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ISSN 2587-3458
e-ISSN 2587-3466

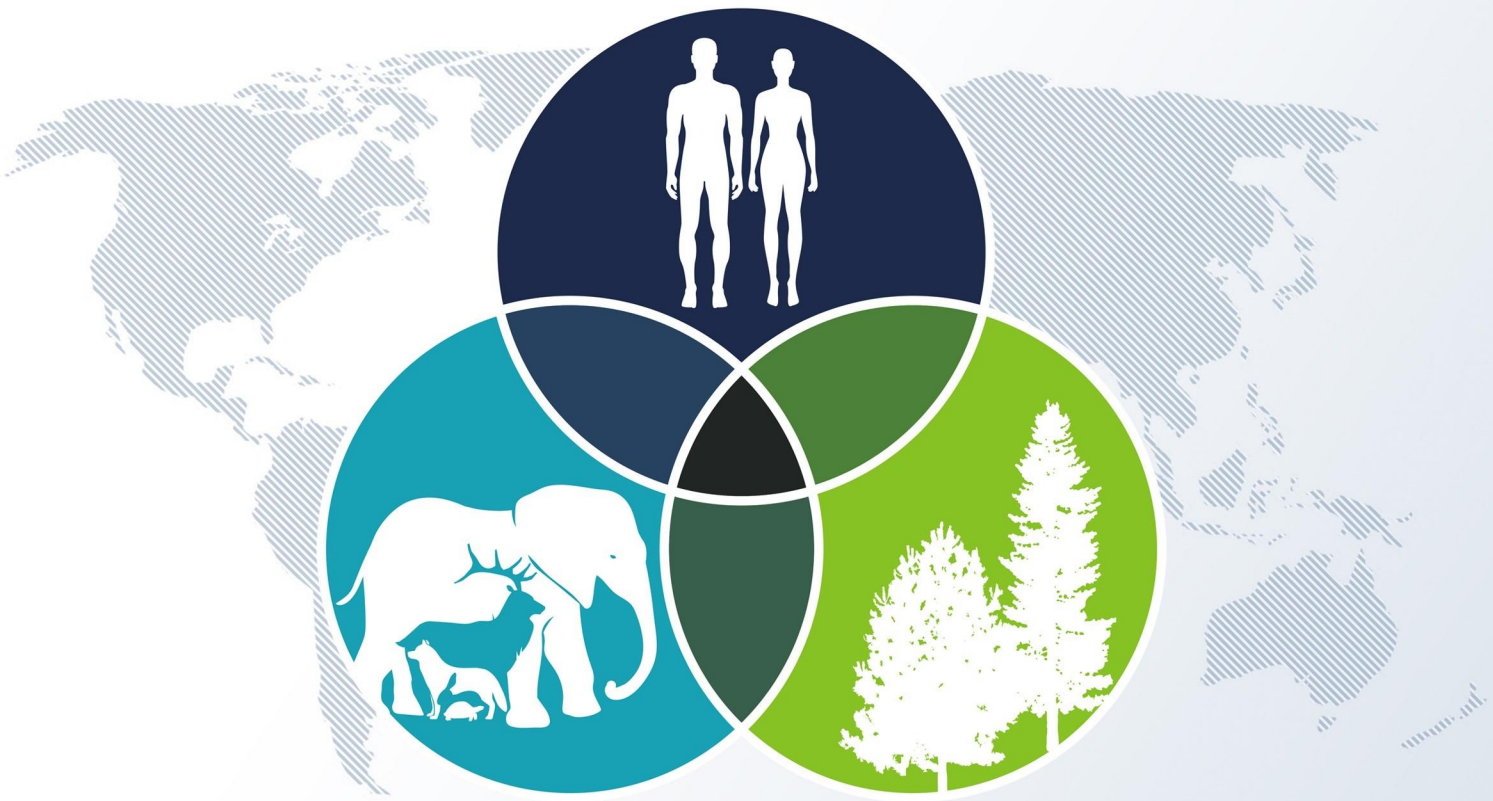


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Category A

OH_&RM ONE HEALTH & RISK MANAGEMENT

THE SCIENTIFIC JOURNAL OF THE
MOLDAVIAN BIOSAFETY AND BIOSECURITY ASSOCIATION



VOLUME 5, ISSUI 2/APRIL 2024



The Moldovan Association for Biosafety and Biosecurity (MDBBA) is a scientific and practical, instructive and educational, non-governmental, apolitical and non-profit professional organization, created in 2017.

The main objective of the association is the development of good practices and culture in the field of biosafety and biosecurity and the promotion of knowledge within professional and research-innovation groups.

Biosafety – includes security principles, technologies and rules to be followed to prevent unintended exposure to pathogens and toxins or their accidental release/leakage.

"Protection of personnel, population from unintended exposure to pathogens/biohazardous material".

Biosecurity - includes a wide spectrum of measures (biosecurity policies, regulatory regime, scientific and technical measures) applied in an organized framework, necessary to minimize risks (prevention of actions, terrorist attacks by the intentional release of pathogens or toxins as well as loss, their theft or misuse).

"Protection and prevention of theft, intentional misuse of pathologies/biohazardous material".

Risk management – is a decision-making process in which the results of risk assessment (the process of estimating workplace hazards) are integrated with economic, technical, social and political principles to generate strategies for risk reduction.

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Quarterly edition

Languages of publication: English, French

Founder: Asociația de Biosiguranță și Biosecuritate din Republica Moldova

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ISSN 2587-3458 (Print)

e-ISSN 2587-3466 (Online)

Edited by: Typography "Print-Caro", Edition: 300 ex.

Registered at the Ministry of Justice with no. 476676, 05th of July, 2017



One Health & Risk Management journal promotor of interdisciplinary collaboration and innovative research



Paata IMNADZE,
Scientific Director at National Center for Disease Control
and Public Health
Professor, *Ivane Javakhishvili* Tbilisi State University
Tbilisi, Georgia

As the Deputy Director General in Science of the National Center for Disease Control and Public Health (NCDC) of Georgia, it is my pleasure to acknowledge the launch of the "One Health & Risk Management" journal.

Established within the Moldavian Biosafety and Biosecurity Association (MDBBA) in 2019, this journal is a significant step forward in promoting interdisciplinary collaboration and innovative research. Its policy aligns with the latest international concepts advocated by the World Health Organization (WHO), the World Organization for Animal Health (WOAH), and the Food and Agriculture Organization of the United Nations (FAO).

In today's complex landscape of global challenges, such as emerging infectious diseases and environmental concerns, there is a growing need for coordinated responses. Thus, the One Health Approach, emphasizing cooperation and transdisciplinary solutions, is more relevant than ever.

This journal provides a space for scholars, practitioners, and policymakers to exchange ideas and contribute to the advancement of knowledge. I am confident that "One Health & Risk Management" will play a crucial role in fostering collaboration between international researchers, and bringing diverse perspectives to the forefront.

On behalf of the NCDC, I am excited about the launch of this important platform and would like to congratulate the Moldavian colleagues on such an achievement.



SYNTHESIS ARTICLE – ARTICLES DE SYNTHÈSE

**MANAGEMENT OF SEXUAL AND REPRODUCTIVE PROBLEMS IN BREAST CANCER SURVIVORS**Iurii ARIAN^{1,2}, Dumitrita BIVOL¹, Daniela MACHIDON¹, Ion DUMBRAVEANU^{1,2}¹Laboratory of Andrology, Functional Urology, and Sexual Medicine, Chisinau, Republic of Moldova²Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

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DOI: 10.38045/ohrm.2024.2.01

CZU: 618.17-008:618.19-006.04

Keywords: breast cancer survivors, reproductive health, sexual health, sexual disorders, sexual therapy.

Introduction. Breast cancer is the most common form of cancer in women and is a threat to female sexuality, considering the mammary glands' significance in sexuality, attractiveness, and sexual satisfaction. Female sexual disorders, which involve disturbances in sexual desire, attraction, arousal, orgasm, and genito-pelvic pain, are common among middle-aged breast cancer survivors (BCS).

Material and methods. A systematic and structured search of the specialty literature was conducted using PubMed, SCOPUS, EMBASE, and Web of Science databases with keywords such as breast cancer survivors, "sexual disorders", and "reproductive health" to find studies relevant to the targeted hypothesis. Works were selected according to the following criteria: full-text articles, publications in English, covering the period 2000-2023.

Results. Patients with sexual dysfunctions may benefit from a variety of treatment approaches, such as pharmaceutical, physical, and psychological ones. Studies show that local strategies, such as vaginal estrogens and DHEA, vaginal moisturizers, aqueous lidocaine, vaginal dilators, intravaginal laser, and radiofrequency, as well as psychotherapeutic strategies, such as cognitive-behavioral therapy and couple's therapy based on sexual health, improve sexual function.

Conclusions. Identifying and managing sexual dysfunction requires a higher level of awareness, interpersonal interaction, and education among physicians and other health care practitioners. To provide appropriate care and prevent the condition from getting more severe, a systematic assessment of sexual function in BCS patients may be helpful in early diagnosis of sexual disorders.

Cuvinte-cheie: supraviețuitoare, cancer mamar, sănătate reproductivă, sănătate sexuală, tulburări sexuale.

MANAGEMENTUL PROBLEMELOR SEXUALE ȘI REPRODUCTIVE LA SUPRAVIEȚUITOARELE CANCERULUI MAMAR

Introducere. Cancerul mamar este cea mai răspândită formă de cancer la femei și reprezintă o amenințare pentru sexualitatea feminină, deoarece marea majoritate a acestora consideră glandele mamare importante în sexualitate, atractivitate și satisfacție sexuală. Tulburările sexuale feminine, care implică perturbări ale dorinței sexuale, atracției, stimulării, orgasmului și durerea genito-pelvină, sunt frecvente la supraviețuitoarele cancerului mamar de vârstă mijlocie.

Material și metode. S-a efectuat o cercetare sistemică și structurată a literaturii de specialitate, utilizând bazele de date PubMed, SCOPUS, EMBASE și Web of Science cu cuvintele-cheie „supraviețuitori ai cancerului de sân”, „tulburări sexuale” și „sănătate reproductivă” pentru a găsi studii relevante ipotezei vizate. Au fost selectate lucrări conform următoarelor criterii: articole integrale, publicații în limba engleză, perioada 2000-2023.

Rezultate. Pacientele cu disfuncții sexuale pot beneficia de o varietate de abordări terapeutice, cum ar fi cele farmaceutice, fizice și psihologice. Studiile arată că strategiile locale, precum estrogenii vaginali și DHEA, cremele hidratante vaginale, lidocaina apoasă, dilatatorele vaginale, laserul intravaginal și radiofrecvența, precum și strategiile psihoterapeutice, cum ar fi terapia cognitiv-comportamentală și terapia de cuplu bazată pe sănătatea sexuală, îmbunătățesc funcția sexuală.

Concluzii. Identificarea și gestionarea disfuncției sexuale necesită un nivel mai înalt de conștiință, interacțiune interpersonală și educație în rândul medicilor și altor practicieni din domeniul sănătății. Pentru a oferi îngrijiri adecvate și pentru a preveni agravarea afecțiunii, o evaluare sistematică a funcției sexuale pentru supraviețuitoarele cancerului mamar poate fi utilă în diagnosticarea precoce a tulburărilor sexuale.

INTRODUCTION

Out of all the cancers that affect women, breast cancer has the highest incidence rate. Based on statistical data, breast cancer has become a leading cause of death among female patients. Fortunately, more women are surviving their diagnoses, likely due to earlier detection and improved treatments. Reports indicate that women with breast cancer have a 5-year survival rate of 76–92% following systemic treatment (1). Consequently, more than three million women have been diagnosed with a history of breast cancer, representing the largest group of cancer survivors in the United States (41% of the total cancer survivor population) (2), with over 2.3 million female cancer survivors under the age of 60 (3). As such, managing the long-term health of survivors has become crucial. Sexual health is one aspect that often suffers after surviving breast cancer, due to anxiety, stress, or changes in body image following breast cancer therapy. Female sexual dysfunction can broadly be classified into three categories: genitopelvic pain or penetration difficulties, orgasmic disorder, and sexual desire or arousal disorder. According to the Diagnostic and Statistical Manual 5th Edition (DSM-5) (4), a woman is diagnosed with a sexual disorder if she experiences recurrent symptoms that last at least six months and cause significant distress. A study involving 83 women who had survived breast cancer for three years used the Female Sexual Function Index (FSFI) and the Female Sexual Distress Scale-Revised (FSDS-R) for assessment. According to the Malay Version of the Female Sexual Function Index-6, 73.4% (n=69) of the 94 female patients (aged 16–65 years) who were married, had a partner, had breast cancer, and had undergone breast surgery reported experiencing sexual dysfunction (5). The frequency of sexual issues remained significant 5 and 10 years after a cancer diagnosis. In another study, Ganz and colleagues found that nearly 760 women who had previously participated in a poll returned for a follow-up survey, with an average interval between survey points of 6.3 years. While physical and emotional functioning had returned to normal, sexual function remained impaired, evidenced by a decrease in sexual engagement with a partner (from 65% to 55%), persistent vaginal dryness, and urinary incontinence (6).

On the other hand, survivorship treatment often

fails to adequately address sexual health issues, and few BCS receive sexual function education and information from cancer specialists (7). Moreover, individuals may feel hesitant or embarrassed to discuss sexual issues with healthcare providers (8). To effectively understand the problems and challenges faced by women and offer solutions, midwives and other healthcare professionals should assess, educate, and advocate for women's sexual health within an environment of trust, intimacy, and attentive communication (9, 10). To improve sexual health after breast cancer, various pharmaceutical and behavioral strategies have been explored. Notably, enhancements in sexual function have been observed in women who participate in cognitive-behavioral therapy (CBT), along with pharmaceutical and non-pharmaceutical remedies.

Psychoeducational programs, improved communication, counseling for women (both individually and in couples), group therapy, and couple therapy have all been shown to enhance well-being and promote a positive body image (9). Community midwives must have knowledge of and training in addressing the experiences of women diagnosed with breast cancer. Women are typically more inclined to express their concerns in environments where they feel relaxed.

In this review, *we aim to analyze* existing studies that explore the management of difficulties among individuals who have survived breast cancer. Our goal is to compile information that enhances understanding and awareness of health for both breast cancer survivors and their healthcare professionals.

MATERIAL AND METHODS

Full-text papers, English-language literature, narrative, and systematic review articles were the criteria used to specifically select bibliographic sources for conducting a systematic and structured search. Regarding publication timeframes, papers published between 2000 and 2023 were included. To find studies relevant to the targeted issue, we utilized databases such as PubMed, SCOPUS, EMBASE, and Web of Science.

After analyzing the bibliographic resources using the following keywords: "sexual disorders," "sexual therapy," "breast cancer survivors," "repro-

ductive health," and "sexual health," we located 160 items. Articles evaluating non-gynecological tumors and those examining men's sexuality were excluded. Ultimately, we selected 30 articles that we deemed appropriate for the proposed research topic.

To elucidate particular and complex topics, additional sources of knowledge were consulted in accordance with the established criteria. Excluded from the bibliography were similar studies, papers that were not fully accessible or aligned with the study's objectives, comments, letters, case reports, case series, and articles lacking sufficient data.

RESULTS

Impact of treatment on sexual health

As survival rates increase, addressing the long-term wellness issues of survivors becomes crucial. The multifactorial etiology of sexual dysfunctions in breast cancer survivors encompasses the deleterious effects of radiation, chemotherapy, endocrine therapy, and surgery on a woman's sexual health and function (fig. 1).

Surgery

Mastectomies or breast-conserving surgeries are currently the most prevalent surgical procedures for breast cancer. Studies have shown that a complete mastectomy impacts sexual function more than breast-conserving surgery (11). Women treated with mastectomy reported greater life disturbance and markedly worse ratings in the areas of body image, role, and sexuality. Even more concerning is that sexual functioning does not improve over time. The psychological effects of breast loss and surgical scars can be severe. Women's breasts are significant secondary sexual traits that influence their self-esteem, body image, sexual enjoyment, and stimulation (12).

Chemotherapy

Chemotherapy treatment can lead to ovarian failure and early menopause, resulting in symptoms such as decreased sexual desire, vaginal dryness, difficulty engaging in sexual activities, and other unpleasant side effects. Ultimately, these issues can reduce the frequency and intensity of orgasms (13). Furthermore, taxanes and anthracyclines may adversely affect general bodily functions, including libido, arousal, and perceptions of attractiveness. Intense fatigue, hair loss, gastroin-

testinal distress, and myelosuppression are common physical side effects of chemotherapy.

Radiation therapy (RT)

Reduced sexual function has been associated with locoregional complications caused by radiation therapy (RT), such as lymphedema, discomfort, and reduced flexibility in the arms and shoulders, as well as chronic breast pain (14 - 17). Additionally, radiation treatment can permanently alter the breast. Pores may become enlarged and more visible, and skin may darken slightly. The skin might become firmer and thicker than before treatment and may exhibit increased or decreased sensitivity. Some side effects may persist long after treatment. A recent study of over 600 patients who underwent implant breast reconstruction found that 219 had previously received RT. Those who underwent RT reported lower health-related quality of life and lower satisfaction compared to those who did not (15). Patients who received postoperative RT showed significant declines across all dimensions (satisfaction with breasts and outcomes, emotional well-being, sexual wellness, and physical wellness).

Endocrine therapy

Hormonal treatment is primarily used to limit tumor progression in individuals with hormone receptor-positive breast cancer. Endocrine therapy inhibits the growth of estrogen-dependent breast cancer cells by reducing estrogen levels in the body or blocking the interaction between estrogen and hormone receptors. This involves taking an aromatase inhibitor (AI) for five years or tamoxifen for five years, followed by an additional five years of either tamoxifen or an AI (18).

These medications have been linked to the development or exacerbation of menopausal symptoms. According to a survey by Morales et al. involving more than 180 women, both agents significantly increased the frequency and intensity of vaginal dryness, night sweats, and hot flashes. The use of aromatase inhibitors often results in vaginal dryness. Unlike hot flashes, vaginal symptoms generally worsen over time (19). Tamoxifen significantly reduced sexual attraction, while aromatase inhibitors (AIs) dramatically increased the risk of dyspareunia, indicating that both agents can negatively impact female sexual health, albeit in different ways. Furthermore, they found that toxicities were substantially more common among younger women.

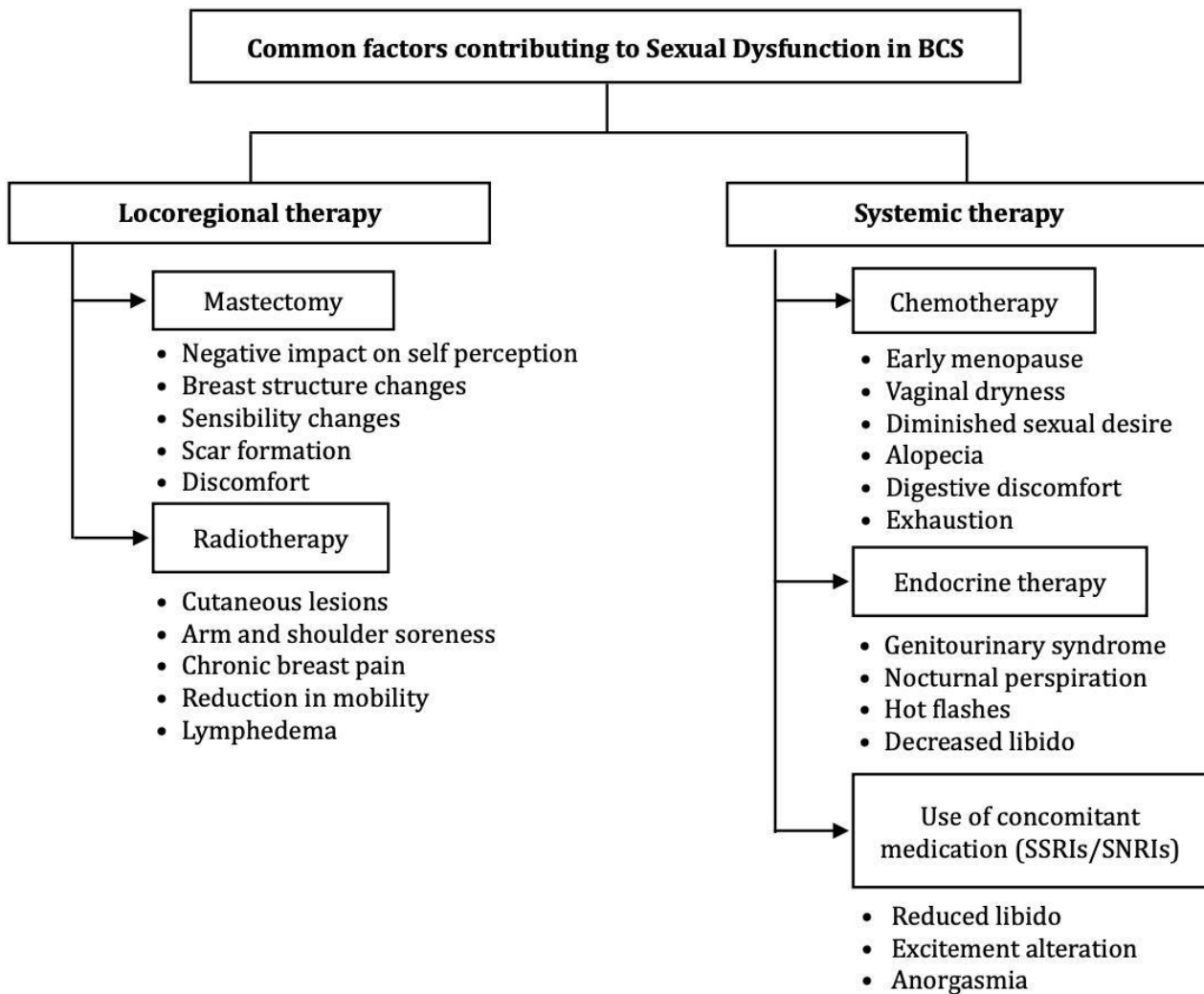


Figure 1. Common factors contributing to sexual dysfunction in BCS.

Management of sexual dysfunction

Patients should be informed about the potential effects of breast cancer treatments on their sexuality and any side effects. This topic should be discussed openly and candidly during visits, and patients should be offered solutions or further information if they need assistance. In some cases, having more treatment options could reduce the likelihood of female sexual dysfunctions (tab. 1).

Vaginal products interventions

The hemodynamic processes involved in the sexual response are significantly influenced by estrogens. Estrogen deficiency not only leads to the shrinkage of the labia majora and minora but also results in decreased clitoral tumescence and insufficient vaginal lubrication. Consequently, up to 50% of affected women experience dyspareunia, and a decline in clitoral sensitivity affects up to 20% of these women. Clinically significant vaginal

atrophy can occur in postmenopausal patients when blood estradiol levels fall below 73 pmol/L. The most effective treatment for vaginal atrophy is the direct application of estrogen medicine (20), a commonly used routine therapeutic option. Numerous meta-analyses have shown that topical estrogen therapy can improve symptoms and signs of vaginal atrophy regardless of the mode of administration (21, 22). Estriol, a low-potency, short-acting estrogen, is irreversibly converted to either estradiol or estrone. It is widely recognized that estriol is safe for endometrial hyperplasia and effective for treating vaginal atrophy (23). Estrogen replacement therapy can be administered through oral pills, transdermal patches, vaginal rings, and transdermal gel. Oral estrogen is converted to estrone in the intestinal environment, which is then transformed to estradiol in the liver, reducing the estrogen level in the serum by about 30%. The serum level of estradi-

ol with the transdermal method is comparable to that of the oral route, as it bypasses the initial liver pass. Research indicates that the use of the transdermal form does not correlate with an increase in breast mammography density (24). Estriol can specifically increase blood flow and the regeneration of the vaginal epithelium, and while it may be less stimulating to the breast and endometrium, this medication is also used for symptoms of genital degeneration. An ultra-low dose of 0.005% estriol vaginal gel effectively treated the symptoms and signs of vulvovaginal atrophy. These outcomes, along with minimal fluctuations in hormone levels during treatment, support the use of an ultra-low dose of 0.005% estriol vaginal gel as a potential treatment for vulvovaginal atrophy in women receiving NSAIDs for breast cancer who require vaginal estrogen therapy. As a final metabolite with a low binding affinity for the estrogen receptor, estriol cannot be converted back into other estrogenic precursors. Additionally, estriol has a preference for binding to β (urogenital) estrogen receptors over α (breast) estrogen receptors, making it a hormone with lower potency and estrogenic potential compared to estradiol (25).

A controlled clinical study evaluated dehydroepiandrosterone (DHEA) as an adjunct hormonal therapy. It has been found that vaginal DHEA is a safe and effective treatment for menopausal women experiencing symptoms of vaginal atrophy. According to several preclinical studies, DHEA is believed to act on the nerves within the vaginal wall fibers, enhancing their sensitivity during sexual activity. This mechanism also explains why intravaginal application of DHEA does not lead to systemic changes in the levels of androgenic hormones or DHEA metabolites, such as testosterone and estradiol. Many of the potential side effects of DHEA can be avoided since it does not cause significant systemic hormonal changes. Furthermore, the local therapeutic effects against vaginal atrophy can be achieved through intravaginal DHEA administration without significant increases in serum estrogen levels, thus minimizing the risk of breast cancer.

Vaginal moisturiser

Vaginal moisturizers are particularly beneficial for individuals when applied vaginally. In a study involving 86 participants, a 12-week therapy using a pH-balanced gel for the vagina was compa-

red to a placebo. Unlike the placebo, the vaginal gel significantly lowered vaginal pH and improved vaginal maturation. Additionally, the treatment reduced dyspareunia and vaginal dryness (26). A similar study on the efficacy of a vaginal moisturizer based on polycarbophils found that applying a polycarbophil-based vaginal moisturizer at least twice a week enhances vaginal dryness, pH, dyspareunia, frequency, and sexual satisfaction, according to clinical trial results.

Unlike lubricants, vaginal moisturizers such as Replens are absorbed by the skin rather than remaining on the surface. The primary purpose of moisturizers is to attract and retain moisture in the skin's tissue. Like lubricants, vaginal moisturizing products have dual functions: they can either increase or decrease desire. Initially, the use of a vaginal moisturizer can alleviate discomfort and itching in the short term. Secondly, regular application of a vaginal moisturizer maintains the softness and flexibility of vaginal tissue, reducing its susceptibility to damage such as tearing, thus helping to restore its integrity. A vaginal moisturizer can also alleviate symptoms of vaginal atrophy and the discomfort caused by vaginal dryness.

Aqueous lidocaine

For individuals experiencing discomfort during penetrative sexual activities, particularly if the pain is localized to the vulvar vestibule, aqueous lidocaine is one potential treatment option. In a randomized experiment involving 46 breast cancer survivors, applying lidocaine topically to the vestibule three minutes before planned penetrative activities, followed by the application of silicone lubricant, led to a reduction in dyspareunia. After open-label use, 90% of the participants reported a return to comfortable penetration (27).

Vaginal dilators

Vaginal dilators can be instrumental in overcoming pelvic floor muscle reflexes, aiding in the increase of vaginal flexibility. Medical professionals often recommend the use of vaginal dilators for this purpose. It is advised that women begin with the smallest, most comfortable size and gradually progress to larger sizes until they reach a level of satisfaction. Dilators function by gently stretching and widening vaginal tissue over time. This process enhances flexibility and can significantly reduce discomfort experienced during sexual activity.

Intravaginal laser and radiofrequency

To enhance the long-term wellness of women with breast cancer, safe and effective non-hormonal treatments are essential. Recently, non-pharmacological therapy alternatives for managing GSM have been suggested, including the use of intravaginal energy-based techniques such as radiofrequency and laser. The short-term data available indicate that both erbium lasers and CO₂ lasers are successful in treating the most prevalent symptoms of GSM. Their primary mechanism of action involves heat effects, which cause collagen fibers in the vaginal epithelial tissue to change shape.

A comprehensive analysis of trials compared the effectiveness of fractional CO₂ laser treatment with standard estrogen therapies such as conjugated estrogens, estriol, and promestriene. The results showed that both treatments were effective in alleviating signs and symptoms associated with genitourinary syndrome of menopause (GSM), as measured by the VAS, VHI, VMI, and FSFI scores (28).

In a randomized trial conducted by Gold et al., the efficacy of vaginal erbium laser treatment versus

hyaluronic acid in women experiencing urogenital atrophy after breast cancer surgery (BCS) was examined. The study included 43 participants who reported symptoms such as dryness, dyspareunia, urgency/dysuria, or recurrent urinary tract infections. Participants were randomly assigned to receive either two sessions of vaginal erbium laser treatment spaced 30 days apart or daily hyaluronic acid vaginal suppositories for 10 days, followed by three times a week for a duration of 12 weeks. After a follow-up period of 12 weeks, both treatment groups showed improvements in the Vaginal Health Index (VHI) without any significant difference in efficacy between the erbium laser and hyaluronic acid treatments (29).

Cognitive behavioral therapy (CBT)

Enhancing sexual well-being in BCS has been achieved through various psychotherapy and educational programs, especially those targeting sexual dysfunction. Sexual counselors, family and marriage psychologists, sex psychologists, and other mental health professionals offer educational courses in cognitive behavioral therapy (CBT), psychological therapies, and other supportive services.

Table 1. The primary therapy for Sexual Dysfunction in BCS.

Local Strategies		Psychotherapeutic Strategies	
Type of treatment	Outcome	Type of treatment	Outcome
Vaginal Estrogen	Better vaginal histology and symptom relief	Cognitive behavioral therapy (CBT)	Rise in sexual activity, remission in urine manifestations
Vaginal DHEA	Increasing sensitivity during sexual intercourse and enhancing sexual pleasure	Couple therapy based on sexual health	Enhance sexual wellness
Vaginal moisturizers	Improvement of vaginal pH, reduction of dyspareunia, vaginal dryness and improvement of sexual pleasure and frequency		
Aqueous lidocaine	Decreased dyspareunia and improvement of pleasant penetration		
Vaginal dilators	Lower discomfort during sexual intercourse		
Intravaginal laser and radiofrequency	Improvement of Genitourinary syndrome, less burning sensations and itching		

In one study, 422 individuals were randomly selected to participate in either cognitive-behavioral therapy (CBT) alone or CBT combined with physical activity. CBT included six weekly group

sessions focused on managing hot flashes and night sweats, as well as addressing issues related to appearance, sexuality, and mental health, alongside relaxation techniques. The physical ac-

tivity component aimed for 2.5 to 3 hours of weekly exercise over 12 weeks. Compared to the control group, patients in the CBT plus physical exercise group exhibited a noticeable increase in sexual activity. Conversely, patients in the CBT alone group experienced a significant and lasting improvement in their urinary symptoms (30). These findings suggest that CBT can be an effective treatment for breast cancer survivors facing sexual health issues.

DISCUSSIONS

Studies have strongly supported the regular use of vaginal moisturizers as an effective means to enhance sexual pleasure and frequency, alleviate dryness and dyspareunia, and improve vaginal pH (26). Additionally, vaginal hormonal products have shown to be more effective than moisturizers in enhancing sexual function (21, 22). It has been established that various counseling and educational techniques, especially those targeting

sexual dysfunction, improve sexual health in BCS (30). Vaginal laser therapy presents an excellent option to repair tissues and prevent estrogen exposure, offering a solution to bridge the gap. The results provide short-term reassurance, indicating that erbium lasers and CO₂ lasers are equally effective in treating the most common symptoms of GSM (28). Furthermore, a study has found that in 90% of breast cancer survivors, painful penetration can be avoided, and severe dyspareunia may be relieved by the application of self-administered lidocaine compresses to the vestibule (27).

Despite the high prevalence of sexual dysfunction and its significant impact, there are no established treatment protocols for affected patients. Considering the complex nature of sexual dysfunction and its profound effects on various aspects of a patient's quality of life, a combination of pharmacological and psychological approaches might offer suitable therapeutic options.

CONCLUSIONS

1. Sexual dysfunction issues have become a clear challenge for patients in this community as more women continue to survive breast cancer diagnosis and treatment. It is becoming increasingly evident that addressing sexual issues is crucial to offering the best possible care to patients with breast cancer.
2. A multidisciplinary team, including oncologists, nurses, psychologists, psychiatrists, and sex therapists, should ideally address concerns related to sexual health. If medical professionals are not equipped to manage this issue, a referral to a sexologist or specialized physician is recommended. The principal areas for future research focus on specific therapeutic options for BCS.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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Date of receipt of the manuscript: 22/12/2023
Date of acceptance for publication: 30/03/2024



RESEARCH ARTICLE – ARTICLES DE RECHERCHE



NEW SPECIES OF STAPHYLINIDAE (COLEOPTERA) FROM THE REPUBLIC OF MOLDOVA REVEALED BY DNA BARCODING AND MORFOLOGICAL ANALYSIS

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DOI: 10.38045/ohrm.2024.2.02

CZU: 595.763:577.212(478)

Keywords: Staphylinidae, barcoding, DNA, newly recorded species, saproxylic species.

Introduction. Saproxylic beetles are important components of biodiversity and are used for monitoring the quality of old-growth forests. Due to the substantial reduction of forested areas and low availability of dead wood, saproxylic beetles are becoming threatened and many small coleopteran species risk disappearing before they are even discovered.

Material and methods. The research was carried out in one natural forest, three natural and two landscape reserves. Coleoptera species were identified using morphological characters and DNA barcoding. Molecular identification established using the GenBank database was confirmed by querying the BOLD Systems database.

Results. The work includes 9 species of coleoptera: *Batrisodes unisexualis*, *Trichonyx sulcicollis*, *Sepedophilus bipunctatus*, *S. constans*, *S. pedicularius*, *Gyrophaena manca*, *Scaphisoma agaricinum*, *Medon rufiventris* and *Hypnogyra angularis* newly identified for the fauna of the Republic of Moldova. Obtained COI sequences were analysed and compared with European species stored in GenBank. From a phylogeographic point of view, the majority of the sequences from the Republic of Moldova are grouped into private haplotypes, that are distributed only in the analysed region. The species *Gyrophaena manca* has 2 haplotypes, one is present in Finland and Germany, another in the Republic of Moldova.

Conclusions. As a result of the research 9 species of saproxylic coleoptera from the studied natural forests were identified as new for the fauna of the Republic of Moldova. Obtained genetic data are available for future phylogeny, phylogeography and conservation biology studies carried out at European level.

Cuvinte-cheie: Staphylinidae, barcodare, ADN, specii noi înregistrate, specii saproxilice.

SPECII NOI DE STAPHYLINIDAE (COLEOPTERA) DIN REPUBLICA MOLDOVA IDENTIFICATE PRIN BARCODAREA ADN-ULUI ȘI ANALIZA MORFOLOGICĂ

Introducere. Coleopterele saproxilice sunt un component important al biodiversității și sunt utilizate pentru monitorizarea stării pădurilor seculare. Din cauza reducerii substanțiale a suprafețelor împădurite și a volumului redus de lemn mort în păduri, coleopterele saproxilice sunt amenințate și multe specii de dimensiuni mici riscă să dispară, chiar înainte de a fi descoperite.

Material și metode. Cercetarea a fost efectuată într-o pădure naturală, trei rezervații naturale și două peisagistice. Speciile de coleoptere au fost identificate folosind caractere morfologice și prin barcodarea ADN-ului. Veridicitatea identificării moleculare cu GenBank a fost confirmată prin interogarea BOLD Systems.

Rezultate. Lucrarea include nouă specii de coleoptere noi pentru fauna Republicii Moldova: *Batrisodes unisexualis*, *Trichonyx sulcicollis*, *Sepedophilus bipunctatus*, *S. constans*, *S. pedicularius*, *Gyrophaena manca*, *Scaphisoma agaricinum*, *Medon rufiventris* și *Hypnogyra angularis*. Secvențele COI obținute au fost analizate și comparate cu cele europene stocate în GenBank. Din punct de vedere filogeografic, majoritatea speciilor secvențiate din Republica Moldova se grupează în haplotipuri private, care sunt distribuite doar în regiunea analizată. Specia *Gyrophaena manca* are două haplotipuri – unul este prezent în Finlanda și Germania, iar altul în Republica Moldova.

Concluzii. În urma studiului realizat au fost identificate nouă specii de coleoptere saproxilice noi pentru fauna Republicii Moldova. Genele obținute au fost depozitate în GenBank și sunt disponibile pentru studiile de filogeneză, filogeografie și conservare a biodiversității la nivel european.

INTRODUCTION

Saproxylic beetles are important components of biodiversity and are used for monitoring the quality of old-growth forests (1, 2, 3). Due to the substantial reduction of forested areas and low availability of dead wood, saproxylic beetles are becoming threatened (4), and many small coleopteran species risk disappearing before they are even discovered.

The research was carried out on the dead wood found in several protected areas, in order to provide new data on the fauna of saproxylic staphylinids in the Republic of Moldova. Dead wood is a preferred microhabitat for predatory and mycetophagous staphylinids and it harbors many small and very agile species from which many are still unknown.

In the fauna of the Republic of Moldova, a total of 262 species of staphylinids, belonging to 13 subfamilies were known until now: Aleocharinae, Habrocerinae, Omaliinae, Oxyporinae, Oxytelinae, Paederinae, Piestinae, Proteininae, Pselaphinae, Scaphidiinae, Staphylininae, Steninae and Tachyporinae (5).

To date, 24 species of staphylinids were identified from dead wood (6, 7, 8). Of these, 21 species belonging to the subfamilies Aleocharinae (3 species), Piestinae (1), Scaphidiinae (2), Staphylininae (7), Tachyporinae (8) are considered saproxylic. The following identified species are saproxylic: *Abemus chloropterus* (Creutzer, 1796), *Astrapaes ulmi* (Rossi, 1790), *Atrecus affinis* (Paykull, 1789), *Dinaraea aequata* (Erichson, 1837), *Gabrius splendidulus* (Gravenhorst, 1802), *Gyrophana joi* Wendeler, 1924, *Gyrophana nana* (Paykull, 1800), *Milichilinus decorus* (Erichson, 1839), *Othius punctulatus* (Goeze, 1777), *Scaphidium quadrimaculatum* Olivier, 1790, *Scaphisoma boleti* (Panzer, 1793), *Sepedophilus immaculatus* (Stephens, 1832), *Sepedophilus littoreus* (Linnaeus, 1758), *Sepedophilus marshami* (Stephens, 1832), *Sepedophilus obtusus* Luze, 1902, *Sepedophilus testaceus* (Fabricius, 1793), *Siagonium humerale* Germar, 1836, *Velleius dilatatus* (Fabricius, 1787), *Tachinus corticinus* Gravenhorst, 1802, *Tachyporus hypnorum* (Fabricius, 1775) and *Tachyporus nitidulus* (Fabricius, 1781) (9).

The aim of the present study was to expand the knowledge regarding the composition of the spe-

cies of saproxylic coleoptera in the Staphylinidae family in the Natural and Landscape Reserves of the country with the identification of new species.

MATERIAL AND METHODS

The research was carried out in natural and landscape reserves, which have an important role in the conservation and regeneration of rare species of flora and fauna in the country.

Prutul de Jos Nature Reserve (45.584188N 28.258056E) is a protected area that includes Lake Beleu and the surrounding floodplains, which preserve and restore the species of flora and fauna in this area. The territory of the reserve is mainly occupied by swamp and floodplain vegetation and the waters of Lake Beleu (10).

Pădurea Domnească Nature Reserve (47.609722N 27.393611E) is a natural floodplain forest dominated by oak, poplar and willow species, located in the Prut River floodplain (11). Currently, floodplain ecosystems are rare and threatened in Europe (3). For the protection of these forest ecosystems, a correct management that will also contribute to the ecological recovery of the floodplain ecosystems is necessary.

Plaiul Fagului Nature Reserve (47.291111N 28.054444E) is covered by natural oak forest in combination with sessile oak, ash, hornbeam and beech. The nature reserve is one of the most representative, well preserved and managed forest ecosystems in the central area of the Republic of Moldova (12).

Pohrebeni Landscape Reserve (47.562778N 28.8875E) is a protected area where the vegetation consists of forests of sessile oak, pedunculated oak and maple. The most widespread forest phytocenoses are sessile with linden and ash, which usually are found at higher altitudes, while sessile associations with hornbeam are more common at lower altitudes (10).

Vila Nisporeni Landscape Reserve (47.009444N 28.175651E) consists of sessile oak, downy oak and pedunculated oak forests (10). Currently it is still heavily affected by natural calamities of the spring of 2017, which broke trees under the weight of the snow.

Natural forest near Vulcănești, Nisporeni district (47.153611N 28.191111E), consists of ses-

sile oak, pedunculate oak, maple, hornbeam and linden species (10).

The material was collected from under the bark of dead and decaying trees that was colonized by ants, affected by molds and fungi that grow on dead wood. The extraction was carried out with the help of the entomological exhaustor or fragments of dead wood were examined in the laboratory using a modified flotation method (13).

Coleoptera species that were difficult to identify based on morphological characters were analysed using DNA barcoding. This technique, which revolutionized the study of biodiversity (14), involves sequencing a fragment of the gene for cytochrome C oxidase I and comparing the obtained sequences to those included in the international databases such as GenBank (NCBI) or BOLD (Barcode of Life System) (15). This approach can lead to the identification of species and the discovery of new species, but it is also an important tool in revealing the phylogeographic and evolutionary models of different organisms.

In the present study, a number of 56 samples (whole larvae or fragments, or damaged specimens) collected from the mentioned Natural and Landscape Reserves were analysed using DNA barcoding. DNA isolation was performed using the ISOLATE II Genomic DNA Kit (Bioline meridian BIOSCIENCE®, London, UK), following the manufacturer's specifications. The amount of genomic DNA isolated from each sample was subsequently used to amplify a fragment from the 5' end of the cytochrome C oxidase I gene, a gene extensively used for animal species identification (16).

The fragment was amplified using the universal COI primers HCO2198 (5'-TAAACTTCAGGGTGA CCAAAAAATCA-3') and LCO1490 (5'-GGTCAA CAAATCATAAAGATATTGG-3') (17) marked with M13 tails. The PCR reaction was performed in a total volume of 50 µL containing genomic DNA template, 1X Green GoTaq® Flexi Buffer, 2.5mM MgCl₂, each dNTP at 0.1 mM, 0.5 µM from each primer and 1 unit of DNA polymerase GoTaq® (Promega, Madison, SUA). Amplification products were isolated from samples that showed clear and visible bands on agarose gel stained with 0.5 µg/mL ethidium bromide. Bands of interest were excised from the gel and amplification products isolated using the Gel/PCR DNA Fragments Extraction Kit (Geneaid, Taiwan), following the

manufacturer's specifications. MacroGen services (Seoul, South Korea) were used for sequencing.

The raw sequences were manually aligned and edited in CodonCode Aligner version 3.7.1 (CodonCode Corporation, Dedham, MA, USA). The corrected sequences were used to interrogate the GenBank and BOLD Systems online databases, in order to identify the species. The GenBank database has a proprietary interface called nucleotide BLAST (Basic Local Alignment Search Tool) that uses an algorithm based on sequence similarity. The interface compares submitted nucleotide sequences with sequences stored in its own database and calculates a statistical significance.

The identity ratio calculated by BLAST represents the percentage of nucleotides that are similar between the subject sequence and the sequences found in the database. BLAST also calculates a degree of overlap which is the percentage of alignment between the sequence of interest and the reference sequences stored in GenBank. Molecular identification established using the GenBank database was confirmed by querying the BOLD Systems database.

In order to perform a basic phylogeographic analysis and to have an accurate picture of how the phylogenetic lines are spread at the European level, all COI sequences corresponding to the six staphylinid species for which we obtained DNA data were downloaded from online databases. The alignment was made in Mega7 (18) and DnaSP v.5 was used to calculate the number of haplotypes (19). The haplotype network identified for each species was reconstructed under a Median Joining algorithm implemented in PopART v1.7 (20).

Several keys were used to identify the coleopteran species according to morphological characters: (21, 22, 23, 24).

RESULTS

As a result of the investigations, this study has reported 9 species of coleoptera new for the fauna of the Republic of Moldova which are grouped as follows: subfamily Tachyporinae with 3 species, family Pselaphinae with 2 species and subfamilies Aleocharinae, Paederinae, Scaphidiinae and Staphylininae with one species each.

In the analysed samples, the molecular identification detected species from the Staphylinidae fa-

mily, subfamilies: Pselaphinae, Tachyporinae, Aleocharinae and Scaphidiinae. The species from the subfamilies: Tachyporinae, Paederinae and Staphylininae were identified using morphological characters.

Family Staphylinidae Latreille, 1802

Subfamily Pselaphinae Latreille, 1802,

Tribe Batrisini Reitter, 1882

Genus *Batrisodes* Reitter, 1881

Batrisodes (Batrisodes) unisexualis Besuchet, 1988

Identification: DNA barcoding.

Collection data and material examined: 1 ex., 20.V.2022, Pădurea Domnească.

Ecology and biology: collected from decaying wood along with ants from genus *Lasius*. Adult sizes 2.3-2.6 mm (22).

Occurrence: Palaearctic. In Europe: Austria, Czech Republic, Continental France, Germany, Poland,

Slovakia, Sweden, Switzerland, the Netherlands and Ukraine (25).

For *Batrisoides unisexualis* species, seven COI sequences were analysed, six of them mined from GenBank, obtained from specimens collected in Belgium and Germany (tab. 1). The haplotype identified in the Republic of Moldova is a distinct one. The other two haplotypes, one common for Belgium and Germany and one distinct for Germany, differ by a single mutation, while the one from Moldova has accumulated four more additional mutations (substitutions) (fig. 1). Four of the observed nucleotide substitutions were synonymous and did not determine differences in the amino acid sequence. Only one causes the change of Alanin (Ala) present at the selected position, in all the samples from Germany and Belgium, to Tyrosine (Tyr).

Table 1. Provenance of the analysed *Batrisoides unisexualis* sequences.

Species	Sequence	Voucher	Place of collection
<i>Batrisodes (Batrisodes) unisexualis</i> Besuchet, 1988	OQ883684	SV5_PD	Republic of Moldova, Pădurea Domnească
	HQ954034	BC_ZSM_COL_01295	Belgium, Blanden, BR Meerdaalbos
	KM439991	BFB_Col_FK_4127	Germany: Rhineland Palatinate, Neuburg, Altrheine
	KM447949	BC_ZSM_COL_00421	Germany: North Rhine-Westphalia, Worringer, Bruch
	KM448359	BFB_Col_FK_8080	Germany: North Rhine-Westphalia, Bergsee
	HQ954018	BC_ZSM_COL_01274	Belgium, Leuven, Blanden, BR Meerdaalbos
	KM444179	BFB_Col_FK_7409	Belgium: West-Vlaanderen, Leuven, BR Meerdaalbos

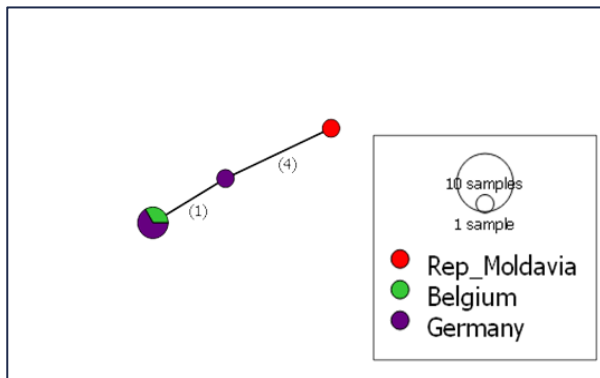


Figure 1. Network analysis for *Batrisoides (Batrisoides) unisexualis*.

Tribe Trichonychini Reitter, 1882

Genus *Trichonyx* Chaudoir, 1845

Trichonyx sulcicollis (Redtenbacher, 1816)

Identification: DNA barcoding.

Collection data and material examined: 1 ex., 20.V.2022, Pădurea Domnească.

Ecology and biology: collected from decaying wood populated by ants using exhauster. Adults are predators. The species occur in dry wood or under the bark of dead stumps, together with ants from genera *Lasius* and *Formica* (25). Adult sizes 2.5-3.5 mm.

Occurrence: In Europe: Austria, Belarus, Belgium, Bulgaria, Central European Russia, Croatia, Czech Republic, Danish mainland, French mainland, Germany, Greek mainland, Hungary, Italian mainland, Lithuania, Norwegian mainland, Poland, Romania, Slovakia, Slovenia, Sweden, Switzerland, The Netherlands, Ukraine and UK (22).

In the case of *Trichonyx sulcicollis*, the number of COI sequences identified/downloaded from international databases is low (tab. 2). Two sequences from Belgium were analysed and they grouped in the same mitochondrial haplotype that is different from the one present in the Re

public of Moldova, in the Pădurea Domnească Reserve by 15 mutations (fig. 2). All observed nucleotide substitutions were synonymous and did not have differences in amino acid sequence.

leotide substitutions were synonymous and did not have differences in amino acid sequence.

Table 2. Analyzed sequences COI of *Trichonyx sulcicollis*.

Species	Sequence	Voucher	Place of collection
<i>Trichonyx sulcicollis</i> (Redtenbacher, 1816)	OQ883692	SV6_PD	Republic of Moldova, Pădurea Domnească
	HQ954018	BC ZSM COL 01274	Belgium, Blanden, BR Meerdaalbos
	KM444179	BFB_Col_FK_7409	Belgium, Blanden, BR Meerdaalbos

In the case of *Trichonyx sulcicollis*, the number of COI sequences identified/downloaded from international databases is low (tab. 2). Two sequences from Belgium were analysed and they grouped in the same mitochondrial haplotype that is different from the one present in the Republic of Moldova, in the Pădurea Domnească Reserve by 15 mutations (fig. 2). All observed nucleotide substitutions were synonymous and did not have differences in amino acid sequence.

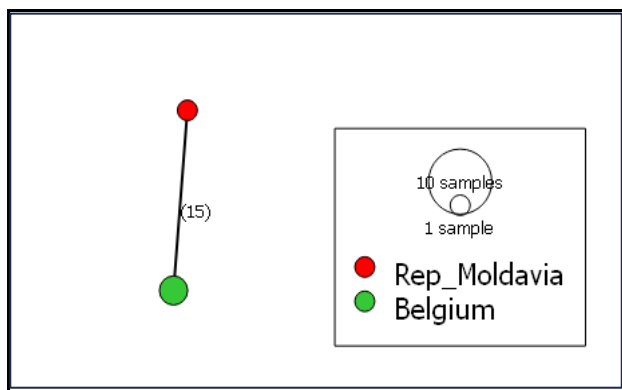


Figure 2. Network analysis for *Trichonyx sulcicollis*.

Subfamily Tachyporinae MacLeay, 1825
Tribe Tachyporini MacLeay, 1825
Genus *Sepedophilus* Gistel, 1856
Sepedophilus bipunctatus (Gravenhorst, 1802)
Synonym: *Tachyporus bipunctatus* Gravenhorst, 1802
Identification: DNA barcoding.
Collection data and material examined: 1 ex., 23.III.2022, Prutul de Jos.

Ecology and biology: species were collected from under damp and mold-affected willow bark using the exhaustor. Corticolous, saproxylic, mycetophagous species, it feeds on the hyphae of fungi that develop on the dead wood of deciduous and coniferous trees (26). Adult sizes 2.0-2.5 mm (25). Occurrence: Palaearctic (25). In Europe: Austria,

Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Danish mainland, Finland, French mainland, Germany, Greek mainland, Hungary, Italian mainland, Latvia, Near East, East, North and South European Russia, Norwegian mainland, Poland, Romania, Slovakia, Sweden, Switzerland, The Netherlands, Ukraine, UK and former Yugoslavia.

In the case of *Sepedophilus bipunctatus*, four haplotypes were identified in 16 COI sequences analysed (tab. 3 and fig. 3). Two of the haplotypes are common to Germany and Finland, while distinct haplotypes appear in the Republic of Moldova and Belgium. These haplotypes differ from each other only by one mutation (fig. 3). The amino acid sequence obtained for the Moldova sample exhibits one silent mutation and three others that change the amino acid order: Tyrosine replaces Histidine – this change is present in our samples from Moldova, but also in samples from Belgium, Germany and Finland; Serine replaces Proline, change that is particular to our samples; and Alanine substitutes Tyrosine not only in our samples, but also in samples from Finland and Germany.

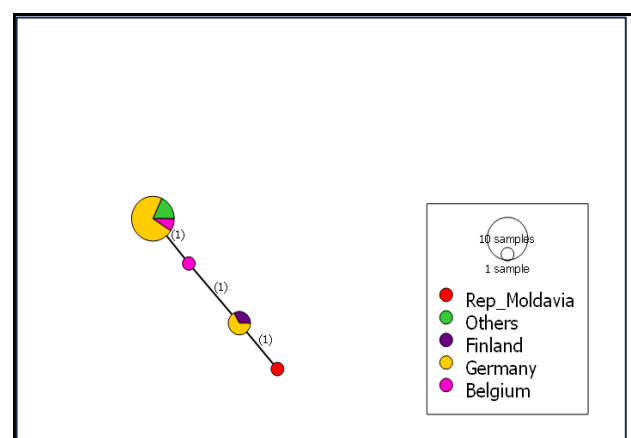


Figure 3. Haplotype network for the species *Sepedophilus bipunctatus*.

Table 3. Analysed sequences COI of *Sepedophilus bipunctatus*.

Species	Sequence	Voucher	Place of collection
<i>Sepedophilus bipunctatus</i> (Gravenhorst, 1802)	OQ883690	SB11_Prutul de Jos	Republic of Moldova, Prutul de Jos
	NC_028611	-	-
	MZ659744	ZMUO.024139	Finland: Ostrobothnia ouluensis, Poellaennokka
	MW259859	ZFMK-TIS-2573928	Germany: North Rhine-Westphalia, Klosterholz
	MW259566	ZFMK-TIS-2573927	Germany: North Rhine-Westphalia, Klosterholz
	KU919616	ZFMK-TIS-2500597	Germany: Thuringia, Wartburgkreis, Neuroth
	KU915356	ZFMK-TIS-2522848	Germany: Saxony-Anhalt, Timmenrode
	KU913802	ZFMK-TIS-5858	Germany: Saxony, Leisnig
	KU911498	ZFMK-TIS-2500628	Germany: Thuringia, Wartburgkreis, Neuroth
	KU911387	ZFMK-TIS-21603	Germany: Rheinland-Pfalz, Thuer
	KU907881	ZFMK-TIS-2500553	Germany: Thuringia, Wartburgkreis, Nesseufer
	KU906618	ZFMK-TIS-2500613	Germany: Thuringia, Wartburgkreis, Neuroth
	KT780676	BMNH 845956	-
	KM446485	GBOL_Col_FK_6206	Germany: Rhineland Palatinate, Scheibenhardt
	KM443565	BFB_Col_FK_7477	Belgium: Vlanderen, Leuven, BR, Meerdaalbos
HQ953492	BC ZSM COL 00539	Belgium, Sint-Genesius-Rode, BR Zonienwoud	

Sepedophilus constans (Fowler, 1888)
 Synonym: *Conosoma constans* Fowler, 1888
 Identification: morphological method.
 Collection data and material examined: 1 ex., (♀), 31.III.2022, Vulcănești (Nisporeni), leg. S. Bacal, det. M. Stan. Ecology and biology: saproxylic species, collected with exhauster from wood of dead oak, mycetobiont, mycetophagus (27). Adult sizes 3.9-4.9 mm (22).
 Occurrence: Austria, Czech Republic, Finland, Germany, Hungary, Italy, Northern European Russia, Norway, Poland, Romania, Slovakia, Sweden, Ukraine and UK.

Sepedophilus pedicularius (Gravenhorst, 1802)
 Synonyms: *Tachyporus pedicularius* Gravenhorst, 1802, *Conosoma pedicularium* (Gravenhorst, 1802), *Conosomus pedicularius* Gravenhorst, 1802, *Tachyporus truncatellus* Gravenhorst, 1806)
 Identification: DNA barcoding.
 Collection data and material examined: 1 ex., 23.II.2022, Vila Nisporeni.
 Ecology and biology: collected using exhauster

from decaying wood. Adult sizes 2.5-3.0 mm.
 Occurrence: Palaearctic. In Europe: Austria, Belarus, Central, North and East European Russia, Czech Republic, Danish mainland, Estonia, Finland, French mainland, Germany, Ireland, Italian mainland, Latvia, Liechtenstein, Northern Ireland, Norwegian mainland, Poland, Romania, Slovakia, Sweden, Switzerland and UK.

In the case of *Sepedophilus pedicularius* in the six analysed sequences which came from Finland, Germany and the Republic of Moldova, three haplotypes were identified, two of which are specific for the Republic of Moldova and Finland, respectively, and one common haplotype in which the sequences from Germany and Finland are grouped. All three haplotypes identified in the samples differ by a single mutation (tab. 4 and fig. 4). In our analysed sample from Vila Nisporeni, one nucleotide substitution is synonymous and did not induce any differences in the amino acid sequence, and another substitution which replaces Methionine with Valine is shared with a sample from Finland.

Table 4. Analysed sequences COI of *Sepedophilus pedicularius*.

Species	Sequence	Voucher	Place of collection
<i>Sepedophilus pedicularius</i> (Gravenhorst, 1802)	OQ883691	SV19_VN	Landscape Rezerve Vila Nisporeni
	KJ962555	ZMUO<FIN>:001221	Finland: Aland Islands, Finstroem, Attboele
	KJ966871	ZMUO<FIN>:005649	Finland: Alandia, Lemland, Bathusfjaerden
	KM448438	GBOL02411	Germany: Bavaria, Schiessplatzheide-Sued
	KU912252	ZFMK-TIS-2521952	Germany: Kiesgruben und Sumpfstellen
	KU915117	ZFMK-TIS-2524706	Germany: Thuringia, Walterslebener Grund

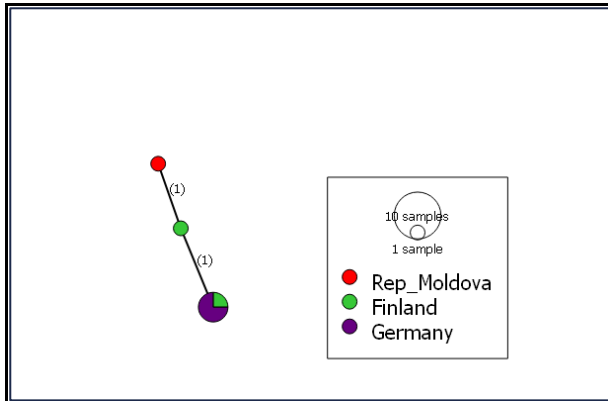


Figure 4. Haplotype network for the species *Sepedophilus pedicularius*.

Subfamily Aleocharinae Fleming, 1821
 Tribe Homalotini Heer, 1839
 Genus *Gyrophæna* Mannerheim, 1830
Gyrophæna manca Erichson, 1839
 Synonyms: *Aleochara angustata* Stephens, 1832, *Gyrophæna angustata* (Stephens, 1832), *Gyrophæna manca* Erichson, 1839, *Gyrophæna puncti-collis* (Hochhuth, 1872)
 Identification: DNA barcoding.

Collection data and material examined: 5 ex., 16.II.2022, Pohrebeni; 2 ex., 20.V.2022, Pădurea Domnească.
 Ecology and biology: mycetophagous species collected from mushrooms growing on decaying dead wood using exhaustor. Adult sizes 1.3-1.6 mm
 Occurrence: Palaearctic (22).

Seven haplotypes were identified in the 13 *Gyrophæna manca* sequences analysed to generate the network presented in Figure 5. In the Republic of Moldova, two haplotypes were identified, one of which is common, also found in samples from Germany and Finland, while the other is distinct and differs by two mutational steps from the common one. The most distant from the common haplotype is the one from Finland (seven mutations) (tab. 5 and fig. 5). From three analysed samples, only one collected in Pădurea Domnească exhibits two mutations in the amino acid sequence, as follows: Proline (Pro) replaces Leucine (Leu), change that is common with a sample from Germany, and one modification private to our sample is the substitution of Glycine (Gly) with Proline (Pro).

Table 5. Analysed sequences COI of *Gyrophæna manca*.

Species	Sequence	Voucher	Place of collection
<i>Gyrophæna manca</i> Erichson, 1839	OQ883685	SV10_Poh	Landscape Reserve Pohrebeni
	OQ883686	SV13_PD	Republic of Moldova, Pădurea Domnească
	OQ883687	SV48_PD	Republic of Moldova, Pădurea Domnească
	MZ660144	ZMUO.028867	Finland: Regio aboensis, Pohja, Fiskars
	MZ657487	ZMUO.028868	Finland: Regio aboensis, Pohja, Fiskars
	MZ656527	ZMUO.028869	Finland: Regio aboensis, Pohja, Fiskars
	KU907280	ZFMK-TIS-2556315	Germany Mecklenburg, Nationalpark, Mueritz
	KM448754	GBOL_Col_FK_0877	Germany: Bavaria, Lusen- und Boehmstrasse
	KM447407	GBOL_Col_FK_0809	Germany: Rhine-Westphalia, NWZ Altwald Ville
	KM443621	GBOL_Col_FK_0832	Germany: Bavaria, Diensthuettenstrasse
	KM441816	GBOL_Col_FK_0825	Germany: Bavaria, Schwarzachstrasse
	KM440835	BC ZSM COL 02540	Germany: Rhineland Palatinate, Lautermuendung
	HQ953314	BC ZSM COL 00312	Germany

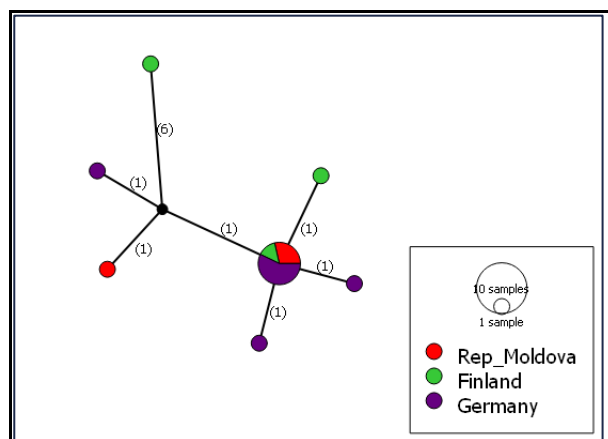


Figure 5. Haplotype network for the species *Gyrophæna manca*.

Subfamily Scaphidiinae Latreille, 1806
 Tribe Scaphidiini Latreille, 1806
 Genus *Scaphisoma* Leach, 1815
Scaphisoma agaricinum (Linnaeus, 1758)
 Synonym: *Silpha agaricinum* Linnaeus, 1758
 Identification: DNA barcoding
 Collection data and material examined: 1 ex., 12.I.2022, Plaiul Fagului; 1 ex., 20.V.2022, Pădurea Domnească.

Ecology and biology: species were collected in the Plaiul Fagului, from under mouldy oak bark. It can be found on mushrooms. The specimens were obtained by the flotation method applied in the laboratory. Adults range in size from 1.7-2.0 mm. Occurrence: Eurasia (25). In Europe: Albania, Austria, Belarus, Belgium, Bosnia and Herzegovina, UK, Bulgaria, Central and East European Russia, Corsica, Croatia, Czech Republic, Danish mainland, East Palaearctic, Estonia, Finland, French mainland, Germany, Greek mainland, Hungary, Ireland, Italian mainland, Latvia, Liechtenstein, Lithuania, Macedonia, North European Russia, Northwest European Russia, Norwegian mainland, Poland, Romania, Slovakia, Slovenia, South European Russia, Spanish mainland, Swe-

Switzerland, The Netherlands, Ukraine and Yugoslavia.

Seven haplotypes were identified in 19 COI sequences analysed for the *Scaphisoma agaricinum*. Three private haplotypes are present in samples collected from Germany. Another private haplotype is present in Finland, while the samples from the Republic of Moldova from Pădurea Domnească and Plaiul Fagului, are grouped into other two private haplotypes. A common haplotype is found in samples from Germany and Belgium (tab. 6 and fig. 6). For our analysed specimens of *S. agaricinum*, all the observed nucleotide substitutions were synonymous and did not induce differences in the amino acid sequence.

Table 6. Analysed sequences COI of *Scaphisoma agaricinum*.

Species	Sequence	Voucher	Place of collection
<i>Scaphisoma agaricinum</i> (Linnaeus, 1758)	OQ883688	SV7_PD	Republic of Moldova, Pădurea Domnească
	OQ883689	SV12_PF	Republic of Moldova, Plaiul Fagului
	HQ953834	BC ZSM COL 00929	Belgium, Sint-Genesius-Rode, Zonienwoud
	KJ963540	ZMUO<FIN>:004481	Finland: Regio aboensis, Turku, Ruissalo
	KJ964524	ZMUO<FIN>:004480	Finland: Regio aboensis, Turku, Ruissalo
	KJ965088	ZMUO<FIN>:004038	Finland: Ostrobotnia kajanensis, Tuohilamminkangas
	KJ966647	ZMUO<FIN>:005909	Finland: Ostrobotnia borealis pars australis, Oulunsalo, Lassilanranta
	KM443518	GBOL_Col_FK_1344	Germany: Rhineland Palatinate, Oberrhein, Altrheine
	KM447364	BFB_Col_FK_5740	Germany, North Rhine-Westphalia, Altwald Ville
	KU906446	ZFMK-TIS-2500635	Germany: Thuringia, Wasungen, Bonndorf, Werra-Aue
	KU908177	ZFMK-TIS-2532952	Germany: Saxony, Klosterbuch/Mulde, NSG Maylust
	KU908615	ZFMK-TIS-2532966	Germany: Saxony, Klosterbuch/, NSG Maylust
	KU908786	ZFMK-TIS-2537173	Germany: Saxony, Klosterbuch, Maylust
	KU911799	ZFMK-TIS-13947	Germany: Thuringia, Beichlingen, Wurmberg
	KU914296	ZFMK-TIS-2519525	Germany: Saxony-Anhalt, Huy, Paulskopf
	KU915341	ZFMK-TIS-6042	Germany: Rhine-Westphalia, Siebengebirge
	KU916539	ZFMK-TIS-13941	Germany: Thuringia, Beichlingen, Wurmberg
	KU917062	ZFMK-TIS-2537172	Germany: Saxony, Klosterbuch, Maylust
	KU919452	ZFMK-TIS-2519522	Germany: Saxony-Anhalt, Huy, Paulskopf

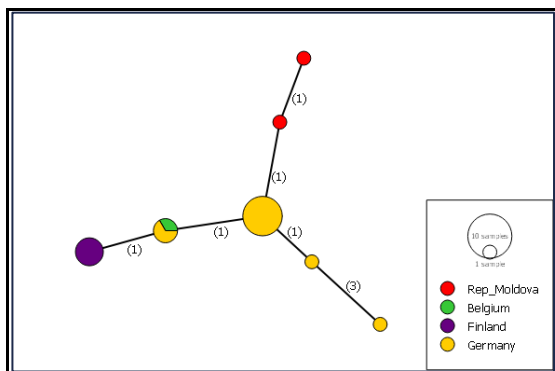


Figure 6. Haplotype network for the species *Scaphisoma agaricinum*.

Paederinae Fleming, 1821
Tribe Paederini Fleming, 1821
Genus *Medon* Stephens, 1833
Medon rufiventris (Nordmann, 1837)
Synonyms: *Lathrobium rufiventris* Nordmann, 1837, *Medon incertus* Mulsant & Rey, 1878
Identification: morphological method.

Collection data and material examined: 2 ex., (1♂, 1♀), 31.III.2022, Vulcănești (Nisporeni), leg. S. Bacal, det. M. Stan. Identified by the aedeagus, sternite VII and VIII.

Ecology and biology: species collected with exhaustor from under the bark of a rotting ash tree. A specimen was extracted in the laboratory by the floatation method. Saprophagous species, feeds on decaying plant matter, quite rare (28). Species associated with dead wood (29). Adult sizes 3.5-4 mm (22).

Occurrence: Austria, Bosnia and Herzegovina, Croatia, Czech Republic, France, Germany, Greece, Hungary, Italy, Lithuania, Middle East, North Africa, Poland, Romania, Slovakia, Slovenia, Spain, Ukraine.

Staphylininae Latreille, 1802

Tribe Xantholinini Erichson, 1839

Genus *Hypnogyra* Casey, 1906

Hypnogyra angularis (Ganglbauer, 1895)

Synonyms: *Xantholinus angularis* Ganglbauer, 1895, *Staphylinus glabra* Gravenhorst, 1802, *Gyrohypnus rotundicollis* Stephens, 1833, *Xantholinus flavipennis* Redtenbacher, 1849, *Lepidophallus bernaboi* Coiffait, 1980, *Hypnogyra bernaboi* Coiffait, 1980, *Hypnogyra flavipennis* Redtenbacher, 1849, *Hypnogyra glabra* (Nordmann, 1837), *Hypnogyra rotundicollis* Stephens, 1833, *Phalacrolinus angularis* (Ganglbauer, 1895), *Phalacrolinus bernaboi* (Coiffait, 1980), *Phalacrolinus flavipennis* (Redtenbacher, 1849), *Phalacrolinus glabra* (Gravenhorst, 1806), *Phalacrolinus rotundicollis* (Stephens, 1833).

Identification: morphological method.

Collection data and material examined: 1 ex., (♂), 08-30.VI.2022, Pădurea Domnească, leg. S. Bacal, det. M. Stan.

Ecology and biology: species was collected using exhaustor, along with ants of the genus *Lasius* from dead oak, infested with mould. Adult sizes 6-7 mm (22).

Occurrence: Austria, Belgium, Bosnia and Herzegovina, Great Britain, Central European Russia, Croatia, Czech Republic, Denmark, Estonia, Eastern European Russia, Finland, France, Germany, Greece, Hungary, Italy, Lithuania, Middle East, Netherlands, North Africa, Poland, Romania, Slovakia, Spain, Sweden, Switzerland and Ukraine.

DISCUSSIONS

Today, the total number of saproxylic staphyli-

nids from seven subfamilies has reached 30 species present in the Republic of Moldova: Staphylininae (11 species), Tachyporinae (8), Aleocharinae (4), Scaphidiinae (3), Pselaphinae (2), Paederinae and Piestinae with only one species for each (9).

The species *Batrisodes unisexualis* was collected from Hungary in 2017 from under the bark of dead trees colonized by ants in the genus *Lasius* (25). Our research confirms the association between the coleopteran species and xylobiont ants.

In Great Britain, *Trichonyx sulcicollis* was collected from under the bark of deciduous trees, and was found in association with *Lasius* and *Formica* ants (25). In our case, the species was collected from dead wood colonized by ants.

The species from the genera *Sepedophilus* (*S. bipunctatus*, *S. constans* și *S. pedicularius*), *Gyrophaena* and *Scaphisoma* are mycetobionts and mycetophages (25, 27). In the present research, the species were collected from dead wood affected by mould and colonized by mushrooms.

Medon rufiventris is considered to be associated with dead wood, quite rare, and usually found in decaying plant matter (28). In the Republic of Moldova, the species was collected from the Vulcănești sessile oak forest in combination with pedunculate oak, birch and beech (10).

Hypnogyra angularis is considered an indicator species of old forests (30). In our research, the species was collected from the Pădurea Domnească natural floodplain forest that includes unique wet habitats in the Republic of Moldova, with old trees and dead wood in various stages of degradation.

From a phylogeographic point of view, most sequences from the Republic of Moldova are grouped into private haplotypes, that are distributed only in the analysed region. The only exception is represented by *Gyrophaena manca* for which two haplotypes have been identified, with one present also in Finland and Germany and the other one, private, found only in the Republic of Moldova.

The COI region was chosen as a barcode because it is sufficiently conserved at species level and is variable enough between species to enable a good taxon identification. Also, the COI barcode fragment is located at the core of energy production within cells and changes in amino acid sequences

that modify the protein structure may affect energy metabolism. In 2016, Pentinsaari and collaborators (31) have revealed that the beetles (Coleoptera) show more amino acid variation, indicating fundamental differences in patterns of molecular evolution in COI. In our analyses we identified some species (*Trichonyx sulcicollis* and *Scaphisoma agaricinum*) with silent mutations in the COI sequences with no differences in amino acid sequence after translation. Considering the

other four analysed species, the identified mutations generate changes in the translated amino acid sequences, from which one is a private variant for Moldova, while the others are shared between different European regions.

The newly identified species confirm the fact that the natural and landscape reserves of the Republic of Moldova are well preserved and rich in terms of biodiversity.

CONCLUSIONS

1. As a result of the research carried out on the saproxylic coleoptera from the dead wood of the Natural Reserves Pădurea Domnească, Plaiul Fagului, Prutul de Jos, the Landscape Reserves Pohrebeni and Vila Nisporeni and the natural forest from Vulcănești (Nisporeni), 9 species, belonging to seven genera and 6 subfamilies, were identified as new for the fauna of the Republic of Moldova: *Batrissodes unisexualis*, *Trichonyx sulcicollis*, *Sepedophilus bipunctatus*, *Sepedophilus constans*, *Sepedophilus pedicularius*, *Gyrophaena manca*, *Scaphisoma agaricinum*, *Medon rufiventris* and *Hypnogyra angularis*.
2. The identification of new species of coleoptera, especially the damaged individuals, was possible thanks to the DNA barcoding, but it is also an important tool in revealing the phylogeographic and evolutionary models.
3. The majority of the sequenced coleopteran species from the Republic of Moldova are grouped into private haplotypes, that are distributed only in the analysed region. The only exception is the species *Gyrophaena manca* with two haplotypes, one present also in Finland and Germany and the other one, found only in the Republic of Moldova.
4. The analysis of the obtained COI sequences and their comparison with stored in GenBank showed that the species identified from the Republic of Moldova are distributed in Europe and in the Palearctic.
5. Thus, as a result of this study, the number of Staphylinidae saproxylic species identified in the Republic of Moldova has reached 30, and they belong to the subfamilies Aleocharinae, Paederinae, Piestinae, Pselaphinae, Scaphidiinae, Scydmaeninae, Staphylininae and Tachyporinae.
6. The genetic data obtained in the present study will contribute to the enrichment of genetic databases, becoming available for future phylogeny, phylogeography and conservation biology studies carried out at European level.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The authors express their gratitude to Dr. Melanya Stan from the “Grigore Antipa” Museum, Bucharest, Romania for identifying the species based on morphological characters. The work

was carried out with financial support from projects 20.80009.7007.02, 22.00208.7007.05/PD1 “Saproxylic Coleoptera from the Republic of Moldova: taxonomy, ecology, zoogeography and importance” and 010701 “Evaluation of the structure and functioning of the animal world and aquatic ecosystems under the influence of biotic and abiotic factors in the context of ensuring ecological security and the well-being of the population”.

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Date of receipt of the manuscript: 22/01/2024

Date of acceptance for publication: 24/03/2024



PARENTAL SOCIO-DEMOGRAPHIC INFLUENCES ON SCHOOL-AGED CHILDREN'S DIETS: A QUANTITATIVE STUDY IN THE REPUBLIC OF MOLDOVA

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DOI: 10.38045/ohrm.2024.2.03

CZU: 613.2.03-053.2:314/316(478)

Keywords: knowledge, attitudes, practices, parents, children's nutrition, nutritional education.

Introduction. The transition from childhood to adulthood significantly impacts overall health and requires adequate nutrition to facilitate physical and cognitive development. Managing children's weight and fostering an environment that promotes healthy eating habits are significantly influenced by parents' knowledge, attitudes, and practices.

Material and methods. In February – March 2023, a quantitative study was conducted, involving 472 parents of school-aged children. The questionnaire, developed according to the Knowledge, Attitudes, and Practices methodology, was completed both online, in electronic format, and on paper.

Results. The results of the KAP study regarding the nutrition of school-aged children show that parents have a significant level of knowledge (76.9±19.07%) about their children's nutrition. Regarding attitude, the score is moderate (59.99±10.7%), indicating that parents may not prioritize healthy eating habits for their children or may not be sufficiently motivated to act. However, there is a low level of practice: 45.43±16.6%. Statistical analysis indicates significant correlations between: gender and knowledge ($\tau=0.175$, $p<0.01$ and $\rho=0.185$, $p<0.01$), gender and attitudes ($\tau=0.111$, $p<0.05$ and $\rho=0.111$, $p<0.05$), age and knowledge ($\tau=-0.055$, $p<0.05$ and $\rho=-0.062$, $p<0.05$), living environment and knowledge ($\tau=0.227$, $p<0.01$ and $\rho=0.240$, $p<0.01$), region and knowledge ($\tau=-0.162$, $p<0.01$ and $\rho=-0.185$, $p<0.01$), indicating the importance of these factors in influencing dietary behavior.

Conclusions. The study's findings reveal a discrepancy between the level of knowledge and the positive attitudes of parents regarding children's nutrition and their practical implementation.

Cuvinte-cheie: cunoștințe, atitudini, practici, părinți, nutriția copiilor, educație nutrițională.

INFLUENȚE SOCIO-DEMOGRAFICE ALE PĂRINȚILOR ASUPRA DIETEI COPILOR DE VÂRSTĂ ȘCOLARĂ: UN STUDIU CANTITATIV ÎN REPUBLICA MOLDOVA

Introducere. Tranziția de la copilărie la vârstă adultă influențează semnificativ starea de sănătate și impune un regim alimentar adecvat pentru a facilita dezvoltarea fizică și cognitivă. Gestionarea greutății copiilor și cultivarea unui mediu care promovează obiceiuri alimentare sănătoase sunt influențate semnificativ de cunoștințele, atitudinile și practicile părinților.

Material și metode. În februarie – martie 2023, a fost desfășurat un studiu cantitativ, ce a inclus 472 de părinți ai copiilor de vârstă școlară. Chestionarul, elaborat conform metodologiei Cunoștințele, Atitudini și Practici, a fost completat atât online, în format electronic, cât și pe suport de hârtie.

Rezultate. Rezultatele studiului CAP, referitoare la nutriția copiilor de vârstă școlară, arată că părinții au un nivel de cunoștințe semnificativ (76,9±19,07%) despre nutriția copiilor lor. În ceea ce privește atitudinea, scorul este mediu (59,99±10,7%), indicând că părinții ar putea să nu acorde suficientă importanță alimentației sănătoase a copiilor sau să nu fie suficient de motivați să acționeze. Cu toate acestea, se observă un nivel scăzut al practicii: 45,43±16,6%. Analiza statistică indică corelații semnificative între: gen și cunoștințe ($\tau=0,175$, $p<0,01$ și $\rho=0,185$, $p<0,01$), gen și atitudini ($\tau=0,111$, $p<0,05$ și $\rho=0,111$, $p<0,05$), vârstă și cunoștințe ($\tau=-0,055$, $p<0,05$ și $\rho=-0,062$, $p<0,05$), mediul de trai și cunoștințe ($\tau=0,227$, $p<0,01$ și $\rho=0,240$, $p<0,01$), regiune și cunoștințe ($\tau=-0,162$, $p<0,01$ și $\rho=-0,185$, $p<0,01$), indicând importanța acestor factori în influențarea comportamentului alimentar.

Concluzii. Concluziile studiului relevă o discrepanță între nivelul de cunoștințe și atitudinile pozitive ale părinților privind nutriția copiilor și implementarea lor practică.

INTRODUCTION

The group of school-age children stands out due to their unique nutritional requirements, which are greater than those of adults, yet their dietary intake must simultaneously meet the needs for energy, recovery, and growth. A distinguishing characteristic of school-age children is their limited autonomy in making dietary decisions. Instead, their diet is influenced by parents, relatives, school personnel, friends, and aggressive marketing (1).

The transitional stage from early childhood to adulthood is critically significant in shaping an individual's overall health. This period, known as "middle childhood" (6-11 years) and "adolescence" (12-18 years), is marked by notable changes in physical and cognitive development, where proper nutrition is essential to support these changes and mitigate any negative impact of nutritional deficiencies or excesses (2).

In the Republic of Moldova, school meals are accessible; however, the quality of nutrition in educational institutions, particularly in rural areas, remains suboptimal. Menus are often overloaded with bakery products and oils but lack fruits, vegetables, and good fats. The Ministry of Health of the Republic of Moldova, in collaboration with the Ministry of Education and Research of the Republic of Moldova, has revised nutrition recommendations for school and preschool institutions. However, the ongoing issue of insufficient funding for children's meals in schools persists (3).

Although there are recommendations for a healthy diet and adequate physical activity in educational institutions in the Republic of Moldova, developed in 2016, and updated specifically for kindergartens in 2023 (4), as well as the "*Guide to Good Practice: Rational Nutrition, Food Safety, and Changing Nutrition Behaviors*" from 2019 (5), there is no clear and consistent message to parents regarding the nutritional quality of foods consumed by children at home. Consequently, children heavily rely on the knowledge and capabilities of others to ensure they receive proper nutrition (1).

Currently, the world is facing a dual challenge of childhood weight issues, with a rising trend in the prevalence of overweight children and a persistent problem of underweight children.

Malnutrition is a pathology that affects children

and adolescents, associated with various negative health outcomes, including delayed growth, impaired cognitive development, low IQ, behavioral problems, and more (6). Longitudinal studies indicate that malnutrition can serve as a precursor to stunted growth. Furthermore, stunting in early life is associated with poor cognitive development, lower rates of school enrollment and academic achievement, and ultimately, decreased productivity and learning potential (7).

Considering that the school-age period is the last and the second most rapid phase of height attainment after early childhood, this stage of life offers a final "window of opportunity" for addressing deficits and potentially achieving growth and cognitive recovery, mitigating its negative consequences for individuals and society (8). Children who remain stunted throughout their school years may face an increased risk of obesity. Growing evidence suggests that infants with rickets and children who experience rapid weight gain in childhood have an elevated risk of being overweight, obese, and developing non-communicable diseases in adulthood (9). This issue becomes increasingly important as many populations experience a secular transition from undernutrition to obesity. Under the significant global influence of the obesogenic environment and due to access to non-traditional foods, excess body weight and obesity among children and adolescents have become a major issue in developed countries, with developing nations following suit (10). According to World Health Organization data from 2018, the prevalence of overweight among adolescents has dramatically increased from 4% in 1975 to 18% in 2016. Currently, 216 million adolescents are overweight. The prevalence of obesity has risen from 0.7% in 1975 to 5.6% in 2016 among girls and from 0.9% to 7.8% among boys, affecting 124 million children in 2016. These figures highlight a concerning global trend of increasing overweight and obesity rates among adolescents (11).

In the Republic of Moldova, unhealthy eating is a significant risk and is classified among modifiable behavioral risks alongside smoking, alcohol consumption, and physical inactivity. According to statistics, nutrition contributes to 20% of the disease burden in the country, significantly impacting the onset of most major chronic diseases (5). A study conducted in 2022 at the School of Pub-

lic Health Management reveals a lack of knowledge and practices regarding nutrition among parents with young children (0-3 years). The research results show that 94% of parents are aware that candies are unhealthy, yet 58% of them introduce sweets into their children's diets by the age of 3 (12).

A study conducted in the Republic of Moldova in 2017, focusing on children aged 10-16 years, revealed the following findings: disturbances in body mass index were identified in 20.7% of cases. This demonstrated a predominance of children with excess weight in 16.2% of cases, compared to underweight children in 4.5% of cases. Of the total cases, overweight accounted for 13.5%, with obesity present in 2.8% of cases. Abdominal obesity was observed in 71.43% of obese children (13).

According to the Health Behavior among School Children studies conducted in 2014, 2017, and 2022 in the Republic of Moldova, the prevalence of overweight teenagers was observed to plateau, maintaining a steady rate of about 11-13% between 2014 and 2017, before rising to 17.2% in 2022 (14). Overall, about a third of adolescents have an abnormal body mass index – 1 in 7 adolescents is overweight and 1 in 5 is underweight. Among male adolescents, a higher prevalence of overweight is observed (14%) compared to females (9%). The mathematical model for diabetes and hypertension predicts that nearly 1,000 cases annually for each condition could be prevented if the prevalence of overweight in the country were to decrease by 5% (15).

The direct consequences of obesity on health encompass conditions such as dyslipidemia, hypertension, and dysglycemia, ultimately leading to the development of metabolic syndrome. A long-term consequence is the onset of arteriosclerosis, which begins in childhood. The clinical manifestations of cardiovascular diseases may not be immediately apparent, representing conditions without evident clinical symptoms. Another study conducted in the Republic of Moldova by Esanu V. et al. asserts that metabolic syndrome is a risk factor in children aged 10-18 years and is positively correlated with the initiation of left ventricular myocardial remodeling (RR: 1.7, 95% CI: 1.3–4.2, $p < 0.05$) (16). In addition to cardiovascular risk factors, overweight and obesity in children also

contribute to the development of type II diabetes, bronchial asthma, sleep apnea, fatty liver disease, gallstones, and menstrual irregularities. Until recently, many of the mentioned health conditions were primarily associated with adults; however, they are now highly prevalent among obese children (17).

Parental knowledge, attitudes, and practices related to health are crucial in managing children's body weight (18). Given the challenges associated with treating obesity, experts recommend that prevention efforts should begin in childhood (19). Furthermore, greater attention should be directed towards parents, who are often the primary caregivers of young children. In this role, parents establish a contextual environment that significantly influences their child's dietary and physical activity habits. Through their understanding of nutrition and feeding practices, parental behaviors profoundly affect their children's eating behaviors (20). These dietary patterns, established during childhood, tend to persist and may even continue into adulthood (21).

The relationship between diet and health is becoming increasingly clear, yet it remains complex, with many unanswered questions. Nonetheless, consumer segmentation and social marketing techniques assist health professionals in understanding their target audience and tailoring specific messages to different segments (22). Furthermore, the data provided by the KAP studies are invaluable. Additionally, these may serve to ground the Product, Price, Placement and Promotion policy within a social marketing program (23).

Given the lack of clear and unified messaging regarding the nutritional quality of foods consumed by school-age children in the Republic of Moldova, we hypothesize that there will be gaps in parental knowledge, attitudes, and practices concerning the dietary habits of their children. By testing this hypothesis, the study aims to assess parents' knowledge, attitudes, and practices regarding the nutrition of school-age children. This evaluation intends to offer insights into the factors influencing parental behaviors related to their children's nutrition and to inform targeted interventions designed to enhance the dietary habits and overall health outcomes of this vulnerable population.

MATERIAL AND METHODS

This quantitative study was conducted to assess the knowledge, attitudes, and practices of parents regarding the diets of school-aged children. It was selective, including 472 parents of school-aged children who participated in the survey by completing an online questionnaire on the Google Docs platform and on paper. The questionnaire, developed in the Romanian language, featured both closed and open-ended questions with multiple response options, drawing on the format of similar studies (24 – 27) and adhering to the KAP methodology (28). It underwent preliminary testing and was adapted based on the results of a pre-test before the final data collection. The data collected from the questionnaire were entered into the SPSS software for statistical processing, utilizing both descriptive (mean, median, standard deviation) and inferential (Spearman's rho, Kendall's tau_b, Chi-Square test) statistics.

The study sample primarily consisted of female parents, who accounted for 78% of the 472 participants included. The majority of respondents were aged between 36 and 45 years, constituting 46% of the sample. When categorized by living environment, 57% resided in urban areas, while 43% lived in rural areas. In terms of education, 35.6% of participants reported having higher education, 5.7% held a Ph.D. degree, 24.3% had completed high school, 19.9% had finished middle school, and 2.1% had primary education. Regarding employment status, 60.6% were employed, and 27.1% were not active in the workforce. The distribution of parents' body mass index (BMI) showed that 46.2% were of normal weight, 31.8% were overweight, and the remainder had various degrees of obesity (Grade I – 12.7%, Grade II – 4.7%, Grade III – 1.7%). Among the children included in the study, there was a nearly equal gender distribution, with 53% male and 47% female. The children were divided into age groups, with 47% in the younger school age group (6-11 years) and 53% in the older school age group (12-14 years). The majority of the children were of normal weight (50.2%), with the rest being underweight (33.3%), overweight (12.1%), and obesity grade I (4.4%).

RESULTS

The results of the KAP study on parents' knowledge, attitudes, and practices concerning

the diet of school-aged children show that the respondents possess a relatively significant level of knowledge ($76.9 \pm 19.07\%$) about their children's nutrition, indicating a solid foundation but also highlighting areas for improvement. However, the attitude score is below average ($59.99 \pm 10.7\%$), suggesting that parents might not prioritize their children's healthy nutrition as highly as necessary or may lack sufficient motivation to act accordingly. The practice score is the lowest ($45.5 \pm 16.6\%$), revealing that despite having positive knowledge and attitudes, parents often struggle to apply what they know and believe about their children's nutrition effectively.

In the context of the nutritional education strategy, it is considered that the percentage of correct responses, optimal practices, or positive/desirable attitudes should be at least 90%. From this perspective, the level of parents' knowledge is nearly satisfactory, but their attitudes and practices require substantial improvement to reach an optimal level of nutritional education.

Table 1 provides an analysis of the correlations between the level of knowledge, attitudes, and practices of parents and their socio-demographic data, as well as the BMI.

Parents' gender: A significant positive correlation was observed between their gender and both their knowledge ($\tau=.175, p<0.01; \rho=.185, p<0.01$) and attitudes ($\tau=.111, p<0.05; \rho=.111, p<0.05$) towards the nutrition of their children. This suggests that women are more involved in promoting a healthy lifestyle for children than men.

Parents' age: It presents a significant negative correlation with knowledge ($\tau=-.055, p<0.05; \rho=-.062, p<0.05$), indicating that older parents tend to have less knowledge about the nutrition and health of their children.

Parents' place of residence: A significant positive correlation was observed between the parent's place of residence and their knowledge about nutrition and health ($\tau=.227, p<0.01$ and $\rho=.240, p<0.01$). However, there was also a significant negative correlation between the place of residence and parents' practices regarding nutrition ($\tau=-.206, p<0.05$ and $\rho=-.206, p<0.05$). As a result, urban areas, while exhibiting higher knowledge levels, pose challenges related to dietary practices.

Table 1. Analysis of correlations between the level of knowledge, attitudes, and practices of parents and their socio-demographic data.

Parents	Spearman's rho (ρ)			Kendall's tau_b (τ)			
	Kn	At	Pr	Kn	At	Pr	
Gender	CC	.175**	.111*	-.089	.185**	.111*	-.090
	Sig. (2-tailed)	.000	.016	.052	.000	.016	.052
Age	CC	-.055*	.077	.000	-.062*	.083	.000
	Sig. (2-tailed)	.178	.072	1.000	.180	.072	1.000
Place of residence	CC	.227**	-.047	-.206**	.240**	-.047	-.206**
	Sig. (2-tailed)	.000	.305	.000	.000	.305	.000
Region of residence	CC	-.162**	.053	.104*	-.185**	.058	.112*
	Sig. (2-tailed)	.000	.212	.015	.000	.212	.015
Education level	CC	.011	.032	.131**	.012	.036	.145**
	Sig. (2-tailed)	.790	.441	.002	.788	.441	.002
Statute	CC	-.005	-.105*	-.018	-.005	-.110*	-.018
	Sig. (2-tailed)	.911	.017	.690	.920	.017	.690
BMI	CC	-.109**	.024	-.023	-.124**	.026	-.025
	Sig. (2-tailed)	.007	.578	.591	.007	.579	.592

** . The correlation is significant at the $p < 0.01$ level.

* . The correlation is significant at the $p < 0.05$ level.

CC - correlation coefficient, Kn - knowledge, At - attitude, Pr - practice

Region of residence: It presents a significant negative correlation with knowledge ($\tau = -.162$, $p < 0.01$ and $\rho = -.185$, $p < 0.01$), indicating that parents in certain regions have less knowledge about the nutrition and health of their children compared to parents in other regions. The Chi-Square test also indicates a significant relationship between parents' knowledge and the region in which they live ($X^2 = 46.424$, $df = 6$, $p < 0.001$). Symmetric measures show a significant negative relationship between parents' knowledge and the region in which they live, with parents in the Northern and Central regions having better knowledge about nutrition and health than those in the South and the municipality of Chisinau.

Parents' education level: A positive correlation was observed between the parent's level of education and their knowledge and attitudes toward the nutrition of school-aged children, as well as a significant positive correlation regarding their nutrition-related practices ($\tau = .131$, $p < 0.01$ and $\rho = .145$, $p < 0.01$). This indicates that parents with higher levels of education have better knowledge about the nutrition and health of their children and are more likely to have positive attitudes and implement this knowledge in the nutrition of their children.

Socio-economic status of parents: The correlation

coefficient was very small ($\rho = -0.005$ to -0.018), indicating an association between the knowledge, attitudes, and practices of parents regarding the nutrition of school-aged children and their socio-economic status. However, this association is relatively weak and not always significant.

Parents' BMI: A significant negative correlation was observed between their BMI and their knowledge about nutrition and health ($\tau = -.109$, $p < 0.01$ and $\rho = -.124$, $p < 0.01$). In other words, parents with a higher BMI have less knowledge about nutrition and health compared to those with a lower BMI.

In accordance with Table 2, which outlines the statistical correlations between children's BMI scalar values and parents' nutritional knowledge, attitudes, and practices, the Pearson correlation coefficient of 0.016 suggests a weak positive correlation between parents' knowledge and children's BMI, with a non-significant p-value of 0.730. This indicates a lack of substantial association between parents' knowledge levels and their children's BMI. In contrast, the Pearson correlation coefficient of -0.154^{**} reflects a statistically significant moderate negative correlation between parents' attitudes and children's BMI at the $p < 0.01$ level ($p = 0.001$). This suggests that an increase in positive parental attitudes is associated

with a decrease in their children's BMI. Similarly, the Pearson correlation coefficient of -0.119^{**} indicates a statistically significant moderate nega-

tive correlation at the $p < 0.01$ level ($p = 0.010$) between parents' practices and children's BMI, implying that favorable parental practices are linked to a reduction in their children's BMI.

Table 2. Statistical correlations between children's BMI scalar values and parents' nutritional knowledge, attitudes, and practices.

Parameters		Parents' knowledge	Parents' attitude	Parents' practices
Children's BMI	Pearson correlation	.016	-.154**	-.119**
	Sig. (2-tailed)	.730	.001	.010

** . The correlation is significant at the $p < 0.01$ level.

DISCUSSIONS

The identified factors – gender, age, place of residence, education level, and parents' BMI – emphasize their pivotal roles in shaping parental knowledge and attitudes toward child nutrition and health.

Our research supports the finding of Arlinghaus et al. regarding a gender disparity in the promotion of healthy lifestyles for children, showing that women are more involved than men (29). This gender gap in health information behavior, with women being more proactive in seeking health-related information, emphasizes the need for gender-sensitive interventions that particularly focus on engaging fathers in promoting healthy behaviors (30).

Thakur et al., in a recent review, emphasize that while nutrition knowledge is crucial, it alone is insufficient to guarantee healthy eating habits in children (31). Other factors, such as age, gender, nutritional status, lifestyle, and parents' education level, also show associations with knowledge and practices.

Our research shows a correlation: parental age is inversely related to knowledge of children's nutrition, suggesting that as parents get older, their comprehension of children's nutrition and health tends to decline. These results imply that age constitutes a significant factor influencing parents' awareness of nutritional and health-related matters concerning their children. This phenomenon may be explained by the limited access to and education on nutrition and health for older generations compared to the more informed younger ones. Additionally, older individuals often adhere to conservative dietary habits, which could affect their levels of nutritional knowledge.

Limited research directly comparing obesity determinants in urban and rural children has yielded conflicting results (32). Our study suggests that parents living in urban environments are more informed about new trends in nutrition and health, thereby possessing higher knowledge levels. However, the dietary practices of parents in urban areas might be influenced by the availability and accessibility of unhealthy fast-food options.

Research consistently shows a strong interconnection between parents' education level and their knowledge of children's nutrition, which is also confirmed in our study. Fadare found that higher levels of a mother's education are positively associated with child nutrition outcomes, suggesting that education can reinforce knowledge to produce better nutrition outcomes for children (33). Xu and Romanos-Nanclares both highlight the influence of parents' food knowledge and healthy-eating attitudes on their children's understanding of food and nutrition, as well as their diet quality and micronutrient intake (34, 35). Thakur further emphasizes the need for holistic behavior change strategies, including supporting food skills, to encourage healthy eating habits among children (31).

Our study aligns with a consistent body of research indicating that individuals with a higher BMI tend to have lower knowledge about nutrition and health compared to those with a lower BMI. This may be due to individuals with a higher BMI often being less concerned about their diet and overall health, leading to less knowledge on these topics. Additionally, parents with a higher BMI may be less inclined to adopt healthy lifestyles and make positive food choices for their

children, which can also affect their knowledge about nutrition and health. Kocaadam-Bozkurt found that children's BMI was negatively correlated with their physical activity and positively correlated with their mothers' and fathers' BMI (36). Vaitkeviciute identified associations between children's BMI and dietary behavior, as well as nutrition-related parenting practices (37). Williams further supported this by showing that families with overweight or obese parents had a less healthy nutrition and physical activity environment (38). Lee found that parental BMI was positively associated with their children's BMI, fat mass, and waist circumference, and negatively associated with their physical fitness (39). These studies collectively underscore the significant influence of parents' BMI on children's nutrition and health.

There are numerous research findings that underscore the significant impact of parents' nutritional knowledge, attitudes, and practices on children's BMI. However, our study found no significant correlation between parental knowledge and children's BMI. Possible explanations for this lack could include other influential factors not considered in the analysis, the complexity of the relationship, or the need for a more comprehensive investigation incorporating additional variables. Conversely, there is a correlation between unfavorable parental attitudes and practices and elevated BMI in children, suggesting that parental habits may influence their children's weight. Vaitkeviciute and Nordin both found that unhealthy dietary behavior and nutrition-related parenting

practices, such as the control of unhealthy food and the use of food as a reward or punishment, were positively associated with children's BMI (37 – 40). Conversely, positive practices like encouragement and a liberal attitude were negatively associated with BMI. Larsen further emphasized the importance of the home food environment, influenced by parental dietary behavior and food parenting practices, in shaping children's dietary behavior and BMI (41). Kocaadam-Bozkurt highlighted the role of parenting in influencing children's dietary intake and BMI, focusing on the importance of balanced nutrition and the family environment (36). These findings and our study results serve as a basis for developing social marketing programs aimed at inducing behavior change regarding nutrition in parents and their children.

LIMITATIONS OF THE STUDY

It is important to note that the data collection method, through self-administration of the questionnaire, has certain limitations, such as the possibility of untrue or idealized responses (e.g., about weight and height) due to factors like the lack of supervision or guidance from the researcher. It is crucial to acknowledge these limitations and strive to mitigate them by incorporating additional data collection methods. Additionally, the current study focused on children aged 6 to 14 years; therefore, future studies should assess how parents understand the importance of healthy nutrition and encourage good eating habits among their adolescents.

CONCLUSIONS

1. The conducted research enabled the assessment of parents' knowledge, attitudes, and practices regarding the nutrition of school-aged children, highlighting the obstacles and issues that make implementing this crucial process challenging in children's development.
2. The parents' knowledge level regarding the nutrition of school-aged children is high, with an average level recorded in the attitudes section and a low level of practice. Concerning the nutritional education strategy, the parents' knowledge level is almost satisfactory, but their attitudes and practices require significant improvement to achieve an optimal level of nutritional education.
3. Our statistical analysis highlights several significant correlations between demographic factors and parental knowledge and attitudes towards children's nutrition. Gender, age, place of residence, and region all play crucial roles in shaping parental understanding and perceptions regarding dietary habits. These findings underscore the importance of considering socio-demographic factors in designing targeted interventions aimed at promoting healthier dietary behaviors among children.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING ACKNOWLEDGEMENT

The study was conducted as part of a Master's research project within the School of Public Health Management.

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ETHICAL APPROVAL

The study does not present ethical risks and does not require examination and approval by a Research Ethics Committee.

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Date of receipt of the manuscript: 05/02/2024

Date of acceptance for publication: 24/03/2024



THE GENETIC BASIS OF GRAM-NEGATIVE BACTERIA RESISTANT TO ANTIMICROBIALS ISOLATED FROM INVASIVE INFECTIONS IN THE REPUBLIC OF MOLDOVA

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DOI: 10.38045/ohrm.2024.2.04

CZU: [616.98:579.84+615.33.015.8+575](478)

Keywords: antimicrobial resistance, resistance enzymes, resistance genes, gram-negative bacilli, carbapenemases.

Introduction. Despite the efforts made and measures taken to combat antimicrobial resistance, alarming levels of resistance in gram-negative bacteria continue to be reported on a global scale. The antimicrobial resistance mechanisms of these bacteria represent the main cause of therapeutic failures.

Material and methods. A retrospective analysis of strains of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* isolated from patients with invasive infections was conducted for the period 2020-2023. Screening for carbapenemase-producing strains was performed based on sensitivity to antimicrobial agents tested using the Vitek-2 compact automated system. Confirmation of resistance mechanisms was achieved through multiplex PCR molecular biology technique.

Results. The analysis of the obtained data indicates high resistance among strains of *K. pneumoniae* to fluoroquinolones (77.2%), while the majority of *E. coli* strains were resistant to penicillins (53.5%). Concerningly, non-fermentative bacilli strains also present alarming figures, with over 60.0% of *P. aeruginosa* strains resistant to penicillins, cephalosporins, fluoroquinolones, carbapenems, and over 80.0% of *A. baumannii* strains resistant to all tested antimicrobial groups. The resistance enzyme OXA-48 was detected in 91.7% of *K. pneumoniae* strains and 15.6% of *E. coli* strains, while the *bla*NDM resistance gene was detected in 15.9% of *P. aeruginosa* isolates, and the *bla*OXA-23 gene was identified in 55.2% of *A. baumannii* isolates.

Conclusions. The rapid identification of multi-drug resistant gram-negative bacilli ensures the success of therapy for infections caused by them, and monitoring resistance profiles is an essential step for subsequent actions to combat antimicrobial resistance.

Cuvinte-cheie: rezistență la antimicrobiene, enzime de rezistență, gene de rezistență, bacili gram-negativi, carbapenemaze.

BAZELE GENETICE ALE BACTERIILOR GRAM-NEGATIVE REZISTENTE LA ANTIMICROBIENE IZOLATE DIN INFECȚIILE INVAZIVE ÎN REPUBLICA MOLDOVA

Introducere. În pofida eforturilor depuse și măsurilor întreprinse în combaterea rezistenței la antimicrobiene, continuă să fie raportate, în context global, nivele îngrijorătoare de rezistență a bacteriilor gram-negative. Mecanismele de rezistență la antimicrobiene ale acestor bacterii reprezintă principala cauză a eșecurilor terapeutice.

Material și metode. A fost efectuată analiza retrospectivă a tulpinilor de *E. coli*, *K. pneumoniae*, *P. aeruginosa* și *A. baumannii* izolate de la pacienți cu infecții invazive, în perioada 2020-2023. Screeningul tulpinilor producătoare de carbapenemaze s-a efectuat în baza sensibilității la preparatele antimicrobiene testate prin sistemul automat Vitek-2 compact. Confirmarea mecanismelor de rezistență a fost realizată prin tehnica de biologie moleculară – PCR multiplex.

Rezultate. Analiza datelor obținute denotă rezistență înaltă a tulpinilor de *K. pneumoniae* la fluorochinolone (77,2%), în timp ce cele mai multe tulpini de *E. coli* au fost rezistente la peniciline (53,5%). Motive de îngrijorare prezintă și tulpinile de bacili nonfermentativi cu peste 60,0% tulpini de *P. aeruginosa* rezistente la peniciline, cefalosporine, fluorochinolone, carbapeneme și peste 80,0% tulpini de *A. baumannii* rezistente la toate grupele de antimicrobiene testate. Enzima de rezistență OXA-48 a fost detectată la 91,7% tulpini de *K. pneumoniae* și 15,6% tulpini de *E. coli*, în timp ce la 15,9% izolate de *P. aeruginosa* a fost detectată gena de rezistență *bla*NDM, iar la 55,2% izolate de *A. baumannii* s-a identificat gena *bla* OXA-23.

Concluzii. Identificarea rapidă a bacililor gram-negativi multirezistenți asigură succesul terapiei infecțiilor determinate de acestea, iar monitorizarea profilurilor de rezistență este etapa esențială pentru acțiunile ulterioare de combatere a rezistenței la antimicrobiene.

INTRODUCTION

The phenomenon of antimicrobial resistance (AMR) is currently extensively addressed among researchers worldwide due to the rapid spread of multi-drug resistant microorganisms and their impact on public health (1, 2).

Despite all global efforts to reduce the incidence of infections caused by multidrug-resistant strains (MDR), this phenomenon has escalated and seems unstoppable. Furthermore, the spread of antimicrobial resistance is still fuelled by irrational prescription and consumption of antimicrobial agents (1, 2).

Invasive infections are usually associated with high mortality due to delayed appropriate antimicrobial therapy, as well as determining the source of infection.

A major challenge for patients with invasive infections has become multidrug-resistant Gram-negative bacilli (GNB), which represent an important target in AMR surveillance and monitoring (1, 2). In recent decades, *Enterobacteriaceae* and non-fermentative GNB have become the main causative pathogens of invasive infections, accounting for 50–75% of all cases of infections and from 15 to 42% of deaths (3).

Extended-spectrum beta-lactamases (ESBLs) are responsible for hydrolyzing penicillins, monobactams, and third-generation cephalosporins, while carbapenemases and metallo-beta-lactamases (MBLs) can hydrolyze drugs from the carbapenem group (4).

Resistance to carbapenems has been primarily conferred by the production of carbapenemases (5). Currently, 350 variants of carbapenemases have been identified and described worldwide, which are clinically significant, with dominant enzymes including KPC, NDM, VIM, IMP, and OXA-48 (6).

Based on numerous studies conducted worldwide, it has been established that characteristic features of multidrug-resistant strains of non-fermentative Gram-negative bacilli, including *P. aeruginosa*, include the production of enzymes such as NDM, IMP, VIM, and OXA. In strains of *A. baumannii*, the presence of MBL, NDM, and OXA enzymes has been described, but these strains usually also co-harbor various extended-spectrum β -lactamases. The OXA-23 enzyme has been most commonly detected in carbapenem-resistant *A.*

baumannii, and globally, high rates of dissemination (77-100%) of various types of OXA enzymes have been reported in this species (7).

Among multidrug-resistant (MDR) and extensively drug-resistant (XDR) isolates of GNB, ESBL-producing strains and carbapenem-resistant isolates (CRE) have become particularly concerning, especially for medical institutions, due to limited treatment options and high mortality rates (3).

Over 19% of healthcare-associated infections are caused by ESBL-producing bacteria. The mortality rate caused by these bacteria is 57% higher in bloodstream infections compared to mortality from infections caused by sensitive strains (4).

The World Health Organization (WHO) has highlighted the species *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae* as a critical priority for the development of new antibiotic options, given that carbapenems, used in the therapy of these infections as a last resort, are no longer effective (1, 2).

For invasive infections, precise diagnosis and initiation of appropriate antimicrobial therapy are essential to increase the patient's chances of survival, thus reducing the high incidence of morbidity and mortality (8).

Molecular-genetic testing of antimicrobial-resistant strains represents a rapid and precise method for detecting the molecular determinants of antimicrobial resistance.

The aim of this study was to determine the resistance profiles and highlight the different variants of carbapenemases detected among *Enterobacteriaceae* and non-fermentative GNB strains isolated from patients with invasive infections in the Republic of Moldova.

MATERIAL AND METHODS

A retrospective cross-sectional study was conducted, including strains of *Enterobacteriaceae* and non-fermentative Gram-negative bacilli resistant to antimicrobial agents, isolated in the 17 laboratories that are part of the national network for epidemiological surveillance of antimicrobial resistance, during the period 2020-2023.

The research included species listed in the global surveillance list of antimicrobial resistance developed by the WHO: *K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *A. baumannii*.

The strains were identified using the Vitek2 Compact automated system (BioMerieux), utilizing GN identification cards. Screening of resistant strains was conducted based on their testing with antimicrobial agents, performed using the disc diffusion method, as well as through automated method using the same Vitek2 Compact system, using AST-N204 cards for Enterobacteriaceae, containing antibiotics: amikacin, amoxicillin / acid clavulanic, ampicillin, cefepime, cefotaxime, ceftazidime, ciprofloxacin, ertapenem, fosfomycin, gentamicin, imipenem, meropenem, nitrofurantoin, norfloxacin, piperacillin / tazobactam, and AST-N222 for non-fermentative GNB, containing antibiotics: amikacin, aztreonam, cefepime, ceftazidime, ciprofloxacin, colistin, gentamicin, imipenem, meropenem, minocycline, pefloxacin, piperacillin, piperacillin / tazobactam, rifampicin, ticarcillin, ticarcillin / acid clavulanic, tobramycin, which are recommended antimicrobials by the EUCAST standard. Interpretation was also conducted based on the EUCAST standard.

Subsequently, resistant strains were tested using phenotypic methods and molecular biology tests to confirm the presence of resistance mechanisms.

The genetic basis of resistant strains isolated from blood was determined through molecular-genetic testing (multiplex PCR).

A database was created in Excel to systematize the obtained information, followed by the subsequent calculation of respective statistical indices, such as relative values for isolates resistant to antimicrobial agents, relative values for strains positive for phenotypic testing, relative values of resistance enzymes detected in strains investigated through confirmation methods (multiplex PCR), confidence interval for calculated values. These indicators were calculated cumulatively for all four years for each isolated pathogen.

The normality of data distribution was tested using the Shapiro-Wilk and d'Agostino-Pearson methods. Multivariate analysis was conducted to determine variables with significant independent correlations. Z-tests and Chi-square tests were performed to determine the statistical significance of observed differences. Values with $p \leq 0.05$ were considered statistically significant.

The respective research conducted surveillance of GNB isolated from blood, and the chosen criteria and methods were justified because blood is a sterile biosubstrate, making it more likely to represent true infections. Additionally, GNB is the group currently showing the highest resistance rates worldwide.

RESULTS

Throughout the years 2020-2023, a total of 734 strains of Enterobacteriaceae and non-fermentative Gram-negative bacilli isolated from blood were investigated, including 46.4% (95% CI 44.3-48.6) strains of *K. pneumoniae*, 11.8% (95% CI 9.7-14.0) strains of *E. coli*, 33.3% (95% CI 31.2-35.5) strains of *A. baumannii*, and 8.5% (95% CI 6.4-10.7) strains of *P. aeruginosa*.

The analysis of antimicrobial sensitivity of the isolated microorganisms during the period 2020-2023 highlighted high resistance among the studied microorganisms to various groups of antimicrobial agents.

In the analysis of resistance data, it was found that *K. pneumoniae* strains exhibited significantly higher levels of resistance compared to *E. coli* strains. The majority of *K. pneumoniae* isolates were resistant to fluoroquinolones (77.2%; 95% CI 75.1-79.4), followed by cephalosporins and penicillins, with 70.5% (95% CI 68.4-72.7) and 68.7% (95% CI 66.6-70.9) resistant isolates, respectively. *E. coli* showed higher resistance to penicillins – 53.5% (95% CI 51.4-55.7) and fluoroquinolones – 44.9% (95% CI 42.8-47.1).

The analysis of resistance profiles in *E. coli* highlights only 1.7% (95% CI -0.5-3.9) of isolates resistant to carbapenems, while resistance to these agents in *K. pneumoniae* strains was recorded at 40.4% (95% CI 38.3-42.6) of isolates, showing a consistent increase over the years of study.

The combined resistance to cephalosporins, fluoroquinolones, and aminoglycosides in *E. coli* strains isolated during the research period was 8.1% (95% CI 6.0-10.3), while multidrug-resistant *K. pneumoniae* strains constituted 63.4% (95% CI 61.3-65.6).

The results of antimicrobial susceptibility testing for non-fermentative Gram-negative bacilli

(*P. aeruginosa* and *A. baumannii*) isolated during the research period showed similarly alarming levels of resistance. Practically, for all groups of tested antimicrobial agents, these microorganisms exhibited resistance in over 50% of isolates. Furthermore, this resistance showed a consistent increase in these indicators each year.

The highest proportion of resistant strains of *P. aeruginosa* was observed for penicillins, with 65.1% (95% CI 63.0-67.3) of resistant isolates, followed by cephalosporins and fluoroquinolones with a resistance of 63.5% (95% CI 61.4-65.7)

strains. Additionally, a high level of resistance was recorded for carbapenems – 60.3% (95% CI 58.2), and combined resistance to cephalosporins, fluoroquinolones, and aminoglycosides in *P. aeruginosa* strains constituted 52.4% (95% CI 50.3-54.6). Of the total isolates of *A. baumannii*, 99.6% (95% CI 97.5-101.8) were resistant to fluoroquinolones, 93.2% (95% CI 91.1-95.4) to carbapenems, and 88.4% (95% CI 86.3-90.6) to aminoglycosides. The proportion of strains resistant to carbapenems, fluoroquinolones, and aminoglycosides concurrently constituted 80.3% (95% CI 78.2-82.5) (fig. 1).

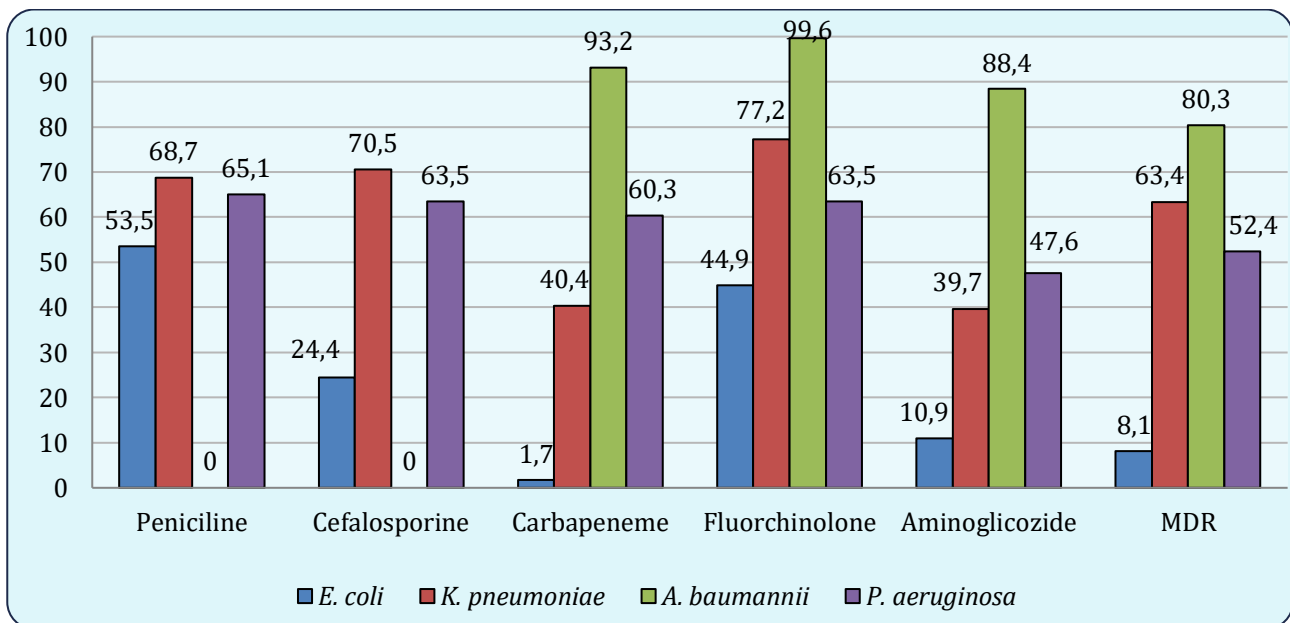


Figure 1. The proportion (%) of *Enterobacteriaceae* and non-fermentative GNB strains resistant to tested antimicrobial groups, 2020-2023.

Based on the antibiotic susceptibility testing, strains suspected to possess resistance mechanisms were selected. The main resistance mechanisms detected in these strains were the production of carbapenemases and extended-spectrum beta-lactamases.

Screening for the production of extended-spectrum beta-lactamases was conducted based on resistance to cephalosporins, and the confirmation of this resistance mechanism was performed only through phenotypic methods (double-disk synergy test, combined disk test). Therefore, this resistance mechanism was not fully elucidated in the respective study.

Screening for the production of carbapenemases by the researched microorganisms was conduc-

ted based on susceptibility to carbapenems (meropenem, imipenem, and ertapenem), and the confirmation of the presence of resistance enzymes was performed through molecular-genetic method – Polymerase Chain Reaction.

Thus, from the isolated strains during the study period, suspected to produce carbapenemases were 54.3% (95% CI 52.2-56.5) of *K. pneumoniae* strains, 96.8% (95% CI 94.5-99.0) of *A. baumannii* strains, 100% (95% CI 97.9-102.2) of *P. aeruginosa* strains, and 1.8% (95% CI -0.4-4.0) of *E. coli* strains.

The PCR test allowed the detection of blaOXA-48, blaKPC, blaVIM, blaIMP, and blaNDM resistance genes in the isolated strains. Thus, the blaOXA-48 gene was present in 91.7% (95% CI 89.6-93.9) of

K. pneumoniae strains, 15.6% (95% CI 13.5-17.8) of *E. coli* strains, and 11.1% (95% CI 9.0-13.3) of *P. aeruginosa* strains. The blaKPC gene was detected in 8.3% (95% CI 6.2-10.5) of *K. pneumoniae* strains and 4.8% (95% CI 2.7-7.0) of *P. aeruginosa* isolates, while the blaNDM gene was recorded in 35.8% (95% CI 33.7-38.0) of *K. pneumoniae* strains, 15.9% (95% CI 13.8-18.1) of *P. aeruginosa* strains, and 1.2% (95% CI -1.0-3.4) of *E. coli* strains. The blaVIM gene was detected only in

P. aeruginosa strains in 12.7% (95% CI 10.6-14.9) of cases. In *A. baumannii* strains, the blaOXA-23 gene was identified in 55.2% (95% CI 53.1-57.4) of isolates, blaOXA-40 in 11.2% (95% CI 9.1-13.4) of isolates, and blaOXA-58 in 20.4% (95% CI 18.3-22.6) of isolates. Additionally, the method identified 2 or even 3 resistance genes simultaneously in the same isolate in 9.0% of cases (95% CI 6.9-11.2) (fig. 2).

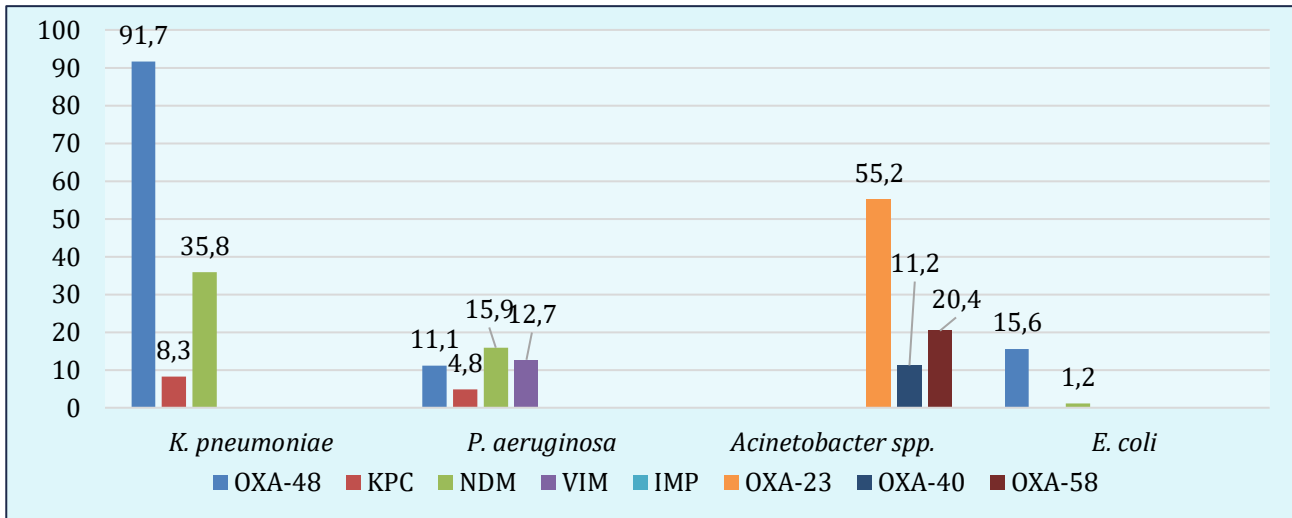


Figure 2. The spectrum of resistance genes detected by PCR method according to the isolated species.

DISCUSSIONS

The study focused on analyzing strains of *Enterobacteriaceae* and non-fermentative gram-negative bacilli resistant as causal agents of invasive infections, and the research results highlighted that *K. pneumoniae* was the main determinant of these infections. Two international studies on invasive infections conducted in two different hospitals in Brazil also found that monomicrobial episodes of invasive infections were largely caused by gram-negative bacteria, selecting those species for research as in the respective work, and also highlighted the prevalence of the *K. pneumoniae* species (9, 10).

Another cross-sectional study conducted in a tertiary hospital in Addis Ababa, Ethiopia found that the majority of bacteria involved in the etiology of invasive infections were gram-negative bacilli (54.2%). Among these microorganisms, *K. pneumoniae* species predominated (32.5%), followed by *Acinetobacter spp.* (20.4%) and *E. coli* (16.5%).

The same consistency in the etiology of invasive infections analyzed was observed in the given

study (4).

Researchers describe *Klebsiella spp.* as the predominant microorganism isolated among gram-negative bacteria in patients with invasive infections from a tertiary care hospital in Malé, Maldives, similar to the research provided. However, in the etiological spectrum of these infections, *E. coli* (8.9%), *Pseudomonas spp.* (12.5%), and *Acinetobacter spp.* (1.5%) follow, which differs from the results obtained in this study (11).

According to the study results, antibiotics from the aminoglycoside group have proven to be the most effective against infections caused by *K. pneumoniae*, which is consistent with the findings of a study conducted at the Laboratory for Research on Hospital Infections (LAPSA FIOCRUZ) in Brazil, where amikacin was the most effective antimicrobial against *K. pneumoniae* (1).

High rates of carbapenem resistance exceeding 90.0% in *K. pneumoniae* may be associated with specific carbapenemase genes that are more com-

monly encountered. Thus, in the same studies conducted in hospitals in Brazil, as mentioned above, the blaKPC resistance gene was most frequently detected in *K. pneumoniae* (1), which does not correspond with the results of this research, where we found that the most commonly detected resistance enzyme in *K. pneumoniae* strains was OXA-48.

Based on prospective surveillance of patients with positive blood cultures at São Rafael Hospital, researchers in Brazil found that the predominant enzymatic variants regarding resistance determinants in multidrug-resistant gram-negative bacilli were SHV, TEM, OXA-1-like, and CTX-M-gp1, while the genes KPC, VIM, OXA-48, NDM, and OXA-23 were characterized as emerging enzymes (8).

In a study conducted on residents of a nursing home in Spain, a significant association was established between the use of medical devices such as venous catheters, urinary catheters, and colonization with carbapenem-resistant GNB. The most commonly isolated pathogen was *K. pneumoniae*, with the blaOXA-48 gene identified. A similar study was conducted in a nursing home in Israel, which also highlighted the prevalence of *K. pneumoniae*. However, the most frequently identified enzyme in these strains was KPC (7).

Similar to the findings in the given study, OXA-48 production has been considered the main mechanism conferring carbapenem resistance among *Enterobacteriaceae* in Tunisia, as well as in Mediterranean countries, which have been considered endemic (12, 13). However, high carbapenem resistance among *A. baumannii* species has typically been associated with the production of OXA-23, OXA-58, and intrinsic carbapenemases such as OXA-51 (12). The same was found in a study conducted by researchers in Brazil, where OXA-23 carbapenemase was the most widespread enzyme detected in *A. baumannii* strains (1, 14, 15).

In contrast to other regions, numerous studies conducted in India have reported isolates of *A. baumannii* responsible for invasive infections, in which the blaNDM gene has been detected (7).

The identification of these resistance enzymes in *A. baumannii* confers resistance to all classes of antimicrobial agents, necessitating the urgent development of new effective antimicrobial agents.

In a study analyzing the mechanisms of resistance in imipenem-resistant *P. aeruginosa* strains responsible for 87 cases of invasive infections in southern Taiwan, Kao et al. found that the most frequently detected carbapenemases were of the VIM type, followed by the OXA type. These findings were consistent with studies conducted in China but differed from those observed in the respective research, where the most frequently detected enzyme in *P. aeruginosa* strains was NDM, followed by VIM (7).

A study conducted by Schäfer et al. at three medical centers in Germany highlighted the prevalence of the blaVIM resistance gene in 30.6% of *P. aeruginosa* strains. Similar data (32% of *P. aeruginosa* strains producing VIM) were recorded by Kateete et al. in a study conducted in Uganda and by Moubareck et al. in research conducted in hospitals in Dubai (7).

An outbreak of extensively drug-resistant *P. aeruginosa* was reported in a tertiary care pediatric hospital in Italy. The study conducted on this outbreak revealed the prevalence of *P. aeruginosa* strains producing the VIM carbapenemase. Furthermore, it was found that the risk of colonization with this species increased with prolonged hospitalization duration (7).

In a study conducted in China, Hu et al. found that all isolates of *E. coli* identified in patients with invasive infections were found to be producers of the NDM enzyme (7). This contradicts the results obtained in the respective research, where the majority of isolated *E. coli* strains were found to carry the OXA-48 enzyme.

Comparable findings to the provided research were obtained in a study conducted in Spain on 121 isolates of carbapenem-resistant *E. coli*, which highlighted the prevalence of the blaOXA-48 gene in 71.9% of strains. The blaVIM gene was detected in 22.3% of strains; the blaKPC gene in 3.3% of strains, the blaNDM gene in 1.7% of strains, and 0.8% of strains harbored the blaIMP gene (16).

A global study highlights carbapenemase NDM as the most commonly identified in isolates of *E. coli*, followed by KPC, whereas studies conducted in Portugal and China highlight the prevalence of the KPC carbapenemase type (16).

CONCLUSIONS

1. Monitoring the resistance profiles of GNB involved in infectious pathology, as well as detecting resistance mechanisms, is indispensable for assessing the spread of AMR and identifying new alternatives in antimicrobial therapy. This process is crucial for implementing national measures to reduce this phenomenon.
2. The results obtained in the study highlight an alarming trend of increasing resistance indices towards most groups of antimicrobial agents. Among these, a high proportion of resistance is noted in *K. pneumoniae* to fluoroquinolones and cephalosporins, in *P. aeruginosa* strains to carbapenems, as well as in *A. baumannii* with extremely high levels of resistance to all tested groups of antimicrobial agents.
3. The investigation of Enterobacteriaceae strains and non-fermentative GNB for the presence of resistance mechanisms has revealed a significant number of strains producing resistance enzymes. Furthermore, in 9.0% of cases, strains with two or more antimicrobial resistance enzymes were recorded.
4. The main recorded resistance mechanisms were the production of carbapenemases, with their types varying from one species to another. Thus, these resistance mechanisms were confirmed in the majority of *A. baumannii* isolates (84.4%), predominantly featuring the OXA-23 carbapenemase, in *K. pneumoniae* and *E. coli* strains - the OXA-48 enzyme, and in *P. aeruginosa* isolates - the NDM metallo-beta-lactamase.
5. The study results, along with the analysis of international studies in this field, point to the need to pay particular attention to GNB, especially regarding the transmission of virulence factors and resistance genes, particularly between species of *K. pneumoniae* and *Acinetobacter* spp.

ETHICAL APPROVAL

Favorable opinion of the Research Ethics Committee of the *Nicolae Testemitanu* State University

of Medicine and Pharmacy, No. 1, dated September 27, 2022.

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Date of receipt of the manuscript: 08/02/2024

Date of acceptance for publication: 28/03/2024



CLINICAL ASPECTS OF PREGNANCY AND CHILDBIRTH IN WOMEN WITH INTRAHEPATIC CHOLESTASIS OF PREGNANCY

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DOI: 10.38045/ohrm.2024.2.05

CZU: [618.3+618.5]:616.36-008.811.6

Keywords: intrahepatic cholestasis of pregnancy, pregnancy, childbirth.

Introduction. Intrahepatic cholestasis of pregnancy (ICP) is a liver pathology that has negative impact on progression of pregnancy and childbirth in affected women. This study aims to assess the clinical characteristics of pregnancy and delivery among women diagnosed with intrahepatic cholestasis of pregnancy.

Material and methods. The study was conducted as a prospective cohort study involving 142 clinical cases, divided into two groups according to the complication of pregnancy with cholestasis gravidarum. Evaluation of pregnancy and childbirth clinical aspects was performed alongside an examination of medical records. Statistical data were processed using IBM Statistics SPSS 21, MedCalc and GraphPad software.

Results. It was determined that every fourth pregnant woman diagnosed with intrahepatic cholestasis of pregnancy also presented with hyperemesis gravidarum during the current pregnancy. Additionally, an elevated prevalence of iron deficiency anemia and gestational diabetes mellitus was detected among pregnant women with this condition. Meconium staining of the amniotic fluid complicated approximately one in every four pregnancies in women with intrahepatic cholestasis gravidarum.

Conclusions. The study findings indicate a notable increase in hepato-biliary conditions and nephrouinary pathology among women with ICP. Hence, a high incidence of hyperemesis gravidarum, along with an elevated frequency of iron deficiency anemia and gestational diabetes mellitus, was found in women with this condition.

Cuvinte-cheie: colestază intra-hepatică de sarcină, sarcină, naștere.

ASPECTELE CLINICE ALE SARCINII ȘI NAȘTERII LA FEMEILE CU COLESTAZĂ INTRA-HEPATICĂ DE SARCINĂ

Introducere. Colestaza intrahepatică de sarcină este o patologie hepatică, care are un impact negativ asupra evoluției sarcinii și nașterii la femeile cu patologie cercetată. Studiul a avut drept scop evaluarea particularităților clinice ale sarcinii și nașterii la femeile cu colestază intrahepatică de sarcină.

Material și metode. Premisa cercetării s-a bazat pe un studiu prospectiv de cohortă a 142 de cazuri clinice, divizate în două loturi, în funcție de complicațiile în sarcină cu afecțiunea colestaza gravidarum. Cercetarea a fost realizată prin aprecierea particularităților sarcinii și nașterii la femeile cu patologia data și analizarea documentației medicale. Datele statistice au fost prelucrate prin intermediul programului IBM Statistics SPSS 21, MedCalc și GraphPad.

Rezultate. S-a constatat că, fiecare a patra gravidă cu colestază intrahepatică de sarcină a fost diagnosticată cu hiperemesis gravidarum în sarcina curentă și s-a depistat o frecvență crescută a anemiei feriprive și a diabetului gestațional în rândul gravidelor cu patologie cercetată. Astfel, la femeile cu colestaza gravidarum, aproximativ fiecare a patra sarcină a prezentat complicații la nivel intrauterin, prin-culoarea meconială a lichidului amniotic.

Concluzii. Rezultatele studiului au demonstrat că ratele afecțiunilor hepato-biliare și nefrouinare au fost semnificativ mai mari la femeile cu colestaza intrahepatică de sarcină, identificându-se o rată crescută de hiperemesis gravidarum, o frecvență crescută a anemiei feriprive și a diabetului gestațional la femeile cu patologia cercetată.

INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is a liver condition, that occurs in pregnancy, with an incidence of 0.5-1% worldwide (1). The incidence of the condition varies widely, depending on several factors. Despite being a significant medical concern, ICP often is overlooked. It can lead to severe complications for both the mother and fetus. However, literature data show that ICP generally follows a favorable course for mothers and yields positive outcomes. In most cases ICP develops towards the end of the second trimester or early in the third trimester, with clinical symptoms and laboratory abnormalities resolving within 2-3 weeks postpartum (2).

However, in recent years, a number of case reports have been published concerning potential fetal complications leading to adverse perinatal outcomes, related to ICP (3). One of the most dangerous complications of cholestasis gravidarum is intrauterine fetal death. At the same time, there is an increased incidence of premature births (spontaneous or iatrogenic) among women whose pregnancies are complicated by ICP (4, 5). However, the rate and nature of fetal risks associated with ICP are not fully understood (6). The authors consider that ICP may be one of the causes of birth complications. Perinatal outcomes are of clinical interest in the management of pregnancy and childbirth in women with cholestasis gravidarum, given the potential for preterm birth, intrauterine fetal death, and postpartum hemorrhage (7, 8).

The purpose of the study was to assess clinical aspects of pregnancy, and childbirth in women with intrahepatic cholestasis of pregnancy.

MATERIAL AND METHODS

The prospective cohort study was conducted at the Department of Obstetrics and Gynaecology, within *Nicolae Testemitanu* State University of Medicine and Pharmacy, during the period 2020-2022.

The representative research sample was calculated using the EpiInfo 7.2.2.6 program, specifically the StatCalc - Sample Size and Power section, for the observational analytical cohort study, based on the following parameters:

- Confidence interval (CI) for a 95.0% level of significance:

- Statistical power - 80.0%;

- The average difference in pregnancy outcomes between women with intrahepatic cholestasis of pregnancy (ICP) and those without is 20.0% (9);

- Group ratio - 1:1.

Result: The calculated value for a 95.0% CI is 44, with a 10.0% non-response rate, yielding n=48.

Thus, according to the obtained data, the recommended sample size for a representative research group should be no fewer than 48 patients.

For the prospective research two groups were created:

- Group A - 71 pregnant women whose pregnancy was complicated by ICP (research group, L1);

- Group B - 71 pregnant women whose pregnancies were not complicated by ICP (control group, L0).

The inclusion criteria for the research group were as follows: pregnant women diagnosed with ICP, with the exclusion of other potential causes of skin pruritus (ICP diagnosis was established based on clinical data and biochemical test results); serum bile acids level $\uparrow 10 \mu\text{mol/L}$; gestational age between 22^o-41^o weeks; patient age ≥ 18 years; informed consent, in written form, for research participation.

Exclusion criteria included women with known liver diseases such as acute viral hepatitis, autoimmune hepatitis, Wilson's disease, primary sclerosing cholangitis, primary biliary cirrhosis, and symptomatic cholelithiasis. Additionally, individuals diagnosed with cytomegalovirus and Epstein-Barr virus were excluded due to their potential to induce liver injury with elevated liver enzyme levels. Other exclusion criteria comprised drug-induced hepatitis, acute fatty liver of pregnancy, pre-eclampsia, HELLP syndrome, congenital thrombophilia, and women diagnosed with epilepsy to exclude the possible influence of antiepileptic medication on intestinal vitamin K absorption.

The diagnosis of ICP was based on anamnestic, clinical and biochemical data, medical documentation (obstetric and medical observation - form no. 96/e).

Statistical data were processed using *IBM Statistics SPSS 21*, *MedCalc* and *QuickCalcs* section of *GraphPad*. Statistical processing of data obtained by applying specific statistical operations: systematization of the material, statistical grouping according to parameters and levels, obtaining primary indicators and statistical data series; calculation of absolute (numbers) and/or relative (percentages) frequencies for nominal or categorical variables, and 95% CI for proportion. For continuous variables, mean values and standard deviations (SD) were calculated, while the median (Me) and interquartile range (Q1; Q3) were determined for characteristics exhibiting non-normal distributions. To compare categorical variables, the χ^2 test with Yates' continuity correction was employed, while the t-test was used to assess statistical differences between the two means.

RESULTS

The age of the pregnant women included in the study ranged from 18-43 years, while 37 women (52.1%; 95% CI: 36.6-71.8%) in L1, compared to the control group - 22 women (30.9%; 95% CI: 19.4-46.9%), were over 30 years of age (χ^2 5.683, $p=0.0171$). The mean age

of the study participants was 29.5±6.3 years [Me 30 (25; 34)] in the research group and 27.3±5.4 years [Me 27 (23; 31)] in the control group ($p=0.029$).

In order to assess the clinical course of pregnancy in women with ICP, it was important to consider their extragenital history. There were assessed 9 indicators, and 21 women were determined with hepato-biliary conditions viz. 29.6% (95% CI: 18.3-41.5%) in the L1 vs L0 - 8 or 11.3% (95% CI: 3.8-19.4%), indicating a significantly higher risk of developing this condition during the current pregnancy (RR 2.6250, 95% CI: 1.2463-5.5290, $p=0.0111$). Similarly, gastrointestinal conditions were present in 10 women (14.1%, 95% CI: 18.3-41.5%) in the L1 vs L0 - 8 women (11.3%, 95% CI: 3.8-19.4%), RR 1.250, 95% CI: 0.5239-2.9823, $p=0.615$. However, nephrourological conditions were determined in 22 women (31.0%, 95% CI: 21.1-43.9%) in the L1 vs L0 - 10 women (14.1%, 95% CI: 5.2-24.6%), RR 2.2000, 95% CI: 1.1244-4.3047, $p=0.0213$. However, no statistically significant difference was found when assessing other indicators of complicated extragenital history between the pregnancies with ICP and those in the control group (Figure 1).

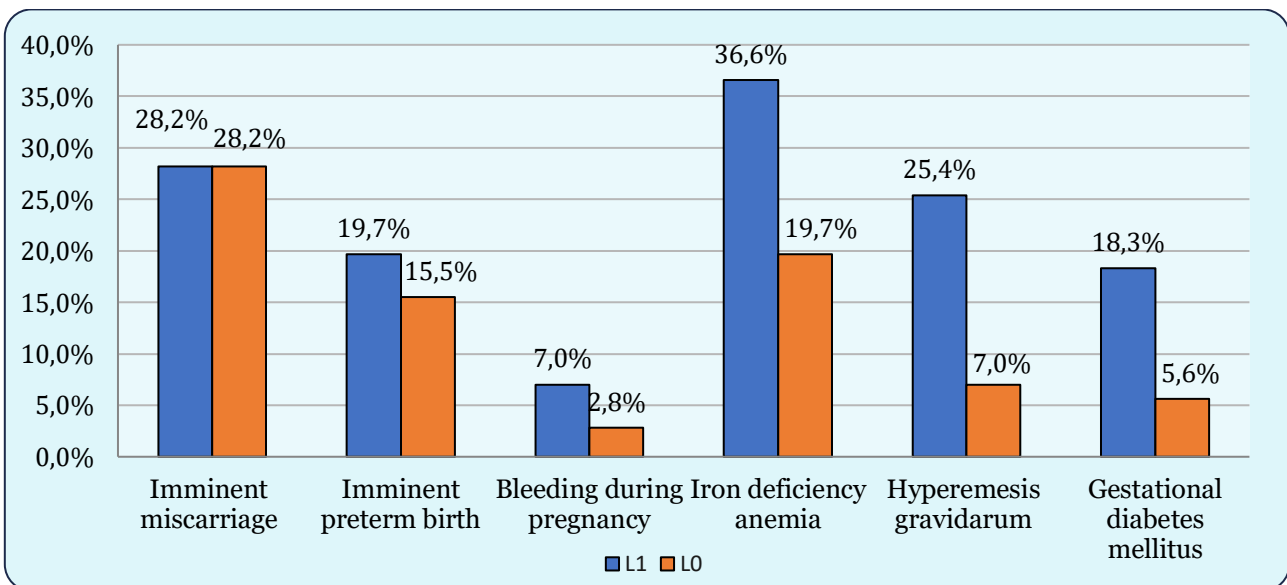


Fig. 1. Pregnancy outcome of pregnant women included in the study (%).

The study found no statistical difference in the number of complicated cases with imminent miscarriages [L1 - 20 women or 28.2% (95% CI: 18.3-38.5%) vs L0 - 20 or 28.2% (95% CI: 18.3-38.5%), χ^2 0.000, $p=1.0000$], imminent preterm birth [L1

- 14 women or 19.7% (95% CI: 9.9-32.8%) vs L0 - 11 or 15.5% (95% CI: 5.2-27.2%), χ^2 0.194, $p=0.6595$], bleeding during pregnancy [L1 - 5 cases or 7.0% (95% CI: 1.2-13.1%) vs L0 - 2 or 2.8% (95% CI: 0-8.5%), χ^2 0.601, $p=0.4382$].

In the analysis of pregnancy complications associated with various hypertensive conditions, 2 cases (2.8%; 95% CI: 0-7.3%) of essential hypertension were found in L1 vs L0 - 5 cases (7.0%; 95% CI: 1.2-14.3%), χ^2 0.601, $p=0.4382$; pregnancy-induced hypertension was detected in 6 cases (8.5%; 95% CI: 2.8-14.5%) in L1 vs L0 - 13 cases (18.3%; 95% CI: 11.0-31.0%), χ^2 2.187, $p=0.1391$, the difference being statistically insignificant. There was no statistically significant dif-

ference in the number of cases complicated with fetal growth restriction [L1 - 3 cases (4.2%; 95% CI: 1.2-11.7%) vs L0 - 3 cases (4.2%; 95% CI: 1.2-11.7%), χ^2 0.000, $p=1.0000$]; oligohydramnios [L1 - 1 case (1.4%; 95% CI: 0-4.4%) vs L0 - 5 (7.0%; 95% CI: 2.4-12.9%), χ^2 1.566, $p=0.2108$]; polyhydramnios [L1 - 3 cases (4.2%; 95% CI: 1.2-11.7%) vs L0 - 3 (4.2%; 95% CI: 1.2-11.7%), χ^2 0.000, $p=1.0000$].

Table 1. Characteristics of pregnant women in the study groups according to term and mode of delivery (%).

Type of birth	Research group, L ₁ n ₁ =71		Control group, L ₀ n ₀ =71		χ^2	p
	abs.	% (95% CI)	abs.	% (95% CI)		
Vaginal birth	41	57.7% (47.4-70.1%)	54	76.1% (67.2-83.1%)	4.580	0.0324
Caesarean section	30	4.3% (29.9-52.6%)	17	2.9% (16.9-32.8%)		

It was determined that every fourth pregnant woman in L1 was diagnosed with hyperemesis gravidarum in the current pregnancy - 25.4% women (95% CI: 18.3-38.0%) compared to 7.0% (95% CI: 2.6-14.3%) in L0, (χ^2 7.471, $p=0.0063$). An increased frequency of iron deficiency anemia was detected among pregnant women in L1 - 36.6% cases (95% CI: 24.7-50.2%), vs L0 - 19.7% (95% CI: 8.0-27.4%), χ^2 4.211, $p=0.0402$. Thus, the frequency of gestational diabetes mellitus among L1 was 13 women, or 18.3% (95% CI: 11.3-28.2%), whereas 4 women were found among L0, or 5.6% (95% CI: 0-12.7%), χ^2 4.277, $p=0.0386$, thus indicating a statistically significant difference.

Hence, the features of childbirth were assessed, we studied and analyzed the term and mode of delivery. On average, delivery occurred at 37.6±2.0 (Me 38: 36.5; 39.2) w.g. in women with ICP, vs L0 - 39.2±1.7 (Me 39.5: 38.4; 40.3) w.g. (95% CI: -2.216 - 0.984, $p<0.0001$). A higher frequency of preterm births was determined among women in L1 - 19 cases (26.8%, 95% CI: 18.1-35.7%), vs L0 - 5 cases (7.1%, 95% CI: 0-11.7%), RR 3.8000, 95% CI: 1.5014-9.6176, $p=0.0048$, despite the predominance of term births both groups. Vaginal delivery was the most common mode of delivery in both study groups: 41 or 57.7% (95% CI: 47.4-70.1%) of cases in L1 and 54 or 76.1% (95% CI: 67.2-83.1%) of cases in L0

(tab. 1). At the same time, a higher frequency of cesarean sections in the research group was observed (χ^2 4.580, $p=0.0324$).

In 13 (18.3%, 95% CI: 11.0-27.0%) of cases in the L1, labour was induced by amniotomy and/or Folley catheter application for cervical preparation followed by amniotomy. In 9 (12.7%, 95% CI: 5.4-21.4%) of cases in L1, labour was induced by prostaglandin administration according to the standard clinical protocol (10). It should be noted that the reason for delivery by caesarean section or induction of labour in 21 (29.5%; 95% CI: 15.5-42.5%) cases in the research group was the presence of severe maternal symptoms and/or increased liver function tests values and/or bile acids levels in these women. Labour was induced by amniotomy or prostaglandin administration in 3 (4.2%; 95% CI: 0-7.3%) cases in L0 and 7 (9.9%; 95% CI: 2.6-18.5%) cases in L1; in all cases (10 women, 14.1%; 95% CI: 6.8-21.4%), induction of labour was indicated in relation to postterm pregnancy.

In the current study, no statistical difference was found regarding birth complications in both groups in terms of prenatal amniotic sac rupture, failure of contraction forces, placental and/or membranal tissue defects, or incidence of soft birth canal lacerations. Nevertheless, in women with cholestasis gravidarum, approximately every 4th pregnancy was complicated with meco-

niium staining of the amniotic liquid (L_1 - 20 cases or 28.2%; 95% CI: 18.3-38.0%, vs L_0 - 9 cases or 12.7%; 95% CI: 4.0-22.5%; χ^2 4.333, $p=0.0374$).

DISCUSSIONS

The data obtained are consistent with data from the literature showing that cholestasis gravidarum more often affects women over the age of 35 years (4). However, a recent study by M. Guszczynska-Losy et.al (2020) reported an average age of 30 years of pregnant women with ICP (11). The present study revealed a statistically significant difference in women's age between two groups. Hence, here exists a heterogeneity between L_1 and L_0 (age); thus, one of the limitations of the study is that the results may not be generalizable to larger populations.

Analyzing the extragenital anamnesis of the women included in the study, it was identified that hepato-biliary and nephrouinary conditions serve as risk factors for ICP, increasing the risk of developing cholestasis gravidarum by 2.6 ($p=0.0111$) and 2.2-fold ($p=0.0213$), respectively. These findings were also supported by other researchers, who reported hepatobiliary conditions such as gallbladder dysfunction, chronic cholecystitis, chronic viral hepatitis C and/or chronic viral hepatitis B as risk factors for ICP (12). Hepatitis C virus infection is considered by some authors as a risk factor for the development of CIS and may be associated with early onset of the pathology (13, 14). Conversely, other authors found no difference in the incidence of hepatitis C virus among pregnant women with cholestasis gravidarum compared with the control group (15). Concurrently, studies have demonstrated an increased risk for developing CIS among pregnant women infected with hepatitis B virus (16).

Hence, the data obtained were confirmed by other study, that highlighted that nephrouinary conditions may be a risk factor for the development of cholestasis gravidarum (11).

Assessing the particularities of delivery in the women included in the study, it was determined that 28.2% of cases of intrahepatic cholestasis of pregnancy were complicated by meconium staining of the amniotic fluid ($p=0.0374$). However,

there is controversy in the literature about the rate of meconium staining of the amniotic fluid among women with ICP, ranging from 12% to 25% according to different studies (2, 17). At the same time, some studies report that in pregnancies complicated by stillbirth, meconium amniotic fluid was reported in about 100% of cases (18, 19). Some authors suggest that the frequency of occurrence of meconium staining amniotic fluid may correlate with maternal serum bile acids values, with a positive interdependence (20, 21). However, the mechanism by which bile acids cause this effect is not fully understood (22). Bile acids are thought to increase fetal colonic motility, which may explain the presence of meconium in the amniotic fluid, although this process may be a side effect of bile acids toxicity (21).

According to literature data, preterm birth accounts for approximately half of perinatal deaths (23). Therefore, the impact of preterm birth on newborn health cannot be ignored. The results of the study revealed a preterm birth rate of 26.8%. These findings can be compared with other studies, which indicate a preterm birth rate of 25-32% in pregnancies complicated by cholestasis gravidarum (1, 24). R. Reid and coauthors reported an increased incidence of preterm birth of up to 36% in women with cholestasis gravidarum. However, a more recent study by Saleh and coauthors reported a preterm birth rate of approximately 44% in women whose pregnancies were complicated by ICP (25, 26). Some authors suggest a correlation between maternal serum bile acids levels and the preterm birth rate in women with ICP. The rate of this complication was significantly higher in pregnant women with ICP and bile acids values $>40 \mu\text{mol/L}$ (2). However, in patients with bile acids levels $<20 \mu\text{mol/L}$, an increase in the rate of preterm birth was not observed (27). Experimental rodent studies have shown that the myometrium of the non-pregnant rat responds to cholic acid administration by increasing contractility, and administration of this bile acid to sheep increases the incidence of spontaneous preterm birth (28). Thus, it has been hypothesized that the myometrium of ICP patients may be more sensitive to the effects of oxytocin (20).

CONCLUSIONS

1. The study results revealed a significant increase in the rates of hepato-biliary conditions and nephrouinary pathology among women with ICP.

2. A high rate of hyperemesis gravidarum in the current pregnancy, along with an increased frequency of iron deficiency anemia and gestational diabetes mellitus, was found in women with intrahepatic cholestasis of pregnancy.
3. Hence, ICP has been shown to have a negative impact on the evolution of labor, which is characterized by increased rates of meconium staining of the amniotic liquid.

CONFLICT OF INTEREST

Nothing to declare.

ETHICAL APPROVAL

The study obtained ethical approval (nr.46, from 28.02.2020) from the Ethics Committee of the *Nicolae Testemitanu* State University of Medicine

and Pharmacy, Chisinau, Republic of Moldova. Written informed consent was obtained from all participants, whereas all methods were carried out in accordance with relevant guidelines and regulations. Study registration number ISRCTN21187408 <https://www.isrctn.com/ISRCTN21187408>.

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Date of receipt of the manuscript: 30/01/2024

Date of acceptance for publication: 28/03/2024

REQUIREMENTS FOR AUTHORS

Rules of drafting

The manuscript (written in English and French) 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 $\frac{1}{3}$ from the volume of the manuscript. The journal reserves the right to make any other formatting changes. Rejected manuscripts are not returned.

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- **MATERIAL AND METHODS**
- **RESULTS**
- **DISCUSSIONS**
- **CONCLUSIONS**

- **CONFLICT OF INTERESTS**
- **ACKNOWLEDGEMENT** (optional)
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- **REFERENCES**

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

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

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- **MATERIAL ȘI METODE**
- **REZULTATE**
- **DISCUȚII**
- **CONCLUZII**
- **CONFLICT DE INTERESE**
- **MULȚUMIRI ȘI FINANȚARE** (optional)
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- **Introducere**
- **Material și metode**
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În rezumat nu vor fi incluse tabele, grafice și note bibliografice; informații care nu sunt prezentate în studiu.

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

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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**
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- **Résultats**
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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.

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

- **РЕЗЮМЕ** (см. требования ниже)
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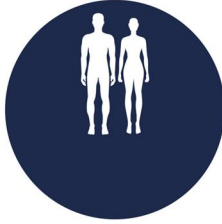
Таблицы. Текст, включенный в таблицы, должен быть написан в Cambria, размер 10 пунктов. Каждая таблица должна сопровождаться заголовком. Они должны вставляться в текст, не превышая ширину страницы. Должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (*например*: таб. 1). Название 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 АМА, 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 точность данных лежит на авторе. Будут цитироваться 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

The *One Health* concept

Human health



The WHO defined health in 1946 as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity", with the later addition of "the capacity to lead a socially and economically productive life".

Animal health



The OIE defines animal welfare in 2008: an animal is in good condition if it is healthy, enjoys comfort, is well fed, is safe, is able to display its innate (natural) behavior and does not suffer from unpleasant conditions such as pain, fear and stress.

Plant and
environmental health



Environmental health refers to those aspects of human health that include the quality of life determined by physical, biological, socio-economic and psycho-social factors in the environment. The interrelationships of people with the environment concern medicine, when an ecological system is in a state of equilibrium, the health of the population prevails.

Globally, the *One Health* concept is a worldwide strategy to expand interdisciplinary collaborations and communications in all aspects related to the health care of humans, domestic animals or wildlife, which can no longer be approached separately, but only jointly.

One Health addresses not only human and animal disease concerns, but also issues related to lifestyle, diet, exercise, the impact of different types of human-animal relationships, and environmental exposures that can affect both populations. In order to achieve the expected effects, it is also necessary to educate the population to make them aware of the risk factors and benefits of prevention, as well as communication and understanding between patients and healthcare providers.

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