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Hot Topics in Microbiology

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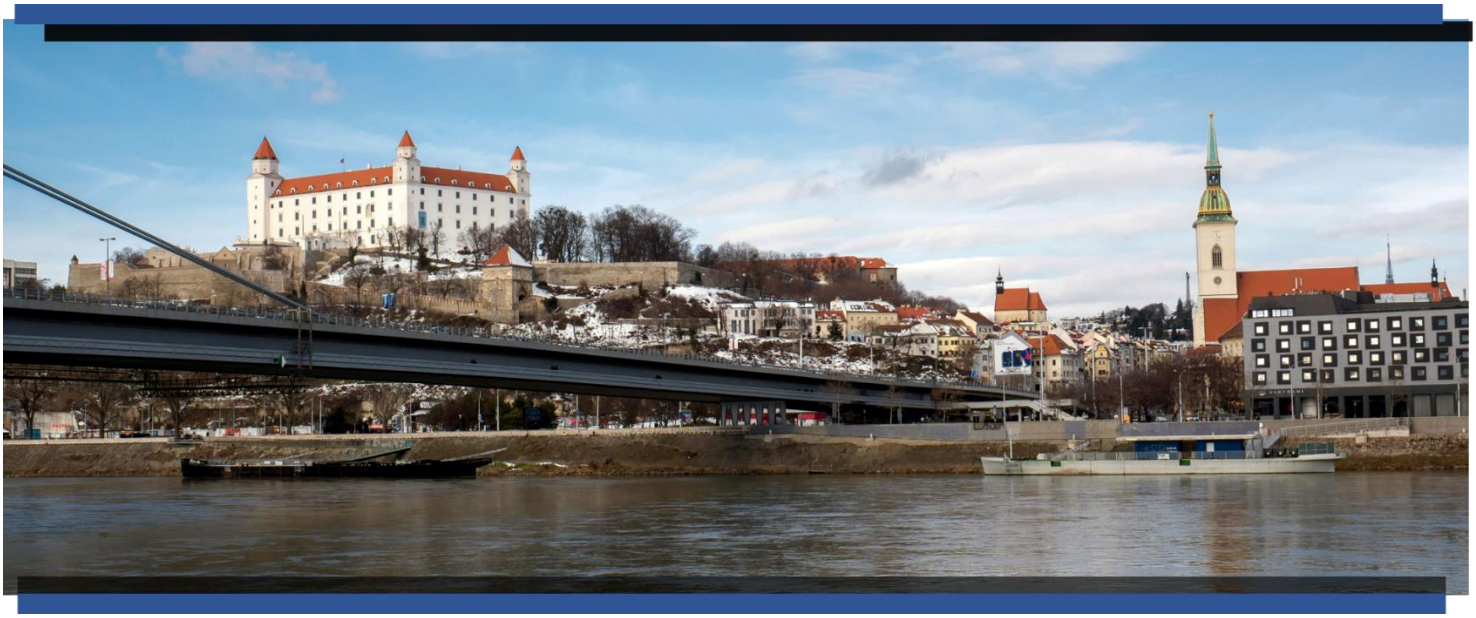

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Contents

KEYNOTE LECTURES

Chemical imaging tools for studying *in situ* microbial physiology

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The vast majority of the microorganisms in environmental and medical microbiomes cannot be grown at all under laboratory conditions, and even the cultured microbes often show substantially different ecophysiological properties in their natural habitat than in the lab. Moreover, it is very challenging to reconstruct complex multispecies microbial assemblies – such as biofilms – for targeted experimental studies. In recent years, advanced chemical imaging technologies, such as Raman microspectroscopy and Nanoscale Secodary Ion Mass Spectrometry (NanoSIMS), have been leveraged to investigate microbial metabolic activites directly *in situ* and in complex samples [1,2]. When used in combination with stable isotope tracers, these cultivation-independent methods reveal fascinating new insights into microbial substrate utilization, niche partitioning, and interactions that will dramatically improve our understanding of microbiome composition and functioning. Moreover, some approaches can be linked with single-cell sorting, and thus they enable the extraction of single microbial cells from complex samples, based on their metabolic activity, for downstream analyses like single-cell genomics or targeted cultivation attempts [3]. This presentation will give an overview of selected chemical imaging techniques along with application examples, which demonstrate the potential of these tools for studying biofilms and other types of complex microbial communities in medical and environmental microbiology.

[1] Berry D.; Mader E.; Lee T.K.; Woebken D.; Wang Y.; Zhu D.; Palatinszky M.; Schintimeister A.; Schmid M.C.; Hanson B.T.; Shterzer N.; Mizrahi I.; Rauch I.; Decker T.; Bocklitz T.; Popp J.; Gibson C.M.; Fowler P.W.; Huang W.E.; Wagner M. Tracking heavy water (D_2O) incorporation for identifying and sorting active microbial cells. Proc. Natl. Acad. Sci. U. S. A. 112:E194–E203 (2015). doi: 10.1073/pnas.1420406112

[2] Ge, X.; Pereira, F.C.; Mitteregger, M.; Berry, D.; Zhang, M.; Hausmann, B.; Zhang, J.; Schintlmeister, A.; Wagner, M.; Cheng, J.-X. SRS-FISH: A high-throughput platform linking microbiome metabolism to identity at the single-cell level. Proc. Natl. Acad. Sci. U. S. A. 119: e2203519119 (2022). doi: 10.1073/pnas.2203519119

[3] Lee K.S.; Palatinszky M.; Pereira F.C.; Nguyen J.; Fernandez V.I.; Mueller A.J.; Menolascina F.; Daims H.; Berry D.; Wagner M.; Stocker R. An automated Raman-based platform for the sorting of live cells by functional properties. Nature Microbiology 4:1035–1048 (2019). doi: 10.1038/s41564-019-0394-9

Biofilms, infection and antimicrobials resistance

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Biofilm-associated infections are a public health concern especially in the context of healthcare-associated infections (HAI). These infections have often as aetiological agents microorganisms resistant to multiple antimicrobials and desinfection agents/ procedures. In this context is urgent to identify the reservoirs potential patogens in the healthcare unit in order to mitigate the impact of HAI. Here we focused on identifying potential pathogens in water, evaluating their susceptibility to antimicrobials and desinfectants on planktonic stage or organized in biofilms. In addition, the role played by biofilm assembly on catheter related bloodstream infections was assessed by a prospective observational study. The aetological agents and their susceptibility to antimicrobials were evaluated. For the most prevalent aetiological agents, staphylococci, whole genome sequecing was performed to confirm the isogenicity of the microorganisms isolated from the central venous catheter and the blood. Since biofilms are multimicrobial communities the biofilm assembly by the aetiological agents of a coinfection bloodstream infection were monitored *in vitro*.

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Efficiency of anidulafungin and tigecycline against *Candida albicans* – *Staphylococcus aureus* polymicrobial biofilms.

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The emerging use of medical devices encounters an increased occurrence of biofilm-related infections. Both the bacterium *Staphylococcus aureus* and the yeast *Candida albicans* are key players in the cause of hospital-acquired infections due to their extreme ability to inhabit and to form mixed species biofilms on diverse host niches especially in immunocompromised individuals. Because of this reason, it is crucial to study and to understand the behavior of these pathogens when coexisting together and to discover a viable option for treatment of not only single species but also mixed species biofilms. In this study, we demonstrate the activity of antifungal drug anidulafungin and an antibiotic tigecycline against dual species *C. albicans* – *S. aureus* biofilms developed in adapted model of intra-abdominal foreign body infection. We provide insight into the pathogenesis of this dual-species biofilm-associated infection. We demonstrate that mixed biofilm infection is characterized by bacterial, but no fungal, dissemination into the vital organs within 24 hours which remains persistent over 21 days. In addition, flow cytometry data reveals significantly greater neutrophil influx upon poly-microbial intraperitoneal device-associated infection in comparison to single species infection. In search for an effective treatment strategy, we display that tigecycline acts synergistically when combined with anidulafungin against device-associated *S. aureus* cells retrieved from *in vivo* polymicrobial biofilms. Moreover, anidulafungin impairs the synthesis of poly-β(1-6)-N-acetylglucosamine (PNAG), a major constituent of *S. aureus* biofilm matrix. Therefore, we hypothesize that the effect of anidulafungin on fungal and bacterial polysaccharides may contribute to the synergism between these two drugs.

Galleria mellonella as an alternative *in vivo* model to study implant-associated infections

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In vivo biofilm models play major role to study biofilm development, morphology, and regulatory molecules involve in biofilm. Due to ethical restrictions, the use mammalian models are replaced with other alternative models in basic research. Recently, we have developed insect infection model *G. mellonella* larvae to study implant associated bacterial and fungal infections. First, the model was established with bacteria-free implants to evaluate the biocompatibility of implants in the larvae. Titanium or stainless steel implants were implanted without any adverse effects over the entire observation period of 5 days compared to controls. Then, stainless steel and titanium implants pre-incubated with *Staphylococcus aureus* or *Candia albicans* were implanted into the larvae to mimic implant-associated infection. For both materials, pre-incubation of the implant with *S. aureus* or *C. albicans* led to significantly reduced survival of the larvae compared to bacteria-free implants. Survival rates of the larvae could not be improved with implant infection situation by the addition of antimicrobial compounds. Whereas the antimicrobial treatment could improve the survival of the larvae in case of planktonic infection of the larvae, confirming the typical characteristics of reduced antibiotic susceptibility of biofilm infections. Additionally, biofilm formation on implant was confirmed by surface electron microscopy and by measuring gene expression of biofilm-related genes, which showed strong biofilm formation and upregulation of autolysin (atl) and *sarA* genes [1]. In conclusion, *G. mellonella* can be used as an alternative *in vivo* model to study implant-associated infections, which may help to reduce animal infection experiments with vertebrates in the future.

[1] Mannala, G. K., Rupp, M., Alagboso, F., Kerschbaum, M., Pfeifer, C., Sommer, U., Kampschulte, M., Domann, E. and Alt, V. (2021) “Galleria mellonella as an alternative in vivo model to study bacterial biofilms on stainless steel and titanium implants”, ALTEX - Alternatives to animal experimentation, 38(2), pp. 245–252. doi: 10.14573/altex.2003211.

Working in synergy to win the battle: a lesson learned from antimicrobial agents

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Antimicrobial peptide dendrimers (AMPDs), such as linear antimicrobial peptides (AMPs), have recently emerged as new potential candidates in combating Gram-negative bacteria. They have a 3D-branched structure with very dense and flexible functional groups [1,2]. AMPDs have been reported for their remarkable activity against Gram-negative *P. aeruginosa*, *A. baumannii* and *E. coli* [3]. G3KL, a third generation AMPD has selective properties that disrupt the bacterial membrane from both sides and accumulates in Gram-negative bacteria, leading to vesicle leakage and cell death, while exhibiting pro-angiogenic properties, leading to the endothelial tubular network formation. However, their clinical use is hindered due to reported toxicity, hemolysis towards mammalian cells and proteolytic degradation *in vitro*. We addressed these shortcomings by covalent coupling of AMDPs to different chitosan derivatives to induce synergistic antibacterial potency [4]. We observed synergistic effects within very low concentrations only when coupling AMPDs to chitosan derivatives covalently . Moreover, AMPD-chitosan conjugates showed extremely little toxicity to mammalian cells and almost no hemolysis to red blood cells. Besides, their half-life has been extended from 6 h to a minimum of 48 h when exposed to human serum.
AMPDs are highly potent tools to be used against the emerging bacteria and acting in synergy is a promissing approach for fighting against multidrug resistant bacteria, which is a worldwide hurdle nowadays.

[1] Kawano, Y.; Jordan, O.; Hanawa, T.; Borchard, G.; Patrulea, V. Are Antimicrobial Peptide Dendrimers an Escape from ESKAPE? Advances in Wound Care 2020, 9(7), 378-395. https://doi.org/10.1089/wound.2019.1113
[2] Patrulea, V.; Borchard, G.; Jordan, O. An Update on Antimicrobial Peptides (AMPs) and Their Delivery Strategies for Wound Infections, Pharmaceutics 2020, 12(9), 840. https://doi.org/10.3390/pharmaceutics12090840
[3] Siriwardena, T. N.; Capecchi, A.; Gan, B.-H.; Jin, X.; He, R.; Wei, D.; Ma, L.; Köhler, T.; van Delden, C.; Javor, S.; Reymond, J.-L. Optimizing Antimicrobial Peptide Dendrimers in Chemical Space. Angewandte Chemie 2018, 130(28), 8619-8623. https://doi.org/10.1002/ange.201802837
[4] Patrulea, V.; Gan, B.-H.; Perron, K.; Cai, X.; Abdel-Sayed, P.A.; Sublet, E.; Ducret, V.; Porroche Nerhot, N.; Applegate, L.A.; Borchard, G.; Reymond, J.-L.; Jordan, O. Synergistic effects of antimicrobial peptide dendrimer-chitosan polymer conjugates against *Pseudomonas aeruginosa*. Carbohydrate Polymers 2022, 280, 119025. https://doi.org/10.1016/j.carbpol.2021.119025

Whats new with the ATCGs – the newest developments in amplicon and (meta)genome sequencing approaches in microbiology

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High throughput sequencing has, over the last few decades, in many ways revolutionized research in, microbiology and microbial ecology. But with the current pace of developments in sequencing-based research methods and sequencing approached, it's becoming increasingly complicated to stay up to date and select the optimal sequencing approach to answer your specific research question. At the JMF (Joint Microbiome Facility) we facilitate the investigation of clinical and environmental microbiomes by offering individualized consulting and state-of-the-art services in study design, microbiome sequencing, bioinformatic analyses, and data interpretation. Additionally, we aim to advance the field of microbiome research by benchmarking existing approaches and developing new technological and analytical strategies. In this lecture, I will give a brief overview of the workflows we use, the methods we have developed and are working on developing further, and introduce you to the state of art approaches for long- and short-read based sequencing and analysis of microbial samples.

INVITED LECTURES

HTmicro HOT TOPICS IN MICROBIOLOGY
Keywords: Resistance, Biofilm, Antimicrobial materials
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How dead is dead in biofilm-associated infections? Investigation by fluorescence in situ hybridization (FISH)

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Medical biofilms are difficult to treat because of their recalcitrance to antibiotics. Common routine diagnostics comprise cultivation and testing for antibiotic susceptibility by determining the minimal inhibitory concentrations (MIC). These tests imply disintegration of the biofilm and growth of the bacteria in vitro.
Therefore, the routine procedures might miss bacteria in stationary phase, presumable persister cells, and give no information about spatial distribution of the viable cells or active biofilm layers.
16S rRNA directed Fluorescence in situ hybridization (FISH) is a culture-independent tool for in situ detection of microbial cells. The signal intensity of FISH correlates with the ribosomal content of the bacteria indicating metabolic activity at the time point of sampling. We employed FISH as a tool to visualize and quantify the activity of antimicrobial substances on in vitro and in vivo grown biofilms. For different modes of action of antimicrobial substances (contact killing, release) we present digital image analysis strategies for quantification of the biofilms and their active parts as well as to analyse the development of antimicrobial resistance towards antibiotics in biofilms versus planktonic cells.

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It is important to think on adaptive response of fungal cells by enhancing the antifungal activity

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With rising fungal resistance to antifungal compounds several new approaches have been used for enhancing the effectiveness of common antifungals. Besides the discovery of novel compounds, the alternative approach known as combination strategy came to the forefront of scientific interest. The combination of known antifungals with other bioactive molecules results in enhancement of antifungal activity and the combination should be able to prevent the activation of adaptive response of the pathogen. Our research was focused on the yeast *Candida albicans* CAF 2-1 and we were interested in the effect of 1,4-dihydropyridine -2,3,5-tricarboxylate, an intermediate of nilvadipine synthesis (in this work assigned as derivative H). The derivative H alone does not show any antifungal activity, but it enhances the activity of fluconazole and voriconazole. Looking at the mechanism that potentiates the activity of azoles we have found that derivative H is the substrate for the CDR1 efflux pump and is able to decrease the expression of the *ERG11* gene even in the presence of fluconazole. The expression of *ALS3* decreased as well and we have concluded that this result could explain the decreased biofilm formation when adding the derivative H to the adherence phase of biofilm formation. The most surprising result was that derivative H was able to prevent the decreasing susceptibility of dispersed biofilm cells to fluconazole and voriconazole. Our results show that even bioactive compounds without antifungal activity are able to enhance the effectiveness of commonly used compounds in antifungal therapy. The most important point of our research was that on the background of increased antifungal activity of fluconazole and voriconazole was the ability of derivative H to modulate the adaptive response of *C.albicans*.

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Novel synthetic antimicrobial and anti-biofilm peptides (SAAPS)-containing coatings to prevent biomaterial-associated infection

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The use of medical devices has grown significantly over the last decades, and has become a major part of modern medicine and our daily life. Infection of implanted medical devices (biomaterials), like catheters, prosthetic heart valves or orthopedic implants, can have disastrous consequences, including removal of the device. For still not well understood reasons, the presence of a foreign body strongly increases susceptibility to infection. These so-called biomaterial-associated infections (BAI) are mainly caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*. Formation of biofilms on the biomaterial surface is generally considered the main reason for these persistent infections, although bacteria may also enter the surrounding tissue and become internalized within host cells [1]. Our work focuses on the development and characterization of novel antimicrobial agents and delivery systems, and their effectiveness in the prevention of BAI and other difficult-to-treat biofilm infections. The scarcity of current antibiotic-based strategies to prevent infections and their risk of resistance development prompted us to develop novel Synthetic Antimicrobial and Anti-biofilm Peptides (SAAPs) based on the primary sequences of the human antimicrobial proteins Thrombocin-1 and LL-37, and to test their potential in the fight against implant-associated and wound infections by multidrug-resistant bacteria. The lead peptide, SAAP-148, kills multidrug-resistant pathogens without inducing resistance, prevents biofilm formation and eliminates established biofilms, and is effective against both acute and established skin infections [2]. Currently, we are developing improved SAAPs. As a next step, we aim to develop antimicrobial coatings, such as a new polymeric supramolecular scaffold material, exerting two important functions: preventing microbial adhesion - by incorporating SAAPs - and thereby preventing biofilm formation, and inducing endogenous (eukaryotic) cells to adhere and propagate, as a first step towards functional tissue repair.

[1] Riool *et al.*, Acta Biomater., 2014; [2] de Breij & Riool et al., Sci. Transl. Med., 2018

Keywords: Resistance, Biofilm, Antimicrobial materials

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Gut microbiome and biofilm associated microorganisms in health and disease.

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Gut microbiota is the most abundant microbial community of human body. Although mostly non-pathogenic microbes reside there, during dysbiosis several opportunistic pathogens like *Staphylococcus* spp., *Klebsiella* spp., *Streptococcus* spp. or *Enterococcus* spp. prevail in the gut of patients with non-transmissive diseases including cancer. However, also gut commensal microorganisms may become pathobionts [1]. Is there any pattern between cancer, autism spectrum disorder, or inflammatory bowel disease? What is the perspective of biofilms in human gut including luminal and mucosal locations, and its potential impact on human health and disease?

[1] Buret, A.G., Motta, JP., Allain, T. *et al.* Pathobiont release from dysbiotic gut microbiota biofilms in intestinal inflammatory diseases: a role for iron?. *J Biomed Sci* **26**, 1 (2019). https://doi.org/10.1186/s12929-018-0495-4

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Persisters and intracellular survivors: as if biofilms were not enough trouble

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Pathogenic bacteria have the capacity to evade the host defence by a variety of ways. A well known strategy is the formation of biofilms, in which bacteria are protected from antibioitcs and host cells such as neutrophils and other phagocytes. Moreover, bacteria can produce phenotypic variants very tolerant to antibiotics, the socalled persister cells. Thirdly, bacteria can survivive within host phagocytes, basically using the cell as a „safe haven".
In recent work we have developed a system to physically isolate persister cells. This allows unequivocal analysis of agents to eradicate persisters, which is essential to fully cure (chronic) infection. We have used this technique to assess the effects of antimicrobial peptides on these cells.
In order to treat intracellular bacteria, it is vital to deliver antimicrobials not only inside a phagocytic cell, but to let the antimicrobials interact with the intracellular bacteria within the cell. Bacteria localized in phagosomes and antimicrobials in separate endocytic vesicles need to be brought in contact with each other within the cytoplasm. To achieve this, we have applied photosensitizers in macrophages in vitro as well as in a zebrafish embryo model. In the embryos, we were able to *in vivo* in real time visualize the release of gentamicin intracellularly in *Staphylococcus aureus* – containing phagocytes, and showed that this resulted in rescue of the embryos from a lethal infection. These techniques and the research findings will help understand the role of these bacterial survival forms in infection, and to develop strategies to eradicate these difficult-to-treat infections.

ORAL PRESENTATIONS

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CEMBO.EU
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Whole genome analysis of bacteria from clinical samples and food industry

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Next generation sequencing (NGS) provide relatively cheap and fast way to study bacterial genomes. In this study we focused on analysis of clinically important strains and strains isolated from food industry. We analysed 68 clinical isolates belonging to genus *Klebsiella* and *Escherichia*, as well as 23 *Listeria monocytogenes* and 27 strains belonging to lactic acid bacteria (LAB). Many of *E. coli* were characterised as uropathogenic and were classified as sequence type (ST) 131, which is the most prevalent ST globally. Furthermore, many of them contained genes providing resistance against various antibiotics including beta-lactams (*CTX-M, TEM, OXA*), aminoglycosides (*AadA, Sat*) and sulphonamides (*Sul*). *K. pneumoniae* strains were selected for whole genome sequencing based on their resistance against carbapenem antibiotics. From 43 *Klebsiella* strains, 60% were characterised as ST-11, 19% as ST-258 and 12% as ST-584. Most of them contained genes *NDM* and *KPC* (carbapenem resistance), as well as other antibiotic resistance genes, such as *Sul* (sulphonamide resistance), *AadA*, *RmtF* (aminoglycoside resistance) and *QuacE delta 1* (quaternary ammonium compounds resistance). *L. monocytogenes* were isolated from sheep farm and meat processing plant according to their persistent phenotype and 52% of these strains were characterised as ST-14. This sequence type is associated with only 0,02% of genome samples deposited in the PasteurMLST database. Strains belonging to LAB, were selected as a perspective starter cultures for traditional Slovak bryndza cheese. We detected some prophages in these strains which could be potentially problematic during starter growth. This potential will be the subject of our further study.

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Development of diagnostic and antimicrobial triggered release systems for wound dressings

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Healthcare-associated infections (HAIs) can be acquired in any healthcare facility. Increasing morbidity, prolonged hospital stay, and increased treatment costs are some of the consequences of HAIs. Thus, there is a critical need for novel antimicrobial strategies and products for emerging antibiotic resistance and the search for cutting-edge infection detection and treatment systems detection systems (theranostics).

This project aims to create rapid screening systems for theranostic antimicrobial devices and to analyze the effectiveness of novel antimicrobial peptides (AMPs)[1] that can be used in such devices for wound dressings.

A novel set of peptides was designed using artificial intelligence, synthesized, and their antimicrobial activities under physiological conditions were analyzed against planktonic *Staphylococcus aureus* JAR060131 and multidrug-resistant *Acinetobacter baumannii* RUH875. The lethal concentration killing 99.9% of the inoculum (*i.e.* $LC_{99.9}$) of these peptides ranged from 0.94-15 μM in the presence of 50% human plasma. The efficacy of selected lead peptides (*i.e.* AMP-038 and AMP-045) and their retro-inverso (RI) variants were also compared to promising AMPs in preclinical/clinical phase of development. Moreover, within 2 hours, 60 μM of AMP-038 and 30 μM of AMP-045 showed more than 3-log reduction against biofilm-encased *S. aureus*.

Because resistance development is one of the major concerns, we assessed whether *S. aureus* and *A. baumannii* developed resistance to the lead peptides. While the MIC of *S. aureus* for rifampicin, and of *A. baumannii* for ciprofloxacin increased ≥4096-fold and >256-fold, respectively, no significant change in MIC was observed when the strains were cultured in the presence of AMP-038(-RI) and AMP-045(-RI) peptides for more than 20 passages.

In conclusion, we showed the antimicrobial and antibiofilm efficacy of the candidate peptides, their RI variants, and their lack of resistance development.

1. Thapa, R. K., Diep, D. B., & Tønnesen, H. H. (2020). Topical antimicrobial peptide formulations for wound healing: Current developments and prospects. Acta Biomaterialia, 103, 52-67.

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Keywords: Resistance, Biofilm, Antimicrobial materials

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Antimicrobial activity of silver complexes

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Microbial resistance is one of the major challenges of the 21st century, posing a serious global threat to public health worldwide. Ensuring effective prevention and treatment of both common and serious infections is becoming more difficult due to the loss of effectiveness of the used drugs. For this reason, it is essential to work on the development of new antimicrobial compounds. One of the alternatives are metal complexes. In our work, we focused on the combination of silver ions with naturally occurring structures such as aminoacids, dipeptides and nicotinamide.

The aim of the work was to determine the antibacterial activity of the silver complexes *in vitro*, defining inhibitory concentrations MIC90 and IC50. The results for G^+ and G^- bacteria were comparable. The impact on biofilm production was observed by crystal violet staining after application of the tested complexes at different stages of biofilm formation. We verified the potential mutagenic activity with the Ames test, it was not demonstrated in any of the tested complexes. We noted an increased production of reactive forms of oxygen, however, damage to the cytoplasmic membrane was not detected.

The results of the study suggest that the tested complexes have potential application in the design of new antibacterial agents. The antibacterial activity of the complexes is likely to depend on their bioavailability, as it correlates with the transporter's affinity for the dipeptide ligand.

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Development of a multiplex mRNA PNA-FISH method to assess *Legionella pneumophila* morphological state within multispecies biofilms in water systems

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Legionella pneumophila represents a particular concern and remains one of the most tracked agents in most anthropogenic water systems, being considered an opportunistic waterborne pathogen. *L. pneumophila* adopts a distinct biphasic life cycle, and it seems that the virulence factors may also be related to the mechanisms of biofilm formation and dispersion. Moreover, the microbial composition, physicochemical parameters, and types of disinfectants used may influence the *Legionella* virulence within the biofilm. Knowledge of the complexity of the ecology and physiology of *Legionella* in cooling tower biofilms hence requires the determination of the expression of such genes, taking into account the location of the individual *L. pneumophila* cells within the biofilm.
Fluorescence *in situ* hybridization using Nucleic Acid Mimic probes (NAM-FISH) is a powerful and versatile technique for bacterial detection [1, 2]. Targeting this technology to mRNA provides information about gene expression in single cells either in isolation or in the biofilm context, recording both spatial and functional information.
In fact, rethinking current *Legionella* research and management paradigms are critical to providing additional insight into *Legionella* proliferation and control strategies. For that, our work will try to clarify the organization and functional development of biofilms and understand the roles that spatial and temporal heterogeneity plays in the *L. pneumophila* virulence status in water systems.

1. Cerqueira, L., et al., DNA mimics for the rapid identification of microorganisms by fluorescence in situ hybridization (FISH). Int J Mol Sci, 2008. 9(10): p. 1944-60.
2. Cerqueira, L., et al., Establishment of a New PNA-FISH Method for Aspergillus fumigatus Identification: First Insights for Future Use in Pulmonary Samples. Microorganisms, 2020. 8(12).

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Eradication of staphylococcal biofilms manifesting up-regulation of the *NorA* gene on newly designed photoactive material

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Staphylococcus aureus belongs to the ESKAPE group and forms strong biofilms on medical devices, which is hard to treat. This study analyzed the effectiveness of polyurethane with a hybrid film composed of clay mineral saponite (Sap) modified with poly(diallyldimethylammonium) (PDDA) and with functionalized photoactive compound phloxine B (PhB) [1]. The material was tested against 24-h biofilms of the standard strain *S. aureus* CCM3953 and methicillin-resistant *S. aureus* L12 (MRSA) before and after irradiation using a green laser (λ = 532 nm, 100 mW, duration of irradiation for 120 s).

S. aureus L12 was confirmed to be resistant to oxacillin, ciprofloxacin, and norfloxacin (MIC>256 μg/mL, MIC>32 μg/mL, *MIC*>256 μg/mL, *respectively, using E-tests)*. The strain CCM3953 was sensitive to all tested antibiotics. The ethidium bromide agar screening method [2] showed a higher efflux activity for *strain* L12 compared to the standard strain CCM3953. The results were confirmed in planktonic cells by quantitative PCR (the $2^{-\Delta\Delta CT}$ method) and demonstrated significantly higher gene expression of the *NorA* gene in strain L12 compared to CCM3853. The expression of the *NorB* and *NorC* genes was not examined. Preliminary test was performed only on standard strain CCM3953. The effectiveness was tested on polytetrafluoroethylene membranes covered with hybrid films prepared in different ratios of PhB/Sap (n_{PhB}/m_{Sap}: 0.5 mmol/g, 1.0 mmol/g, and 1.5 mmol/g), demonstrating 96.83%, 99.92%, and 100% inhibition of after photodynamic inactivation (PDI). The efficacy of polyurethane with modified surface containing PhB/Sap in ratio (n_{PhB}/m_{Sap}) 1.5 mmol/g showed more, than 99.70% inhibition of CFU/mL after PDI for CCM3953 and even against MRSA strain L12.

Results confirmed the potential of modified polyurethane in eradication of MRSA biofilm even that with up-regulated *NorA* gene.

[1] Pribus, M., Budzák, Š., Slušná, L. P., Baranyaiová, T. Š., Jankovič, Ľ., Mészáros, R., & Bujdák, J. (2022). Luminescence of Reichardt's dye in polyelectrolyte-modified saponite colloids. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *642*, 128663. https://doi.org/10.1016/j.colsurfa.2022.128663.

[2] Martins M, McCusker MP, Viveiros M, Couto I, Fanning S, Pagès JM, Amaral L. A Simple Method for Assessment of MDR Bacteria for Over-Expressed Efflux Pumps. Open Microbiol J. 2013 Mar 22;7:72-82. doi: 10.2174/1874285801307010072. PMID: 23589748; PMCID: PMC3624690.

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Prevalence and characterization of *Staphylococcus aureus* in medical students during their studies

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Staphylococcus aureus is a part of a healthy human microbiome and colonizes approximately 20-30 % of the population. In some cases, *S. aureus* can be the cause of infection, especially in immunocompromised persons. Healthcare workers and medical students can be colonized and spread *S. aureus* in the hospital environment including their patients.
Pre-clinical and clinical medical students were tested for *S. aureus* colonization. Pre-enriched nasal swabs in BPW and fingerprints on B-P medium were taken. Identification of *S. aureus* was made by PCR and confirmed *S. aureus* were tested for the presence of genes encoding enterotoxins and virulence factors. Antibiotic susceptibility tests were performed by the disk diffusion method. Typing of *S. aureus* strains was performed by *spa*-typing.
A total of (47/104) 45.2% and (14/43) 39.5% (for now, still in process) nasal carriers were found in pre-clinical and clinical students respectively. Contamination on the students' fingers was (11/104) 10.6%, and (4/43) 9.3%. The most frequent antibiotic resistance was to Clindamycin (5/147) and Erythromycin (17/147). There were also detected enterotoxin and virulence genes. Strains belonged mostly to *spa* types t10060, t085 t084.

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Analysis of *Streptococcus agalactiae* prophages

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Streptococcus agalactiae (Group B Streptococcus, GBS) is a leading cause of invasive bacterial infections in new-borns and is also responsible for diseases in older and immunocompromised adults. Prophages represent an important factor contributing to genome plasticity and adaptive evolution. The aim of this work was to characterize prophages of human GBS isolates. Genome sequences of 27 isolates revealed 53 prophages, which were divided into eight groups (A-H) according to a comparative analysis. PCR based prophage identification confirmed high lysogenic status among 123 GBS strains with the highest prevalence of group A (71%) and satellite group B (62%). Prophages of other groups were detected in a lesser extent. Functionality of prophages were tested by Mitomycin C induction with positive results for groups A, C, D, E and F. D2 prophages of isolates KMB-572 and KMB-669 showed the highest induction capacity. Since conventional microbiological methods were insufficient, we quantified and tested the abilities of the induced phages to infect GBS strains and form lysogens by ddPCR. During amplification of phages f-572-D2 and f-669-D2 we observed a decrease of free phage DNA in medium. However, we confirmed functionality of the phages by their integration into the genomes of two strains. After 24h cultivation phage f-572-mitC4 was able to incorporate into 7% of KMB-555 and 16% of KMB-865 cells. Phage f-669-mitC4 was present in 9% of both host strains. Since no lytic phage infecting *S. agalactiae* has been identified, the research focuses on study and isolation of tempered phages and their subsequent genetic modification.

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Microfluidic fabrication of Dhvar5-chitosan nanogels: A way to fight orthopedic device-related infections

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Orthopedic Device-Related Infections (ODRIs) are a major medical challenge, particularly due to the involvement of biofilm-encased and multidrug-resistant bacteria[1]. Current therapies, based on antibiotic administration, have proven to be inefficient[2]. Consequently, there is a need for antibiotic-free alternatives[3]. Antimicrobial peptides (AMPs) are a promising solution due to their broad-spectrum of activity, high efficacy at very low concentrations, and low propensity to induce resistance[4]. We aim to develop a new AMP-based chitosan nanogel coating to prevent ODRIs. Chitosan was functionalized with norbornenes (NorChit) through the reaction with carbic anhydride[5]. Then, the cysteine-modified AMP Dhvar5 was covalently conjugated to NorChit (NorChit-Dhvar5), through a thiol-norbornene photoclick chemistry, under UV-photoactivation[5]. Characterization was done by Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance spectroscopy (NMR) analyses, and a successfully functionalization of chitosan with norbornenes and posterior Dhvar5 immobilization was proved. For NorChit-Dhvar5 nanogels production, the NorChit-Dhvar5 solution (0.15% w/v) and Milli-Q water were injected separately into a microfluidic system with rates of 1 µL/min and 10 µL/min, respectively. The nanogels were characterized regarding size and shape using Nanoparticle Tracking Analysis. The nanogels antibacterial properties were assessed, against four relevant microorganisms, in phosphate buffer (PBS), for 6 hours. . The obtained NorChit-Dhvar5 nanogels, presented round-shaped, and ~100 nm. NorChit-Dhvar5 nanogels in a concentration of 10^{10} nanogels/mL were capable of reducing the initial inoculum of *Staphylococcus aureus* by 99%, *Escherichia coli* by 90%, *Pseudomonas aeruginosa* by 99% and *S. aureus* MRSA by 90%, having high potential to prevent antibiotic-resistant infection in the context of ODRIs.

[1] Muthukrishnan G., Masters E. A., Daiss J.L., Schwarz E.M. Mechanisms of Immune Evasion and Bone Tissue Colonization that make *Staphylococcus aureus* the Primary Pathogen in Osteomyelitis. Curr. Osteoporos. Rep. 2019, 17: 395–404. doi: 10.1007/s11914-019-00548-4.
[2] Siljander M.P., Siljander M.P., McQuivey K.S., Fahs A.M., Galasso L.A., Serdahely, K.J., Karadsheh M.S. Current Trends in Patient-Reported Outcome Measures in Total Joint Arthroplasty: A Study of 4 Major Orthopaedic Journals. J. Arthroplasty, 2018, 33:185-94. doi: 10.1016/j.arth.2018.06.034.
[3] Sinha R., Shukla P. Antimicrobial Peptides: Recent Insights on Biotechnological Interventions and Future Perspectives. Protein Pept. Lett., 2019, 26: 79–87. doi: 10.2174/0929866525666181026160852.
[4] Huan Y., Kong Q., Mou H., Yi H. Antimicrobial Peptides: Classification, Design, Application and Research Progress in Multiple Fields. Front. Microbiol., 2020, 11:582779. doi: 10.3389/fmicb.2020.582779.
[5] Alves P.M., Pereira R.F., Costa B., Tassi N., Teixeira C., Leiro V., Monteiro C., Gomes P., Costa F. and Martins M.C.L. Thiol−Norbornene Photoclick Chemistry for Grafting Antimicrobial Peptides onto Chitosan to Create Antibacterial Biomaterials. ACS Appl. Polym. Mater. 2022, 4: 5012−5026. doi:10.1021/acsapm.2c00563

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Microplastics and their influence on the development of antibiotic resistance

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Microplastics represent a new source of pollution in the environment. Their occurrence has been recorded in water, soil, seas, and oceans [1]. When researching microplastics, it was soon discovered that they can interact with environmental components, such as pollutants or microorganisms [2]. Antibiotic resistant bacteria can also be a part of the microplastics biofilm [3]. The effect of microplastics on resistant bacteria is still relatively unknown. In this work, we monitored the influence of selected microplastics on the development of mutations leading to ciprofloxacin resistance in model bacteria *Salmonella* Typhimurium using a modified Ames test [4,5]. Acrylonitrile butadiene styrene (ABS), polylactic acid (PLA) microplastics, both 0.09 and 0.125 mm in size, and glitter from polyethylene terephthalate (PET) were used. Microplastics were added to the bacterial culture in different dosages from 5 to 50 mg. From the results, it can be concluded that most of the microplastics used and their concentrations did not have a significant impact on the development of mutations leading to resistance to ciprofloxacin. Frequency of resistant mutants RI was at the same level or lower as the control with no added microplastics. We observed a slight increase in RI with addition of 10 mg of ABS (0.09 mm), 5, 10 and 50 mg of PLA (0.09 mm) and 5 mg of glitter, but the ratio of RI to the control did not exceed the value of 2.90. In the next work, the set of microplastics will be expanded to include other types of materials.

[1] Zhang, S.; Wang, J.; Liu, X.; Qu, F.; Wang, X.; Wang, X.; Li, Y.; Sun, Y. Microplastics in the environment: A review of analytical methods, distribution, and biological effects. TrAC Trends Anal. Chem. 2019, 111, 62–72, doi:10.1016/j.trac.2018.12.002.
[2] Zettler, E.R.; Mincer, T.J.; Amaral-Zettler, L.A. Life in the "Plastisphere": Microbial Communities on Plastic Marine Debris. Environ. Sci. Technol. 2013, 47, 7137–7146, doi:10.1021/es401288x.
[3] Liu, Y.; Liu, W.; Yang, X.; Wang, J.; Lin, H.; Yang, Y. Microplastics are a hotspot for antibiotic resistance genes: Progress and perspective. Sci. Total Environ. 2021, 773, 145643, doi:10.1016/j.scitotenv.2021.145643.
[4] Birošová, L.; Mikulášová, M.; Vaverková, Š. Phenolic Acids from Plant Foods Can Increase or Decrease the Mutation Frequency to Antibiotic Resistance. J. Agric. Food Chem. 2007, 55, 10183–10186, doi:10.1021/jf072009r.
[5] Birošová, L.; Mikulášová, M. The mechanism of ciprofloxacin resistance in dihydrogen peroxide-induced mutants of Salmonella enterica subsp. enterica serovar typhimurium consists mainly in mutations in gyrA gene and less in mutations affecting ciprofloxacin uptake. Folia Microbiol. (Praha). 2008, 53, 368–372, doi:10.1007/s12223-008-0057-3.

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Optimization of photodynamic inactivation on biofilms formed by *Candida albicans* and *Staphylococcus aureus*

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Mixed biofilms formed by *Candida albicans* and *Staphylococcus aureus* are causing great problems in the healthcare sector due to their resistance to available drugs. Therefore, research is currently focused on finding alternative methods for their eradication, such as photodynamic inactivation (PDI).
The main objective of our study was to optimize the PDI method in 48-h single- and dual-species biofilms of *C. albicans* and *S. aureus* to achieve the highest possible inhibition after PDI. Experiments were carried out in the presence of methylene blue (MB) with different concentrations (0.25; 0.5 and 1 mM) and different pre-incubation periods (2; 4 and 16 h). The biofilms were irradiated with a red laser (190 mW/cm^2, λ 660 nm) for 1 min. The resulting PDI effect was determined by using CFU/ml. The ratio of MB and leucomethylene blue (LMB) in the samples was also measured by UV-vis spectroscopy.
According to the results, the optimal conditions for PDI in dual biofilms were determined to be 16-h pre-incubation period in the presence of 0.25 mM MB. Overall, these results suggest a reduction in the survival of *S. aureus* biofilm cells by 2.08-log$_{10}$ and 1.6-log$_{10}$ in standard and resistant strains, respectively. In dual biofilms, the reduction was 1.91-log$_{10}$ and 0.6-log$_{10}$ of the standard and resistant strain, respectively. The UV-Vis analysis demonstrated an increase in LMB concentration with a longer incubation period, especially for biofilms formed by *C. albicans*, which agreed with the low efficiency of PDI determined to a biofilm cell inhibition by 0.4-log$_{10}$ and 0.1-log$_{10}$.
Therefore, LMB formation could thus contribute to the lower efficiency of PDI in yeast. The optimal conditions for PDI testing were estimated to be a 16-hour pre-incubation period and 0.25 mM MB.

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Role of *ERG6* gene in *Candida glabrata* antifungal resistance

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Candida glabrata is an opportunistic fungal pathogen associated with high mortality in immunocompromised patients. Clinical isolates of *C. glabrata* often exhibit high level of resistance to antifungal drugs - azoles and echinocandins [1]. In the presented work we analyzed the effect of *CgERG6* gene deletion on *C. glabrata* antifungal resistance. The *ERG6* gene is involved in ergosterol biosynthesis, an essential structural component of fungal plasma membrane [2]. Our results show that the *CgERG6* gene deletion leads to increased resistance of *C. glabrata* to azoles. qRT-PCR analysis revealed significantly higher expression of genes encoding drug efflux pumps (*CgCDR1, CgCDR2, CgSNQ2*) and the transcriptional regulator (*CgPDR1*) in the absence of *CgERG6* gene. The analysis of the *Cg*Cdr1 efflux pump activity using rhodamine-6G indicates higher rate of efflux in the *Cgerg6Δ* strain. Increased levels of mRNA of the genes encoding efflux pumps together with the results of rhodamine-6G efflux can explain the observed resistance of *Cgerg6Δ* strain to azoles. *Cgerg6Δ* strain also exhibits decreased susceptibility to polyenes. Sterol analysis showed decreased amount of ergosterol in *Cgerg6Δ* strain. Accumulation of sterol intermediates in the plasma membrane of the *Cgerg6Δ* strain can be responsible for the observed resistance to polyenes. On the contrary, we showed that deletion of *CgERG6* gene leads to susceptibility of *C. glabrata* to echinocandins. Decreased expression of *FKS1* and *FKS2* genes in the strain lacking the *CgERG6* gene might explain the observed susceptiblity to echinocandins. Taken together, our results underscore the importance of the *CgERG6* gene in *C. glabrata* antifungal resistance.

[1] Hassan Y, Chew SY, Than LTL. *Candida glabrata*: Pathogenicity and Resistance Mechanisms for Adaptation and Survival. J Fungi (Basel). 2021 Aug 17;7(8):667. doi: 10.3390/jof7080667.
[2] Jordá T, Puig S. Regulation of Ergosterol Biosynthesis in *Saccharomyces cerevisiae*. Genes (Basel). 2020 Jul 15;11(7):795. doi: 10.3390/genes11070795.

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In vitro activity of avocado oil extracts against planktonic cells and biofilm formation of *Arcobacter*-like species

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Arcobacter-like species are Gram-negative curved rods. Various species are regarded emerging food pathogens and can cause of human gastrointestinal disease. *Arcobacter*-like has been isolated from a range of sources including wastewater, meat, and seafood. Detection and identification of *Arcobacter*-like species by biochemical methods are problematic, and therefore molecular biological methods are used. These bacteria are able to form biofilm structures. Biofilm formation ability results in higher resistance to antimicrobial substances. Vegetable oils have been tested for antimicrobial effects. The effects of avocado oil have been described for e.g. *Pseudomonas aeruginosa*.
The aim of the study was to test *in vitro* effect of an avocado oil water extract on cell survival and biofilm formation. Minimum inhibitory concentrations (MIC) were determined and growth curves were measured using the RTS-1C bioreactor. The biofilm formation ability at different concentrations of extract was assessed by the modified Christensen method.
Significant inhibition of *Arcobacter*-like species was especially observed up to a extract concentration of 90%. The exception was *A. thereius* LMG 24488, for which the MIC value of 45 % was determined. *Arcobarter*-like strains grew mainly at concentrations below 50%. The highest biofilm formation was measured at concentrations of 0.00–0.35 %. *A. defluvii* LMG 25694 produced the most of biofilm in all tested concentrations of avocado oil extract. On the contrary, the lowest amount of biofilm was observed for *A. cryaerophilus* CCM 7050.
The results indicate that avocado oil extract has an antimicrobial effect that can affect the growth and survival of *Arcobacter*-like species.

[1] Hanan, M.; Musarat, A.; Syed, R.A.; Wafaa, H.B.H. Prospects of using Avocado oil for attenuating quorum sensing regulated virulence, bio-filming formation and its antibacterial and antioxidant activities. bioRxiv 2018. https://doi.org/10.1101/486928.

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The antimicrobial activity of Graphene Quantum Dots

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One of the most common complications related to implantation of a biomaterial is biomaterial-associated infection (BAI). BAI is predominantly caused by the commensal bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis*, which can become pathogenic in the presence of a biomaterial. These infections may lead to chronic inflammation and in severe cases, loss of implant function. Biomaterial surfaces coated with antimicrobials are a promising strategy for prevention of BAI, but the use of antibiotics is discouraged due to resistance development. Graphene quantum dots (GQD) have good antimicrobial properties with low cytotoxicity and might provide an alternative for antibiotics to protect against infection. GQD consist of a single layer of carbon atoms in a honeycomb-like structure with photoactivation properties. Upon photoactivation, GQD can produce reactive oxygen species (ROS) which can kill bacteria. The aim of our study was to test a novel GQD coating for its antimicrobial activity against *S. aureus*. The coating consisted of several alternating layers of GQD and polymer applied on glass slides. To test the antimicrobial activitiy, we used the Japanese Industrial Standard (JIS) assay where we photoactivated the GQD coating with 450nm blue light. Surprisingly, the GQD coating showed promising antimicrobial activity with, as well as without photoactivation. The properties of the polymer are likely responsible for the antimicrobial activity without photoactivation. With photoactivation, the GQD further enhanced the antimicrobial activity, which resulted in complete killing of the bacteria. Taken together, these data show that the GQD coating has promising (dual) antimicrobial activity against *S. aureus*.

Biofilm mediated migration across the non-nutritive solid surface

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A novel bacterial migration and motility phenomenon related to biofilms growing at the air/liquid interface was observed and investigated. Due to the experimental setup, this motility differed substantially from the previously described swarming, twitching, gliding, sliding, or surfing. The aforementioned describes the motility of bacterial cells over nutritive surfaces immersed in or covered by liquid or solid nutrient media and described by standard motility assays. However, using a regular microscope glass slide, we showed how an array of bacteria could migrate vertically from a biofilm up the non-nutritive but slightly moist solid surface. The glass slide was partially immersed in nutrient media, causing the growth of substantial biofilm at the air/liquid interface, but the surface across which the bacteria were moving was never in contact with nutrient media and exposed to air. Observations revealed that capillary forces initiated the cell migration, and when a biofilm was formed at the air/liquid interface, the EPS production facilitated the movement. The observed mobility seems to be the property of many bacterial species, regardless of their morphological differences. In addition, biofilm-mediated migration was observed both for pure bacterial cultures and mixed species, in which cooperative migration and grouping by species were observed. Finally, the distinguishable experimental setup used in this study could enable the motility described here to be classified as a previously undescribed cellular movement type.

[1] Ivankovic T, Haddad U, Kushmaro A, Dekic S, Cevid J, Percela M, Hrenovic J. Capillary bacterial migration on non-nutritive solid surfaces. Archives of Industrial Hygiene and Toxicology 71 (2020) 251-260.

MRSA infection in elderly patients from nursing homes

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Frequent hospitalizations of geriatric patients can be a risk for seniors placed in nursing homes, due to the possibility of transmission of resistant strains of bacteria from the hospital settings to these facilities. The main objective of our study was to determine the prevalence of *S. aureus* and its methicillin-resistant form − MRSA in nursing home residents (n=242; 59-100 years) in comparison with controls: non-institutionalized volunteers (n=182; 18-86 years). Our results showed that *S. aureus* was present in the nasal cavity of residents in 123 cases out of 242 samples examined. In the control group we identified *S. aureus* in 54 cases out of 182 samples tested. Subsequently, six carriers of MRSA strains were detected in the control group, while 29 cases of MRSA were detected in the elderly group. We found a statistically significant difference in the prevalence of *S. aureus* and MRSA between study groups, using Pearson's chi-square test, with the odds of *S. aureus* being almost 2.5 times higher in elderly individuals compared to the control group (50.83 % vs 29.67 %; OR= 2.45; CI 95 %; 1.63 - 3, 67; $p<0.0001$). Similarly, the chances of incidence of MRSA in seniors housed in nursing homes were almost 4 times higher than in non-institutionalized volunteers (3.3 % vs 11.98 %; OR= 3.9937; CI 95 %; 1.6214 - 9.8372; $p=0.001295$). In conclusion, our study suggests that the prevalence of potentially high-risk MRSA strains could increase in the setting of elderly care facilities compared to the general population.

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Farnesol down-regulates the expression of genes involved in resistance to Fluconazole in *Candida auris*

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Candida auris is considered a serious fungal pathogen frequently exhibiting a high resistance to a wide range of antifungals. The overexpression of genes coding for the efflux pumps belonging to the ABC (*CDR1, CDR2*) and MFS (*MDR1*) superfamilies, together with the overexpression or point mutations of the *ERG11* gene, are the main mechanisms responsible for azole resistance in *Candida* spp. In this study, a combination of the quorum-sensing molecule farnesol (FAR) and fluconazole (FLU) was tested on FLU-resistant *C. auris* isolates (*C. auris* S and *C. auris* R) compared to the susceptible *C. auris* H261. qPCR was performed to investigate possible changes in the *CDR1*, *CDR2*, *MDR1*, and *ERG11* expression after FAR plus FLU treatment in all the *C. auris* isolates. The results showed that FAR modulates genes involved in azole resistance. When FAR was added to the cells in combination with FLU, a significant decrease in the expression of the *CDR1* gene was observed in the resistant *C. auris* isolates. FAR seems to block the Cdr1 efflux pump triggering a restoration of the intracellular content of FLU. These results were supported by observed increasing accumulation of rhodamine 6G by *C. auris* cells. Moreover, *C. auris* treated with FAR showed an *ERG11* gene down-regulation. Overall, these results suggest that FAR is an effective modulator of the Cdr1 efflux pump in *C. auris* and, in combination with FLU, enhances the activity of this azole, which might be a promising strategy to control infections caused by azole-resistant *C. auris.*

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Testing of antimicrobial effect of hydrogel matrix based on Gum Karaya resin supplemented by the phage preparation on methicillin-resistant *Staphylococcus aureus* strains on animal model

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Introduction

Gum karaya (GK) is a natural polysaccharide with great potential in the treatment of chronic skin and soft tissue infections (SSTIs). The polysaccharide-based hydrogels keep a moist environment and stimulate faster moist wound healing. Supplementing this material with phage preparation can increase its antimicrobial effects.

Methodology

Four pigs (20 wounds, 5x5cm) were used in the experiment. The wounds were infected with *Staphylococcus aureus* (ST22). The first sampling verified a successful infection. The other 4 samplings monitored the treatment. A piece of tissue was taken as well as an impression of the wound surface. The tissue was processed and 10 µl of the suspension was added to the BA and MH with oxacillin. The impression was transferred from BA to MH. The plates were cultured at 37°C for 48 h. Then the colonies were counted, and numbers were converted to tissue weight (CFU/g). The impressions were scored quantitatively using a 1-4 scale.

Results

Testing showed a positive effect of the combination of GK with phage in both tissue and surface wounds. During the experiment, there was a gradual reduction of bacterial numbers in the tissues and in the surface impressions. The wound was gradually shrinking by the formation of healthy tissue.

Conclusion

GK-based hydrogels have been tested *in vitro,* where they showed an antimicrobial effect. These experiments in an animal model show that the effect is also demonstrated directly on the infected wound. This material could be a promising candidate for the treatment of chronic wounds.

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Occurrence of resistance coliform bacteria, enterococci and their resistant genes in surface water and sediment originating from Slovakia

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In recent years, resistance to antibiotics has been overshadowed by the SARS-COVID-19 pandemic. It is important to say, that the problem of antimicrobial resistance has not gone away. Moreover, it is more than likely the outbreak of this pandemic could have contributed to the spread of resistance, for example through the increased prescription of antibiotics. This may have led to an increase of resistant determinants in the environment or food chain. Antibiotic resistance is not only concerning the hospital environment, but it is closely related to any anthropogenic activity. In recent study, samples of surface water and its sediments were investigated, originating from south of central Slovakia. Total coliform bacteria counts were higher in sediments compared to corresponding water samples. Resistance coliform bacteria were predominantly found in sediment samples in compared to corresponding surface water samples. Resistant coliform isolates from surface waters and sediments were mainly identified as *E. coli*, *Citrobacter* spp., and *Enterobacter* spp. More than half of the resistant isolates showed multidrug resistance and almost half showed overproduction of efflux pumps. The most frequently occurring resistant genes was bla_{TEM}. The genes bla_{SHV} and bla_{OXA}, bla_{NDM} and *tetA* were also observed. Enterococci were mainly detected in sediment samples, and resistance to ampicillin and vancomycin was mostly recorded. Resistant isolates were identified as *E. faecalis*, *E. durans* and *E. faecium* and showed resistance to ampicillin and most of them resistance to vancomycin, however resistance gene *vanA* was present in only 4 strains. More than half showed overproduction of efflux pumps.

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Setting treatment by using hepatitis C virus genotyping for a narrow group of prison inmates

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Hepatitis C is an infectious, blood-borne disease caused by hepatitis C virus (HCV) which belongs to RNA viruses of the *Flaviviridae* family. Eight major genotypes and eighty-six subtypes have been identified worldwide.

Specific HCV genotypes/subtypes affect the clinical course of infection and also differ in the response to antiviral treatment. Lately, combinations of direct-acting antivirals (DAAs) are used as compared to the previously utilized interferon (IFN) therapy. Since certain genotypes may be resistant to therapy with a particular combination of DAAs, HCV genotyping plays an important role in determining the treatment. A lower response rate to the 3D regimen or elbasvir/grazoprevir therapy was proven for genotype 1a. In addition, a failure of Vosevi pangenotypic combination was shown in individuals infected with genotypes 1a and 3.

We tested sera of a narrow group of prisoners, sent from Ilava prison in the Slovak Republic. The RNA was isolated with PureLink™ RNA Mini Kit. Subsequently, VERSANT® HCV Genotype 2.0 Assay (LiPA) was used to determine specific genotypes. This assay is based on reverse hybridization, in which biotinylated DNA PCR products are hybridized to immobilized oligonucleotide probes that are specific for the 5′UTRs and core regions of the HCV genotypes. We detected 3 different genotypes/subtypes within this group of prisoners. Knowing genotypes helped doctors to set up the right treatment. Efficacy of the treatment was shown by testing sera from treated prisoners. In conclusion, HCV genotyping is necessary not only for the treatment, but also for controlling the spread of the virus.

[1] Manns, M. P., Buti, M., Gane, E., Pawlotsky, J. M., Razavi, H., Terrault, N., & Younossi, Z. (2017). Hepatitis C virus infection. Nature Reviews Disease Primers, 3(1). https://doi.org/10.1038/nrdp.2017.6

[2] Hedskog, C., Parhy, B., Chang, S., Zeuzem, S., Moreno, C., Shafran, S. D., Borgia, S. M., Asselah, T., Alric, L., Abergel, A., Chen, J. J., Collier, J., Kapoor, D., Hyland, R. H., Simmonds, P., Mo, H., & Svarovskaia, E. S. (2019). Identification of 19 Novel Hepatitis C Virus Subtypes—Further Expanding HCV Classification. *Open Forum Infectious Diseases*, *6*(3). https://doi.org/10.1093/ofid/ofz076

[3] Skladaný, L., Oltman, M., Fraňková, S., Dražilová, S., Husa, P., ŠPerl, J., Hejda, V., Urbánek, P., Adamcová-Selčanová, S., Janičko, M., Kristian, P., Kupčová, V., Rác, M., Schréter, I., Virág, L., Liptáková, A., Ondrášová, M., & Jarčuška, P. (2020). Chronic hepatitis C virus infection in the Czech Republic and Slovakia: an analysis of patient and virus characteristics. International Journal of Public Health, 65(9), 1723–1735. https://doi.org/10.1007/s00038-020-01496-y

[4] https://ictv.global/sg_wiki/flaviviridae/hepacivirus/table1

P. aeruginosa grown in printable cystic fibrosis model biofilms suitable for high-throughput screening of antibiotics interactions

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Bacteria within a biofilm are up to 1,000-fold more tolerant to antibiotics and are inherently insensitive to the host's immune response. This is particularly relevant in cystic fibrosis (CF). Mucus accumulates in the lungs of CF patients because of impaired respiration. Bacteria build up in this environment, with *Pseudomonas aeruginosa* dominating. Once *P. aeruginosa* colonizes the lungs, it acquires a mucoid phenotype, rendering the infection resistant to antibiotics. This makes chronic lung infection in CF an impossible disease to treat, which results in high mortality. With no clear explanation for the extreme resistance of CF-Biofilm to antibiotics, it has been suggested that interactions of antibiotics with alginate and other extracellular polysaccharides (EPS) in the biofilm are essential for *P. aeruginosa's* tolerance to antibiotics. We develop simplified 3D biofilm models with features relevant to mimicking lung conditions in CF patients. These models allow us to investigate the specific interactions and structural changes of the biofilm matrix that offer protective mechanisms for *P. aeruginosa* when exposed to antibiotics. We hypothesize that we can improve existing therapies for eradicating microorganisms in mucoid biofilms by understanding and controlling how antibiotics interact with the EPS. To achieve this, commercially obtained bacterial-like alginate, eDNA, and *P. aeruginosa* cells are mixed with extracted EPS (Pel and Psl) from *P. aeruginosa* mutants to create bioinks. The bioinks are printed as gel beads for high-throughput screening. Live-dead fluorescence and (cryo) scanning electron microscopy are used to determine the growth of bacteria and structural changes in the EPS.

[1] Hollmann B. Perkins M. and Walsh D. (2021). Biofilms and their role in pathogenesis. CBS, University of Nottingham, Immunology.org
[2] Jones, C.J., and Wozniak, D. I. (2017). Psl Produced by Mucoid *Pseudomonas aeruginosa* Contributes to the Establishment of Biofilms and Immune Evasion, MBio 8(3)
[3] Colvin, K. M. Gordon, V. D. Murakami, K. Borlee, B. R. Wozniak, D. J. Wong, G. C. Parsek, M. R. (2011).The pel polysaccharide can serve a structural and protective role in the biofilm matrix of *Pseudomonas aeruginosa*, Plos Pathog 7(1)

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Preparation of antimicrobial polyurethane films.

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Hybrid nanomaterials based on layered silicates modified with organic molecules and functionalized with organic dyes have been extensively studied due to their interesting properties, such as fluorescence, light-harvesting, photosensitization, antibacterial properties, etc. [1, 2] This work is aimed at the interaction of phloxine B (PhB) with layered silicate saponite (Sap). Before, the surface of Sap particles had to be modified with poly(diallyldimethylammonium) (PDDA) cations. The modification of Sap was necessary due to the negative charge of the Sap surface and photosensitizer molecules. The cation-exchange capacity of Sap is 0.87 ± 0.05 mmol·g^{-1} [3], and the loading of PDDA was 1.5 mmol·g^{-1}, to change the Sap surface charge to positive. The adsorption of PhB on the PDDA/Sap complex was achieved from the PhB solution. The prepared complex was stirred at room temperature, filtered through a Teflon filter, and washed with distilled water to remove unadsorbed PhB by vacuum filtration. Liquid transparent precursors of the two-component polyurethane resin were mixed and the mixture was applied on the surface of PhB/PDDAC/Sap thin film. Finally, the prepared film was dried at ambient temperature in the flow of air and let to harden for 24 h. The material prepared in this way was characterized and used for anti-microbiological tests.

[1] Bujdák, J.; Jurečeková, J.; Bujdáková, H.; Lang, K.; Šeršeň, F. Clay Mineral Particles As Efficient Carriers of Methylene Blue Used for Antimicrobial Treatment. Environmental Science & Technology 2009, 43, 6202. doi:10.1021/es900967g
[2] Bourakadi, K.E.; Merghoub, N.; Fardioui, M.; Mekhzoum, M.E.M.; Kadmiri, I.M.; Essassi, E.M.; Bouhfid, R. Chitosan/polyvinyl alcohol/thiabendazoluim-montmorillonite bio-nanocomposite films: Mechanical, morphological and antimicrobial properties. Composites Part B: Engineering 2019, 172, 103. doi:10.1016/j.compositesb.2019.05.042
[3] Utracki, L.A.; Sepehr, M.; Boccaleri, E. Synthetic, layered nanoparticles for polymeric nanocomposites (PNCs). Polym. Adv. Technol. 2007, 18, 1. https://doi.org/10.1002/pat.852

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Bioactive Glass S53P4 as a non-antibiotic antimicrobial strategy to treat Biomaterial Associated Infections

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Medical device (biomaterial)-associated infections (BAIs) present a difficult and perplexing problem. Staphylococci are the most common bacteria to cause these infections. BAI are generally challenging to treat because of antibiotic tolerance or resistance. The development of biofilms on the biomaterial surface adds to phenotypic tolerance to antimicrobial agents and may even lead to persistence and antimicrobial resistance (AMR), both of which increase the risk of treatment failure and recurring infections. In this context, Bioactive Glass (BAG) S53P4 has been demonstrated to have good antimicrobial properties and might provide an alternative for antibiotics to prevent or even help treat infections. BAG is a synthetic silica based material with excellent chemical and mechanical property able to bind to the host bone tissue. Moreover, the contact of bioactive glass with biological fluids results in the increase of osmotic pressure and pH due to the leaching of ions from the surface, thus making the surrounding environment hostile to microbial growth. BAG is manufactured in the form of granules, powder and cream. To assess the relative antimicrobial potency of these BAG forms, their antimicrobial activity was evaluated by performing bactericidal concentration assays against *Staphylococcus aureus*. BAG powder and cream exhibited high bactericidal activity compared to the granules. This study presents important considerations for future research and suggest that application of BAG S53P4 cream on implants may contribute to prevention of infection.

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Antimicrobial peptide-grafted PLGA-PEG nanoparticles to fight bacterial wound infections

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The topical application of antimicrobial peptides (AMPs) to treat chronic wound infections (CWI) is not yet effective due to the loss of activity *in vivo*. This work explores the immobilization of the AMP MSI-78(4-20) to poly(D,L-lactide-co-glycolide)-polyethylene glycol (PLGA-PEG) nanoparticles (NPs), a strategy that reduces AMP aggregation and promotes faster action compared to the conventional AMP encapsulation. MSI-78(4-20) is a derivative of MSI-78 that shows equivalent antimicrobial performance, with improved selectivity towards bacterial cells [1]. In this work, MSI-78(4-20) was grafted to PLGA-PEG NPs through a thiol-maleimide Michael addition. Different ratios of PLGA-PEG/PLGA-PEG-Maleimide (Mal) were tested, and the formulation containing 40% PLGA-PEG-Mal displayed the best colloidal properties and the highest AMP content, as evidenced by NPs zeta potential (+8.6±1.8mV) and AMP quantification (326µg/mL). AMP-NPs proved to be as effective as the free AMP with a minimal inhibitory concentration of 8-16µg/mL against *Pseudomonas aeruginosa* and 32-64µg/mL against *Staphylococcus aureus*. In addition, AMP grafting shortened the time to kill from 1-2h to 15min for *P. aeruginosa* and from 6-8h to 0.5-1h for *S. aureus*. When tested in Simulated Wound Fluid, AMP-grafted NPs showed enhanced antimicrobial activity against *S. aureus* while the opposite effect was observed against *P. aeruginosa*. Importantly, AMP-NPs at a concentration of 16 and 32µg/mL caused no cytotoxic effects on human foreskin fibroblasts with respect to their metabolic activity. To sum up, our findings support that AMP-PLGA-PEG NPs represent a promising approach to manage CWI.

1. Monteiro, C., et al., *A 17-mer Membrane-Active MSI-78 Derivative with Improved Selectivity toward Bacterial Cells.* Mol Pharm, 2015. **12**(8): p. 2904-11.

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Unravelling the mechanism of action of Antimicrobial Peptides conjugated to nanoparticles

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Antimicrobial peptides (AMP) are a promising alternative to conventional antibiotics and their conjugation to nanoparticles has been used to improve stability and protection against degradation. However, little is known about the mechanism of action of these conjugates.

Here we explore the interaction of AMP conjugated poly(lactide-co-glycolide acid)-polyethylene glycol nanoparticles (AMP-NPs) with giant unilamellar vesicles (GUVs) mimicking Gram-positive (GP) and Gram-negative (GN) bacterial membranes using confocal microscopy.

Fluorescently labelled NPs are produced by nanoprecipitation and functionalized with MSI-78(4-20)[1]. AMP-NPs with an average size of 140 nm and a surface charge of 12.2 ± 1.1 mV, maintain the activity of the free AMP with a minimal inhibitory concentration of 8-16μg/mL against *Pseudomonas aeruginosa*, and 32-64μg/mL against *Staphylococcus aureus*.

Fluorescently labelled GUVs are produced by electroformation of a mixture of 1,2- dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylglycerol (POPG) to mimic GP bacteria, while a mixture of DOPC, POPG, and lipopolysaccharide (LPS) is used to mimic GN bacteria.

Bare NPs do not interact with GUVs maintaining their original shape, while AMP and AMP-NPs exhibit signs of interaction. The AMP induces deformation and aggregation of GUVs before disrupting their membrane, while the AMP-NPs induce these effects only in GUVs mimicking GN bacteria. On GUVs mimicking GP bacteria, AMP-NPs disrupt the membrane completely without inducing deformation and aggregation. Moreover, AMP-NPs interact at lower concentrations and at a faster rate than the AMP.

AMP-NPs accumulate at GUVs membrane, most probably due to electrostatic interactions between the negatively charged GUVs and positively charged AMPs.

[1] Monteiro C, Pinheiro M, Fernandes M, Maia S, Seabra CL, Ferreira-da-Silva F, Reis S, Gomes P, Martins MC.
A 17-mer Membrane-Active MSI-78 Derivative with Improved Selectivity toward Bacterial Cells. Mol. Pharm 2015, 12 (8), 2904–2911.

Keywords: Resistance, Biofilm, Antimicrobial materials

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How to study biofilms of fastidious organisms?

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In recent decades *Bartonella quintana* seems to be an underdiagnosed cause of infective endocarditis. During the two world wars, it was commonly known to cause trench fever. The only known vector for the Gram-negative bacterium *B. quintana* is body lice (*Pediculus humanus corporis*), therefore it is often found in the homeless and people with poor hygiene. Most cases of *Bartonella* endocarditis remain blood-culture-negative, which makes this entity difficult to diagnose with conventional methods. Correct diagnosis of the causing microorganism is essential for an effective treatment. In case of *B. quintana* treatment should be performed with a combination of *doxycycline* and a second antimicrobial (*rifampin* or *gentamicin*). We perform Fluorescence in situ hybridization (FISH) in combination with 16S rRNA gene sequencing on a routine basis to identify pathogens in explanted heart valves. In the last 5 years, our laboratory was able to diagnose *B. quintana* caused endocarditis in 19 patients from different hospitals in Germany. In most cases we found extensive biofilms with differential ribosome content indicating activity of the microorganisms under empirical therapy for culture negative endocarditis.

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Efficiency of phage preparation STAFAL® to carrier and clinical strains of *Staphylococcus aureus*

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Staphylococcus aureus is an important causative agent of human infections or an agent that may asymptomatically colonize the body. In addition, there is an increasing number of strains resistant to antibiotics. Therefore, efforts to find new strategies for the treatment of infections or decolonization of carriers are growing. One of the most promising alternatives is phage therapy. However, the use of phages is limited by the low number of accessible phage preparations and by 'a fear of unknown'. In this study, we evaluated the efficiency of STAFAL® phage preparation (registered in the Czech and Slovak republic) on 111 *S. aureus* carrier strains and on 81 clinical isolates from the bloodstream (35) or skin and soft tissue infections (46). The susceptibility of strains to phage preparation was determined by spot assay. STAFAL® was effective in 142 strains in total (74 %), while 82 strains (74 %) were susceptible in the carrier strain group, 28 (80 %) in the hemoculture strain group, and 32 strains (70 %) in the skin and soft tissue infection group. Susceptibility to the preparation was significantly higher in methicillin resistant *S. aureus* (MRSA) strains (31 (94 %)) than in methicillin susceptible *S. aureus* (MSSA) strains (111 (70 %); $p < 0.01$) and also in strains resistant to erythromycin compared to strains susceptible to erythromycin ($p < 0.05$). The high susceptibility of *S. aureus* strains *in vitro* indicates a good potential of STAFAL® in the decolonization or treatment of infections caused especially *S. aureus* strains reistant to antibiotics.

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Photodynamic inactivation effectively eradicates *Candida albicans* and *Candida auris* biofilms despite its interference with efflux and stress response genes

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Employment of photodynamic inactivation (PDI) can effectively eradicate microorganisms, including biofilms of *Candida albicans* and *Candida auris*. Resistance does not affect PDI and its efficacy; however, some differences in susceptibility have been observed. PDI was tested against 24-h biofilms formed by 3 clinical isolates of *C. auris*, one standard strain, and one clinical isolate of *C. albicans* using methylene blue (0.25mM, 1mM) irradiated with a red laser (λ=660nm, 190 mW/cm^2). Changes in the expression of efflux genes (*CDR1, CDR2, MDR1*) and genes involved in oxidative stress (*CAP1, MRR1, SOD1-3, GPX2, GLR1*) were determined before/after PDI by qPCR. Reactive oxygen species were measured by chemiluminescence. The highest inhibitory effect was achieved in all strains after irradiation for 300s. The irradiated group showed growth inhibition of more than 90% and 70% in *C. auris* and *C. albicans*, respectively. Relative changes in the expression of efflux pump genes were observed in all isolates. The genes *CDR1* and *CDR2* were overexpressed in all *C. auris* isolates (up to 4.8-fold, 6.6-fold, respectively), while up-regulation of *MDR1* (up to 124.1-fold) occurred only in all isolates after PDI. Up-regulation of *CAP1* (up to 2.3-fold) and *SOD2* (up to 3.9-fold) occurred in *C. albicans*, while *SOD3*, *GPX2* and *GLR1* have been down-regulated compared to the control. The amount of detected H_2O_2 increased greatly after PDI. PDI can be used effectively, even for highly resistant biofilms formed by *C. auris* and *C. albicans*. PDI modulates the expression of all efflux genes, *CAP1* and *SOD2*, which implies how cells are trying to defend against the oxidative stress produced by PDI.

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Rationally designed antimicrobial peptides show antimicrobial activity against Gram-negative and Gram-positive bacteria in a 3D human skin equivalent model.

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With the emergence of multidrug-resistant strains there is an imminent threat towards the "post antibiotics era", as our arsenal of effective antibiotics dwindles. Wound site infections, particularly in skin, apart from having numerous challenges to treat due to biofilm formation and multidrug resistance, can have debilitating effects on patients' health. There is an urgent need for new antimicrobials.

Antimicrobial peptides (AMPs), have been hailed as potent alternative antimicrobials[1]. Using a machine learning approach, peptides with antimicrobial activity were identified[2]. After initial *in vitro* screening for antimicrobial activity, four top hit peptides were selected: AMP-038, AMP-045 and their retro-inverso variants (RI). *In vitro* skin models pose many similar properties as normal human skin, here this model was used to study skin infection and treatment. Skin equivalents were prepared using the immortal human keratinocyte cell line hTert/KER-CT. The equivalents were infected with inoculum suspensions of *Acinetobacter baumannii* RUH875 or *Staphylococcus aureus* JAR060131 for 90 minutes, followed by peptide treatment for four hours. At 30 µM concentrations of AMP-038 and AMP-045, neither planktonic nor adherent cells of *S. aureus* and *A. baumannii*, survived on treated skin equivalents. Moreover, at 10 µM concentrations of AMP-038, AMP-045 and their retro-inverso variants, the planktonic *S. aureus* cells show no survival. In conclusion, novel AMPs, identified using a new machine learning method, show broad-spectrum antimicrobial activity. Next, the cell migration rate after wounding and healing capacity of these peptides using an *in vitro* scratch assay will be evaluated. Additionally, using a tube formation assay the angiogenic properties will be tested on endothelial cell lines.

1. Baltzer SA, Brown MH. Antimicrobial peptides: promising alternatives to conventional antibiotics. J Mol Microbiol Biotechnol. 2011;20(4):228-35. doi: 10.1159/000331009. Epub 2011 Sep 2. PMID: 21894027.
2. Dutch Scientific Council (NWO) LIFT program (729.001.024).

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Antifungal compounds cause cell surface remodelling in *Neurospora crassa*

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The presence of antifungal compounds in the environment of fungal cells causes stress conditions. The immediate response of fungi utilizes the evolutionary conserved stress-adaptation mechanisms, which triggers the rearrangement of cell surface structures to minimize the impact of antifungals. In this work, we focused on the response of model filamentous fungus *Neurospora crassa* to the presence of antifungal azoles and its comparison to the reactions to echinocandins. Using real-time PCR, we analyzed the expression of genes encoding proteins involved in ergosterol/sphingolipid biosynthesis and phospholipid remodelling of the plasma membrane, and proteins remodelling glucans, mannanes and biosynthesizing chitin of the cell wall. The response to azoles causes noticeable increase in expression of genes encoding ergosterol biosynthesis enzymes, though we observed the slight increase in expression in all genes, the products of which act on the plasma membrane. Surprisingly, the azoles impacted the expression of genes encoding proteins involved in cell wall arrangement, even chitin synthases, which we verified also at the level of saccharide. Expectedly, the response to echinocandins was based on increased chitin synthesis. Yet, the fungal cells reacted more broadly as genes encoding ergosterol biosynthesis enzymes were affected (increase in expression). To conclude, there is a possibility that the cell surface structures undergo changes to maximize the protection of *N. crassa* from antifungals, partially independent from the mechanism of action of a compound.

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Combination of molecular analysis and imaging of the microbiome: visualization and spatial distribution of microorganisms in infectious disease

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Communities of microorganisms for example in the gastrointestinal tract are closely connected to stages of health and disease in humans and animal models. It has only lately become apparent that not only the composition of the microbial communities is relevant but also their spatial organisation. Key microbial species are directly associated with the host epithelial surfaces or even invasive within the host tissue. MG-FISH (Microbiome-guided (MG) FISH) synergistically combines FISH with Next Generation Sequencing (NGS), so that microorganisms (bacteria and fungi) can both be identified and localized. We amplify microbial DNA out of methacrylate-embedded samples with a very high sensitivity and without contamination. Therefore, we interpret FISH and sequencing results together, which allows for a very high technical and diagnostic accuracy. FISH gives the microbiome data a spatial dimension in the context of the intact sample: Quantity and distribution of the microorganisms involved; Localization at and in the tissue; Identification of key pathogens.

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Gut microbiota resistome of pediatric oncology patients with febrile neutropenia.

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Leukemic patients undergo therapy including also broad-spectrum antibiotics. In healthy gut, commensal bacteria prevent pathogens from its colonization. Rapidly increasing rate of multidrug resistance render prophylaxis and empiric therapy ineffective in the cancer setting [1]. Resistance can develop in neutropenic patients in two ways - by horizontal transmission or by selective pressure exerted by broad-spectrum antibiotics used in prophylaxis. Multidrug-resistant bacteria include representatives of the *Enterobacteriaceae* family, *Pseudomonas aeruginosa*, *Staphylococcus*, *Streptococcus*, or *Enterococcus* [2]. These species are able to form biofilms, which may increase the severity of infections in immunocompromised patients [3]. Biofilm-producing organisms are more resistant to antimicrobial agents than non-biofilm-producing ones [4]. The aim of the study was to analyze gut microbiome and identify antibiotic resistance encoding genes carried by gut bacteria. In our research, we analyzed samples from pediatric patients from the Department of Haematology and Oncology of NUDCH in Bratislava. Metagenomic approach followed by paired-end massive parallel sequencing on Illumina platform was used for the identification of gastrointestinal resistome and subsequent resistance to groups of antimicrobials in patients with febrile neutropenia (FN). We found out that the gut microbiome of patients with FN after hematopoietic stem cell transplantation (HSCT) was enriched in genes encoding resistance to several antimicrobial groups, such as aminoglycosides or macrolides, before transplantation. The most frequently occurring genes conferring resistance to these antibiotics (*aac(6')-li*, *ant(6)-la*, *msr(C)*) are mainly carried by the genera *Enterococcus* and *Streptococcus*, what correlates with the increased abundance of these bacteria in neutropenic patients.

[1] Gudiol C, Carratalà J. Antibiotic resistance in cancer patients. Expert Rev Anti Infect Ther. 2014 Aug;12(8):1003-16. doi: 10.1586/14787210.2014.920253. Epub 2014 May 16.
[2] Gustinetti G, Mikulska M. Bloodstream infections in neutropenic cancer patients: A practical update. Virulence. 2016 Apr 2;7(3):280-97. doi: 10.1080/21505594.2016.1156821.
[3] Chen M, Yu Q, Sun H. Novel strategies for the prevention and treatment of biofilm related infections. Int J Mol Sci. 2013 Sep 6;14(9):18488-501. doi: 10.3390/ijms140918488.
[4] Dunne WM Jr. Bacterial adhesion: seen any good biofilms lately? Clin Microbiol Rev. 2002 Apr;15(2):155-66. doi: 10.1128/CMR.15.2.155-166.2002.

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Whole genome analysis of *Listeria monocytogenes* strains isolated from food-processing environment

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Listeria monocytogenes is gram-positive, facultatively anaerobic, non-spore forming bacterium which causes listeriosis. It is one of the most dangerous food-borne zoonotic pathogens. Due to these characteristics, the frequency of its occurrence in food must be monitored. Increased attention should be paid to persistent strains that are able to continuously contaminate resulting food products. In our work, we have analysed 16 strains of *L. monocytogenes*. Average coverage of sequenced genomes was 51 with average contig number of 35 and length 2,9 Mbp. We divided these strains into six unique groups using cgMLST analysis based on 1748 genes. Each *L. monocytogenes* strain contained one to four prophages. The most frequent site of prophage integration into the bacterial genome were tRNA genes, followed by genes encoding a competence transcription factor and an esterase. The prophages integrated into the esterase genes are incomplete prophages with a genome size of 12 Kbp and 19 protein encoding genes. They have no homology to any published prophages in the world databases. The tRNA integrated prophages are complete prophages, they possess the genome size on average 40 Kbp with 60 protein-coding genes. The homology to several database prophages was observed for these phages. Results obtained in our study could be used for a construction of phage-based preparations for biocontrol of pathogen growth in food products and food processing environments.

[1] Véghová, A.; Minarovičová, J.; Koreňová, J.; Drahovská, H.; Kaclíková, E. Prevalence and tracing of persistent *Listeria monocytogenes* strains in meat processing facility production chain. Journal of Food Safety. 2017, 37, 12315. https://doi.org/10.1111/jfs.12315

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Characterization of *E. coli* bacteriophages suitable for phage therapy

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Urinary tract infections (UTIs) belong to the most common bacterial infections, affecting 150 million people each year worldwide. The uropathogenic *Escherichia coli* (UPEC) strains are very common causative agents of this condidion, they are responsible for 80% of all cases. Infections are commonly treated with antibiotics, but the problem is the increasing incidence of resistant bacteria. The phage therapy is one of the alternative treatment options. To increase the host spectrum and reduce the likelihood of developing phage-resistant strains of bacteria, cocktails composed of multiple phages are most often used in therapy. The aim of the presented work was the isolation and characterization of bacteriophages infecting UPEC strains.

We isolated bacteriophages from waste water. We established the host phage specificity in a panel of 80 clinical strains of *E. coli*. We established a one-step growth curve and the adsorption of phages to host strains. We characterized both phage and bacterial genomes using next-gen sequencing.

We isolated 17 bacteriophages, which belonged to *Myoviridae, Siphoviridae, Drexlerviridae, Autographiviridae* and *Podoviridae* families. We found that up to 81% tested strains were susceptible to infection with at least one phage, phages infected mainly the B2 and D phylogroup strains. A phage cocktail composed of six phages was prepared and it confirmed good efficacy against four of the five tested strains in both LB and artificial urine medium. We found that phages adsorbed well to host strains of *E. coli*. The acquired results will be used

in the preparation of cocktails for UTIs phage therapy.

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Monitoring the detoxification response of filamentous fungi in the presence of antifungal compounds

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Aspergillus fumigatus is the most common human fungal pathogen, causing severe aspergillosis. Due to the high incidence of resistant strains the treatment of aspergillosis is often complicated. Invasive aspergillosis is the most lethal fungal infection, affecting over 300,000 people a year, with a mortality rate ranging from 30–80 % (Gonzalez-Jimenez, 2020). Azole drugs, amphotericin B as well as echinocandins are used for fungal therapy in clinical practice. The CDC reports that azole resistance was as high as 19% in some parts of the world in 2019. The frequent causes of azole resistance are the mutations in *cyp51A* often combined with tandem repeats in promotor resulting in increased production of ergosterol biosynthetic pathway enzymes. These mechanisms may be accompanied by the increased activity of efflux pumps. In this work, we verified the susceptibility of the isolate *A. fumigatus* CCF 6367 with a confirmed mutation L98H in *cyp51A*. The isolate was sensitive to voriconazole, prochloraz caspofungin and terbinafine. It has shown reduced sensitivity or resistance to other azole drugs. Newly synthesized silver complexes, AgSD, AgGLY, AgINA, AgGLYASP and AgNAD had shown antifungal potential. The silver complex AgNAD had shown the highest antifungal activity despite the lowest silver content among silver complexes. The increased expression of *A. fumigatus* CCF 6367 detoxification phase I genes *cyp651*, *cyp652* and *cyp653* of after treatment with azole compounds was not observed. In the presence of azoles, the increased expression of the *cyp51A*, *cyp51B*, *erg25A*, *erg3* and *srbA* genes was observed and, conversely, decreased expression of *atrR* and *cdr1*was evaluated.

[1] GONZALEZ-JIMENEZ Irene et al. 2020. *A Cyp51B Mutation Contributes to Azole Resistance in Aspergillus fumigatus.* In Journal of fungi (Basel, Switzerland) [online] Vol. 6 (4): 315, doi:10.3390/jof6040315

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Potential biofilm-forming bacteria revealed in gut-specific microbiome of *Ixodes ricinus* nymphs.

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Ixodes ricinus harbours a diverse group of native microorganisms, including fungi and bacteria. Microbial biofilms, either bacterial or fungal, are essential for colonization and successful symbiotic relationships of microbes and their arthropod hosts [1]. The association and effects of tick-borne pathogens on ticks' gut biofilm formation are well studied, however, the mutualistic biofilm-forming microbes do not get much attention. Biofilm formation and its homeostasis in the tick gut may play an important role in tick nutrition and immunity [2].

The aim of our research was to identify the diversity and activity of potential biofilm-forming bacteria and fungi in the gut of *Ixodes ricinus* nymphs originating from a laboratory colony maintained at the Institute of Zoology, SAS.

Total RNA isolated from tick gut was used for the construction of sequencing libraries, with the coding RNA enrichment approach. The quality of obtained libraries was verified by chip electrophoresis. Prepared paired-end libraries were sequenced using the Illumina NextSeq550 platform, bioinformatically processed and finally taxonomically classified with Kraken2 [3].

Over 500 bacterial and over 30 fungal genera have been detected, among which, multiple are able to form biofilm. The most prevalent bacterial genera were *Bacillus* spp., *Spirosoma* spp., *Cutibacterium* spp. and *Pseudomonas* spp. Also, multiple biofilm-forming fungi including *Malassezia* spp., *Fusarium* spp., *Candida* spp. and *Kluyveromyces* spp., were detected. The potential of biofilm formation associated with physical and chemical protection from environmental stress including antimicrobial compounds [4] needs to be further elucidated and may be vital for establishment of successful symbiosis with the host [5].

[1] Estrada-Peña A, Cabezas-Cruz A, Obregón D. Resistance of Tick Gut Microbiome to Anti-Tick Vaccines, Pathogen Infection and Antimicrobial Peptides. Patogény. 2020 Apr 22;9(4):309. doi: 10.3390/pathogens9040309.

[2] Abraham NM, Liu L, Jutras BL, Yadav AK, Narasimhan S, Gopalakrishnan V, Ansari JM, Jefferson KK, Cava F, Jacobs-Wagner C, Fikrig E. Pathogen-mediated manipulati on of arthropod microbiota to promote infection. Proc Natl Acad Sci U S A. 2017 Jan 31;114(5):E781-E790. doi: 10.1073/pnas.1613422114. Epub 2017 Jan 17.

[3] Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol. 2014;15(3):R46. Published 2014 Mar 3. doi:10.1186/gb-2014-15-3-r46

[4] Bronnec V, Alexeyev OA. In vivo model of Propionibacterium (Cutibacterium) spp. biofilm in Drosophila melanogaster. Anaerobe. 2021;72:102450. doi:10.1016/j.anaerobe.2021.102450

[5] Kim JK, Kwon JY, Kim SK, Han SH, Won YJ, Lee JH, Kim CH, Fukatsu T, Lee BL. Purine biosynthesis, biofilm formation, and persistence of an insect-microbe gut symbiosis. Appl Environ Microbiol. 2014 Jul;80(14):4374-82. doi: 10.1128/AEM.00739-14. Epub 2014 May 9. PMID: 24814787; PMCID: PMC4068690.

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The antifungal and anti-aflatoxigenic effect of essential oils from *Juniperus communis*

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In recent years, the popularity of essential oils grew rapidly as a possible natural alternative to the synthetic food preservatives. The full antimicrobial and antifungal potential of essential oils is still under scientific investigation, but recent studies show promising results. These characteristics of essential oils can be used in the product protection in agriculture and food industry. It is known that a contamination of grains by filamentous fungi is one of the major safety problems in agricultural products, because of the production of mycotoxins. Contaminated feed represents a risk for animal and human health as well as an economic losses risk for farmers. Thus it is very important to search for new methods of antifungal control in grains during the storage. From the wide variety of essential oils we focused on fermented and nonfermented oil from *Juniperus communis*. An antifungal activity of the oil was tested on various fungal species, e.g. *Aspergillus sp., Fusarium sp., Alternaria alternata* and *Penicillium purpurogenum.* Aflatoxins belong to the most harmful mycotoxins produced by filamentous fungi. Therefore, we insisted on testing the oil´s inhibition effect on the production of aflatoxins by *Aspergillus parasiticus,* which is one of the most common contaminants and aflatoxin-producing species in grains.

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Occurrence of antibiotic resistant bacteria in samples of flours and powders

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In recent years, bacteria-contaminated flour has been registered as the cause of several alimentary illnesses which have highlighted the microbiological risk associated with its consumption [1]. Microbiological quality concern is also raising due to increased consumer demand for plant powders, while some of them can be consumed without prior thermal processing [2]. Based on the above stated facts, we have focused on occurrence of antibiotic resistant coliform bacteria and enterococci in flour and plant powder from Slovak retail.

Our results indicated presence of both total and antibiotic resistant coliform bacteria and enterococci in the flour and powder samples. Lower numbers of total as well as resistant bacteria were detected in flours compared to plant powders. Coliform bacteria isolates were predominately identified as *Klebsiella spp*. (38 %) and *Enterobacter spp*. (25 %). Ampicillin resistance appeared in 97 % of isolates followed by chloramphenicol resistance (22 %) and tetracycline resistance (17 %). The presence of the bla_{SHV} gene was confirmed in 13 % of isolates. The *tetA* and *tetE* genes were present in 25 % of isolates of coliform bacteria. The presence of enterococci was detected only in plant powders. Antibiotic resistant strains were identified as *Enterococcus casseliflavus* (70 %), *E. gallinarium* (20 %) and *E. faecium* (10 %). Despite the isolates showing resistance to vancomycin, the presence of the *vanA* gene was not detected. Majority of antibiotic resistant isolates belonged to the group of medium biofilm producers. None of these isolates showed efflux pumps overproduction.

[1] Harris, L. J.; S. Yada. Flour and cereal grain products: foodborne illness outbreaks and product recalls [Tables and references]. Flour & Cereal Grains – Outbreaks and Recalls. 2019. Available at: https://ucfoodsafety.ucdavis.edu/sites/g/files/dgvnsk7366/files/inline-files/271162.pdf
[2] Losio, M.-N.; Dalzini, E.; Pavoni, E.; Merigo, D.; Finazzi, G.; Daminelli, P. A survey study on safety and microbial quality of "gluten-free" products made in Italian pasta factories. Food Control 2017, 73, Part B. https://doi.org/10.1016/j.foodcont.2016.08.020

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Surveillance of enterovirus spread in Slovakia

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Enteroviruses are positive-sense single-stranded RNA viruses and are associated with several human diseases. They mainly infect new-borns, young children and people with immunodeficiency. Infections caused by enteroviruses can target various parts of human body including gastro intestinal tract, upper and lower respiratory tract, heart and nervous system. Genus Enterovirus consist of polioviruses, coxsackievirus, echovirus, rhinovirus, and enterovirus EV-71 and EV-D68. Samples used in this study were isolated from patients and from sewage waters from all over Slovakia. We implemented diagnostics on L20B and RD cell cultures, as well as molecular-biological methods such as RT-PCR, rRT-PCR and next generation sequencing (NGS). Out of 172 samples, 57% had positive signal for enteroviruses (59 samples from sewage water and 39 clinical samples), which were further analysed for poliovirus presence. Average age of patients diagnosed with enterovirus was 16 years, with prevalence of men of 17%. Furthermore we observed higher occurrence of positive samples in months June - October. We managed to detect four polioviruses, which were then diagnosed as PV3 serotype with Sabin genotype. These samples were isolated from sewage water, which could indicate growing number of poliovirus cases despite of global eradication program. Further we are working to implement modern diagnostics of enteroviruses EV-71 and EV-D68 and new diagnostic method for surveillance of coxsackievirus A16 and A6, which incidence is growing.

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Keywords: Resistance, Biofilm, Antimicrobial materials

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Characterization of lytic bacteriophages active against carbapenem-resistant *Klebsiella pneumoniae* strains

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Three phages were isolated from the wastewater treatment plants Petržalka (Bratislava) and Žilina between June and September 2021. Their host ranges were determined against 8 clinical carbapenem-resistant *Klebsiella pneumoniae* strains belonging to 6 sequence (STs) and 5 capsular K locus types. Lytic activity was restricted to *K. pneumoniae* strains as we did not observe any activity against representatives of *Enterobacteriaceae* such as *Escherichia, Enterobacter* and *Cronobacter* sp. Individual isolates were active against 2 to 6 indicator strains, their mixed lytic activity cover entire set of all 8 indicator strains. Phages were stable within a pH range 4 – 10 and there was no significant loss of active phage particles after 1 h of incubation at the temperatures from 4° to 50° C. All isolates exhibited a latent period of 40 to 50 min and the burst size from 5 – 25 phage particles per cell. The 43646 bp long genome of phage Pet-KP684 exhibited 95% similarity with Klebsiella phage vB_KpnP_NER40 (*Autographiviridae*). Phage Pet-KP940 possesses 168283 bp genome similar to 99 % to vB_KpnM_BovinicusUrsus phage (*Myoviridae*). Pet-KP931 phage had DNA 46803 bp long related with 98% similarity to Pseudomonas phage PSA28 (*Siphoviridae*). All three phages in cocktail fully inhibited growth of indicators bacteria within 2 to 10 hours, suggesting their potential as a tool for the treatment of carbapenem-resistant *K. pneumoniae* strains.

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Biofilm forming microorganisms of the gut of pediatric oncology patients.

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Biofilm-forming microorganisms are associated with human diseases and can cause serious infections that can lead to death. Bloodstream infections represent a very serious threat to children undergoing hematopoietic stem cell transplantation (HSCT). Bacteremia or fungemia are associated with high mortality of patients. Between gut bacteria and bacteria detected in bloodstream genetic similarity at bacterial strain level was determined [1]. It may indicate a potential bacterial translocation through a disrupted gut-blood barrier. The aim of our study was to investigate the composition of gut microbiome before HSCT, one week after HSCT and one month after HSCT of 11 pediatric oncology patients. Bacterial and fungal composition was determined by shot-gun metagenomic sequencing and bioinformatic analysis using Silva database. Several main biofilm-producers including *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., *Enterobacter* spp. were identified. The same bacteria (*Enterococcus faecium*, *Enterococcus faecalis*, *Enterobacter cloacae*) were identified also in nose and tonsils of these patients. *Enterococcus faecium* and *Enterococcus faecalis* are well-known multi-resistant species causing complications in patient's healing [2]. Additionally, investigation of the gut fungome revealed the presence mainly of *Saccharomyces cerevisiae*, *Malassezia restricta* or *Yarrowia lipolytica*, however their presence in gut microbiota did not correlate with the other body sites. Our study suggests presence of main bacterial, as well as fungal biofilm producers in gut microbiota of pediatric oncology patients. Further investigation of the biofilm-forming properties should be carried out. This is a first step towards the determination of the gut microbiota potential of biofilm involvement in posttransplantational complications of pediatric oncology patients.

[1] Tamburini, F. B., Andermann, T. M., Tkachenko, E., Senchyna, F., Banaei, N., & Bhatt, A. S.(2018). Precision Identification of Diverse Bloodstream Pathogens in the Gut Microbiome. Nature Medicine, 24(12), 1809. https://doi.org/10.1038/S41591-018-0202-8

[2] Benamu, E., & Deresinski, S. (2018). Vancomycin-resistant enterococcus infection in the hematopoietic stem cell transplant recipient: an overview of epidemiology, management, and prevention. F1000Research, 7. https://doi.org/10.12688/F1000RESEARCH.11831.1

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