BOOK OF ABSTRACTS

Symposium: Young Scientists and the Future of Laboratory Medicine

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BOOK OF ABSTRACTS

Polymers: secret heroes of future medicine

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Polymers are a special type of material consisting of a high number of repeating units that can be engineered to possess specific chemical, physical and biological properties. Over the past decades, polymers have gained significant positions in a wide range of biomedical applications, including drug delivery systems, gene therapy, wound healing, tissue engineering, and even in the development of implantable devices. It is widely accepted that polymer properties such as chemical structure, reactivity, and charge, together with the interfacial properties of their self-assemblies, including size, shape, surface chemistry, and biodegradation, are key parameters that influence and direct their interactions with cells and biological membranes and tissues.[1] Thanks to these unique features, polymers are at the forefront of developing next-generation therapeutics, offering new possibilities for personalized medicine.

Among these advanced materials, poly(2-oxazolines) are promising biomedical polymers with excellent biocompatibility, structural stability, hydrophilicity, and versatility. In our research, we focused on the preparation and application of poly(2-isopropenyl-2-oxazoline) (PIPOx) in different fields of biomedicine. PIPOx, prepared by atom transfer radical polymerization, has gained significant attention due to non-toxicity, immunomodulation properties[2] and the possibility of post-polymerization modifications via pendant reactive 2-oxazoline group on the side chain. PIPOx has been used for the preparation of thermosensitive or cationic polymers, drug conjugates, hydrogels, brush-like materials, and polymer coatings available for drug and gene delivery, tissue engineering, blood-like materials, antimicrobial materials, and many others.[3]

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Therapeutic potential of bacteriophage endolysin for infections caused by *Streptococcus agalactiae*

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Bacterial biofilms are widespread in the environment, and bacteria within these biofilms exhibit high resistance to antibiotics and host immune defenses, leading to significant environmental health issues. The extensive use of antibiotics has led to a crisis due to antimicrobial resistance (AMR), complicating the clinical management of bacterial infections and posing a significant global public health challenge. The ability of many opportunistic bacteria to form biofilms, coupled with resistance to antibiotics, complicates efforts to control widespread infections, resulting in high risks of negative outcomes and economic costs.

Streptococcus agalactiae (Group B Streptococcus, GBS) is a major neonatal pathogen and commonly colonizes the gastrointestinal and urogenital tracts in adults. The treatment of GBS infections is often hindered by the recurrence of multi-resistant strains. The irrational use of traditional antimicrobial drugs has exacerbated bacterial resistance, prompting the search for novel antimicrobial substances.

Bacteriophages, the most prevalent biological entities on Earth, have evolved unique lytic systems, including endolysins. Endolysins are promising compounds that efficiently combat bacteria, including multidrug-resistant strains and biofilms, with a low probability of subsequent emergence of stable endolysin-resistant phenotypes. Endolysin EN534, derived from prophage A2 of the human isolate *Streptococcus agalactiae* KMB-534, features a modular structure with two terminal catalytic domains (amidase_3 and CHAP) and a central binding domain (LysM). This endolysin demonstrated lytic activity against various GBS serotypes, *Bacillus subtilis*, *Lactobacillus jensenii*, and *Escherichia coli*, while sparing beneficial vaginal lactobacilli. The protein remains stable between 20°C and 37°C, and its activity is enhanced by calcium ions within a pH range of 5.0 to 8.0. Time-lapse fluorescence microscopy revealed the exolytic activity of recombinant endolysin EN534-C on a *S. agalactiae* CCM 6187 substrate. EN534-C shows promise as a potential antimicrobial agent for treating *S. agalactiae* biofilms and infections.

Epicatechin: Green tea molecule in the fight against microbial pathogens

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Catechins, a class of bioactive flavonoids present in green tea and various other food sources, are recognized for their multiple biological activities beneficial for human health. This class includes eight different types of catechins that are currently the focus of intensive research, particularly for their antimicrobial, anticarcinogenic, antioxidant, and immunomodulatory effects. Among these, epicatechin is one of the primary catechins in green tea, yet remains one of the least studied. Our research investigated the antibacterial activity of epicatechin on a range of Gram-positive and Gram-negative bacteria, including clinical and environmental isolates. Our results indicated that epicatechin exhibited stronger efficacy against Gram-positive strains aligning with general observations on catechin antimicrobial properties[1]. We further explored the potential synergy between epicatechin and various antibiotics, focusing on *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA). Notably, epicatechin showed promising co-effect with cefoxitin against MRSA. Given the immunomodulatory activity of catechins in general, we examined the effect of epicatechin on the cellular immune response of *Galleria mellonela* larvae (great wax moth). To assess antibacterial potential of epicatechin *in vivo,* we infected *Galleria mellonella* larvae with MRSA and observed that treatment with high doses of epicatechin extended larvae survival within 24 hours after infection. The safety of high doses of epicatechin used in experiments was confirmed by toxicity assay and mutagenicity was excluded by Ames assay.

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Effective options for monitoring bacterial phytopathogens in vegetable production

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Effective management of bacterial phytopathogens in vegetable production is essential to ensure food safety, minimize crop losses, and reduce the environmental impact of agricultural practices. Bacterial diseases in vegetables can cause significant economic losses and hinder sustainable production, especially within intensive farming systems. This research aimed to identify monitoring strategies that not only improve early detection but also support integrated approaches to disease management, ultimately contributing to sustainable vegetable production and increased resistance to bacterial diseases. We tested several innovative and effective options for monitoring of bacterial phytopathogens, focusing on user-friendly methods that enable early, accurate and cost-effective pathogen detection in vegetable crops a/or seeds. The findings provide insights into the selection and application of appropriate monitoring techniques (PCR, multiplex PCR, LAMP methodologies) tailored to the non-scientific skills of farmers in agriculture.

This work was supported by the grant INTERREG 304011X035.

The Application of Oxford Nanopore Sequencing in the Metagenomic Analysis of Whey and Environmental Samples from a Bryndza Manufacturing Facility

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Slovak bryndza cheese, traditionally made from unpasteurized sheep's milk, is a signature product across Slovakia and holds EU protected geographical status. During the aging of unpasteurized cheese curd, microbiota undergoes dynamic changes that influence bryndza cheese's unique taste and aroma. Its typical composition includes lactococci, lactobacilli, leuconostocs, streptococci, yeasts, and filamentous fungi. To maintain its characteristic flavour, using standard commercial bacterial cultures is discouraged. Producers using unprocessed sheep's milk require specific cultures for aging control, while those working with pasteurized milk seek new cultures to enhance the flavour of Slovak bryndza cheese and similar cheeses. The study aimed to analyze the bacterial and fungal metagenome of air, swab, and whey samples from the bryndza cheese production environment, focusing on microbial composition correlations. This involved amplifying the 16S rDNA gene for bacteria and the 28S rDNA gene (NL1/NL4 primers) for fungi. A sequencing library was created using the MinION platform (Oxford Nanopore Technologies) with the Ligation Sequencing Kit (SQK-LSK109), and sequences were processed and visualized via bioinformatics tools. In the air samples, the dominant bacterial genera were *Lactococcus*, *Brevibacterium*, *Deinococcus*, *Acinetobacter*, and *Staphylococcus*, with the fungal genus *Aspergillus*. Swab samples showed dominance of *Lactococcus*, *Leuconostoc*, *Psychrobacter*, and *Pediococcus*, along with fungal genera *Kluyveromyces*, *Malassezia*, *Psilocybe*, and *Aspergillus*. In whey samples, the main bacteria were *Lactococcus* and *Leuconostoc*, with fungi *Aspergillus*, *Kluyveromyces*, *Candida,* and *Pichia.* Gram-negative psychrotrophic bacteria such as *Acinetobacter* and *Psychrobacter* can also occasionally occur as microbial food contaminants. Nanopore sequencing revealed the microbiome composition of air, swabs, and whey, with metagenomic analysis highlighting key bacterial and fungal strains for bryndza cheese safety. *Leuconostoc* and *Lactococcus*, which were especially abundant in whey, are essential to bryndza cheese ripening. Additionally, *Candida*, *Pichia*, and *Kluyveromyces* yeasts, prevalent in whey and swabs, support bryndza cheese maturation

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Staphylococcus spp.: A Challenge in Food Safety.

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Food quality and safety are major global challenges. During food production, contamination often occurs with ubiquitous bacteria of the genus *Staphylococcus*. Staphylococcal food poisoning (SFP) is caused by ingestion of food containing staphylococcal enterotoxins (SEs) produced by enterotoxigenic *Staphylococcus* species, especially *S. aureus*. Moreover, if these bacteria are antibiotic resistant, the risk of poisoning increases, also the possibilities of their spread and survival in the environment. Our study focused on determining SE production (types A-E), the presence of associated genes, and identifying selected mechanisms of antibiotic resistance in 69 *Staphylococcus* isolates from human (36%), animal (16%) and food samples (48%). Half of the isolates produced one or more SEs, with 20% being coagulase-negative staphylococci. Seventy-five percent of the isolates showed resistance to at least one tested antibiotic, mainly clindamycin (45%), penicillin (36%), and erythromycin (35%). We also monitored efflux pumps overproduction, ethidium bromide accumulation rates, and biofilm formation. Most isolates (63%) were moderate biofilm producers, while 33% demonstrated a very high level of biofilm production. Selected isolates were also tested for virulence using the *Galleria mellonella* model. To address challenges in this issue, we are implementing a mass spectrometry method to lower detection costs compared to the ELISA method. By gradually improving this methodology, we have been able to detect one of the five most commonly occurring SEs in food samples. Our research underscores the need for a One Health approach, integrating aspects of veterinary medicine, human health, and environmental protection.

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Forming a safer world: *Caenorhabditis elegans* in endocrine disruptor research

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Endocrine disruptors are substances that interfere with the hormonal system, leading to various health issues in living organisms. Bisphenol A (BPA), a prevalent endocrine disruptor, is associated with reproductive, metabolic, and neurological disorders. In response to these concerns, BPA has been substituted with bisphenol S (BPS). Recent studies have shown that BPS adversely affects both humans and wildlife. The aim of our study was to assess the impact of varying concentrations of BPA and BPS on specific biological traits of the nematode *Caenorhabditis elegans (C. elegans)*. We evaluated the effects of these chemicals by utilizing the N2 strain and mutant strain *bli-1* and exposing them to various concentrations of BPA and BPS (0,1; 0,5; 1 and 5 µM). Our findings indicate that both chemicals statistically significantly impaired hatching, habituation to anterior touch, and body length in both strains. The mutant nematode, with altered cuticle collagen, showed increased sensitivity to BPA and BPS, highlighting the cuticle's crucial role in protecting against toxic agents. The findings indicate that BPS, although regarded as a safer alternative for BPA, may still pose significant risks to human health and environment. Our research demonstrates that even supposedly safer substitutes for BPA can adversely affect living organisms. It emphasizes the need for rigorous testing and regulation of these chemicals to protect public health and the environment. Our research indicated that bisphenols exerted a more pronounced effect on the *C. elegans bli-1* strain, which has a mutation in a gene responsible for producing one of the collagens in the nematode cuticle. This illustrates the significance of this structural component in shielding the nematode from detrimental substances.

Analysis of Trends in NT-proBNP Testing in the last 5 Years and Evaluation of NT-proBNP Values in an Epidemiological Context

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N-terminal pro–B-type natriuretic peptide (NT-proBNP) is a key biomarker in the diagnosis and monitoring of heart failure (HF) and is a critical predictor of hospitalisation and mortality. It helps not only in assessing clinical status, but also in early disease detection, significantly influencing patient prognosis. Given the increasing prevalence of cardiovascular diseases, monitoring of NT-proBNP testing trends is vital for evaluating diagnostic strategies and the quality of healthcare. This study is aimed to analyze the frequency of the NT-proBNP test over the past five years and to evaluate the findings in an epidemiological context.

We performed a retrospective analysis of the data from the laboratory information system. The study included all patients over 18 years of age tested for NT-proBNP due to suspected HF from 2019 to 2023. The patients were classified into three age groups: under 50 years, 50-75 years, and over 75 years. Based on NT-proBNP levels, patients were further classified into four categories: low (<125 ng/L), moderately elevated (125-300 ng/L), high (300-900 ng/L), and very high (>900 ng/L).

From 2019 to 2023, 11,236 NT-proBNP tests were performed in 9,117 patients. There was a significant increase in the number of patients with suspected HF and NT-proBNP tests, with an upward trend in both sexes ($p<0.001$). In 2023, the diagnostic threshold for acute HF was met by 12% of patients under 50 years of age (450 ng/L), 22% of patients aged 50-75 years (900 ng/L), and 35% of patients over 75 years (1800 ng/L).

Our study indicates a significant increase in the frequency of the NT-proBNP test from 2019 to 2023, highlighting a growing interest in the diagnosis of HF. This trend underscores the need for regular monitoring of biomarkers such as NT-proBNP to improve the prognosis of patient and optimise treatment outcomes.

Pharmacogenetic testing as a part of the personalised pharmacotherapy of patients with cardiovascular diseases

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Currently, cardiovascular diseases such as heart failure (HF) and pulmonary arterial hypertension (PAH) are treated with a "one-size-fits-all" approach. However, there is known interindividual variability in relation to the patient's drug response, therapeutic outcomes and adverse effects. Genetics play a key role in drug biotransformation, making pharmacogenetic testing a valuable tool for personalised medicine and the optimisation of treatment. This study aimed to perform pharmacogenetic testing on patients with cardiovascular diseases in Slovakia, identify actionable drug-gene pairs, and demonstrate the potential for optimising drug therapy based on genetic results. In a retrospective observational study, 101 patients with cardiovascular diseases (heart failure *n* = 68; pulmonary arterial hypertension, *n* = 21; others, *n* = 12) treated at the National Institute of Cardiovascular Diseases in Bratislava underwent pharmacogenetic testing of selected gene alleles of *CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4,* and *VKORC1*, using qPCR. We identified heterozygotes and homozygotes for variant alleles, finding that 98% of patients carried at least one gene polymorphism associated with altered gene function. Among the 143 different drugs administered to these patients, 53% were biotransformed by CYP450 enzymes, most commonly *CYP3A4* (42%), followed by *CYP2D6/2C9* (13%), *CYP2C19* (10%), and *CYP1A2* (9%). Pharmacogenetic recommendations for therapy adjustments exist for 20 drugs according to US guidelines (CPIC) and 15 according to Dutch guidelines (DPWG). The most frequently used CYP-metabolised drugs included pantoprazole (37%), metoprolol (32%), and warfarin (22%). Allele frequencies in our population were comparable to the general European population, with exceptions of *CYP1A2*1F (rs762551) and CYP2D6*3* (rs35742686) (our sample vs European estimate 71.2% vs. 29.7%, p<0,0001; 1.8% vs. 0.5%, p<0,0001; respectively). The practical application of pharmacogenetic recommendations is illustrated in a case study. This study highlights the potential for pharmacogenetic testing to optimalise treatment in patients with cardiovascular diseases, supporting the integration of personalised medicine into cardiovascular care.

Does latent toxoplasmosis in pregnant women associate with parametric data of newborns?

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According to some studies women with latent toxoplasmosis have longer pregnancy and have more sons[1,2]. The aim of our study is to evaluate and verify the effect of latent toxoplasmosis in pregnant women on sex, some parametric data of their newborns and length of their pregnancy. We examined 2258 pregnant women for toxoplasmosis. Serum samples were screened for specific IgM and IgG antibodies by enzyme immunoassay (EIA) and total antibodies by the complement fixation test (CFT). We also determined the avidity of IgG antibodies (EIA) and analysed IgA antibodies (EIA) in selected cases. We obtained parametric data of newborns retrospectively by requesting data after birth. Out of all the women, 249 (11.0%) had a latent infection, and 2009 (89.0%) were Toxoplasma-negative. The number of boys and girls born to mothers with and without Toxoplasma was equal (CI for $p < 0.05$, $\chi 2 = 0.08$, $p = 0.773$ for $p < 0.05$). Additionally, there were no differences in the length of pregnancy between Toxoplasma-infected and uninfected women (CI for $p < 0.05$, $p = 0.964$ for $p < 0.05$), nor were there any differences in birth weight or length in both groups of mothers. Similarly, the birth weight and length of newborns were unaffected by the Rh factor of either group of mothers ($\chi 2 = 0.59$ for $p = 0.05$). The impact of toxoplasmosis in pregnant women on the sex of the newborns and their parametric data, as well as the impact on the length of pregnancy, was not confirmed by us.

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Chemotactic response of *Caenorhabditis elegans* - a promising method for early cancer diagnosis

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Early diagnosis of cancer is crucial for the success of its treatment. The tiny nematode *Caenorhabditis elegans* can distinguish a wide range of volatile organic compounds through its olfactory receptors, that is used in various types of chemotactic tests. In the field of cancer diagnostics, there is an increasing interest in the research of non-invasive screening methods that could detect cancer based on the analysis of volatile organic compounds in the patient's urine. The main goal of our thesis was to determine whether there is a statistically significant difference in the behavior of helminths *C. elegans* exposed to the urine of cancer patients and healthy individuals, as well as to determine the diagnostic utility of the used test (sensitivity, specificity, as well as positive and negative predictive value of the used test and its effectiveness). After synchronizing the developmental stages, we exposed the nematodes to the urine of cancer patients and healthy individuals. Subsequently, we calculated the chemotactic index for each urine sample. Statistical analysis showed a significant difference in the chemotactic response of helminths exposed to urine samples from cancer patients compared to healthy volunteers ($p < 0.05$) and the test showed high sensitivity (86 %), specificity (88 %), positive (83 %) and negative predictive value (83 %) and test effectiveness (86 %). Our findings demonstrate that *C. elegans* could be a promising tool for early cancer diagnosis in the future, offering a non-invasive and cost-effective method.