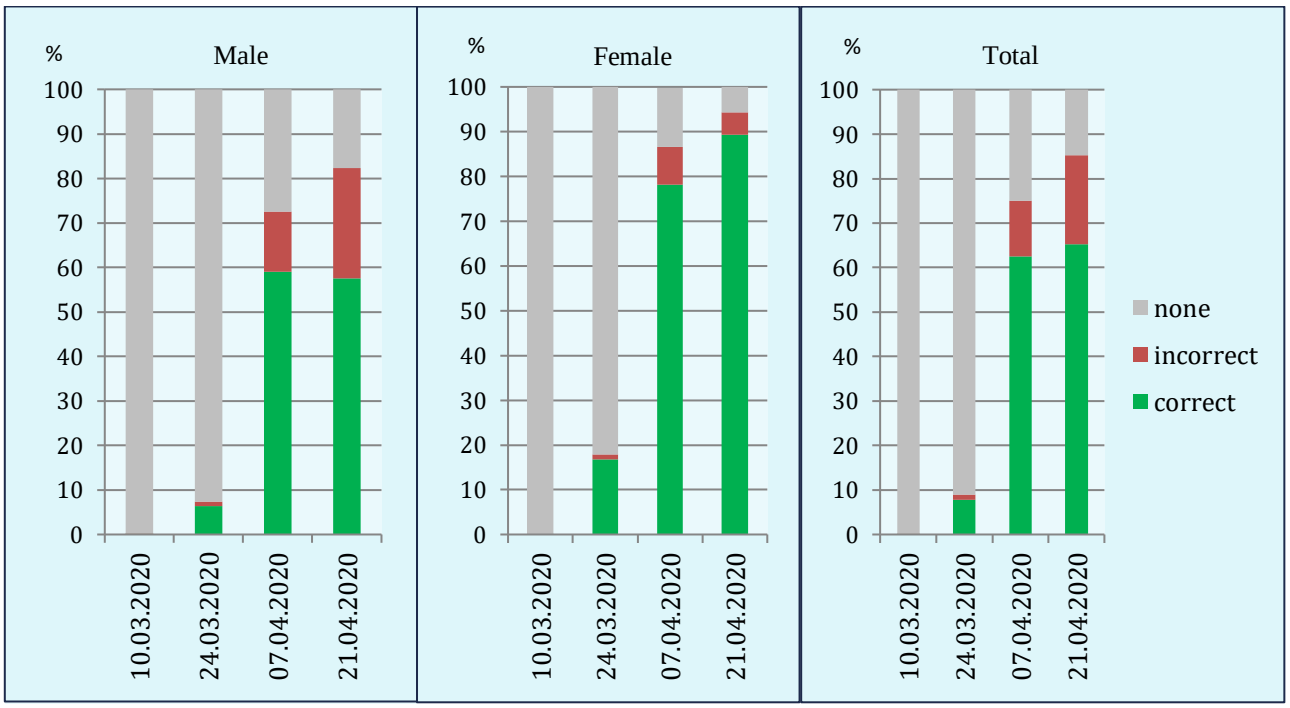


Figure 2. Distribution of mask wearing rates by gender.

**DISCUSSIONS**

The containment measures have of paramount importance in the control of the COVID-19 pandemic. There are studies arguing that the containment measures are necessary to take the outbreak under control and these should be administrated as soon as possible. Many countries have initiated COVID-19 containment measures due to the pandemic (13-16). The first COVID-19 wave seen outside Hubei, China, where the outbreak was first detected, declined due to aggressive non-pharmaceutical control measures. The efficiency of containment measures was seen when the spread of pandemic was prevented to a great extent with second strict measures (5, 16, 17). It was identified that the lockdown administrated firstly in Lombardy, Italy and then the whole country and the lockdown in Spain prevented the spread of disease to a certain extent. It was reported in another study that the lockdown had a positive effect particularly in central and southern regions of Italy (18, 19). The containment measures taken in Turkey are as follows: schools were vacationed on 16 March 2020, a curfew circular was issued to those with chronic illnesses aged 65 and over on 21 March 2020, city entrance and age restriction measures were taken on 03 April 2020, and the first two days curfew was announced on 10 April 2020 (20, 21). While containment measures were administrated partially in Turkey, there are some studies suggesting that only very strict restrictions caused a decline in virus spreading (7).

In this study, 76,04% decrease was found in the number of people passing through pedestrian crossings on 07 April 2020 compared to 10 March 2020. Although the number of case was increasing in Turkey, an increase was determined in the number of people using the above-mentioned pedestrian crossings on 21 April 2020 (22). The duration of protection measures plays an important role in fighting against the spread of disease in terms of compliance with control measures (23). It is also urged in our study that long term protection measures have a negative effect on the attitudes of people to go out. In a study conducted in Australia on compliance with home quarantine for pandemic influenza, 94.1% of the participants stated that they were willing to comply with quarantine at home, and avoided public events and meetings at very high rates. In addition, it was identified in this study that men complied with the home quarantine less than women (24). In this study, the rate of men going outside, which was higher even in the pre-pandemic period, increased even more during the pandemic process. The women were observed to comply with the home quarantine more.

In this study, containment measures and wearing mask behaviours of people were investigated. Wearing a mask protects people who wear masks and the people around them, thus, the use of face masks has a critical importance in reducing the spread of disease (18, 25). The face masks should be worn carefully on mouth and face, and should be tied safely (26). In this research, the appropriate mask-wearing of people was determined to be 7.8% on 24 March 2020, 62.5% on 7 April 2020 and 65.2% on 21 April 2020. It was observed that women wore masks at a significantly higher rate than men at these periods, and their appropriate mask-wearing was also at higher rates. In Large Scale International Poll Survey made with 29.000 people from different countries during COVID-19 pandemic, the mask-wearing rates according to countries are as follows: Italy 81%, France 34%, Germany 20% China 83%, Japan 77% (27). 86% of 160 adult participants in Taiwan reported that they used medical masks for several times during a day (28). In a study conducted on primary school students in China, while 51.60% of the students showed a good behaviour in wearing a mask, a significant difference was not found between the mask-wearing behaviours of male and female students (29). Another research carried out in England on 2.025 participants regarding the relationship between the rate of wearing a face mask during COVID-19 pandemic and demographic, health and psychological variables, showed that the face mask wearing rate was 16.7%, and the rate of wearing a mask in males was concluded to be higher than in females (30). An investigation conducted on 2.459 participants, reported that men considered wearing a mask as shameful, being an indicator of weakness, stigmatizing, as they felt negative emotions while wearing masks, thus did not comply with the necessity of wearing masks as much as women. The same study urged that men did not believe that the pandemic would affect them severely (31). In another study carried out in Hong Kong in 2017, when there was no pandemic, less than one-fifth of the participating family members reported that they always wore face masks in inflammatory diseases

and respiratory tract infections, while men stated that they used the mask less frequently (32). The mask-wearing behaviours were determined by observation in our study rather than a questionnaire. The rates of men's not complying with the rule of mask-wearing and their general mask-wearing rates are in accordance with the literature.

This study has some limitations. First, although there are some criteria, the assessment in this observational study is subjective. Secondly, instant video recordings of pedestrian crossings were examined. The passage of people is dynamic and constantly changing. Third, the study was conducted at the beginning of the pandemic. Results cannot be generalized for a pandemic that lasts more than one year. Moreover, the small sample size in a single city can be considered as another limitation.

## CONCLUSIONS

1. Compliance to mask-wearing and control measures was higher at the beginning of the pandemic.
2. In the records reviewed, women were less likely to be seen at the pedestrian crossing than men before the pandemic. After the pandemic started, women adapted to containment measures more than men, thus being more restricted. Women behaved more responsibly. In addition, a high percentage of women wore and used masks correctly.
3. About 40 days after the pandemic started, people wore the mask less correctly. It is considered that the decrease in wearing a mask and complying with containment measures despite a rapid increase in COVID-19 case numbers in Turkey may be related to the decrease in disease risk perception.

## CONFLICT OF INTERESTS

Authors declare that there is no conflict of interest. No informed consent was received.

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# ANTIMICROBIAL ACTIVITY OF NOVEL 1-[(2,4-(DI-TERT-BUTYLPHENOXY))-3-DIALKYLAMINO-2-PROPANOL] DERIVATIVES

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***Keywords:*** *aminopropanol derivatives, antimicrobial activity, bacteria, biofilm.*

***Introduction.*** *The microbial biofilm-forming ability is one of the major aspects of the emerging issue of antibiotic resistance, which makes them tolerant to antibiotics and host defense systems and other external stresses, thus contributing to persistent chronic infect ions. A series of relevant studies confirmed the high efficiency of aminopropanol derivatives as potential antibacterial and antifungal agents. This present study was aimed to evaluate the antimicrobial activity of new 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives on the planktonic bacterial/fungal cells and biofilms.*

***Material and methods.*** *The minimum inhibitory concentrations (MIC) of the new compounds were determined by a standard method, along with their effects on biofilms estimated via the gentian violet adsorption-desorption assay.*

***Results****. The KVM-219 compound showed the most pronounced effect on planktonic bacterial and fungal cells. The MIC values ranged between 0.78 µg/mL to 12.5 µg/mL, depending on the microbial strain. The KVM-316 compound exhibited the strongest inhibitory effect on biofilms, thus preventing their formation by S. aureus (96.1%), E. coli (57.2%), and P. aeruginosa (96.1%).*

***Conclusions.*** *The 15 newly synthesized 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives revealed marked antibacterial and antifungal effects on planktonic microorganisms. Most of these compounds showed a strain-specific inhibition of biofilm formation by at least 50% for S. aureus 222, E. coli 311, P. aeruginosa 449 and C. glabrata 404 strains.*

***Cuvinte cheie:*** *derivați de aminopropanol, activitate antimicrobiană, bacterii, biofilm.*

**ACTIVITATEA ANTIMICROBIANĂ A NOILOR DERIVAȚI DE 1 - [(2,4-(DI-TERT-BUTYLPHENOXY)) - 3-DIALKILAMINO-2-PROPANOL]**

***Introducere.*** *Scopul studiului l-a constituit evaluarea efectelor antimicrobiene ale derivaților 1 - [(2,4 (di-terț- butylphenoxy)) - 3- dialkylamino-2-propanol] asupra celulelor planctonice bacteriene/fungice și asupra biofilmelor.*

***Material și metode.*** *Concentrațiile minime inhibitorii (CMI) ale compușilor noi au fost determinate printr-o metodă standard, activitatea antibiofilm a fost testată prin absorbția violetului de gențiană pe structuri formate pe plăci de polistiren, urmată de resolubilizare cu solvent organic și resazurină ca indicator redox.*

***Rezultate.*** *Efectul cel mai pronunțat asupra celulelor planctonice bacteriene și fungice l-a demonstrat compusul KVM -219, CMI 0,67 µg/ml - 12,5 µg/ml, în funcție de microorganism, iar asupra biofilmelor - compusul KVM-316. KVM-316 a prevenit formarea biofilmelor de către S. aureus (96,1%), E. coli (57,2%) și P. aeruginosa (96,1%).*

***Concluzii.*** *Cei 15 derivați nou sintetizați ai 1 - [(2,4- (di- tert-butylphenoxy)) - 3- dialkylamino -2-propanol] au prezentat efecte antibacteriene și antifungice pronunțate asupra microorganismelor planctonice. Majoritatea acestor compuși au inhibat în mod specific formarea biofilmelor de către tulpinile clinice S. aureus 222, E. coli 311, P. aeruginosa 449 și C. glabrata 404 cu cel puțin 50%.*

## INTRODUCTION

Over the decades, antimicrobial agents have been considered the primary suppressing means for bacterial infections. However, their irrational use led to the emergence and spread of antimicrobial-resistant strains (1, 2). The antimicrobial drug resistance has led to a decrease of infection prevention and control measures and lowered the therapeutic effectiveness thus resulting in a prolonged patient's hospital stay and increased treatment costs. The antibiotic resistance has been regarded nowadays as a major threat to public internal safety across many countries. Therefore, a global action plan to overcome antimicrobial resistance has been developed by World Health Assembly (3). In 2017, WHO presented a list of 12 bacterial species that pose a threat to human health, which are classified into three categories of pathogens, namely critical, high and medium priority, according to the urgency of need for new antibiotics (4). The goal of the global action plan is to ensure, for as long as possible, continuity of successful treatment and prevention of infectious diseases by effective and safe medicines that are quality-assured, used in a responsible way and accessible to all who need them. To achieve this goal, five strategic objectives have been set out: to improve awareness and understanding of antimicrobial resistance; to strengthen knowledge through surveillance and research; to reduce the incidence of infection; to optimize the use of antimicrobial agents; to develop the economic case for sustainable investment that takes account of the needs of all countries; and to increase investments in new medicines, diagnostic tools, vaccines and other interventions (3). Considering all of the above mentioned, the most promising approach is to search for potentially novel antimicrobial agents for combatting antimicrobial resistance.

The microbial biofilm-forming ability is one of the major aspects of the emerging issue of antibiotic resistance, which makes them tolerant to antibiotics and host defence systems and other external stresses, thus contributing to persistent chronic infections (5). Several studies confirmed the high efficiency of aminopropanol derivatives as potential antibacterial and antifungal agents, which actually drew our interest to compounds of this group (6, 7, 8).

The purpose of the present study was to evaluate the antimicrobial activity of new 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives.

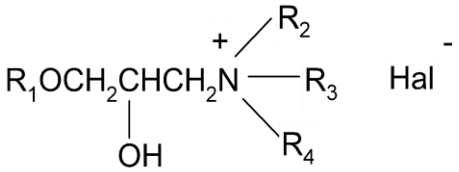
## MATERIAL AND METHODS

### Bacterial strain and growth conditions

The study was conducted on the gram-positive (*Staphylococcus aureus subsp. aureus* (ATCC® 25923™), *S. aureus* 222 (*MRSA*)) and gram-negative (*Escherichia coli* (ATCC® 25922™), *E. coli* 311, *Pseudomonas aeruginosa* (ATCC® 27853 ™), *P. aeruginosa* 449) bacterial strains, and yeasts (*Candida albicans* NTCC 885/653, *C. glabrata* 404). The bacterial strains were subcultured at 37°C in Mueller-Hinton broth (HiMedia™ Laboratories Pvt Ltd) and Tryptic Soy Broth (TSB) (Merck Millipore) (pH 7.3), yeasts – at 30-32°C in Saburo dextrose broth (HiMedia™ Laboratories Pvt Ltd) (pH 5.6).

### Chemicals

The new derivatives of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] were first synthesised at the Institute of Organic Chemistry NAS of Ukraine. These compounds were synthesized by using the same procedure (9). Its general structural formula is shown in Figure 1.




$R_1$ – (2,4-di-tert-butylphenyl); $R_2$,$R_3$ – alkyl, dialkyl, cycloalkyl; $R_4$ – methyl, benzyl, 4-nitrobenzyl, 4-metoxybenzyl, 4-chlorobenzyl, 4- fluorobenzyl, 4-methylbenzyl; Hal – Cl-, (I-).

Figure 1. Structural formula of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] new derivatives.

**Minimum Inhibitory Concentration (MIC) determination**

Synthesized compounds antimicrobial activity (**I – XV**) was tested by the twofold serial dilution method (10, 11) against gram-positive (*S. aureus* ATCC 25923) and gram-negative (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853) bacteria, and yeasts (*C. albicans* NTCC 885/653). Inoculum density was 1-2×$10^5$ CFU/mL culture medium (bacteria) and 1-2×$10^4$ CFU/mL (yeasts). The 96-well microtiter plates with bacterial cultures were incubated at 35-37°C for 18-24 h, while yeasts – at 30-32°C for 24-48 h. Mueller-Hinton broth and Saburo dextrose broth were used for minimal inhibitory concentration (MIC) determination. The lowest compound concentration inhibiting the microbial growth was considered as the MIC. All assays were performed in triplicate for control of culture growth (as a positive) and cultural media (as a negative).

**Quantitative biofilm assay**

The anti-biofilm activity of the tested compounds was determined by using the microtiter plate for biofilm formation assay described by O'Toole (12). The overnight cultures were diluted 100-fold with fresh TSB medium (bacteria) or Saburo dextrose broth (yeasts). Cell suspensions (100 µL) were transferred into individual wells of sterile polystyrene 96-well plate. The anti-biofilm effect was estimated by growing strains in media with or without test compounds (2.0×MIC) at 37°C for 24 hours. After incubation, the media were discarded, and plates were rinsed thrice with distillate water to remove nonadherent cells. Adherent cells were stained for 10-15 min with 0.1% crystal violet. The dye was extracted with ethanol for 15 min to quantify biofilm formation. The optical density was measured at 630 nm via the Absorbance Microplate Reader (model ELx800, BioTek, USA). The measurements were performed in six replications and repeated for at least three times; the values were then averaged.

**Statistical Analysis**

Newman-Keils (ANOVA) and Kruskal-Wallis criteria were used to assess the results via the STATISTICA, version 6.0 (StatSoft. Inc., USA) (13). Data are presented as M±m, where M is the mean value and m is the standard error of the mean.

## RESULTS

The studies of antibacterial and antifungal activities confirmed that 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] new derivatives caused inhibitory effect on *S. aureus* and *C. albicans*, with the MIC values ranging from 0.78 to 3.75 µg/mL and from 1.56 to 20.0 µg/mL, respectively. These compounds revealed no antibacterial effects against gram-negative bacteria, except for the **VII** and **IV** compounds, the MIC value for *E. coli* reaching to 12.5 µg/mL and 20.0 µg/mL, respectively (tab. 1, 2).

Table 1. Antimicrobial activity (MIC, µg/mL) of new 1-(2,4-(di-tert-butylphenoxy)-3- dialkylamino-2-propanol derivatives.

| Compound | *S. aureus subsp. aureus* (ATCC® 25923™) | *E. coli* (ATCC® 25922™) | *P. aeruginosa* (ATCC® 27853™) | *C. albicans* NTCC 885/653 |
|---|---|---|---|---|
| KVM-190 (**I**) | 2.5 | >20.0 | >20.0 | 3.75 |
| KVM-266 (**II**) | 5.0 | >20.0 | >20.0 | 7.5 |
| KVM-267 (**III**) | 5.0 | >20.0 | >20.0 | 7.5 |
| KVM-316 (**IV**) | 5.0 | 20.0 | >20.0 | 5.0 |
| KVM-251 (**V**) | 3.12 | >20.0 | >20.0 | 12.5 |
| KVM-327 (**VI**) | 1.56 | >20.0 | >20.0 | 6.25 |
| KVM-219 (**VII**) | 0.78 | 12.5 | >20.0 | 1.56 |
| KVM-220 (**VIII**) | 2.5 | >20.0 | >20.0 | 3.75 |
| KVM-269 (**IX**) | 5.0 | >20.0 | >20.0 | 7.5 |
| KVM-268 (**X**) | 5.0 | >20.0 | >20.0 | 7.5 |
| KVM-221 (**XI**) | 7.5 | >20.0 | >20.0 | 5.0 |
| KVM-222 (**XII**) | 3.75 | >20.0 | >20.0 | 2.5 |
| KVM-319 (**XIII**) | 1.56 | >20.0 | >20.0 | 20.0 |
| KVM-317 (**XIV**) | 5.0 | >20.0 | >20.0 | 5.0 |
| KVM-318 (**XV**) | 1.56 | >20.0 | >20.0 | 5.0 |

Table 2. Structure of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives in correspondence with antibacterial and antifungal activity.

| Compound | Substituents | | | | | MIC, μg/mL | |
|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | Hal | *S. aureus* | *C. albicans* |
| **I** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | $CH_3$ | $CH_3$ | $CH_2C_6H_5$ | Cl- | 2.5 | 3.75 |
| **II** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | $CH_3$ | $CH_3$ | $CH_2(C_6H_4)$-4-$NO_2$ | Cl- | 5.0 | 7.5 |
| **III** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | $CH_3$ | $CH_3$ | $CH_2(C_6H_4)$-4-$OCH_3$ | Cl- | 5.0 | 7.5 |
| **IV** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | $CH_3$ | $CH_3$ | $CH_2(C_6H_4)$-4-Cl | Cl- | 5.0 | 5.0 |
| **V** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | $CH_3$ | $C_6H_{11}$ | $CH_2C_6H_5$ | Cl- | 3.12 | 12.5 |
| **VI** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | $CH_3$ | $C_6H_{11}$ | $CH_2(C_6H_4)$-4-Cl | Cl- | 1.56 | 6.25 |
| **VII** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | ($C_4H_8$) | | $CH_3$ | I- | 0.78 | 1.56 |
| **VIII** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | ($C_4H_8$) | | $CH_2C_6H_5$ | Cl- | 2.5 | 3.75 |
| **IX** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | ($C_4H_8$) | | $CH_2(C_6H_4)$-4-$NO_2$ | Cl- | 5.0 | 7.5 |
| **X** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | ($C_4H_8$) | | $CH_2(C_6H_4)$-4-$OCH_3$ | Cl- | 5.0 | 7.5 |
| **XI** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | ($C_6H_{12}$) | | $CH_3$ | I- | 7.5 | 5.0 |
| **XII** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | ($C_6H_{12}$) | | $CH_2C_6H_5$ | Cl- | 3.75 | 2.5 |
| **XIII** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | [$(CH_2)_2CHCH_3(CH_2)_2$] | | $CH_2(C_6H_4)$-4-$NO_2$ | Cl- | 1.56 | 20.0 |
| **XIV** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | [$(CH_2)_2CHCH_3(CH_2)_2$] | | $CH_2(C_6H_4)$-4-$CH_3$ | Cl- | 5.0 | 5.0 |
| **XV** | 2,4-[$(CH_3)_3C$]$C_6H_3$ | [$(CH_2)_2CHCH_3(CH_2)_2$] | | $CH_2(C_6H_4)$-4-F | Cl- | 1.56 | 5.0 |

2,4-[$(CH_3)_3C$]$C_6H_3$ –- 2,4-di-tert-butylphenyl radical.

The most pronounced effect was found in the **VII** compound. The MIC value against *S. aureus* was 0.78 μg/mL, *E. coli* – 12.5 μg/mL, and for *C. albicans* – 1.56 μg/mL.

Considering that biofilms are the main mode of microbial existence, the evaluation of their susceptibility to the tested compounds was of great scientific interest. Our clinical trials tested the following microbial strains: *S. aureus* 222, *E. coli* 311, *P. aeruginosa* 449, and *C. glabrata* 404.

The obtained results demonstrated that all the tested compounds, except for the **XIII** compound, prevented *S. aureus* biofilm formation. The use of 2.0×MIC decreased the mass of *MRSA* 222 biofilm by 82.5% up to 100 % compared to the untreated culture (tab. 3).

Table 3. Antibiofilm activity (%) of new 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives.

| Compound | ***S. aureus* 222** | ***E. coli* 311** | ***P. aeruginosa* 449** | ***C. glabrata* 404** |
|---|---|---|---|---|
| | Control 100.0±3.6 | Control 100.0±1.2 | Control 100.0±9.6 | Control 100.0±0.9 |
| KVM-190 **(I)** | 17.5±1.1* | 36.1±0.8* | 249.1±15.1* | 261.3±6.6* |
| KVM-266 **(II)** | 0.2±0.1* | 31.6±5.4* | 150.3±6.1* | 119.5±6.6 |
| KVM-267 **(III)** | 0.7±0,4* | 85.9±0,5* | 129.9±3.1* | 160.0±43.8* |
| KVM-316 **(IV)** | 12.7±0.2* | 42.8±2.1* | 3.9±0.2* | 83.2±2.6 |
| KVM-251 **(V)** | 1.2±0.2* | 127.8±1.7* | 164.1±9.6* | 251.3±1.5* |
| KVM-327 **(VI)** | 0.3±0.2* | 77.6±1.7* | 61.1±4.3* | 3.0±2.4* |
| KVM-219 **(VII)** | 88.8±1.5 | 88.0±1.5 | 37.2±0.2* | 111.8±0.0 |
| KVM-220 **(VIII)** | 0.2±0.2* | 77.7±0.4* | 174.9±3.7* | 77.5±2.5 |
| KVM-269 **(IX)** | 0.2±0.1* | 91.1±1.7* | 69.7±3.3* | 9.6±1.9* |
| KVM-268 **(X)** | 0.1±0,0* | 92.8±0.4 | 145.5±1.6* | 3.8±1.9* |
| KVM-221 **(XI)** | 12.4±4.0* | 111.4±0.7* | 145.5±3.2* | 187.5±7.5* |
| KVM-222 **(XII)** | 0.0±0.0* | 98.1±1.6 | 131.1±3.2* | 161.9±13.0* |
| KVM-319 **(XIII)** | 51.4±2.9* | 93.7±1.1 | 106.2±3.1 | 17.3±3.3* |
| KVM-317 **(XIV)** | 0.6±0.6* | 106.9±1.8* | 63.0±4.9* | 1.3±0.9* |
| KVM-318 **(XV)** | 0.4±0.3* | 96.3±0.1 | 79.1±1.7* | 11.5±5.1* |

*$p<0.05$ in comparison with control.

At the same time *E. coli* biofilms were less susceptible to 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives. Among all tested derivatives, the **I**, **II** and **IV** compounds showed the most pronounced inhibitory effects (63.9%, 68.4% and 57.2%, respectively, $p<0.05$).

The 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives almost did not inhibit *P. aeruginosa* biofilm formation. Only two compounds, namely the **IV** (96.1%) and **VII** (62.8%) exhibited the most pronounced inhibitory effect, whereas the **VI** (38.9%), **IX** (30.3%), **XIV** (37.0%) and **XV** (20.9%) derivatives showed a lesser activity. Unlike these ones, the **I**, **II**, **III**, **V**, **VIII**, **X**, **XI**, **XII**, and **XIII** compounds even stimulated the *P. aeruginosa* biofilm formation.

Thus, according to the study results the most pronounced inhibitory effect on biofilms formation were found for the **IV** compound, with a 96.1% decrease in *S. aureus* biofilm mass, 57.2% – *E. coli* and 96.1% – *P. aeruginosa.*

As regarding the *C. glabrata* biofilm formation, a pronounced inhibitory effect was demonstrated by the **VI**, **IX**, **X**, **XIII, XIV** and **XV** compounds (82.7%-98.7% biofilm mass decrease).

## DISCUSSIONS

The comparative study of the antibacterial and antifungal activity of 1-[(2,4-(di-tert-buty-lphenoxy))-3-dialkylamino-2-propanol] derivatives with various substituents in the molecular structure showed that substitutes close to the amino fragment might affect the antimicrobial activity of the tested compounds (tab. 2).

By introducing the N-benzyl dimethylamine (**I**); N-benzyl pyrrolidine (**VIII**) and N-benzyl hexamethyleneamine (**XII**) groups to the structure of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] at the amino fragment, an inhibitory effect towards *S. aureus* and *C. albicans* ranging between 2.5-3.75 μg/mL was registered.

The addition of the substituents on the benzylic radical in the 4-position, namely the nitro group (**II, IX**), methoxy group (**III** and **X**) and chlorine group (**IV**) exhibited an increased inhibitory activity of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives. Their MIC values for *S. aureus* and *C. albicans* ranged between 5.0-7.5 μg/mL.

Another heterocyclic substituent (4-methylpiperidine) inserted to the 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] molecular derivatives, viz. **XIV** and **XV,** did not significantly affect their antifungal activities compared with **II** compound**.** At the same time, the antibacterial activity of **XV** compound (MIC 1.56 μg/mL) towards *S. aureus* increased, compared to the **II** compound (MIC 5.0 μg/mL).

Upon the insertion of a nitro group at the 4-position to the benzylic radical, viz. the **XIII** compound**,** it showed a reduced antifungal activity (MIC 20.0 μg/mL) in comparison with **XII** compound (2.5 μg/mL).

The replacement of one of the methyl groups for cyclohexyl in the molecule of the **V** compound led to a decrease of its antifungal activity (MIC 12.5 μg/mL), while its inhibitory effect towards *S. aureus* remained nearly unchanged (MIC 3.12 μg/mL) compared with the **I** compound (MIC 2.5 μg/mL).

The introduction of the chlorine at the 4-position led to completely different consequences: the MIC value of the **VI** compound showed a two-fold decrease in comparison with the MIC values of the **I** and **V** compounds (*S. aureus* – 1.56-3.12 μg/mL; *C. albicans* – 25.0-12.5 μg/mL). However, this decrease was not statistically significant.

The addition of N-methylpyrrolidine fragment to the amino group allowed to increase the antibacterial and antifungal activity of the **VII** derivative by 3.2 and 2.4 times, respectively (compared with **I** and **VIII** compounds). The MIC values towards *S. aureus* and *C. albicans* decreased to 0.78 μg/mL and 1.56 μg/mL, respectively.

The replacement of hexamethylenamine pyrrolidine radical in the **XI** derivative was accompanied by a decreased inhibitory activity (compared to the **VII** compound), whereas the MIC values towards *S. aureus* and *C. albicans* were 5.0-7.5 μg/mL.

This present study demonstrated that both tested derivatives 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] were able to suppress the gram-positive and gram-negative bacteria in fungal (*C. glabrata*) planktonic microorganisms and biofilms formation processes. The comparative structure-activity analysis showed that the inhibitory effect depended not only on the molecular structure and position of the substituents of

1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] derivatives, but also on the microbial strain used.

Thus, the trials carried out on planktonic and bio film cultures demonstrated that the newly synthesized derivatives of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] exhibited an inhibitory effect against bacteria and fungi.

## CONCLUSIONS

1. The studies on planktonic microorganisms demonstrated that the newly synthesized derivatives of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] have antibacterial and antifungal effects. This research showed that the anti-biofilm effects of the most evaluated compounds could specifically reduce the biofilm formation ability of *S. aureus* 222, *E. coli* 311, *P. aeruginosa* 449 and *C. glabrata* 404 by at least 50%, depending on the nature of the substituents used in their molecules.
2. The newly synthesized derivatives of 1-[(2,4-(di-tert-butylphenoxy))-3-dialkylamino-2-propanol] represent a promising class of chemical compounds, which might lead to the development of novel antimicrobial agents intended for the treatment of many infectious diseases. Further researches are required to study the broad activity spectrum of compounds with the most pronounced antimicrobial action, as well as their antibacterial and antifungal mechanisms, acute toxicity and efficacy in vivo.

## CONFLICT OF INTERESTS

Authors have no conflict of interests to declare.

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# BIOMASA DE STREPTOMICETE – ADITIV EFICIENT ÎN ALIMENTAȚIA TINERETULUI AVICOL

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***Keywords:*** *streptomyces biomass, young poultry, gross weight gain, specific consumption.*

**STREPTOMYCES BIOMASS – EFFECTIVE ADDITIVE IN POULTRY NUTRITION**

***Introduction.*** *The breeding of domestic poultry is an important source to soupplement the human needs in animal proteins with a high biological value. Therefore, knowledge and guidance of the factors that influence poultry production is a guarantee of increasing these productions, both in terms of quantity and quality.*

***Material and methods.*** *The investigation was aimed to administer the 0.05% and 0.1% streptomyces biomass in the recipe of combined fodder, intended for the feeding of young poultry of the Argintie de Adler breed, in order to identify the effectiveness of its administration.*

***Rezults.*** *The supplementing of streptomyces biomass in the proportion of 0.1% in the recipe of combined feed intended for feeding the poultry of the Argintie de Adler breed, contributed to a 5.1% gross weight gain and a lower specific consumption by 9.9% in chickens from the experimental batch, compared to those of the control batch.*

***Conclusions.*** *The assessment of the dynamic indices of growth and development in chickens of the Argintie de Adler breed, as a result of supplementation of nutrition recipes with streptomyces biomass, has established a sure way to boost the productivity.*

***Cuvinte cheie:*** *biomasă de streptomicete, tineret avicol, spor în greutate, consum specific.*

***Introducere.*** *Creşterea păsărilor domestice reprezintă o sursă importantă de asigurare a omului cu proteine animale, de o înaltă valoare biologică. De aceea cunoaşterea şi gestionarea factorilor care influenţează producţiile de păsări, constituie o garanţie a sporirii acestor producţii, atât din punct de vedere cantitativ, cât şi calitativ.*

***Material şi metode.*** *Investigaţiile au avut ca obiectiv administrarea în reţeta de nutreţ combinat, destinată alimentaţiei tineretului avicol de rasa Argintie de Adler, a biomasei de streptomicete, în proporţie de 0,05% şi 0,1%, cu scopul identificării efecienţei acesteia.*

***Rezultate.*** *Suplimentarea reţetei de nutreţ combinat destinat alimentaţiei puilor de găină, de rasa Argintie de Adler, cu biomasă de streptomicete, în proporţie de 0,1%, a favorizat obţinerea unui spor în greutate mai mare cu 5,1% şi un consum specific mai mic cu 9,9%, la puii din lotul experimental, comparativ cu cei din lotul martor.*

***Concluzii.*** *Urmărind dinamica indicilor de creştere şi de dezvoltare a puilor de găină de rasa Argintie de Adler, în urma suplimentării reţetelor de nutriţie cu biomasă de streptomicete, a fost stabilită o tendinţă sigură de îmbunătăţire a acestora.*

## INTRODUCERE

Creşterea păsărilor are ca scop obţinerea de carne şi de ouă într-un timp scurt şi cu o eficacitate economică maximă. Explozia demografică, îmbogăţirea substanţială a volumului de cunoştinţe privitor la alimentaţia raţională a omului, precum şi alte cauze de ordin social-economic, au dus la intensificarea creşterii păsărilor şi la elaborarea tehnologiilor, care să amplifice capacităţi de producţie (1).

Actinomicetele, inclusiv genul cel mai răspândit în natură Streptomyces, ies în evidenţă printre microorganisme, datorită capacităţii lor de a forma multiple substanţe biologic active: antibiotice, vitamine, enzime, lipide, aminoacizi, fitohormoni etc., care stimulează creşterea şi dezvoltarea animalelor şi a păsărilor de fermă. Din considerentele date, acestea sunt utilizate cu prepoderabilitate în una dintre cele două ramuri de bază ale agriculturii - zootehnia (2, 3, 4, 5, 6).

Produsele de sinteză microbiană, obţinute din streptomicete, sunt utilizate în zootehnie sub formă de biomasă, care suplinesc raţia furajeră de bază a animalelor şi a păsărilor, ceea ce duce la optimizarea metabolismului, fortificarea sistemului imun şi la creşterea productivităţii. În plus, biomasa de streptomicete, pe lângă valoarea nutritivă, posedă şi proprietăţi antioxidante şi antistres, activitate antimicrobiană şi imunomodulatoare, care favorizează digestia alimentelor, absorbţia lor în ţesuturile tractului digestiv şi inhibă dezvoltarea microflorei patogene în intestine. Biomasa obţinută pe baza streptomicetelor este sigură, inofensivă, ecologic pură şi contribuie la o absorbţie mai eficientă a substanţelor nutritive biologic active din furaje, ce influenţează pozitiv rezistenţa şi productivitatea animalelor şi a păsărilor agricole (7, 8, 9).

Reieşind din cele menţionate anterior, investigaţiile efectuate au avut ca scop includerea în reţetele de nutreţ combinat, destinat alimentaţiei tineretului avicol, a biomasei de streptomicete, produsă de tulpinile *Streptomyces fradiae* CNMN-Ac-11, prelevate din solurile Moldovei, pentru stabilirea influenţei acesteia asupra performanţelor de creştere şi de dezvoltare a puilor de găină.

## MATERIAL ŞI METODE

Investigaţiile efectuate, privind utilizarea biomasei de streptomicete în alimentaţia puilor de găină şi influenţa acesteia asupra performanţelor de creştere, au fost organizate în cadrul SRL „Avicola Sărătenii-Vechi", s. Sărătenii Vechi, r-nul Teleneşti, din Republica Moldova.

Materialul biologic a fost reprezentat de puii de rasa Argintie de Adler, în vârstă de o zi.

Metodele de lucru utilizate, pentru obţinerea indicatorilor planificaţi, au fost cele indicate în literatura de specialitate.

Cercetările au fost realizate pe parcursul a 7 săptămâni, pe trei loturi de pui (un lot martor şi două loturi experimentale) pe un efectiv de 150 de pui, care au fost distribuiţi aleatoriu în trei loturi, a câte 50 de pui fiecare.

Datele primare obţinute (tab. 1) în urma studiilor efectuate au fost prelucrare statistic, cu determinarea criteriului de semnificaţie.

Tabelul 1. Schema experimentului.

| Loturile | Materialul biologic | Cap. | Caracterul alimentaţiei |
|---|---|---|---|
| **Martor** | tineret avicol de 1 zi | 50 | Nutreţ combinat (NC) |
| **Experimental I** | tineret avicol de 1 zi | 50 | NC + 0,05% biomasă de streptomicete |
| **Experimental II** | tineret avicol de 1 zi | 50 | NC + 0,1% biomasă de streptomicete |

Conţinutul energetic şi proteic al reţetelor de nutreţuri combinate, administrate puilor în cadrul experimentului, a fost administrat în funcţie de vârsta acestora. Astfel, reţetele de nutreţ combinat asigură necesarul acestora cu energie şi proteină, dar şi alţi nutrienţi, specifici vârstei (tab. 2). Pe parcursul investigaţiilor s-a determinat:

- ✓ greutatea corporală a tineretului de găină, la diferite perioade de creştere;
- ✓ sporul mediu zilnic al tineretului de găină pentru aceleaşi perioade;
- ✓ consumul specific;
- ✓ viabilitatea efectivului.

Tabelul 2. Structura şi valoarea nutritivă a reţetei din experiment.

| Specificare | Cota de includere, % | |
|---|---|---|
| | vârsta 0-21 zile | vârsta 22-49 zile |
| Porumb | 54,1 | 50,5 |
| Grâu | 11,0 | 18,5 |
| Şrot de soia | 28,1 | 24,0 |
| Făină de peşte | 3,0 | 3,0 |
| Ulei | 1,0 | 1,0 |
| Premix | 1,0 | 1,0 |
| Monocalciu Fosfat | 1,8 | 2,0 |
| **TOTAL** | **100** | **100** |
| EM, kcal/kg furaj | 2850 | 2688 |
| PB, % | 21,9 | 19,4 |
| Celuloză, % | 3,1 | 3,3 |

În scopul stabilirii performanţelor de creştere, pentru puii din experiment s-au creat condiţii identice de întreţinere (în baterii cu cuşti, condiţiile de microclimat şi igienice au fost asigurate conform normelor prevăzute pentru această categorie, şi cât mai aproape de condiţiile de exploatare din unităţile avicole).

**REZULTATE**

Performanţele de creştere ale puilor de găină sunt caracterizate, în general, prin evoluţia greutăţii corporale, aceasta fiind şi obiectivul de bază al investigaţiilor, care indică gradul de dezvoltare a puilor.

În ceea ce priveşte dinamica greutăţii corporale s-a stabilit, că aceasta a fost mai mare în lotul experimental II de pui, cărora li s-a administrat biomasa de streptomicete în proporţie de 0,1%, evoluţia acestora fiind prezentată în Figura 1.

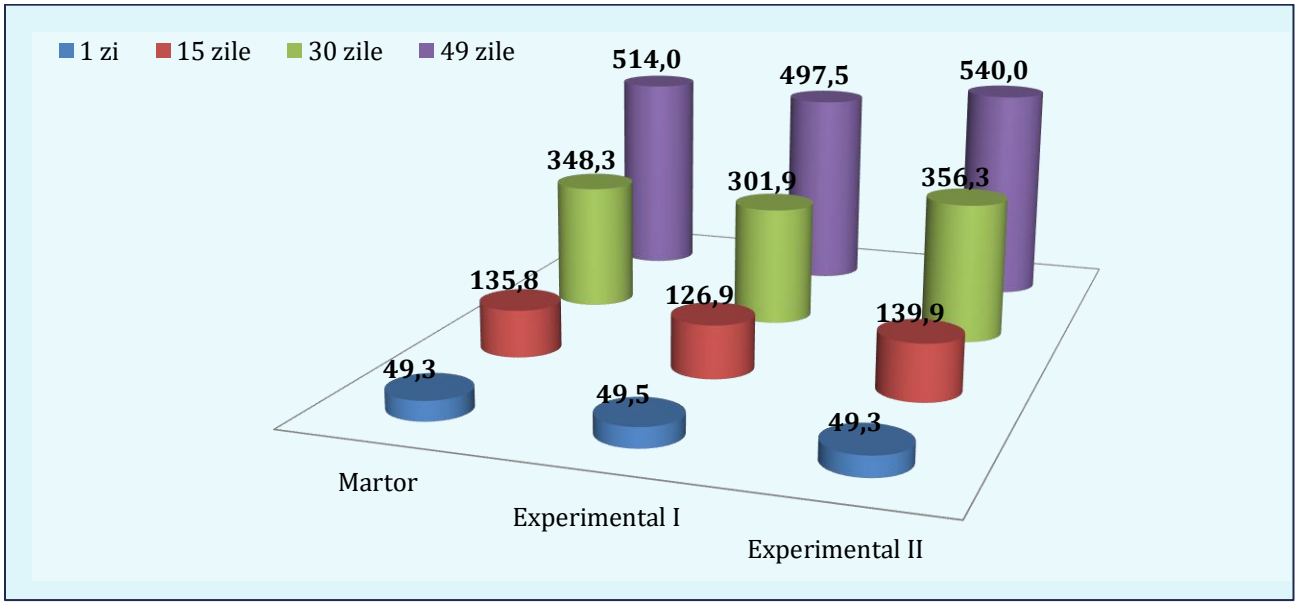


Figura 1. Evoluţia greutăţii corporale (grame) a puilor din experiment.

Datele prezentate anterior ne demonstrează că rezultatele cele mai bune, privind greutatea corporală, au fost înregistrate în lotul experimental II, unde acest indice a alcătuit 540,0 g. la finele perioadei experimentale, ceea ce este cu 5,1% mai mare, comparativ cu rezultatele puilor din lotul martor.

Eficienţa creşterii şi dezvoltării tineretului avicol, pe lângă greutatea corporală, o reprezintă şi in dicele consumului specific, care, de asemenea, a evoluat diferit pe parcursul investigaţiilor, valorile acestuia fiind prezentate în Figura 2.

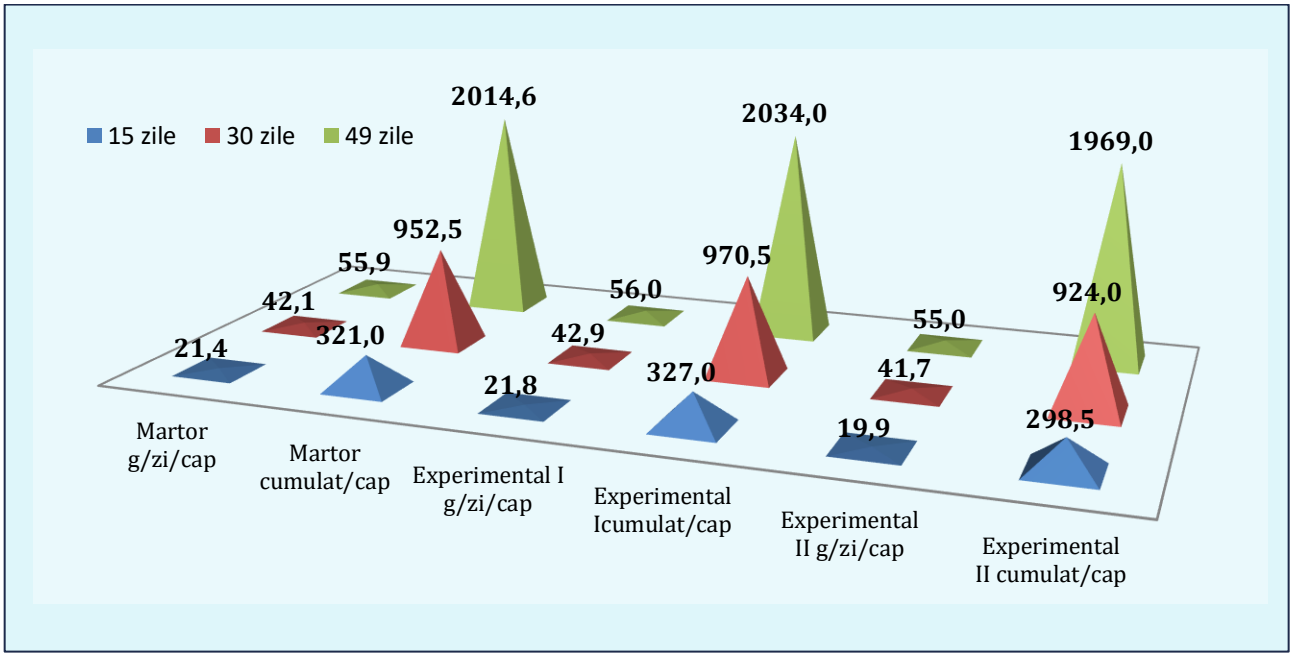


Figura 2. Evoluţia consumului de furaje a puilor din experiment.

Din datele prezentate anterior observăm că, consumul cumulat de furaje, pe parcursul perioadei de investigaţii, a fost mai mic în lotul experimental II, unde acest indice a constituit 1969,0 g. faţă de 2034,0 g. în lotul experimental I şi 2014,6 g. în lotul martor.

În baza rezultatelor obţinute în experiment au fost determinaţi şi ceilalţi indici, propuşi pentru cercetare, iar rezultatele totalizate a investigaţiilor sunt prezentate în continuare (tab. 3).

Tabelul 3. Rezultatele administrării biomasei de streptomicete în alimentaţia puilor de găină.

| Nr. gr. | Loturile | Greutatea corporală la vârsta 49 zile | | Sporul zilnic, g. | | Consumul specific | | % de menţinere |
|---|---|---|---|---|---|---|---|---|
| | | g | % | g | % | kg | % | |
| 1 | Martor | 514,0±6,1 | 100 | 9,5 | 100 | 4,3 | 100 | 98 |
| 2 | Experimental I | 497,5±6,4 | 96,8 | 9,1 | 95,7 | 4,5 | 104,6 | 100 |
| 3 | Experimental II | 540,0±5,8** | 105,1 | 10,0 | 105,2 | 3,9 | 90,1 | 100 |

** B = 0,99

Datele prezentate în tabel, ne indică faptul că, puii din lotul experimental II au înregistrat o creştere de 5,1% în greutatea corporală, comparativ cu puii din lotul martor. La fel şi sporul mediu zilnic a înregistrat valori mai mari în lotul experimental II, cu un procent de 5,2%, faţă de puii din lotul martor.

Indicele consumului specific, însă, a consemnat valori mai mici în lotul experimental II, fapt ce indică un consum mai eficient a furajului, fiind mai redus cu 9,9%, comparativ cu puii din lotul martor.

## DISCUŢII

Cercetările noastre evidenţiază faptul că, sistemul intensiv de dezvoltare a tineretului avicol rămâne una dintre principalele metode pentru creşterea acestora. Pentru a obţine rezultate bune de creştere şi de dezvoltare a puilor de găină, unităţile avicole trebuie să asigure o furajare corespunzătoare exigenţelor şi potenţialului genetic, al materialului biologic aflat în exploatare, prin utilizarea preparatelor biologic active, care favorizează metabolismul şi respectiv, influenţează productivitatea şi bunăstarea acestora.

Rezultatele investigaţiilor efectuate au demonstrat că, utilizarea în calitate de preparat furnizor de substanţe biologic active a biomasei de streptomicete influenţează pozitiv indicii productivi la puii de găină.

## CONCLUZII

1. Urmărind dinamica indicilor de creştere şi de dezvoltare a puilor de găină, în urma suplimentării reţetelor de nutreţ, cu biomasă de streptomicete, a fost stabilită o tendinţă sigură de îmbunătăţire a acestora.
2. Materialul biologic utilizat în cercetare nu a manifestat situaţii de stres pe parcursul investigaţiilor, ceea ce rezidă în adaptarea puilor la regimul experimental, concomitent cu manifestarea unor performanţe de creştere.
3. Suplimentarea reţetei de nutreţ combinat, destinat alimentaţiei puilor de găină, cu biomasă de streptomicete, în proporţie de 0,1%, a favorizat obţinerea unui spor în greutate de 5,1% şi a unui spor mediu zilnic de 5,2% la puii din lotul experimental II, comparativ cu cei din lotul martor
4. Consumul de furaje la 1 kg spor în greutate, de asemenea a fost favorizat de biomasa de streptomicete inclusă în furaje, care a arătat valori ce indicau 9,9% consum mai mic, în favoarea puilor din lotul experimental II.

## CONFLICT DE INTERESE

Autorul nu declară conflict de interese.

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# PARASITIC NEMATODES IN POTATOES OF DIFFERENT VARIETIES AND THEIR INTERRELATIONS WITH SOME ARTHROPODS

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***Keywords:*** *potato, nematodes, arthropods, nematophagous, phytophagous.*

***Introduction.*** *Potato (Solanum tuberosum L.) is susceptible to infestation with an associated complex of different species of parasitic and saprophytic nematodes, bacteria, fungi, and arthropods, which diminish the quality of the product.*

***Material and methods.*** *10 varieties of potatoes were investigated (Agata, Albastriu-mov, Bella rosa, Concorde, Desiree, Irga, Iagodca, Roko, Romano, Sprinter), cultivated on the territory of the Republic of Moldova. The extraction of nematodes and mites was performed using the Baermann funnels, modified by Nesterov.*

***Results.*** *Multiannual researches on the degree of potato infestation have shown that tubers are preferred by various arthropods (Acaridae), Agriothes spp. (Elateridae), Gryllotalpa gryllotalpa, (Grillotalpidae) and Sciaridae spp. (Sciaridae), which form different interactions with the parasitic nematodes of tubers (Ditylenchus destructor, D. dipsaci).*

***Conclusions.*** *Solanum tuberosum infested by parasitic nematodes D. destructor in association with saprophytic nematodes and dry rot are colonized by nematophagous (mites – 80%) and phytophagous arthropods (wireworms – 40%; mole cricket – 50%; flies – 40%). Among the researched arthropods, Rhizoglyphus echinopus were more frequently found, which together with other species actively consume the primary and secondary parasitic nematodes, their mortality constituting up to 90%. In the traumatized by some arthropods potatoes, with the soil, secondary parasitic nematodes, also penetrate, carrying bacterial and fungal infections, subsequently stimulating the total rot of potato tubers.*

***Cuvinte cheie:*** *cartof, nematode, artropode, nematofage, fitofage.*

## NEMATODELE PARAZITE LA CARTOFII DE DIFERITE SOIURI ŞI INTERRELAŢIILE CU UNELE ARTROPODE

***Introducere.*** *Cartoful (Solanum tuberosum L) este susceptibil la infestare cu un complex asociat de diferite specii de nematode parazite, saprofite, bacterii, ciuperci şi artropode, ceea ce îi diminuează din calitatea lui ca produs.*

***Material şi metode.*** *Au fost cercetate 10 soiuri de cartofi (Agata, Albăstriu-mov, Bella rosa, Concorde, Desiree, Irga, Iagodka, Rocko, Romano, Sprinter) cultivate în Republica Moldova. Extragerea nematodelor şi a acarienilor s-a efectuat cu utilizarea pâlniilor Baermann, modificate de Nesterov.*

***Rezultate.*** *Cercetările multianuale, privind gradul de infestare a cartofilor, au demonstrat că tuberculii sunt preferaţi de diferite artropode: Agriotes spp. (Elateridae), Gryllotalpa gryllotalpa, (Grillotalpidae) şi Sciaridae spp. (Sciaridae), care stabilesc interrelaţii diverse cu nematodele parazite (Ditylenchus destructor, D. dipsaci) ale culturii.*

***Concluzii****. Solanum tuberosum, infestat de nematodul parazit Ditylenchus destructor, în asociere cu nematodele saprofite şi cu putregaiul uscat, este colonizat de artropodele nematofage (acarieni – 80%) şi fitofage (viermii-sârmă – 40%; coropişniţe – 50%; musculiţe – 40%). Printre artropodele cercetate, mai frecvent a fost atestat Rhizoglyphus echinopus (Fumouze & Robin, 1868), care, împreună cu alte specii, consumă activ nematodele parazite primare şi pe cele secundare, mortalitatea acestora constituind până la 90%. În cartofii traumaţi de unele artropode, odată cu solul, pătrund şi nematodele parazite secundare, purtătoare ale infecţiilor bacteriene şi fungice, stimulând ulterior putrefacţia totală a tuberculilor.*

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is the third most important food crop plant of the world, producing high yields of nutritionally valuable food in the form of tubers, which has captured the attention of many researchers. Because the tubers are susceptible to infestation with an associated complex of different species of parasitic and saprophytic nematodes, bacteria, fungi, and arthropods, it is important to know their impact on the host organism. Primary parasitic nematode *Ditylenchus destructor* (Thorne, 1945) creates pathways in tubers for secondary parasitic invertebrates such as: saprophytic nematodes, bacterial and fungal infections, these being vectors of infections for susceptible potato varieties. As a result, the resistance of potato crop is diminished and their complete putrefaction occurs (1).

The nematode *D. destructor* is widespread in all countries where potatoes are growing. For example, in Romania, in the process of studying the level of infestation with the nematode *D. destructor* and other pathogens, which cause diseases of the seed potato of 4 varieties – Desiree, Ostara, Dahlia, Alka, it has been established that the most susceptible to both the *D. destructor* and the pathogenic species of microorganisms are the varieties Desiree and Ostara (2). The frequency of the attack with *D. destructor* was 44.8% and 27.38%, respectively, and the Dahlia and Alka varieties were more resistant, the frequency of the attack constituted 5.26% and 4.10%, respectively. In the Republic of Belarus, in all agro-climatic zones, there have been cases of crop loss of up to 43%, due to infestation with the *D. destructor* nematode of food and seed potatoes (3).

In phases 1, 2 of ditylenchosis the primary monotypic population of tubers is infested only with the population of a single species of nematode – *D. destructor* (females, males, larvae of different ages) (4). In such tubers, parasitic nematodes do not cause obvious external symptoms of ditylenchosis and only by removing the shell, small stains on the surface of the tuber pulp appear low visible. In phases 4-5 of ditylenchosis, the tissue invaded by different secondary invertebrate organisms becomes evidently rotten (5). Ditylenchosis disease of tubers initially manifests by cracking the shell, similar to dry rot, caused by *Fusarium* spp.

The purpose of the research was the study of the species of nematophagous and phytophagous arthropods, as well as the complex of infections associated with potato tubers of 10 varieties, during the autumn-winter-spring periods.

## MATERIAL AND METHODS

The researches were carried out during 2014-2019 years. The research was conducted on 10 varieties of potatoes *(*Agata, Albastriu-mov, Bella rosa, Concorde, Desiree, Irga, Iagodca, Roko, Romano, Sprinter), which were cultivated in the north, south and central areas of the Republic of Moldova. For each variety, 15-20 potatoes tubers per sample were researched. The primary and secondary parasites of tubers have been determined in advanced phases of ditylenchosis. The extraction of nematodes and mites was performed using the Baermann funnels, modified by Nesterov (6). The density of nematodes extracted from the tubers affected by ditylenchosis was calculated by applying the De Grisse chamber. The fixed preparations were carried out according to the method proposed by Seinhorst (7). The stereoscopic microscope was used to establish morpho-physiological changes in the infested potatoes.

Statistical analysis was performed using MS Excel program.

## RESULTS

### *Nematodes associated with rot*

It was determined that all the potatoes varieties collected during the autumn-winter-spring periods were infested with parasitic nematodes *Ditylenchus destructor* (advanced phases of ditylenchosis, 4-5) in combination with *D. dipsaci*, saprophytic nematodes, bacteria and fungi (fig. 1).

The results of the research showed a wide spread (100%) of the obligate primary parasite *D. destructor* in all investigated 10 potato varieties. The varieties of Albastrui-mov, Dessiree and Irga differed in frequency and density of infestation. The most frequent (100%) *D. destructor* is associated with dry rot caused by *Fusarium* spp. and less frequent (60%) with wet rot caused by *Erwinia* spp.

### *Nematophagous arthropods – mites*

It was established that the studied potatoes tubers (Agata, Albastriu-mov, Bella rosa, Concorde, Desiree, Irga, Iagodca, Roko, Romano, Sprinter) infested with parasitic nematodes *D. destructor*,

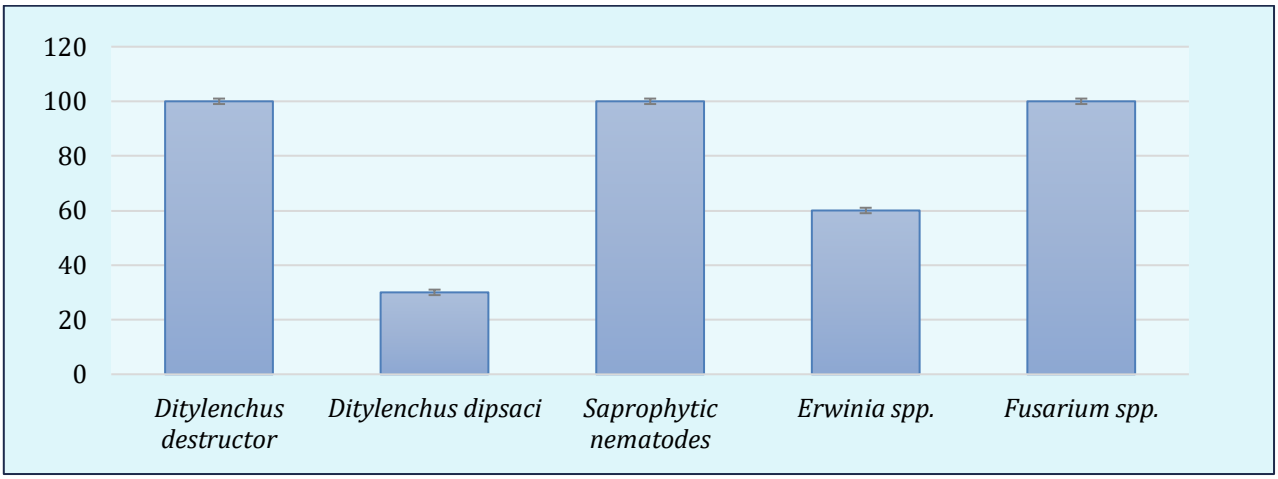



Figure 1. Frequency of parasitic and saprophytic nematodes, of dry and wet rot (%) in the investigated potato varieties.

*D. dipsaci*, saprophytic nematodes, bacteria and fungi, were also colonized by different species of mites (fam. Acaridae), which have been commonly found in all the researched varieties (fig. 1, 2).

It should be mentioned the fact that the potato tubers infested by *D. destructor* in association with saprophytic nematodes and dry rot were colonized by nematophagous (frequency 80%, different species of mites) and phytophagous arthropods (wireworms – 40%; mole cricket – 50%; flies – 40%) (fig. 2).

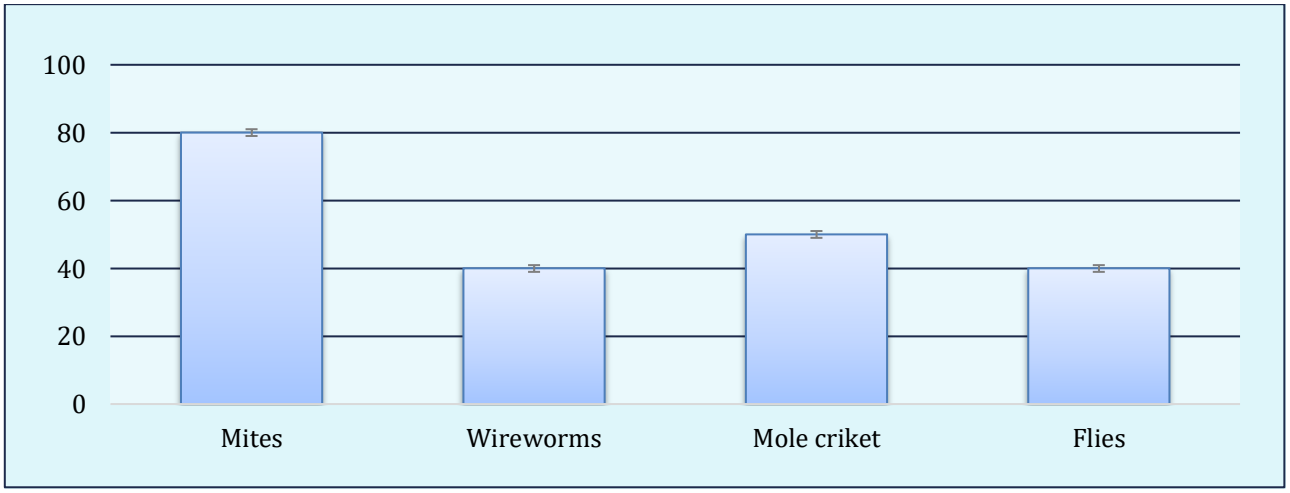



Figure 2. Frequency of nematophagous and phytophagous arthropods (%) in the investigated potato varieties.

An increased density of mites (hundreds of individuals along with their eggs/potato tubers) was detected in 5 of the investigated varieties - Agata, Bella rosa, Desiree, Irga, Roko. In the study process, for the first time, cases of active mite's attack on primary (*D. destructor, D. dipsaci*) and secondary (facultative, most of them of the order Rhabditida) parasitic nematodes have been observed. The latter (females, males, larvae, eggs) are devoured and consumed by mites (fig. 3). Under such conditions, the mites lay eggs, from which the larval forms develop, which then leads to the post-embryonic development cycle.

All mite species present in potatoes tubers infested with nematodes and microorganisms proved to be active predators. It has been established that mites attack nematodes (from families*:* Anguinidae, Cephalobidae, Neodiplo-gasteridae, Rhabditidae etc.) usually, in the region of median intestine, along the reproductive tract (ovary, oviduct in this area the holes are obser-

ved, through which the content of the organs is absorbed, first of all the gonad with the nematode eggs (fig. 4 (3a, 3b)). High mortality up to 90% both of primary and secondary parasitic nematodes has often been observed in such suspension (fig. 4 (1b)).

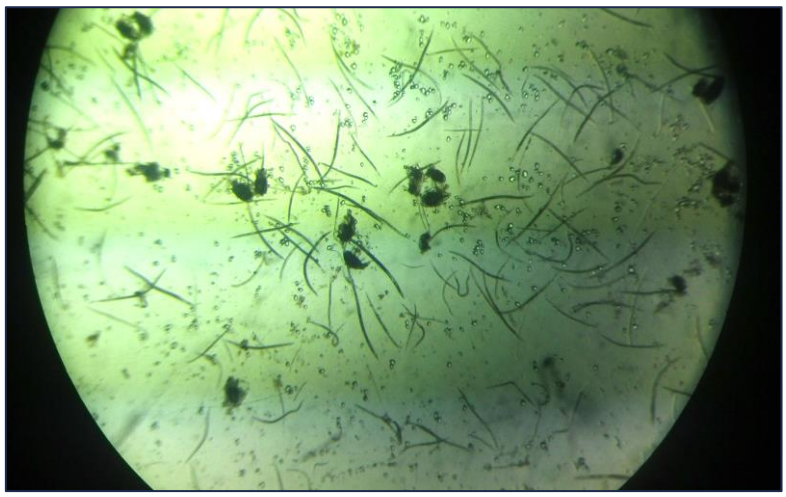


Figure 3. The nutritional process of mites with parasitic and saprophytic nematodes, which were extracted from infested potatoes by *D. destructor, D. dipsaci*, in advanced phases of ditylenchosis.

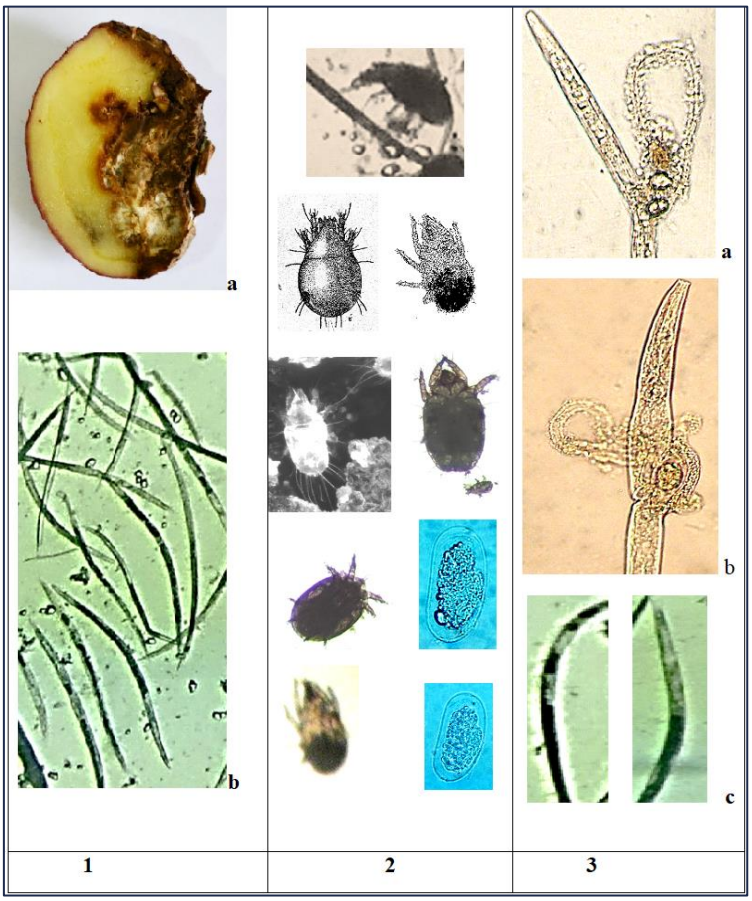



Figure 4. 1a – Potato tuber (section) infested by *D. destructor* in association with secondary parasites (saprophytic nematodes, microorganisms, nematophagous mites); 1b – nematodes *D. destructor*, *D. dipsaci*, devoured by mites; 2 – species of nematophagous mites and their eggs; 3 – different species of dead nematodes after being in contact with mites (a, b – saprophytes; c – devoured portions of *D. destructor*).

There have been observed cases, when in the infested potatoes, in which the density of mites accounted for hundreds of mature individuals/ tuber, all species of nematodes disappeared, as well as the bacterial and fungal infections. As a result, portions of the tuber with completely macerated substrate remained, containing only empty cells. Also, the starch granules disappeared in these phases of ditylenchosis. In such tubers, the passing from the infested to the non-infested portion and the extension of nematological, bacterial and fungal infections was not observed. Only a clear differentiation of not infested and infested portion of potatoes was observed, which demonstrates the cessation of parasitic, fungal and bacterial infection.

Mites were detected in the investigated potato tuber varieties (a significant number), which belong to the species of *Rhyzoglyphus echinopus* F. & R., family Tyroglyphidae (fig. 4.2). It is a widespread phytophagous species, with a 0.4-0.5 mm body length.

Loads of eggs deposited by mites in the potatoes tubers was also observed. A female lays about 800 eggs on average. A generation might develop over 30 days. Their development occurs at a humidity of more than 60%, being very sensitive to this factor. It should be mentioned, that the tubers contain an increased amount of water, depending on the potato variety (between 77% – variety Albastriu-mov and 83% – Agata variety), but in the infested potatoes by primary parasites the amount of water increased with 5-6%, creating favourable conditions for existence not only for mites, but also for bacteria, fungi and saprophytic nematodes.

***Phytophagous arthropods***

Wireworms of click beetles (family Elateridae). The research results showed that potato harvest at some of the analysed varieties (Agata, Bella rosa, Desiree, Roko, Sprinter, Concorde), originating from field culture, has been quite affected by some harmful phytophagous arthro-pods, such as wireworms of the click beetles (fig. 5b, 5c). Wireworms depreciate qualitatively the potato tubers due to the galleries, which are dug into the pulp. During the laboratory studies of the affected portions, it was observed that soil penetrated into those galleries (fig. 5a) made by the wireworms, thus favouring the colonization with bacterivore saprophytic nematode species.

**a** **b** **c**

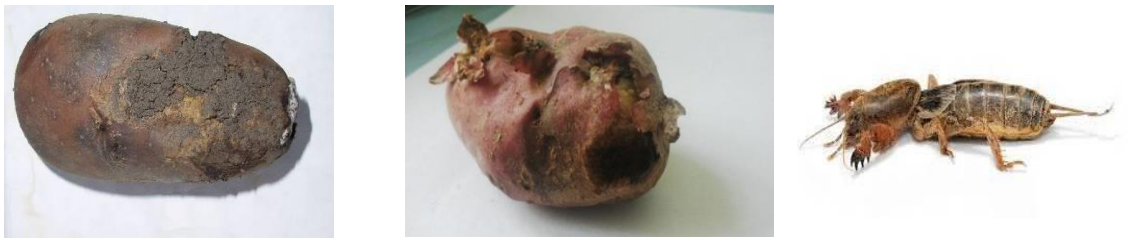
Figure 5. Potatoes attacked by wireworms of click beetles in association with saprophytic nematodes and mites (a; b) (c – wireworms, exterior aspect).

The laboratory investigation of the soil extracted from pulp galleries of the affected tubers demonstrated the presence of nematodes that eliminate bacterial and fungal infections of the order Rhabditida, families: Rhabditidae, Cephalobidae and order Dorylaimida, family Dorylaimidae (sp. *Mesodorylaimus bastiani*). In addition, crowds of mites were detected in the tested soil.

***Mole cricket (Gryllotalpa gryllotalpa L.)***

Among the phytophagous arthropods in some of the investigated varieties (Agata, Desiree, Irga, Roko, Iagodca), the presence of the common mole cricket (*G. grillotalpa*, family Grillotalpidae) has been established (fig. 6c). The observations made on individual lots in *Roko* potatoes variety determined 100% of mole cricket attack (fig. 6a, 6b). Soil penetrates the mechanically traumatized

tubers by mole cricket, in which the bacterial saprophytic nematodes were frequent found, which brought microorganisms on the body or intestine and cause both dry rot (*Fusarium* spp.) and wet rot (*Erwinia* spp.).



a b c

Figure 6. a, b – Potatoes tubers infested by *D. destructor* (phases 3, 4 of ditylenchosis) in association with secondary parasites and pests (with cavities formed by mole cricket); c – *G. grillotalpa* L. (exterior aspect).

Under laboratory conditions, most of the harvested tubers, affected by mole cricket bites, over a storage interval of 1.5-2.0 months at room temperature, turned into waste caused by the rot (fig. 7c). The insects produced huge cavities (fig. 7b) in the potato tubers, causing qualitative and quantitative deterioration of the potato crops, thus being transformed into waste.


a b c

Figure 7. Roko variety potatoes (from field experiments, obtained from a mother tuber): a – healthy potatoes; b – traumatized by mole cricket; c – traumatized by mole cricket after being kept in laboratory conditions for over 2-3 months at room temperature – 100% infestation of wet rot.

### *Flies* (Sciaridae spp.)

In the researches carried out on 10 varieties of potatoes, flies were detected only in some of them such as Agata, Deziree, Iagodca, Roko, which were selected from the field crops, with primary and secondary parasites and some pests. The affected tubers are characterized by perforated surfaces, whereas the flies' larvae were frequently detected in the damaged tissue (fig. 8c). It has been determined that these belong to the Sciaridae family.

It was determined that the tubers associated with Sciaridae spp. occur in the advanced stages of ditylenchosis, phases 4-5, when these are in the process of decomposition. A series of parasites and pests might colonize such tubers, such as phytoparasitic nematodes, saprophytes, micro-organisms and mites. Sciaridae spp. usually lives in wet soil, attacks plant roots and feeds with their living tissue.

## DISCUSSIONS

In the conditions of the Republic of Moldova, a wide spread (100% frequency) of tubers obligatory primary parasite – *D. destructor* in all 10 researched varieties was observed. Resear-ches has shown an association of parasitic nematodes *D. destructor* and *D. dipsaci* (frequency 30%) only in the varieties Albastrui-mov, Bella rosa, Desiree. In our opinion, this association displays a local character. Nematode *D. destructor* is frequently (100%) accompanied by secondary parasitic nematodes (saprophytes), most being of the


Figure 8. a – Roko variety potatoes infested by *D. destructor* in association with saprophytic nematodes, microorganisms, mites, flies; b – holes in the pulp of the tuber through which Sciaridae spp. penetrated; c – larva; d – imago.

order Rabdithida. A frequent association (100%) of the nematode *D. destructor* with dry rot caused by *Fusarium* spp. was determined. The wet rot *Erwinia* spp was found less frequently (60%) in the investigated varieties. It was observed that both primary parasitic nematodes and secondary saprophytic nematodes are not associated with wet rot and commonly leave the environment.

Our research reported an 80% frequency of mites, namely in the varieties of Agata, Albastriumov, Bella rosa, Concorde, Desiree, Irga, Iagodca, Roko. It has been determined that mites are common in tubercles infested with *D. destructor* in association with saprophytic nematodes and dry rot. Also, it was observed cases of active attack of mites on parasitic and saprophytic nematodes. Similar researches on nematode-mite interrelationships have been followed by other authors (8), who observed some processes regarding the perforation and nutrition of mites of the species *Tyrophagus putrescentiae* with the nematodes of the species *Rhabditis* spp., *Cephalobus* spp., *Heterodera orizae*. According to some researches (9) special importance is given to the mites of the genus *Caloglyplus* (Acarina: Acaridae), which shows a high efficacy in decreasing the density of parasitic nematodes. All stages of these mites attack the egg mass, laid by females, juvenile forms and females of the species *Meloidogyne, Globodera rostochiensis and Heterodera schachtii*, as well as endo- and ectoparasitic migratory species: *Paratylenchus* spp., *Pratylenchus* spp., *Tylenchorhynchus* spp. It has been established that the species *Hypoaspis sculifer* (family Gamasidae) attack the phytophagous nematode *Tylenchorhynchus dubius*, reducing their populations up to 68% (10). The extensive researches of some authors (11, 12, 13) determined that the mites of the species *T. putrescentiae* and *Hypoaspis calcuttaensis* are both nematophagous, consuming invasive larvae of the species *Meloidogyne javanica* and consumers of harmful fungi of plants: *Fusarium, Alternaria, Mucor* etc. Researchers from Florida (Orlando) (14) observed that the mite *Coleoscirus simplex* (Ewing) (Cunaxidae: Coleoscirinae) colonizes greenhouse pot cultures of root knot nematodes (*Meloidogyne* spp.), where it preys on vermiform nematodes and soil arthropods. This is the first report of nematophagy in a cunaxid mite. Previous researches (15) have shown that potatoes tubers infested with *D. destructor* (phases 3-4-5 of ditylenchosis) are always colonized by various species of nematophagous mites from the family Acaridae, more common being individuals of the species *Rhyzoglyphus echinopus*. The phytophagous mite *R. echinopus*, also called bulb mite, infests both field plants and those stored in the warehouses (10, 14). They attack nematodes in the region of the gonad and oviduct with eggs that they consume actively, causing the mortality of both parasitic and saprophytic forms. Some species of mites (Gamasidae sp.) devour and use for food the parasitic species *D. dipsaci* and *D. destructor,* the daily norm being up to four small nematodes. At the same time, the presence of mites is important in regulating the density of nematodes under natural conditions (14). In our researches, all mite species present in potatoes tubers infested with nematodes and microorganisms proved to be active predators of phytophagous and saprophytic nematodes.

The harvested tubers are attacked by some phytophagous arthropods, such as wireworms of click beetles (fam. Elateridae), mole cricket

(family Grillotalpidae) and flies (fam. Sciaridae).

The research results showed that 40% of the analysed varieties (Agata, Concorde, Roko, Sprinter) were attacked by the wireworms of click beetles. According to the experimental data of some authors (16), among the click beetles, the species of the genus *Agriotes (*family Elateridae) are widely spread in the Republic of Moldova. The presence of 6-8 wireworms per 1m$^2$ causes tuber damages up to 60%, and the installation of various pathogens in the galleries left, favours the rot of tubers, leading to crop losses of up to 50% (17). In the process of laboratory investigations of the affected parts, it was observed that soil penetrates into the galleries through the pulp, which favours the colonization with bacterivore saprophytic nematode species of the order Rhabditida, these being active decimators of microbial infections. Similar results were obtained by other researchers (18), where potato tubers affected by wireworms are often subsequently infested by pathogenic bacteria and fungi, which cause dry and wet rot.

In monoculture conditions, the mole cricket (family Grillotalpidae) attack (frequency 50%) was also observed namely on the varieties Albastruimov, Dessiree, Irga, Roko, Sprinter. It is a widespread polyphagous species in Europe, as well as in the Republic of Moldova, especially in conditions of increased humidity or on irrigated lots. In the superficial horizon of the soil (0-20cm) a single female lays up to 500 eggs (19). Like the wireworm, the mole cricket cause tuber damage through mechanical damage, with the possibility of penetrating the soil with bacterivorous saprophytic nematodes, accompanied by various microorganisms, which stimulate the appearance of rot (*Fusarium* spp., *Erwinia* spp.) that over a period of storage turn the tubers into waste.

In the potato tubers, cultivated in monoculture condition, the presence of Sciaridae spp. (frequency 40%) was detected in the varieties Agata, Deziree, Iagodca, Roko infested by the nematode *D. destructor* in combination with the saprophytic nematodes and microorganisms, in the 4-5 phases of ditylenchosis. These could be transmitters of both *D. destructor* nematodes (larvae, eggs) and various microbial infections. According to some researches (20), eggs and larvae of the nematode *D. dipsaci* were detected in the intestines of flies from the Drozophilidae family. According to the authors, the transmission of parasitic nematodes from one deposit to another might occur due to the flies.

## CONCLUSIONS

1. *Solanum tuberosum* infested by *Ditylenchus destructor* in association with saprophytic nematodes and dry rot are colonized by nematophagous (frequency 80%, different species of mites) and phytophagous arthropods (wireworms – 40%; mole cricket – 50%; flies – 40%).
2. Potatoes tubers are preferred by different arthropods (Acaridae), *Agriothes* spp. (Elateridae), *G. gryllotalpa* (Grillotalpidae), Sciaridae spp. (Sciaridae), which form different interactions with the parasitic nematodes of tubers. Among the studied arthropods, *Rhizoglyphus echinopus* (Fumouze & Robin, 1868) were more frequently found, which together with other species (*Agriothes* spp. (Elateridae), *G. gryllotalpa* (Grillotalpidae), Sciaridae spp. (Sciaridae)), actively consume the primary (*D. destructor, D. dipsaci*) and secondary parasitic nematodes (saprophytes, most being of the order Rhabditida), their mortality accounting for up to 90%.
3. The Sciaridae spp. might carry the eggs of primary parasitic nematode species in potatoes from warehouses. Some phytophagous arthropods might damage potatoes, thus the secondary parasitic nematodes from the soil might penetrate and bring bacterial and fungal infections, subsequently stimulating the total rot of potatoes tubers.

## CONFLICT OF INTERESTS

The author does not declare any conflict of interest.

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# ANTIBACTERIAL SUSCEPTABILITY OF *E. COLI* STRAINS ISOLATED FROM RAW MILK

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***Keywords:*** *Escherichia coli, strains, microorganisms, raw milk, antibiotic resistance, susceptibility.*

***Introduction.*** *The processing of most raw milk products can lead to contamination with unwanted microflora due to poor sanitation and hygienic conditions. The inadequate antibiotic use over the past decades has led to the emergence and wide spread of bacterial populations, particularly of Escherichia coli, which developed resistance to antibacterial drugs.* ***Material and methods****. Raw milk samples were obtained from clinically healthy cows on farms from Kiev and Poltava regions to identify E. coli, Staphylococcus spp., Enterococcus spp. isolates. Antimicrobial susceptibility testing was performed using the EUCAST disk diffusion method and MU on "Determination of microbial susceptibility to antibacterial drugs".* ***Results****. The examined milk samples revealed the presence of E. coli, Staphylococcus spp. and Enterococcus spp. isolates, which proves poor sanitary and hygienic conditions of milk production process. Escherichia coli isolates were found susceptible to Ampicillin/sulbactam, Cefoxitin (100%), Meropenem, Tobramycin (100%), Netilin, Tigecycline, Nitroxoline (100%), Gatifloxacin, and Nitrofurantoin (100%). The studied E. coli isolates were found resistant to Ampicillin (100%), Imipenem, Tetracycline, and Doxycycline (100%). 41.7% of isolates of Staphylococcus epidermidis, Staphylococcus aureus were found resistant to Oxacillin, of which 90% were resistant to Benzylpenicillin and 20% to Rifampicin.* ***Conclusions.*** *The circulation of antibiotic-resistant Enterobacteriaceae strains among farm animals is a major problem requiring a strategy development aimed to prevent the emergence and spread of antibiotic resistance worldwide.*

***Cuvinte cheie:*** *Escherichia coli, tulpini, microorganisme, lapte crud, rezistență la antibiotice, sensi-bilitate.*

**SENSIBILITATEA LA ANTIMICROBIENE A TULPINILOR DE *E. COLI* ISOLATE DIN LAPTE CRUD**

***Introducere.*** *Prelucrarea produselor din lapte crud poate duce la contaminarea cu microfloră nedorită din cauza condițiilor sanitaro-igienice precare. Utilizarea inadecvată a antibioticelor în ultimele decenii a dus la apariția și răspândirea pe scară largă a populațiilor bacteriene, în special a Escherichia coli, care a dezvoltat rezistență la preparatele antibacteriene.* ***Material și metode.*** *Probele de lapte crud au fost obținute de la vaci sănătoase, din punct de vedere clinic, de la fermele din regiunile Kiev și Poltava, din care au fost isolate și identificate E. coli, Staphylococcus spp., Enterococcus spp. Testarea sensibilității la antimicrobiene a fost efectuată utilizând metoda disc difuziometrică recomandată de EUCAST și MU privind „Determinarea sensibilității microbiene la preparatele antibacteriene".* ***Rezultate.*** *Probele de lapte examinate au relevat prezența izolatelor de E. coli, Staphylococcus spp. și Enterococcus spp., ceea ce elucidează condițiile sanitaro-igienice precare ale procesului de producție a laptelui. Izolatele de E. coli au fost sensibile la Ampicilin/sulbactam, Cefoxitin (100%), Meropenem, Tobramicin (100%), Netilin, Tigeciclin, Nitroxolin (100%), Gatifloxacin și Nitrofurantoin (100%). Izolatele studiate de E. coli au prezentat rezistență la Ampicilin (100%), Imipenem, Tetraciclin și Doxiciclin (100%). 41,7% din izolatele de Staphylococcus epidermidis, Staphylococcus aureus au fost rezistente la Oxacilin, dintre care 90% au fost rezistente la Benzilpenicilin și 20% la Rifampicin.* ***Concluzii.*** *Circulația tulpinilor de Enterobacteriaceae rezistente la antibiotice printre animalele de fermă prezintă o problemă majoră care necesită dezvoltarea strategiei menită să prevină apariția și răspândirea rezistenței la antibiotice în întreaga lume.*

## INTRODUCTION

The production of high-quality raw milk depends on various factors related to both genetics and physiological condition of the dairy cattle, as well as on the product manufacturing technology. Moreover, individual factors might have a remote impact on milk quality and safety. Thus, the use of antibiotics for therapeutic purposes in lactating animals can significantly affect the antibiotic-resistant properties of microorganisms found in milk and serve as one of the pathways for the spread of antibiotic-resistance genes in the environment (1). Furthermore, the antibiotic resistance of the same microbial strains, isolated from animals kept in the same room, may differ depending on the type of antibiotics used to treat cows at different stages of production. A research on raw drinking milk on retail sale in England revealed pathogenic agents or signs of poor zoosanitary guidelines in almost half of the samples studied (2). This problem occurs regard-less of the level of livestock farming and dairy industry development (3). More than 150 antibiotics are used in the production of livestock products used for human consumption and 90% of them are natural products of bacteria, fungi and semi-synthetic substances obtained as a result of natural products modification or even synthesis (4). The most widely used antimicrobial agents used in treatment of productive animals are β-lactams, tetracyclines, aminoglycosides, lincosa-mides, macrolides, and sulfonamides (5, 6). Close to ANVISA (7). Microorganisms isolated from lactating cows show resistance to both natural and synthetic antibiotics. *Escherichia coli* isolated from cattle rectum exhibited high resistance to ampicillin (59.09%) and tetracycline (43.43%) (8). Special attention should be paid to the commensal microbiota (*Escherichia coli*, *enterococci*). These bacteria can also acquire antimicrobial resistance due to the selective pressure and may act as reservoirs for antimicrobial resistance and virulence genes within the environment, as well as in food and agricultural animals, which are likely to transmit resistance to pathogenic bacteria (9). Previous researches suggest that *E. coli* may generally enhance the mutation rates of target cells contributing to antibiotic resistance (10). *Staphylococci* were found the most common pathogens isolated from milk samples taken from cows with clinical and subclinical mastitis across several countries. *Staphylococcus aureus* is the main pathogen of this genus, being responsible for up to 40% of all mastitis cases in some geographic regions (11). A thorough under-standing on antibiotic resistance mechanisms is paramount to developing new strategies for preventing the emergence of resistance (12).

## MATERIAL AND METHODS

Milk samples (32) were obtained from clinically healthy cows from the farms of the Kiev and Poltava regions. Culture media were prepared and controlled according to ISO 11133:2014 Microbiology of food, animal feeding stuffs and water. Preparation, production, storage and performance testing of culture media. The nutrient media, commercial tests, and discs with antimicrobial drugs manufactured by HiMedia were used within the study. Isolation and identification of *E. coli* used the appropriate *ISO* 16649-2:2014 (*ISO* 16649-2:2001, ITD) Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of -glucuronidase-positive *Escherichia coli*. Part 2. Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl-*D*-glucuronide. Isolation and identification of *Staphylococcus spp.* was carried out in accordance with ISO 6888-1: 1999 / Amd 1: 2003. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium - Amendment 1: Inclusion of precision data. Isolation and identification of *Enterococcus spp.* was carried out in accordance with SSU 8534: 2015 Food products. Method for detection and determination of Enterococci (8534: 2015 Food products Method for detection and enumeration of Enterococci). Antimicrobial susceptibility testing was performed using the EUCAST disk diffusion method and MU on "Determination of microorganisms susceptibility to antibacterial drugs" (MHU 2009) (13, 14). The study results were recorded and interpreted via an Automatic Colony Counters Scan® 500 manufactured by INTERSCIENCE.

## RESULTS

The present study examined milk samples obtained from clinically healthy cows from livestock complexes located in the Kiev and Poltava regions. The results of bacteriological studies showed that *E. coli* and *Enterococcus spp. s*trains were found in 100% of raw milk samples; thus,

*Staphylococcus spp*. isolates – in 100%, including 87.5% of *Staphylococcus epidermis* and 12.5% of coagulase-positive *Staphylococcus aureus*. It should be noted that among *Staphylococcus* strains, 41.7% of isolates were resistant to Oxacillin, of which 90% were resistant to Benzylpenicillin and 20% to Rifampicin (fig. 1).

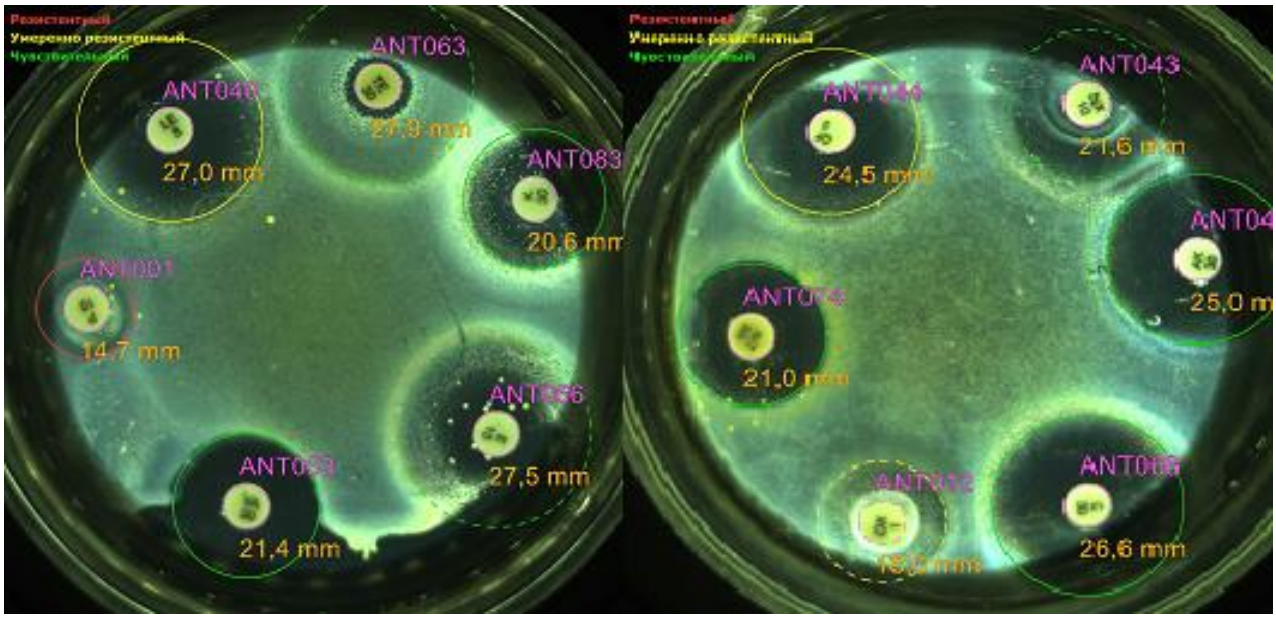


Figure 1. Antibiotic susceptibility of *Staphylococcus aureus* strains isolated from raw milk. ANT001 Benzylpenicillin (r), ANT012 Oxacillinum (r), ANT040 Levofloxacin (ATU), ANT043 Norfloxacin (s), ANT044 ofloxacin (ATU), ANT045 Amikacin (s), ANT053 Vancomycin (s), ANT056 Erythromycin (r), ANT063 Tetracyclinum (r), ANT068 Chloramphenicol (s), ANT074 Nitrofurantoin (s), ANT083 Kanamycin (r); «s» – sensitive; «r» – resistant; « ATU » – Area of Technical Uncertainty.

Antibiotic sensitivity in 24 *E. coli* strains isolated from raw milk was studied to beta-lactams from the groups of penicillins (semi-synthetic and inhibitor-protected drugs), cephalosporins (I-IV generations), carbarpenems; as well as the *E. coli* sensitivity to aminoglycosides (I-III generations), tetracyclines, quinolones (I-IV generations), Nitrofurantoin and Chloramphenicol.

*E. coli* sensitivity to the group of semi-synthetic penicillins, namely to Ampicillin, Piperacillin, Ticarcillin, Ampicillin/sulbactam, Ticarcillin/clavulanic acid was also studied. The research results showed that 100% of the studied cultures showed resistance to Ampicillin (fig. 2). 57.1% of strains were resistant to Piperacillin, 14.3% were moderately resistant, and 28.6% of the studied cultures were sensitive. The studied cultures exhibited resistance to Ticarcillin in 50%, 14.3% and 35.7% respectively.

Most of the studied isolates showed sensitivity to Ampicillin/sulbactam viz. in 95.8% of cases, thus showing resistance in 4.2% of *E. coli* isolates. 75% of isolates showed resistance to Ticarcillin/clavulanic acid, 4.2% - moderate resistance, and 20.8% - sensitivity (tab. 1).

The antibiotic susceptibility of *E. coli* isolates to the group of cephalosporins was studied, namely to Cefalotin, Cephalexin, Cefazolin (1st generation); to Cefaclor, Cefoxitin, Cefuroxime, Cefamandole (2nd generation); to Cefixim, Cefoperazone, Cefotaxim, Ceftriaxone, Ceftazi-dime (3rd generation); to Cefepim (IV generation) (tab. 1). No susceptible *E. coli* isolate was identified to Cefalotin from first-generation cephalosporins; 37.5% of isolates were moderately resistant and 62.5% of strains were found resistant. However, 70.8% and 71.4% were sensitive to Cephalexin and Cefazolin, respectively; 29.2% and 7.2% of *E. coli* isolates showed resistance; and 21.4% of *E. coli* isolates were moderately resistant to Cefazolin.

The *E. coli* isolates showed ambiguous sensitivity to the second- generation cephalosporins. 100% of *E. coli* isolates were susceptible to Cefoxitin. 25%, 83.3% and 70% of E. coli isolates exhibited

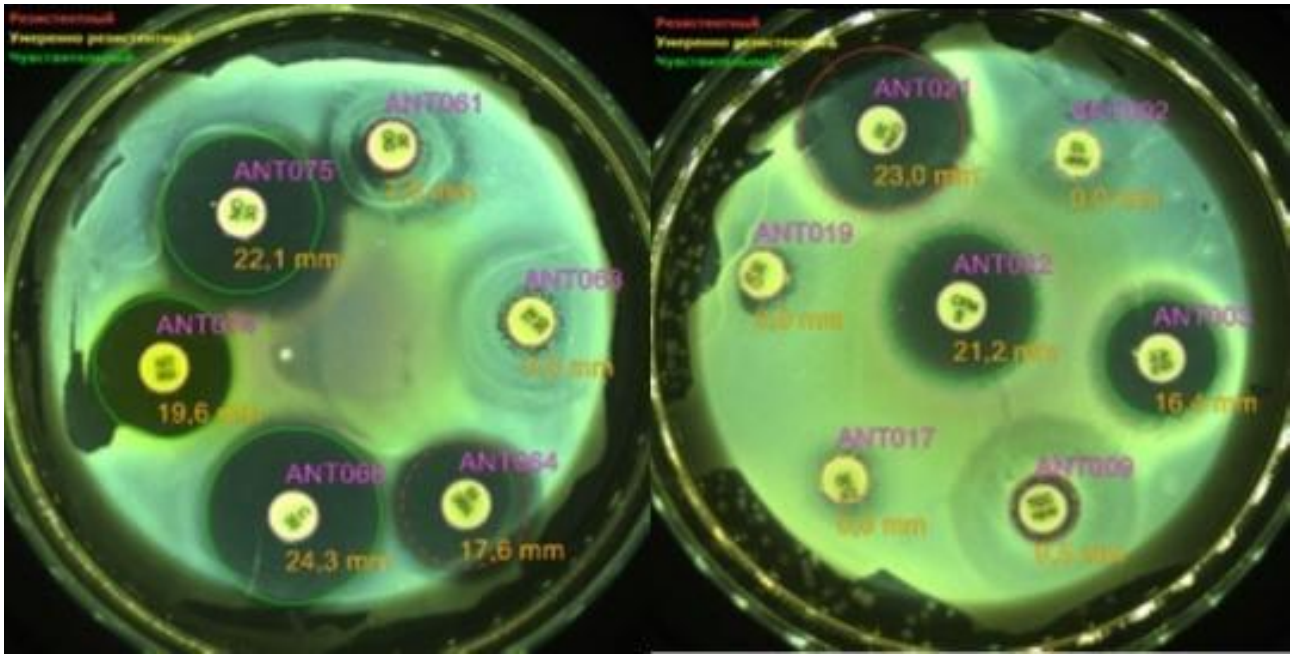


Figure 2. Antibiotic susceptibility of *E. coli* strains isolated from raw milk. ANT002 Ampicillin (r), ANT003 Ampicillin/sulbactam (s), ANT009 Ticarcillin/clavulanic acid (r), ANT017 Cefaclor (r), ANT019 Cefalexin (r), ANT021 Cefepim (r), ANT022 Cefixim (s), ANT061 Doxycycline (r), ANT063 Tetracyclinum (r), ANT064 Tigecycline (r), ANT068 Chloramphenicol (s), ANT074 Nitrofurantoin (s), ANT075 - Nitroxoline (s); «s» – sensitive; «r» – resistant; « ATU » – Area of Technical Uncertainty.

Table 1. Antimicrobial susceptibility *of E. coli* isolates (n=24).

| ***Antibiotic*** | ***Cultures*** | | |
|---|---|---|---|
| | ***s*** | ***ATU*** | ***r*** |
| **Penicillins** | | | |
| **Ampicillin** | - | - | 100% |
| **Ampicillin/sulbactam** | 95.8% | - | 4.2% |
| **Ticarcillin** | 35.7% | 14.3% | 50% |
| **Ticarcillin / clavulanic acid** | 20.8% | 4.2% | 75% |
| **Piperacillin** | 28.6% | 14.3% | 57.1% |
| **Cephalosporins** | | | |
| **Cefalotin (I)** | - | 37.5% | 62.5% |
| **Cephalexin (I)** | 70.8% | - | 29.2% |
| **Cefazolin (I)** | 71.4% | 21.4% | 7.2% |
| **Cefaclor (II)** | 25% | 37.5% | 37.5% |
| **Cefoxitin (II)** | 100% | - | - |
| **Cefuroxime (II)** | 83.3% | - | 16.7% |
| **Cefamandole (II)** | 70 % | 10 % | 20% |
| **Cefixim (III)** | 87.5% | - | 12.5% |
| **Cefoperazone (III)** | 40% | 20% | 40% |
| **Cefotaxim (III)** | 58.3% | 16.7% | 25% |
| **Ceftriaxone (III)** | 83.4% | 8.3% | 8.3% |
| **Ceftazidime (III)** | 64.3% | 28.6% | 7.1% |
| **Cefepim (IV)** | 8.4% | 20.8% | 70.8% |
| **Carbapenems** | | | |
| **Imipenem** | 20% | - | 80 % |
| **Meropenem** | 90% | - | 10% |

*Note*: «s» – sensitive; «r» – resistant; « ATU » – Area of Technical Uncertainty

sensitivity to Cefaclor, Cefuroxime, Cefamandole, respectively, and were resistant in 37.5%, 16.7%, 20% of isolates. 37.5% of isolates were moderately resistant to Cefaclor and 10% to Cefamandole.

The isolates showed sensitivity to Cefixim, Cefoperazone, Cefotaxim, Ceftriaxone, Ceftazidime in 87.5%, 40%, 58.3%, 83.4%, and 64.3%, respectively; resistance – in 12.5%, 40%, 25%, 8.3%, and 70.8% of cases. 8.4% strains were susceptible to Cefepim, moderately resistant – 20.8%, and resistant in 70.8% of *E. coli* isolates.

The studied isolates also showed ambiguous sensitivity to carbapenems viz. 20% of isolates were sensitive to Imipenem and 90% – to Meropenem, being resistant in 80% and 10% of strains, respectively (tab. 1).

*E. coli* isolates were found sensitive to Gentamicin, Kanamycin, Tobramycin, Netilin, Amikacin from the aminoglycoside group in 75%, 16.6%, 100%, 95.8% and 66.7%, respectively (tab. 2). It should be noted the high occurrence of isolates susceptible to Tobramycin (100%) and Netilin (95.8%); whereas a greater amount of isolates were resistant (41.7%) and moderately resistant (41.7%) to Kanamycin.

Table 2. Antimicrobial susceptibility of *E. coli* isolates (n=24).

| Antibiotic | Cultures | | |
|---|---|---|---|
| | s | m | R |
| ***Aminoglycosides*** | | | |
| **Gentamicin (I)** | 75% | - | 25% |
| **Kanamycin (I)** | 16.6% | 41.7% | 41. 7% |
| **Tobramycin (II)** | 100% | - | - |
| **Netilin (II)** | 95.8% | - | 4.2% |
| **Amikacin (III)** | 66.7% | - | 33.3% |
| ***Tetracyclines*** | | | |
| **Tetracycline** | 16.7% | - | 83.3% |
| **Doxycycline** | - | - | 100% |
| **Tigecycline** | 80% | - | 20% |

*Note*: «s» – sensitive; «r» – resistant; «ATU» – Area of Technical Uncertainty

83.3%, 100% and 20.0% of the investigated *E. coli* isolates were resistant to the drugs of the tetracyclines group (Tetracycline, Doxycycline and Tigecycline), respectively. 16.7% of them showed sensitivity to Tetracycline and 80% of strains to Tigecycline (tab. 2).

The investigated *E. coli* isolates were sensitive to quinolones (tab. 3).

Table 3. Antimicrobial susceptibility of *E. coli* isolates (n=24).

| Antibiotic | Cultures | | |
|---|---|---|---|
| | s | m | R |
| **Quinolones** | | | |
| **Nalidixic acid (I)** | 83.33% | 8.33% | 8.33% |
| **Nitroxoline (I)** | 100 % | - | - |
| **Norfloxacin (II)** | 87.5% | - | 12.5% |
| **Ciprofloxacin (II)** | 83.3% | 12.5% | 4.2% |
| **Ofloxacin (II)** | 62.5% | 25% | 12.5% |
| **Lomefloxacin (II)** | 41.7% | 45.8% | 12.5% |
| **Pefloxacin (II)** | 60% | - | 40% |
| **Gatifloxacin (IV)** | 95.8% | - | 4.2% |
| **Other antibiotics** | | | |
| **Nitrofurantoin** | 100% | - | - |
| **Chloramphenicol** | 50% | - | 50% |

*Note*: «s» – sensitive; «r» – resistant; «ATU» – Area of Technical Uncertainty

100% of the tested cultures were susceptible to Nitroxoline and 83.33% to Nalidixic acid 87.5%, 83.3%, 62.5%, 41.7%, 60% of *E. coli* isolates were susceptible to fluoroquinolones of the II genera

tion, viz. Norfloxacin, Ciprofloxacin, Ofloxacin, Lomefloxacin, Pefloxacin, respectively. 95.8% of the studied *E. coli* isolates were susceptible to the fourth-generation fluoroquinolone, namely Gatifloxacin.

Sensitivity to Nitrofurantoin was proved in 100 % and to Chloramphenicol in 50% of *E. coli* isolates tested (tab. 3).

## DISCUSSIONS

Therefore, the present research revealed the presence of *Escherichia coli, Staphylococcus spp*. (*Staphylococcus epidermidis, Staphylococcus aureus*) *Enterococcus spp*. isolates in all the samples of raw milk, which proved the non-compliance with the sanitary and hygienic conditions for milk production processing.

Most *Escherichia coli* isolates were found resistant to semisynthetic penicillins; 100% of the isolated cultures were resistant to Ampicillin. *Escherichia coli* isolates were selectively susceptible to inhibitor-protected penicillins, thus 95.8% of the cultures were sensitive to Ampicillin/sulbactam and 20.8% of the cultures were sensitive to Ticarcillin / clavulanic acid. *Escherichia coli* isolates were predominantly susceptible to cephalosporins.

Furthermore, the isolates showed no sensitivity dependence to the group of cephalosporins, belonging to specific generation type. At the same time, 100% of the studied isolates were susceptible to Cefoxitin and no susceptible *E. coli* isolates were detected to Cefalotin. It should also be noted the low percentage of strains sensitive to Cefepim (IV).

Moreover, there was no clear sensitivity proved to antimicrobial drugs from the carbapenem group. Most isolates showed resistance to Imipenem and were sensitive to Meropenem.

The tested *E. coli* isolates were generally susceptible to Aminoglycosides, viz. to Tobramycin (second-generation aminoglycosi-des) in 100% and to Netilin in 95.8% of isolates. At the same time, 16.6% of isolates were susceptible to Kanamycin.

The studied *E. coli* isolates were predominantly resistant to Tetracyclines and only 16.7% of the isolates were susceptible to Tetracycline. At the same time, 80% of the studied strains showed sensitivity to Tigecycline (the first-generation antibiotic of the glycylcycline group).

The tested *E. coli* isolates were also predo-minantly susceptible to quinolones and fluoroquinolones, particularly to the high activity of Nitroxoline, Nalidixic acid, and Gatifloxacin. Nitrofurantoin was also highly active against *E. coli* isolates, while only 50% of the studied *E. coli* isolates were susceptible to Chloramphenicol.

41.7% of *Staphylococcus epidermidis* isolates were resistant to Oxacillin, of which 90% were resistant to Benzylpenicillin, – 20% to Rifampicin, thus indicating an inappropriate use of antibacterial drugs in animals for disease control, prevention, and treatment.

## CONCLUSIONS

1. The study results revealed the presence of *E. coli, Staphylococcus spp., and Enterococcus spp*. isolates in 100% of milk samples obtained from clinically healthy cows on livestock farms from Kiev and Poltava regions of Ukraine.
2. *Escherichia coli* isolates were predominantly resistant to semi-synthetic penicillins.
3. High percentage of *Escherichia coli* isolates were found resistant to Tetracycline (80%) and Doxycycline (100%).
4. *Escherichia coli* isolates exhibited susceptibility to Ampicillin / sulbactam, Cefoxitin (100%), Meropenem, Tobramycin (100%), Netilin, Tigecycline, Nitroxoline (100%), Gatifloxacin, and Nitrofurantoin (100%). The tested *Escherichia coli* isolates were resistant to Ampicillin (100%), Imipenem, Tetracycline, and Doxycycline (100%).
5. *Staphylococcus spp*. strains included *Staphylococcus epidermidis* (87.5%) and *coagulase-positive Staphylococcus aureus* (12.5%); 41.7% of *Staphylococcus spp*. isolates were resistant to Oxacillin, of which 90% were resistant to Benzylpenicillin and 20% to Rifampicin.

## CONFLICT OF INTERESTS

All authors declare no competing interests.

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# BIOCHEMICAL COMPOSITION CHANGES OF GRAM-NEGATIVE MICROORGANISMS UNDER THE ACTION OF NEW CHEMICAL COMPOUNDS

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***Keywords:*** *antimicrobial activity, antioxidant-enzyme activity, chemical compounds, CT, SOD.*

***Introduction.*** *It is known that the enzymes catalase (CT) and superoxide dismutase (SOD) are actively involved in protecting cells of microbial pathogens against the biochemical factors produced by macrophages in vivo, in order to stop infection. Therefore, this study was aimed to quantify the parameters of the antioxidant-enzyme activities within the reference cultures under the action of selected new chemical compounds.*

***Material and methods.*** *Cu (II) coordination compounds, Co (II) and Zn (II) were included as "in vitro" study material, as well as aromatic propenones synthesized at the Department of Inorganic Chemistry, at State University of Moldova. The antimicrobial effects were tested on three gram-negative reference strains. The enzymatic activity of SOD and the CT activity was determined to assess the pathogen-induced changes in the antioxidant status under the action of newly tested compounds.*

***Results.*** *The new chemical compounds showed a significant decrease in the antioxidant enzymatic activities of SOD and CT, induced in all reference strains used, which indicates an intense oxidative stress generated by the tested compounds.*

***Conclusions.*** *The specific activity of new chemical compounds on primary antioxidant enzymes, which represent pathogenicity factors of infectious agents, allows us to assume the benefits of in vivo effect of new native substances, as well as to recommend the selected compounds for further biomedical tests.*

***Cuvinte cheie:*** *activitate antimicrobiană, enzime cu activitate antioxidantă, compuși chimici, CT, SOD.*

**MODIFICĂRILE CONȚINUTULUI BIOCHIMIC AL MICROORGANISMELOR GRAM-NEGATIVE SUPUSE ACȚIUNII COMPUȘILOR CHIMICI NOI**

***Introducere.*** *Se știe că enzimele catalaza (CT) și superoxid dismutaza (SOD) se implică activ în protecția microorganismelor patogene contra factorilor biochimici, produși de macrofage in vivo pentru a stopa infecția. Prin urmare, considerăm utilă cuantificarea parametrilor ce reflectă activitatea enzimelor antioxidante în culturile de referință la acțiunea compușilor chimici nou selectați.*

***Material și metode.*** *În calitate de obiecte de studiu in vitro au servit compușii coordinativi ai Cu (II): Co(II), Zn(II), dar și propenonele aromatice sintetizate la Catedra de chimie anorganică a Universității de Stat din Moldova. Efectele antimicrobiene au fost testate pe 3 tulpini de referință Gram-negative. A fost determinată activitatea enzimelor SOD și CT pentru a aprecia modificările statutului antioxidant al culturilor de microorganisme patogene sub influența compușilor nou testați.*

***Rezultate.*** *Compușii chimici noi induc în toate culturile de referință testate o reducere semnificativă a nivelului de activitate a enzimelor antioxidante SOD și CT, ceea ce indică prezența unui stres oxidativ intens, generat de compușii testați.*

***Concluzii.*** *Acțiunea specifică a compușilor chimici noi asupra enzimelor antioxidante primare, care constituie unul dintre factorii de patogenitate a agenților infecțioși, permite presupunerea unui eventual efect benefic in vivo al noilor substanțe autohtone și recomandarea compușilor selectați pentru teste biomedicale.*

## INTRODUCTION

Bioinorganic chemistry is a field that studies the role of metals in biology, which has opened new avenues for scientific research of coordination compounds. A large number of compounds are regarded as being of major biological importance. Some metals are essential for biological functions, being a part of the enzymes and cofactors involved in a range of various processes (1).

The pharmacological activity of metal compounds depends on the metal ion, its ligands and the integral compound structure. These factors are responsible for the metal complexes to reach the specific target site within the body. It is known that certain metal ions enter the bacterial cells and inactivate their enzymes. Some metal ions might cause the formation of peroxide, which leads to bacterial death (2).

A series of publications have studied the particularities of action of new antimicrobial compounds, showing various biochemical composition changes in the cells of microbial pathogens subjected to toxic antimicrobial activity. It is known that the enzymes catalase (CT) and superoxide dismutase (SOD) are actively involved in protecting cells of pathogenic microorganisms against biochemical factors produced by macrophages *in vivo* to stop infection (3, 4, 5, 6).

Superoxide dismutase is involved in neutralizing the superoxide radical. The active synthesis and the increased activity of superoxide dismutase contributes to the microbial survival under conditions of oxidative stress. High biomass content of superoxide dismutase is a biological marker of moderate oxidative stress, whereas the decrease of enzyme activity below normal is an indicator of deep oxidative stress. Catalase activity contributes to the elimination of hydrogen peroxide formed due to both normal processes and influence of harmful factors. It has been determined that the catalase activity exhibits a significant ten-time increase in case of oxidative stress in different cell types. Thus, an increased amount of catalase and superoxide dismutase produced by the pathogenic microorganisms help in neutralizing the hydrogen peroxide and superoxide radical of the immune cells, thereby avoiding the microbial death. The significant decrease in the activity of these two important protective factors leads to a greater vulnerability of bacteria *in vivo*, which might be an argument for conducting further biomedical researches to promote these valuable compounds (7).

The performed studies represent a valuable argument to select and study the particularities of action of new chemical compounds as substances that produce biochemical changes on the reference strains of pathogenic microorganisms. These studies represent an argument for conducting biomedical research in the further promotion of these valuable compounds.

## MATERIAL AND METHODS

Cu (II) coordination compounds, Co (II) and Zn (II) were included as "in vitro" study material, as well as aromatic propenones synthesized at the Department of Inorganic Chemistry (at State University of Moldova). The antimicrobial effects were tested on 3 gram-negative reference strains, *Escherichia coli* ATCC 25922, *Shigella sonnei* ATCC 25931, *Salmonella enterica* (S. Abony ГИСК03/03). These strains derive from two culture collections recognized as material suppliers of biological quality for performance studies: American Type Culture Collection (ATCC, USA) and the State Collection of Microbial Pathogens of the State Scientific Research Institute for Standardization and Control of biological medical preparations „L. A. Tarasevici" (ГИСК, Russian Federation).

The superoxide dismutase (SOD) activity was determined based on the principle of the enzyme ability to inhibit the photochemical reduction of nitroblue tetrazolium according to the method of Giannopolitis and Ries in 1977 (8) with subsequent modifications.

The sample in the amount of 100 mg is cold lysed with extractive buffer (2 mL buffer K, Na-phosphate, 50 mM, pH 7.8 + 20 µL phenylmethylsulfonyl fluoride solution of 100 mM). The homogenised tube shall be passed into the Ependorf tube and centrifuged for 5 min. at 12 000 g. The reactant mixture is prepared with the use of 100 µl bacterium extract, to which 0.5 mL solution of 0.05% nitroblue tetrazole, 0.9 ml buffer K, Na-phosphate, 50 mM, pH 7.8 and 20 µL solution of 0.24% EDTA. For each sample, prepare two identical working tubes as described above. One of the test tubes is placed in the dark and serves as a witness of darkness. The second test tube is exposed to light. In addition, the blank samples are pre-

pared, of which the bacterial extract is missing which are intended to perform the calculation of the maximum amount of formazan formed. Control swabs contain instead of bacterial extract, 100 µL tampom K, Na-phosphate 50 mM with pH 7.8.

The reaction is initiated by adding 20 µL of 0.025% riboflavin solution (added to all test tubes included in the study). Dark-control and experience-witnessed screws shall be placed in place away from the rays of light. The other test tubes shall be placed below 2 illuminating lamps with a power of 18W for 15 min. The reaction is stopped by disconnecting the light. The optical density shall be read at the wavelength of 560 nm immediately after the disconnection of the light, and in case there is a time interval between disconnection and measurements, the samples shall be placed in the dark.

As a unit of activity SOD is considered the amount of enzyme, which can inhibit the reaction of reduction of nitroblue tetrazole to 50%. In order to calculate the value of the optical density corresponding to a SOD activity unit, the value E corresponding to the maximum level of formation of the formazan shall be divided by 2 and shall be considered to be equal to 50% inhibition.

The calculation of the SOD activity at the mass unit shall be carried out according to the formula:

A=(a-V-X)/m, where a = 1- ($E_{sample}$ρ0.5)/ ($E_{formazan/2}$) A – the activity of the SOD enzyme; V – volume of extract; X – dilution of the extract; It's$_{sample}$ – the optical density measured for the sample; It's$_{formazan}$ – the optical density of the sample with maximum formation of formazan.

The calculation can also be made in SOD activity values according to the quantitative formation index of the formazan. The following calculation formula applies to this:

F=(ΔE-X)/7.2-m, where F – the amount of formazan formed at the unit of mass; ΔE – the difference between the optical density of the sample with the maximum formation of the forman and that of the sample to be investigated; X – dilution of bacterial extract; 7.2 – the value of the extinction coefficient of the forman at the wavelength of 560 nm, in $mM^{-1}cm^{-1}$; m – the absolutely dry mass of the sample.

The catalase (CT) was determined via the spectrophotometric method proposed by Aebi in 1984. This method is aimed to determine the rate of decomposition of hydrogen peroxide by the catalase contained in the sample, which subsequently yields water and oxygen (9).

The sample in the amount of 100 mg is cold lysed with extractive buffer (2 mL buffer K, Na-phosphate, 50 mM, pH 7.8 + 20 µl phenylmethyl-sulfonyl fluoride solution of 100 mM). The homogenised tube shall be passed into the Ependorf tube and centrifuged for 5 min. at 12 000 g. The reactant mixture is prepared by adding 2.95 ml of 0.6 M hydrogen peroxide to the sample (30 µl). The control sample is prepared analogously, but does not contain hydrogen peroxide. Ultraviolet sample spectrophotometry is performed at 240 nm with an interval of 100 s.

Calculation of catalase activity per gram of dry matter is done according to the formula:

A=(2.3t-lg(E1/E2))-X)/m, where: A – the activity of catalase in conventional units per g of dry matter; E1 and E2 – the value of the initial optical density and above 100 s, X – the dilution of the sample; t – reaction time; m – mass of the sample.

## RESULTS

The activity of SOD and CT enzymes was determined to assess changes in the antioxidant status of microbial pathogenic strains under the influence of newly tested compounds.

The study results reflecting the activities of the antioxidant enzymes SOD and CT on the reference *Shigella sonnei* ATCC 25931 strain, when treated with new chemical compounds are presented in Figure 1.

When treated with furacillin, there was a decrease with 41.4% in catalase activity and with 50.0% in superoxide dismutase activity, compared to their activity in biomass of *Shigella sonnei* under normal conditions. Under the action of new chemical compounds, the SOD activity in culture accounted for 17.9-48.7% of the normal pathogenic activity. The catalase activity was 19.5-44.8% of the typical activity in the intact culture. The values of the primary antioxidant enzyme activity in the cell lysate, obtained from the culture treated with furacillin and with MIC of the selected new chemical compounds, confirm the high-intensity oxidative stress within the culture of *Shigella sonnei* ATCC 25931 when treated with new antibacterial chemical compounds.

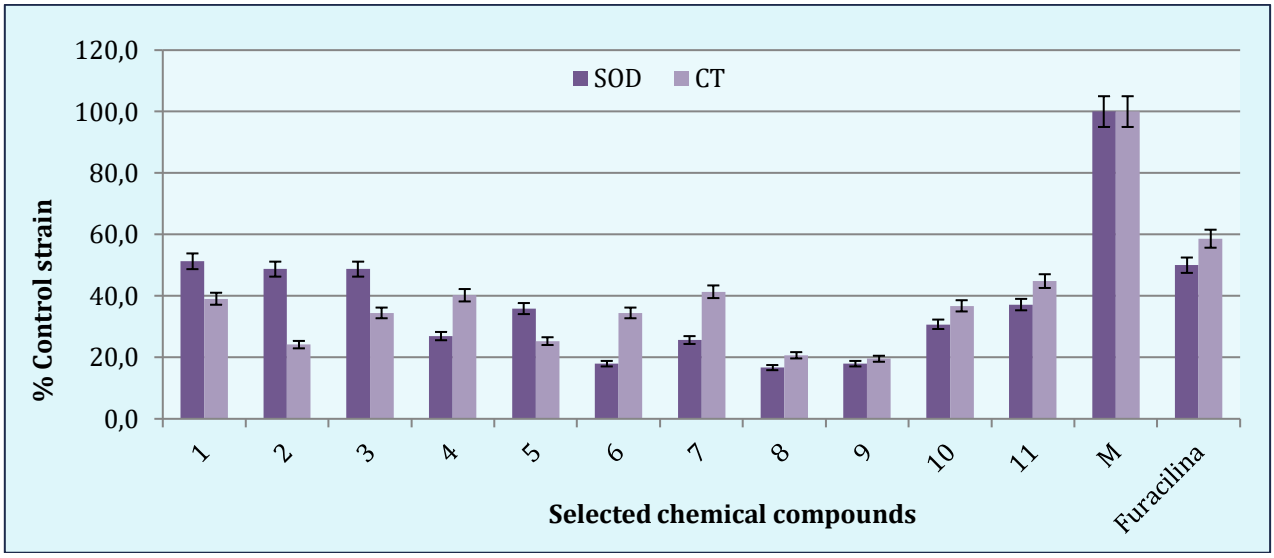


Figure 1. Changes in the antioxidant enzyme activity in the standard *Shigella sonnei* ATCC 25931 strain under the action of new chemical compounds: 1 - $C_{38}H_{38}Cu_2N_{14}O_{10}S_4$; 2 - $C_{42}H_{42}Cu_2N_{14}O_{12}S_4$; 3 - $C_{44}H_{40}Cl_2Cu_2N_{14}O_4S_6$; 4 - $C_{15}H_{19}ClCuN_4O_2S$; 5 - $C_{15}H_{19}CuN_5O_5S$; 6 - $C_{15}H_{17}ClCuN_4OS$; 7 - $C_{15}H_{19}CuN_5O_5S$ (2,5); 8 - $C_{15}H_{19}CuN_5O_5S$(3,4); 9 - $C_{15}H_{19}CuN_5O_5S$ (2,4); 10 - $C_{18}H_{22}Cl_2Cu_2N_8S_2$; 11 - $C_9H_{11}ClCuN_4S$; M – untreated culture.

Two chemical are particularly highlighted, namely di (μ-S) -bis {nitrate- [2-picolidene-4-(3,4-dimethylphenyl) thiosemicarbazido- (1-)] copper} tetrahydrate and di (μ-S) -bis {nitrate - [2-picolidene-4- (2,4-dimethylphenyl) thiosemicarbazido - (1-)] copper} tetrahydrate, which exhibited the most significant reduction in both SOD and CT activities. The values obtained by treating the pathogenic strains with these two compounds were 5 times lower than the antioxidant enzyme activity in the lysate of the control culture.

The study results reflecting the activity of the antioxidant enzymes SOD and CT on the reference culture *Escherichia coli* ATCC 25922 when treated with new chemical compounds are presented in Figure 2.

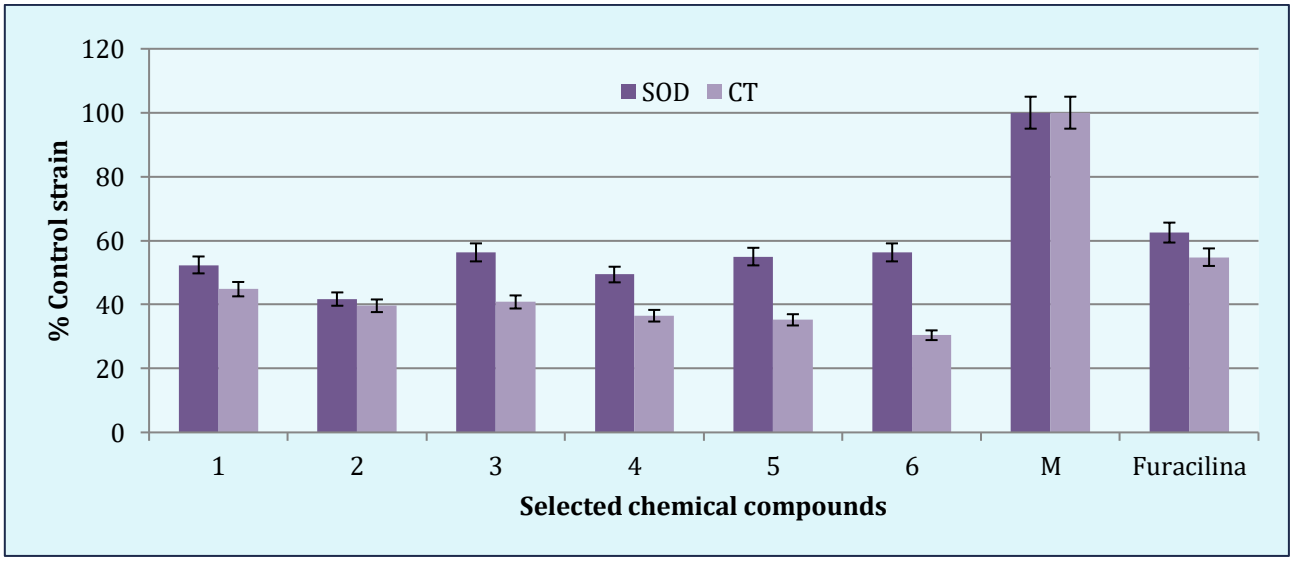


Figure 2. Changes in the antioxidant enzyme activity in the standard *Escherichia coli ATCC 25922* strain under the action of new chemical compounds:1 - $C_{38}H_{38}Cu_2N_{14}O_{10}S_4$; 2 - $C_{44}H_{40}Cl_2Cu_2N_{14}O_4S_6$; 3 - $C_{46}H_{46}Cu_2N_{18}O_{10}S_6$; 4 - $C_{46}H_{42}Cu_2N_{18}O_{10}S_4$; 5 - $C_{18}H_{22}Cl_2Cu_2N_8S_2$; 6 - $C_9H_{11}ClCuN_4S$; M- untreated culture.

Under the action of furacillin, there was a 45.2% and a 37.5% reduction in catalase and superoxide dismutase activities, respectively, compared to the activity of CT in *Escherichia coli* biomass obtai-ned under normal conditions. The SOD activity accounted for 41.7-56.3% of the reference *E. coli* SOD activity, when treated with new chemical compounds, thus showing a statistically significant difference ($p<0.01$). The catalase activity exhibited lower values, being 30.4-44.8% of the typical activity in intact culture. As in the case of SOD, the statistically significant differences between the enzyme activity in the control biomass and in the biomass treated with furacillin or the selected compounds were at the same level of significance. The obtained results confirmed a high-intensity oxidative stress in the *Escherichia coli* ATCC 25922 strain under the action of new antibacterial chemical compounds. As regarding the reference culture, we cannot highlight any of the six selected compounds, since their action on the antioxidant enzyme activity was very similar from case to case.

The study results reflecting the activity of the antioxidant enzymes SOD and CT on the reference culture *Salmonella enterica* (*S. Abony* ГИСК 03/03 y) under the action of new chemical compounds are presented in Figure 3.

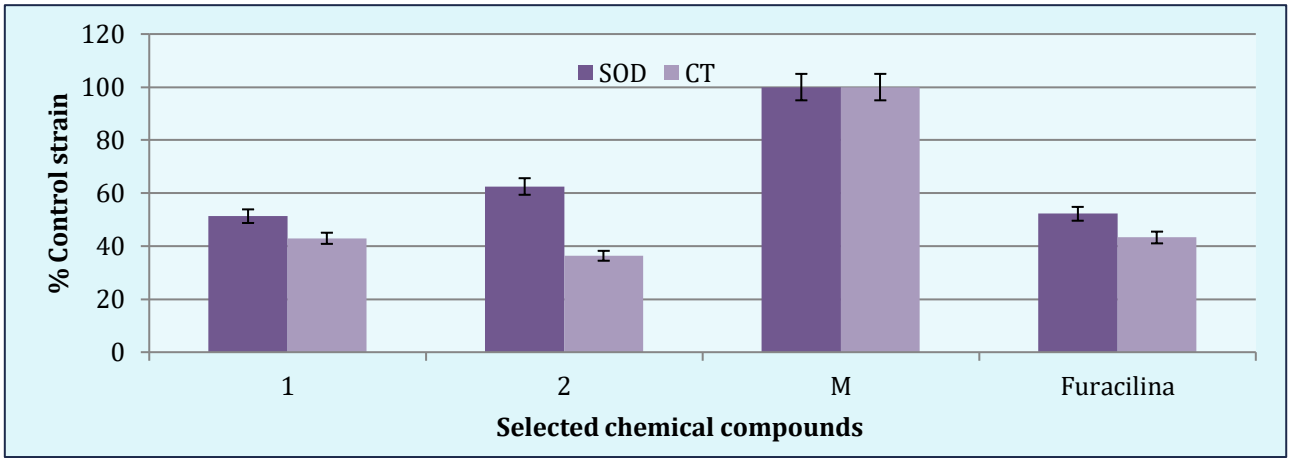


Figure 3. Changes in the antioxidant enzyme activity in the standard *Salmonella enterica (S. Abony ГИСК 03/03y)* strain when treated with new chemical compounds: 1 - $C_{38}H_{38}Cu_2N_{14}O_{10}S_4$; 2 - $C_{44}H_{40}Cl_2Cu_2N_{14}O_4S_6$; M- untreated culture.

Under the action of furacillin, there was a decrease to 56.7% in catalase and to 47.8% in superoxide dismutase activities, compared to the CT and SOD in *Salmonella* biomass obtained under normal conditions. The SOD activity accounted for 51.3% and 62.5% of the normal activity of salmonella strain under the action of new chemical compounds. The catalase activity level was 36.4% and 43.0%, which was lower than the typical activity level in intact culture. These fluctuations confirmed the high-intensity oxidative stress for the culture of *Salmonella enterica* (*S. Abony* ГИСК 03/03 y) under the action of new antibacterial chemical compounds.

## DISCUSSIONS

Under the action of new chemical compounds, all the reference cultures tested, showed a significant reduction in the activity of the antioxidant enzymes superoxide dismutase and catalase, which indicated an intense oxidative stress gen erated by the tested compounds.

The same phenomenon was observed when treating the microbial pathogens with furacillin. This substance is known for its antibacterial mechanism that consists in the formation of amino derivatives due to the reduction of nitrofural 5-nitrogroup under the action of bacterial flavonoid activity. The amine derivatives formed, conformationally alter the proteins and eventually lead to cell death.

Since there is a decrease in the activity of both catalase and superoxide dismutase, we can assume the alteration of the specific conformation of the proteins, including the above- mentioned enzymes. However, further research is required to confirm this assumption.

Hence, the new antibacterial compounds used on

microbial pathogenic strains, produce an oxide tive stress within these microorganisms, being associated with the accumulation of free radicals, lower total antioxidant capacity, a reduced expression and protective antioxidant enzymes activity.

## CONCLUSIONS

1. One of the mechanisms of action of the newly tested compounds is based on the significant decrease in the activity of first-line antioxidant enzymes superoxide dismutase and catalase. Thus, under the action of new chemical compounds the activity of these two enzymes decreased below 30% of their activity in untreated biomass.
2. Hence, we might assume the preserving of the antibacterial effect observed *in vivo*, whereas the enzymes are regarded as the key elements in the protection of microbial pathogens against the activity of the host immune system.
3. The specific action of new chemical compounds on primary antioxidant enzymes, which is one of the pathogenicity factors of infectious agents, allows assuming the beneficial *in vivo* effect of new native substances and recommending these selected compounds for further biomedical tests.

## CONFLICT OF INTERESTS

Nothing to declare.

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CASE PRESENTATION – STUDIU DE CAZ – PRESENTATION DE CAS CLINIQUE – ПРЕЗЕНТАЦИЯ СЛУЧАЕВ ИЗ КЛИНИЧЕСКОЙ ПРАКТИКИ

MEDICAL SCIENCES

OPEN ACCESS

# ANALYSIS OF CLINICAL AND MOLECULAR GENETIC CHARACTERISTICS OF WISKOTT-ALDRICH SYNDROME AND X-LINKED THROMBOCYTOPENIA

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***Keywords:*** *Wiskott Aldrich syndrome, X-linked thrombocytopenia, immunodeficiency.*

***Introduction.*** *Wiskott-Aldrich syndrome is a rare X-linked disorder characterized by microthrombocytopenia, eczema, and recurrent infections. It is caused by mutations of the WAS gene which encodes the WAS protein (WASp) – a key regulator of actin polymerization in hematopoietic cells. Mutations within the WASp gene result in a wide heterogeneity of clinical disease, ranging from 'classical WAS' to mild asymptomatic thrombocytopenia (X-linked thrombocytopenia [XLT]), or congenital neutropenia (X-lined neutropenia [XLN]).*

***Case presentation.*** *This present paper reports a phenotypical and laboratory description of two children diagnosed with WAS and one child diagnosed with XLT. The first case was a six months old male with septicemia, thrombocytopenia, eczema and petechial rash. The second case was a 2 years old boy presenting with complaints of recurrent infections, eczema and thrombocytopenia with small platelet size. The third case was a 16 years old boy who presented with thrombocytopenia and recurrent sinopulmonary infections.*

***Conclusions.*** *Due to a wide spectrum of clinical findings, the diagnosis of WAS/XLT should be considered in any male patient presenting with petechiae, bruises, and congenital or early-onset thrombocytopenia associated with small platelet size.*

***Cuvinte cheie:*** *sindromul Wiskott-Aldrich, trombocitpenie X-linkată, imunodeficiență.*

**ANALIZA CARACTERISTICILOR CLINICE ŞI MOLECULAR-GENETICE ALE SINDROMULUI WISKOTT-ALDRICH ŞI TROMBOCITOPENIA X-LINKATĂ**

***Introducere.*** *Sindromul Wiskott-Aldrich este o afecțiune rară X-linkată, caracterizată prin microtrombocitopenie, eczeme și infecții recurente. Acesta este cauzat de mutații ale genei WAS, care codifică proteina WAS (WASp) - un regulator cheie al polimerizării actinei în celulele hematopoietice. Mutațiile din gena WASp generează o eterogenitate largă a bolii clinice, variind de la „WAS clasic" la trombocitopenie asimptomatică ușoară (trombocitopenie X-linkată [XLT]) sau la neutropenie congenitală (neutropenie X-linkată [XLN]).*

***Prezentarea cazului.*** *Este raportată descrierea, fenotipică și de laborator, a doi copii diagnosticați cu WAS și a unui copil diagnosticat cu XLT. În primul caz, un băiat în vârstă de șase luni, cu septicemie, trombocitopenie, eczemă și erupții de tip peteșii. În al doilea caz, un băiat de 2 ani, care a prezentat acuze de infecții recurente, eczemă și trombocitopenie, cu dimensiune mică a trombocitelor. Iar în al treilea caz, un băiat de 16 ani, care s-a adresat cu acuze de infecții sinopulmonare recurente și trombocitopenie.*

***Concluzii.*** *Datorită spectrului larg de manifestări clinice, diagnosticul WAS/XLT trebuie luat în considerare la orice pacient de sex masculin, care prezintă erupții de tip peteșii, echimoze și trombocitopenie congenitală sau cu debut precoce, asociată cu o dimensiune mică a trombocitelor.*

## INTRODUCTION

The Wiskott-Aldrich syndrome (WAS, OMIM 301000) is a rare X-linked immunodeficiency syndrome originally described by Dr. Alfred Wiskott in 1937 and Dr. Robert Aldrich in 1954 as a familial disease, characterized by the classic triad of severe immunodeficiency, microthrombocytopenia, and eczema. The incidence of WAS is estimated at between 1 in $10^5$ and 1 in $10^6$ cases per live birth and WAS makes up approximately 3% of all Primary Immunodeficiencies Disorders in the European Society for Immunodeficiency registry (1).

The gene responsible for WAS is located on the short arm of the X chromosome at Xp11.22–p11.23. The *WAS* gene encodes the WAS protein (WASp), which is a cytosolic multidomain 502-amino acid protein expressed within the cytoplasm of nonerythroid hematopoietic cells. WASp is involved in actin polymerization and associated coupling of receptor engagement, signaling events, and cytoskeletal rearrangement (2). Mutations within the WASp gene result in a wide heterogeneity of clinical disease, ranging from 'classical WAS', where babies present in the first year of life with a severe persistent thrombo-cytopenia, opportunistic and increased frequency of respiratory tract infections and early development of autoimmunity (3), to mild asymptomatic thrombocytopenia (X-linked thrombocytopenia [XLT]), or congenital neutropenia (X-lined neutropenia [XLN]) (1). X-linked thrombocytopenia (XLT), shares similar bleeding risk from thrombocytopenia but is not associated with other significant clinical features and is generally managed conservatively (4).

In WASP-deficient cells, the formation of the Immunological Synapse (IS) in T cells and T Cell Receptor (TCR)-dependent activation, the cyto-toxic activity of CD8+ T cells and Natural Killer (NK) cells and the suppressor activity of Naturally occurring Regulatory T (nTreg) cells are all impaired. Motility, adhesion and migration of B cells are also defective. Additionally, the lack of WASP affects podosome formation, motility and T cell priming in Dendritic Cells, as well as podosome and phagocytic cup formation in macrophages (5).

Low number of platelets and thrombocytopenia are universal features of WAS, usually present in the first year of life and typically causing petechiae, easy bruising, spontaneous or prolonged bleeding. Life-threatening bleeding episodes, particularly gastrointestinal or intracranial bleeding, have been reported in 10–30% of patients (4). Patients with WAS are at a higher risk of developing autoimmunity, the most frequent being hemolytic anemia (36%), followed by vasculitis (including cerebral vasculitis; 29%), arthritis (29%), neutropenia (25%), inflamma-tory bowel disease (9%), and IgA nephropathy (3%). Henoch–Schönlein-like purpura, dermatomyositis, recurrent angioedema, and uveitis have also been reported in some patients (5). Furthermore, WAS patients often develop hematological malignancies often associated with Epstein-Barr virus infection and of poor prognosis (6). The tumor incidence in WAS is estimated to be 13–22% with a mean age of onset of 9.5 years and with poor prognosis. WAS patient tumors include non-Hodgkin lymphoma, EBV positive and EBV negative lymphoma, Hodgkin lymphoma, Burkitt lymphoma, and less frequently myelodysplasia, acute lymphoblastic leukemia, myelomonocytic leukemia, and nonhematopoietic malignancies (7, 8).

The immunodeficiency in WAS involves T cells, and is associated with both quantitative and qualitative defects in T cells. Humoral immune responses are abnormal: patients with WAS exhibit variable levels of serum IgM, normal to high levels of serum IgA, and high levels of serum IgD, IgG, and IgE (2). Despite the presence of normal numbers of neutrophils, monocytes, and other phagocytes, functional abnormalities may also be present in patients with WAS. Chemotaxis, the initiation of degranulation, the formation of a functional respiratory burst, and antibody-medi-ated phagocytosis may be impaired in patients with WAS (9).

With regard to treatment, hematopoietic stem cell transplantation (HSCT) is the current accepted curative approach for patients with WAS. Patients with WAS, who undergo unrelated donor HSCT at younger ages (less than 5 years of age), have outcomes that are comparable to those associated with the use of matched-sibling donor HSCT (3). At present, gene therapy in WAS patients offers remarkable possibilities. The first gene therapy study in WAS used a γ-retroviral vector, with WASp expression driven via the viral promoter (10). Restoration of T proliferative responses, polyclonal T-cell repertoires, natural killer cell cytotoxicity and antibody responses to vaccination

were demonstrated post treatment, and platelet counts were either within the normal range (43%) or significantly higher than pregene therapy (57%) (11). Another trial used a self-inactivating lentivirus vector for *WAS* gene correction in which a 1.6 kb fragment of the proximal promoter of the *WAS* gene is used to express the full-length coding sequence of the human *WAS* gene in cells of the hematopoietic lineage. Consequently, there has been an improvement at 24 months in eczema, the frequency and severity of infections, bleeding tendency, autoimmunity, reduction in disease-related days of hospitalization and improvement in immunological and haematological parameters (12, 13). This vector has also been applied to adult WAS patient (14).

## CASE PRESENTATION

Patients were referred to the Human Molecular Genetics Laboratory at the Institute of Mother and Child, for genetic counselling. In order to establish the diagnosis, the WAS clinical scoring system was used, which ranges between 0 and 5, including the presence of thrombocytopenia, eczema, immunodeficiency, autoimmunity, and malignancy. Immunological investigations were performed in order to identify changes suggestive of the presence of the disease in suspected patients. The number of T-cell receptor excision circle (TREC)/kappa-deleting recombination excision circle (KREC) copies were quantified by qPCR and were related to the albumin control gene.

The first case was a 6-month-old male patient with a WAS score equal to 3 which is considered "classic" WAS. He was the first child born from nonconsanguineous parents. He was admitted to the IMSP Institute of Mother and Child with septicemia, thrombocytopenia, eczema and petechial rash. The patient also presented with restraint in physical development (petty malnutrition) and motor-hypotonicity. Besides those mentioned above, the patient also revealed respiratory insufficiency, cytomegalovirus hepatitis, signs of cardio-circulatory insufficiency, disseminated intravascular coagulation. During the hospitalization, the condition of the child was severe, presenting cardio-circulatory insufficiency, marked hepatomegaly, myotonia, and psychomotor retardation. Quantitative serum immunoglobulin tests detected a low serum level of IgM (0.3 g/L) and IgA (0.2 g/L), elevated IgE (767.6 kU/L), low level of CD4(+) T cells (9%), elevated CD8(+) T cells (59%). Treatment is directed mainly at control of bleeding through transfusions of blood and platelets, and control of infections with antibiotics and Immunoglobulin replacement.

The second case was a 2-year-old boy presenting with complaints of recurrent infections, eczema and thrombocytopenia with small platelet size (WAS clinical score – 3). The mother of the patient had a brother who died at a young age and no clear diagnosis was made at that time. Analysis of serum immunoglobulins revealed a low serum level of IgM (0.2 g/L), normal IgA (1.39 g/L) and normal IgG (7.18 g/L), low level of CD3(+) (63%), low level of CD4(+) T cells (32%), elevated CD8(+) T cells (28%), elevated CD16(+) (32%). Quantification of TREC/KREC copies showed low TREC and KREC levels.

The third case was a 16-year-old boy who presented with thrombocytopenia and recurrent sinopulmonary infections. In the present case, the WAS clinical score was 2, which rather suggested the presence of XLT. Immunological investigations indicated a normal immunoglobulin profile, low level of CD4(+) T cells (29,8%) and elevated CD8(+) T cells (36,6%). TREC and KREC copy counts revealed low TREC levels while KREC level was within the normal range.

## DISCUSSIONS

During the period 2016-2020, WAS was clinically suspected in 5 patients, all of Caucasian ethnicity. The diagnosis was genetically confirmed in 3 patients.

Diagnosing Wiskott-Aldrich syndrome can be difficult and is often overlooked or confused with other more common conditions. The presence of family history makes the diagnosis easier. The mean age at the time of diagnosis is 24 months in patients whose family members are unaffected by this syndrome previously (15).

For the purpose of performing the genetic test, genomic DNA was isolated from peripheral blood leukocytes by using the Salting-out method. In order to examine mutations on the WAS gene, direct sequencing was performed on the ABI 3500 DX Genetic Analyzer (Applied Biosystems) for all 12 coding regions of the *WAS* gene listed in Table 1. Data were analyzed using the bioinformatic software "Sequencing Analysis Software v6.0".

Table 1. Primer sequences for Sanger sequencing (16, 17, 18).

| Primer | Forward/ Reverse | Sequence (5′-3′) | Product length (bp) |
|---|---|---|---|
| ***WAS gene Exon 1*** | Forward | GGTCTAAGCAGTCAAGTGG | 498 |
| | Reverse | GGAAGGGTGGATTATGACG | |
| ***WAS gene Exon 2*** | Forward | TACCCCTGACCAGACTCCAC | 237 |
| | Reverse | GGTTTGGGGGTTGAGAACT | |
| ***WAS gene Exon 3*** | Forward | CTCCCACCCTACACCTCTCC | 164 |
| | Reverse | TTCCCATCTCCTCTCCACAC | |
| ***WAS gene Exon 4*** | Forward | CTCACTTGGGGTGTGGAGAG | 219 |
| | Reverse | ACCTCTGCCCAACTTCCTTT | |
| ***WAS gene Exon 5*** | Forward | AAGGAATCAGAGGCAAAGTG | 248 |
| | Reverse | GGGAAGATGGAATGTGTAGA | |
| ***WAS gene Exon 6*** | Forward | GTGGCAGGGCTGTGATAACT | 223 |
| | Reverse | GCTCGTCCATCCACATACCT | |
| ***WAS gene Exon 7*** | Forward | TACCTCCATGACCATCCAACA | 380 |
| | Reverse | CCATCCTTCCATTCACTCAGC | |
| ***WAS gene Exon 8-9*** | Forward | CAAGAGGTTTCACTATGAAGG | 534 |
| | Reverse | GCGTATCTTAGCTATGAGCTGC | |
| ***WAS gene Exon 9*** | Forward | CCTGGCCTTTTTCCTCCT | 208 |
| | Reverse | AGAAGGGAGCGTATGGAAGC | |
| ***WAS gene Exon 10*** | Forward | GCTTCAGTCAGGAGTTGGTC | 580 |
| | Reverse | TCCTGACTTAGACGGGACAC | |
| ***WAS gene Exon 11*** | Forward | GGGAGAAATGCTCCTTTCC | 291 |
| | Reverse | GTTAATGCTGTCAAACAGATG | |
| ***WAS gene Exon 12*** | Forward | TTAACCAGACAGGAAGCAAT | 593 |
| | Reverse | CTTGAGTGAAGAGAACTGAGA | |

Genetic analysis of *WAS* gene was performed in the first patient, and subsequently in the patient's mother. The direct sequencing analysis showed A-to-G transition at complementary nucleotide 274 (c.274-2 A>G), located in intron 2 (fig. 1a). Molecular analysis of the *WAS* gene performed in the patient's mother revealed no mutation. Thus, taking into consideration that family history analysis did not reveal the presence of relatives with clinical features of Wiskott-Aldrich syndrome, and DNA analysis of the patient's mother did not disclose any mutation, we could assume that the mutation found in the patient appeared de novo. The severe phenotype of the patient correlated with the presence of an aberrant protein.

In the second case, genetic analysis for the detection of a mutation of *WAS* gene showed a pathogenic mutation – c.391 G>A (p. E131K) in exon 3 (fig. 1b). Direct sequencing performed in the patient's mother confirmed that she is a heterozygous carrier of the same mutation. Family history analysis revealed the presence of a family member (the maternal uncle) with clinical signs similar to Wiskott Aldrich syndrome, who died in childhood.

In the third case, molecular genetic analysis performed through Sanger sequencing of the *WAS* gene revealed two mutations – c.57 G>T (p. Q19H) in the first exon, and c.136 C>A (p. L46M) in the second exon (fig. 1c). The presumed impact on the patient phenotype was investigated on The Ensembl Variant Effect Predictor and as a result, c.57 G>T (p. Q19H) mutation had a severe phenotypic effect, while the impact of c.136 C>A (p. L46M) mutation was moderate. Considering the patient's age, the WAS clinical score, the immunological picture and the data of the molecular genetic analysis we can suggest the presence of XLT.

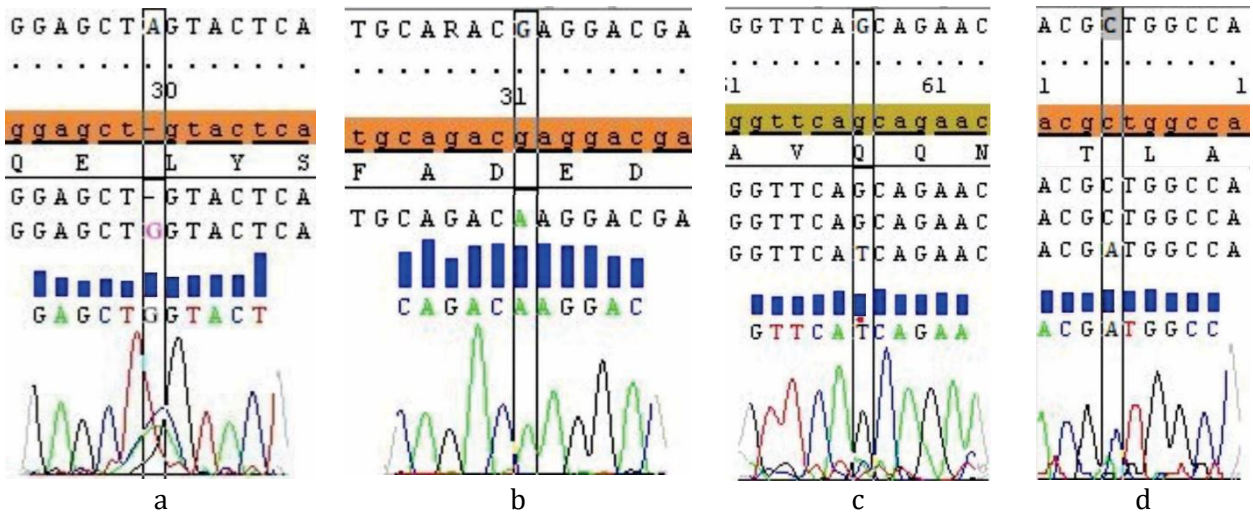


Figure 1. Sequencing identification of mutations in the *WAS* gene. Wave overlay denotes the presence of the mutation in labeled nucleotide.

a. Mutation c.274-2 A>G located in intron 2 of *WAS* gene in the first case
b. Mutation c.391 G>A located in exon 3 of *WAS* gene in the second case
c. Mutation c.57 G>T located in exon 1 of WAS gene in the third case
d. Mutation c.136 C>A in exon 3 of WAS gene in the third case

## CONCLUSIONS

1. Due to a wide spectrum of clinical findings, the diagnosis of WAS/XLT should be considered in any male patient presenting with petechiae, bruises, and congenital or early-onset thrombocytopenia associated with small platelet size.
2. Obtaining a definitive and specific molecular diagnosis for a patient with clinically suspected Wiskott-Aldrich syndrome is important because it ensures more effective medical management, allows early access to standard care, avoiding invasive investigations and significant impact on the evolution of the disease.

## CONFLICT OF INTERESTS

The authors declare no conflict of financial or non-financial interests.

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EXPERTS' OPINIONS – OPINII ALE EXPERȚILOR – AVIS DES EXPERTS
МНЕНИЯ ЭКСПЕРТОВ



# EUROPEAN BIOSAFETY ASSOCIATION (EBSA) – STRENGTHENING BIOSAFETY AND BIOSECURITY REGIONALLY AND GLOBALLY

**Thomas BINZ,** PhD, President of the European Biosafety Association (EBSA)

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The European Biosafety Association has been founded in June 1996. The association comprises over 400 members from most of the European countries and from other countries all over the world. EBSA strives to the following objectives:

- Establishment and communications of best biosafety and biosecurity practices amongst its members and encouragement of dialogue and discussions on developing issues
- Representation of the collective interests of its members in all areas relating to biosafety and biosecurity
- Support of emerging legislation and standards, with the objective of ensuring the prevention of harm to man or the environment from biological substances or materials.
- Coordination of actions with national biosafety organizations and other stakeholders to ensure that advocacy efforts are directed efficiently
- Expand participation in global initiatives

To ensure the realization of its objectives and to further foster opportunities to enhance national, regional and global biosafety, EBSA has held, since the foundation, every year, except for 2001, a scientific conference and, since more than a decennia, annual preconference training courses and events. Furthermore, EBSA executive members regularly participate at the scientific conference of the numerous partner organizations.

### *Annual conferences: dispersion of scientific knowledge in biosafety and biosecurity*

Importantly, EBSA needed to install operational bodies to maintain the annual scientific conference and the preconference training activities.

The annual conference is regularly organized by an EBSA working group, the Conference Program working group (CPWG), in collaboration with a committee on site of the location where the conference takes place (Local organizing committee, LOC). The main task of the CPWG is the planning of the scientific programme of the annual conference. Furthermore, the working group takes care of scientific issues like e.g. the response to consultative documents, the review of articles and setting up liaisons with other organizations and enforcing bodies in relation to scientific affairs.

Since the first conference in London in 1997 to the last virtual online conference in October 2020, a total of 23 scientific conferences have taken place. Among many other topics related to biological safety, selected topics were: safety in microbiological diagnostic laboratories, infectious waste management, use of microbiological safety hoods, biosafety of animal by-products, safety of CRISPR-Cas9, risk related to viral gene therapy vectors, safety of cell cultures, biosecurity related topics, occupational health issues, arthropod containment, risk assessment and sustainability issues.

### *Training and Education*

The preconference training events are set up by the education and training working group (ETWG). The group met initially at the Ljubljana Conference 2010 to review the work programme passed down by the EBSA council. The aim is to strengthen the role EBSA plays in improving biosafety competency and skills among members and to the whole biosafety community and providing information to non-specialists. One major task is to look specifically to align the pre-conference workshops with the CEN CWA 16335 Biosafety professional competence and identify different ways to provide training. Developments in the competency of biosafety advice are driving the urgency of this. Further objectives include the identification of the needs for biosafety/biosecu

rity education and training at different levels; basic life sciences, biosafety professionals in small and large organizations, the identification of training possibilities, pre-conference workshops, workshops in conjunction with events other than EBSA conferences, separate workshops, university arranged courses etc. and identification of training methodology developments and implement if applicable. Since its creation, EBSA has delivered, among other topics, and is still delivering training on these subjects:

- ISO 35001 – An introduction into the biorisk management standard
- Gene therapy: main approaches and biosafety issues
- Blend your biosafety training – how to produce and use educational technologies
- Biological Risk Assessment – how safe are we in our labs if we apply the risk-based approach according to the new WHO Biosafety manual?
- Disinfection and disinfectants: overview of the biocide regulation and validation procedures
- How to convince and influence for biosafety and biosecurity
- Biorisk Management meets quality management
- Incident & accident investigation: how to apply root cause analysis
- Assessing risks and benefits of viral vectors: understand the underlying Risk assessment
- Cell Culture biosafety: from Bench to Body
- Movement of biological material: all you need to know to ensure safe transport
- Introduction to audits and inspections – a comprehensive and practical introduction
- Biosafety Officer – from basic to expert in one day
- Biological waste management, a clear and practical approach

### *Networking/liaisons: focal points*

Focal Points are EBSA designated subject matter experts (SME), who are the first point of contact for their topic(s). They cover areas that are considered important from an EBSA perspective and/or a biosafety/biosecurity perspective. They represent EBSA in specialist for a provide information to the EBSA membership. EBSA, to date, maintains the following focal points:

- *Transport of hazardous biological materials*: advise EBSA members on changes in regulations in the transport of infectious substances, clinical specimens, GMO's
- *Biological Toxin and Weapons Convention (BTWC)/European Chemical, Biological, Radiological, Nuclear (CBRN) protection*
- *Liason World Health Organisation*
- *Liason World Organisation for Animal Health*
- *Diversity and Inclusion:* advise EBSA on issues of diversity and inclusion, promote EBSA in new areas and regions and monitor diversity at EBSA
- *Containment of arthropods*: provides information on how to handle infected and exotic arthropods under biosafety containment

### *Projects*

EBSA closely observes the development of biosafety-relevant legislation in the European Union. Recently, there have been several regulatory developments that have been introduced where EBSA sees a need to emphasize regulatory issues that may affect biosafety procedures in diagnostic research laboratories or production facilities.

Therefore, working group for each issue/regulatory document(s) have been constituted and position paper have been issued. This is related to :

- Consequences of the EU legislation on Animal By-Products for the scientific research sector
- Revision of the EU biological agents directive 2000/54/EC, the EU directive 2019/1833
- Biocide product regulation (work in progress)
- ***Cooperation and partner organizations***
- EBSA maintains numerous ties to its 19 national and regional partner organizations throughout the world. All EBSA resources can be found on the website: https://www.ebsaweb.eu/

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## EVENTS/ANNIVERSARIES – EVENIMENTE/ANIVERSĂRI – ÉVÉNEMENTS/ANNIVERSAIRES – СОБЫТИЯ/ЮБИЛЕИ

# Academicianul Stanislav GROPPA – savant notoriu și manager talentat

*Adevărata ştiinţă nu e abstracţiune goală, ci acţiune şi viaţă.*

Francesco de Sanctis

Stanislav Groppa s-a născut la 15 mai 1956, în satul Verejeni, raionul Ocniţa. Absolvent al Universităţii de Stat de Medicină şi Farmacie „Nicolae Testemiţanu", la vârsta de 29 de ani devine doctor în ştiinţe medicale şi prorector, la 35 - doctor habilitat şi la 39 de ani i se conferă titlul de profesor universitar. În 2007, devine membru corespondent al Academiei de Ştiinţe a Moldovei, în 2008 este ales în calitate de academician coordonator al secţiei medicale a Academiei de Ştiinţe a Moldovei şi în 2012 - membru titular al AŞM. În 2014 deţine funcţia de vicepreşedinte al AŞM, iar în 2019 - de prorector pentru activitatea de cercetare a USMF „Nicolae Testemiţanu".

Aria de preocupări ştiinţifice ale profesorului Stanislav Groppa este vastă şi este axată pe cercetările în domeniul neurologiei stărilor urgente şi pe studiul patologiei neuroereditare. Investigaţiile vizează câteva direcţii consacrate studiului factorilor ce determină apariţia bolilor cerebrovasculare, a degenerescenţelor cerebrale şi a mecanismelor patogenice de neurocitoprotecţie şi de plasticitate cerebrală, precum şi al elaborării metodelor de tratament şi de profilaxie.

O mare parte a eforturilor Academicianului Stanislav Groppa au fost concentrate asupra pregătirii şi creşterii viitoarelor generaţii de medici. În acest sens a susţinut şi a încurajat accesul la un număr impresionant de granturi de cercetare şi stagii de formare profesională, mai ales în marile centre din Europa, precum şi participarea multor tineri neurologi la manifestări ştiinţifice internaţionale.

Pe parcursul carierei a fost distins cu numeroase premii, care certifică, în mod incontestabil, dedicaţia, efortul depus în activitatea sa prodigioasă, consacrată sprijinului plenar al celor afectaţi de diverse probleme neurologice. Printre premiile şi distincţiile acordate, amintim Titlul de „Om Emerit", Ordinul „Gloria Muncii", Medalia „Nicolae Testemiţanu", Ordinul „Cuviosul Paisie Velicikovski", Medalia „Nicolae Milescu Spătarul" a AŞM.

Academicianul Stanislav Groppa este şi va rămâne un pilon reprezentativ al mediului academic naţional, graţie contribuţiei personale, deosebit de importante, la dezvoltarea învăţământului medical şi a activităţii de cercetare din Republica Moldova.

**Mulţi ani prosperi, Domnule Stanislav Groppa!**

Cu profund şi deosebit respect, consiliul de redacţie al Revistei *One Health & Risk Management*

# Academicianul Aurelian GULEA – adevărat pilon al cercetării ştiinţifice

*Vârsta devine un merit, sau chiar un admirabil merit, numai când ai foarte multe alte merite.*

Lucian Blaga

Calea vieţii academicianului Aurelian Gulea este impresionantă, fiind bogată în multiple realizări ştiinţifice redutabile. Perseverenţa, creativitatea, capacitatea enormă de a cunoaşte lucruri noi şi de a atinge scopul urmărit sunt doar câteva dintre calităţile care îi definesc personalitatea.

Din anii 1970 şi până în prezent, viaţa şi activitatea sa se asociază cu Universitatea de Stat din Moldova. Deşi, pe parcurs a avut mai multe oferte tentante, a rămas un patriot fidel al Alma Mater – Facultatea de Chimie şi Tehnologie Chimică a Universităţii de Stat din Moldova, căreia i-a dedicat cinci decenii de activitate şi realizări.

Academicianul Aurelian Gulea a demonstrat aptitudini distinse de abordare originală a diverselor probleme ştiinţifice, afirmându-se ca un cercetător de talie internaţională. Domnia Sa a excelat prin rezultatele ştiinţifice valoroase pe care le-a obţinut în domeniul chimiei anorganice şi coordinative fiind autorul a circa 850 de lucrări ştiinţifice în reviste de specialitate recunoscute în circuitul international. Rezultatele cercetărilor ştiinţifice au fost evaluate la justa lor valoare la prestigioase expoziţii şi saloane de inventică internaţionale din Belgia, Polonia, Malta, Spania, Franţa, SUA, Canada, Elveţia, Rusia, România, Coreea de Sud etc.

Distinsul acad. A. Gulea este deţinătorul numeroaselor titluri onorifice de talie mondială: Membru al Academiei de Ştiinţe din New York, SUA; Membru de Onoare al Consiliului Institutului de Relaţii Internaţionale UNESCO, Paris etc. Printre distincţiile sale cele mai valoroase se numără: Meritul Inventiv al Regatului Belgiei în grad de Comandor şi de Mare Ofiţer; Medalia de Aur pentru Servicii eminente aduse Cauzei Progresului - ICEPEC, Bruxelles; Medalia „Marie-Sklodowska Curie" în domeniul Chimiei - Varşovia; Ordinul „Gloria Muncii"; titlul onorific de „Om Emerit în Ştiinţă" – Republica Moldova etc.

Domnia Sa a inspirat aprecierea şi profunda consideraţie a comunităţii academedice naţionale şi internaţionale, colegilor, studenţilor şi doctoranzilor graţie calităţilor inerente - erudiţia înnăscută, verticalitatea, exigenţ, responsabilitate, profesionalism şi capacitate de muncă asiduă. La acest prag al vieţii îi dorim multă sănătate, pace în suflet, forţă de muncă şi realizări remarcabile.

**Mulţi ani prosperi, Domnule Academician Aurelian GULEA!**

Cu profund şi deosebit respect, consiliul de redacţie al Revistei *One Health & Risk Management*

# Profesorul Valentin GUDUMAC la 80 de primăveri

*Cum se poate defini omul, dacă nu prin faptele care îl leagă de ceilalți oameni.*

Marius Torok

Vorbind despre personalitatea profesorului Valentin Gudumac, ţinem să apreciem calităţile dumnealui deosebite, care îl plasează în rândul intelectualilor de prestanţă ai Republicii Moldova. Subliniem erudiţia, înaltul profesionalism, omenia, cumsecădenia, spiritul de iniţiativă, compatibilitatea etică şi sobrietatea de savant, prin care se impune în mediul academic şi în cel universitar.

Formarea personalităţii dlui Valentin Gudumac îşi are originea la baştină, în educaţia aleasă, oferită de dragii săi părinţi. S-a născut la 4 mai anul 1941, în comuna Parcova, raionul Edineţ într-o familie de oameni oneşti şi harnici, moştenind cele mai frumoase calităţi, fiind mereu o persoană atentă şi respectuoasă faţă de oameni.

Anii de studenţie au înscris o pagină aparte în biografia dlui Valentin Gudumac. Anume atunci a fost pusă temelia viitorului succes şi a capacităţii de afirmare, viaţa studenţească contribuind substanţial la acumularea şi diversificarea cunoştinţelor, necesare pentru a începe o carieră profesională. A activat în calitate de asistent universitar la Catedra biochimie, apoi ca lector superior, conferenţiar, profesor universitar şi şef la catedra Diagnostic de Laborator Clinic. În perioada anilor 1970-1991 a participat la mai multe stagii ştiinţifice la Institutul de Medicină „I. Secenov", dar şi la Institutul Central de Perfecţionare a Medicilor din Moscova. Iar în 1994 a susţinut teza de doctor habilitat în Medicină.

Activitatea de profesor de elită şi de cercetător notoriu i-a fost apreciată, de-a lungul anilor, printr-o serie de distincţii şi diplome, acordate de diverse instituţii ştiinţifice, fundaţii şi foruri, la nivel naţional şi internaţional: Om Emerit al Republicii Moldova, Inventator de Elită al României cl. I, ordinul „Gloria Muncii" etc., multiple medalii de aur, de argint şi de bronz la saloanele internaţionale de invenţii de la Bruxelles, Belgia („EUREKA"); Pittsburgh, SUA (INPEX); Iaşi, Bucureşti şi Cluj-Napoca, România; Geneva, Elveţia; Sevastopol, Ucraina („Novoe vremea").

În pragul trecerii în deceniul al nouălea, îi dorim profesorului Valentin Gudumac viaţă lungă, alături de familie şi de cei dragi şi apropiaţi, putere de muncă creativă, astfel încât să prospere ştiinţa din Republica Moldova, prin noi lucrări şi studii, în care să manifeste acelaşi simţ al obiectivităţii şi al moderaţiei, în căutarea adevărului ştiinţific.

**Mulţi ani prosperi, Domnule Valentin GUDUMAC!**

Cu profund şi deosebit respect, consiliul de redacţie al Revistei *One Health & Risk Management*

# Cătălina CROITORU – un exemplu al dedicației și tenacității

*Perseverența este cheia succesului.*

John Lubbock

James Matthew Barrie spunea: „Secretul fericirii nu este să faci ceea ce îți place, ci să-ți placă ceea ce trebuie să faci", stare definitorie și pentru conferențiarul universitar Cătălina Croitoru, fapt care a motivat-o să înceapă, în 2019, lucrul în echipa noastră, implicându-se cu entuziasm în lansarea și elaborarea unei noi reviste științifice - *One Health & Risk Management*.

Cătălina Croitoru s-a născut în satul Bleșteni, raionul Edineț. În 1986 a absolvit școala din satul natal și a fost înmatriculată la Școala medicală din orașul Bălți, pe care a absolvit-o în 1989 cu diplomă cu mențiune. După obținerea studiilor medicale incomplete, se angajează în calitate de asistentă medicală în secția Neurochirurgie a Spitalului Clinic Republican nr. 1, unde, pe parcursul a 8 ani, se dedică plenar activității sale, paralel făcându-și studiile la secția cu frecvență redusă a Facultății de Zootehnie a Universității Agrare de Stat din Moldova. În perioada 1997-2003, studiază la Universitatea de Stat de Medicină și Farmacie „Nicolae Testemițanu", Facultatea Medicină Preventivă. În această perioadă, pentru merite deosebite la învățătură, obține Bursa municipală (2001) și Bursa de merit SOROS de gradul III (2003). În 2005 obține diploma de masterat și diploma de licență, urmând studiile de doctorat și susținerea tezei de doctor în medicină (2012), devenind Laureată a Concursului Național „TEZA DE DOCTOR DE EXCELENȚĂ A ANULUI 2012" (gradul II) în domeniul științelor vieții.

Este autor și coautor a 157 lucrări științifice și metodico-didactice, inclusiv 1 monografie monoautor, 3 monografii colective, 6 capitole în monografii internaționale, 2 compendii, coautor a 3 manuale, 14 indicații/elaborări/recomandări metodice și a două ghiduri. Cătălina Croitoru a participat la peste 100 de manifestări științifice în țară și peste hotare. Este autor a 16 certificate de inovator și 2 – de drept de autor. A participat în 4 proiecte naționale și 7 internaționale. A obținut o medalie de bronz la expoziția de invenții.

Este membră a Societății Igieniștilor din Republica Moldova, fondatoare și membră a Asociației de Biosiguranță și Biosecuritate din Republica Moldova, precum și membră a societăților și asociațiilor din străinătate: Societatea Igieniștilor din România, Societatea de Medicina Muncii din România, Federația Internațională a Asociațiilor de Biosiguranță (IFBA), Asociația de Biosiguranță din Europa (EBSA).

Doamna conferențiar Cătălina Croitoru este un Om al cetății, care face totul cu pasiune, cu generozitate pentru cei din jur, mereu activă, implicată în diverse activități sociale, științifice și de voluntariat, desfășurate cu studenții, un om atent la nevoile tuturor.

**Mulți ani prosperi, Doamna Cătălina CROITORU!**

Cu profund și deosebit respect,
colegii consiliul de redacție al Revistei științifice *One Health & Risk Management*

## REQUIREMENTS FOR AUTHORS

**Rules of drafting**

The manuscript (written in Romanian, English, French and Russian) should be in accordance with the guidelines published in: *Uniform Requirements for Manuscripts Submitted to Biomedical Journal (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). The manuscripts should be written in font Cambria, size 11 points, spaced at 1.0, fully justified alignment, fields 2 cm on all sides. All pages must be numbered consecutively (in the right bottom corner) and continuously. Abbreviations should be explained at first occurrence in the text and should not be excessively used. The manuscripts must not exceed the number of words (without the title, affiliation, abstract and references): review articles – 4,500 words; research articles – 3,000 words; expert opinions – 2,500 words; case presentation – 1,700 words; experimental and clinical notes – 1,300 words; book reviews and presentations – 2,000 words; teaching articles – 4,000 words. The volume of tables and figures should not exceed ⅓ from the volume of the manuscript. The journal reserves the right to make any other formatting changes. Rejected manuscripts are not returned.

**All manuscripts submitted for publication should be accompanied by two abstracts: in the language of origin of the article and English.**

**Title and authors**

The title should be as short as possible (maximum – 120 signs with spaces), relevant for the manuscript content. The names of the authors should be written in full: name, surname (*e.g.:* Jon JONES). Affiliation should include: Department/Unit/Chair, University/Hospital, City, Country of each author. Beneath the affiliation, the author`s details and contact information – e-mail address (*e.g.:* corresponding author: Jon Jones, e-mail: jon.jones@gmail.com).

**The structure of the manuscript**

The manuscript should comprise the following subheadings (capitalized):

- **SUMMARY**
- **INTRODUCTION**

(will reflect the topicality and the general presentation of the problem studied, purpose and hypothesis of the study)

- **MATERIAL AND METHODS**
- **RESULTS**
- **DISCUSSIONS**
- **CONCLUSIONS**
- **CONFLICT OF INTERESTS**
- **ACKNOWLEDGEMENT** (optional)
- **ETHICAL APPROVAL** (specify the presence or absence of a positive opinion from the ethics committee: no, date, institution ad informed consent)
- **REFERENCES**

The **summary** should contain 1,600 signs with spaces:

- **Introduction**
- **Material and methods**
- **Results**
- **Conclusions**
- **Key words:** 3-5 words

The summary should not include tables, charts, and bibliographic notes; information not included in the article.

**Figures.** The text included in figures should be written in font Cambria, 10 point. Each figure should be accompanied by a heading and legend. They should be numbered with Arabic numerals and placed in parentheses (*e.g.:* fig. 1). Both the title (*e.g.* Figure 1) and legend are centred, below the figure.

**Tables.** The text included in tables should be written in font Cambria, 10 point. Each table should be accompanied by a heading. Tables should be inserted into the text and adjusted to the width of the page. The tables are numbered in Arabic numerals and mentioned in body text in parentheses (*e.g.* tab. 1). The title of the table is centred on the top of the table (*e.g.* Table 1).

**References** are numbered in the order they appear in the paper. The reference sources are cited at the end of the article by using AMA style and will include only the references cited within the text (the reference is numbered within round parentheses). The in-text citations that appear more than once are numbered similarly as in the first citation. The number of references should not exceed 50 sources. The scientific authors are responsible for the accuracy of their writings. The reference list should include only those references that have been consulted by the authors of the manuscript. The elements of the reference sources are written exactly in accordance with the requirements.

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**Reguli de tehnoredactare**
Pregătirea manuscrisului (elaborat în limbile română, engleză, franceză și rusă) va fi în conformitate cu instrucțiunile publicate în: *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). Manuscrisele trebuie să fie cu font Cambria, dimensiune 11 puncte, spațiat la interval 1,0, aliniere justificată, câmpurile 2 cm pe toate laturile. Toate paginile trebuie să fie numerotate consecutiv (în colțul de jos, în partea dreaptă) și să includă nume-rotarea continuă a paginilor. Abrevierile trebuie să fie explicate la prima apariție în text și nu trebuie utilizate excesiv. Manuscrisele nu trebuie să depășească (fără a număra titlul, afilierea, rezumatul și refe-rințele): pentru articole de sinteză/referate – 4500 de cuvinte; pentru articole de cercetare – 3000 de cuvinte; pentru opinii ale experților – 2500 de cuvinte; prezentare de caz și imagini din practica clinică/laborator – 1700 de cuvinte; note experimentale și clinice – 1300 de cuvinte; recenzii și prezentări de carte – 2000 de cuvinte; articole didactice – 4000 de cuvinte. Volumul tabelelor și figurilor nu trebuie să depășească ⅓ din volumul manuscrisului. Revista își rezervă dreptul de a face orice alte modificări de formatare. Manuscrisele respinse nu sunt returnate.

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Titlul ar trebui să fie cât mai scurt posibil (maximum - 120 de semne cu spații), elocvent pentru conținutul manuscrisului. Numele autorilor vor fi scrise deplin: prenume, nume de familie (*ex:* Ion RUSU). Afilierea va include: Secția/Departamentul/Catedra, Universitatea/Spitalul, Orașul, Țara pentru fiecare autor. Se vor menționa obligatoriu, mai jos, datele autorului corespondent și informațiile de contact – adresa de e-mail (*ex:* autor corespondent: Ion Rusu, e-mail: ion.rusu@gmail.com).

**Structura manuscrisului**
Manuscrisul va cuprinde următoarele subtitluri (scrise cu majuscule):

- **REZUMAT** (vezi cerințele mai jos)
- **INTRODUCERE** (se va reflecta actualitatea și prezentarea generală a problemei studiate, scopul și ipoteza studiului)
- **MATERIAL ȘI METODE**
- **REZULTATE**
- **DISCUȚII**
- **CONCLUZII**
- **CONFLICT DE INTERESE**
- **MULȚUMIRI ȘI FINANȚARE** (optional)
- **APROBAREA ETICĂ** (se va specifica prezența sau lipsa avizului pozitiv de la comitetul de etică: nr, data, instituția și acordul informat)
- **REFERINȚE**

**Rezumatul** va conține până la 1600 de semne cu spații și va cuprinde:

- **Introducere**
- **Material și metode**
- **Rezultate**
- **Concluzii**
- **Cuvinte cheie**: 3-5 cuvinte

În rezumat nu vor fi incluse tabele, grafice și note bibliografice; informații care nu sunt prezentate în studiu.

**Figuri.** Textul inclus în figuri trebuie să fie scris cu font Cambria, dimensiune 10 puncte. Fiecare figură trebuie să fie însoțită de titlu și legendă. Ele vor fi numerotate cu cifre arabe și vor fi menționate în text în paranteze (*ex:* fig. 1). Titlul (*ex:* Figura 1) și legenda figurii trebuie să fie scrisă centrat, sub figură.

**Tabele**. Textul inclus în tabele trebuie să fie scris cu font Cambria, dimensiune 10 puncte. Fiecare tabel trebuie să fie însoțită de titlu. Tabelele vor fi inserate în text, fără a depăși lățimea unei pagini. Ele vor fi numerotate cu cifre arabe și vor fi menționate în text în paranteze (*ex:* tab. 1). Titlul tabelului va fi poziționat deasupra tabelului centrat (*ex:* Tabelul 1).

**Referințele** trebuie să fie numerotate în ordinea apariției în text. Citarea sursei de referință va fi conform stilului *AMA*, plasată la sfârșitul articolului și va include doar referințele citate în text (menționând numărul de referință în paranteză rotundă). Dacă aceeași referință este citată de mai multe ori, ea va fi trecută în text cu același număr ca la prima citare. Numărul total de referințe nu va depăși 50 de surse. Acuratețea datelor ține de responsabilitatea autorului.

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**Tous les manuscrits à publier doivent être accompagnés par deux résumés: dans la langue originale et en anglais.**

**Titre et auteurs**

Le titre doit être le plus court que possible (maximum – 120 signes avec espaces), éloquent pour le contenu du manuscrit. Les noms des auteurs seront écrits complets: prénom, nom (*ex:* Albert LEBRUN). Quant à l'affiliation, on devra indiquer: Section/ Département/Chaire, Université/Hôpital, Ville, Pays – pour chaque auteur. Les données de l'auteur correspondant et les coordonnées – adresse e-mail (*ex:* auteur correspondant: Albert Lebrun, e-mail: albert.le-brun@gmail.com*)* seront obligatoires ci-dessous.

**Structure du manuscrit**

Le manuscrit comprendra les sous-titres suivants (avec lettres majuscules):

- **RÉSUMÉ** (voir les exigences ci-dessous)
- **INTRODUCTION** (reflétera l'actualité et la présentation générale du problème étudié, le but et l'hypothèse de l'étude)
- **METHODES**
- **RESULTATS**
- **DISCUSSIONS**
- **CONCLUSIONS**
- **CONFLIT D'INTERETS**
- **REMERCIEMENTS ET FINANCEMENT**
- **APPROBATION ÉTHIQUE** (préciser la présence ou l'absence d'avis favorable du comité d'éthique: no, date, institution et consentement éclairé)
- **REFERENCES**

Le **résumé** contiendra 1600 signes avec espaces:

- **Introduction**
- **Méthodes**
- **Résultats**
- **Conclusions**
- **Mots clés**: 3-5mots.

Le résumé ne comprendra pas des tableaux, graphiques et des notes bibliographiques; des informations non présentées dans l'étude.

**Figures.** Le texte inclus dans les figures doit être écrit avec police Cambria, taille 10 points. Chaque figure doit être accompagné par un titre et une légende. Ceux-ci seront numérotés avec des chiffres arabes et mentionnés dans le texte entre parenthèses (*ex:* fig. 1). Le titre (*ex:* Figure 1) et la légende de la figure doivent être centrés, au-dessous de la figure.

**Tableaux.** Le texte inclus dans les tableaux doit être écrit avec police Cambria, taille 10 points. Chaque tableau doit être accompagné par un titre. Les tableaux seront numérotés avec des chiffres arabes, mentionnés dans le texte entre parenthèses (*ex:* tab. 1), et seront insérés dans le texte, sans dépasser la largeur d'une page. Le titre du tableau sera placé au-dessus du tableau, centré (*ex:* Tableau 1).

Les **références** doivent être numérotées dans l'ordre où elles apparaissent dans le texte. La citation de la source de référence sera de style *AMA*, placée à la fin de l'article et n'inclura que des références citées dans le texte (mentionnant le numéro de référence entre parenthèses rondes). Si la même référence est citée plusieurs fois, elle sera transmise dans le texte avec le même numéro que celui de la première citation. Le nombre total de références ne dépassera pas 50 sources. La responsabilité pour l'exactitude des données est à la charge de l'auteur. Il faut indiquer dans le manuscrit seulement les références vraiment consultées par les auteurs. Les composants des sources de référence doivent être rédigés strictement selon les exigences.

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## ТРЕБОВАНИЯ ДЛЯ АВТОРОВ

**Правила составления**

Подготовка рукописи (разработанной на румынском, английском, французском и русском языках) будет осуществляться в соответствии с инструкциями, опубликованными в: *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). Авторы должны использовать шрифт Cambria, размер 11 точек, с интервалом 1,0, выравнивание по ширине, поля 2 см со всех сторон. Все страницы должны быть пронумерованы последовательно (в правом нижнем углу) и включать непрерывную нумерацию страниц. Сокращения должны быть объяснены при первом появлении в тексте и не должны использоваться чрезмерно. Объем рукописей не должен превышать (без названия, принадлежности, резюме и литературы): для обзорных статей/рефератов – 4500 слов; для научных статей – 3000 слов; для экспертных заключений – 2500 слов; для презентации случаев из клинической/лабораторной практики – 1700 слов; для экспериментальных и клинических заметок – 1300 слов; для рецензий и презентаций книг – 2000 слов; для учебных статей – 4000 слов. Объем таблиц и рисунков не должен превышать ⅓ от объема рукописи. Журнал оставляет за собой право вносить любые другие изменения форматирования. Отклоненные рукописи не возвращаются.

**Все рукописи, представленные для публикации, должны сопровождаться двумя резюме: на языке оригинала статьи и на английском языке.**

**Название и авторы**

Название должно быть как можно короче (максимум – 120 знаков с пробелами), но достаточно информативным для содержания рукописи. Фамилии авторов будут написаны полностью: имя, фамилия (*например:* Иван ИВАНОВ). Принадлежность будет включать: Отделение/ Департамент/Кафедра, Университет /Больница, Город, Страна для каждого автора. Данные соответствующего автора и контактная информация – адрес электронной почты (*например:* контактная информация: Иван Иванов. e-mail: ivan.ivanov@gmail.com) будут обязательно ниже.

**Структура Рукописи**

Рукопись будет включать в себя следующие подзаголовки (они должны быть заглавными):

- **РЕЗЮМЕ** (см. требования ниже)
- **ВВЕДЕНИЕ** (будет отражать актуальность и общее представление изучаемой проблемы, цель и гипотезу исследования)
- **МАТЕРИАЛЫ И МЕТОДЫ**
- **РЕЗУЛЬТАТЫ**
- **ДИСКУССИИ**
- **ВЫВОДЫ**
- **КОНФЛИКТ ИНТЕРЕСОВ**
- **БЛАГОДАРНОСТИ И ФИНАНСИРОВАНИЕ**
- **ЭТИЧЕСКОЕ ОДОБРЕНИЕ** (указать наличие или отсутствие одобрения со стороны комитета по этике: №, дата, учреждение и информированное согласие)
- **ЛИТЕРАТУРА**

**Резюме** должно содержать 1600 знаков с пробелами и будет включать в себя следующие подзаголовки:

- **Введение**
- **Материалы и методы**
- **Результаты**
- **Выводы**
- **Ключевые слова:** 3-5 слов

Резюме не должно включать таблицы, диаграммы и библиографические заметки, информацию, не представленную в исследовании.

**Рисунки** (графики, диаграммы). Текст, включенный в рисунки, должен быть написан в Cambria, размер 10 пунктов. Каждый рисунок должен сопровождаться заголовком и описанием. Название (*например:* Рисунок 1) и описание рисунка должны быть вписаны по центру, в низу рисунка. Они должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (*например:* рис. 1).

**Таблицы.** Текст, включенный в таблицы, должен быть написан в Cambria, размер 10 пунктов. Каждая таблица должна сопровождаться заголовком. Они должны вставляться в текст, не превышая ширину страницы. Должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (*например:* таб. 1). Название таблицы должно располагаться над таблицей в центре (*например:* Таблица 1).

**Литература.** Источники должны быть пронумерованы в порядке их появления в тексте. Ссылки на источники должны быть в стиле *AMA*, помещены в конце статьи и включать только источники, цитируемые в тексте (упоминание номера источника в круглых скобках). Если один и тот же источник цитируется несколько раз, он будет передан в тексте с тем же номером, что и первый раз. Общее количество источников не должно превышать 50. Ответственность за точ-ность данных лежит на авторе. Будут цитиро-ваться только те источники, с которыми ознакомились авторы рукописи. Компоненты справочных источников должны быть написаны строго в соответствии с требованиями.

Дополнительная информация на :http://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/editing_guidelines

## Conceptul *One Health*

**Sănătatea umană**

OMS a definit în 1946 sănătatea ca fiind „o stare pe deplin favorabilă atât fizic, mintal cât şi social, şi nu doar absenţa bolilor sau a infirmităţilor", cu o completare ulterioară „capacitatea de a duce o viaţă productivă social şi economic".

**Sănătatea animală**

OIE defineşte bunăstărea animalelor în 2008: un animal este în bună stare dacă este sănătos, se bucură de confort, este bine hrănit, se află în siguranţă, poate să îşi manifeste comportamentul înnăscut (natural) şi nu suferă din cauza unor stări neplăcute, precum durere, frică şi stres.

**Sănătatea plantelor și mediului**

Sănătatea mediului se referă la acele aspecte ale sănătăţii umane ce includ calitatea vieţii determinată de factorii fizici, biologici, socio economici şi psiho sociali din mediul ambiant. Interrelaţiile omului cu mediul preocupă medicina, atunci când un sistem ecologic este în stare de echilibru, prevalează starea de sănătate a populaţiei.

La nivel global conceptul *One Health* este o strategie mondială de extindere a colaborărilor interdisciplinare și a comunicărilor în toate aspectele legate de îngrijirea sănătății oamenilor, animalelor domestice sau a faunei sălbatice, care nu mai poate fi abordată separat ci doar în comun.

*One Health* se referă nu numai la preocupările legate de bolile ce apar la oameni și animale, ci și la aspecte legate de stilul de viață, dietă, exercițiu, impactul diferitelor tipuri de relații om-animal și expuneri de mediu care pot afecta ambele categorii populaționale. Pentru a se atinge efectele scontate este nevoie și de o educație a populației care să conștientizeze factorii de risc și beneficiile prevenției, dar și de comunicare și înțelegere între pacienți și furnizorii de servicii de sănătate.

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