



Dear Colleagues,

On behalf of the European Biotechnology Thematic Network Association (EBTNA), we are delighted to invite you to the European Biotechnology Congress 2026 (EuroBiotech 2026), a premier international event bringing together the multifaceted impacts and future potential of biotechnology.

This prestigious congress will be held in Varna, Bulgaria, from 24–25 April 2026, and is organized by EBTNA in collaboration with leading academic and scientific institutions. The congress provides a unique platform for scientists, academics, clinicians, and industry leaders from diverse disciplines to redefine the boundaries of biotechnology and to foster new interdisciplinary collaborations.

We cordially invite you to participate in EuroBioTech 2026 and to contribute to an inspiring scientific exchange in one of the most vibrant cities on the Black Sea coast.

Sincerely,

Prof. Munis Dundar
President of EBTNA

Prof. Svetlana Fotkova Georgieva
Local Chair

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BEST ORAL PRESENTATION AWARDS**

1st Place

Our experience in orthobiology: from PRP to exosomes

YEVHEN HOLIUK

2nd Place

(Real-World External Validation of BrainScan Artificial Intelligence for Intracranial Hemorrhage Detection on Non-Contrast Head Computed Tomography

IVAN SITKINOV

3rd Place

CAPN5 and CAPN13 as Potential Molecular Targets in Lung Adenocarcinoma

NURDENIZ NALBANT

3rd Place

A patient with dual phenotype: Coffin–Siris Syndrome Type 6 and Swyer Syndrome

PEMBE SAVASH

Honorable Mention

High-risk human papillomavirus genotypes and vaginal microbiota alterations in women from Northern Cyprus

BEHICH KOYUTOURK

BEST POSTER PRESENTATION AWARDS

1st Place

Accelerated Antimicrobial Susceptibility Testing in Patients with UTIs: A Comparative Study of the Laser Light Scattering vs. Disk Diffusion Method

MILENA YANCHEVA RUPCHEVA

2nd Place

Accumulation of uranium by aquatic plants from Southern Portugal: Phytofiltration potential of contaminated water

PAULO FAVAS

3rd Place

Association of Common Cardiovascular Genetic Variants with Coronary Artery Disease in a Bulgarian Case–Control Cohort

KRISTIYANA VITANOVA

3rd Place

Assessment of diets integrated with olive oil polyphenols in rainbow trout: gene expression study set-up and optimization

MARTINA TORRICELLI

3rd Place

Catechin-rich hazelnut green husk extract: antimicrobial activity and DNA gyrase interaction

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INVITED SPEAKER ABSTRACTS

DOI: 10.2478/ebtj-2026-0010

Alpha-Mannosidosis: Therapeutic strategies

Bellini Baldelli S., Fiorani F., Albi E., Beccari T., Codini M.

University of Perugia

Alpha-mannosidosis is a rare lysosomal storage disorder with an autosomal recessive inheritance caused by mutations in the gene encoding the lysosomal enzyme α -Dmannosidase.

So far, 190 variants in 230 patients have been identified and partially characterized at the biochemical level. As in other lysosomal storage diseases, there is no relationship between genotype and phenotype in alpha-mannosidosis. Enzyme replacement therapy is currently the most effective therapy for lysosomal storage diseases, including alpha-mannosidosis. An alternative therapy is bone marrow transplantation. The primary indication for hematopoietic stem cell transplantation in patients affected by alphanmannosidosis is the preservation of neurocognitive function and prevention of early death.

The results obtained from a Phase I-II study and a Phase III study provide evidence of positive clinical effect of the recombinant enzyme in patients with alpha-mannosidosis. Most recently, in Europe, enzyme replacement therapy has been approved for use in adults, adolescents, and children with mild-moderate forms of alpha-mannosidosis. The next step will be to develop the production of a BBB-crossing recombinant alpha-mannosidase. Recently it has been demonstrated partial correction of the globally distributed storage lesions by infusion of a high dose of adeno-associated virus 1-feline alpha-mannosidase into the cerebrospinal fluid via the cisterna magna in the cat brain at different ages, corresponding to different stages of disease progression. Interestingly, significant improvements in clinical parameters were observed, and partial correction was observed in many tissues. All together, what has been shown so far indicates the progress that has been achieved in the treatment of alpha-mannosidosis and indirectly for other lysosomal diseases.

Keywords: Alpha-mannosidosis, lysosomal storage disorders, enzyme replacement therapy

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Bacterial cellulose: synthesis, properties, and applications

Iza Radecka¹, Habib Khan¹, Abhishek Gupta¹, Zinnia Mansoor¹, Siting Guo¹, Marek Konop², Grazyna Adamus³, Marek Kowalczyk³, Fidelity Tchuente-Magaia¹

¹Faculty of Science & Engineering University of Wolverhampton, Wulfruna

Street, Wolverhampton, WV1 1LY, UK

²Medical University of Warsaw, Department of Experimental Physiology and Pathophysiology, A. Pawińskiego 3C 02-106 Warsaw, Poland

³Centre of Polymer and Carbon Materials, Polish Academy of Sciences, M. Curie-Skłodowskiej 34, 41-819 Zabrze, Poland

Biodegradable polymers are produced by a diverse group of microorganisms from renewable resources under controlled conditions. These polymers play a significant role in mitigating negative environmental impacts and can contribute to sustainability across various industries.

Bacterial cellulose (BC) is an extracellular polymer produced by Komagataibacter spp. BC is a unique natural polymer showing desirable properties such as chemical purity, significant mechanical strength, water holding capacity, biodegradability and biocompatibility. Additionally, BC can be chemically altered to form BC composites with customised properties making this bacterial polymer a very attractive versatile biomaterial for a variety of applications.

Microbial BC synthesis includes cultivation under static or agitated conditions. The choice of the cultivation method impacts on the microstructure and properties of BC. In the static cultures, a thick gelatinous pellicle of BC is formed on the surface of the growth medium. However, in the agitated culture, BC is produced in the form of sphere-like or irregular mass with significantly denser fibres compared to the BC produced in static conditions.

Selection the cultivation method depends on its final application. Produced BC is fully characterised and, if needed, modified according to the proposed application.

This talk will give detailed insights into biosynthesis of BC and its physicochemical properties. Additionally, it will provide an updated overview relevant to sustainable and cost-effective production of this biopolymer, along with examples of the most recent applications ranging from biomedical applications to agricultural applications.

Keywords: Komagataibacter spp., bacterial cellulose, sustainability, biomaterials, BC composites

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Beyond Biotics: Bulgarian Science for Innovative Postbiotic Solutions

Svetla Danova¹, Nikoleta Atanasova¹, Lili Dobрева¹, Petar Donchev¹, Radoslav Abrashev¹, Ekaterina Krumova¹, Maria Angelova¹, Greta Naydenova², Apostol Apostolov², Dragomir Yankov²

¹Stephan Angeloff Institute of Microbiology (IMicB), BAS; 26, Acad. Bontchev Str

²Institute of Chemical Engineering- (ICHe) BAS-1113Sofia, Bulgaria;

Background: We present a scientific report that discuss the question: **WHY BIOTICS?** From the oldest biotechnology to personalized medicine: or how the beneficial microbiota in fermented products works to promote health. The rapid evolution of the Biotics has shifted scientific focus beyond traditional “probiotic strains” to “postbiotics platforms”. Postbiotics represent a paradigm shift from live microbial therapy to defined bioactive compounds, enabling precision, safety, and reproducibility.

Results: The Laboratory of “LAB and Probiotics”- IMicB-BAS, has been investigating the microbial diversity of lactic acid bacteria (LAB) from various ecological niches for 25 years. The long-term goal is to characterise and enable their PROBIOTIC use at the forefront of health, as a guarantee of food safety and scientific proven first generation correcting dysbiosis, in the microbiome. In the focus are original Bulgarian strains, proving their probiotic potential and health benefits Pro-Meta-Para and Postbiotics - the necessary discussion on the road to pharmabiotics and the scientific challenges in response to consumers’ inherent desire for health, well-being, and longevity. The joint project involving IMicB and IChE-BAS, developing green technologies for production and application of active Bulgarian Pro/postbiotics, based on byproducts from bio- and agro-production.

Conclusion: Our findings confirm that Bulgarian probiotic strains are a promising source of postbiotics with significant functional potential. The study underscores the need for further in-depth research, including clinical validation, to support the development of standardized and evidence-based postbiotic formulations. The joint scientific effort has the capacity to play a leading role in advancing innovation.

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Keywords: Probiotics, Postbiotics, Lactic Acid Bacteria (LAB), Microbiome, Pharmabiotics

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From Single-Omics to Multi-Omics Integration: A Machine Learning Framework for Inherited Retinal Disease Classification

Ahmad Jainul Abidin¹, Kristjana Dhuli¹, Sara Feizyab¹, Matteo Bertelli^{1,2,3}

¹MAGI EUREGIO, Bolzano, Italy

²MAGI'S LAB SRL, Rovereto (TN), Italy

³MAGISNAT, Atlanta Tech Park, Peachtree Corners, 30092 GA, USA

Background: Inherited retinal dystrophies (IRDs) present significant diagnostic challenges due to their genetic and phenotypic heterogeneity. While single-omics analyses have provided valuable insights, integrating multi-omics approaches with machine learning (ML) holds promise for improved disease classification accuracy. This study evaluates single-omics methodologies and advances toward a comprehensive multi-omics classification framework for IRDs.

Methods: Single-omics analyses were performed independently: (1) Proteomic analysis of 500 samples with imputed plasma profiles, employing pathway activation scores; four ML algorithms were compared to identify the optimum classification approach; (2) Genomic and clinical data from 527 patients were analyzed using kernel density estimation and protein-protein interaction networks to assess pathway-level mutational burden. (3) An integrative multi-omics framework combining genomic and metabolomic data with advanced ML methods is being developed to leverage complementary information across data types.

Results: In the proteomic analysis, 82 proteins were selected based on expression frequency and categorized into ten relevant biological pathways. Logistic Regression achieved optimal classification performance (accuracy 0.73, F1-score 0.73). Genomic analysis revealed significant mutational burden in retinoid cycle pathways, with gene variant combinations (ABCA4, RDH12) and (ABCA4, RBP3) identified as unique IRD biomarkers. Findings from both omics layers converged on retinoid metabolism, suggesting that multi-omics integration capturing genotype-phenotype relationships across molecular scales may enhance diagnostic precision.

Conclusion: Single-omics approaches demonstrate moderate classification performance and reveal distinct molecular insights. The progression toward multi-omics integration represents a promising strategy for enhanced IRD classification, potentially capturing complex disease mechanisms across multiple biological layers.

Keywords: Inherited Retinal Disease, Multi-Omics, Machine Learning, Disease Classification, Precision Medicine

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Halophytes: new crops for adaptation to climate change

Oscar Vicente¹, Monica Boscaiu²

¹Institute for the Conservation and Improvement of Valencian Agrobiodiversity (COMAV). Universitat Politècnica de València, Valencia (Spain)

²Mediterranean Agroforestry Institute (IAM). Universitat Politècnica de València, Valencia (Spain).

Climate change effects, including increasing temperatures, altered precipitation patterns, and more frequent and intense extreme weather events, are already affecting crop performance in many regions. Drought and soil salinisation are the two interconnected environmental factors that most severely reduce crop yields worldwide. As freshwater resources become scarcer and salinity expands across irrigated a

coastal lands, conventional crops – generally salt-sensitive – are increasingly unable to maintain stable productivity.

In this context, the domestication and commercial cultivation of halophytes – salt-tolerant plant species – offer a promising strategy for climate-resilient agriculture. The development of “saline agriculture” based on halophytic crops that can grow in saline soils and use brackish or seawater irrigation could help maintain agricultural production on marginal lands while reducing pressure on freshwater resources, therefore contributing to adapting agricultural systems to future environmental conditions.

Several halophytic species show strong potential as new crops. As a food crop, quinoa (*Chenopodium quinoa*) is a pseudocereal with high nutritional value and notable tolerance to salinity and drought. Other edible halophytes, such as *Salicornia* species, are increasingly explored as vegetable crops, whereas *Atriplex lentiformis* represents a promising forage crop. Oil-producing halophytes, including *Salicornia bigelovii*, also offer opportunities for saline-based oilseed production. Beyond food and feed uses, halophytes have potential as minor crops: ornamentals, medicinal plants, or industrial crops for biofuel and biomass production. Additionally, many halophytes can provide ecosystem services, including phytoremediation of saline or polluted soils and biological desalination.

Overall, integrating halophytes into agricultural systems could enhance agrobiodiversity and resilience to climate-driven environmental stresses, supporting sustainable agriculture under increasingly saline and water-limited conditions.

Keywords: saline agriculture, food security, drought, salt stress, agrobiodiversity

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Implementation of a GPU Accelerated and AI Integrated NGS Workflow

Jurgen Kaftalli¹, Sara Feizyab², Matteo Bertelli^{1,2,3}

¹MAGI'S LAB SRL, Rovereto (TN), Italy

²MAGI EUREGIO, Bolzano, Italy

³MAGISNAT, Atlanta Tech Park, Peachtree Corners, 30092 GA, USA

Background: Validate an upgraded clinical bioinformatics workflow ("PipeMAGI v2") designed to address the computational bottlenecks and interpretative challenges associated with high-throughput Next-Generation Sequencing (NGS).

Methods: The legacy CPU-based pipeline was migrated to a GPU-accelerated architecture using Nvidia Parabricks, incorporating DeepVariant and GATK HaplotypeCaller. Analytical quality control was enhanced through algorithmic contamination detection, kinship verification, and strand bias filtering. A dual-algorithm strategy for Copy Number Variant (CNV) calling, utilizing ExomeDepth and CoNVaDING, was implemented. Furthermore, two machine learning (ML) models, and a rule-based automated sample classification algorithm were deployed: a logistic regression model to predict the

necessity of Sanger confirmation and a "Smart Reporting" classifier to assess variant interpretation complexity, as well as an automated workflow that assigns sample-level clinical classes from variant and patient metadata and flags non-actionable or data-incomplete cases as 'Complex' for expert review.

Results: Preliminary validation indicates that the GPU-accelerated workflow significantly reduces secondary analysis latency while maintaining high concordance with standard benchmarks. The dual-CNV calling strategy revealed critical batch-effect vulnerabilities in normalization-based algorithms when applied to external samples. The code skeleton, designed with modularity in mind, is shared at: [github. repo](#). The ML-based filtering for Sanger confirmation achieved a high predictive value, potentially reducing confirmatory load by >90%.

Conclusion: PipeMAGI v2 represents a robust, scalable framework for clinical genomics, effectively integrating hardware acceleration and artificial intelligence to enhance diagnostic efficiency and accuracy.

Keywords: GATK, Parabricks, CNV, GPU

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Into lake water: molecular and microbiological insights from a One Health perspective

G.Vita¹, S. Primavilla¹, F. Agnetti¹, A. Felici¹, E. Radicchi¹, M. Croce², S. Ricci², M. D'Incau³, A.F.M. Marino⁴, D. Cruciani¹, S. Crotti¹, M. Ciullo¹, M. Biagetti¹, M. Torricelli¹

¹IZSUM, PG

²Regione Umbria

³CNRLL/IZSLER, BS

⁴CETOX/IZSSicilia, PA

Waterborne infections are increasingly recognized as a public health concern in a One Health perspective. *Toxoplasma gondii* could act as passive carrier and environmental sentinel. Inland waters as soil, wastewater and groundwater may be contaminated by resistant oocysts. Due to the lack of harmonized and standardized detection methods, environmental contamination is underestimated. The major detection probability is related to surface waters and wet seasons. Few systematic studies have assessed environmental risk factors for *Leptospira* spp.

This work aims to evaluate the presence of these pathogens in Umbrian lakes, assessing also microbial flora. After method optimization and Limit Of Detection in matrix assessment, DNA extraction and qPCR were performed on 17 water samples each of about 1 L, and related pellets (16 from Trasimeno Lake' different sites, 1 from Piediluco lake, collected on summer and winter). Water samples DNA was extracted using DNeasy PowerWater Kit, while DNA from filtered pellets was extracted with QIAamp DNA Mini-Kit. Duplex qPCR assays targeted B1 or LipL32 genes and internal controls. Standard microbiological analyses of lake water were performed according to ISO methods, and some strains were typed with MALDI-TOF.

Among analyzed samples, one pellet and one water sample, collected from Trasimeno Lake respectively in December 2025

and February 2026, resulted positive to *Leptospira* spp (≥ 38 Cq over 45 Cq). Water microbiological analyses revealed several pathogens of public health or zoonotic concern across all sites. Wide microorganism's spectrum could require a shotgun metagenomic in depth analysis. Despite the sporadic molecular detection of *Leptospira* spp, its presence, also in traces, in the lacustrine environment deserves further investigation.

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Keywords: *Toxoplasma gondii*, *Leptospira* spp, lacustrine environment, qPCR/dPCR, microbiological assay

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MAGI Deep Learning Based Classification of Inherited Retinal Diseases Using Retinal Imaging

Sara Feizyab¹, Matteo Bertelli^{1,2,3}

¹MAGI EUREGIO, Bolzano, Italy

²MAGI'S LAB SRL, Rovereto (TN), Italy

³MAGISNAT, Atlanta Tech Park, Peachtree Corners, 30092 GA, USA

Background: Inherited retinal diseases (IRDs) present heterogeneous phenotypes and genetic causes, often requiring extensive and costly testing. Multimodal retinal imaging captures complementary structural and functional signals that may support earlier hypothesis generation on the most likely causative gene. This project proposes an explainable deep-learning framework to assist clinicians by prioritizing candidate genes from retinal imaging, as a support tool to streamline genetic testing and interpretation.

Methods: We will develop a multimodal deep-learning pipeline using retinal imaging modalities, including color fundus photography (CFP), fundus autofluorescence (FAF), near-infrared reflectance (NIR), and optical coherence tomography (OCT). The training set will be composed of cases with confirmed molecular diagnoses, enabling supervised learning. Two prediction tasks are planned: (1) binary classification for selected genes (mutation present/absent), and (2) multi-class candidate ranking to output Top-K genes per patient. The system will incorporate explainability modules (e.g., saliency/attention-based maps) to highlight image regions that contribute most to predictions, supporting clinical interpretability and quality control. The pipeline will be implemented with reproducible data handling, preprocessing, and model-training steps, compatible with integration into existing bioinformatics and clinical workflows.

Results: As this is an ongoing project, results are not yet available. The planned outputs include per-patient probability estimates and/or Top-K ranked candidate genes, accompanied by visual explanation maps and model confidence indicators to guide expert review.

Conclusion: This proposal aims to deliver an explainable AI tool for IRDs that supports gene prioritization from multimodal retinal imaging, potentially reducing diagnostic time and focusing downstream genetic testing while preserving clinical oversight.

Keywords: Deep Learning, Inherited Retinal Disease, Retinal Imaging, OCT, Explainable AI

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MAGISNAT Microbiome Pipeline: Data Processing and Interpretation

Ekaterina Chueva¹, Jurgen Kaftalli¹, Matteo Bertelli^{1,2,3}

¹MAGI'S LAB SRL, Rovereto (TN), Italy

²MAGI EUREGIO, Bolzano, Italy

³MAGISNAT, Atlanta Tech Park, Peachtree Corners, 30092 GA, USA

Background: The human oral microbiome consists of bacterial communities, which colonise several microhabitats. Interactions with the microbiome are proven to influence the quality of life for the host. For example, they are linked to such processes as vitamin synthesis, response to inflammation, and others. In this regard, our oral microbiome test aims to provide insights into the general well-being of the human taking the test.

Methods: We developed an oral microbiome pipeline to analyse shotgun metagenomic data from a buccal mucosa sample. The test provides species-level information, focusing on microbial diversity, identification of non-beneficial microbial networks, and the microbial pathways that influence the host.

Results: The microbiome pipeline provides a comprehensive overview of the microbial community present in the buccal mucosa and connects it to the well-being. A methodology to compare an incoming sample to the reference cohort is considered as well.

Conclusion: Shotgun metagenomic sequencing enables the provision of a comprehensive species-level analysis of the oral microbiome.

Keywords: Oral Microbiome, Bioinformatics Pipeline, Complex Systems Analysis, Shotgun Metagenomic Sequencing

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Modulating high-molecular glucansucrase in search of improved product profile for novel prebiotics

Stanimira Angelov^{a1}, Viktoria Nedkova¹, Veselin Bivolarski¹, Tonka Vasileva¹, Ilia Ilie^{v2}

¹Department of Biochemistry and microbiology, Faculty of Biology, Paisii Hilendarski University of Plovdiv, Plovdiv, Bulgaria

²Centre of Technologies, Plovdiv University, Plovdiv, Bulgaria

Glucosyltransferases from the glucoside hydrolase family 70 (GH70) catalyze the synthesis of α -D-glucans with diverse linkage types and branching patterns, using sucrose as the sole donor substrate. Their ability to transfer glucosyl units to a wide range of carbohydrate and non-carbohydrate acceptors underscores their potential as sustainable biocatalysts for industrial glycosylation processes. Advances in enzyme engineering have further expanded opportunities to fine-tune glucansucrase function for obtaining polymers and oligosaccharides with desirable prebiotic features. The dual-domain

glucansucrase URE 13-300 from *Leuconostoc mesenteroides* URE 13 serves as a valuable model for exploring structure–function relationships within GH70 enzymes. The successful expression of the full enzyme in recombinant strain *Escherichia coli* BL21 enabled targeted mutagenesis for specificity and activity optimization. This study examines the role of the E2332 residue, positioned near the transition-state stabilizer in the second catalytic domain (CD2), through site-saturation mutagenesis using randomized NNK codons. The obtained library resulted in three highly active mutants, where the glutamate was substituted with leucine, serine and threonine, respectively. All of them retained the wild-type temperature optimum (30 °C), however exhibited a pH shift from 5.3 to 6.5 and higher specific activities relative to the native enzyme. Moreover, mutant variants displayed higher specific enzyme activities. Variants E2332S and E2332T produced altered oligosaccharide profiles, suggesting modified transglucosylation specificity, while E2332L yielded a glucan with markedly enhanced water-retention capacity. This glucan supported robust growth of multiple *Lactobacillus* strains when used as the sole carbon source. These results demonstrate that targeted amino acid substitution in glucansucrase URE 13-300 can modulate catalytic parameters and product architecture, offering a promising route for the development of tailor-made glucans with potential prebiotic and industrial applications.

Acknowledgements: This study was supported by the European Union- NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0001-C01, DUECOS.

Keywords: site-saturation mutagenesis, glucansucrase, α -glucans, transferase reaction

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Non Conventional Yeasts as Next Generation Probiotics, Prebiotics, and Postbiotics

Ivana Marova, Michaela Kubalova, Paula Vecerikova, Vojtech Bures, Martina Husova, Jan Cvecek

Faculty of Chemistry, Brno University of Technology, Brno, Czech Republic

Non-conventional yeasts (NCYs)—all yeast species outside the widely used *Saccharomyces cerevisiae*—have emerged as powerful candidates for next-generation probiotic, prebiotic, and postbiotic applications. NCYs possess extraordinary genetic and functional diversity, enabling them to thrive in harsh conditions, metabolize diverse substrates, and produce valuable metabolites.

This work focused on exploring several unconventional yeast species. Extracts of yeast cultures from the genera *Rhodotorula*, *Metchnikowia* and *Yarrowia* were used as sources of prebiotic compounds to support the growth and metabolic activity of probiotic bacteria and to create stable synbiotic systems. Probiotic strains (*L. acidophilus*, *L. plantarum*, *L. delbrueckii*, *L. rhamnosus*, *B. bifidum*, *B. breve*, *B. adolescentis*, and *B. longum*) were cultivated in these extracts, and their growth

was compared with that in standard MRS medium. To evaluate the probiotic potential of these yeasts, several parameters were determined, including viability at low pH, antioxidant and antimicrobial activity, lactic acid production and biofilm formation. Immunomodulatory function will be tested using human macrophage cell lines. The prebiotic potential of the studied yeasts will be assessed through analysis of yeast-derived polysaccharides (e.g. beta-glucans, mannans) and effects of cell-wall fragments on the growth and metabolism of probiotic bacteria. The postbiotic effects of NCYs will be related to homogenates of inactivated yeasts containing a rich array of postbiotic compounds, such as short-chain fatty acids, antioxidants, antimicrobial peptides, organic acids and vitamins. The safety and stability of postbiotic molecules will be evaluated using cytotoxicity testing following artificial digestion. Based on the results, NCYs can be considered interesting and highly-functional next-generation prebiotic, probiotic and postbiotic platforms. Their superior robustness, metabolic flexibility, and ability to produce diverse bioactive compounds position them at the forefront of future innovations in food, health, and therapeutics.

Keywords: probiotics, prebiotics, postbiotics, non-conventional yeasts

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Polygenic Risk Score in Lipedema: A Machine Learning Approach to Risk Stratification

Luca Ferrari¹, Eglantina Kalluçi², Ornella Gordani², Xhilda Dhamo², Matteo Bertelli^{1,3,4}

¹MAGI'S LAB SRL, Rovereto (TN), Italy

²Department of Applied Mathematics, Faculty of Natural Sciences, University of Tirana, Tirana, Albania.

³MAGI EUREGIO, Bolzano, Italy

⁴MAGISNAT, Atlanta Tech Park, Peachtree Corners, 30092 GA, USA

Background: Multifactorial genetic diseases exhibit a polygenic architecture in which numerous common single-nucleotide polymorphisms (SNPs) contribute modestly to overall disease risk. Conventional Polygenic Risk Scores (PRS) rely on genome-wide association study (GWAS) effect sizes, which are often unavailable or poorly transferable for rare or understudied conditions. This study presents a GWAS-independent pipeline for PRS construction and demonstrates its application in lipedema.

Methods: Individual-level genotypic data from 314 subjects (208 cases and 106 controls) were analyzed using a workflow based on biologically preselected variants associated with relevant physiological pathways. The standardized pipeline included quality control, alignment, variant calling, annotation, and genotype filtering. Feature selection was performed using a linear Support Vector Machine (SVM), ranking variants by absolute model weights to identify the most informative predictors. Ridge regression with L2 regularization was subsequently applied to derive stable PRS coefficients.

Results: Model performance was evaluated using area under the curve (AUC), accuracy, F1 score, and Nagelkerke's pseudo- R^2 . Analysis of PRS distributions and predictor importance supported model interpretability and highlighted the contribution of weak but cumulative genetic signals. The final model achieved an AUC of 0.87 and a Nagelkerke's pseudo- R^2 of 0.13, indicating meaningful capture of aggregated genetic risk in lipedema.

Conclusion: This GWAS-independent pipeline provides a transparent and clinically interpretable strategy for PRS development in multifactorial diseases lacking large GWAS datasets. The combined SVM-ridge approach proved robust and adaptable, showing encouraging performance in lipedema and potential applicability to translational risk stratification in other complex conditions.

Keywords: Polygenic Risk Score, Multifactorial Diseases, GWAS, Machine Learning, Lipedema

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Rethinking Psychiatric Risk Detection: AI Based Non-EEG Predictive and Preventive Framework

Farhad Nassehi, Refika Dilara Aşçı, Ege Kırıcı, Andaç Budak, Osman Eroğul

TOBB University of Economics and Technology

Social anxiety disorder (SAD) is a condition characterized by intense fear of social interactions, potentially leading to significant distress and impaired ability to function in daily life. This research focuses on developing a wearable technology solution for predicting anxiety attack states in individuals diagnosed with SAD. Previous studies have also mentioned the detection of psychiatric disorders such as SAD with an Electroencephalogram (EEG). It has been reported that detection can be made from different physiological parameters such as heart rate and body temperature, but there is a lack of an effective method for prediction. This study aims to calm down the SAD-diagnosed patients with sound and vibration stimulation after predicting the moment just before anxiety from PPG and ECG signals recorded during the one-minute rest state before the anxiety. 30 Patients participated in the experiment which took a total of 4 minutes, including 2 minutes of rest-rest data and 2 minutes of rest-task data. The selection of participants was conducted based on evaluations performed by a specialist doctor, who confirmed scores exceeding 80 on the Liebowitz scale. ECG and PPG records were taken in both stages. At the same time, the State-Trait Anxiety Inventory questionnaire was applied to the participants for the trait anxiety, pre-experiment and post-experiment situations. An article search was conducted for the extraction of important features to be used in signal analysis and feature selection methods were mutually used for the final selection. Selected features were used as input to the Naïve Bayes machine learning algorithm. The results show that the ECG and PPG features that differed between sessions could predict the anxiety state with an 84.24% accuracy rate on av-

erage. The anxiety prediction and relaxation system that will improve the life quality of individuals diagnosed with SAD has been developed with the Naïve Bayes algorithm.

Keywords: Machine learning; AI; Psychiatric Disorder

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Subcritical water extraction as a novel approach for obtaining bioactive extracts from *Echinacea purpurea*

Petko Denev¹, Desislava Teneva¹, Manol Ognyanov¹, Mariya Pimpilova¹, Ani Petrova¹, Bela Vasileva², Milena Georgieva², Kamelia Hristova-Panusheva³, Natalia Krasteva³, Georgi Dimitrov¹

¹Laboratory of Biologically Active Substances-Plovdiv, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Plovdiv, Bulgaria

²Institute of Molecular Biology "Acad. R. Tsanev", Bulgarian Academy of Sciences, Sofia, Bulgaria

³Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: Subcritical water extraction (SWE) is a promising green alternative to conventional organic solvent-based extraction methods. Despite advantages such as environmental compatibility, high extraction efficiency, and tunable selectivity, its industrial application remains limited.

Methods: This study investigated, for the first time, the efficiency of SWE for extracting hydroxycinnamic acids (chicoric and caftaric acids) from *Echinacea purpurea* roots as a green alternative to conventional hydroalcoholic extraction. Extractions were performed at different temperatures (100°C–170°C) and durations (5–30 min). The obtained extracts were characterized by HPLC, and their biological activity was evaluated in HT29 cells after 24 and 48 h of treatment.

Results: Both extraction temperature and duration significantly influenced the recovery of hydroxycinnamic acids from *Echinacea* roots. Optimal conditions were identified at 108–110°C for 8–10 min. Increasing the temperature to 170°C enhanced overall extract yield by up to 50%, but reduced the levels of chicoric and caftaric acids due to thermal degradation. Biological evaluation demonstrated that SWE extracts induced stronger cellular responses in HT29 cells after 24–48 h of treatment. Comet assay results indicated greater DNA interaction, while FACS cell-cycle analysis revealed more pronounced modulation of cell-cycle distribution, suggesting that SW extracts are enriched in biologically active compounds and exert stronger regulatory effects on cell proliferation.

Conclusion: SWE represents an efficient and environmentally friendly technology for producing *Echinacea purpurea* root extracts standardized to hydroxycinnamic acids, with reduced extraction time and without the use of toxic organic solvents. The resulting extracts demonstrate comparable or enhanced biological activity relative to conventional extracts. Therefore, SWE-derived *Echinacea* extracts show strong potential for application in the food, pharmaceutical, and cosmeceutical in-

dustries in the development of drugs, dietary supplements, and functional foods.

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Keywords: Subcritical Water Extraction, Echinacea purpurea, Nutraceuticals Pharmaceuticals

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Comparative evaluation of cell-biosensors based spatial and temporal fingerprinting of biotoxicity at Bay of Vlora

Ariola Bacu^{1,2}, Danja Dollaku¹, Adela Sinani¹, Sindi Ymet¹, Robert Marks^{3,4}

¹Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania

¹Academy of Sciences of Albania, Albania

²Avram and Stella Goldstein-Goren Department of Biotechnology Engineering, Ben-Gurion University of the Negev, Be'er-Sheva 84105, Israel

³The Ilse Katz Center for Nanoscale Science and Technology, Ben-Gurion University of the Negev, Be'er Sheva 84105, Israel

Gulf of Vlora, of particular economic importance for Albania, represents a complex natural ecosystem under the influence of urban and industrial pollution. Several spots are investigated by the National Environmental Monitoring Program, which does not include the biotoxicity assessment of waters. In order to distinguish the temporal pattern of toxicity seawater were sampled in November 2023-May 2025-September 2025 at nine hot-spots in shallow waters, and were assessed using bioluminescent *Vibrio fischeri* according to ISO11348-3:2007 via BioFix Lumi-10 reader. Also *E. coli* strains K802NR, TV1061, DPD2794, DPD1718, DPD2544, and DPD2511 were used to determine toxicity in waters and sediments (September-November 2025). All strains contain a plasmid-based fusion of the *E. coli* *lasI*, *grpE*, *recA*, *recA*, *fabA*, and *katG* promoters, respectively, with the *Photobacterium luminescens* LuxCDABE reporter operon. The bioluminescent toxicity response of the reporter strains was acquired over 10 h period using Ascent microplate reader (ThermoFischer) set to kinetic mode at 15 min intervals between readings. The induction factor was calculated as a ratio of the maximum RLU of the test to the average of the negative control. Heatmaps of toxicity were prepared to verify the spatial pattern of toxicity. In parallel, key physicochemical parameters of waters were measured to explore potential correlation to biotoxicity. Results display the impact on toxicity of the climate change within a three-year period, also the seasonal variation reaching the peak in May 2025. Clear differences were evidenced among water and sediment toxicity, with the last being within the accepted limits. As the luminescence of *V. fischeri* is impacted by salinity of marine waters, the use of *E. coli* strains was important. Variations in the sensitivity of *E. coli* strains suggested the presence of diverse contaminant types and concentrations, with *E. coli* strains TV1061 and DPD2511

less sensitive, the rest above the accepted levels, and DPD1718 and DPD2544, induced above the accepted levels in all cases. Nine out of nine spots were classified as having high toxicity by 1-3 of the biosensors, even though physicochemical and biological parameters showed no correlations. Results highlighted the importance of the evaluation of toxicity as an independent indicator of the quality of waters alongside with those suggested by WFD.

Acknowledgement: This work was supported by the Academy of Sciences of Albania, Project BioSensToxicWater of the Centre for Biotechnology & Genetics, 2025-2027.

Keywords: cytotoxicity, bioluminescent bio-reporting bacteria, physicochemical parameters

ORAL PRESENTATION ABSTRACTS

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Agro-Industrial By-Products As Substrate for Pro-, Post- And Metabiotics - Characterization and Use

Dragomir Yankov¹, Greta Naydenova¹, Apostol Apostolov¹,
Lili Dobрева², Nikoleta Atanasova², Svetla Danova²

¹Institute of Chemical Engineering - Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., bl. 103, Sofia, zip 1113

²Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev str., bl. 26, Sofia, zip 1113

In view to decrease the use of sugars as substrate in various fermentations, research is underway to develop methods to convert different agro-industrial by-products to fermentable sugars. Lignocellulosic substrates are the largest source of fermentable sugars (RS) for bioconversion to valuable compounds. Carbohydrates from cellulosic feedstocks such as distiller's dried grains with solubles (DDGS), spent coffee grounds (SCG), sunflower seed shells (SSS), and sunflower de-oiled cake (SDC) are abundant and promising agro-industrial by-products to be further converted to fermentable sugars.

In the present study, these by-products were characterized as possible source for sugars for biotechnological processes. The composition of the above-mentioned waste materials was determined and compared before and after hydrothermal or dilute-acid pretreatment. The resulting hydrolysates, containing different reducing sugars and proteins, were used as carbon and nitrogen sources, replacing costly components in the fermentation media. The applicability of the obtained hydrolysates was tested in fermentative lactic acid production as an example.

All investigated waste materials contain enough lignocellulose, which can be used as a source of reducing sugars. Except for sunflower meat, the hydrolysis of other materials results in more than 70% of RS. There are minimal differences in sugar content after acid and enzyme hydrolysis. The difference is that, in the case of SSS, the enzyme hydrolysis liberates additional amounts of galactose and mannose.

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Key words: Renewable resources, Agro-industrial by-products, Pretreatment, Lactic acid production

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Biotechnology Shaping Tomorrow's Circular and Sustainable Packaging

Fideline Tchuenbou-Magaia¹, Siting Guo¹, Eglantina Kalluci², Evgeni Ivanov³, Vladimir Georgiev³, Todor Batakliiev³, Matteo Berterlli⁴, Iza Radecka¹

¹Faculty of Science & Engineering University of Wolverhampton, Wulfruna Street, Wolverhampton, WV1 1LY, UK

²Department of Mathematics, Faculty of Natural Sciences, University of Tirana, Albania

³Institute of Mechanics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. Block 4, 1113 Sofia, Bulgaria

⁴MAGI EUREGIO, Bolzano, Italy

Biotechnology offers transformative solutions for reducing reliance on fossil-based materials, positioning it as a key driver in advancing circular and sustainable packaging systems. Polylactic acid (PLA) and bacterial cellulose (BC) are biotechnology-derived polymers that can be sustainably produced from food- and agricultural waste through microbial fermentation. Their favourable physicochemical properties make them strong candidates to replace or complement fossil-derived plastics in packaging, particularly when functionalised or integrated into composite systems. In this regard, cellulose nanocrystals (CNCs) serve as key enablers for the development of advanced materials for smart packaging, owing to their ability to enhance the mechanical and barrier performance of polymer matrices while promoting the uniform dispersion of secondary conductive nanofillers. The talk will discuss current research on CNCs and PLA composite development for packaging, highlighting key challenges, opportunities, and future directions.

CNCs were produced from bacterial cellulose grown on food-waste media and compared with those from standard media, while PLA composites containing CNCs, graphene nanoplatelets (GNPs), or carbon nanotubes (CNTs) were fabricated by melt extrusion and characterised a range of analytical techniques.

The results demonstrate that CNCs enhance the performance and processability of PLA composite filaments by acting as effective dispersion aids and network organisers. They minimise GNP and CNT agglomeration and facilitate the formation of a uniform, continuous hybrid network within the PLA matrix, resulting in improved electrical, thermal, and rheological properties. Spearman's correlation matrix and Hierarchical Cluster Analysis identify clear property groupings: electrical conductivity clusters with thermal-transport variables due to shared conductive-network effects, while elongation, toughness, yield strain, and critical strain form a coherent ductility cluster. These ductility measures are strongly inter-correlated

and negatively associated with storage modulus, reflecting the classic stiffness–ductility trade-off in filled thermoplastics. Together, this correlation structure provides actionable guidance for designing high-performance, safe, and application-specific PLA-based packaging materials.

Keywords: Bacterial cellulose, PLA, cellulose nanocrystals, packaging, sustainability

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CAPN5 and CAPN13 as Potential Molecular Targets in Lung Adenocarcinoma

Nurdeniz Nalbant¹, Ece Ozdemir¹, Ece Tolgay², Elif Ulker Akyildiz³, Sehime Gulsun Temel^{1,4}

¹Department of Translational Medicine, Bursa Uludag University, Bursa, Turkey

²Faculty of Medicine, Bursa Uludag University, Bursa, Turkey

³Department of Pathology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey

⁴Department of Medical Genetics, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey

Background: Non-Small Cell Lung Cancer (NSCLC) accounts for approximately 85% of all lung cancer cases, with lung adenocarcinoma (LUAD) being its most common histological subtype. Despite advances in chemotherapy, immunotherapy, and targeted therapies, treatment resistance and poor survival remain major clinical challenges. Calpains are calcium-dependent cysteine proteases involved in cancer-related processes such as cellular signaling, apoptosis, and cell cycle regulation. However, the roles of CAPN5 and CAPN13 in NSCLC remain largely unexplored.

Methods: Transcriptomic expression analyses of CAPN5 and CAPN13 were performed using The Cancer Genome Atlas Lung Adenocarcinoma (TCGA-LUAD) dataset through the UALCAN platform. To validate the bioinformatic findings, CAPN5 and CAPN13 expression levels were analyzed by RT-qPCR in formalin-fixed paraffin-embedded (FFPE) LUAD tumor tissues and matched adjacent normal lung tissues obtained from 48 patients.

Results: Bioinformatic analysis revealed significant upregulation of CAPN5 and CAPN13 in LUAD tumor tissues compared with normal lung tissues. Gene dependency analysis based on CRISPR–Cas9 screening data suggested a potential role of these genes in lung cancer cell viability. RT-qPCR analysis confirmed the significant overexpression of both genes in LUAD tumor samples relative to matched normal tissues.

Conclusion: CAPN5 and CAPN13 are significantly overexpressed in LUAD tumor tissues and may represent potential molecular targets for targeted therapeutic strategies in lung adenocarcinoma.

Keywords: Lung Adenocarcinoma; Calpains; CAPN5; CAPN13

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Clinical Evaluation Of The Pediatric Trauma Support System In Pediatric Trauma Patients

Dimitar Dachev¹, Elean Zanzov¹, Kety Tokmakova², Stoyan Lupanov³, Biser Ivanov³, Borislav Isakov³, Nikolay Mavrev³, Penka Stefanova³

¹Medical University - Plovdiv - Bulgaria, Department of Propedeutics of Surgical Diseases, Department of Pediatric Surgery

²Medical University - Plovdiv - Bulgaria, Department of Orthopedics and Traumatology

³Medical University - Plovdiv - Bulgaria, Department of Propedeutics of Surgical Diseases, Department of Pediatric Surgery

Background: Pediatric trauma is a leading cause of morbidity and mortality worldwide. Although validated scoring systems such as the Glasgow Coma Scale (GCS), Revised Trauma Score (RTS), Pediatric Trauma Score (PTS), and Shock Index (SI) are widely used, their real-time integration into emergency workflows remains limited. The Pediatric Trauma Support System (PTSS) is a digital Clinical Decision Support System (CDSS) for children aged 0–18, developed based on protocols from Oxford Children's Hospital, Bambino Gesù Hospital, and Johns Hopkins Children's Center.

Methods: We conducted a prospective observational study of 100 consecutive pediatric trauma patients presenting to the Emergency Department at UMHAT “St. George”, Plovdiv, Bulgaria over four months. PTSS recorded demographic data, physiological parameters (RR, SpO₂, HR, BP, capillary refill, temperature), injury mechanism, and anatomical severity, and automatically calculated RTS, PTS, SI, and triage categories. Outcomes included ICU admission, hospitalization, surgical interventions, imaging use, and adverse events.

Results: Of the 100 patients, 10 were critically ill (RTS <6) and required ICU care, 30 were hospitalized for observation, and 60 were discharged home. Patients with mild trauma (GCS 15, RTS 7.84, SI 0.81) experienced no complications. Critically ill patients (GCS 7, RTS 4.21, SI 1.57) were hemodynamically unstable and correctly identified by PTSS. The system demonstrated 100% sensitivity for detecting critical cases, guided selective imaging, and appropriately directed surgical interventions. Correlations between PTSS-derived indices and clinical outcomes confirmed its predictive validity.

Conclusions: PTSS provides objective, reliable, and clinically applicable decision support in pediatric trauma care. It enables early identification of high-risk patients, optimizes triage, reduces unnecessary imaging, and supports resource allocation. These findings support PTSS as a practical tool for emergency settings.

Keywords: Pediatric trauma, Clinical decision support system

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Evaluation of antioxidant, antidiabetic, anti-inflammatory and toxicity potential of *Cestrum diurnum* L. Leaf Extract in a diabetic Mouse Model

Attya Bhatti

Background: Type 2 Diabetes Mellitus (T2DM) has emerged as a significant global metabolic disorder. It is characterized by chronic hyperglycemia resulting from impaired insulin secretion by pancreatic β -cells and increased insulin resistance in peripheral tissues, leading to dysregulated glucose homeostasis. Although several pharmacological interventions are available for the management of T2DM, their long-term use is often associated with adverse effects and limited therapeutic efficacy. Consequently, there is increasing interest in the exploration of plant-derived bioactive compounds as alternative therapeutic agents. *Cestrum diurnum*, an ornamental shrub widely cultivated in different regions of the world, has a long history of use in traditional medicine for the treatment of various ailments.

Objective: The present study aimed to evaluate the antidiabetic potential of *Cestrum diurnum* leaf extract through phytochemical, biochemical, and in vivo experimental analyses.

Methods: Phytochemical screening and biochemical assays were performed to identify bioactive compounds and assess the in vitro activities of the ethanolic extract of *Cestrum diurnum* leaves. An in vivo study using BALB/c mice was also conducted to evaluate its therapeutic effects on glucose intolerance. Experimental diabetes was induced using a high-fat diet and streptozotocin, after which mice were treated with graded doses of the extract, followed by biochemical and histopathological analyses.

Results: Administration of *Cestrum diurnum* leaf extract resulted in a significant reduction in hyperglycemia and improved glycemic regulation in the diabetic mouse models. Histopathological examination of liver and kidney tissues revealed marked improvements in tissue architecture, indicating a protective and restorative effect of the extract on cellular morphology.

Conclusion: This study demonstrates that *Cestrum diurnum* leaf extract exhibits significant antidiabetic, antioxidant, and anti-inflammatory activities, likely attributable to the presence of pharmacologically active phytochemicals. These findings suggest that *Cestrum diurnum* may serve as a promising natural source for the development of novel therapeutic agents for the management of T2DM.

Keywords: *Cestrum diurnum*, Type 2 Diabetes Mellitus, Phytotherapy, Biochemical Analysis, Diabetic Mice Model, Histopathological analysis, Median lethal dose LD50, Acute toxicity assay, Dose related response

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From Flowers to Residual Biomass: Nutritional and Chemical Analysis of *Helichrysum italicum*

Katya Peycheva, Neli Markova, Veselina Panayotova, Diana A. Dobрева, Rositsa Stancheva, Tatyana Hristova, Tonika Stoycheva, Albena

Merdzhanova

Department of Chemistry, Medical University of Varna

Helichrysum italicum (*H. italicum*) is a Mediterranean halophyte shrub with bright yellow flowers characterized by a strong curry-like aroma. The essential oils from *H. italicum* are widely used in cosmetic and pharmaceutical applications due to their anti-inflammatory and antiallergic properties, while the flowers themselves have potential as culinary seasonings, flavoring agents, and natural food preservatives. In this study, we evaluated the nutritional composition and chemical analysis of *H. italicum* flowers and solid waste biomass. The flowers were predominantly composed of carbohydrates (>80% dry weight), followed by minerals and essential elements ($6.31 \pm 0.95\%$ dw), proteins ($0.87 \pm 0.03\%$ dw), and lipids ($3.11 \pm 0.53\%$ dw). They contained appreciable amounts of Fe, Zn, Ca, and K. The solid waste biomass contained significantly lower carbohydrate ($37.4 \pm 0.3\%$ dry weight) and higher protein and lipid contents. Fatty acid profile of *H. italicum* flowers and waste showed similar distribution: polyunsaturated followed by saturated and monounsaturated fatty acids. The most abundant fatty acids in the flowers were linoleic and palmitic acid, while in solid waste biomass – linoleic and α -linolenic acid. These results highlight the nutritional richness and bioactive potential of *H. italicum* flowers and solid waste biomass, supporting their application in functional foods, nutraceuticals, and health-promoting products.

Keywords: *Helichrysum italicum*, proximate composition, fatty acids, mineral content

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High-risk human papillomavirus genotypes and vaginal microbiota alterations in women from Northern Cyprus

Behich Koyutourk¹, Mufit Cemal Yenen², Mahmut Cerkez Ergoren¹

¹Molecular Microbiology Laboratory, Near East University Hospital, Nicosia, Cyprus

²University of Kyrenia Hospital, Department of Gynecology and Obstetrics, Kyrenia, Cyprus

Background: High-risk human papillomavirus (HPV) infection, especially by HPV-16 and HPV-18, is the main causative agent in cervical carcinogenesis. Recent studies have shown that the composition of vaginal microbiota is an important factor in modulating the persistence of HPV infection and oncogenic progression. Although there have been studies on the relationship between vaginal microbial profiles and HPV genotypes in different populations, no comprehensive study has been conducted on this relationship in Cypriot women. This study therefore provides novel regional epidemiological and microbiological data, contributing to the understanding of HPV-microbiota interactions within the Mediterranean population.

Methods: A total of 100 women were stratified into HPV-nega-

tive (n = 34), HPV-16-positive (n = 33), and HPV-18-positive (n = 33) groups. HPV DNA was extracted using the QIAamp DNA Mini Kit and genotyped by PCR-based molecular assay. Vaginal microbiota profiling was performed using a multiplex real-time molecular STD panel. Comparative frequency analysis was conducted to assess differences in microbial distribution across genotype groups.

Results: HPV-16 infection demonstrated a distinct dysbiotic profile compared with HPV-18 and HPV-negative groups. *Gardnerella vaginalis* was detected in 71.4% of HPV-16-positive women in the analysed subgroup, compared with 50% of HPV-negative controls and 0% of HPV-18-positive patients. HPV-16 cases also showed increased polymicrobial colonization, including *Ureaplasma spp.* and *Mycoplasma hominis*. In contrast, HPV-18 infection exhibited minimal microbial imbalance. These findings indicate a genotype-specific microbial signature, with HPV-16 strongly associated with anaerobe-dominant dysbiosis.

Conclusion: The findings of the present investigation show the existence of genotype-related differences in the vaginal microbiota of individuals infected with HR-HPV. The prevalence of *Gardnerella vaginalis* in HPV-16-infected individuals indicates a possible synergistic effect. The combination of molecular HPV typing and microbiota typing is a promising biotechnological approach in the prevention of HPV-related diseases.

Keywords: Human papillomavirus, Vaginal microbiota, Genotype-specific dysbiosis, Precision prevention

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Innovation in the Age of Generative AI: From Observing the Innovation Ecosystem to Visions

Kosi Gramatikoff

Medical University Varna

This presentation explores how generative AI is reshaping the nature, structure, and pace of innovation. Moving beyond a tool-centric view, it frames innovation as a systemic, recursive ecosystem—less a “lightbulb moment” and more an interconnected grid of capabilities, feedback loops, and enabling infrastructures. Drawing on historical contrasts between exploratory and formalized knowledge creation, the talk situates today’s AI moment within a longer arc of scientific and technological evolution. An integrated framework of innovation is introduced through four core pillars and a recursive loop architecture that defines emerging AI-driven innovation cycles. These cycles emphasize continuous iteration between hypothesis generation, modeling, validation, and application—accelerated by generative AI systems acting as active collaborators rather than passive tools. The presentation further outlines a normative architecture for future innovation ecosystems, grounded in five guiding principles, and demonstrates practical implications through AI-assisted hypothesis generation and biomedical modeling use cases. Particular attention is given to the convergence of wet and dry lab paradigms, the role of digital twins, and the identifica-

tion of disease modules as a new frontier in systems biology. Finally, the talk presents seven forward-looking visions, including AI as a lab partner, human–AI cognitive alignment, the emergence of a shared human–AI language, and the redefinition of creativity as a central organizational value. These visions collectively point toward a future in which innovation is co-evolved by humans and machines, culminating in a broader aspiration: the democratization and freedom of knowledge.

Keywords: Human–AI collaboration, AI lab partner, Cognitive alignment, Human–AI interface (HMI), Human–AI language, Augmented intelligence, Hypothesis generation, Computational modeling, Wet lab vs. dry lab integration, Digital twins, Omics data integration, Disease modules

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Interactive Moodle-Based Pre Simulation Lesson on Endodontic Access Cavity Preparation for Third Year Dental Students: A Preliminary Satisfaction Study

Angelina Kirkova-Bogdanova¹, Nikolay Simeonov², Elena Stavreva², Dimitar Kolev³, Neshka Manchorova²

¹Department of Medical Informatics, Biostatistics and E-learning, Faculty of Public Health, Medical University—Plovdiv, Plovdiv, Bulgaria

²Department of Operative Dentistry and Endodontics, Faculty of Dental Medicine, Medical University—Plovdiv, Plovdiv, Bulgaria

³Department of Logopaedics, Faculty of Public Health, Health Care and Sports, SWU “Neofit Rilski”, Blagoevgrad, Bulgaria

Background: Simulation-based education plays an increasingly important role in dental curricula, supporting the acquisition of psychomotor and cognitive skills in a controlled, low-risk environment. Learning management systems such as Moodle can further enhance this process by providing structured, interactive learning units that prepare students for hands-on training on high-fidelity simulators. This study describes the development and preliminary evaluation of an interactive Moodle lesson used as pre-simulation preparation in Endodontics for third-year dental students at the Medical University of Plovdiv. **Methods:** An interactive learning unit titled “Endodontic Access Cavity Preparation” was developed using the Lesson activity in Moodle. The learning unit included clearly defined objectives, nonlinear navigation paths, and multimodal content (text, images, and short video clips), and embedded formative questions to maintain interactivity. It was implemented in both Bulgarian- and English-taught groups of third-year dental students. Student satisfaction was assessed via an anonymous electronic questionnaire following lesson completion. Data was exported and analyzed quantitatively using IBM SPSS Statistics version 23.

Results: Results indicate high levels of student satisfaction across both language groups. Participants reported that the structure and multimodal presentation made the material interesting and engaging, and the nonlinear pathways supported a sense of personalization and choice in learning. Students also stated that the lesson was suitable for self-directed preparation

before working with a high-technology dental simulator and helped them better understand key steps in endodontic access cavity preparation. No significant difference in satisfaction levels was observed between Bulgarian- and English-speaking cohorts.

Conclusion: Integrating interactive Moodle lessons as preparatory tools for simulation-based training in dental education positively influences student engagement and learning satisfaction. The approach supports personalized learning and serves as an effective bridge between theoretical instruction and practical application on high-fidelity simulators.

Keywords: Moodle, simulation-based learning, conservative dentistry, endodontics, dental education, interactive e-learning

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Maternal Vitamin D Status association with Clinico-laboratory Characteristics in Pregnant Women Aged 30–40 Years

Irina Nakashidze¹, Luka Khalvashi¹, Nino Kedelidz¹, Ahishtan Febrian Nishanthan¹, Nameera Parveen Shaik¹, Aleena Parveen Shaik¹, Jumber Ungiadze¹, Salome Glonti¹, Tamar Peshkova¹, Elisabetta Albi², Tommaso Beccari²

¹Batumi Shota Rustaveli State University, Batumi, Georgia

²University of Perugia, Perugia, Italy

Vitamin D is widely recognized as a central regulator of numerous physiological processes in the organism. Vitamin D status is critically important in pregnant women of all ages as it is actively involved in several processes. At the molecular level, it regulates the transcription of numerous genes. Maintaining adequate status is crucial to supporting a healthy pregnancy, optimal fetal growth, and long-term offspring health. Our study aimed to investigate the association between vitamin D status and clinical and laboratory characteristics in pregnant women. The study was conducted in the Adjara population region (Georgia). According to the study, different results were revealed depending on vitamin D status, both in clinical and laboratory characteristics. Our findings suggest an important link between maternal vitamin D levels and various characteristics.

Keywords: Vitamin D status; pregnancy; pregnant women; clinico-laboratory characteristics; maternal health

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Modern approaches to the diagnosis, risk stratification, and treatment of wild mushroom intoxications

Kaloyan Mihalev¹, Ivelin Iliev¹, Nadya Agova¹, Galina Yaneva², Tsonka Dimitrova², Djeni Cherneva², Svetlana Georgieva¹

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Varna, Varna, Bulgaria

²Department of Biology, Faculty of Pharmacy, Medical University of Varna, Varna, Bulgaria

Background: Wild mushroom poisoning remains a significant global toxicological challenge due to the diversity of toxins and

clinical syndromes. Early diagnosis is critical, particularly in amatoxin intoxication, where delayed symptom onset is associated with severe organ damage and high mortality.

Methods: A systematic literature review was conducted using PubMed/MEDLINE, EMBASE, and Web of Science, covering studies published between January 2008 and March 2025. Studies addressing diagnosis, risk stratification, and clinical management in human mushroom poisoning were included and analysed using a narrative synthesis approach.

Results: A total of over 52 studies were analysed. Urinary amanitin detection demonstrated high diagnostic reliability, while LC-MS/MS and LAMP assays provided highly sensitive toxin and species identification. Risk stratification models, including CLIF-OF and machine learning algorithms, showed strong prognostic performance (AUC up to 0.90). Combined therapy with silibinin and penicillin G significantly reduced treatment failure compared to monotherapy. Extracorporeal detoxification techniques and liver transplantation improved survival, with pooled mortality estimated at approximately 2.87%.

Conclusion: The diagnostic paradigm in mushroom poisoning is shifting toward an integrated model combining clinical, toxicological, and molecular approaches. Effective management requires early recognition, dynamic risk assessment, and timely therapeutic escalation. Future progress depends on standardized clinical algorithms and multicenter prospective studies to optimize treatment strategies and improve patient outcomes.

Keywords: mushroom; fungi; poisoning; treatment

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Moodle-Based Interactive Quiz as Assessment as Learning in Pre-Simulation Training on Endodontic Access Cavity Preparation for Third-Year Dental Students

Angelina Kirkova-Bogdanova¹, Elena Stavrev², Nikolay Simeonov², Dimitar Kolev³, Neshka Manchorova²

¹Department of Medical informatics, Biostatistics and E-learning, Faculty of Public Health, Medical university - Plovdiv, Plovdiv, Bulgaria

²Department of Operative Dentistry and Endodontics, Faculty of Dental Medicine, Medical University - Plovdiv, Plovdiv, Bulgaria

³Department of Logopaedics, Faculty of Public Health, Health Care and Sports, SWU "Neofit Rilski", Blagoevgrad, Bulgaria

Background: Multifactorial genetic diseases exhibit a polygenic architecture in which numerous common single-nucleotide polymorphisms (SNPs) contribute modestly to overall disease risk. Conventional Polygenic Risk Scores (PRS) rely on genome-wide association study (GWAS) effect sizes, which are often unavailable or poorly transferable for rare or understudied conditions. This study presents a GWAS-independent pipeline for PRS construction and demonstrates its application in lipedema.

Methods: Individual-level genotypic data from 314 subjects (208 cases and 106 controls) were analyzed using a workflow based on biologically preselected variants associated with relevant physiological pathways. The standardized pipeline inc-

cluded quality control, alignment, variant calling, annotation, and genotype filtering. Feature selection was performed using a linear Support Vector Machine (SVM), ranking variants by absolute model weights to identify the most informative predictors. Ridge regression with L2 regularization was subsequently applied to derive stable PRS coefficients.

Results: Model performance was evaluated using area under the curve (AUC), accuracy, F1 score, and Nagelkerke's pseudo- R^2 . Analysis of PRS distributions and predictor importance supported model interpretability and highlighted the contribution of weak but cumulative genetic signals. The final model achieved an AUC of 0.87 and a Nagelkerke's pseudo- R^2 of 0.13, indicating meaningful capture of aggregated genetic risk in lipedema.

Conclusion: This GWAS-independent pipeline provides a transparent and clinically interpretable strategy for PRS development in multifactorial diseases lacking large GWAS datasets. The combined SVM-ridge approach proved robust and adaptable, showing encouraging performance in lipedema and potential applicability to translational risk stratification in other complex conditions.

Keywords: Moodle, assessment as learning, simulation-based learning, conservative dentistry, formative quiz

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Our experience in orthobiology: from PRP to exosomes

Yevhen Holiuk

Institute of Traumatology and Orthopedics NAMS of Ukraine

The analysis of our research include 1,640 patients with osteoarthritis and avascular necrosis of the hip and knee, meniscus tears and shoulder injuries, who were treated at the Institute of Traumatology and Orthopedics of the NAMS of Ukraine in the period from 2015 to 2025. We used autologous products from peripheral blood, bone marrow and adipose tissue and allogenic MSCs and exosomes.

We found that the patients with the 1st and 2nd stages of hip and knee osteoarthritis had good results after LR-PRP and LP-PRP ($p \leq 0.05$). The patients with stage 3 knee osteoarthritis needed additionally bone marrow or adipose tissue concentrates. The comparative analysis of orthobiologic treatment patients with meniscal injuries demonstrated its effectiveness in the short term and in the long term, which is reflected in the improvement of joint function and a significant reduction of pain. We found that the quality of life of the patients with hip and knee avascular necrosis with preoperative preparation by intra-articular injection of autologous peripheral blood concentrates demonstrated better results than in the group with single intra-osseous injection of the mononuclear fraction of bone marrow aspirate ($p < 0.05$). An analysis of quality of life was conducted depending on the Ficat stage and reliable indicators of improvement in quality of life, as well as analgesic effect and improvement in joint function in patients with stages 1 and 2 of avascular necrosis were established.

Allogenic hP-MSCs and exosomes were well-tolerated, with no severe adverse events observed. Significant improvements in WOMAC and VAS scores were noted in the MSC group compared to the Control group at both 6 and 12 months. MRI analysis revealed no significant differences in cartilage thickness or optical density index between the groups. Serum cytokine analysis showed a significant decrease in interleukin-2 (IL-2) levels, indicating an anti-inflammatory effect of hP-MSCs.

Keywords: Orthobiology, osteoarthritis, avascular necrosis, joint diseases, regenerative medicine, mesenchymal stem cells, exosomes

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Proteomic maturation of articular cartilage reveals shared pathways with osteoarthritis

Giulia Giuffredi

University College Dublin

Articular cartilage (AC) is a connective tissue whose extracellular matrix (ECM), composed mainly of type II collagen and aggrecan, provides tensile strength and resistance to compression. Due to its avascular and sparsely cellular nature, AC has limited turnover and repair capacity, making it vulnerable to degeneration. Tissue remodelling during growth is essential for long term joint function, yet the underlying protein level adaptations remain poorly characterised. Here, label free mass spectrometry was used to profile the ECM proteome of goat AC, a large animal model relevant to human joint biology, to define molecular changes during cartilage maturation.

AC was collected at seven postnatal stages from neonatal to adult ($n = 3$ per stage) under UCD ethical approval (AREC 21 22). Full thickness distal first phalanx samples were cryopulverised and extracted using a guanidine HCl protocol to recover soluble and insoluble matrix fractions. Peptides were digested with trypsin and analysed by LC MS MS. Data were processed in MaxQuant using the Capra hircus proteome. Label free quantification values were filtered, log transformed and analysed in R. Proteins were classified with MatrisomeDB; differential abundance was assessed with limma and pathway enrichment with Reactome.

A total of 799 proteins were identified, including 157 ECM components. Principal component analysis revealed a clear developmental trajectory from neonatal to adult tissue. Early stages were enriched in collagens and proteoglycans such as COL9A1, COL11A1 and ACAN, whereas regulators and structural proteins including COL6A1, FN1, CILP and HTRA1 increased with age. Enrichment analysis indicated declining collagen biosynthesis after mid juvenile stages and increased elastic fibre formation, crosslinking and glycosaminoglycan metabolism. Cartilage maturation involves functional reorganisation rather than replacement of matrix components, with a shift from ECM assembly in early development to structural stabilisation and maintenance in adulthood.

Keywords: Articular cartilage, proteomics, extracellular matrix, development, mass spectrometry, goat, cartilage maturation, osteoarthritis

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Real-World External Validation of BrainScan Artificial Intelligence for Intracranial Hemorrhage Detection on Non-Contrast Head Computed Tomography

Ivan Sitnikov^{1,2,5}, Eray Halil^{1,2,5}, Neli Atanasova^{3,5}, Petra Popova^{3,5}, Kostadin Kostadinov⁴, Penka Atanasova^{1,2,5}

¹Department of Neurology, Medical University of Plovdiv, Plovdiv, Bulgaria

²Clinic of Neurology, University Hospital "St George", Plovdiv, Bulgaria

³Department of Radiology, University Hospital "St George", Plovdiv, Bulgaria

⁴Department of Social Medicine and Public Health, Medical University of Plovdiv, Plovdiv, Bulgaria

⁵Strategic Research and Innovation Program for the Development of MU-Plovdiv (SRIPD-MUP), EU-NextGenerationEU, Plovdiv, Bulgaria

Background: Artificial intelligence (AI) tools may improve rapid detection of intracranial hemorrhage on non-contrast head computed tomography (CT). However, their external validity in routine clinical practice remains insufficiently established. We evaluated the diagnostic performance of BrainScan CT, an AI-based radiology software, in an independent real-world cohort of patients undergoing emergency CT for suspected acute stroke.

Methods: This retrospective study included 1,419 non-contrast head CT scans from 1,260 consecutive patients. Intracranial hemorrhage was present in 120 scans (8.5%). Independent interpretation by two board-certified radiologists served as the reference standard. Diagnostic performance was assessed using sensitivity, specificity, predictive values and McNemar's test. Additional analyses evaluated performance by hemorrhage location and imaging artefacts.

Results: BrainScan CT detected hemorrhage in 116 cases (96.7%) with a median confidence score of 1.00 (IQR 0.96–1.00). Sensitivity was 96.7% and specificity 99.8%, with positive and negative predictive values of 98.3% and 99.7%, respectively. No significant systematic disagreement with radiologist interpretation was observed (McNemar $p = 0.68$). Detection remained high across anatomical regions, although sensitivity was lower for occipital hemorrhages.

Conclusion: BrainScan CT demonstrated excellent diagnostic performance and strong agreement with radiologist interpretation for detection of intracranial hemorrhage on non-contrast head CT. The tool may support triage and workflow in acute stroke imaging but should complement, not replace, expert radiological assessment.

Keywords: Artificial intelligence; Computed tomography; Intracranial hemorrhage; Stroke.

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S100 calcium binding protein A8 in adipose-bone axis mediated

immuno-metabolic crosstalk in breast cancer

Ilhaam Ayaz Durrani, Peter John*, Attya Bhatti*

Department of Biomedicine, Atta ur Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST), Islamabad, Pakistan, 44000.

*Corresponding Author

Background: Bone metastasis in advanced primary breast cancer patients is a global health concern. Bone marrow adipose tissue (BMAT) provides a cultivating ground for tumour spread, however the underlying molecular mechanisms are not yet fully understood.

Methods: Present study employed an *in silico* approach to identify bone lesion (BL) associated differentially expressed genes (DEGs) in breast cancer (BC) by employing the GEO2R tool on GEO database sourced microarray expression data. To this BL DEGs list, DEGs associated with BMAT were applied. Common DEGs were obtained and subjected to protein-protein interaction (PPI) and functional enrichment analysis using STRING 12.0. Subsequently, the immuno-metabolism associated genes' list derived from GeneCards was mapped. Immune filtration was estimated using TIMER 2.0.

Results: A total of 16 BL in BC associated genes were found to be commonly dysregulated for BMAT. These were mainly enriched for immune associated terms including immune system and neutrophil degranulation. Amongst these genes, *S100 calcium binding protein A8 (S100A8)* was found to be immuno-metabolic in nature. It associated negatively with M0 and M1 macrophages but positively with M2 highlighting pro-tumorigenic potential in BC.

Conclusion: Hence, targeting *S100A8* may provide a therapeutic window into the BMAT mediated immuno-metabolic crosstalk to address bone metastasis in BC.

Keywords: Type 2 diabetes mellitus, osteoarthritis, breast cancer, bone metastasis, adipose tissue

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Synthesis, Biological Activity and Development of Elastic Liposomal Formulations of 4-(2,4-Dichlorobenzamido)-Benzohydrazide Derivatives as Potent Cholinesterase Inhibitors

Halil Şenol¹, Furkan Çakır¹, Ahmet Gökhan Ağgöl², Ayşe Merve Şenol³, Hümeysra Şahin Bektay⁴, Mustafa Gökçe⁵, Feyzi Sinan Tokalı⁶

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bezmialem Vakıf University, 34093 Fatih, Istanbul, Türkiye

²Department of Biochemistry, Faculty of Pharmacy, Ağrı İbrahim Çeçen University, 04100 Ağrı Türkiye

³Department of Medical Services and Techniques, Program of Medical Laboratory Techniques, University of Health Sciences, 34668, Istanbul, Türkiye

⁴Department of Pharmaceutical Technology, Faculty of Pharmacy, Bezmialem Vakıf University, 34093 Fatih, Istanbul, Türkiye

⁵Department of Pharmacology, Faculty of Pharmacy, Istanbul University-Cerrahpaşa, 34500 Büyükdere, Istanbul, Türkiye

⁶Department of Material and Material Processing Technologies, Kars Vocational School, Kafkas University, 36100 Kars, Türkiye

This study presents the design synthesis biological activity and nanoformulation of novel 4-(2,4-dichlorobenzamido)-benzohydrazide derivatives as potent cholinesterase inhibitors with therapeutic relevance for Alzheimer's disease. A rational medicinal chemistry approach supported by computational modeling was employed to develop a focused compound series targeting acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).

All synthesized derivatives exhibited strong nanomolar inhibition against AChE (2.57–5.06 nM) and BChE (3.08–4.95 nM), significantly outperforming donepezil. Compounds 5l and 5y were identified as lead candidates based on their superior IC₅₀ values. Molecular docking and MM-GBSA analyses revealed favorable binding interactions within the catalytic sites of both enzymes, which were further supported by 250 ns molecular dynamics simulations demonstrating stable protein–ligand complexes.

To enhance biocompatibility and therapeutic performance, elastic liposomal nanoformulations (F-5l and F-5y) were developed. The optimized vesicles showed nanosized distributions (66.08–74.98 nm), low polydispersity, and physicochemical stability at 4 °C. In vitro cytotoxicity studies on SH-SY5Y neuroblastoma and HUVEC healthy endothelial cells demonstrated that liposomal encapsulation significantly reduced toxicity toward healthy cells while maintaining or improving antiproliferative activity against cancer cells, leading to a marked increase in selectivity index.

Density functional theory (DFT) calculations further clarified electronic characteristics and supported structure–activity relationships.

Overall, this integrated medicinal chemistry and nanobiotechnology strategy highlights the translational potential of elastic liposomal systems for selective cholinesterase inhibition and neurodegenerative drug development.

Keywords: Alzheimer's disease; Cholinesterase inhibitors; Elastic liposomes; Cytotoxicity; Molecular docking; MD simulation.

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The Impact of Water Temperature Increase on the Growth Performances in *Mytilus Galloprovincialis* Reared in The Aquaculture of Butrint Lake

Dritan Arapi¹, Rigerta Sadikaj², Ariola Bacu²

¹Research Centre of Flora and Fauna, Faculty of Natural Sciences, University of Tirana, Albania

²Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania

The aim of this study was to provide information on the damage of some indicators of the growth performance in mussels, caused by the excessive increase in water temperature in summer season. The study was carried out in a mussel “population” that was reared in the panels of a fixed suspended culture, in Manastiri area, during 2024s. Samplings of mussels were car-

ried out without selection in the months of April-May and July-August, from three parts of the ropes. For each month, no less than 90 molluscs were sampled.

The highest measured value for temperature, in the surface layer of water (0.25 m) was 29.4 °C (August). The increase in the average water temperature in the 0-3.0m layer, from 19.03 °C to 25.52 °C, had caused the reduction in the average oxygen content from 7.30 mgO₂/l to 4.58 mgO₂/l. The average values of the SGR%-index, for the reared stock of *Mytilus galloprovincialis*, were 0.298±0.015 and 0.196±0.020. The regression and correlation analysis confirmed the significance of this differences. The average values of the Kn-factor for the reared mussels, in two time intervals were: Kn=0.871, in the Summer season and Kn=1.041, in the Spring season. The average values calculated for the meat yield according to four months, were: 0.241±0.0107 in April; 0.276±0.0151 in May; 0.231±0.0135 in July and 0.207±0.0150 in August. All analytical indicators of mussel growth, which were evaluated, manifested a reduction in values under conditions of a significant increase in water temperature.

Keywords: mussel, aquaculture, climate change, specific growth rate.

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Change-aware artificial intelligence for longitudinal medical image classification: a scoping review

Mehmet Sait Dunder

Department of Medical Imaging Techniques, Halil Bayraktar Health Services Vocational School, Erciyes University, Kayseri, Türkiye

Longitudinal medical imaging detects disease-related anatomical or functional alterations that are frequently imperceptible in single-time-point assessments. This scoping study assesses whether machine-learning and deep-learning models that explicitly incorporate temporal changes enhance illness picture classification, prognosis, or treatment-response prediction.

Recent research was reviewed in the fields of neuroimaging, ophthalmology, cancer, respiratory imaging, and musculoskeletal radiology. Qualified research employed paired or serial illness images, change maps, Siamese architectures, recurrent or transformer temporal models, self-supervised longitudinal pretraining, or the integration of radiomics with deep learning. The synthesis emphasized methodological patterns, documented performance improvements compared to baseline single-image models, and identified obstacles to clinical application.

In several disease domains, change-aware models typically surpassed static-image methods when longitudinal pictures were accessible and temporally synchronized. In Alzheimer's disease, sequential MRI-based deep learning and temporally organized machine-learning models enhanced the prediction of mild cognitive impairment conversion and produced progression-sensitive biomarkers. In retinal disease, longitudinal self-supervised fundus/OCT models captured progression dynamics and enhanced the prediction of early diabetic re-

tinopathy or age-related macular degeneration compared to models developed from the ground up. In oncology, Siamese and multi-task networks that use pre- and post-treatment MRI enhance response prediction compared to using pre-treatment or post-treatment pictures independently. Comparable trends were observed in glaucoma progression, alterations in osteoarthritis severity, tuberculosis/COVID-19 monitoring, and overall disease severity assessment. The primary benefit seems to stem from modeling intra-patient variation, therefore minimizing inter-patient anatomical discrepancies and highlighting biologically significant progression or regression. Nonetheless, the evidence is varied due to uneven follow-up intervals, absent visits, registration inaccuracies, scanner/domain shifts, restricted external validation, and variable management of leakage at the patient level.

Longitudinal, change-sensitive medical image analysis is a promising avenue for enhanced disease categorization and prognosis. The clinical value will rely on standardized temporal datasets, strong alignment and harmonization, elucidated change location, and prospective validation.

Keywords: longitudinal imaging; deep learning; disease progression; medical image classification; change detection

POSTER PRESENTATION ABSTRACTS

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Accelerated Antimicrobial Susceptibility Testing in Patients with UTIs: A Comparative Study of the Laser Light Scattering vs. Disk Diffusion Method

Milena Yancheva Rupcheva^{1,2}, Kostadin Kostadinov^{2,3}, Radoslav Tassev^{1,2}, Petya Markova⁴, Violeta Zheleva⁵, Yana Tsvetkova⁶, Maria Atanasova^{1,2}, Michael M. Petrov^{1,2}

¹Department of Medical Microbiology and Immunology, Prof. Dr. Elissav Yanev, Faculty of Medicine, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria;

²Research Institute of Medical University of Plovdiv (RIMU), Bulgaria;

³Department of Social Medicine and Public Health, Faculty of Public Health, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria

⁴Department of Paediatrics, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria;

⁵Second Department of Internal Medicine, Nephrology Section, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria;

⁶Faculty of Medicine, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria

Background: Rapid diagnostics are essential for managing urinary tract infections (UTIs) amid rising antibiotic resistance. This study evaluates the reliability of laser light scattering (LLS) technology, which monitors real-time bacterial growth kinetics, for antimicrobial susceptibility testing (AST) compared to the standard Bauer-Kirby disk diffusion method (DDM).

Methods: A total of 89 microbial isolates from urine samples of hospitalised patients with symptomatic UTIs were analysed. LLS was used for the initial screening for bacterial growth and subsequent AST analysis of the positive samples. Identification of isolates was performed using MALDI-TOF mass spectrometry after agar-plate culturing. Positive samples underwent rapid phenotypic AST via Alifax system (based on laser light scattering). The AST results (obtained in 3-6 hours) were statistically compared with DDM results (obtained in 24 hours) utilising categorical agreement and Cohen's Kappa coefficient.

Results: Among 89 isolated microorganisms, Gram-negative enterobacteria dominated, primarily *Escherichia coli* (42.7%) and *Klebsiella pneumoniae* (29.2%). A total of 18 antibiotics were tested for each strain. The comparative analysis confirmed almost perfect inter-method reliability (Overall Percentage Agreement 97.34%, Cohen's Kappa 0.9475), achieving 99.67% sensitivity and 100% specificity for detecting resistance. The limited discrepancies predominantly involved *E. coli* isolates tested against amoxicillin-clavulanate, highlighting a need for cautious interpretation with this combination. The LLS method reduced turnaround time by nearly 20 hours compared to DDM.

Conclusion: Laser light scattering technology demonstrates high analytical concordance with traditional DDM while drastically reducing AST turnaround time. This technology facilitates same-day targeted antibiotic therapy, which in turn could optimise patient outcomes and antimicrobial stewardship, thus holding potential as a cornerstone in precision microbiology for managing UTIs.

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Keywords: laser light scattering, urinary tract infections

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Accumulation of uranium by aquatic plants from Southern Portugal: Phytofiltration potential of contaminated water

Paulo Favas^{1,2}, João Pratas³

¹University of Trás-os-Montes e Alto Douro (UTAD), School of Life Sciences and the Environment, Vila Real, Portugal

²University of Coimbra, MARE – Marine and Environmental Sciences Centre / ARNET, Department of Life Sciences, Coimbra, Portugal

³Faculty of Sciences and Technology, University of Coimbra, Portugal

The capacity of several aquatic plants to accumulate uranium (U) has been well documented, supporting their potential use in the phytofiltration of U-contaminated water. This study evaluates the phytofiltration potential of native aquatic flora growing in waters from the uraniumiferous region of Nisa, southern Portugal. Plant and water samples were collected at selected sites near U-mineralized areas. The sampled flora included submerged, free-floating, and rooted emergent species. Plant material was thoroughly washed with fresh water to remove sediment and other foreign matter. When appropriate, samples were separated into roots and aerial tissues. After drying, plant samples were ground into a homogeneous powder for analysis. Water samples were filtered, acidified, and cooled immediately after collection. Uranium concentrations in water and plants were determined by fluorometry. Chemical analysis of stream water showed that U was present in surface waters at concentrations ranging from 0.63 to 59.42 µg/L, while pH ranged from 5.20 to 6.74. The mean U concentration in water (5.97 µg/L) was markedly higher than the estimated global riverine U flux values (0.2-0.6 µg/L). At one sampling site, U exceeded the WHO provisional guideline value for drinking water (30 µg/L). Plant analysis revealed substantial U bioaccumulation in

several species, at levels far above those measured in ambient water. In general, the highest U concentrations were found in submerged species, whereas the lowest occurred in free-floating species. Among submerged plants, the highest concentration was recorded in *Callitriche stagnalis* (314.11 mg/kg DW). In rooted emergent species, U appeared to be preferentially partitioned into rhizomes/roots, with the highest value observed in *Baldellia ranunculoides* rhizomes (10.68 mg/kg DW). The abundance of Callitricheaceae members, together with their biomass, relatively high bioproductivity, and capacity to accumulate several toxic elements simultaneously, highlights their potential for phytoremediation methodologies. Other species with high U uptake, such as *Lemna gibba*, *Fontinalis antipiretica*, *Riccia fluitans*, and *B. ranunculoides*, may also be suitable for monoculture or combined phytoremediation systems inspired by natural communities.

Keywords: *Callitriche stagnalis*; Macrophytes; Natural attenuation; Phytoremediation; Rhizofiltration

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Advances in the Separation and Purification of Optically Pure Enantiomers by Chiral Chromatography

Vasil Yotov¹, Katya Peycheva², Albena Merdzhanova², Veselina Panayotova²

¹Medical Student, Medical University of Varna

²Department of Chemistry, Medical University of Varna

The production of optically pure enantiomers is essential in pharmaceutical development, where small structural differences can lead to major variations in therapeutic activity and safety. Chiral chromatography has become one of the most powerful biotechnological tools for achieving high-resolution enantiomeric separation. This review summarizes recent advances in chromatographic techniques used for the purification of chiral compounds, with emphasis on biocompatible and sustainable approaches. Key developments include the design of recombinant protein-based chiral selectors, immobilized enzyme stationary phases, and polysaccharide-derived chiral columns that offer improved selectivity and robustness. Emerging technologies such as supercritical fluid chromatography, microfluidic chiral separation platforms, and multidimensional chromatographic workflows further enhance efficiency, scalability, and solvent reduction. Together, these innovations demonstrate the expanding role of chiral chromatography in producing enantiomerically pure pharmaceuticals and highlight its importance for precision medicine and modern biotechnological manufacturing.

Keywords: biotechnology, chromatography, enantiomerically pure pharmaceuticals

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Ancient Salt, Modern Solutions: Biotechnological Potential of

Halophiles from Miocene Deposits in Central Europe

Sona Brestovicova, Jakub Milcak, Zuzana Lorkova Kellnerova, Lea Nosalova, Lenka Malinovicova, Mariana Kolesarova, Jana Kiskova, Peter Pristas

Department of Microbiology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Safarik University in Kosice, Srobarova 2, 04001 Kosice, Slovakia

Background: The halophilic bacteria inhabiting high-salinity environments have evolved multiple molecular adaptations, allowing them to survive under high salt, low nutrient availability, and high-pressure conditions. These adaptations encompass diverse mechanisms, including the synthesis of compatible solutes and other bioactive metabolites, as well as specialized enzymes that are stable under industrial conditions.

Methods: The combination of the cultivation approach followed by genetic and genomic analyses was used to analyze the isolates from brines. Standard biochemical approaches were used to identify hydrolytic activities, and the combination of Raman spectroscopy and gas chromatography was used for polyhydroxyalkanoate characterization.

Results: Over 200 bacterial strains were isolated from brines associated with Miocene salt deposits in Slovakia, Ukraine, and Romania. Members of the Pseudomonadota class predominated in all samplings; however, species-level comparisons revealed minimal overlap between different sampling sites. Nearly 30% of isolated taxa are probably representatives of new species, as evidenced by 16S rRNA and genomic analyses. A broad spectrum of adaptations to hypersaline environments was detected, from genome reduction to specialized metabolite production. The isolates exhibited significant biotechnological potential, including the production of industrially relevant enzymes such as laccases, proteases, amylases, cellulases, and lipases. Some isolates demonstrated the capacity to synthesize polyhydroxyalkanoates, predominantly polyhydroxybutyrate, highlighting their promise as sustainable biopolymer producers.

Conclusion: Halophilic microbiota preserved within ancient Miocene salt deposits (approximately 10 to 12 million years old) represents a significant, largely untapped, and unique reservoir of biotechnological potential.

Keywords: Halophilic bacteria, brine, diversity, extremozymes, polyhydroxyalkanoates

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Application of CT scanning in Forensic education

Antoaneta Fasova¹, Mirena Sotirova², Teodora Gudelova², Atanasios Topalidis³, Slavi Delchev¹

¹Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University Plovdiv, Bulgaria

²Department of Forensic Medicine and Deontology, Faculty of Medicine, Medical University Plovdiv, Bulgaria

³Faculty of Medicine, Medical University Plovdiv, Bulgaria

CT scans are extensively used in forensic medicine training to provide non-invasive visualization, 3D anatomical modeling, and objective documentation of injuries. They enhance education by allowing students to study trauma, locate foreign bodies, analyze skeletal, dental, or decomposed remains without destroying evidence.

Virtual autopsy is not implemented in Bulgarian forensic medicine departments, representing a novel approach despite its established global use. Introducing this innovative, combined method for post-mortem examinations will enhance the accuracy of determining the cause of death, documenting injuries, and reconstructing the mechanisms involved. The current project aims to combine CT scans with a 3D anatomical visualization table Sectra, enabling the creation of three-dimensional images of anatomical structures. The database created could be used for training medical students, specialists, and PhD students in forensic medicine and imaging diagnostics, with a comprehensive level of their competence.

Key applications in Forensic education: virtual autopsy training: students learn to use 3D-CT data to visualize injuries and plan autopsies, which is vital for forensic pathology education. **Trauma and injury analysis:** CT scans offer clear visualization of fractures, air embolism in stab wounds, and gunshot wound paths, aiding in understanding cause of death. **Forensic anthropology:** CT is used for age, sex, and stature estimation, as well as identification of victims in decomposed or burned states. **Case documentation and presentation:** students learn to use digital, reproducible images for court presentations, which are often more objective than traditional autopsy photographs. **Pediatric trauma recognition:** CT is crucial for identifying signs of child abuse.

Benefits in education: non-destructive analysis: allows detailed study of cases without disturbing the body, preserving evidence. **Enhanced visualization:** 3D rendering helps in understanding complex, multi-layered trauma.

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Keywords: education, virtual autopsy, post-mortem CT examination, 3D scanning

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Artificial Intelligence-Assisted Imaging Interpretation in Extremity and Pelvic Trauma: Implications for Orthopedic Surgical Decision-Making

Keti Tokmakova¹, Valentin Petrov¹, Petar Molchovski¹, Penka Stefanova-Peeva², Dimitar Dachev²

¹Department of Orthopaedics and Traumatology, Medical University of Plovdiv, Plovdiv, Bulgaria

²Department of Propedeutics of Surgical Diseases, Department of Pediatric Surgery, Medical University of Plovdiv, Plovdiv, Bulgaria

Background: Extremity and pelvic injuries represent a substantial proportion of trauma-related morbidity and frequently require urgent orthopedic surgical intervention. Diagnostic inaccuracies or delayed imaging interpretation may significantly influence surgical prioritization and operative planning. Artificial intelligence (AI)-assisted imaging interpretation has emerged as a promising adjunct to conventional radiologic assessment in trauma settings, offering the potential to improve diagnostic accuracy and support clinical decision-making.

Methods: A structured review of the literature was conducted using PubMed, Web of Science, and Scopus databases, focusing on studies published between 2018 and 2025. Eligible studies evaluated AI-based imaging analysis for upper extremity, lower extremity, and pelvic trauma. Particular emphasis was placed on diagnostic performance, external validation, clinical applicability, and integration into orthopedic workflows.

Results: AI algorithms demonstrate high sensitivity and specificity for fracture detection across multiple anatomical regions, including distal radius, proximal humerus, hip, femoral shaft, tibial plateau, ankle, pelvic ring, and acetabular injuries. Emerging applications include automated fracture detection and classification, CT-based injury segmentation, and AI-supported decision tools for surgical planning. These technologies may support rapid triage, reduce diagnostic errors, and assist orthopedic surgeons in prioritizing surgical management in acute trauma settings. Despite encouraging performance metrics, important limitations remain, including dataset heterogeneity, limited external validation, regulatory considerations, and challenges related to integration into real-world clinical workflows.

Conclusions: AI-assisted imaging interpretation holds significant potential to enhance diagnostic accuracy and support orthopedic surgical decision-making in extremity and pelvic trauma. Future research should prioritize prospective validation studies, multicenter data harmonization, and structured implementation frameworks to facilitate safe and effective clinical adoption.

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Keywords: artificial intelligence, trauma imaging, clinical decision support

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Assessment of diets integrated with olive oil polyphenols in rainbow trout: gene expression study set-up and optimization

M. Torricelli¹, R. Branciarì², M. Trabalza Marinucci², E. Radicchi¹, G. Vita¹, M. Croce³, S. Ricci³, E. Giovannini³, A. Fratto¹, M. Biagetti¹, A. Di Paolo¹, A. Manfrin⁴, F. Agnetti¹

¹IZSUM, PG

²UNIPG, PG

Aquaculture is a growing sector due to the high demand of nutritive and beneficial food for consumers. Together with an ever-growing population, recovery of by-products for feed integration is a sustainable aim, in the green transition era. This project wants to evaluate in rainbow trout (*Oncorhynchus mykiss*) reared in outdoor tanks of about 120 specimens each, the effects of diets integrated with polyphenols extracted from olive oil by-products. Polyphenols are rich in bioactive molecules, chemically characterized, with known antimicrobial, anti-inflammatory, antioxidant, and immune-modulating properties. Differential gene expression will be assessed by RTqPCR in 3 categories of 9 specimens each, particularly the control, the low-dose polyphenol-treated, and the high-dose polyphenol-treated groups, targeting genes of immune-inflammatory, oxidative stress and metabolic pathways, normalized with β -actin house-keeping gene.

Prior to conducting experimental runs, assay optimization is generally crucial. Primer sets were designed by means of Primer3-BLAST for different genes encoding; immunity mediators; superoxide dismutase 1; glutathione reductase; catalase; glutathione peroxidase; alkaline phosphatase; fatty acid-binding protein; fatty acid desaturase 6; elongation of very long-chain fatty acids protein 5. Amplicon length and standard parameters have been considered together with the presence of an intron in the corresponding genomic DNA, to avoid contamination and amplification bias. RNA extraction was performed with Trizol[®], followed by Qubit[®] fluorometer quantification and RTqPCR.

For assay optimization, amplification was performed using SuperScript[®]III Platinum[®]SYBR[®] Green One-Step and an ideal concentration (200 nM) of primer forward/reverse as of template. Targets' amplifications resulted efficient and repeatable, and melting curve pointed out primer sets specificity. Gene expression and transcriptomic studies will be implemented in the next future.

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Keywords: nutrigenomic, RTqPCR, circular economy, *Oncorhynchus mykiss*

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Association of Common Cardiovascular Genetic Variants with Coronary Artery Disease in a Bulgarian Case-Control Cohort

Kristiyana Vitanova^{1,2}, Teodora Tsvetanova³, Delyan Georgiev^{1,2}, Valentina Peycheva^{1,2}, Martin Georgiev^{1,2}, Arman Postadzhiyan⁴, Margarita Apostolova³, Radka Kaneva^{1,2}

¹Genome Diagnostics Laboratory, Department of Medical Chemistry and Biochemistry, Medical Faculty, Medical University of Sofia, Sofia, Bulgaria, 1431

²Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Medical Faculty, Medical University of Sofia, Sofia, Bulgaria, 1431

³Institute of Molecular Biology "Rumen Tsanev", Bulgarian Academy of Sciences, Sofia, Bulgaria, 1113

Background: Coronary artery disease (CAD) is a complex, multifactorial condition influenced by both genetic and environmental factors. The estimated heritability of CAD ranges from 40–60%, and numerous common genetic variants contribute to disease susceptibility through pathways involved in lipid and triglyceride metabolism, vascular function, and inflammation. This pilot association study investigates common variants in cardiovascular genes in patients with CAD and healthy controls.

Methods: Seventeen patients with CAD and a 257 controls were included. Targeted next-generation sequencing (NGS) with the TruSight[®] Cardio Sequencing Kit (Illumina, San Diego, CA, USA) was performed. Common variants (MAF > 0.05) in cardiovascular genes were assessed, and their distribution between cases and controls was evaluated. Data processing was performed using BCFtools 1.23 and PLINK 1.9, and association analysis was conducted using Fisher's exact test with the Haldane–Anscombe correction.

Results: Several potential associations were found. Significant differences were observed for variants in genes involved in lipid and triglyceride metabolism, including *PCSK9* (rs35574083, $p=0.0003$), *APOA4* (rs147610191, $p=0.01$), *APOB* (rs373477107, $p=0.001$), *GPIHBP1* (rs61747644, $p=0.01$), and *CETP* (rs5883, $p=0.002$). Associations were found also in genes related to vascular structure and function, such as *TGFBR2* (rs2228048, $p=0.006$) and *MYH11* (rs794728667, $p=0.01$); and cardiac structure, such as *DPP6* (rs3734960, $p=0.02$) and *TRIM63* (rs35123100, $p=0.003$).

Conclusion: This pilot study revealed some interesting associations of common variants with CAD in Bulgarian patients. Some of the genes, including *PCSK9*, *APOB*, and *CETP* have been previously reported in GWAS of CAD and lipid traits. For variants detected in *APOA4*, *GPIHBP1*, *TGFBR2*, *MYH11*, *DPP6*, and *TRIM63* there is limited evidence for direct CAD association and further investigation in larger cohorts is needed.

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Keywords: genetic variants, coronary artery disease, next-generation sequencing

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Attitudes Toward AI-Based Personalized Wellness Services in the Context of Promoting Preventive Health: A Case Study of the Adjara

Giorgi Gogitidze¹, Guguli Turmanidze¹, Tommaso Beccari²

¹Grigol Robakidze University, Tbilisi 0159, Georgia

²University of Perugia, Perugia, Italy

Contemporary trends in digital health and preventive medicine have increased interest in using artificial intelligence to

develop and promote personalized wellness services. Recent trends indicate that AI-based systems enable the development of recommendations tailored to individual needs, lifestyles, and health-related behaviors, which may be regarded as an effective tool for promoting preventive health. The aim of our study was to examine attitudes toward AI-based personalized wellness services in Adjara. A quantitative study was conducted using a questionnaire survey, with 200 respondents. Based on the findings of the present study, it is noteworthy that the development of health-oriented, innovative, and preventive wellness services may represent a promising direction that could significantly create new opportunities in wellness tourism.

Keywords: artificial intelligence, wellness services, personalization, preventive health, Adjara

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Blood hematology and biochemical monitoring in show jumping horses

Rădulea Alexandros, Turbatu Raluca Mihaela, Brăslăușu Elena Daniela, Ignătescu Roxana Mariana, Ioniță Carmen

University of Agronomic Sciences and Veterinary Medicine Bucharest, Romania Faculty of Veterinary Medicine Bucharest

Streets are physical or recreational challenges designed to test the speed, agility and endurance of an animal or a team that must complete them, usually timed, through jumping and balance. Horse show jumping mainly consists of fences (smaller, foldable) and fences/obstacle courses (larger, solid) used in show jumping races. Other obstacles include water jumps, open ditches or hedged ditches: they are designed to test the agility and endurance of a horse. Hurdles can range in height from 1.03 m to over 1.53 m. The training of show jumping horses requires a holistic, long-term approach that balances physical conditioning, mental preparation and, most importantly, maintaining their health. A successful competition horse is a “happy athlete”, which means that its training, diet and rest must be managed to avoid injuries and exhaustion. Physical conditioning should begin at least 3 months before the start of the season, with an emphasis on gradual and progressive training. Usually, show jumping horses have good health, their daily training allows them to be in great sporting shape, any ailments being the result of inadequate nutrition in quantity and quality, in this regard, checking the ration, feed assortments and water must be done periodically. Following hematological and biochemical laboratory tests on blood samples from 5 horses, alterations in blood parameters were found, which allows the identification of hidden diseases and their early treatment.

Keywords: obstacle course; training; feed; metabolic profile

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Catechin-rich hazelnut green husk extract: antimicrobial activity and DNA gyrase interaction

Pelin Kiraz¹, Gökem Güngör², Mehmet Özbil², Meltem Yeşilçimen

Akbaş¹

¹Gebze Technical University, Department of Molecular Biology and Genetics, Kocaeli, Türkiye

²Gebze Technical University, Institute of Biotechnology, Kocaeli, Türkiye

Background: The utilization of plant-derived phytochemicals contributes to global sustainability goals by the valorization of agricultural by-products and reducing environmental waste. Plants contain various phytochemicals with antimicrobial properties. Hazelnut (*Corylus avellana*) is one of the most widely consumed nuts globally; however, the hazelnut processing industry generates more than 300 thousand tons of green husk waste annually. This study aims to explore the antimicrobial potential of these husks as a natural source of bioactive compounds.

Methods: A methanolic extract was obtained from hazelnut green husks and analyzed for its phenolic contents. The antimicrobial activity of the extract was evaluated against *Staphylococcus aureus* strains (ATCC 25923 and ATCC 29213) using agar well diffusion and broth microdilution assays. Ciprofloxacin was used as a comparative standard. Additionally, molecular docking simulations were performed to assess the binding affinity of catechin to DNA Gyrase, a key antibacterial target.

Results: The extract was found to contain high amounts of catechin (0.5527 mg/mL). In agar well diffusion assays, the extract showed inhibition zone diameters of 12-6 mm for ATCC 25923 and 16-6 mm for ATCC 29213, while ciprofloxacin (1 µg/mL) yielded 12 mm for both. MIC₉₀ values for the extract were 6250 µg/mL and 6263 µg/mL, compared to 710 µg/mL and 720 µg/mL for ciprofloxacin. Molecular docking revealed that catechin had higher binding affinities (-9.03 kcal/mol and -9.12 kcal/mol) against DNA Gyrase subunits than ciprofloxacin (-7.94 kcal/mol and -7.18 kcal/mol).

Conclusion: These findings suggest that hazelnut green husk represents a promising natural source of catechin with significant antimicrobial potential. The valorization of this agricultural waste offers a viable alternative to conventional antibiotics and supports environmental sustainability.

Keywords: Antimicrobial, DNA Gyrase, hazelnut green husk, plant extract, *Staphylococcus aureus*

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Chromatin condensation, survival rate and morphological evidence of AL-PCD at fine root cells of *T.aestivum* under stress

Nadiel Çadri¹, Bamela Tafa¹, Aja Pollo¹, Uejda Llaka¹, Ariola Bacu^{1,2}

¹Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania

²Academy of Sciences of Albania, Albania

Studying AL-PCD in plants is crucial for plant development especially in climate change conditions. This study aimed to establish workflows investigating AL-PCD phenomena in wheat (*T.aestivum* L.) under stress conditions (heat, Ethanol and NaCl treatment).

Plant material was prepared following Hogg *et al.*, 2011. For heat treatment, Five-day-old seedlings were exposed to 55°C, then returned to 23°C for 6 hours before analysis. Ethanol treatment: Five-day-old seedlings were placed in 6-cm Petri dishes, incubated at room temperature for 6 hrs. NaCl treatment: Five-day-old wheat seedlings were incubated in 6-cm Petri dishes for 5 min and were transferred to 6-cm Petri dishes containing 5 ml of ddH₂O and kept in constant light, room temperature for 24 hrs. To assess chromatin condensation, 2mm of fine roots were stained in DAPI (1µg/ml), visualized in Fluorescence Inverted Microscope (10X-40X) with OptikaProview to measure fluorescence intensity, alive/dead cells and cell morphology across 5 samples X 5 standard squares (controls and treated). Same samples were used to score alive/dead cells, assess membrane damage and lesion formation. Results showed chromatin condensation at stressed material, with HT material reaching 2X fluorescence than controls. Also, other treatments exceeded control levels. No significant differences were found between the two cultivars under heat stress. Root cell survival declined progressively across ethanol, salinity and heat treatments, causing membrane damage and necrotic lesions up to threefold larger than normal cells.

In conclusion, the workflows effectively assess AL-PCD under abiotic stress and support selection of resistant cultivars.

Keywords: AL-PCD, chromatin condensation, fluorescence, cell morphology

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Clinical and paraclinical research in turkey chicks raised in a semi-intensive system

Dumitrescu (Teodorescu) Dana- Maria, Ioniță Carmen, Turbatu Raluca Mihaela, Purdoi Letiția, Ignătescu Roxana Mariana, Brăslas Elena Daniela, Ioniță Lucian

University of Agronomic Sciences and Veterinary Medicine Bucharest, Romania Faculty of Veterinary Medicine Bucharest

In a private microfarm, at the request of the owner, we performed a clinical examination and blood sampling for biochemical and hematological examination in turkeys and turkey poults; we collected blood from 10 turkey poults (age 21 days). The clinical manifestations observed in turkeys and turkey poults can be considered "technopathies Pendulous goiter is a condition that inhibits growth and impairs yield; the etiology is not fully elucidated; it seems to be a deficiency in pyridoxine or excessive food intake causes the glandular stomach to expand. At a percentage of 0.5-1% we observed unilateral and/or bilateral infraorbital sinusitis; to discover the etiology of this condition which is usually infectious, we took blood samples from 2 males and 2 females and performed a Leukocyte Formula which was not modified; so air currents, poor ventilation led to the appearance of this condition. Locomotor disorders and conditions are isolated cases...but they do exist; we found lameness, we observed lack of movement, lateral decubitus and "curved claw position". Locomotor disorders are due to some

dysmineralosis, dysvitaminosis (in B2), etc. Biochemically, we determined: total pro-teins, cholesterol, glucose, ASAT, ALAT, GGT, uric acid and creatinine; we found hypoalbuminemia, hypoglycemia, increased ALAT activity: the rest of the parameters are normal. Hypo-albuminemia can be the expression of an Inflammatory syndrome in response to exogenous or endogenous stress factors, which can alter protein metabolism or hepatic synthesis disorders. Increased ALT in meat turkeys is often related to nutritional or metabolic imbalances. Hypoglycemia in 21-day-old (3-week-old) turkey poults is a critical metabolic condition, often related to environmental stress, malnutrition or an underlying disease.

Keywords: stress; turkeys; pendulous goiter; ventilation; techn

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Clinical evaluation of oral cavity diseases in domestic cats: a study of 10 cases

Turbatu Raluca Mihaela, Fernoagă Cristina, Brăslas Elena Daniela, Vlăgioiu Adriana Daniela, Ioniță Carmen

University of Agronomic Sciences and Veterinary Medicine Bucharest, Romania Faculty of Veterinary Medicine

Oral cavity diseases represent common pathologies in feline medicine and can significantly affect the health and welfare of domestic cats. This study evaluated the clinical presentation and prevalence of oral diseases in a population of ten feline patients examined at the "Prof. Dr. Alin Bîrțoiu" University Veterinary Emergency Hospital in Bucharest. For each case, the diagnostic protocol consisted of anamnesis, clinical examination, and complementary investigations when required. The evaluation included both extra-oral and intra-oral examination of oral tissues, teeth, gingiva, and mucosal structures in order to identify inflammatory, infectious, or proliferative lesions. In selected cases, additional investigations were performed to confirm the diagnosis, including blood analyses, PCR testing for infectious agents such as feline calicivirus, feline herpesvirus, *Mycoplasma spp.*, and *Chlamydia spp.*, as well as imaging techniques when indicated.

The results showed that oral diseases affected cats of different ages and both sexes, although certain tendencies were observed. Among the ten patients, males were predominant (7 cases). Age distribution indicated a higher frequency of oral pathologies in older animals, with four cats older than 12 years. However, three cases were also identified in the 0-2 year age group, showing that oral diseases may also occur in young animals.

Periodontal disease was the most frequently diagnosed condition, affecting approximately 50% of the evaluated cats. Chronic gingivostomatitis represented the second most common disorder, while eosinophilic granuloma complex was less frequent but clinically relevant. The most common clinical signs included halitosis, gingival inflammation, sialorrhea, dysorexia, oral discomfort, and lesions located

at the mucocutaneous junction of the upper lip in cases of feline eosinophilic granuloma complex.

These findings highlight the importance of early diagnosis, thorough clinical examination, and appropriate therapeutic management, including local and systemic treatment when required, in order to improve the health and quality of life of affected feline patients.

Keywords: Feline oral diseases; periodontal disease; gingivostomatitis; veterinary dentistry; feline stomatology

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Comparative analysis of the antioxidant activity of extracts from three Black Sea bivalves

Rositsa Stancheva, Veselina Panayotova, Katya Peycheva, Diana A.

Dobрева, Tatyana Hristova, Tonika Stoycheva, Neli Hristova, Albena Merdzhanova

Department of Chemistry, Faculty of Pharmacy, Medical University of Varna, Varna, Bulgaria

Marine bivalves are known to accumulate various secondary metabolites that may exhibit antioxidant properties. This study investigates the antioxidant properties of 70% and 95% ethanol extracts from the tissues of three Black Sea bivalve species: *Mytilus galloprovincialis*, *Donax trunculus*, and *Chamelea gallina*. The antioxidant potential was evaluated using four spectrophotometric assays based on different chemical mechanisms: DPPH and ABTS (radical scavenging) and FRAP and CUPRAC (reducing power). The results indicate that the extraction of antioxidant compounds was influenced by both the species and the ethanol concentration used. In the 70% ethanol group, the highest activity was measured in *M. galloprovincialis* (1.75 mM TE/g via ABTS). Within the 95% ethanol extracts, *C. gallina* showed the highest values, particularly in the CUPRAC assay (5.24 mM TE/g). These variations suggest that the biochemical composition of the samples responds differently to solvent polarity, likely reflecting differences in species-specific diets and environmental conditions. Statistical analysis identified specific relationships between the chemical components and the observed activity. Total phenolic content correlated most strongly with the ABTS results ($r = 0.9040$), while total carotenoids showed a higher correlation with the FRAP assay ($r = 0.9130$). These findings suggest that phenolic and carotenoid compounds contribute to the antioxidant capacity through different chemical pathways.

In conclusion, the data show that these Black Sea bivalves contain compounds with measurable antioxidant activity, particularly when extracted with 95% ethanol. Such results provide a basis for further research into their potential use as ingredients in functional food or biotechnological applications.

Keywords: Black Sea bivalves; Antioxidant capacity; Ethanol extraction; Phenolic compounds; Carotenoids

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Determination of the content of some heavy metals in walnut (*Juglans Regia*) fruits in various villages of mountainous Adjara

Nino Kedelidze^{1,2}, David Baratashvili², Nani Gvarishvili²

¹Institute of Phytopathology and Biodiversity, Batumi Shota Rustaveli State University, Batumi, Georgia

²Department of Biology, Faculty of Natural Sciences and Health Care, Batumi Shota Rustaveli State University, Batumi, Georgia

To detect heavy metals and radioactive substances, samples from 6 control plants (walnut fruits) were collected from different villages in Khulo Municipality. The content of mercury and arsenic is almost equal (0.001-0.01 µg/kg) in the fruits of the varieties selected for research in different villages of Khulo municipality. The fruits of form No. 2 are distinguished by their relatively high cadmium content (0.02±0.003 µg/kg), which is found in the village of Vernebi. Form No. 2 (0.12 µg/kg) is also distinguished by the highest lead content, while form No. 4 (0.03 µg/kg) is distinguished by the lowest content in the village of Kvatia. The highest copper content was also recorded in form No. 4 (11.2 µg/kg), while form No. 2 (5.4 µg/kg) stands out for this element in the village of Pushrukauli. The highest zinc content was observed in form No. 5, distinguished in Pushrukauli (35.5 µg/kg), while the lowest content was observed in form No. 1, distinguished in Vernebi (19.6 µg/kg). Form No. 1, which is found in the village of Vernebi, stands out for its high (6.78±13.8 µg/kg) cesium content. The low level is observed in the village of Pushrukauli, in the form of No. 5 (0.00±14.7 µg/kg). Form No. 2 stands out in the village of Vernebi with its high strontium content (5.40±21.9 µg/kg). As the research results showed, the content of some heavy metals and radioactive substances varies, both at the level of different villages and forms.

Keywords: Walnut (*Juglans Regia*), heavy metals, fruit, Adjara, content.

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Digital Whole-Slide Imaging in pathology education: scanned histological slides as a platform for Electronic Learning

Elena Poryazova^{1,2,4}, Daniel Markov^{1,2}, Veselin Chonov¹, Galabin Markov³, Antoaneta Fasova⁵, Nadya Penkova⁵, Elena Daskalova⁵, Zdravka Harizanova⁵, Ferihan Popova⁵

¹Department of General and Clinical Pathology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria;

²Department of Clinical Pathology, Pulmed University Hospital, Plovdiv, Bulgaria

³Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria;

⁴Clinical and Experimental Morphology Division, Research Institute at Medical University of Plovdiv, Medical University of Plovdiv, Plovdiv, Bulgaria

⁵Department of Anatomy, Histology and Embryology, Medical University of Plovdiv, Plovdiv, Bulgaria

The transition from conventional light microscopy to digital, whole-slide imaging (WSI) has created a mature technological

foundation for scalable e-learning in morphology-based disciplines such as histology and pathology. WSI enables complete digitization of glass slides into navigable, multi-resolution “virtual slides” that can be stored, shared, annotated, and repeatedly used for teaching and assessment, thereby extending access beyond the microscope room and enabling structured self-directed learning.

A defining advantage of digitized slide repositories is standardization: all learners can work with the same high-quality images, avoiding variability due to slide fading, broken coverslips, limited microscope availability, or restricted lab hours. Evidence synthesis in medical education indicates that virtual microscopy is commonly preferred by learners and is associated with improved academic performance compared with traditional light microscopy in many settings (meta-analysis pooled effect size demonstrating higher exam performance).

We present the project-oriented concept of Medical University Plovdiv into an academically framed implementation and education model. The central aim is to transform archived histology/pathohistology teaching slides into high-resolution digital images, store them securely within institutional infrastructure, and deploy them via an authenticated virtual platform that supports annotations, search, curriculum alignment, and integration with formative and summative assessment.

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Keywords: education, pathology, histology, whole-slide imaging, virtual microscopy

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Distinct Gut Microbiome Signatures in Patients with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

Ralitsa Nikolova^{1,2,3,4}, Deyan Donchev^{4,5}, Katya Vaseva^{1,4}, Ivan N. Ivanov^{4,5}

¹Department of Medical Microbiology and Immunology “Prof. Dr. Elissay Yanev”, Faculty of Medicine, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria;

²Division of Innovative Diagnostic Methods, Research Institute at Medical University of Plovdiv, 4002 Plovdiv, Bulgaria

³Laboratory of Microbiology, University Hospital St. George, 4002 Plovdiv, Bulgaria

⁴Strategic Research and Innovation Program for the Development of Medical University of Plovdiv (SRIPD-MUP), 4002 Plovdiv, Bulgaria

⁵National Reference Laboratory for Control and Monitoring of Antimicrobial Resistance, Department of Microbiology, National Center of Infectious and Parasitic Diseases, 26 Yanko Sakazov Blvd., 1504 Sofia, Bulgaria

Background: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a chronic multisystem illness that is frequently accompanied by gastrointestinal symptoms. Growing evidence suggests distinct gut microbiome profiles in affected individuals. The objective of the current

study was to investigate the gut microbiome features of patients with (ME/CFS).

Methods: Following strict inclusion criteria, 75 ME/CFS patients and 37 healthy controls aged 18-65 were selected. Gut microbiome composition was profiled by full-length 16S rRNA gene sequencing with the Microbial Amplicon Barcoding Kit (Oxford Nanopore Microbial Amplicon Barcoding Kit) followed by taxonomical classification with the Emu database v3.4.5. Profiles were assessed using non-phylogenetic diversity metrics and ordination. Beta diversity was assessed using Bray–Curtis distances in QIIME2 with multivariable testing adjusting for age, BMI, and gender. Differential abundance analysis (DAA) was performed using ANCOM-BC at genus and species levels. Microbial co-abundance networks and multivariable association models were applied to explore relationships between taxa and clinical questionnaire scores related to anxiety and depression.

Results: Beta diversity analysis revealed significant differences in gut microbial community composition between ME/CFS patients and controls (PERMANOVA $p=0.021$), without significant dispersion differences (PERMDISP $p=0.112$). Multivariable PERMANOVA confirmed that disease status remained significantly associated with microbiome composition after adjusting for age, BMI, and gender ($R^2=1.8\%$, $p=0.016$). No significant effects of age, BMI, or gender were observed, and no interaction between BMI and disease status was detected. DAA identified several taxa significantly altered in ME/CFS patients, including increased abundance of *Phascolarctobacterium* (LFC=1.44, $p=0.024$) and *Subdoligranulum* (LFC=1.01, $p=0.002$). Conversely, reduced abundance was observed for taxa such as *Prevotella* (LFC=−1.72, $p=0.011$) and *Holdemanella* (LFC=−1.52, $p=0.0005$), indicating disease-associated shifts in microbial composition.

Conclusion: These findings demonstrate a significant association between ME/CFS and alterations in gut microbiome structure, independent of major demographic and metabolic covariates, with specific taxa contributing to community-level differences.

Keywords: gut microbiome, dysbiosis, Myalgic Encephalomyelitis, Chronic Fatigue Syndrome

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Effect of Salinity and Drought on *Spartina patens* in Mediterranean Coastal Wetlands

Adrián Sapiña-Solano¹, Mario X. Ruiz-González², Oscar Vicente³, Monica Boscaiu¹

¹Mediterranean Agroforestry Institute (IAM), Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain, mobosnea@eaf.upv.es

²Department of Plant Production and Forest Resources, University of Valladolid, Avda. Madrid 57, 34004 Palencia, Spain

³Institute for Conservation and Improvement of Valencian Agrobiodiversity (CO-MAV), Universitat Politècnica de València, Camino de Vera s/n, 46022 Valen-

Coastal wetlands are ecosystems of high ecological value because of their biodiversity and the ecosystem services they provide. However, they are threatened by anthropogenic pressure, climate change, and the spread of invasive species, which can alter plant and soil microbial communities. In the Albufera Natural Park (Spain), the expansion of *Spartina patens*, a halophytic grass native to the Atlantic coasts of North America, represents an ecological threat. This species forms dense mats that restrict the development of native vegetation, modifying plant community structure and reducing biodiversity.

This study analyses the tolerance mechanisms of *S. patens* and its mycorrhizal colonisation under drought and salt stress. Morphological parameters, photosynthetic pigments (chlorophylls *a* and *b*, carotenoids), osmolytes (proline, total soluble sugars, glycine betaine), ions (Na^+ , Cl^- , K^+ , Ca^{2+}), oxidative stress markers (MDA, H_2O_2), antioxidant enzyme activities (superoxide dismutase, catalase, glutathione reductase, ascorbate peroxidase) and antioxidant metabolites (total phenolic compounds, flavonoids) were evaluated. In addition, mycorrhizal colonisation structures (arbuscules, vesicles and hyphae) were quantified under salinity and water stress.

Spartina patens reduced leaf number from 400 mM NaCl onwards, whereas the root-to-shoot ratio increased significantly at 800 mM NaCl. Proline accumulation was observed, and the species restricted the transport of Na^+ and Cl^- to the aerial parts, maintaining high K^+ levels in leaves. All treatments reduced mycorrhizal colonisation; however, the percentage of arbuscules increased under 200 mM NaCl, which could enhance the invasive capacity of *S. patens* and consequently disturb ecosystem balance.

Keywords: coastal wetlands, drought, mycorrhizae, salinity, *Spartina patens*

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Effect of Thrombolytic Therapy on Mitochondrial Respiration and HIF-1 α Levels in Patients with Ischemic Stroke

Maria Kazakova^{1,2}, Eleonora Kovacheva^{1,2}, Maria Gevezova^{1,2}, Margarita Koeva³, Valentina Mihaylova^{1,2}, Vasilka Kormova³, Emanuela Kostadinova³, Yulia Kostadinova³, Victoria Sarafian^{1,2}

¹Department of Medical Biology, Medical University-Plovdiv, 4000 Plovdiv, Bulgaria

²Research Institute, Medical University-Plovdiv, 4000 Plovdiv, Bulgaria

³Neurology Ward, University Hospital "Pulmed", 4000 Plovdiv, Bulgaria

Background: Ischemic stroke (IS) is characterized by mitochondrial dysfunction caused by reduced oxygen supply to the brain. Cellular adaptation to hypoxia involves activation of hypoxia-inducible factor-1 alpha (HIF-1 α), a key regulator of metabolic and inflammatory responses. Thrombolytic therapy restores cerebral blood flow and its impact on mitochondrial

bioenergetics and hypoxia-related signaling remains insufficiently understood. The aim of the present study was to evaluate mitochondrial respiratory function and plasma HIF-1 α levels in patients with IS before and after thrombolytic therapy.

Methods: A total of 62 samples of patients with IS were included in the study - patients before (n=31) and after thrombolytic therapy (n=31). Mitochondrial function was assessed using the Seahorse analyzer (Agilent). Plasma concentrations of HIF-1 α were determined using enzyme-linked immunosorbent assay (ELISA).

Results: Mitochondrial functional analysis revealed a significant increase in basal respiration and spare respiratory capacity after thrombolytic therapy compared to values measured before treatment. These findings suggest improved mitochondrial bioenergetic capacity following reperfusion. In contrast, plasma HIF-1 α levels were significantly decreased after thrombolysis compared to levels measured at admission, indicating a reduction in hypoxia-related signaling following restoration of blood flow.

Conclusion: Thrombolytic therapy in IS patients is associated with improved mitochondrial respiratory function and reduced circulating HIF-1 α levels. These findings suggest that restoration of cerebral perfusion may contribute to normalization of cellular bioenergetics and attenuation of hypoxia-induced molecular responses.

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Keywords: ischemic stroke, thrombolysis, mitochondrial respiration, Seahorse analysis, HIF-1 α , hypoxia

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Effects of antibiotic and corticosteroid therapy on hypertrophic scar development in a rabbit ear experimental model

Ancuța Diana Larisa¹, Vaduva Mariana^{1,2}, Caraș Iuliana¹, Coman Cristin^{1,2,3}

¹"Cantacuzino" National Military-Medical Institute for Research and Development, Bucharest, Romania

²Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania

³Center of Excellence in Translational Medicine, Fundeni Clinical Institute, Bucharest, Romania

Background: Hypertrophic scars (HTS) represent a pathological outcome of wound healing characterized by excessive collagen deposition and persistent inflammation. Animal models are essential for understanding the biological mechanisms involved in abnormal scar formation and for evaluating therapeutic strategies. This study aimed to investigate the effects of antibiotic prophylaxis and corticosteroid treatment on inflammatory response and hypertrophic scar formation in a rabbit ear experimental model.

Methods: Three adult *New Zealand White* rabbits were included in the study. Standardized auricular wounds were created to induce hypertrophic scarring. Animals were assigned to three treatment conditions: combined dexamethasone and amoxicillin (ID1), amoxicillin alone (ID2), and dexamethasone alone (ID3). Each animal also had an untreated contralateral control wound. Clinical monitoring, hematological analysis, and inflammatory indices, including neutrophil-to-lymphocyte ratio (NLR), were evaluated at baseline and during the 21-day follow-up period. After euthanasia on day 28, tissue samples were collected for histological examination using Masson's Trichrome staining to assess collagen organization, cellularity, and scar architecture.

Results: Animals receiving combined dexamethasone and amoxicillin showed a controlled inflammatory response with normalization of hematological parameters by day 21. The amoxicillin-only group demonstrated moderate but persistent inflammation. In contrast, the dexamethasone-only group developed severe systemic inflammatory responses, characterized by progressive increases in NLR and neutrophil counts. Histological analysis confirmed marked differences between groups. The dexamethasone-only group exhibited severe hypertrophic scarring with excessive collagen deposition, hypercellularity, and intense neovascularization, frequently associated with inflammatory or necrotic foci. The amoxicillin-treated group showed a more organized dermal structure and reduced scar elevation compared with controls.

Conclusion: The results highlight the importance of infection control in wound healing and demonstrate that corticosteroid treatment without adequate antibiotic prophylaxis may exacerbate inflammatory complications and hypertrophic scar formation. The rabbit ear model proved suitable for investigating the interaction between inflammation, infection, and fibrotic scar development.

Keywords: hypertrophic scar, wound healing, rabbit ear model, inflammation, antibiotic prophylaxis

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Efficiency and efficacy of video monitoring of wild boars' motion in endemic area of african swine fever in Romania

Oana Tanase¹, Mihaela Anca Dascalu¹, Florica Barbuceanu², Florentina Daraban Bocaneti¹

¹"Ion Ionescu de la Brad" Iasi University of Life Science, M Sadoveanu Alley 8, 700490 Iasi, Romania

²Institute for Diagnosis and Animal Health, Dr Staicovici 63, Sector 5, Bucharest 050557, Romania

African swine fever is a viral disease of pigs and wild boars that causes high mortality in the affected animals, resulting in significant socio-economic impact in many countries. This study aimed to establish the efficiency and efficacy of video monitoring by evaluation of the video monitoring activation and the frequency of wild boars' motion during the February-November 2025 period, using a thermal network mini hybrid bullet

camera installed in a feeding area from Victoria village, Iași County (47°38'14.16"N 27°56'35.12"E), Romania. During this period, the camera was actively recording the presence of wild boars in March, May and June, with 3 nocturnal and 6 diurnal motion detections. Based on the data collected during the monitoring period, there were approximately 8 adult boars and 16 wild piglets, with the gender unable to be established. No cases of hyperthermia were observed among the wild boars; all those observed were feeding and moving normally. No wild boar carcasses were found in the study area. Video monitoring is an efficient tool in surveillance programs, providing real-time information.

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Keywords: wild boars, motion, hyperthermia

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Endoparasite infections in domestic cats in Southern Romania: preliminary epidemiological data from a coprological assessment

Anca Marian Zisopol, Emanuel Mitrea, Ioan Liviu Mitrea, Mariana Ionita

Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Bucharest, Romania

Endoparasites in cats are of significant relevance for both animal and public health, since they can affect the animal health and, in addition, some of them have a zoonotic potential. Therefore, the present study aimed to investigate the occurring and epidemiology of endoparasite infections in cats, Romania. For this, 244 fecal samples collected from domestic cats of different age groups, lifestyle, living conditions, and habitat, from 10 premises in six counties, Southern Romania, were subjected to parasitological investigations by using a qualitative sodium chloride flotation method and microscopy. Overall, 41.8% (95% CI: 35.54 - 48.27) of the tested samples were positive for at least one endoparasite infection, 32.8% (95% CI: 26.93 - 39.07) were single and 9.0% (95% CI: 05.73 - 13.34) were concurrent infections. Significantly higher infection rates were registered for young animals (<1 year old), stray, outdoor, and rural cats, respectively. Among the identified species, the most prevalent was *Toxocara cati* (37.7%), followed by *Ancylostoma tubaeforme* (5.3%) and *Toxascaris leonina* (3.3%); a lower frequency was registered for *Capillaria aerophila*, *Dipylidium caninum*, *Cystoisospora* spp. Age, lifestyle, living conditions and habitat were identified as potential risks for various parasites. In conclusion, the findings emphasize a high prevalence and diversity of endoparasitic fauna, including zoonotic species, in the investigated cats and highlight the need for increasing awareness on the associated risks.

Keywords: domestic cats, endoparasites, epidemiology, Southern Romania

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From biofilms to biotherapeutics: harnessing *Streptococcus salivarius* bacteriocins for next-gen oral health

Kremena Yordanova^{1,2}, Ralitsa Galabinova¹, Stanimira Angelova^{1,2}, Tonka Vasileva^{1,2}, Ilia Iliev^{1,2}

¹Paisii Hilendarski University of Plovdiv, Faculty of Biology, Department of Biochemistry and Microbiology

²Centre of Technologies, Paisii Hilendarski University of Plovdiv

The stability of the oral microbiome is a complex process that is influenced by several factors, such as mode of birth, diet, and intake of antimicrobial agents or probiotics. The human oral microbiome plays an exceptional role in human health throughout life. It is also closely linked to the health of the human gastrointestinal tract and maintaining it in a favorable state. Throughout a person's life, their oral microbiome undergoes a series of changes influenced by lifestyle, diet, hygiene, and the intake of various medications and probiotic preparations. To study these relationships and influences, we used oral samples from a group of children aged 3 to 6 years in different regions of Bulgaria. Potential strains were selected using morphological and biochemical identification methods. The selected strains were grown on BHI broth and Mitis Salivarius agar media. Molecular identification was performed by 16S rRNA sequencing. Eighty strains were isolated, twelve of which were identified as *Streptococcus salivarius*. Phenotypic and metabolic profiling was performed using 10 different prebiotic oligosaccharides and 10 different fibers to assess metabolic diversity among the new strains *S. salivarius*. The results revealed notable strain-specific differences in metabolic activity, particularly in the utilization of carbohydrate sources relevant to oral biofilm ecology. Furthermore, three of them were able to synthesize glucan and six strains showed antimicrobial activity against *E. coli*, *Staphylococcus aureus* and *Micrococcus luteus*. Selected *Streptococcus* strains expressed the ability for mucin adhesion. These promising results demonstrate the ability of these novel strains to be candidates for future oral probiotics.

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Keywords: *Streptococcus salivarius*, oral microbiota, prebiotic oligosaccharides, fibers, antagonistic activity, probiotics

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PV-Negative Cervical LSIL Cytology Associated with Atypical Endometrial Hyperplasia Exhibiting PTEN/PAX2 Loss and High AMHR2 Expression: A Complex Case

Neli Doneva¹, Ekaterina Uchikova¹, Nikoleta Parahuleva¹, Mariana Parahuleva⁴, Mariya Koleva², Anna Mihailova³, Emral Kyosebekirov⁵, Angelina Mollova-Kysebekirova²

¹Department of Obstetrics and Gynecology, Faculty of Medicine, Medical University of Plovdiv

²Department of General and Clinical Pathology, Faculty of Medicine, Medical University of Plovdiv

³Department of Healthcare Management, Faculty of Public Health, Medical University of Plovdiv

⁴Universitätsklinikum Gießen und Marburg Standort Marburg, Marburg, Germany

⁵Department of Anesthesiology and Intensive Care Medicine, Medical University of Plovdiv

Background: Low-grade squamous intraepithelial lesion (LSIL) detected on cervical cytology is most commonly associated with human papillomavirus (HPV) infection and is typically considered a low-risk abnormality. HPV-negative LSIL findings are less common and may raise diagnostic uncertainty. The association of HPV-negative LSIL with atypical endometrial hyperplasia (AEH) exhibiting high-risk molecular features is unusual and can create diagnostic complexity.

Case report and results: We report a 36-year-old woman presenting with abnormal uterine bleeding. Histopathological examination of endometrial curettage revealed atypical endometrial hyperplasia. Immunohistochemistry demonstrated loss of Phosphatase and Tensin Homolog (PTEN) and *Paired Box 2* (PAX2) expression and strong diffuse positivity for Anti-Mullerian hormone receptor type 2 (AMHR2), findings that may indicate an increased risk of malignant transformation. Cervical cytology revealed a low-grade squamous intraepithelial lesion (LSIL), while HPV PCR testing was negative for all high-risk genotypes. The presented results are part of an ongoing research project approved by the institutional ethics committee (approval P-KHE-4/28.10.2024).

Materials and Methods: A diagnostic evaluation was performed including liquid-based cervical cytology and high-risk human papillomavirus (HR-HPV) detection using a polymerase chain reaction (PCR)-based assay. Endometrial sampling was obtained by uterine curettage and evaluated histopathologically using hematoxylin and eosin staining. Immunohistochemical analysis for PTEN, PAX2, and AMHR2 was performed on formalin-fixed, paraffin-embedded tissue sections to assess molecular alterations in atypical endometrial glands.

Conclusion: This case highlights a rare and diagnostically complex gynecologic scenario involving HPV-negative LSIL cytology associated with atypical endometrial hyperplasia exhibiting PTEN/PAX2 loss and high AMHR2 expression. Integrated morphologic and molecular evaluation is essential for accurate diagnosis and appropriate risk stratification.

Keywords: atypical endometrial hyperplasia, LSIL, HPV, PTEN, PAX2, AMHR2

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Impact of AI-Assisted Radiographic Interpretation on Orthopedic Surgeons' Workflow and Decision Confidence in Acute Polytrauma Care: A Cross-Sectional Study

Keti Tokmakova¹, Valentin Petrov¹, Petar Molchovski¹, Ralitsa Raycheva², Penka Stefanova-Peeva³, Dimitar Dachev³

¹Department of Orthopaedics and Traumatology, Medical University of Plovdiv, Plovdiv, Bulgaria

²Department of Social Medicine and Public Health, Faculty of Public Health, Medical University of Plovdiv, Plovdiv, Bulgaria

³Department of Propedeutics of Surgical Diseases, Department of Pediatric Surgery, Medical University of Plovdiv, Plovdiv, Bulgaria

Background: Acute polytrauma care requires rapid radiographic interpretation under conditions of substantial time pressure and cognitive workload. Artificial intelligence (AI)-assisted imaging systems have demonstrated promising diagnostic performance in musculoskeletal trauma imaging. Their impact on orthopedic surgeons' workflow experience and occupational perceptions remains insufficiently characterized. This study aimed to evaluate the relationship between workload perceptions, occupational strain, and the perceived impact of an AI-assisted radiographic interpretation system among orthopedic surgeons.

Methods: A single-center cross-sectional study was conducted following real-world clinical implementation of an AI-assisted radiographic interpretation system in a tertiary trauma center. Ethical approval was obtained. Orthopedic surgeons completed a structured questionnaire assessing workload perceptions, occupational strain, diagnostic confidence, and perceived impact of AI support during clinical practice. Responses were recorded and associations between workload-related variables and evaluation of the AI system were explored.

Results: A total of 26 orthopedic surgeons participated in the study. Most participants reported increased diagnostic confidence and reduced concern regarding missed findings when using AI support. Perceived availability of sufficient time for radiograph review was positively associated with reduced concern about missed findings. Fear of missed findings during busy shifts was negatively correlated with perceived improvement in job satisfaction associated with AI use. Overall professional satisfaction was positively associated with AI system satisfaction.

Conclusions: AI-assisted radiographic interpretation appears to enhance orthopedic surgeons' diagnostic confidence and reduce concern regarding missed findings. The perceived benefits of AI are influenced by organizational context and workflow integration. Given the relatively small sample size, these correlations should be interpreted as exploratory findings requiring confirmation in larger prospective studies.

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Keywords: artificial intelligence, trauma radiography, polytrauma, orthopedic surgery, surgeon well-being, clinical implementation

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Impact of ejaculation frequency on semen quality in dogs: Comparison of 24 hour and 96 hour collection Intervals

Andra-Elena Nica

University of Agronomic Sciences and Veterinary Medicine of Bucharest

Understanding the impact of ejaculation frequency on canine semen quality is essential for optimizing collection protocols used in breeding and assisted reproductive technologies. This study evaluated semen characteristics from five clinically healthy adult dogs collected at two intervals: every 24 hours and every 96 hours. The analysis focused on sperm concentration, progressive motility, abnormal motility, normal morphology, and abnormal morphology. Semen collected at 24-hour intervals exhibited markedly reduced sperm concentration and lower progressive motility, consistent with previously documented declines in total sperm output and motility under intensive collection regimens. Furthermore, dogs sampled every 24 hours showed a significant increase in abnormal motility and a corresponding reduction in morphologically normal sperm, aligning with evidence that higher ejaculation frequency elevates morphological defects and reduces overall sperm quality. In contrast, semen obtained following 96-hour intervals maintained higher concentration, stronger progressive motility, and a greater proportion of morphologically normal sperm, reflecting the stabilizing effect of longer recovery periods on sperm parameters. These findings underscore that shortened collection intervals adversely affect key fertility indicators, while extended intervals support improved seminal quality. This study provides practical guidance for breeders and reproductive specialists seeking evidence-based semen collection schedules that preserve sperm integrity and maximize reproductive outcomes.

Keywords: Ejaculation frequency, Canine semen quality, Semen collection interval

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Levetiracetam as a First Choice Treatment in Canine Neuro-pathology: Findings From a Case Series

Buscă M. C., Roșca M., Codreanu A. M. Cristian A., Fernoagă C.

Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Bucharest, Romania

Epileptic seizures are among the most common neurological conditions in veterinary practice. Although phenobarbital remains the traditional first-line treatment, Levetiracetam has gained increasing attention due to its distinct SV2A-mediated mechanism of action and favorable pharmacological profile.

The aim of this study was to evaluate the efficacy of levetiracetam used as a first-line therapy in the control of seizures in dogs. The study included 25 canine patients presenting with seizure episodes over an eight-month period at the Veterinary Emergency Hospital "Prof. Dr. Alin Birtoiu", Bucharest. All patients underwent clinical and neurological examinations,

evaluation based on owner-recorded videos, hematological and biochemical testing, SNAP 4DX testing, and bile acid measurements (pre- and post-prandial). In selected cases, brain magnetic resonance imaging was performed. Patients were classified according to seizure etiology, including idiopathic epilepsy, post-traumatic seizures, intoxication-related seizures, structural brain abnormalities, portosystemic shunts, and infectious causes.

Levetiracetam administered as monotherapy achieved complete seizure control in 20 of the 25 patients (80%). In 5 cases (20%), complete seizure control was achieved only after the addition of phenobarbital as adjunct therapy. Among patients diagnosed with idiopathic epilepsy and normal diagnostic findings (n=11), 8 patients (72.7%) achieved complete seizure control with levetiracetam monotherapy. In dogs with structural brain lesions (n=3), two patients responded favorably to monotherapy, while one required phenobarbital. Overall, complete seizure control was achieved in all patients included in the study.

The results of this study suggest that levetiracetam may represent an effective first-line therapeutic option in the management of seizure disorders in dogs. Its favorable pharmacological profile and ability to control seizures as monotherapy in the majority of cases support its consideration as a potential alternative to traditional antiepileptic drugs in veterinary neurology.

Keywords: levetiracetam, canine epilepsy, seizures, antiepileptic drugs, veterinary neurology

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Lipid Metabolism and Inflammation-Related Biomolecules Are Associated with Clinical Severity in Autism Spectrum Disorder

Maria Gevezova^{1,2}, Maria Kazakova^{1,2}, Iliana Pacheva^{4,5}, Michael Maes^{2,3}, Nikolay Mehterov^{1,2}, Zdravko Ivanov^{4,5}, Elena Timova^{4,5}, Stefka Spassieva⁶, Erhard Bieberich⁶, Ivan Ivanov^{4,5}, Victoria Sarafian^{1,2}

¹Department of Medical Biology, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria

²Research Institute, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria

³Sichuan Provincial Center for Mental Health, Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu 610072, China

⁴Department of Paediatrics, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria

⁵Paediatrics Clinic, St. George University Hospital, 4002 Plovdiv, Bulgaria

⁶Department of Physiology, University of Kentucky, Lexington, KY 40536, USA

Background: Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental condition with complex and still incompletely understood etiology and pathogenesis. Increasing evidence suggests that disturbances in lipid metabolism, inflammatory signaling and mitochondrial dysfunction may contribute to the molecular background of ASD. However, the relationship between these molecular alterations and the clinical characteristic of the disorder remains insufficiently explored.

The aim of the present study was to investigate potential associations between biomolecules related to lipid metabolism and inflammation and the clinical severity of ASD.

Methods: The study included a cohort of children with idiopathic ASD and age-matched healthy controls (HC). Diagnosis and clinical severity were assessed using the Autism Diagnostic Observation Schedule (ADOS). Molecules involved in sphingolipid metabolism and inflammatory pathways as sphingomyelin phosphodiesterases (SMPD1 and SMPD5), ceramide synthases (CerS1 and CerS6), cyclooxygenase-2 (COX-2), and chitinase-3-like protein 1 (YKL-40) were analyzed.

Results: Altered molecular profiles in ASD patients compared with HC were observed. Increased levels of biomarkers associated with lipid metabolism and inflammatory responses were detected. Furthermore, significant correlations were noted between CerS1, CerS6, and COX-2 levels and ADOS scores. Multiple regression analysis revealed that 48.0% of the variance in the total ADOS score could be explained by the combined effects of COX-2, CerS1, CerS6, and age.

Conclusion: These findings provide new insights into molecular alterations associated with ASD and suggest that biomarkers related to lipid metabolism and inflammatory pathways may contribute to the clinical heterogeneity and severity of the disorder.

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Keywords: autism spectrum disorder; ceramide synthases; sphingomyelin phosphodiesterases; lipid metabolism; inflammation; COX-2; YKL-40.

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Monitorization of wild boars circulation in endemic area of African swine fever disease in north-eastern Romania

Florentina Daraban Bocaneti¹, Mihaela Anca Dascalu¹, Florica Barbuceanu², Oana Tanase¹

¹"Ion Ionescu de la Brad" Iasi University of Life Science, M Sadoveanu Alley 8, 700490 Iasi, Romania

²Institute for Diagnosis and Animal Health, Dr Staicovici 63, Sector 5, Bucharest 050557, Romania

African swine fever disease is a transmissible infection, resulting in important economic impact. The aim of study was to evaluate the circulation of wild boars and to detect the hyperthermic individuals in an endemic area of African swine fever disease. A DHI-TPC-DF1241-T Thermal Network Mini Hybrid Bullet camera was installed in a feeding area from Victoria village, Iași County (47°38'14.16"N 27°56'35.12"E). During February - November 2025 period, the camera detected actively the circulation of both adult and young individuals, more

frequently during the night time. Moreover, during the aforementioned period, none of the animals detected in the visual registration area of the camera was hyperthermic. This data reinforces the need of continuous monitoring of area where continuous movement of wild boars exists, with early identification of infected animals.

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Keywords: boars, monitorization, camera

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Nanostructure of lipid bilayers from simultaneous ellipsometry imaging and QCM-D

G. Rouire¹, G. Perino¹, B. Barletti¹, M. Hijazi¹, A. Shahi^{1,2}, R. Leonetti^{1,2}, I. Vilgrain³, M. Maccarini¹, D. K. Martin¹

¹Université Grenoble-Alpes, CNRS, Grenoble INP, LMGP-UMR 5628, France

²Institut Laue Langevin, Grenoble, France

³Université Grenoble Alpes, INSERM U13, CEA-IRIG; BGE-Biomics, France

Within the ERC-funded Energion project, we aim to develop a biologically engineered energy storage platform in which ion gradients generated by membrane co-transport proteins act as a sustainable voltage source and biological supercapacitor. In this study, we focus on the fundamental role of lipid composition in optimizing the incorporation and functionality of the sodium/proton antiporter protein NhaA within lipid bilayers. Understanding the contribution of individual lipid species is essential, as the stability and performance of membrane-protein-based energy systems critically depend on the physicochemical properties of the supporting lipid matrix. Here, we report the real-time monitoring of bilayer formation and subsequent protein reconstitution using a uniquely integrated instrument that combines quartz crystal microbalance with dissipation monitoring (QCM-D), imaging ellipsometry (IE), and optical microscopy. By tuning lipid composition to modulate bilayer rigidity, hydration, and electrostatic environment, we identify conditions that promote stable protein integration while preserving membrane integrity. These findings establish transferable design principles for more complex lipid architectures and constitute a fundamental step toward the realization of sustainable, membrane-based biobattery platforms.

Keywords: Energy storage, biobattery, NhaA, Cardiolipin, transport protein

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Optimisation of cultivation media for controlled recombinant enzyme expression and enhanced ϵ -caprolactone production

Štefánia Hrončeková¹, Marek Bučko¹, Alica Vikartovská¹, Viera Illeová², Tomáš Molnár²

¹Department of Glycobiotechnology, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia

²Department of Chemical and Biochemical Engineering, Institute of Chemical and Environmental Engineering, Faculty of Chemical and Food Technology,

Slovak University of Technology, Bratislava, Slovakia

Background: Uncontrolled basal expression of recombinant enzymes in *Escherichia coli* reduces metabolic stability and biotransformation efficiency. Variability in complex media composition critically affects expression control and process performance. This study evaluated different commercial organic nitrogen sources and developed an optimised medium enabling tightly regulated enzyme expression for efficient ϵ -caprolactone production.

Methods: Recombinant *E. coli* BL21(DE3) cells expressing a three-enzyme cascade were cultivated in complex and semi-defined media. Seventeen organic nitrogen sources were screened for their impact on basal expression and catalytic performance. Cell growth and metabolite production were monitored spectrophotometrically and analysed by liquid chromatography, and enzyme expression was assessed by gel electrophoresis. Whole-cell biotransformations were performed in shake flasks and scaled up to 0.5 L bioreactors. Substrate conversion and ϵ -caprolactone formation were quantified by gas chromatography.

Results: Significant basal expression was detected with eight of the tested commercial nitrogen sources. The semi-defined medium enabled tight regulation of recombinant expression and eliminated detectable leaky expression. In shake flasks, complete conversion of 4 mM substrate yielded 0.879 mol mol⁻¹ with a specific productivity of 0.71 mmol h⁻¹ g⁻¹. Scale-up maintained full conversion at 4 mM (0.83 mol mol⁻¹; 0.81 mmol h⁻¹ g⁻¹). At 8 mM, conversion remained complete (0.76 mol mol⁻¹; 0.74 mmol h⁻¹ g⁻¹), increasing total product formation, whereas 12 mM reduced performance. Compared with Terrific Broth, the novel semi-defined medium improved both stability and cascade efficiency.

Conclusion: Medium composition critically influences recombinant expression control and biocatalytic performance. The developed semi-defined medium provides a robust and scalable platform for controlled enzyme production and efficient ϵ -caprolactone biotransformation.

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Keywords: bioreactor cultivation, cascade reaction, medium optimisation, process scale-up, recombinant expression

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Pediatric Trauma Severity Assessment and Prognosis Using Automated Scoring Systems

D. Dachev¹, E. Zanzov², K. Tokmakova³, S. Lupanov¹, B. Ivanov¹, B. Isakov¹, N. Mavrev¹, P. Stefanova¹

¹UMHAT "St George", Plovdiv, Bulgaria - Department of Pediatric Surgery

²UMHAT "St George", Plovdiv, Bulgaria - Department of Plastic surgery

³UMHAT "St George", Plovdiv, Bulgaria - Department of Orthopedics and Traumatology

Background: Trauma is a leading cause of morbidity and mortality in children. Early assessment of injury severity is critical for triage, prognosis, and clinical management. Trauma scores such as the Revised Trauma Score (RTS), Pediatric Trauma Score (PTS), and Shock Index (SI) provide objective measures of physiological status and risk of complications.

Materials and Methods: A retrospective analysis included 100 pediatric patients registered in the Pediatric Trauma Support System (PTSS). Patients were stratified by age: ≤ 2 years ($n=15$), 2-6 years ($n=39$), 6-12 years ($n=34$), and 12-18 years ($n=12$). RTS, PTS, and SI were calculated for each patient. Six children sustained severe abdominal trauma requiring surgical or conservative management.

Results: In severe cases, PTS ranged 5-6/12, RTS 6-7.11, and SI averaged 1.38. In children ≤ 2 years, PTS dropped to 9/12 even with minor head contusions, RTS 7.84, and SI 1.27, demonstrating high sensitivity to mild injuries. For ages 2-6 and 6-12, PTS was 10-11/12, RTS 7.84, and SI 0.8-1.11. Adolescents (12-18 years) showed similar values. Combined use of RTS, PTS, and SI improved early identification of severe injuries and allowed age-adapted interpretation.

Discussion: Automated calculation via PTSS streamlines clinical assessment, reduces calculation errors, and supports rapid decision-making in emergency pediatric trauma care. Age-specific physiological adjustments are crucial for accurate interpretation of trauma scores.

Conclusion: RTS, PTS, and SI are reliable indicators for early evaluation of pediatric trauma severity.

Integration into automated systems like PTSS enhances triage efficiency, prognosis accuracy, and clinical decision-making.

Abbreviations: RTS - Revised Trauma Score; PTS - Pediatric Trauma Score; SI - Shock Index; PTSS - Pediatric Trauma Support System

Keywords: pediatric trauma, trauma scores, RTS, PTS, Shock Index, PTSS, prognosis, triage

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Probiotic and postbiotic lactic acid bacteria from Bulgarian dairy products: antioxidant activity

Radoslav Abrashev¹, Nikoleta Atanasova², Lili Dobрева², Maria Angelova¹, Svetla Danova², Ekaterina Krumova¹

¹Department of Mycology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

²Department of General Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: Lactic acid bacteria (LAB), despite their aerotolerant nature, are susceptible to reactive oxygen species (ROS)-induced oxidative stress (OS). To mitigate the damaging effects of OS, LAB have evolved various defense mechanisms. The antioxidant potential of LAB pro-

biotics and their cell-free supernatants (CFS) have garnered significant attention in both research and application. This study aimed to evaluate the antioxidant activity of selected Bulgarian LAB strains.

Methods: Over 50 candidate-probiotic LAB strains and the resulting postbiotics (cells and CFS) were assessed for antioxidant activity using the DPPH and ABTS assays.

Results: A group of 10 LAB exhibited strong ABTS radical scavenging activity, comparable to the controls. Washed cells from six strains showed an inhibitory effect ranging from 50%

to 70%, approaching the level of vitamin C. They also demonstrated DPPH scavenging potential, although to a lesser extent than observed with the ABTS assay. Many of tested 45 postbiotic samples displayed significant antioxidant activity, with values exceeding those of the probiotics. Specifically, 24 samples exhibited substantial ABTS scavenging activity, ranging from 60% to 80%. DPPH free radical inhibition was less pronounced.

Conclusions: The results demonstrate that viable LAB cells and their CFS derived from Bulgarian dairy products possess significant antioxidant activity. This activity was strain- and test-dependent and influenced by the culture medium. These findings support the potential health benefits of Bulgarian-originated LAB.

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Keywords: Lactic acid bacteria, probiotic, postbiotic, antioxidant activity

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Probiotic potential of Bulgarian lactic acid bacteria for functional food applications

Lili Dobрева¹, Nikoleta Atanasova¹, Petar Donchev¹, Radoslav Abrashev¹, Ekaterina Krumova¹, Maria Angelova¹, Greta Naydenova², Apostol Apostolov², Dragomir Yankov², Svetla Danova¹

¹Stephan Angeloff Institute of Microbiology, BAS; 26, Acad. G. Bontchev Str. 1113 Sofia, Bulgaria;

²Institute of Chemical Engineering, BAS; 21 Acad. G. Bontchev Str. Sofia, Bulgaria

Background: Lactic acid bacteria (LAB) from traditional fermented products are well known as beneficial microorganisms. This study aims to evaluate the probiotic potential of Bulgarian lactobacilli as additives for functional products.

Results: A total of 31 LAB strains were isolated from traditionally prepared *katak*, yogurt, cheese and sauerkraut. They were identified at the species level from different genera within the *Lactobacillales*. Classical microbiological and molecular genetic methods were used to assess biological activity and probiotic potential in accordance with *in vitro* EFSA criteria. Several strains were selected based on ability to inhibit the growth and biofilm formation of food-associated and clinically significant pathogens, as well as spoilage fungi. The biochemical and ge-

netic basis of their antagonistic potential is being investigated in terms of their future application as biopreservatives. The spectrum of antibiotic susceptibility was assessed in order to prevent transfer of resistance genes. Along with their ability to survive simulated gastrointestinal tract (GIT) conditions, these properties define them as promising candidate probiotics.

Conclusion: Compliance with EFSA safety criteria, combined with their probiotic characteristics, makes them suitable for incorporation into functional foods. The obtained data provide a scientific basis for the selection of appropriate lactobacilli with high added value for functional food development with enhanced health benefits.

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Keywords: lactic acid bacteria, probiotics, functional foods, antimicrobial activity, biopreservation

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Prognostic assessment of congenital and acquired cervical spinal lesions in dogs

Fernoagă Cristina, Turbatu Raluca Mihaela, Neagu, Alexandru Gabriel, Gheorghe Andreea, Tudor Niculae

University of Agronomic Sciences and Veterinary Medicine Bucharest

Spinal cord injuries at the cervical level are a major cause of neurological dysfunction in dogs and can arise from either congenital abnormalities or acquired pathological conditions. From a clinical point of view, lesions may lead to cervical pain, ataxia, tetraparesis, or tetraplegia.

The aim of this study was to characterize the clinical profiles of dogs affected by cervical C1–C5 lesions and to evaluate the reliability of prognostic indicators by comparing predicted outcomes with the actual clinical evolution of the patients. The study was conducted in three consecutive phases. The first phase included a retrospective analysis of 57 canine patients consulted at the "Prof. Dr. Alin Birțoiu" University Veterinary Emergency Hospital in Bucharest. The factors analyzed included age, race, gender, body type, lesion type, and etiology (congenital or acquired).

In the second phase, prognostic evaluation was performed using neurological grading based on standardized scales (from 0 to 5), patient history, duration and severity of clinical signs, magnetic resonance imaging findings, and additional diagnostic indicators.

Finally, the predicted prognosis was compared with the actual clinical evolution of patients over a three-month follow-up period. The results showed that acquired cervical lesions represented most cases (79%), while congenital lesions were more frequently observed in younger animals. Acquired conditions were identified in all medium-sized dogs, whereas congenital lesions were predominantly observed in small and very large purebred breeds. The French Bulldog was the most frequent-

ly affected breed, and intervertebral disc disease accounted for approximately 65% of cervical C1–C5 lesions. In addition, prognostic predictions were found to be more consistent with the clinical outcome in congenital conditions than in acquired lesions.

These findings emphasize that prognosis is influenced by both the etiology of cervical spinal lesions and the moment of presentation, particularly in dogs requiring surgical intervention, underlining the importance of early diagnosis.

Keywords: Canine cervical spine; cervical lesions C1–C5; prognosis; neurological grading; congenital disorders

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Routine Usage of Indocyanine Green Fluorescence by Younger Surgeons in Patients with Acute and Chronic Cholecystitis During Laparoscopic Cholecystectomy

Georgi Daskalov, Petur Uchikov, Julian Dichev

Department of Special Surgery, Medical University Plovdiv, Plovdiv, Bulgaria

Background: Laparoscopic cholecystectomy (LC) has become the gold standard for the treatment of benign gallbladder diseases. Indocyanine green (ICG) fluorescence is a safe and non-toxic method for visualization of the extrahepatic biliary tree. In this study, we investigated whether the routine use of ICG by younger surgeons (with less than 5 years of experience) can shorten operative time and reduce the conversion rate.

Methods and Materials: Eighty-seven patients underwent laparoscopic cholecystectomy between August 2024 and March 2026. They were divided into two groups: 39 in the ICG group and 48 in the control group. Both groups were compared with regard to duration of surgery and conversion rate.

Results: The mean operative time in the ICG group ($n = 39$) was 61.1 ± 22.6 minutes, compared with 87.6 ± 27.9 minutes in the control group ($n = 48$). There was a statistically significant reduction in operative time in the ICG group ($p < 0.001$). Seven patients (14.6%) were converted to open cholecystectomy in the control group, while none were converted in the ICG group. There was a statistically significant difference between the two groups ($p = 0.02$).

Conclusion: In recent years, ICG imaging has proven to be a useful method for visualization of critical anatomical structures during LC, especially when used by younger surgeons. It leads to a significant reduction in operative time and conversion to open surgery. The results of our study are encouraging, but they need to be confirmed in larger groups of patients.

Keywords: Laparoscopic Cholecystectomy, Indocyanine green

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Screening toxicity in marine samples with different cell-sensor methodologies-Towards improved accuracy

Adela Sinani¹, Sindi Ymet¹, Danja Dollaku¹, Ariola Bacu^{1,2}, Robert Marks^{3,4}

¹Department of Biotechnology, Faculty of Natural Sciences, University of Ti-

rana, Albania

²Centre for Biotechnology & Genetics, Academy of Sciences of Albania

³Avram and Stella Goldstein-Goren Department of Biotechnology Engineering, Ben-Gurion University of the Negev, Be'er-Sheva 84105, Israel

⁴The Ilse Katz Center for Nanoscale Science and Technology, Ben-Gurion University of the Negev, Be'er Sheva 84105, Israel

Marine pollution represents a growing environmental concern in coastal areas influenced by urban, industrial, and tourism-related activities in Albania. Cytotoxicity assessment using *whole-cell bioluminescent bacterial biosensors* offers a sensitive approach. Seawater and sediment samples were collected in September 2025 from Durres Bay at eight pre-determined hot-spots, and biotoxicity were assessed using three different methods: *Vibrio fischeri* (according to ISO11348-3:2007); *E. coli* strain DPD2794, and DPD2544 (portable BioFix-Lumi10 method); *E. coli* strain DPD2794, and DPD2544 (luminescence Multimode Plate Reader). Both *E. coli* strains contain a plasmid-based fusion of the promoters with the *Photobacterium luminescens* LuxC-DABE reporter operon consisting of five structural genes that encode the heterodimeric luciferase enzyme and the biosynthesis enzymes responsible for producing the luciferase substrate, tetradecanal, in an ATP- and NADPH-dependent manner. The induction factor was calculated as a ratio of the maximum RLU of the test to the average of the negative control. In parallel, key physicochemical parameters of waters (pH, temperature, salinity, conductivity, turbidity, nitrates, nitrites, ammonium, phosphates, heavy metals and dissolved oxygen) and phytoplankton *chl a* were measured to explore potential correlation to biotoxicity. Results showed higher values of toxicity based on *V. fischeri*, while *E. coli* strains luminescence did not depend on the reading method. Clear differences were evidenced among water and sediment toxicity, indicating heterogeneous distribution and/or accumulation of contaminants. Variations in the sensitivity of *E. coli* strains suggested the presence of diverse contaminant types and concentrations. As the bioluminescence of *V. fischeri* is impacted by salinity/conductivity of marine waters, the use of *E. coli* strains was important. The absence of strong correlations of cytotoxicity with physicochemical and biological parameters emphasizes the fact that the last is influenced by multiple interacting factors, as well as the importance of conducting this assessment alongside with other WFD requirements for water quality evaluation.

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Keywords: cytotoxicity, bioluminescent bio-reporting bacteria, physicochemical parameters

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Surgical management of first compartment forestomach impa-

ction caused by corn ingestion in an alpaca

Cătălin Nicolae, Alexandru Dutulescu, Hassan Srour, Mario Codreanu, Iuliana Codreanu

Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Bucharest, Romania

Background: South American camelids have a three-compartment forestomach, with the first compartment being the main site of fermentation and feed storage. Impaction of this compartment is uncommon in alpacas but may occur after inappropriate feeding, dehydration, or impaired gastrointestinal motility. Severe impaction can compromise digestive function and may require surgical treatment when medical management is unsuccessful.

Methods: A 4-year-old female alpaca weighing 61.3 kg was presented with markedly reduced appetite, low water intake, apathy, weight loss, intermittent constipation, and occasional mucus-covered feces. The animal frequently adopted sternal recumbency. Clinical examination revealed mild hypothermia, absence of forestomach sounds, slight enlargement of the left flank, firm consistency on palpation, and dullness on percussion. The owner reported recent corn grain feeding before onset of clinical signs. Blood analysis and preanesthetic preparation were performed before induction of general anesthesia and endotracheal intubation. A left flank laparotomy through the paralumbar fossa provided access to the first forestomach compartment. Gastrotomy was performed, and impacted corn material obstructing the sacculi was manually removed, followed by lavage and routine closure.

Results: The impacted material was successfully evacuated and the sacculi structures were cleared. No intraoperative complications were observed. Postoperative treatment included antimicrobial and anti-inflammatory therapy, fluid support, and gradual reintroduction of soft feed. Clinical condition progressively improved, with recovery of appetite and normalization of fecal output.

Conclusion: Left flank gastrotomy was an effective treatment for severe first compartment forestomach impaction in this alpaca and allowed successful restoration of digestive function.

Keywords: alpaca; forestomach impaction; corn ingestion; gastrotomy; camelid surgery

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The Impact of African Swine Fever on Domestic Pigs and Wild Boar Populations in Romania

Mihaela Anca Dascalu¹, Florentina Daraban Bocaneti¹, Florica Barbuceanu², Oana Tanase¹

¹"Ion Ionescu de la Brad" Iasi University of Life Science, Mihail Sadoveanu Alley 8, 700490 Iasi, Romania

²Institute for Diagnosis and Animal Health, Dr Staicovici 63, Sector 5, 050557, Bucharest, Romania

African swine fever (ASF) is a severe viral disease that has caused substantial reductions in both domestic pig and wild boar

populations worldwide, significantly impacting the pig production sector and rural communities, particularly in regions where pork constitutes a key dietary protein source.

Following the first confirmed case of ASF in Romania on July 31, 2017, official statistics from the National Institute of Statistics and Eurostat show a decline in the domestic pig population from approximately 4.4 million heads in 2016 to around 2.9 million heads in 2025. In contrast, wild boar populations, estimated at approximately 96,000 individuals in 2016, decreased to around 63,000 in 2025, remaining highly variable due to the lack of precise national census data and the effects of hunting pressure and disease dynamics, based on data from the Ministry of Environment, Waters and Forests.

Overall, these trends highlight the long-term impact of African swine fever on both domestic livestock production and wild boar populations in Romania, emphasizing the challenges of disease control at the wildlife–livestock interface. Furthermore, they underline the economic vulnerability of small-scale pig farming systems and the increasing dependence on biosecurity measures and coordinated surveillance strategies to prevent further spread of the disease.

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Keywords: ASF, pig, wild boar

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The role of Artificial Intelligence in training medical students and pathology residents

E. Poryazova^{1,2,4}, D. Markov^{1,2}, V. Chonov¹, I. Bivolarski¹, D. Serteva¹, G. Markov³, A. Stoyanov³, S. Delchev⁵

¹Department of General and Clinical Pathology, Faculty of Medicine, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria

²Department of Clinical Pathology, Pulmed University Hospital, 4002 Plovdiv, Bulgaria

³Faculty of Medicine, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria

⁴Clinical and Experimental Morphology Division, Research Institute at Medical University of Plovdiv, Bulgaria

⁵Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv, Bulgaria

The rapid maturation of digital pathology and artificial intelligence (AI) is reshaping how microscopic pathology is practiced, validated, and taught. Whole-slide imaging (WSI) enables the conversion of glass slides into high-resolution digital files that can be stored, shared, annotated, and examined at multiple magnifications, while AI-based image analysis can provide quantitative outputs (e.g., cell counting, hotspot detection, immunohistochemistry scoring), supporting standardization and reproducibility. Contemporary evidence shows that virtual microscopy can be equivalent or advantageous to conventional light microscopy for learners, with meta-analytic data indicating favorable educational outcomes and broad acceptance among students and trainees.

At the institutional level, a sustainable transition to digital pat-

hology education requires coordinated investment in scanners, storage, network infrastructure, visualization software, content management, and secure access—often supported by cloud-enabled delivery models that facilitate distance learning and interdisciplinary teaching.

This study outlines a practical framework for implementing a digital pathology education platform that integrates WSI and AI-enabled image analysis into undergraduate and postgraduate pathology training, with a specific focus on the needs and strategic direction of a Bulgarian academic and clinical environment.

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Keywords: education; pathology; whole-slide images; virtual microscopy; artificial intelligence; image analysis; residency training

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Ultrasound-Guided Punctures and Ultrasonography Accuracy in Prostatic Masses/Collections – Causes of Urinary Retention – in Dogs

Andrei-Mario Codreanu, Teodora Furtună, Valerica Constantinescu, Stelian Bărităreanu

Faculty of Veterinary Medicine, University of Agriculture Science and Veterinary Medicine Bucharest, Romania

Introduction: Prostatic disorders are a common cause of lower urinary tract obstruction in intact male dogs and may result in partial or complete urinary retention. Ultrasonography is most frequently used for evaluating prostatic morphology and identifying pathological changes within the gland. In addition, real-time ultrasonographic guidance enables minimally invasive procedures such as diagnostic puncture or therapeutic drainage of prostatic collections.

Materials and Methods: The aim of study was identification of prostatic changes associated with urinary retention in dogs and to evaluate the usefulness of ultrasound-guided puncture for diagnostic and therapeutic purposes. The study included 32 male dogs presented with dysuria, stranguria, or acute urinary retention. All animals underwent abdominal ultrasonographic examination using 7.5–10 MHz microconvex or linear probes, with evaluation of prostate size, contour, capsular margins, echogenicity, and the presence of focal lesions or fluid collections. When masses or collections were detected, ultrasound-guided puncture was performed under aseptic conditions using a fine needle to obtain samples for cytological and bacteriological analysis or a syringe needle to perform therapeutic drainage.

Results: Benign prostatic hyperplasia was the most frequent finding, observed in 50% (n=16) of cases and characterized by symmetrical enlargement and heterogeneous echotexture. Prostatic cysts were identified in 28.1% (n=9) of dogs, ranging from small intraparenchymal microcysts to large supracenti-

metric cavitory structures with anechoic content. Prostatic abscesses were detected in 15.6% (n=5) of cases, appearing as fluid collections with internal echoes and irregular walls. Suspected prostatic neoplasia was observed in 6.3% (n=2) of dogs associated with irregular margins and heterogeneous parenchyma. Ultrasound-guided puncture allowed accurate sampling or drainage without major complications.

Conclusion: Ultrasonography combined with ultrasound-guided puncture represents a valuable diagnostic and therapeutic approach for the management of prostatic diseases causing urinary retention in dogs.

Keywords: urinary retention, prostatic cysts, ultrasound-guided puncture, benign prostatic hyperplasia, ultrasonography

FULL TEXTS

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A patient with dual phenotype: Coffin–Siris Syndrome Type 6 and Swyer SyndromePembe Savash^{2,5}, Behich Koyutourk¹, Havva Cobanogullari², Neşe Akcan³, Ali Cenk Ozay⁴, Mahmut Cerkez Ergoren¹¹Near East University Hospital, Medical Genetics Diagnosis Laboratory, Nicosia, 99138, Cyprus²Eastern Mediterranean University, Faculty of Arts and Sciences, Department of Biological Sciences, Famagusta, Cyprus³Near East University Hospital, Department of Child Health and Diseases, Nicosia, 99138, Cyprus⁴Cyprus International University, Nicosia, Cyprus⁵Adagen Medical Centre, Nicosia, Cyprus.**Abstract**

Coffin–Siris syndrome type 6 (CSS6) is a rare developmental disorder defined by heterozygous pathogenic mutation in the *ARID2* gene. Patients with CSS6 have intellectual disability of variable degree, coarse facial and body features, thin hair, skeletal growth delay, and congenital anomalies. Due to similar characteristics with other syndromes, there have been misdiagnoses of CSS. Thus, a detailed genetic evaluation is imperative. Here, we present a 19-year-old female Turkish patient with a history of abnormal eye movements, arachnodactyly, delayed speech and language development, gonadal dysgenesis, hypoplastic uterus, growth delay and paediatric tall stature, nystagmus and mental deterioration. The initial assessment primarily indicated Swyer syndrome, which was substantiated by a 46, XY karyotype. FISH analysis indicated the existence of SRY gene on Y chromosome. Thus, whole-exome sequencing (WES) planned to check sexual development genes. WES identified a heterozygous nonsense variant in *ARID2* (NM_152641.3:c.202C>T, p.Gln68) classified as likely pathogenic according to ACMG guidelines. This finding confirmed the diagnosis of autosomal dominant Coffin–Siris syndrome type 6. This case expands the clinical spectrum of CSS6 by illustrating a presentation that initially mimicked Swyer syndrome. The report emphasises the diagnostic value of whole exome sequencing in atypical clinical scenarios and highlights the need for genetic counselling and parental testing.

Keywords: Coffin–Siris syndrome type 6, *ARID2*, gonadal dysgenesis, whole exome sequencing, Swyer syndrome mimic

Introduction

Coffin–Siris syndrome (CSS) is a rare congenital condition characterised by variable developmental delay, coarse facial features, hypotonia, it also includes differences in growth, and defects of the fifth digits and nails of hands & feet (Tsurusaki et al., 2012). To date, multiple genetic subtypes of CSS have been identified, most are caused by pathogenic variants of genes that encode components of the SWI/SNF chromatin remodelling complex. Coffin–Siris syndrome type 6 (CSS6, OMIM #617808) is a CSS caused by heterozygous variants in *ARID2*, which encodes a subunit of the polybromo-associated BAF (PBAF) complex (Chérot et al., 2018).

The phenotypic heterogeneity of the clinical presentation of CSS6 includes intellectual disability, sparse hair on the scalp, coarse facial features, and congenital malformations. Because of the rarity of the condition and its phenotypic overlap with other developmental disorders, it is typically hard to diagnose during the diagnostic workup (Clark et al., 2018; Pandey et al., 2025). In particular, patients with gonadal dysgenesis, excessive height, and growth disturbances are at risk of misclassification due to other diagnoses such as Swyer syndrome.

Herein, we present a 19-year-old female patient with several congenital anomalies and gonadal dysgenesis, which was suspected to be Swyer syndrome. Whole exome sequencing revealed a novel nonsense mutation in *ARID2* to make the diagnosis of CSS6. The present case illustrates the diagnostic challenge of syndromic overlap presentation and highlights the need for high-density genomic analysis of rare genetic disorders.

Case Presentation

A 19-year-old girl patient, born to consanguineous parents, presented for genetic evaluation due to multiple congenital anomalies and developmental delay. The patient exhibited abnormal eye movements, nystagmus, arachnodactyly, dyspnea, delayed speech and language development, specific learning disability, growth delay, tall height, and mental decline. Other results included hypoplasia of the uterus, gonadal dysgenesis, abnormal development of the breast, and nasal bone defects.

The patient was born at term after an uncomplicated pregnancy with infantile presentation of symptoms. Family history revealed a cleft lip in two maternal uncles. There was no such

history of syndromic features in first-degree relatives. The first investigations were standard cytogenetic analysis and these revealed a normal 46, XY karyotype. In view of the gonadal dysgenesis and stature, the clinical working diagnosis was Swyer syndrome. The extensive distribution of other anomalies prompted further genetic investigations.

Whole-exome sequencing (CentoXome® Solo, CENTOGENE Rostock, Germany) was performed on genomic DNA obtained by extraction from peripheral blood. Analysis of the sequencing data revealed the presence of a heterozygous nonsense variant in *ARID2* (NM_152641.3:c.202C>T, p.Gln68*), resulting in a premature stop codon. The *ARID2* variant was classified as likely pathogenic (ACMG Class 2). In addition, no clinically relevant variants were identified in other genes traditionally associated with gonadal dysgenesis, including *SRY*, *SF-1*, *SOX9*, *NR5A1*, *DHH*, *WT1*, *WNT4*, *CBX2*, *MAP3K1*, *GATA4*, *FOXL2*, *ZFPM2*, *TSPYL1*, *SUPT3H*, and *OTOG*.

Based on the identified variant and alignment with clinical characteristics, a diagnosis of Coffin–Siris syndrome type 6 (CSS6; OMIM #617808) was assigned. Genetic counselling was recommended, and parental carrier testing was suggested to determine whether the variant was inherited or *de novo*.

Results

Whole exome sequencing (WES) achieved >99% coverage of the targeted coding regions at ≥20× depth. The detection revealed a heterozygous nonsense variant in *ARID2* (NM_152641.3:c.202C>T, p.Gln68), which generates a premature stop codon (Table 1). This loss-of-function mutation is consistent with the established disease mechanism for *ARID2*. According to ACMG guidelines, it was classified as likely pathogenic (Class 2). The variant is not present in population databases of large size (gnomAD, ESP, 1000 Genomes), suggesting that it is extremely rare or emerging. In silico analysis revealed a deleterious impact, and the nucleotide position is highly conserved.

Additional variants

Two heterozygous variants were identified in metabolic genes, reported as incidental findings:

- *AMT* (NM_000481.3:c.992G>A; p.Arg331Gln): predicted deleterious (SIFT, MutationTaster) and classified as likely pathogenic. This gene is associated with nonketotic hyperglycinemia when biallelic pathogenic variants are present. In this patient, the single heterozygous variant is unlikely to be clinically relevant.
- *GAA* (NM_000152.3:c.1828G>A; p.Ala610Thr): classified as pathogenic and associated with Pompe disease. Similar to *AMT*, the heterozygous status suggests a carrier state without clinical manifestation.

Additionally, due to the initial suspicion of Swyer syndrome, genes associated with disorders of sex development (DSD) and gonadal dysgenesis (i.e., *SRY*, *SOX9*, *NR5A1*, *DHH*, *WT1*, *WNT4*, *CBX2*, *MAP3K1*, *GATA4*, *FOXL2*, *ZFPM2*, *TSPYL1* and *SUPT3H*) were specifically examined. No pathogenic variants were detected in those genes, thus excluding their role

as causative in this case.

Finally, the clinical features of the patient, including gonadal dysgenesis, growth abnormalities, intellectual disability, and multiple congenital anomalies, are consistent with Coffin–Siris syndrome type 6 (CSS6) due to pathogenic variants in *ARID2*. The other two identified variants (*AMT* and *GAA*) were incidental and did not explain the patient's phenotype.

Discussion

Coffin–Siris syndrome (CSS) is a genetically and clinically heterogeneous group of diseases caused by pathogenic variants in genes coding for subunits of the SWI/SNF chromatin remodelling complex. In particular, heterozygous variants in *ARID2* have been associated with Coffin–Siris syndrome type 6 (CSS6) (Tsurusaki et al., 2012; Chérot et al., 2018). While coarse facial features, hypoplastic or missing fifth fingernails or toenails, developmental delay, intellectual disability and sparse scalp hair remain as typical clinical features, atypical findings and considerable phenotypic variability can complicate the diagnosis (Wang et al., 2022; Schrier Vergano, 2024).

In the present case, the patient had multiple congenital anomalies, delayed neurocognitive development, gonadal dysgenesis, arachnodactyly, intellectual disability and growth retardation, which initially raised the suspicion of Swyer syndrome. Swyer syndrome (46,XY complete gonadal dysgenesis) is typically characterised by absent or striated gonads, primary amenorrhoea and female external genitalia (McCann-Crosby et al., 2014). Swyer syndrome may be misclassified when overlapping features such as gonadal dysgenesis or tall stature are present. Similar diagnostic challenges have been reported in the literature where other *ARID*-related disorders or CSS6 were initially confused with syndromes such as Swyer, Cornelia de Lange and Noonan (Wang et al., 2022). This emphasises the need for confirmation at the molecular level using next generation sequencing (NGS) in atypical cases.

The diagnosis of the disease was made by identifying a heterozygous nonsense variant in *ARID2* (NM_152641.3:C.202C>T; p.Gln68)*. This variant is classified as probably pathogenic according to the ACMG guidelines. This finding is consistent with previous reports indicating that loss-of-function *ARID2* variants are an important pathogenic mechanism in CSS6 (Kalia et al., 2017; Schrier Vergano, 2024). Importantly, no additional pathogenic variants were detected in genes typically associated with disorders of sex development (e.g. *SOX9*, *SRY*, *NR5A1*, *WT1*, *DHH*), further supporting the *ARID2* gene as the causative gene. This case expands the known spectrum of symptoms associated with CSS6 and emphasises that the disease can sometimes resemble gonadal dysgenesis, a rare and possibly underappreciated aspect of the disease.

Interestingly, the discovery of two heterozygous variants in *GAA* and *AMT*, which were incidental findings and discovered by whole-exome sequencing, highlights the complexity of whole-exome sequencing (WES). Although these variants are categorised as likely pathogenic or pathogenic in the context

Table 1. Genetic variants were detected in the patient.

Gene	Transcript (NM)	Variant (cDNA)	Protein Change	Zygosity	Classification	Clinical Relevance
ARID2	NM_152641.3	c.202C>T	p.(Gln68*)	Heterozygous	Likely Pathogenic (Class 2)	Causative variant for Coffin–Siris syndrome type 6
AMT	NM_000481.3	c.992G>A	p.(Arg331Gln)	Heterozygous	Likely Pathogenic (Class 2)	Variant in gene linked to hyperammonemia, but incidental
GAA	NM_000152.3	c.1828G>A	p.(Ala610Thr)	Heterozygous	Pathogenic (Class 1)	Variant in gene linked to Pompe disease, incidental finding

of metabolic diseases, they are unlikely to contribute to the patient’s phenotype in the heterozygous state. Although broad genomic testing is helpful in detecting such incidental findings, genetic counselling and careful interpretation of these variants is crucial (Kalia et al., 2017).

From a clinical point of view, the diagnostic value of WES in patients with atypical and overlapping syndromic features is emphasised by this case. Based on karyotyping and conventional cytogenetic analysis, the disease was initially diagnosed as Swyer syndrome, but a definitive molecular diagnosis was achieved by WES. Recent studies have shown that early use of WES in patients with congenital anomalies or developmental delay has confirmed a higher yield of diagnosis with rates of 40-50% reported (Clark et al., 2018; Pandey et al 2025). In this case, accurate diagnosis prevents unnecessary treatment steps such as prophylactic gonadectomy due to the risk of malignancy, which would not be relevant in CSS6 and is associated with Swyer syndrome.

This case also emphasises the clinical importance of accurate genetic diagnosis. Appropriate developmental and educational support, informed reproductive counselling for families and targeted surveillance for associated families is achieved through early detection of CSS6. In particular, assessment of relapse risk is guided by recognition of the inheritance pattern (autosomal dominant, typically de novo). In addition, reporting such atypical presentations contributes to a broader knowledge of the phenotypic variability of CSS6, which is needed to refine clinical diagnostic criteria and promote detection in undiagnosed patients.

Conclusion

This case study emphasises the importance of considering ARID2-associated CSS6 in the differential diagnosis of patients with complex congenital anomalies and gonadal dysgenesis. In addition, this case expands the clinical spectrum of CSS6 to include phenotypic features similar to Swyer syndrome. This underscores the critical role of whole-exome sequencing in elucidating atypical or overlapping clinical presentations and emphasises the importance of collecting molecular and clinical data to better understand genotype-phenotype correlations in ARID2-associated disorders. Finally, early

and accurate diagnosis helps to ensure appropriate treatment, avoid misdiagnosis and provide useful information for genetic counselling and family planning.

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In-silico identification of candidate miRNAs affecting chemotherapy response via somatic variants in miRNA binding sites of drug metabolism-related genes with mitochondrial function

Berkcan Doğan^{1,2,*}, Şehime Gülsün Temel^{1,2}

¹Bursa Uludağ University, Institute of Health Sciences, Department of Translational Medicine, 16059, Bursa, Türkiye

²Bursa Uludağ University, Faculty of Medicine, Department of Medical Genetics, 16059, Bursa, Türkiye

*Corresponding author: Dr. Berkcan Doğan (PhD)

Postal Address: Bursa Uludağ University, Faculty of Medicine, Department of Medical Genetics, 16059, Nilüfer, Bursa, Türkiye

Phone: +90 (224) 294 0000 / 54398

E-mail: berkcandogan@uludag.edu.tr

ORCID: 0000-0001-8061-8131

Abstract

Genetic alterations affecting drug metabolism and post-transcriptional regulation significantly influence the inter-individual variability in chemotherapy responses. The impact of variants in genes involved in mitochondrial function on chemotherapy resistance remains largely unknown. Our aim is to identify candidate miRNAs associated with chemotherapy response via an integrative *in-silico* analysis of somatic variants within miRNA-binding sites of drug-metabolism-related genes with mitochondrial function. The ADME genes were obtained from the PharmaADME Consortium and the mitochondrial function-related genes were obtained from the Mitocarta(v.3.0) inventory. Somatic variants located in the miRNA-binding sites of the selected genes were obtained from the SomamiR(v.2.0) database, which is based on experimentally validated miRNA target sites, and common variants affecting miRNA-target interactions were identified. The chemotherapy response of miRNAs carrying somatic variants was assessed in different cell lines using the ncRNA Drug database. A total of 298 ADME genes and 1,136 mitochondrial function-related genes were retrieved and integrated and overlapping genes were selected for subsequent analyses. Somatic variants in miRNA binding sites of transcripts were identified based on experimental data. Ten variants in 4 genes (*ALDH1B1*, *ALDH9A1*,

GPX1, *SOD2*) were revealed and 46 miRNAs were associated with transcripts. Drug response profiles (sensitive vs. resistant) were evaluated across multiple chemotherapeutic agents using the criteria ($p < 0.005$ and $|\log FC| > 2$). Based on these thresholds, 4 miRNAs (hsa-miR-10a-5p, hsa-miR-10b-5p, hsa-miR-363-5p and hsa-miR-526b-5p) were associated with sensitivity to ceritinib, fluorouracil and paclitaxel, while exhibiting resistance to 4-hydroxytamoxifen, tamoxifen (alone and in combination with ethanol or fulvestrant) and vemurafenib. These response patterns were observed in breast, lung, ovarian, colorectal and melanoma-derived cell lines. These miRNAs may serve as translational biomarkers to predict chemotherapy response across different cancer types. These findings provide a translational perspective for patient stratification and the development of precision chemotherapy strategies targeting drug response heterogeneity, which is further supported by further wet-lab validation studies.

Keywords: drug metabolism, mitochondria, microRNAs, chemotherapy response, *in-silico* analysis.

Introduction

The drug metabolism genes are a group of genes involved in drug absorption, distribution, metabolism and excretion (ADME) (1, 2). These genes are involved in the pharmacokinetic journey of a drug within the body from absorption to excretion (2). According to the PharmaADME consortium, ADME genes consist of 32 core genes and 266 extended genes (3-5). These genes are classified based on their function in the pharmacokinetic process, including phase I and II drug-metabolizing enzymes, drug transporters and modifiers (3). Genetic alterations affecting drug metabolism and post-transcriptional regulation significantly influence the drug responses (3, 6).

Mitochondrial functional genes play a central role in regulating cellular oxidative stress, energy metabolism and apoptosis (7). In particular, mitochondrial DNA (mtDNA) encodes 37 genes essential for normal mitochondrial function (8). However, other mitochondrial proteins are encoded by nuclear genes (9). Alterations in any of 400 mtDNA- or nuclear-encoded mitochondrial genes (mitochondrial functional genes) can contribute to drug resistance (10). However, the impact of genetic variants in mitochondrial functional genes on chemotherapy resistance remains largely unclear.

MicroRNAs (miRNAs) are small, non-coding RNA molecules that play a critical role in fundamental cellular and biological functions, such as proliferation, apoptosis and development (11-13). Moreover, miRNAs regulate gene expression by binding to their mRNA target site(s) and genetic variation in these regions can disrupt this interaction and prevent miRNA binding. As a result, the target gene escapes repression, potentially leading to dysregulated expression profiles (14).

Alterations at miRNA binding sites can enable chemotherapy-target evasion by releasing drug-target genes from miRNA-mediated repression, leading to their overexpression and reduced drug efficacy (15). Additionally, such variants can promote survival and resistance by affecting genes involved in

drug metabolism and apoptosis (15, 16). Therefore, identifying these binding-site variants is crucial to serve as predictive biomarkers of chemotherapy resistance.

In this study, we aimed to investigate the potential for chemotherapy response of somatic variants in miRNA-binding sites of ADME genes with mitochondrial function.

Material and Methods

Retrieval of ADME and mitochondrial gene sets

The 298 ADME genes are defined by the PharmaADME Consortium (3-5). The 1,136 mitochondrial functional gene set retrieved from the MitoCarta (v.3.0) database, including annotations of their sub-mitochondrial compartments and pathway annotations (9).

Identification of miRNA-binding site alterations

The miRNA-binding site alterations were obtained from the SomamiR(v.2.0) database, which is based on experimentally validated miRNA target sites (17). To determine the alterations, we used datasets derived from high-throughput technologies such as PAR-CLIP (photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation), HITS-CLIP (high-throughput sequencing of RNA isolated by cross-linking immunoprecipitation), and CLASH (crosslinking, ligation and sequencing of hybrids). Further downstream analyses were conducted using the variants that overlapped across all sequencing data.

Functional assessment of miRNA variants in chemotherapy response

The chemotherapy response of miRNAs carrying somatic variants was assessed using the ncRNADrug database (18). This database provides comprehensive search capabilities across published papers, the Gene Expression Omnibus (GEO) database, and extracted information from the NCI-60, TANRIC, Genomics of Drug Sensitivity in Cancer (GDSC) and Cancer Cell Line Encyclopedia (CCLE) projects. MiRNA patterns associated with chemotherapy response were identified in the GEO repository using criteria of $|\log FC| \geq 2$ and $p < 0.0001$ and showing significant dual effects (both resistance and sensitivity) were excluded.

Results

Identification of overlapping ADME and mitochondrial genes

Overlapping genes between the 298 ADME genes and the 1,136 mitochondrial functional genes were identified. A total of 36 overlapping genes, including *ABCB6*, *ABCB7*, *ABCB8*, *ADHFE1*, *ALDH1B1*, *ALDH2*, *ALDH3A2*, *ALDH4A1*, *ALDH5A1*, *ALDH6A1*, *ALDH7A1*, *ALDH9A1*, *CAT*, *CBR3*, *CYB5R3*, *CYP11A1*, *CYP11B1*, *CYP11B2*, *CYP24A1*, *CYP27A1*, *CYP27B1*, *DHRS1*, *DHRS2*, *DHRS4*, *DHRS7B*, *EPHX2*, *GPX1*, *GPX4*, *GSR*, *GSTK1*, *GSTZ1*, *HAGH*, *MGST1*, *MGST3*, *SOD1* and *SOD2*, were selected for further downstream analyses (Figure 1).

Determination of miRNA-binding site variants in common genes

By integrating CLASH-seq and CLIP-seq datasets, we identified miRNA-binding site variants in mRNA and circRNA tran-

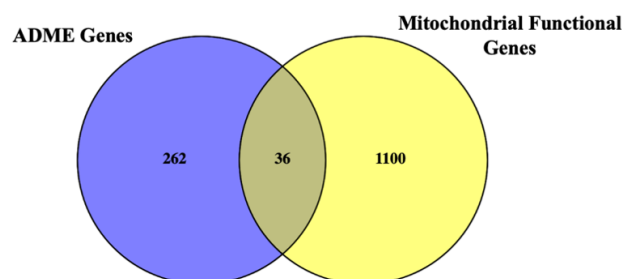


Figure 1. Venn diagram of overlapping ADME and mitochondrial functional genes.

scripts, respectively. A total of 10 common somatic variants were detected across 4 transcripts in 4 different genes, including *ALDH1B1*, *ALDH9A1*, *GPX1* and *SOD2*, which were associated with dysregulated miRNA-mRNA interactions. Among these genes, *ALDH1B1* showed the highest number of variants ($n=5$). The identified variant details are listed in Table 1.

Table 1. Common miRNA-binding site variants identified in CLASH-seq and CLIP-seq datasets.

Characterization of miRNAs associated with common variants

We identified 46 miRNAs associated with 10 shared variants across common transcripts, including hsa-miR-10a-5p, hsa-miR-10b-5p, hsa-miR-92b, hsa-miR-99b, hsa-miR-100, hsa-miR-153-5p, hsa-miR-328, hsa-miR-342-3p, hsa-miR-363-5p, hsa-miR-526b-5p, hsa-miR-568, hsa-miR-578, hsa-miR-609, hsa-miR-620, hsa-miR-653-5p, hsa-miR-1270, hsa-miR-1252-5p, hsa-miR-1273f, hsa-miR-1289, hsa-miR-3147, hsa-miR-3198, hsa-miR-3605-3p, hsa-miR-3619-3p, hsa-miR-4294, hsa-miR-4309, hsa-miR-4434, hsa-miR-4455, hsa-miR-4483, hsa-miR-4516, hsa-miR-4531, hsa-miR-4535, hsa-miR-4635, hsa-miR-4640-5p, hsa-miR-4679, hsa-miR-4684-5p, hsa-miR-4726-5p, hsa-miR-4776-5p, hsa-miR-5703, hsa-miR-6508-3p, hsa-miR-6724-5p, hsa-miR-6745, hsa-miR-6756-5p, hsa-miR-6766-5p, hsa-miR-6770-5p, hsa-miR-6772-5p and hsa-miR-6880-5p. These miRNAs were further analyzed to evaluate their potential impact on chemotherapy response.

Functional impact of variants on chemotherapy response

We identified 4 uniquely patterned candidate miRNAs associated with chemotherapy response across multiple cancer types. These miRNAs were derived from both RNA sequencing and microarray-based datasets in the GEO database. In total, their expression profiles were evaluated across 8 different chemotherapeutic agents in breast, lung, ovarian, colorectal and melanoma cancer cell lines. Among these, tamoxifen showed the strongest association with the candidate miRNAs. The 4 candidate miRNAs and related chemotherapy response signatures across different cancer types ($|\log FC| > 2$ and $p < 0.005$) are pre-

Table 1. Common miRNA-binding site variants identified in CLASH-seq and CLIP-seq datasets.

Transcript ID	circRNA ID	Gene	Variant
NM_000692	hsa_circ_0087093	ALDH1B1	chr9:g.38396464C>T
			chr9:g.38396465G>A
			chr9:g.38396467G>A
			chr9:g.38396474C>T
			chr9:g.38397032G>C
NM_000696	hsa_circ_0015071	ALDH9A1	chr1:g.165662620T>A
			chr1:g.165665083C>T
NM_201397	hsa_circ_0065638	GPX1	chr3:g.49358153C>A
			chr3:g.49358112A>C
NM_001024466	hsa_circ_0004662	SOD2	chr6:g.159682581A>C

Table 2. Candidate miRNAs and their chemotherapy response signatures in different cancer types ($|\log\text{FC}|>2$, $p<0.005$).

Compound	miRNA	Pattern	logFC	p-value	FDR	Method	GEO
4-Hydroxytamoxifen+Tamoxifen	hsa-miR-10a-5p	R	2.823	2.28E-05	1.00E-03	Array	GSE28267
4-Hydroxytamoxifen+Tamoxifen	hsa-miR-10b-5p	R	2.728	1.97E-04	7.00E-03	Array	GSE28267
Ceritinib	hsa-miR-10a-5p	S	-2.899	9.59E-04	3.50E-02	RNA-seq	GSE81486
Ethanol+Tamoxifen	hsa-miR-10a-5p	R	2.723	3.46E-05	3.00E-03	Array	GSE28267
Ethanol+Tamoxifen	hsa-miR-10b-5p	R	2.435	5.85E-04	1.90E-02	Array	GSE28267
Fluorouracil	hsa-miR-10b-5p	S	-2.267	1.07E-26	4.14E-25	RNA-seq	GSE190951
Paclitaxel	hsa-miR-363-5p	S	-8.303	1.74E-08	2.08E-06	Array	GSE43598
Tamoxifen	hsa-miR-10a-5p	R	2.711	1.72E-06	8.87E-04	Array	GSE159979
Tamoxifen+Fulvestrant	hsa-miR-10a-5p	R	7.038	5.41E-10	3.49E-07	Array	GSE159979
Vemurafenib	hsa-miR-526b-5p	R	9.421	4.63E-10	1.11E-08	RNA-seq	GSE116134

FDR: False Discovery Rate; GEO: Gene Expression Omnibus; R: Resistance; S: Sensitive.

sented in **Table 2**, including compound/agent, miRNA pattern, p-value, logFC and cancer type.

Table 2. Candidate miRNAs and their chemotherapy response signatures in different cancer types ($|\log\text{FC}|>2$, $p<0.005$).

Discussion

Chemotherapy resistance remains a major limitation to treatment efficacy and reduces overall survival in patients (19). The development of resistance is a multifactorial, dynamic process involving alterations, especially for post-transcriptional regulatory mechanisms (19, 20). Although ADME-associated genes and mitochondrial functional pathways are known to contrib-

ute to drug resistance, the impact of somatic variants located within miRNA-binding regions of these genes has remained largely unexplored. In this study, we aimed to shed light on the potential role of somatic variants affecting miRNA-binding sites in ADME genes with mitochondrial function and their association with chemotherapy response across multiple cancer types. Our integrative analyses identified 4 candidate miRNAs, including hsa-miR-10a-5p, hsa-miR-10b-5p, hsa-miR-363-5p and hsa-miR-526b-5p, which may function as potential biomarkers associated with chemotherapy sensitivity and resistance across diverse cancer contexts.

MiRNAs are key post-transcriptional regulators and their in-

involvement in cancer drug resistance is tightly linked to genomic and genetic alterations (21). Currently, extensive studies have increasingly focused on elucidating the molecular mechanisms underlying chemotherapy resistance in different cancer cell lines using high-throughput technologies. In particular, comprehensive integration of transcriptomic, genomic and miRNA profiling studies has provided significant insights into the regulatory roles of miRNAs in modulating resistance to various chemotherapeutic agents across multiple cancer types (22-27). Previous studies have demonstrated the involvement of miR-10a-5p and miR-10b in chemotherapy resistance across different cancer types. Xiong et al. reported that miR-10a-5p was significantly upregulated in gemcitabine-resistant pancreatic ductal adenocarcinoma cell lines, thereby promoting chemoresistance by directly targeting TFAP2 (28). Similarly, Attathikhun et al. demonstrated that miR-10b expression was elevated in sorafenib-resistant non-small cell lung cancer models and contributed to resistance development by inhibiting the p53-associated resistance pathway (21). To the best of our knowledge, no comprehensive studies have investigated the direct roles of hsa-miR-363-5p and hsa-miR-526b-5p in chemotherapy resistance mechanisms across cancer types. Therefore, these candidate miRNAs may represent novel regulatory molecules associated with drug response and the development of drug resistance. Further functional and mechanistic studies are required to elucidate their molecular targets, signaling pathways and potential contributions to the progression of chemotherapy resistance.

In conclusion, these candidate miRNAs may serve as translational biomarkers and key regulators of chemotherapy response across different cancer types. Furthermore, these findings provide a translational perspective for patient stratification and the development of precision chemotherapy strategies targeting drug response heterogeneity, which is further supported by further wet-lab validation studies.

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The Impact of Water Temperature Increase on the Growth Performances in *Mytilus Galloprovincialis* Reared in the Aquaculture of Butrint Lake

Dritan Arapi¹, Rigerta Sadikaj², Ariola Bacu²

¹Research Centre of Flora and Fauna, Faculty of Natural Sciences, University of Tirana, Albania

²Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania

Abstract

The aim of this study was to provide information on the damage of some indicators of the growth performance in mussels, caused by the excessive increase in water temperature in summer season. The study was carried out in a mussel "population" that was reared in the panels of a fixed suspended culture, in Manastiri area, during 2024s. Samplings of mussels were carried out without selection in the months of April-May and July-August, from three parts of the ropes. For each month, no less than 90 molluscs were sampled.

The highest measured value for temperature, in the surface layer of water (0.25 m) was 29.4 °C (August). The increase in the average water temperature in the 0-3.0m layer, from 19.03 °C to 25.52 °C, had caused the reduction in the average ox-

ygen content from 7.30 mgO₂/l to 4.58 mgO₂/l. The average values of the SGR%-index, for the reared stock of *Mytilus galloprovincialis*, were 0.298±0.015 and 0.196±0.020. The regression and correlation analysis confirmed the significance of this differences. The average values of the Kn-factor for the reared mussels, in two time intervals were: Kn=0.871, in the Summer season and Kn=1.041, in the Spring season. The average values calculated for the meat yield according to four months, were: 0.241±0.0107 in April; 0.276±0.0151 in May; 0.231±0.0135 in July and 0.207±0.0150 in August. All analytical indicators of mussel growth, which were evaluated, manifested a reduction in values under conditions of a significant increase in water temperature.

Keywords: mussel, aquaculture, climate change, specific growth rate.

Introduction: Anthropogenic climate change has become a major environmental driver, strongly affecting marine biodiversity and ecosystem functioning, often interacting with other human-induced stressors (Gissi et al., 2021; Garrabou et al., 2022). While European countries such as Spain, Italy, France and the Netherlands are still significant producers, European aquaculture mussel production peaked at 600 000 t in the late 1990s and has been declining ever since to 431,000 t in 2022 (Soliño and Figueras, 2025). The decline in EU mussel production is the result of multiple factors, primarily environmental, with diseases, spat depletion, harmful algal blooms, poor water quality, predation, pollution and climate change being the main causes (Portacci et al., 2025). The detrimental effects of marine heatwaves (MHW) seem to be playing a major role in the decline of European mussel production, especially in the Mediterranean Sea (Garrabou et al., 2022).

The experiments showed that mussels can survive in the temperature range of 3–25 °C, but do not survive at 30 °C. For the mussels the optimal temperature was 8 °C at low food conditions and 15 °C at high food condition. *M. edulis* grew less when oxygen concentration was reduced. This effect was more pronounced at higher temperatures, possibly due to increased oxygen demand. High temperature and low oxygen concentration combined reduced clearance rate in *M. edulis* (Kamermans et al., 2022). Tang and Riisgård (2018) found that mussels exposed to oxygen concentrations decreasing from 9 to 2 mg O₂/l resulted in a slow but significant reduction in the respiration rate at 13.5±0.6 °C. At oxygen concentrations below 2 mg O₂/l *M. edulis* responded by gradually closing its valves, resulting in a rapid decrease of filtration rate, concurrent with a rapid reduction of respiration rate. These observations indicated that *M. edulis* is no longer able to maintain its normal aerobic metabolism at oxygen concentration below 2 mg O₂/l.

The high temperatures disrupt the optimal physiological processes in the molluscs. This can lead to physiological stress, metabolic impairment, and even mortality, especially during prolonged heatwaves (Brancchetti et al., 2024). In recent years, mass mortality events linked to high summer temperatures

have also increased in the Mar Grande, further damaging mussel production (Arduini et al., 2023). In the period between 2015 and 2020 mass mortality events had happened in Albania, in the mussel farm of Butrinti Lagoon. The result was the almost complete failure of the production.

The aquaculture of Mediterranean mussel (*M. galloprovincialis*) in Butrinti Lake (the Albanian coast of Ionian Sea) started in 1968, in 80 concrete "panel" structures (Peja et al., 1996). These structures were installed at the depth from 6 m to 10 m, in three areas of the lake (Manastir, Pallavraq and Butrint) (Figure



Figure 1. A "panel" type system, with concrete structures, for the cultivation of mussel (*M. galloprovincialis*) in Butrint Lake

1). The maximal production was 4920 ton/yr, in 1989. The highest value of exports to EU countries was 700 ton/yr and was realized in 1987. With the interruption of exports, for sanitary reasons, after the 90s, the mussel was commercialized in the domestic market, and the production was reduced (150-2000 ton/yr) (ASPBM, 2010).

The aim of this study was to provide information on the damage of some indicators of the growth performance in mussels, caused by the excessive increase in water temperature in summer season.

Materials and methods:

The study was carried out in a mussel "population" that was reared in the panels of a fixed suspended culture, in Manastiri area (Figure 1), during 2024s. Samplings of mussels were carried out without selection in the months of April-May and July-August, from three parts of the ropes; in the upper 1/3, in the middle and in the lower 1/3 parts. For each month, no less than 90 molluscs were sampled. Each individual was cleaned from detritus and biofouling before measurements. Measurements were made for shell length (TL , cm), live weight (W , g) and meat weight (w , g). The total shell length was measured to the nearest 0.01 mm using a digital caliper. Each mussel was weighed for live weight (W , g). After, using a scalpel the wet tissue (meat) was removed from the shell and weighted individually (w , g) to the nearest 0.01 g (Kovacic et al., 2017). An electronic balance was

used to do the weightings.

The concentration of dissolved oxygen (mgO_2/l) and the water temperature ($^{\circ}C$) were measured at the sampling points using the oxy-thermometric probe HQ40d. The measurements were carried out in the vertical direction, in 10 horizons (0.25m, 0.50m, 0.75m, 1.00m, 1.25m, 1.50m, 1.75m, 2.00m, 2.50m and 3.00m) of the lake depth 0-3 m, for two-time intervals of the rearing cycle (April-May and July-August).

The specific growth rate index (SGR), according to total length of the shell (TL , cm): This growth parameter was estimated for the time-interval from 3 April to 28 May, as well as for the time-interval from 12 July to 30 August. Was used the formula according to Welker et al. (2019):

$$SGRTL = [(\ln TL_2 - \ln TL_1) / (t_2 - t_1)] * 100.$$

TL_1 was the average total length (cm) of the sample's mollusks on the 3 April and 12 July and TL_2 was the average total length of the sample's molluscs in 28 May and 30 August, respectively. The variable $t_2 - t_1$ was the number of days between the 3 April and 28 May as well as between 12 July and 30 August.

The calculation of the relative condition factor (Kn) was carried out after preliminary calculation of the values of the two coefficients (a and b) in the length-weight allometric relationship (Kuriakose, 2017), separately for two-time intervals (April-May and July-August) of the rearing cycle. The formula for calculation was according to Zorica and Čikeš Keč (2013):

$$Kn = W/aTL^b$$

W - live weight of the mollusk (g); TL - Total length of the mollusk (cm); b - exponent or allometric coefficient; a - intercept.

The meat yield ($MY\%$): We used the formula according to Çelik et al. (2012) to calculate this growth parameter:

$$MI\% = (w, g / W, g) * 100$$

Were: w - wet meat weight (g) and W - live (total) weight of the mollusc

The calculation of growth parameters (a, b, Kn , $SGRTL$ and $MY\%$) and the biometric data processing were conducted using IBM SPSS Statistics 21.0 (2019). A One-way ANOVA analysis of variance and the Student's t -test were used to compare the

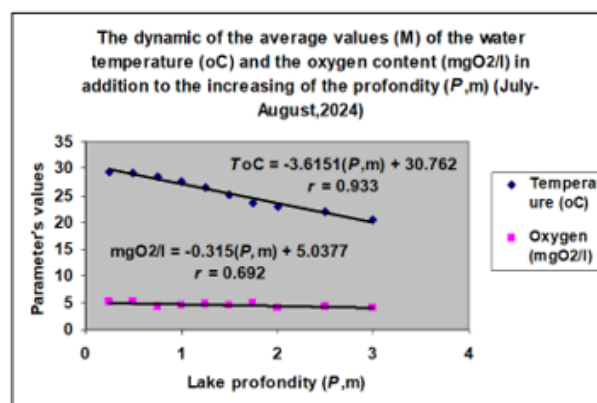


Figure 2. The values of the coefficients in the relationships $P, m - T^{\circ}$ ($r=0.933$) and $P, m - O_2, mg/l$ ($r=0.692$), in the time interval July-August.

means values of the growth parameters,between two time-intervals of mussel's rearing cycle.Were tested the significance

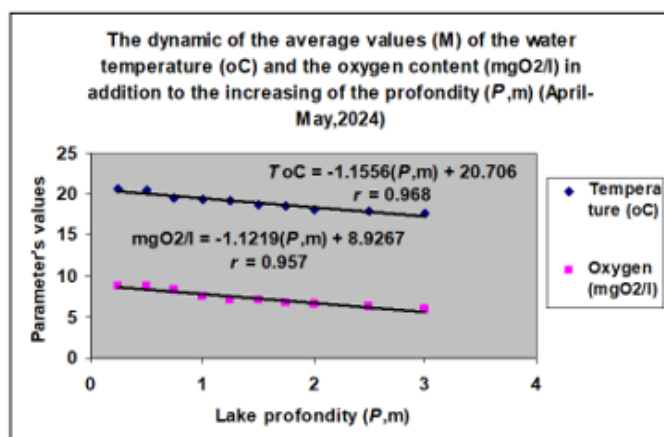


Figure 3.The values of the coefficients in the relationships $P, m-T^{\circ}$ ($r=0.968$) and $P, m-O_2, mg/l$ ($r=0.957$), in the time interval April-May.

levels ($P<0.05$) of the differences between the average values of the calculated parameters (Zar,2010).

Results:

-The relationship between the depth (P_m) and two indicators of water quality, temperature ($^{\circ}C$) and dissolved oxygen content (mgO_2/l):

Figures 2 and 3 demonstrate the isometric relationship between water depth in the area of Manastir (one of the Butrinti lake's area where are located the panels of the mussel cultivation plant),on the one hand,and the water temperature as well as dissolved oxygen content,on the other hand,for two time intervals of the rearing cycle,July-August and April-May.

In the two time intervals, a negative relationship is proven between water depth and temperature as well as depth and oxygen concentration.However, in the spring season, the water layer near the surface (0-3m) tended to be isothermic, while in the summer season this layer show the features of thermal "stratification".This fact was proven after analysis of variance (ANOVA), comparing the average values of water temperature at two depths, 0-1.5 m and 1.5-3.0 m, for the two time intervals. In the April-May time-interval, the respective average values of the water temperature, according to the indicated depths, were $19.68 \pm 0.77^{\circ}C$ and $18.05 \pm 0.42^{\circ}C$.The comparison of these values did not prove the existence of significant differences ($t=1.8; P>0.05$).The situation of the average values of the water temperature, for the July-August time interval was $27.75 \pm 1.63^{\circ}C$ at a depth of 0-1.5m and 22.18 ± 1.37 , at a depth of 1.5-3.0m.A significant difference was proven between these two values ($t=2.7; P<0.05$).The highest measured value for temperature was $29.4^{\circ}C$ ($P=0.25cm$;August).

The increase in the average water temperature in the 0-3.0m layer,from $19.03^{\circ}C$ (April-May time interval) to $25.52^{\circ}C$ (July-August time interval) (1.34 times),had caused the reduction

in the average oxygen content from $7.30 mgO_2/l$ to $4.58 mgO_2/l$ (1.59 times).The regression and correlation analysis confirmed that the rate of reduction in the values of dissolved oxygen content was higher in the July-August time-interval ($b=8.926$) compared to the April-May time-interval ($b=5.037$).The lowest measured value for oxygen was $4.05 mgO_2/l$ ($P=3.0m$;August). Our estimates confirmed the existence of a significant difference ($t=2.50; P<0.05$) between the average value of the oxygen content for the April-May time interval ($7.30 \pm 1.031 mgO_2/l$) and the average values of this indicator for the July-August time interval ($4.58 \pm 0.401 mgO_2/l$).

-The specific growth rate index (SGR):

The specific growth rate index is a growth parameter that measures the growth rates (in %) between two time intervals (from t_1 to t_2).The difference in the height of the columns, in the dia-

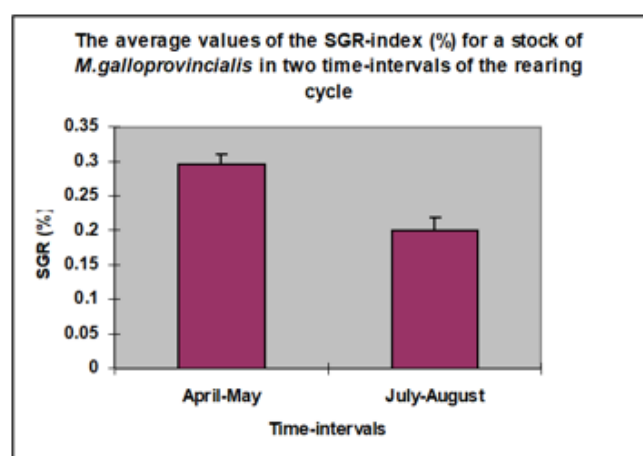


Figure 4.The differences in growth rates,according to the estimarion of the SGR-values,in *M.galloprovincialis* (Bivalvia),reared in Butrinti Lake,in two time-intervals of the production process.

gram of Figure 4,confirms the differences in the growth rate of *M. galloprovincialis* between two time intervals (April-May and July-August) of the rearing cycle.The average values ($M \pm SD$) of the specific growth rate index (SGR%),for the reared stock of *M.galloprovincialis*,in two time-intervals of rearing cycle were respectively: 0.298 ± 0.015 (Var%=5.07) (April-May) and 0.196 ± 0.020 (Var%=10.05) (July-August).

The regression and correlation analysis confirmed the significance of the differences ($t=4.08; P<0.05$) between the average value of the SGR(%)-index in reared mussels,for the time interval April-May and the average values of this growth parameter, for the time interval July-August.This fact proves that the growth rate of mussels in the Summer season has slowed down significantly compared to the Spring season.

-Relative condition factor (K_n):

According to the protocol that was applied,we initially calculated the values of the two coefficients (a and b) in the allometric length-weight relationship and, further, these values were used to calculate the values of the relative condition factor (K_n) in reared mussels, separately for two time intervals, April-May

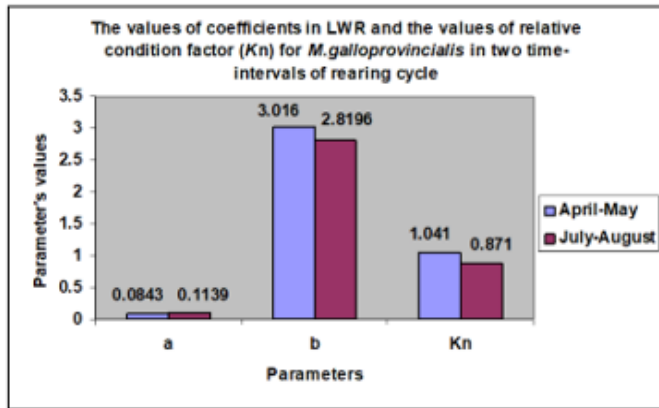


Figure 5. The values of some analytical growth indicators (*a*, *b* and *Kn*) for a reared stock of *M. galloprovincialis* (Bivalvia) in two time-intervals of rearing cycle.

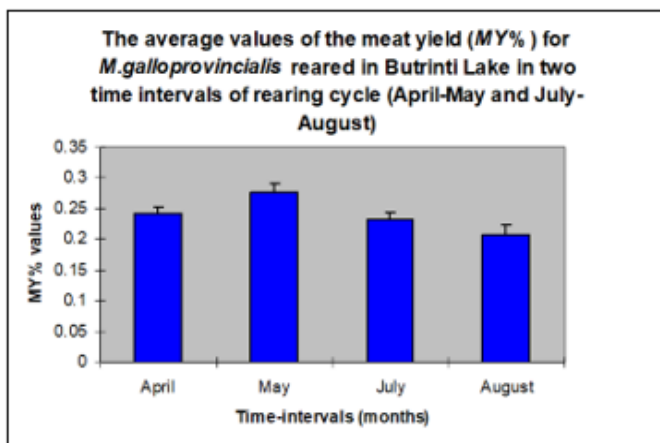


Figure 6. The monthly differences in growth characteristics, according to the average values of MY%, in *M. galloprovincialis* (Bivalvia) reared in Butrinti Lake.

and July-August (Figure 5)

The values of the allometric coefficient (*b*) at LWR ($b=2.819$; July-August and $b=3.016$; April-May) showed that the growth of reared mussels in the Spring season was isometric with a positive allometric trend ($3.0 \rightarrow 3.5$), while in the Summer season it was negative allometric (<3.0). This is another fact that proves the damage to the growth processes in the hottest season of the year. Being basic variables in the calculation of the values of the relative condition factor (*Kn*), the above-mentioned “in time” differences in the values of *a* and *b* are reflected in the values of this condition indicator. The average values of the *Kn*-factor for the reared mussels, in two time intervals were: $Kn=0.871$, in the Summer season and $Kn=1.041$, in the Spring season. Expressed in %, these values prove that the condition of the mussels in the months of July-August was as much as 83.7% of the condition they had in the months of April-May.

-The meat yield (MY%):

The meat yield (MY%) is an analytical parameter for the quality of the mollusk as a product for human consumption. High-

er values of this parameter testify to the higher content of soft tissues in the total (or live) weight of the mollusk. According to the four months, the average values ($M \pm SD$) resulted from the calculation of the meat yield (MY%) of mussels reared in the aquaculture of Butrinti Lake, were: 0.241 ± 0.0107 (Var%=4.44) in April; 0.276 ± 0.0151 (Var%=5.47) in May; 0.231 ± 0.0135 (Var%=6.34) in July and 0.207 ± 0.0150 (Var%=7.24) in August. Not only the reduction of the monthly average value of MY% but also the increase in the value of the biometric indicator Var% confirms the deterioration of the meat content in mussels, from the beginning of April to the end of August. The calculation of the *t*-test of the Student, in the comparison between the average values of the MY%-parameter, for the months of April and August, proved that the changes were at the limits of “significant difference” ($t=1.89$; $n=7$; $P<0.05$).

Discussion:

European aquaculture mussel production of the species *Mytilus galloprovincialis* is in decline. This decrease can be due to multiple stresses linked to climate change. Mussel growth performances were significantly different in relation to the depth. Due to the high temperatures recorded in July (max. 31.01°C at the surface) the reared mussels did not reach the requested condition index for the marketing. Moreover, mussels reared on the surface resulted more vulnerable to air exposure (Calabrese et al., 2025). Several authors have conducted assessments on the thermal regime and oxygen content in the water of Butrint Lake. Bushati et al. (2012) conducted measurements in Butrint Lake for temperature and dissolved oxygen, at depths of 1m and 3m. They recorded minimum and maximum values of 6.1°C and 29.8°C for temperature, as well as $5.1\text{ mgO}_2/\text{l}$ and $11.4\text{ mgO}_2/\text{l}$ for the oxygen content. The average values were: $17.7 \pm 6.2^\circ\text{C}$ and $8.4 \pm 1.2\text{ mgO}_2/\text{l}$. These authors had noted that in the Summer season the water temperature reached the limit of 30°C , while the oxygen content was quite low. The Spring season was considered the best for mussel growth.

Kolitari et al. (2013) had found that in 2011, in Butrinti Lake there was a gradually growing temperature and this leads to catastrophic temperature in July. These authors pointed out that the water temperatures in depth 1m changed more than in depth 3 or 5 m during the months. This happens because temperatures of surface are in influence of air temperatures. Then in depth 3 and 5 m the temperatures had a stability during the months, except June. Also, were proven significant differences between the minimum value ($2.3\text{ mgO}_2/\text{l}$) and the maximum value ($9.96\text{ mgO}_2/\text{l}$), of the oxygen content. We have not found situations where the oxygen content was as low as the minimum measured by Kolitari et al. (2013), but we must admit that, although mussels are quite tolerant to hypoxia, the general deterioration of water quality causes at the same time an increase in their sensitivity to hypoxic stress. In the conditions of water quality damage from eutrophication, the intensity and duration of hypoxic situations have increased significantly, causing damage to mussels, which are often forced to be exposed to air or subjected to fluctuations in the oxygen content in the water, which

are characteristic of the summer season (Dias and Rosenberg, 1995).

It should be noted that short-term thermal crises caused by heatwaves have also occurred in Lake Butrint in 2015 and 2017 (MBZHR, 2018). In the summer of 2015, the water temperature in the surface layer of the lake had reached 33°C, while in the same season in 2017, a record temperature of 40°C was registered. In this year, 70% of the mussels in the aquaculture system had died. This was a typical case of production failure, caused by a heatwave. Heatwaves are causing catastrophic failures in mussel production, with recent examples showing nearly total, or up to 90%, losses in major production areas like Greece in 2024. These failures are driven by water temperatures exceeding 30°C, which are lethal to mussels (<https://www.google.com/search> the failure of mussel production). In recent years, studies on the negative impact of global warming on production levels in mussel aquaculture have increased significantly. Recent studies confirmed that the occurrence of recurrent extreme marine heatwaves along the Mediterranean coasts during the 2015–2019 period caused mass mortality events in invertebrates, including corals, sponges, bryozoans and mussels (Garrahou et al., 2022). Considering that extreme temperatures will likely intensify in the future, further efforts are needed to design new farming strategies, such as an IMTA system in an offshore wind farm, to overcome this challenge (Calabrese et al., 2015). Mussels of the genus *Mytilus* are one of the most important economic and ecologically relevant bivalves in European coastal areas, with the Mediterranean species *M. galloprovincialis* limiting its expansion by temperatures <9 °C (Fly et al., 2015), and showing lethal temperatures between 26 and 28 °C (Jones et al., 2009).

Our evaluations of several parameters that measure mussel growth proved that in general heatwaves, acting at relatively short intervals during the summer season, cause the reduction of values for such analytical indicators as SGR(%), MY(%) and Kn. Peharda et al. (2007) had found that, in an aquaculture plant of the Adriatic Sea, the length of the mussel (*M. galloprovincialis*) in the March-May time-interval had increased on average by 6.4±2.2 mm, while during the July-September time-interval the average increase was 1.5±1.2 mm. According to this authors growth rates were lowest during the summer months (July and August) when water temperature ranged between 21 °C and 24 °C, and highest during March and April, when the water temperature was as low as 10 °C. Mussels exhibited intermediate growth rates during all other periods. The observed pattern of mussel growth can partially be explained by the temperature dependence of mussel growth rate. The optimal growth of bivalves calculated from the most common bivalve, which are oysters, clams, scallops, and mussels, occurs at an average temperature of 18°C, with a range of 12–24°C. Higher temperatures can slow growth and increase mortality (Acquaafredda et al., 2019; Arrieche et al., 2020). Heatwaves can cause significant physiological stress in marine organisms, leading to differences in growth and even declines in population (Masanja et al., 2023).

Conclusion:

In the conditions of systematic recurrence of spontaneous heatwaves, the negative impact of which on the growth and production of aquaculture products, including bivalve molluscs, has been proven by many studies, it is necessary to carry out technical, but also biotechnological interventions, to reduce the negative effects. It should be noted that cultivation in the lagoon, due to the limited depth and the more rapid changes in water parameters, compared to the sea, offers fewer opportunities for technical modifications of the equipments and renewal of technologies for the rearing of the mollusks. The aquaculture of mussels (*M. galloprovincialis*) should increasingly be directed to the sea, integrating intensive practices in production technologies as well as including biotechnological methods of genetic improvement.

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