

## Tackling Pandemics: Strategies for Prevention, Preparedness and Response



Abstract Book



## Oral Presentations

### **OP1. Epidemiological Surveillance of SARS-CoV-2 in a Cohort of Portuguese Schools**

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#### Background

Implementing viral surveillance in schools enables to detect transmitted variants and facilitates prompt public health measures. The EUCARE project has emerged to provide evidence to guide measures to be taken in the context of epidemics of SARS-CoV-2 variants, with a focus on school cohorts. This was an interventional study with a crossover design, which included bi-weekly screening using the Lolli method, i.e. combined saliva samples for each class, and the application of socio-demographic and psychological questionnaires. This study aims to characterize the SARS-CoV-2 epidemic in schools, the different variants in circulation, identify and characterize transmission clusters and analyze their effect on the school population.

#### Methods

We collected pooled saliva samples from 143 classes in 9 schools from Amadora and Cascais, involving 1,226 participants in two different time periods (from March to June 2023 and again from September 2023 to March 2024). Individual samples from classes that tested positive in the pool analysis were collected the day after. In both cases, saliva samples were analysed using qPCR (BD MAX ExK TNA-3 kit targeting Genes N1 and N2). Positive individual samples ( $Ct \leq 28$ ) underwent sequencing using Oxford Nanopore technology. The obtained genomic sequences were analysed with the Pangolin COVID-19 Lineage Assigner Tool for variant classification.

#### Results

The study conducted 2,171 tests, of which 1,516 were pooled tests. Among these pooled tests, 5.5% (83/2171) have positive results. Of the 655 individual tests, 8.8% (58/655) of participants tested positive, with 10 cases occurring in pairs in the same class, which can indicate potential transmission clusters. Among the 58 positive cases, 23 were successfully sequenced, revealing several recombinant variants of Omicron, including XBB.1 and BA.2 (specifically BA.2.75 and BA.2.10.1). Several of the variants detected in our school study were not detected by national surveillance efforts.

#### Conclusion

Pooled saliva screening using the Lolli method was effective in early detection of SARS-CoV 2 in schools. This study highlights the importance of rapid and simple screening methods for epidemiological monitoring, especially in high contact environments such as schools. These methods can also be applied to future outbreaks of other respiratory viruses.

## **OP2. Vector-Borne Diseases: the reason behind its increase**

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Mosquitoes (Culicidae), with over 3,726 species, significantly impact global health by transmitting vector-borne diseases (VBDs) like malaria and dengue fever, causing around 700,000 annual deaths. Their introduction into new regions, facilitated by global trade, human activities, climate change and urbanization, enables rapid spread to new areas where they may transmit new pathogens or participate in the transmission cycles of existing indigenous pathogens, contributing to the spread of VBDs. Monitoring invasive species is crucial for disease control, yet existing biodiversity databases have limitations, including data gaps and validation issues. Our study analyzed the global distribution of non-native mosquito species that serve as vectors for human diseases, tracking their first records in new regions and identifying their origins and introduction pathways through a comprehensive review of scientific literature. We found that 45 mosquito species, corresponding to 24.2% of all mosquito species known to transmit pathogens in the wild, have been introduced to non-native regions somewhere in the world. Of these, 28 (62.2%) were recorded as introduced for the first time after 1950. After 1900 the number of introductions showed a rapid increase, with nearly half of all first records occurring after 1950. We also found that in general the earlier the species has been recorded globally, the newer regions it has spread into. However, this does not yet apply to a large number of newly emerging species, with introduction records known only for one or two regions. Species native from Africa and Asia dominate intercontinental species exchange, while Europe and South America primarily receive introduced species. Post-1900 colonization trends show a shift in origin, with Asia, Australia, and the Americas contributing the most introduced species. By analyzing the temporal and geographic distribution patterns of these introduced species, our study provides valuable insights into the potential spread of disease and supports ongoing disease prevention and control efforts.

### **OP3. SARS-CoV-2 viral entry: the role of the N-terminal and internal fusion peptides in membrane fusion**

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SARS-CoV-2 is an enveloped virus that emerged in late 2019 and quickly spread worldwide, giving rise to a devastating pandemic. This virus enters host cells through a membrane fusion process, mediated by the spike glycoprotein (S-protein), which facilitates the fusion of the viral and host membranes. A crucial domain of the S-protein, known as the fusion peptide (FP), is responsible for inserting into and disturbing the host membrane, facilitating fusion. However, the precise identity of the FP within the S-protein remains unclear, with two possible regions being proposed: the N-terminal FP (nFP) and the internal FP (iFP). To provide insight onto this matter, we combined computational and experimental approaches to characterize and compare the effects of the two proposed FPs. Our results indicate that the iFP has a stronger affinity for the membrane and exhibits greater hydrophobicity compared to the more amphipathic nFP, which tends to remain at the membrane-water interface. Moreover, the iFP causes higher membrane perturbation than the nFP, inducing lipid mixing and vesicle content leakage. These findings suggest that the nFP may play a role in interacting with the membrane more superficially, possibly facilitating the deeper insertion of the iFP into the membrane, where the latter induces a large effect on the membrane properties and promotes membrane fusion. By highlighting the roles of the nFP and iFP in the entry of SARS-CoV-2, our study provides important insights into the virus's fusion mechanism.

#### **OP4. Rapid Therapeutic Development Using AI-Driven Protein Design: Targeting SARS-CoV2 as a Proof of Concept**

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COVID-19 highlighted that prevention and preparedness will be crucial to mitigate the impact of future pandemics. Predicting the next outbreak is nearly impossible, making rapid strategies for producing effective therapeutics essential within the first months of an emerging pandemic.

This work demonstrates the use of computational protein design tools to design de novo tailor-made proteins that bind viral targets, focusing on SARS-CoV2 as proof-of-concept. By leveraging AI-driven tools, such as RFdiffusion, ProteinMPNN, and AlphaFold 2, combined with physics-based approaches including analysis with the Rosetta software suit and molecular dynamics simulations, we designed thousands of miniproteins targeting the SARS-CoV2 Spike protein receptor-binding domain (RBD), attempting to block viral entry and halt the infection.

Initial rounds of design and computational metrics analysis led to a pool of 8 candidates that were produced in *Escherichia coli* with high yields. Biophysical characterization revealed that these proteins were stable, withstanding extreme thermal conditions and reversibly maintaining their secondary structure. Functionally, a subset of these proteins demonstrated binding to RBD and neutralization of SARS-CoV2 infection. A second generation of proteins was designed, focusing on optimizing the protein-target interface. Computational metrics for this second design cycle significantly improved, which translated into a predictably higher binding affinity. From 17 produced and characterized proteins, nearly all were capable of binding to RBD.

This research highlights computational protein design as a powerful tool for rapidly developing therapeutics in response to emerging viral threats. By designing de novo tailor made proteins, we illustrate a forward-thinking and adaptable strategy for viral preparedness, with the potential for rapid deployment as part of a pandemic response framework.

**OP5. A new broad-spectrum antiviral strategy against SARS-CoV-2**

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*The Abstract corresponding to this presentation cannot be disclosed.*

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### **OP6. Improving vaccine response in the elderly through modulation of the hormonal immune axis**

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From clinical assays to diagnosis and therapeutics, the understanding of women's biology has lagged behind men's. This chasm is particularly impactful in the elderly, where deficient knowledge on the role of sex in aging immune system is compounded by increased susceptibility to infections and decreased vaccine responsiveness. Identifying and understanding molecular mechanisms underpinning sexual dimorphic immune aging is vital to provide critical insights on how to manipulate such differences through the development of personalized and inclusive treatments, to better treat men and women throughout their life.

We propose to have identified a novel immunoregulatory hormone whose production is regulated in a sex- and age-dependent manner. Resorting to human samples from men and women healthy donors we found that the expression of the hormone receptor by immune cells is fine-tuned according to their cellular differentiation state, suggesting that interaction between the hormone and its receptor might underpin an immunoregulatory network encompassing cellular, sex and age stratifiers. Functionally, in vitro supplementation with physiological serum concentrations of the hormone decreases the expression of the exhaustion marker PD-1 and of the transcription factor FoxP3, while potentially increasing T cell migratory capability towards inflammation sites by upregulating the chemokine receptor CCR2.

Uncovering a novel immunomodulatory hormone, could provide critical insights on how to manipulate sex differences towards the development of tailored treatments to boost men's immune responses to infectious diseases and cancer and to improve vaccine responsiveness in the elderly.



## Poster Presentations

### **P1. Exploring *Leptospira* spp. in urban biofilms, soil, and water in Lisbon: an environmental study**

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Leptospirosis is a re-emerging zoonosis with global distribution whose etiological agents are pathogenic *Leptospira* species. It is estimated that there are over 1,000,000 reported human cases and more than 60,000 deaths annually worldwide. These bacteria can be transmitted through direct or indirect contact with the urine of infected reservoirs. Rodents, as the main natural reservoir hosts, are primarily responsible for its spread, but other animals can also contribute to its transmission. Leptospire can survive in the environment, including water, soil, and biofilms, which are complex ecosystems that support microbial communities. There are few studies on the environmental burden of leptospirosis in Lisbon despite ECDC report recording 43 cases.

This work aims to investigate the presence of *Leptospira* spp. in urban biofilms across the Lisbon metropolitan area by collecting the samples at following areas: Cascais; São Pedro and Queluz. Biofilms will be collected from abiotic surfaces, riverbanks, and waste accumulation sites. Alongside, paired soil and water samples within a 1-meter radius will be collected. Samples will be analysed by qPCR for the detection of pathogenic *Leptospira*, followed by its isolation and further molecular and phenotypic characterization.

This study will contribute to a better understanding of *Leptospira* diversity in the environment, helping to develop strategies for leptospirosis control and prevention.

Expected outcomes of this study include: 1) Identifying *Leptospira* spp. in Lisbon's urban biofilms, water and soil; 2) Genotypically and phenotypically characterizing the isolates; and 3) Comparing the species diversity with global studies. These results will enhance the understanding of leptospire biology and ecology, highlighting biofilms as potential alternative environmental reservoirs for leptospire.

**Funding:** Fundação para a Ciência e a Tecnologia (FCT), Portugal, through funds to GHTM (UID/04413/2020) and LA-REAL (LA/P/0117/2020)

## **P2. Designing de novo biopharmaceuticals to combat Zika virus infection with AI and physical methods**

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In the past decades, the world has struggled with recurrent viral outbreaks, with viruses from diverse families demonstrating pandemic and epidemic potential. One of those viruses is the Zika virus. The envelope protein has a pivotal role in the viral entry of this virus into host cells. It comprises three structural ectodomains (DI, DII, and DIII) and a transmembrane region. DIII is an immunoglobulin-like domain that contains receptor binding sites and is responsible for the initial binding of the virus to a host cell. Within the DIII structure, relevant epitopes are targeted by the immune system and may therefore be the focus for antiviral strategies, including the use of protein design to generate tailor-made biopharmaceuticals. Protein design has emerged as a powerful tool for customizing protein properties like stability and binding affinity. In this work, we are designing monobodies (Mobs), small proteins derived from the human fibronectin type-III domain (FN3) that target the DIII. State-of-the-art protein design tools, such as RFdiffusion and ProteinMPNN, were implemented to generate de novo Mob-like structures and increase their binding affinity to the target. Additionally, protein structure prediction tools AlphaFold2 and Rosetta were used to extract confidence metrics based on features such as the i\_pTM score, shape complementarity, the number of buried unsatisfied hydrogen bonds present at the interface and the estimated binding free energy. The structures of the designs passing the established thresholds for all selected metrics were then predicted using AlphaFold2 and those presenting a root-mean-square deviation (RMSD) equal to or below 1.5 Å from the final structures obtained with Rosetta were elected the most promising designs. As a final in silico control, molecular dynamics simulations were conducted to assess the stability of the most promising designs unbound and bound to DIII. A total of 11 designs passed the selection process based on the confidence metrics and the RMSD filter. Molecular dynamics simulations of the unbound Mobs showed promising results regarding the predicted stability and the ability of designs to maintain their conformational fold. The simulations of the complexes also presented promising results concerning the stability of the Mob-target interactions. This comprehensive approach seeks to redefine strategies in combating the Zika virus, holding the potential to enhance preparedness against emerging viral threats.

### **P3. The role of wood shavings in the spread of *Aspergillus section Fumigati*: From carpentries environments to zoonosis risks in poultry farms**

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**Introduction:** Wood dust is not solely an occupational hazard for carpenters, since this material is commonly used as bedding for poultry industry. Wood shavings are among the various contaminants affecting poultry production, and may be a reservoir of pathogens. Thus, wood-based litter might contribute to a potential risk of zoonosis. *Aspergillus section Fumigati*, is included in the fungal priority pathogens list published by the World Health Organization, and has already been detected in woodworking environments. This study aims to perform a contamination assessment to *Aspergillus section Fumigati* in both carpentries and poultries.

**Methods:** The sampling campaigns was conducted in 6 carpentries from the Lisbon Metropolitan Area and in 14 poultries pavilions from Madeira Island, Portugal. Settled dust samples from carpentries (n=17) and wood shavings used as animals bedding material were collected from poultry pavilions (n=47).

**Results:** Regarding carpentries, 17 *Aspergillus sp.* were isolated from all settled dust samples inoculated in DG18 and incubated at 37°C, belonging all of them (100%) to section *Fumigati*. In what concerns poultries, from the 57 *Aspergillus sp.* isolates recovered, 40 belong to section *Fumigati* (70.2%).

**Discussion/Conclusion:** Data presented in this study evidence wood shavings as a potential reservoir of *Aspergillus section Fumigati* contamination in carpentries and in poultry pavilions. Future studies should consider the potential of wood shavings in fungal dissemination between these two sectors enabling Health Services to prioritize interventions in these specific settings to protect workers, animals, and consumers.

#### **P4. PLANET4HEALTH - Four case studies to assist translation of science to policy to mitigate adverse effects of environmental degradation and climate change on planetary health**

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The PLANET4HEALTH project is investigating the primary environmental factors contributing to health issues by examining the complex relationships between environmental degradation, climate change, and the health of humans, animals, and ecosystems.

The central concept of the PLANET4HEALTH involves employing a comprehensive yet diverse range of methodological approaches, including One Health, data analysis, environmental and climate sciences, machine learning and artificial intelligence, social, political, and economic sciences, as well as co-creating interoperable technologies and tools with various end users.

These approaches are applied to four case studies developed to address either emerging environmental health threats or the drivers of environmental degradation that lead to a wide range of health problems:

1. Vector-borne diseases in the Iberian Peninsula;
2. Air pollution and its adverse health effects in South Africa;
3. Food contamination arising from water and soil contamination in Central Europe; 4. Mental wellbeing in the environmental and climate context.

PLANET4HEALTH aims to achieve:(a) a significant amount of new or newly reconstructed and systematized health datasets; (b) enhanced knowledge and understanding of environmental and climate factors influencing disease, disorders, and health problems (both human and animal); (c) novel data and knowledge platforms accessible to all; and (d) improved understanding of the pathways for efficient and prompt science-policy and science-society interaction.

**Funding:** European Commission grant 101136652. The five Horizon Europe projects, GO GREEN NEXT, MOSAIC, PLANET4HEALTH, SPRINGS, and TULIP form the Planetary Health Cluster.

## **P5. Providing a better knowledge and comprehension of climate and environmental drivers of sand fly-borne diseases - The CLIMOS Project**

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Sand fly-borne diseases, including leishmaniasis and phleboviruses, represent a major public health and veterinary concern. CLIMOS involves universities, research centers and ministries of health from 16 countries within and outside Europe and aims to characterize the climatic, environmental, demographic and epidemiological characteristics associated with the presence and abundance of sand flies and domestic animal infection rates at different geographic scales across Europe and neighboring countries. These data will feed into mathematical epidemiological-climate prediction models of realistic human-induced climate change scenarios to help develop an early warning system for infection and disease designed for public use seeking to better prepare for current and future impacts of climate and environmental change on human and animal health.

**Funding:** CLIMOS consortium is co-funded by the European Commission grant 101057690 and UKRI grants 10038150 and 10039289. The six Horizon Europe projects, BlueAdapt, CATALYSE, CLIMOS, HIGH Horizons, IDAlert and TRIGGER form the climate change and health cluster.

## **P6. Development of a platform to screen and experimentally validate computationally designed anti-viral proteins**

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In response to the COVID19 pandemic, great efforts were made to develop new treatments and vaccines in record time. However, this pandemic highlighted the difficulties in facing emerging viral spreads and evidenced the need to develop preparedness strategies.

Even though the conventional display-based approaches in biopharmaceuticals development present throughput advantages, cost and time associated to it is very high. Moreover, these approaches fail to assess the stability of hit binders, which is mandatory for the development of lead biopharmaceuticals. Computational design serves as a mean to generate and screen millions of candidates, narrowing to thousands of designs based on predicted binding affinity and physical stability. Still, the thousands of generated outputs requiring experimental validation impose the development of a screening platform to rapidly assess binding activity and design stability.

For this, it was established a recombinant production and evaluation platform, divided in two protocols according to their throughput and scale. The first protocol fully functions in multi-well plate format and warrants screening up to 96 designs per run, assessing stability through thermal denaturation (differential scanning fluorimetry, DSF) and aggregation propensity (turbidity and dynamic light scattering, DLS) analysis, and binding activity by surface plasmon resonance spectroscopy (SPR). While affording a higher throughput, this protocol merely allows for ranking stable binders. The second protocol allows a lower throughput (<20 designs per run). Yet, besides enabling the stability and binding activity based design ranking, it generates sufficient protein amounts for additional downstream analysis, including structural characterization by far-UV circular dichroism, DLS and analytical SEC. Additionally, the lead candidates can be further assessed in in vitro viral neutralization and cytotoxicity assays. This platform has already been implemented in the screening of >100 computational designs targeting different viral proteins.



## **P7. Thermal Regulation of Immune Response in Human Lymphoid Organs**

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Fever is a cardinal response to infection, and an evolutionarily conserved mechanism across all evolution. The increase in core body temperature of 1C–4C during fever is associated with improved organism survival and the resolution of many infections by reducing viral replication and increasing bacterial susceptibility to complement-mediated lysis. Nonetheless, these effects of fever on pathogen fitness are insufficient to explain the pleiotropic effects of fever in infection outcomes, as many pathogens are known to be unaffected or grow better at higher temperatures. Raising the poorly explored possibility that fever protective effects can be enacted through modulation of the immune response. We have started to explore how fever-like temperatures affect the responses of T and B cells in secondary lymphoid organs, where protective immune responses are orchestrated.

Cells were isolated from human tonsils and flow cytometry techniques were employed to immunophenotype tonsillar CD4+ T cell populations after temperature challenge at moderate fever temperature of 39°C for 16 hours. Exposing cells to experimental hyperthermia in vitro is a powerful tool, which allows the discrimination between the effects of inflammatory mediators observed in natural fever and the effects of fever on the immune system by itself.

Results show that 39°C fever temperatures do not alter CD4+ T cells' gross parameters, such as cell survival, activation and subset distribution within the follicles. Instead, temperature appears to exert selective effect in particular tonsillar CD4+ T cell functions, such as the modulation of the cytokine production profile, which is accompanied by an increase in mitochondrial mass.

Our results show that fever modulates the function of tonsillar CD4+ T cells in a highly selective manner. Moreover, modulation of the CD4+ T cell cytokine production is accompanied by an increase of mitochondrial mass, which suggests that metabolic pathways may be implicated in resetting the CD4+ T cell cytokine profile in response to fever. Our work unlocks several possibilities in the healthcare sector, where the immunostimulatory effects of fever can be explored as a non-toxic, readily available resource to treat and manage infections and other diseases.

## **P8. Occurrence of *norA* and other multidrug efflux pump determinants across the *Staphylococcus* genus**

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**Background:** Multidrug efflux pumps (MDR EPs) play a major role in the emergence of antimicrobial resistance (AMR), particularly multidrug resistance phenotypes. *NorA* is a main native MDR EP in *Staphylococcus aureus*. Its encoding gene is part of the *S. aureus* core genome, but less is known about the occurrence of *norA* and other MDR EPs across the *Staphylococcus* genus. This study aims to analyze the presence of *norA* across staphylococci and identify other putative MDR EP determinants in seven pathogenic staphylococcal species.

**Methods:** The *norA* nucleotide sequences from 61 *Staphylococcus* species were retrieved from public databases and aligned. Other MDR EP genes were searched in public databases for *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, *Staphylococcus hominis* and *Staphylococcus pseudintermedius*. Putative genes encoding homologues of *S. aureus* main MDR EPs were also BLAST searched in complete genomes for each species, while putative *S. coagulans* MDR EP genes were BLAST searched against *S. pseudintermedius* sequences.

**Results:** The *norA* phylogenetic tree follows the phylogenetic relations within the *Staphylococcus* genus highlighting the presence of *norA* in the early branching of the genus. Over 40 putative MDR transporter genes were identified for *S. epidermidis*, *S. haemolyticus* and *S. lugdunensis*; 38 for *S. hominis*, 32 for *S. pseudintermedius* and 21 for *S. coagulans*.

**Conclusions:** Our results suggests that *norA* is part of the staphylococcal core genome. The identification of main MDR EP determinants in other staphylococcal species of clinical relevance opens new avenues for the study of their impact on AMR.

**Funding:** Project BIOSAFE (LISBOA-01-0145-FEDER-030713, PTDC/CAL-EST/30713/2017) funded by FEDER and by Fundação para a Ciência e a Tecnologia (FCT, Portugal). Further support by FCT to GHTM (UID/04413/2020); LA-REAL (LA/P/0117/2020); DREBI project (2022.07931.PTDC); SSC (CEECINST/00042/2021/CP1773/CT0009) and PhD Grants 2021.05063.BD (CF), 2023.02437.BD (ML) and UI/BD/154472/2022 (MA).

## **P9. Leadership Skills for Public Health Extreme Events: The Case of Intensive Care and Emergency Service**

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Healthcare organizations, as complex adaptive systems (CAS) [1,2], often operate in complex, uncertain environments, which can hinder preparedness for unexpected events due to reliance on reductionist cause-and-effect models [1-3]. Recognizing the importance of “One Health” perspective, this study explores the leadership skills essential for managing extreme events like pandemics in Intensive Care Units and Emergency Services. This study aims to identify key leadership competencies necessary for pandemic response in healthcare, analyzing data from semi-structured interviews using Gioia's methodology [4]. Leadership was assessed across transformational [5], distributed [6], and humble [7] leadership dimensions. Five core dimensions of Leadership in Health Emergencies (HEL) emerged: (1) Balancing command and shared decision-making; (2) Achieving an ambidextrous balance; (3) Fostering interconnected actions; (4) Cultivating transcendent awareness; and (5) Leveraging relational capital. Effective HEL involves, paradoxically, managing dual, often antagonistic skills to ensure both stability and adaptability. HEL requires balancing reliability with flexibility, encouraging adaptive responses that unify teams and enable responsive pandemic preparedness. This study adds to the literature by identifying competencies crucial for effective leadership during health emergencies, fostering resilience and adaptive capabilities within healthcare settings.

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## **P10. High antimicrobial resistance and genetic diversity of *S. pseudintermedius* associated with skin and soft-tissue infections in companion animals in Lisbon, Portugal**

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**Background:** *Staphylococcus pseudintermedius* is the main agent of skin and soft-tissue infections (SSTIs) in companion animals, for which antimicrobial resistance (AMR) is a growing concern. We characterized a collection of *S. pseudintermedius* causing SSTIs, establishing the main clonal lineages and AMR traits.

**Methods:** The collection comprised 155 *S. pseudintermedius* collected from SSTIs in companion animals over five years at two laboratories in Lisbon. Susceptibility profiles were established by disk diffusion for 28 antimicrobials from 15 classes. All isolates were typed by PFGE-Smal typing and representative isolates were further typed by MLST.

**Results:** Forty-eight isolates (31.0%) were methicillin-resistant (*mecA+*, MRSP). Multidrug resistant (MDR) phenotypes were detected in 45.2% of isolates, corresponding mainly (>95%) to MRSP. Only 12.3% of isolates were susceptible to all antimicrobials tested. In total, we detected 43 AMR profiles. The 155 isolates were distributed within 129 PFGE clusters, grouped by MLST in 42 clonal lineages, 25 corresponding to new STs. ST71 was the most frequent lineage found, but other relevant lineages, including ST258, are described for the first time in Portugal.

**Conclusions:** This study reveals high rates of MDR profiles and MRSP associated with SSTIs in companion animals. The high clonal diversity of this collection, linked to a significant AMR burden, reinforces the need for continuous surveillance of this pathogen.

**Funding:** Project BIOSAFE funded by FEDER/FCT (Portugal), Grant LISBOA-01-0145-FEDER 030713, PTDC/CAL-EST/30713/2017. Further support by FCT to GHTM (UID/04413/2020), LA-REAL (LA/P/0117/2020), CM (UI/BD/151061/2021, doi:10.54499/UI/BD/151061/2021), SSC (CEECINST/00042/2021/CP1773/CT0009, doi:10.54499/CEECINST/00042/2021/CP1773/CT0009), ML (2023.02437.BD), MA (UI/BD/154472/2022) and CF (2021.05063.BD).

### **P11. Evaluation of SARS-CoV-2 infection in a sialic acid deficient cell model**

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**Background:** Sialylation, the addition of sialic acid to glycans, plays a critical role in numerous cellular functions and has been implicated in modulating viral infections, including coronaviruses. Five years after the onset of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) pandemic, the molecular mechanisms that influence differential patient response, remain poorly understood. This study aimed to evaluate the impact of sialylation as a viral entry mediating agent.

**Methods:** Using a cell line with disrupted sialic acid biosynthesis by GNE knockout, we developed an ACE2+ cell line, in which were conducted infection assays and analysis of its glycan expression by lectin staining and glycomics.

**Results:** The infection assays revealed that SARS-CoV-2 infection is increased by hyposialylation. Moreover, it also shows that SARS-CoV-2 infection can be altered by supplementation with ManNAc, an investigational drug and precursor of the sialic acid pathway. The glycomics' results confirmed the cellular hyposialylation and unveil an increase in oligomannose content in the hyposialylated cell lines.

**Conclusions:** These findings provide, for the first time, an ACE2+cell model for evaluating the impact of sialylation in infectivity by ACE-dependent viruses. It also enlightens the possible susceptibility of patients with conditions associated with sialic acid defects, including cancer and rare diseases, to SARS-CoV-2.

**P12. Identification of efflux inhibitors through a drug repurposing strategy in *Candida albicans***

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*The Abstract corresponding to this poster presentation cannot be disclosed.*

### **P13. Fractionation and identification of compounds from medicinal plant extracts from Guinea-Bissau with biological activity against *Schistosoma mansoni***

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Neglected Tropical Diseases (NTDs) are prevalent in tropical and subtropical regions. Schistosomiasis, also known as bilharzia, is caused by parasites of various species of trematodes from the *Schistosoma* genus. The World Health Organization (WHO) has focused efforts on controlling *Schistosoma mansoni* in countries with high prevalence. Since ancient times, people have turned to natural resources for the treatment of diseases, and traditional medicine remains relevant in the production of plant-based medicines. The objective of this study was to identify bioactive compounds in plant extracts from Guinea Bissau with potential activity against *Schistosoma mansoni*.

**Method:** Twenty-seven extracts were tested on five bacteria (*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*) to evaluate their antimicrobial activity, using both solid and liquid media, as well as thin-layer chromatography (TLC) techniques for compound separation.

**Results:** Extracts from *Z. leprieuri* and *P. peduncularis* showed promising antibacterial results, suggesting the presence of bioactive compounds with therapeutic potential. These findings highlight the importance of continuing research into the application of medicinal plants in the fight against parasitic and bacterial diseases, especially in tropical countries with limited resources.

**Discussion and Conclusion:** The results obtained in this study underline the significance of traditional medicine in the discovery of new bioactive compounds with potential therapeutic applications. The promising antibacterial activity observed in the tested plant extracts supports the potential use of these resources in combating infectious diseases such as schistosomiasis and other bacterial infections.

#### **P14. Genetic diversity of the capsid protein-coding region from HIV-1 circulating in Angola: implications for the primary resistance to the novel capsid inhibitor lenacapavir**

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**Background:** The HIV-1 pandemic is still claiming around 630 thousand lives worldwide. This is even more egregious in Sub-Saharan Africa, where the first cases emerged. Here, an increased genetic diversity is common, resulting in a bigger risk of resistance to antiretroviral (ARV) drugs. Upon the growing pressure of ARV drug resistance, the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (USFDA), Lenacapavir (LEN) approved a new compound in August of 2022. This is a potent long acting molecule with an innovative target, HIV-1's capsid.

**Methods:** 243 blood samples from naïve HIV-infected patients admitted to the General Hospital of Benguela, were collected from August 2016 to January 2017 and stored in Whatman™ Indicating FTA® Elute Micro Cards (Cytiva, USA). They were submitted to two protocols of nested PCR followed by Sanger sequencing to originate HIV-1 capsid-encoding sequences. Phylogenetic analysis was done using a Maximum Likelihood tree and a recombination analysis was employed when genetic divergence was evident. Finally, a resistance mutation assessment was conducted to evaluate the primary resistance to LEN.

**Results:** The viral sequences showed a great diversity of pure subtypes and recombinant forms. Subtype C was the most common pure subtype (31,25%) followed by G (6,25%), A1 (5%), F1 (3,75%), A2 (1,25%), A6 (1,25%) and H (1,25%). Regarding recombinant forms, URFs were the most common (37,5%) followed by CRFs 02\_AG (2,5%), 14\_BG (2,5%), 18\_cpx (2,5%), 45\_cpx (2,5%) and 124\_cpx (2,5%). Resistance-wise, only one mutation, T107A, was detected, conferring low-level resistance to LEN.

**Conclusions:** These results reinforce Angola's molecular epidemiology as a transition between the highly diverse central Africa and the subtype-C-dominated southern Africa. They also reveal the viability of using LEN for the treatment and prevention of HIV infections in this region, considering the low level of primary resistance found.



## **P15. Enhancing the Accessibility of Public Health Information for Migrant Communities during Pandemics: The Role of AI-Powered Language Translation Technologies**

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**Background:** During the COVID-19 pandemic, it was key that critical health information be accessible to all. However, stakeholders struggled to communicate this information to migrant communities facing language barriers. AI-powered translation technologies (e.g. Google Translate) became essential tools to provide equitable access to health information. While these technologies offer the potential for a more timely and inclusive health messaging, their uncritical use may lead to misinterpretations and information loss, putting migrants at increased risk of harm.

**Aims:** This study aimed to:

- Examine how AI translation technologies were used to provide equitable access to health information during COVID-19.
- Identify ways to improve pandemic-related health communication for migrant communities in Portugal.

**Methods:** The study analyzed the public health information available on the official websites of Portuguese health services during the COVID-19 pandemic to determine when and how health information was provided in languages other than Portuguese. Additionally, an online questionnaire designed with Qualtrics was distributed among migrants in Portugal to gather responses on when and how they used translation technologies to understand public COVID-19 guidance. Qualitative and quantitative analyses were conducted using ATLAS.ti, with responses coded around recurring themes.

**Results:** Findings show that public health information was mainly available only in Portuguese, with minimal availability of human-supervised translations. Instead, translations generated by automatic translation technologies without human oversight were used, often without disclaimers. Questionnaire data indicated that migrants heavily relied on translation technologies to access COVID-19 messaging and faced challenges such as mistrust in translation accuracy and lack of verification strategies.

**Conclusions:** The study stresses the urgent need for a more critical use of translation technologies in health messaging during pandemics, including dedicated leadership for translation efforts during prevention, preparedness and response; and training for healthcare professionals on mitigating the risks of information loss when using translation technologies.

**P16.**José Gonçalves<sup>1</sup><sup>1</sup>NOVA School of Science and Technology

The rising prevalence of environmental contaminants, notably microplastics, and the spread of antimicrobial-resistant (AMR) bacteria present critical challenges to public health and pandemic preparedness. Cold plasma (CP) technology, particularly dielectric barrier discharge cold plasma (DBD CP), has emerged as a promising tool to counter these threats by inactivating microbial pathogens and degrading pollutants. This study evaluates the efficacy of lab-scale DBD CP systems for wastewater treatment, targeting the inactivation of both gram-positive and gram-negative bacteria. Two prototype DBD CP generators were developed, each capable of treating 100 mL samples, achieving significant bacterial reduction after brief exposure. Based on these results, a larger prototype was designed and scaled to accommodate 2 L samples, with ongoing testing against high-priority ESKAPE pathogens and AMR genes (ARGs) to further assess its effectiveness. Findings highlight CP's potential as an innovative approach to control microbial loads in water systems, contributing to novel strategies aimed at mitigating AMR spread.

Future work will expand CP applications to address pathogens associated with microplastics in aquatic environments, acknowledging microplastics' role as carriers of pathogenic microorganisms and ARGs. Effective CP treatment could enable simultaneous reduction of pathogens in water and within the plastisphere. This approach supports the One Health framework, emphasizing the interconnection between ecosystem and human health as central to pandemic prevention. Furthermore, incorporating microplastic pollution into public health priorities promotes a holistic strategy to pandemic preparedness, underscoring the need for integrative methods to safeguard public health.

## **P17. Computational design and experimental validation of antiviral biologics targeting influenza A hemagglutinin**

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The emergence of viral pandemics highlights the need for preparedness strategies. Influenza A, the causative agent of flu, presents a high pandemic potential. This study focused on developing innovative biopharmaceuticals to combat influenza A by targeting hemagglutinin (HA), crucial for viral entry into host cells. The strategy was based on the computational design and experimental validation of Virus-Targeting Antibody-like scaffolds (ViTAls), capable of binding HA to block virus entry. Monobodies (Mobs), small and highly stable proteins derived from fibronectin type III domain that can be engineered to bind with high specificity and affinity to virtually any target protein, were used as ViTAls. Mobs were computationally designed using two strategies: the 'grafting' strategy, where a stable Mob was grafted with segments of loops from an antibody against HA; and the 'library' strategy, where a large pool of Mob and Mob-like motifs were selected and docked onto an epitope. In both strategies, the binding loops were subsequently re-designed to increase their binding affinity, and relevant computational metrics were used to filter the results. Selected monobodies were analyzed through molecular dynamics simulations, which confirmed their structural stability. All Mobs were successfully expressed, purified, and characterized. The designs exhibited different degrees of thermal stability and aggregation profiles. Far-UV circular dichroism (CD) was used to evaluate the secondary structure, revealing similar spectra across the Mobs, consistent with  $\beta$ -sheet folds. The Mobs functional characterization was estimated by binding activity assays using fluidics free surface plasmon resonance. Notably, five Mobs appeared to interact with HA, four of which are particularly stable, being promising therapeutics. This work contributes to developing and validating an innovative strategy that can be applied to tackle various viruses, including influenza A.

## **P18. Molecular characterization of clinical isolates of the *Mycobacterium abscessus* complex circulating in Lisbon**

Maria Almeida<sup>1</sup>, Miguel Viveiros<sup>1</sup>, Diana Machado<sup>1</sup>

<sup>1</sup>IHMT/NOVA

**Background:** The *Mycobacterium abscessus* complex is an emerging pathogen responsible for severe human disease. It includes three subspecies closely related: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *bolletii*. *M. abscessus* is a pathogen known for its broad resistance to antibiotics such as macrolides, aminoglycosides, beta-lactams, and tetracyclines. The resistance profile varies within subspecies. Although *M. abscessus* evolved as an environmental bacterium to survive hostile environments is now in transition to a pathogenic bacterium.

**Methods:** In this study, we characterize genotypically a panel of clinical isolates of *M. abscessus* complex, collected during 2007 and 2022 in the Lisbon metropolitan health area. We identified the isolates at the subspecies level using the commercial identification assay. Genotype NTM-DR and by *erm* (41) DNA sequencing. Further, we used Genotype NTM-DR to determine molecular resistance to macrolides and aminoglycosides. We analysed the genetic diversity using the M13 fingerprinting method.

**Results and discussion:** The results showed that *M. abscessus* subsp. *abscessus* is the most frequent subspecies in circulation, followed by *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *bolletii*. Genotypically, the results showed that the drug resistance differs among the three subspecies. Our population is genotypically diverse at the local level indicating a low epidemiological relationship. Due to the lack of therapeutic options, it is important to differentiate between the subspecies as they may respond differently to the therapy.

Considering our epidemiological context, the prevalence of *M. abscessus* complex in the environment requires a follow-up. Our knowledge regarding national epidemiology can increase by studying strains from other regions of Portugal.

**Conclusions:** Microorganisms can (re)emerge with multidrug resistance or spread rapidly to other regions, causing epidemics. We recommend a deeper study of the mechanisms of drug resistance to the current drugs used in the therapy of *M. abscessus* and the development and implementation of new and more efficient therapeutic strategies, to prevent the emergence of *M. abscessus* drug resistance and dissemination.

## **P19. mosquitoWEB – Contributing for mosquito-borne arbovirois preparedness**

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Several *Aedes* mosquito species are currently expanding their geographic range globally. Among them, *Aedes aegypti* and *Aedes albopictus* are of greater concern – these species are highly associated with humans, readily occupy new territories, and are efficient vectors of several viruses, namely dengue, Zika, and chikungunya. Outbreaks have occurred in several European countries where *Aedes albopictus* is established. On Madeira Island, a dengue outbreak took place seven years after the initial detection of *Aedes aegypti*.

mosquitoWEB ([www.mosquitoweb.ihmt.unl.pt](http://www.mosquitoweb.ihmt.unl.pt)) is a Citizen Science project aimed at the early detection of invasive mosquitoes in Portugal through reports from citizens on mosquito sightings. The mosquitoWEB team then identifies photographs or sent specimens, and feedback is provided to the citizens. New locations of invasive mosquito species are promptly reported to the Health Authority.

To date, submissions to mosquitoWEB have enabled the first detection of *Aedes albopictus* in the municipalities of Lisbon and Oeiras. The districts of Lisbon, Setúbal, and Faro have the highest number of submissions. In Faro, *Aedes albopictus* is the most frequently reported mosquito species. So far, no specimens of *Aedes aegypti* have been detected on mainland Portugal.

*Culex pipiens*, an autochthonous species, is, as expected in Portugal, the most frequently detected by citizens. This species is a vector of the West Nile virus in Europe, including mainland Portugal, and therefore should not be overlooked.

In this way, mosquitoWEB, besides being a highly cost-effective tool for the early detection of invasive species and the monitoring of native species, also contributes to the promotion of health literacy, particularly in the area of vector-borne diseases.

**P20. Analysis of potentially aflatoxigenic fungal contamination in peanuts and maize in Sub-Saharan Africa: Focus on Mozambique (2018 to 2024)**

Mariamo Jaime Machado Parruque<sup>1</sup>; E. Cambaza<sup>2</sup>; L. Rodrigues<sup>3</sup>; J. Silva<sup>4</sup>; T. Magaia<sup>1</sup>

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*The Abstract corresponding to this poster presentation cannot be disclosed.*

## **P21.**

Mariana Alberti Gonçalves

COVID-19 was declared a pandemic due to its global spread. Although everyone is susceptible to SARS-CoV-2, vulnerability is unequal, as protection from the virus and its social impacts depends on living conditions and access to rights, goods, and services. In this context, Popular Health Education is fundamental in facing the crisis, promoting individual and collective empowerment through dialogue and critical thinking, aiming to transform local realities. Simultaneously, Health Literacy plays a crucial role by strengthening individuals' knowledge, motivation, and skills to access, understand, and use health information, enabling informed decision-making. Together, these approaches help not only to prevent disease and promote health but also to reduce inequalities, improving the quality of life, particularly in vulnerable contexts. This study aimed to develop and evaluate educational activities focused on training community health surveillance agents to combat COVID-19. A module was created for the course "Health Education for Community-Based Surveillance of COVID-19 in vulnerable areas in Rio de Janeiro," part of an epidemiological surveillance project conducted in partnership between the Oswaldo Cruz Foundation (Fiocruz) and community leaders from Rio de Janeiro. Activities focused on epidemiological surveillance and quality management for community health surveillance were implemented. The methodology included participatory research, fieldwork, and qualitative data analysis. The target group consisted of 24 participants, including community leaders and health professionals, all residents of the communities studied. The activities included virtual and in-person classes, emphasizing critical reflection on prevention, health promotion, and the social determinants of health related to COVID-19. Five models of prevention leaflets and four posters on COVID-19 detection were produced. Questionnaires were also applied, discussion circles were held, videos were made available, and guidance on the use of digital graphic design tools was provided. Approximately 500 leaflets were distributed, and the posters were displayed during field activities. Among the participants, 95.8% considered the leaflets important for fieldwork, and 95.5% rated the posters as essential to the project. In conclusion, the activities fostered reflections on COVID-19 within the communities involved, contributing to community empowerment and the transformation of local realities.

## **P22. Galleria mellonella research hub: a new in vivo infection model at GHTM/IHMT-NOVA**

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**Background:** *Galleria mellonella* represents a sustainable invertebrate infection model that is gaining interest for pathogenicity and infection, host-pathogen interaction, and pharmaco-toxicological assays. Here we describe (i) the implementation of a *G. mellonella* colony at GHTM/IHMT-NOVA to provide standardized larvae for the research community and (ii) the optimization of virulence assays for relevant human pathogens.

**Methods:** *G. mellonella* was acquired from a commercial house at the last larval stage and the species was confirmed by COI sequencing. The four life cycle stages are reared and maintained in the dark, at 28°C, with a high-nutrition diet. Infection assays were optimized for reference strains of four major pathogens: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae* and *Escherichia coli*.

**Results:** A *G. mellonella* colony was established with optimized parameters such as diet and routine maintenance. In-house reared *G. mellonella* had a life cycle of 31-34 days (egg to adult). Each female laid on average > 1,500 eggs, with an egg-hatching rate of ~34%. Currently, the colony has high productivity (~20,000 larvae/generation). Compared to the commercially acquired larvae, in-house reared larvae are healthier and more resistant to *S. aureus* infection. Our optimized infection protocols (pre-incubation conditions, bacterial inoculum, infection procedures) enabled the ranking of the virulence potential of the four pathogens, as follows: *P. aeruginosa* >>*S. aureus* ~ *E. coli* >>*N. gonorrhoeae*.

**Conclusions:** This work represents an expansion of the animal infection models available at GHTM/IHMT-NOVA for collaborators and the research community. Ongoing work focuses on expanding this model to fungal and parasite pathogens, as well as additional functional assays, such as pharmaco-toxicological studies.

**Funding:** Fundação para a Ciência e a Tecnologia through funds to DREBI Project Ref. 2022.07931.PTDC (doi: 10.54499/2022.07931.PTDC); GHTM (UID/04413/2020); LA-REAL (LA/P/0117/2020); UI/BD/154472/2022 (MA); 2022.07931.PTDC (JN) and CEECINST/00042/2021/CP1773/CT0009 (SSC)



### **P23. Soluble ACE2 as a decoy for SARS-CoV-2**

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COVID-19 highlighted the need for effective therapies against SARS-CoV-2 and potential future coronaviruses. Soluble Angiotensin-Converting Enzyme 2 (sACE2) is a promising therapeutic agent to block viral entry by binding to the SARS-CoV-2 Spike protein receptor binding domain (RBD). We compared sACE2 produced in three expression systems: plant cells (*Nicotiana benthamiana* leaves, pACE2), animal cells (HEK293, hACE2), and bacteria (*Escherichia coli*, eACE2). Each system offers distinct advantages in terms of cost, scalability, and glycosylation patterns, which affect protein function and stability. Binding affinity to RBD was evaluated by surface plasmon resonance (SPR), and virus neutralization assays were performed to assess the ability of the different sACE2 variants to block viral entry. Despite lacking glycosylation, eACE2 showed similar neutralization efficiency and binding affinity, as compared to the other sACE2 variants. While the secondary structure, analyzed by far-UV circular dichroism, was similar for all sACE2 variants, eACE2 exhibited slightly lower thermal stability, assessed by differential scanning fluorimetry. Molecular dynamics simulation analysis demonstrated that eACE2 in solution is quite flexible, displaying a displacement of the N-terminal domain in relation with the C-terminal domain. Moreover, this behavior is maintained when eACE2 is bound to RBD and the eACE2/RBD interaction is quite stable despite the absence of glycans in this complex. Our findings highlight that eACE2 can be a viable, cost-effective option for therapeutic development, enabling large-scale production without compromising efficacy. This research offers valuable insights into optimizing protein-based therapies, reinforcing the potential of sACE2 as a treatment for COVID-19 and other viral infections, while paving the way for more accessible therapeutic solutions through alternative production strategies.

## **P24. Master in Field Epidemiology by blended-learning approach designed for students from West African Lusophone countries: Lessons Learned and Challenges**

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### **INTRODUCTION**

In 2020, the European and Developing Countries Clinical Trials Partnership (EDCTP) and Africa CDC announced funds for training of field epidemiologists in sub-Saharan Africa. A consortium including African and European Universities, National Institutes of Public Health (NIPH) and Research Centers (list of partners below) developed a project proposal to implement a Master in Field Epidemiology (MFE) offered through blended-learning from the University of Cabo Verde (UniCV) to serve students from three countries: Cabo Verde (CV), Guiné-Bissau (GB) and São Tomé & Príncipe (STP). This poster shares experiences, lessons learnt and challenges faced along the implementation MFE.

### **OVERVIEW OF THE MASTER PROGRAM**

The MFE program was inspired in an Advanced Field Epidemiology Training Program (FETP) model from the Center for Disease Control and Prevention (CDC/ United States).

The two-year MFE was accredited at the UniCV and the Higher Education Accreditation Authorities in CV (in 2021). Students were recruited through calls made with the support of NIPH and Ministries of Health of CV, GB and STP. A total of 15 students were enrolled (6 from CV, 6 from GB, and 3 from STP) from 55 applications received from all three countries. The students remained in their countries during the training. Similar to other advanced FETP, the theoretical teaching and field training were designed for students to attain critical competencies in the 1st Year of the MFE and included subjects such as: epidemiology, biostatistics, outbreak investigation, scientific communication, surveillance evaluation, teaching others, climate change, One Health, public health legal frameworks and public health leadership. Field training included placements in local health system sites with support of the NIPH and of locally selected supervisors. The course followed a blended learning methodology.

By the end of the 1st year all students had successfully completed the course work, qualifying as advanced trained field epidemiologists. While preparing for the more academic thesis expected to complete the MEF, students and teachers got together in a full-time one-week seminar to share achievements, difficulties and next steps to complete the MEF.

### **CONCLUSIONS**

The MFE was successfully established and is formally accredited in CV and all conditions and resources are in place to continue providing Advanced FETP training for students from CV, GB, and STP and also to students from other countries. This MFE contributes to enhance the workforce of Field Epidemiologists in Lusophone African countries, together with programs already running in Mozambique and Angola. It complements the frontline and the soon to be introduced intermediate FET coordinated by the NIPH in CV contributing to strengthen its central role as coordinator of the FET Program.

## **P25. A novel class of antibiotics against Multidrug-resistant *Klebsiella pneumoniae* – a key tool for the next pandemics**

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The increasing global trend in bacterial antimicrobial resistance may lead to the next pandemics, mainly because there is a shortage of new antimicrobials. Among clinically concerning pathogens for development of new antibiotics are multidrug-resistant *Klebsiella pneumoniae* (MDR-Kp), classified by WHO as a critical priority. To respond to this urgent need, we developed a candidate metallic compound (IR1), evaluated its antimicrobial activity against MDR *K. pneumoniae* and explored its mode of action.

The antimicrobial activity of IR1 was tested against representative MDR-Kp by microdilution assays, viable cells counting and time-kill assays. IR1 permeation across cell envelope was evaluated by checkerboard assays with polymyxin B (PMB) and RND efflux pump inhibitor (PA $\beta$ N) and by ICP-AE Spectroscopy. IR1 ability to induce membrane permeability was assessed by fluorescence microscopy (SYTO9/PI). To test for resistance development against IR1 and potentially explore its mode of action, MDR-Kp were exposed to increasing IR1 concentrations and resistant mutations identified by WGS. Additionally, IR1 cytotoxicity was assessed in a mammalian cell line using MTT assays.

IR1 exhibited high bactericidal activity and was able to completely eradicate MDR Kp within 2 hours at 8xMIC. Moreover, IR1 had the ability to prevent the formation of biofilms. The compound acted synergistically with PMB (FIC=0.375), but its activity was not affected by Pa $\beta$ N. Moreover, IR1 increased membranes permeability to SYTO9 and was detected across all cellular fractions. IR1-resistant mutants developed after 6 passages and had

4xMIC. Missense mutations were mainly in transport-associated proteins specialized for metabolites uptake and energy production, localized in the inner membrane. Moreover, at active concentration IR1 was non-cytotoxic against mammalian cells (cell viability >70%).

We showed that IR1 had a high bactericidal and biofilm prevention activity against MDR-Kp. Our results suggest that IR1 activity might be associated to cell envelope permeabilization and binding to inner membrane transporters. This new compound holds potential as a pivotal tool in combating infections by MDR-Kp, which are potential drivers of future pandemics.

## **P26. Fungi under the Microscope: Exploring Health Risks Associated with Contamination in Schools**

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**Background:** Young children are particularly vulnerable to illness due to their developing immune systems and close interactions in school settings. These crowded settings can harbor various microbial agents, including potentially pathogenic fungi. While research has predominantly concentrated on bacterial and viral pathogens, fungal exposure— especially to thermophilic fungi—remains an under-researched area that may contribute to respiratory and immune-related issues in children, particularly amid increasing incidences of illness.

**Objective:** This poster presents preliminary results from research on the microbial characterization of school environments, emphasizing the presence of thermophilic and other fungi that may pose health risks to children. The aim of the study is to identify potentially pathogenic fungi in the air and on surfaces within schools and to explore the relationship between fungal exposure and the respiratory symptoms or infections commonly experienced by school-aged children.

**Methodology:** Air and surface samples were collected from classrooms in urban and rural schools, recording environmental factors such as ventilation, humidity, and hygiene protocols. Identification and quantification of potential pathogenic fungal species, such as *Aspergillus fumigatus*, were conducted. Health data, including reported respiratory symptoms, absenteeism due to illness, and existing respiratory conditions, will be gathered through surveys of parents and school health records to evaluate the impact of fungal exposure on children's health.

**Expected Results:** The research is expected to reveal a correlation between fungal contamination levels in school environments, seasonality and reported respiratory symptoms in children. These results aim to support the development of practical recommendations for improved cleaning protocols and ventilation strategies in schools to mitigate health risks for children.

**Relevance:** Given the rising concerns about health impacts due to climate change and its influence on fungal proliferation, this study advocates for the integration of fungal exposure assessments into standard health and safety protocols in schools. The results will inform actionable recommendations for improved cleaning practices, ventilation systems, and regular monitoring of fungal contaminants to protect children's health effectively.

**P27. A new antiviral strategy against influenza A vírus**

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*The Abstract corresponding to this poster presentation cannot be disclosed.*

## **P28. Computational design and validation of ACE2-based inhibitors against SARS-CoV-2**

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In the quest to contain the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), targeting viral entry became a promising therapeutic approach [1, 2]. Here, triple helical bundle antiviral proteins (ARMYs) were computationally designed and optimized to target Spike protein receptor-binding domain

(RBD) and block it from binding angiotensin-converting enzyme 2 (ACE2). Therefore, the design strategy considered the molecular details of the RBD-ACE2 binding, by incorporating the two adjacent  $\alpha$ -helices from ACE2, which accounts for most of the interactions with the RBD, into the backbone scaffolds. Aiming to stabilize the two interacting helices, a short loop and a third helix were inserted into the scaffold. For the interface design, the amino acid sequences were designed to optimize target binding, folding and stability. This resulted in five candidate proteins. Further structural validation was performed using structural prediction methods. Experimental assays were performed and demonstrated that four of the five designed proteins (ARMYs 1-4) effectively bound to the RBD with nanomolar affinity, comparable to the ACE2 affinity for Spike and successfully blocked SARS-CoV-2 infection. Thus, these protein designs showed great potential as viral therapeutics. Further molecular dynamics simulations of the designs were performed in the unbound and the bound state to the RBD to assess their stability and the key interactions between these and the RBD that may have affected the binding affinity, respectively. As expected, ARMY 5 demonstrated a distinct dynamic behavior from the others. The knowledge gained from this work led to a new workflow for anti-SARS-CoV-2 design and validation.

### References

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