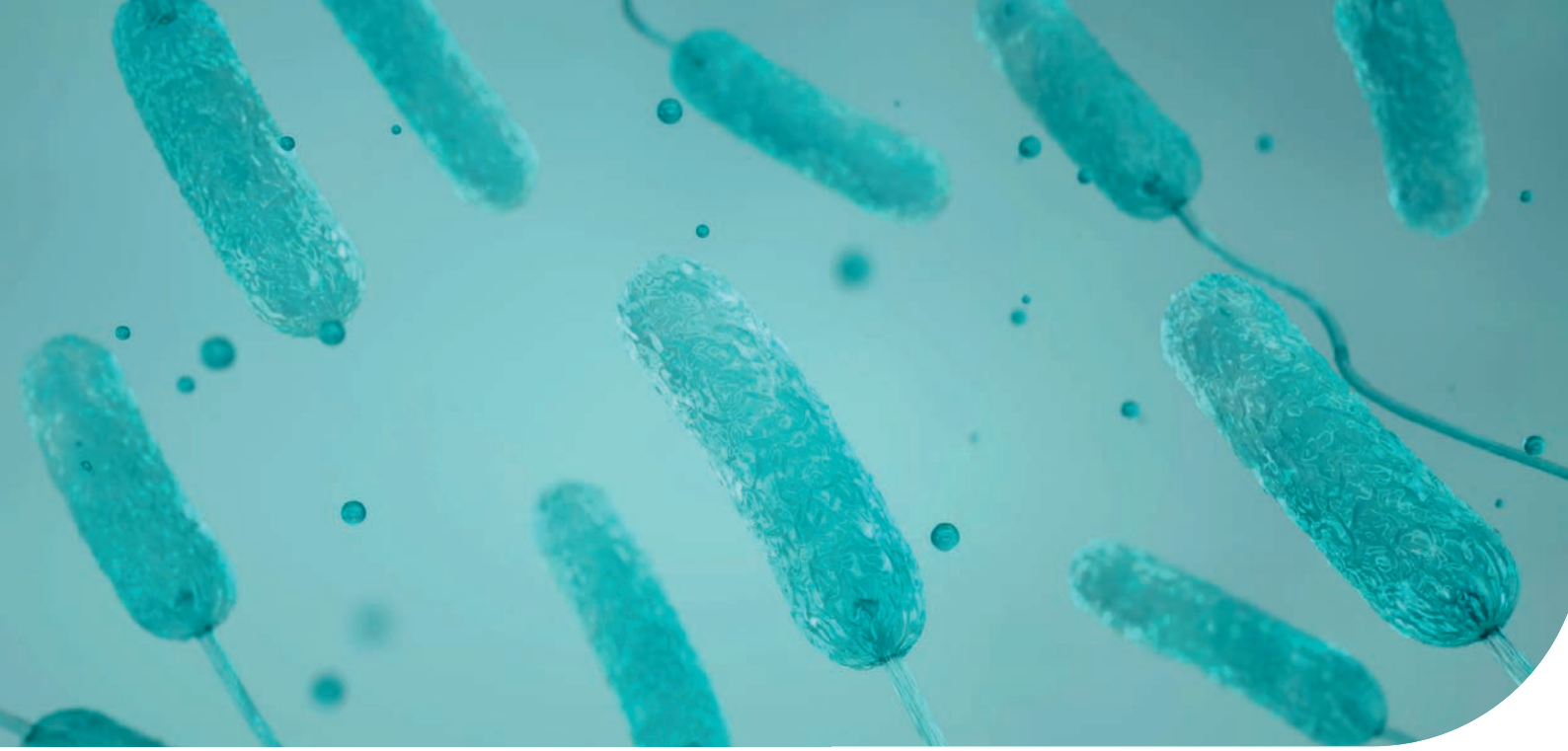

11th International Conference on *Legionella*

Commemorating 50 years of *Legionella* discovery

University of Zurich, Main Campus, Switzerland

Monday September 7 to Friday September 11, 2026

Abstract Book

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NUMBERING LEGEND

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RS01* / P101* Discovery of antiphage defence systems targeting a novel bacteriophage of *Legionella* pneumophila

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Recent work in the Ensminger lab has identified and characterized the first bacteriophage infecting *L. pneumophila*, called LME-1. Investigation of the interaction between LME-1 and *L. pneumophila* has already revealed multiple mechanisms by which *Legionella* defends itself against bacteriophages, including insight into the acquisition of a key *Legionella* virulence determinant (*lag*-1). In a subset of *L. pneumophila* strains, I have observed an additional layer of previously uncharacterized, robust antiphage defence. Using reverse genetics, I have recently localized this defence to an integrative and conjugative element (ICE). Bioinformatic analysis shows that other *L. pneumophila* ICEs consistently encode predicted defence genes, suggesting they may be rich reservoirs for a diversity of antiphage defences. Overall, this work expands the understanding of how *Legionella* interacts with phages and suggests that antiphage defence represents another advantageous trait provided by the mobile genetic elements that drive the diversity and plasticity of *Legionella* genomes. Finally, it reinforces the idea that bacteriophages are an important evolutionary force driving the adaptation of *Legionella* to its environment, despite the historical lack of isolated *Legionella* phages.

**Student Paper*

RS02 Single-cell spatial metabolomics during *Legionella* pneumophila infection reveals specific metabolic signatures in human macrophages

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Infection by intracellular pathogens generates a spectrum of metabolic states in host cells that bulk analyses cannot resolve. This is particularly relevant for *Legionella pneumophila* (*Lp*), which manipulates the metabolism of intrinsically heterogeneous macrophages through injected Type IV Secretion System (T4SS) effectors. To address this, we developed a Mass Spectrometry Imaging (MSI)-based metabolomics pipeline to characterize metabolic changes in *Lp*-infected human primary macrophages at the single-cell level. This label-free approach integrates epifluorescence microscopy, metabolomics, and a newly developed computational pipeline (SpatialMetProfiler) enabling simultaneous detection of hundreds of known and unknown metabolites and their single-cell annotation, identification, and spatial mapping. After optimizing the full workflow, we applied SpatialMetProfiler to compare the single-cell metabolomic profiles of *Lp*-WT-infected macrophages with non-infected cells, an avirulent T4SSdeficient mutant (*Lp*-Δ*dotA*), and heat-killed bacteria, to distinguish the metabolic signatures induced by T4SS effectors from those triggered by immune activation upon bacterial sensing. We found an infection-based metabolic remodelling. Specifically, this rewiring was driven by T4SS effector activity and combined broad metabolite suppression with a glycolytic shift, whereas the sensing of bacterial components alone was insufficient to reprogram host metabolism. Notably, single-cell resolution uncovered a layer of metabolic heterogeneity inaccessible to bulk analyses: infection reshaped the metabolic diversity of the macrophage population, reducing the broad diversity of resting cells while T4SS effector activity gave rise to distinct infection-associated metabolic states. Because effector delivery varies stochastically from cell to cell, this heterogeneity may help explain why some macrophages restrict bacterial growth while others become permissive. This spatially resolved single-cell approach therefore provides a platform to dissect how *L. pneumophila* reprograms the metabolism of human macrophages and why individual cells differ in their ability to control the pathogen, opening infection-associated metabolic states as candidate targets for host-directed therapies against Legionnaires' disease.

RS03 *Legionella* effector AnkX puts the brakes on IMPDH2 filaments

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Legionella pneumophila, the causative agent of Legionnaires' disease, employs more than 300 effector proteins to subvert host cell processes. A central regulator of host metabolic homeostasis is inosine 5'-monophosphate dehydrogenase type 2 (IMPDH2), which catalyses the rate-limiting step of *de novo* GTP biosynthesis by converting IMP to XMP. In this study, we identify human IMPDH2 as a novel target of the *Legionella* phosphocholinase AnkX. We demonstrate that AnkX covalently modifies IMPDH2 within its regulatory Bateman domain through the attachment of a phosphocholine moiety. The Bateman domain binds ATP and GTP according to their intracellular abundance, thereby modulating the conformation and catalytic activity of IMPDH2. AnkX-mediated post-translational modification is specifically promoted in the ATP-bound state of IMPDH2. Although phosphocholination does not directly alter catalytic activity, it severely impairs the ability of IMPDH2 to assemble into filaments and larger macromolecular structures known as cytoophidia. In cells, IMPDH2 reversibly forms filaments and cytoophidia, providing an additional layer of enzymatic regulation. By shifting the equilibrium from filamentous assemblies towards smaller octameric species, AnkX-mediated phosphocholination sensitises IMPDH2 to negative feedback inhibition by GTP binding and induces the disassembly of pre-existing cytoophidia in infected host cells. Consequently, phosphocholination affects IMPDH2 filament assembly, cytoophidia dynamics and regulatory responsiveness. In addition, it promotes the relocalisation of IMPDH2 to the *Legionella*-containing vacuole. Intriguingly, unlike the established AnkX substrate Rab1, phosphocholinated IMPDH2 is resistant to the *Legionella* dephosphocholinase Lem3. Collectively, these findings reveal a previously unrecognised mechanism of metabolic host–pathogen interaction in which a bacterial effector hijacks the structural regulation of a central metabolic hub to facilitate infection.

RS04∗ Subversion of IMPDH2 oncoprotein activity by *Legionella* pneumophila effector AnkX

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Legionella pneumophila, a ubiquitous environmental bacterium, is the causative agent of a severe pneumonia termed Legionnaires' disease and employs a conserved mechanism to infect protozoa as well as human alveolar macrophages. To manipulate host cellular pathways in favor of bacterial survival and growth, *L. pneumophila* delivers ca. 300 different "effector proteins" to the host cell using the Icm/Dot type IV secretion system. Recently, we identified the oncoprotein inosine 5'-monophosphate dehydrogenase type 2 (IMPDH2) as a novel target of the *L. pneumophila* effector protein AnkX. IMPDH2 is crucial for cell metabolism and catalyzes the conversion of inosine 5'-monophosphate (IMP) to xanthosine 5'-monophosphate (XMP), the first and rate-limiting step of the *de novo* guanine nucleotide synthesis pathway. The homotetrameric IMPDH2 enzyme assembles into macromolecular filament bundles termed "cytoophidia" under certain cellular conditions such as low GTP levels or treatment with inhibitors, as a mechanism of activity regulation. We observed that *L. pneumophila* infection caused disassembly of IMPDH2 cytoophidia induced in host cells with the IMPDH2 inhibitor mycophenolic acid (MPA). Intriguingly, this effect was dependent on the bacterial effector protein AnkX, which covalently modified IMPDH2 by attachment of a phosphocholine group and caused disassembly of ATP-induced IMPDH2 filaments *in vitro*. Depletion or inhibition of IMPDH2 in the host cells impaired intracellular growth of *L. pneumophila*. AnkXmediated phosphocholination altered the allosteric regulation of IMPDH2 by GTP and its distribution in the host cell and might thereby modulate local GTP levels. By studying this novel covalent modification of IMPDH2 and its functional consequences, we will improve our understanding of the complex regulation of this crucial enzyme, and its physiological and infection biological implications.

∗Student Paper

RS05∗ CRISPR-Cas12a detection of *Legionella* cell-free DNA for the diagnosis of Legionnaires' Disease

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Legionnaires' disease (LD) is a severe form of community-acquired pneumonia and is a welldocumented occurrence worldwide. However, due to inconsistent testing, this illness often goes undiagnosed leading to empiric treatment and excess use of broad-spectrum antibiotics. Legionnaires' Disease cannot be clinically or radiographically distinguished from other types of pneumonia, so current diagnostics are heavily reliant on sputum samples and test for the presence of *Legionella* bacteria using culture-based or PCR-based techniques. However, these methods have limited applicability in population groups who have difficulty providing sputum, such as older or intubated patients. Many countries also rely on urinary antigen testing for the diagnosis of LD, but these tests can only detect a narrow scope of *Legionella* species, making the true burden of this disease vastly under-represented. Latest advancements in the field of microbial diagnostics employ clustered, short palindromic repeats (CRISPR) and CRISPR associated protein (Cas) enzyme complexes for the detection of pathogen nucleic acids in human blood samples. Previous work from our group has shown that *Legionella* cellfree DNA is present in the blood and urine of patients with Legionnaires' Disease. We propose that *Legionella* cell-free DNA circulating in the human bloodstream can be used as a non-invasive biomarker for the diagnosis of Legionnaires' disease. Our group has designed and optimised a CRISPR-Cas12a-based assay for the detection of *Legionella* DNA in human samples. We have now begun clinical validation of this assay using patient blood samples from the ADEPT study and pleural fluid samples from the PEACE study. Both cohorts recruit patients admitted with pneumonia to Christchurch Hospital, Canterbury, New Zealand. The availability of a non-invasive test for Legionnaires' disease would bolster testing, leading to improved patient outcomes, a better understanding of the disease epidemiology, and slowing the rate of antimicrobial resistance development due to more targeted antibiotic use.

∗Student Paper

M01 50 years of the perfect replication vacuole

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Shortly after the identification of the causative agent of Legionnaires disease, Marcus Horwitz and coworkers developed a human monocyte culture model that demonstrated *Legionella pneumophila* resides in a unique membrane-bound compartment. As verified by extensive downstream work, the *Legionella*-containing vacuole (LCV) avoids entry into the host endo-lysosomal system and is surrounded by rough endoplasmic reticulum (RER) and host-derived mitochondria. LCV biogenesis has been a focus of several decades of genetic and biochemical analyses that often raise more questions than answers. Combined work from several labs, using varied genetic approaches, showed that pathogenesis and intracellular growth of the microorganism require assembly of the multiprotein Icm/Dot type IV secretion system. The key players, however, are the translocated substrates that drive LCV formation and biogenesis, which have been identified by both bioinformatic and direct biological screens for translocation. After 20 years of identifying the complete set of these effector proteins in multiple *Legionellacae,* we now have an excellent biochemical appreciation for the bewildering kaleidoscope of activities that target host membrane trafficking pathways. However, providing molecular detail for Horwitz's original observations has been challenging. Equally problematic has been a lack of explanation for the existence of a large cadre of negative regulators called metaeffectors. This talk will argue that the LCV comprises two distinct structures that undergo a developmental shift driven largely by metaeffectors. The first structure involves rearrangement of tubular ER, selected for speed of construction and for avoidance of host cell antimicrobial activities. Optimal intracellular growth, however, requires deconstruction of this rapidly established compartment by metaeffectors, followed by association with the organelles originally hypothesized by Horwitz. Therefore, Icm/Dot effectors can be divided into Stage I and Stage II classes, with metaeffectors driving the transition from Stage I to Stage II.

M02 Where is Legio, a not so simple question in the environment?

Yann Héchard

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Legionella is an environmental bacterium that was first identified 50 years ago. It has been extensively studied because of its ability to cause legionellosis, but much less attention has been paid to understanding its ecology in natural environments. *Legionella* is naturally present in aquatic environments and, depending on the species, may also occur in soil. However, where exactly can *Legionella* be found in the environment? What types of water bodies harbor it? Are there other important environmental reservoirs? It is well established that L. pneumophila can grow within hosts such as amoebae under laboratory conditions. However, what is known about these interactions in natural environments? Which amoebae are the most suitable hosts, if any? Are there other important environmental hosts? Finally, what roles do environmental factors, particularly temperature, play in the ecology and growth of *Legionella*? Our presentation will review the current state of knowledge from the literature. In addition, new insights derived from publicly available high throughput sequencing datasets will enhance our understanding of *Legionella* ecology. Finally, examples from our ongoing efforts to investigate amoeba–*Legionella* interactions in the environment will be given. In conclusion, more questions remain than answers, and further research is needed to better understand which environments are most favorable to *Legionella* growth. Such knowledge should ultimately contribute to improved strategies for preventing legionellosis.

S01 Two Chemical-Free Approaches to *Legionella* Control in Building Water Systems: Dissolved Oxygen Reduction and Charged-Membrane Filtration

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Legionella growth in building water systems causes healthcare and community-acquired Legionnaires' disease. Control often relies on oxidant injection, which creates disinfection byproducts, accelerates corrosion, adds monitoring and regulatory burden, and can fail when water use is low. Here we examine two approaches that control *Legionella* growth without added oxidants, each using a different mechanism and point of intervention. The first, dissolved oxygen (DO) reduction, is a newer approach with direct peer-reviewed *Legionella* data. Because *L. pneumophila* is an obligate aerobe, it cannot sustain respiration or stay culturable when DO is held below 0.3 mg/L. In a published hot water model colonized with *L. pneumophila*, an inline gas transfer membrane contactor reduced concentrations by over 1 log versus feed water (Krause 2024), and non-tuberculous mycobacteria were reduced similarly. This offers residual control of pathogen growth. The second, charged-membrane point-of-entry filtration, is a mature technology applied at the building inlet to lower incoming *Legionella* load. Electroadsorptive media capture *Legionella* by electrostatic attraction rather than pore size, enabling whole building treatment with low pressure loss. These electropositive media have served EPA virus concentration for decades, with growing water treatment use; cartridges challenge tested under NSF P-231 at the USEPA Test and Evaluation Facility showed over 3 log removal. Efficiency varies with water chemistry, flow, and loading, and long-term in-building data remain limited. Filtration lowers incoming load but offers no residual control. We compare both with ultraviolet (UV) disinfection and chemical controls including chlorine, chlorine dioxide, monochloramine, and copper-silver ionization. UV inactivates organisms within the reactor but leaves no residual and cannot reach downstream colonization. Pairing filtration with UV or DO reduction may improve protection without corrosion or byproducts. We weigh efficacy, monitoring, and environmental impact, placing each chemical-free option within a layered control program, or "defense in depth."

S02 U.S. Cruise-associated case surveillance of Legionnaires' disease—2016–2025

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Cruise ships can present multiple opportunities for *Legionella* exposure because they contain complex potable water systems, hot tubs, decorative water features, and other devices that can aerosolize water and grow *Legionella* if not properly maintained. The U.S. Centers for Disease Control and Prevention (CDC) conducts nationwide surveillance for Legionnaires' disease (LD), including cases associated with travel on cruise ships, and routinely notifies cruise lines of cases in persons who traveled aboard a vessel during their exposure period. We analyzed confirmed LD cases reported to CDC with onsets during January 1, 2016 – December 31, 2025, among persons with at least one overnight stay aboard a cruise ship before illness onset. Cases on river cruises were excluded. For cases with onset before 2020, cruise-associated exposure was defined as travel during the 10 days before symptom onset. Beginning in 2020, the case definition was revised to extend the exposure period to 14 days. During 2016-2025, 232 cruise-associated cases were reported. The annual number of cases increased from 20 in 2016 to 39 in 2018, declined during 2019—2021 to an average of 8.7 and then increased again beginning in 2022, reaching the highest level in 2025 (46). Of all cruise-associated cases, 26 (11.2%) occurred among non-U.S. residents and were reported by the respective countries or by cruise ship physicians; these cases most commonly occurred in residents of Canada (27%) or the United Kingdom (19%). Twenty-eight cruise-associated clusters (defined as two or more cases with travel on the same ship in 12 months) were identified; 11 (39%) clusters included non-U.S. residents, accounting for 15 cases. Prompt reporting to CDC of detailed cruise exposure information, together with coordination among multinational public health agencies, can support LD case identification, cluster detection, and response activities.

M03 How Treatment Reshapes the Water Microbiome and *Legionella* Presence

Pierre Foucault1, Ana Elena Perez-Cobas2,3, Madalina Ababii4, Jesus Marin Miret1, Sebastien

Wurtzer5, Laurent Moulin5, Carmen Buchrieser1 and Laura Gomez-Valero1 *1. Institut Pasteur, Université de Paris, Unité Biologie des Bactéries Intracellulaires, 75015 Paris, France. 2. Department of Microbiology, Ramón y Cajal Institute for Health Research (IRYCIS), Ramón y Cajal University Hospital, Madrid, Spain. 3. CIBER in Infectious Diseases (CIBERINFEC), Madrid, Spain. 4. Research Unit Bacterial Communication and Anti-infectious Strategies (UR CBSA), Rouen Normandy University, Normandy University, 55 rue Saint-Germain, 27000 Evreux, France 5. Direction de La Recherche, du Développement Et de La Qualité de L'Eau, Eau de Paris, 33 Avenue Jean Jaurès, 94200, Ivry-Sur-Seine, France* Ensuring the microbiological safety of drinking water is a major public health priority. Opportunistic waterborne pathogens such as *Legionella* spp. can persist in drinking water systems despite disinfection, posing a risk to vulnerable populations through the inhalation of contaminated aerosols. Despite the increasing incidence of legionellosis driven by climate change and population aging, our understanding of *Legionella* community dynamics in drinking water systems remains limited. Most studies are constrained in either spatial (e.g., individual buildings) or temporal (e.g., one-month sampling) coverage, focus exclusively on bacterial communities, or lack the taxonomic resolution needed for reliable species-level identification To address these gaps, we characterized the composition and dynamics of the water microbiome— particularly *Legionella* communities—in two water sources (river and groundwater) supplying the drinking water system of Paris, along with the corresponding water after treatment. Samples were collected monthly over six months, and a metabarcoding analysis was performed to characterize the bacterial and eukaryotic communities. *Legionella* was further analyzed using genus-specific primers to achieve high taxonomic resolution at the species level. Our results revealed a vast and previously unrecognized diversity of *Legionella*, particularly in natural water sources. The detected *Legionella* species were broadly distributed across the phylogenetic tree and differed between the two sources as well as between source and treated water. Water treatment drastically reduced overall *Legionella* diversity. However, key species such as *L. pneumophila*—the primary agent of clinical cases—were detected in both sources and persisted after treatment, suggesting that its adaptability to artificial environments may contribute to its high clinical incidence. The persistence of *L. pneumophila* after treatment underscores a potential public health concern.

S03* / P102* Comparative genomic analysis reveals distinct population structure in *Legionella* anisa

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Legionella anisa is one of the most frequently detected non-pneumophila species in engineered water systems (EWS). Ecologically, *L. anisa* inhabits niches distinct from those of *L. pneumohila*, favouring cold-temperature environments. Despite its prevalence in the environmental systems, *L. anisa* has low virulence potential as evident by its association with few clinical cases. However, it is still a concern in healthcare facilities (HCFs), where vulnerable populations are at increased risk of infection. The genomic diversity and population structure of *L. anisa* remain poorly characterized. The goal of this study was to perform complete genome characterization for new *L. anisa* (n=4) isolates recovered from a HCF in Quebec, Canada. We further investigated the population structure of this species by performing comparative genomic analyses of the isolates from this study together with publicly available *L. anisa* genomes. Genome-wide phylogenetic analysis revealed the presence of three distinct clades separated by substantial genetic divergence (~500 SNPs). The Quebec isolates forming a tightly clustered group with isolates from Europe and North America, suggesting a clonal lineage. Comparative pangenome analysis indicated moderate core genome conservation accompanied by a highly variable accessory genome (~50%), consistent with the dynamic genome characteristics of *Legionella* species. The isolates characterized in this study harbored multiple plasmids encoding genes associated with conjugation, heavy metal resistance, and other stressrelated functions, suggesting potential roles in environmental persistence. Previous studies have shown that *L. anisa* can proliferate within protozoan host cells, although outcomes vary depending on the host species. Our isolates showed efficient proliferation within *Acanthamoeba castellanii*, but not within *Vermamoeba vermiformis*, under the conditions tested. Together, these findings underscore the genomic diversity of this previously understudied *Legionella* species and provide a framework for future investigations into its environmental persistence and potential pathogenicity.

**Student Paper*

M04 Ending Legionnaires' Disease: An Audacious or Achievable Goal?

Janet E. Stout

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Proactive prevention strategies to control Legionnaires' disease have been implemented in the 50 years since the disease was discovered. However, cases continue at an unacceptable rate in the U.S. and worldwide. The number of cases per 100,000 population has been virtually unchanged in the United States in recent years, from 3.04 in 2018 to 2.9 in 2025. Similar numbers are reported for the EU/EEA. In most countries, *Legionella* remains an important waterborne pathogen. In the U.S., industry self-regulation with voluntary standards has failed to significantly impact the case rate. Epidemiological investigations have revealed new sources and new approaches to prevention. Emphasis on education from industry and professional organizations to raise awareness has included certification courses on water management approaches to prevention. This includes the 2025 WHO Protocol on Water and Health. These efforts make the audacious goal of ending Legionnaires' disease achievable!

M05 Pulmonary and extrapulmonary legionellosis: diagnostic challenges and unresolved questions

Sophie Jarraud

University of Lyon, Lyon, France

Abstract not received yet.

M06 *Legionella* Diagnostics: From Culture to Metagenomics

Valeria Gaia

EOC - IDISI/EOLAB - Legionella Reference Center - Bellinzona - Switzerland

Legionnaires' disease (LD) is a growing public health concern, with reported cases in Switzerland and across Europe rising significantly in recent years. As the *Legionella* genus has diversified — now comprising around 70 species — traditional clinical paradigms are being challenged. Long-held beliefs that the disease is exclusively non-contagious or limited solely to certain host profiles are being reconsidered in light of evidence of probable person-to-person transmission and evolving epidemiological trends. Currently, the diagnostic landscape is dominated by the urinary antigen test (UAT), which is used in 90% of European cases due to its speed and simplicity. However, the UAT is primarily limited to L. pneumophila serogroup 1 and often requires confirmatory 'boiling procedures' to resolve non-specific reactions. Although culture remains the gold standard for strain isolation, antimicrobial susceptibility testing and source tracing, its clinical utility is hampered by low sensitivity and long turnaround times. The transition to molecular diagnostics represents a significant step forward; real-time PCR on respiratory samples offers superior sensitivity, enabling the identification of a further 18–30% of cases compared with rapid antigen tests (UATs) and cultures. Looking to the future, next-generation metagenomic sequencing (mNGS) is emerging as a powerful tool for diagnosing polybacterial infections and extrapulmonary manifestations. Furthermore, next-generation UATs targeting ribosomal proteins promise to extend detection beyond L. pneumophila serogroup 1. The shift from traditional culture to integrated metagenomic approaches is essential for modern surveillance and for improving clinical outcomes in an increasingly complex scenario.

S04 Evaluation of the national enhanced *legionella* surveillance system in England, 2025

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2. Acute Respiratory Infections Unit, UK Health Security Agency, United Kingdom
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The National Enhanced *Legionella* Surveillance System (NELSS), managed by UK Health Security Agency (UKHSA), combines clinical, laboratory, and epidemiological data from England and Wales to support long-term surveillance, cluster detection, and timely outbreak response. We report the first formal evaluation of NELSS since 2017, conducted following transition to a new case management system, to inform internal improvements within UKHSA. Using ECDC guidelines, a mixed-methods approach was used to evaluate surveillance attributes, including representativeness, sensitivity, completeness, validity, timeliness, and acceptability. Quantitative analysis included all laboratory-confirmed Legionnaires' disease (LD) cases in England from 1977 to 2025. Qualitative insights were obtained through an online survey and semi-structured interviews with stakeholders across national and regional teams, capturing user experiences and perceptions of system performance. A total of 12,550 confirmed cases were identified. NELSS showed strong representativeness, with incidence patterns (higher rates among males and those aged ≥50 years), consistent with other European surveillance systems. Case ascertainment was high (100%) and aligned with case management system records. However, cluster detection appeared overly sensitive, with 12.5% (n=29/233) of potential cluster alerts associated with recorded outbreaks requiring public health action. Data completeness and validity were high (>70% completeness and >98% of values within expected ranges). Timeliness improved following recent automation, reducing manual data entry. However, users reported that the median of 7 days (IQR 4-13) between completion of the enhanced surveillance form and cluster notification was insufficient for effective public health action. Qualitative findings highlighted that while the system captures rich data, users reported usability challenges and gaps in cross-team communication. NELSS is a robust and valued surveillance system for LD surveillance in England. Refining cluster detection definitions and improving timeliness are key priorities. Further automation, streamlined workflows, and enhanced communication across teams and stakeholders will strengthen its capacity for rapid outbreak detection and response.

M07 *Legionella* and its secreted phospholipases: a universe in a nutshell

Antje Flieger

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Phospholipases are a diverse class of enzymes produced by both eukaryotic hosts and microbial pathogens. Recent years have provided major insights into how bacterial phospholipases are activated, localized, and deployed during infection. While some act as potent membrane destructors, others subtly reprogram host signaling pathways. In the pneumonia-causing pathogen *Legionella* pneumophila, phospholipases represent an exceptionally diverse arsenal. The genome encodes at least 15 phospholipases A (PLA), three phospholipases C, and one phospholipase D. The PLAs fall into three major families: GDSL, patatin-like, and PlaB-like enzymes. This remarkable repertoire must be precisely synthesized, secreted, and activated to function at the right place and time during infection. Tight control of enzyme activity is particularly critical because phospholipases capable of damaging host membranes can also threaten the bacterium itself. Consequently, L. pneumophila has evolved sophisticated mechanisms that prevent self-inflicted damage while ensuring activity at the pathogen–host interface. In this talk, I will highlight a selection of these elegant regulatory strategies, illustrating how spatial and temporal control of phospholipase activity drives the *Legionella* infection cycle and may reveal new opportunities for antimicrobial intervention.

S05∗ / P103∗ Exploring the biphasic lifecycle of *Legionella* through characterization of L. pneumophila protein Lpg1968

Ms. Tiffany Penner[1], Ms. Jenny Phung[1], Mr. Andrew J. Gierys[1], Dr. Neil Lorente Cobo[1], Mr. Tyler Gislason[1], Dr. Ann Karen Brassinga[1], Dr. Gerd Prehna[1]

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Legionellae are Gram-negative intracellular pathogens of protozoa naturally found in freshwater environments, and in concentrated levels within piped water systems of which aerosols may be inhaled by humans resulting in a severe pneumonia termed Legionnaires' disease (LD). As infection occurs in the host, the bacteria initiate its biphasic lifecycle of which the switch from replicative to transmissive phase is tightly governed by numerous regulators. In particular, the transcription factor PsrA which responds to *in vitro* changes in long chain fatty acid concentrations, but the mechanism of fatty acid regulation is unknown. Interestingly, comparative transcriptomic analyses of the parental and isogenic Δ*psrA* mutant strain showed that the only gene that was significantly upregulated from 93 differentially expressed genes was *lpg1968.* Thus, a molecular and biophysical approach was taken to understand the function of Lpg1968. Using X-ray crystallography, the crystal structure of Lpg1968 was determined of which function is putatively a hydrolase enzyme. Using the structure, we perform *in silico* docking to discover putative substrates and test the contribution of Lpg1968 to the ability of *Legionella* to persist within host cells. Preliminary results using nanoDSF suggest that Lpg1968 is stabilized *in vitro* by numerous small molecules relevant to the biology of *Legionella*. Characterization of Lpg1968 will lead to a better understanding of the role of this protein during infection of host cells and therefore the regulation of *Legionella*'s biphasic lifecycle.

∗Student Paper

S06∗/ P006∗ Capsule-mediated lethality in *Legionella* longbeachae is associated with host immune dysregulation and increased NET formation

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Bacteria of the genus *Legionella* are intracellular Gram-negative pathogens that can cause Legionnaires' disease, a severe atypical pneumonia. *Legionella longbeachae* has emerged as an important pathogen responsible for outbreaks worldwide and exhibits increased lethality and higher replication rates than other *Legionella* species in murine infection models. Genomic and transcriptomic analyses identified capsule biosynthesis genes (*ctrC* and *ctrD*) in *L. longbeachae*. More recently, the capsule was shown to be required for virulence, although the mechanisms underlying its contribution to disease pathogenesis remain incompletely understood. Therefore, this study investigated the role of the capsule in *L. longbeachae* pathogenicity. By comparing mutant strains lacking *ctrCD* with capsule-producing strains, we observed that the capsule is essential for infection-associated lethality, as its absence dramatically reduced mortality in infected animals. We found that both encapsulated and non-encapsulated bacteria induced an early depletion of alveolar macrophages that persisted throughout infection. However, only the encapsulated strain induced a transient increase in pulmonary IL-6 and TNF-α production. Despite only a modest increase in bacterial replication, capsule expression impaired the recruitment of inflammatory monocytes and monocyte-derived macrophages, resulting in defective restoration of the pulmonary myeloid compartment. At later stages of infection, encapsulated bacteria induced increased NET formation in lung tissue and were associated with enhanced lung injury, as evidenced by morphometric analyses, bronchoalveolar lavage protein leakage, and elevated LDH levels. In tissue culture experiments, capsule expression increased epithelial cell infection and directly promoted NET formation in neutrophils, suggesting potential mechanisms contributing to tissue damage. Collectively, our findings indicate that capsule-mediated virulence is driven primarily by dysregulation of host immune responses rather than enhanced bacterial replication. We propose that defective myeloid replenishment impairs restoration of pulmonary homeostasis, promoting tissue damage and mortality. Increased NET formation and enhanced epithelial cell infection may further contribute to capsule-associated pathogenicity and warrant further investigation.

∗Student Paper

M08 Expanding role of the *Legionella* pneumophila type II secretion system

Nicholas Cianciotto

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The type II secretion system (T2SS) mediates a two-step process in which proteins to be secreted (substrates) are first transited across the inner membrane and into the periplasm by the Sec or Tat translocon and then, after folding into their tertiary conformation are recognized and carried across the outer membrane by the multi-protein T2SS apparatus. Genes encoding the T2SS are conserved in all species of *Legionella*. Based on earlier mutant analyses, the T2SS has diverse and important roles in the ecology and pathogenesis of *Legionella pneumophila* (*Lp*), promoting intracellular infection of multiple types of amoebae, biofilm formation, planktonic survival at low temperatures, sliding motility, intracellular infection of macrophages and epithelia, bacterial growth and tissue damage in the infected lung, and subversion of the host's immune response. During intracellular infection, the T2SS facilitates the replicative phase in the *Legionella*-containing vacuole, although some T2SS substrates can translocate into the host cell cytoplasm. Proteomic analysis of culture supernatants indicates that the *Lp* T2SS secretes nearly 120 proteins, including many different types of degradative enzymes that cleave proteins, lipids, carbohydrates, and nucleic acid as well as many novel proteins. Recently characterized *Lp* T2SS phenotypes and substrates include i) mucin degradation and utilization, mediated in part by the chitinase ChiA, ii) growth in low-iron conditions, as linked to the novel IrsA, iii) serum- and polymyxin-resistance, associated with the LPS-deacetylase PdaA, and iv) a putative LasB-like metalloprotease, ecto-ATP diphosphohydrolase, 5′-nucleotidase, glutaminase, and kinase. We have also recently uncovered novel evidence for the T2SS secreting antibiotic-inactivating enzymes, including a penicillin acylase and cephalosporinase. The T2SS is evolutionarily related to the type IV pilus (T4P) apparatus, and as an interesting sidenote, we also recently found that the major pilin of the *Lp* T4P fosters bacterial growth in low-iron conditions, independently of its role in piliation.

M09 Protein sociology of *Legionella* pneumophila PPIase Mip and infection interference

Michael Steinert

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The peptidyl-prolyl *cis/trans* isomerase (PPIase) Mip (macrophage infectivity potentiator) of the intracellular pathogen *Legionella pneumophila* is involved in bacterial virulence regulation, lung tissue degradation and immune evasion. On the pathogen side, the 24 kDa protein Mip interacts with the bacterial proteins SspB, Lpc2061, and FlaA, promotes flagellation and bacterial mobility, which is inhibited by PPIase inhibitor FK506. On the host side, homodimeric Mip enables bacterial transmigration across tissue barriers and is known to bind collagen. Since further Mip interactions with host cells and human lung tissue proteins are largely unknown, we developed a new coimmunoprecipitation approach, applied RFDiffusion and ProteinMPNN with respective protein fragments, and modelled the structure, binding interface, and dynamics of homodimeric Mip with the identified partner acetyl-CoA carboxylase 1 (ACACA). The application of the macrolide FK506, docking simulation and subsequent coimmunoprecipitation experiments confirmed the interaction and demonstrated that Mip inhibits the enzymatic activity of Acetyl-CoA Carboxylase (ACACA), which may open new paths for infection interference.

S07 Macrophage Dysfunction in VEXAS Syndrome

Dr. Isabelle De Malliard[1], Dr. Benjamin De Valence[1], Prof. Patricia Doublet[2], Prof. Sophie Jarraud[3], Dr. Abdelrahim Zoued[4]

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VEXAS syndrome is a rare acquired autoinflammatory disorder caused by somatic mutations in the *UBA1* gene in hematopoietic progenitor cells, leading to dysfunction of the ubiquitin–proteasome system. In addition to systemic inflammation, several clinical studies have reported an increased susceptibility of VEXAS patients to severe intracellular bacterial infections, particularly *Legionella pneumophila*, suggesting an underlying defect in innate immune responses. To investigate the cellular mechanisms responsible for this susceptibility, we used THP-1 macrophages carrying the UBA1 mutation as an in vitro model of VEXAS syndrome. Intracellular replication assays revealed a significant increase in bacterial replication in mutant macrophages following infection with *L. pneumophila*. UBA1 vexas macrophages displayed impaired endosomal maturation together with reduced TNF-α production during infection, highlighting defects in both phagosome processing and inflammatory responses that are likely to promote bacterial survival. These findings provide experimental evidence that the vexas mutation compromises macrophage antibacterial functions and support the concept that VEXAS syndrome represents not only an autoinflammatory disease but also an acquired immunodeficiency characterized by impaired control of intracellular bacterial infections.

M10 Innate immune detection and restriction of *Legionella* pneumophila.

Sunny Shin

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Legionella pneumophila and other intracellular bacterial pathogens pose a unique set of challenges for the immune system. While eukaryotic organisms possess evolutionarily conserved innate immune sensors and signaling pathways to sense and respond to infection, intracellular pathogens likewise possess evolutionarily conserved mechanisms to disrupt, evade, or modulate cellular signaling networks. Thus, while the mechanisms of PRR activation by isolated PAMPs are reasonably wellunderstood, how the immune system successfully generates responses against pathogens like *L. pneumophila* that utilize virulence factors to shut off cellular responses is less clear. *L. pneumophila* deploys virulence factors that disrupt key cellular processes such as host protein synthesis, raising the question of how a robust immune response can be generated during bacterial infection. I will discuss our recent findings elucidating how the immune system overcomes bacterial virulence activities in order to generate a robust antimicrobial response.

M11 Structural elucidation of the essential *Legionella* pneumophila effector SdhA

Sagar Bhogaraju

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The intracellular bacterium *Legionella* pneumophila (LP) secretes around 300 effector proteins into host cells via the dot/icm T4SS. Significant functional redundancy exists within this repertoire of effectors, such that deletion of one effector often does not severely impact LP replication and survival. SdhA was previously shown to be essential for LP intracellular replication by inhibiting host cell apoptosis and blocking innate immune sensing pathways. Here, we determined the cryo-EM structure of SdhA to 3.2 Å overall resolution, paving the way for further structural studies of this important effector with host cell interactors. SdhA displays significant structural similarity to the paralog SidH at the N-terminus but is negatively charged at the region which in SidH interacts with tRNA. Using interaction proteomics, we also found that SdhA can bind specific host proteins in HEK cells. Infection experiments conducted using primary macrophages suggests that SdhA is playing an essential role in bacterial infection at least partly through interacting with these host factors.

S08/ P104 SidL is an adenylyltransferase that modifies the host glycolytic metabolite 3-phosphoglycerate

Dr. Joshua Black[1], Dr. A. Maxwell Burroughs[2], Dr. Edrees Rashan[3], Dr. Charles Nosal[1], Ms. Katerina Romanov[1], Dr. Marco Catipovic[1], Ms. Catherine Valadez[1], Dr. L. Aravind[2], Dr. Kevin Hicks[4], Dr. Caren Freel Meyers[1], Dr. Tamara O'Connor[1], Dr. Matthew Vander Heiden[3], Dr. Rachel Green[1]

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The large effector arsenal of *Legionella pneumophila* enables it to target multiple aspects of host cell biology and focused study of individual effectors has been a rich source of novel biochemistry and insight into host-pathogen relationships. Here, we biochemically characterize the purported translation inhibitor SidL/Ceg14 (Lpg0437) *in vitro* and redefine it as a glycolysis-targeting enzyme that modifies the metabolite 3-phosphoglycerate (3PG) with adenosine monophosphate (AMP) to produce the previously unknown molecule 2-AMP-3-phosphoglycerate (2-AMP-3PG). Reflecting its *in vitro* biochemistry, ectopic expression of SidL in mammalian cells generates 2-AMP-3PG, reducing cellular 3PG levels and downstream glycolytic intermediates. Consistent with this disruption of glycolysis, SidL inhibits the nutrient-responsive translation regulator mTORC1 and we propose that this indirectly decreases translation, explaining the initial identification of SidL as a translation-targeting effector. In an infection model, we observe SidL-dependent synthesis of 2-AMP-3PG in human macrophages infected with *L. pneumophila*, with its production peaking at early stages of the infection cycle. Current efforts are underway to understand how *L. pneumophila* uses SidL and 2- AMP-3PG to its benefit. To our knowledge, this is the first report of a bacterial effector that chemically modifies a host glycolytic intermediate, uncovering a new modality by which pathogens can interface with host metabolism.

S09 Cryo-EM structure of *Legionella* pneumophila 3-methylcrotonyl-CoA carboxylase reveals substrate-induced filamentation

Dr. Radha P. Somarathne[1], Ms. Riti Shrestha[1], Ms. Mishghan Zehra[1], Mr. Cole Brodeur[1], Dr. Anthony D. Shumate[1], Prof. David Bhella[2], Dr. Wing-Cheung Lai[1], Dr. Amit Meir[2], Prof. Clarissa Durie[1]

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Legionella pneumophila exhibits a biphasic lifecycle, alternating between intracellular host-associated replication and environmental growth, in planktonic and biofilm-associated niches. These transitions expose *Legionella* to distinct nutrient availability and require metabolic remodelling. Like many other intracellular pathogens, *Legionella* relies on amino acids as a primary energy source. Among these, leucine catabolism depends on methylcrotonyl-CoA carboxylase (MCC), an essential biotindependent carboxylase. In humans, MCC overexpression is linked to cancer, and its deficiency is linked to inborn metabolism errors. In many pathogens, MCC is an essential enzyme, making it a promising drug target. Hence, understanding its structure and function has broad biological and biomedical relevance. Here, we report the highest resolution cryo-EM structure of MCC to date, obtained by purification of the endogenous holoenzyme from *L. pneumophila*. This EM map allowed identification of MCC's components by the machine-learning tool ModelAngelo, also independently confirmed by mass spectrometry. MCCLp presented the canonical heterododecameric architecture, composed of six β subunits stacked between two α-subunit trimers. Furthermore, Cryo-EM classification revealed multiple states, allowing us to visualize MCC assembly transition and substrate-bound conformations. Intriguingly, substrate binding enhanced MCC filament formation in vitro. Although MCC filamentation has been observed in eukaryotic enzymes and linked to negative-feedback regulation, this is, to our knowledge, the first observation of substrate-enhanced filamentation in a bacterial MCC. We propose that this filamentation may be important for cellular processes, linking nutrient sensing, metabolic remodelling, cell division and biphasic transitions.

M12 Manipulation of the nucleoli by a powerful Dot/Icm T4SS protease effector

Vikrant H. Kashyap[1], Daniel C. Lockwood[1], Alejandro Fernandez-Vega Granado[2], Arshad Rizvi[1] , Flavia Viana[1], Serena Varile[2] , Guan-Bo Wang[1], Mohammad Arefian[3], Jack Penny[3], Raissa Wibawa[4], Isabel Rodríguez-Escudero[2], Elizabeth L. Hartland[4], Seyed M. Jafarnejad[5], Ben C. Collins[3] , Victor J. Cid[2], Gunnar N. Schroeder 1

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Legionella pneumophila translocates more than 300 effector proteins through the Dot/Icm type IV secretion system into a host cell to rewire cellular processes. Bioinformatics has transformed our ability to identify conserved domains and predict functions of effectors; however, the actual activities of most effectors remain to be experimentally determined. We characterised a family of peptidase C58-like T4SS effectors encoded in a wide variety of *Legionella* species. Functional analysis of the most conserved member of the family, LegA7, revealed that the protease cleaves itself and, upon ectopic expression, has profound effects on the physiology of eukaryotic cells, inducing changes to, for example, ER structure, Golgi apparatus and nucleus, without causing cell membrane permeability or lysis. Substrate trapping experiments with the inactive enzyme in combination with mass spectrometry yielded a large number of interactors involved in ribosome biogenesis and function. Subsequent cleavage assays identified Nucleolin as protease substrate, prompting us to investigate its effect on ribosomal RNA synthesis and the nucleoli. This revealed not only a disruption of rRNA transcription but also a complete loss of the nucleoli and accumulation of nucleolar proteins in the cytoplasm, in turn leading to a translation shutdown. During infection, we also observed disruption of the nucleoli; however, this did not depend on the effector, likely because redundant effectors act. Based on our data we propose that LegA7 is a new nucleomodulin effector that is deployed together with other effectors by *Legionella* to modulate the nucleoli and ribosome biogenesis in host cells.

S10 Using mitochondrial signals and AI to predict *Legionella* pneumophila replication within human macrophages

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1. Microbiology Department, Institut Pasteur, Paris, France

Accurately tracking dynamic state transitions is crucial for modeling and predicting biological outcomes, as it captures heterogeneity of cellular responses. To build a model to predict bacterial infection in single cells, we have monitored in parallel infection progression and metabolic parameters in thousands of human primary macrophages infected with the intracellular pathogen *Legionella pneumophila*. By combining live-cell imaging with a tool for classifying cells based on infection outcomes, we were able to trace the specific evolution of metabolic parameters linked to distinct outcomes, such as bacterial replication or cell death. Our findings revealed that early changes in mitochondrial membrane potential (Δψm) and in the production of mitochondrial Reactive Oxygen Species (mROS) are associated with macrophages that will later support bacterial growth. We used these data to train an explainable machine-learning model and achieved 83% accuracy in predicting *L. pneumophila* replication in single, infected cells before bacterial replication starts. Our results highlight backtracking as a valuable tool to gain new insights in host-pathogen interactions and identify early mitochondrial alterations as key predictive markers of success of bacterial infection.

M13 Blame the phage – on the unexpected origins of Legionnaires' disease.

Alexander W. Ensminger

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Despite obvious potential applications and extensive searching, the growing consensus over the first 50 years of *Legionella* research has been that these bacteria largely avoid phage infections. Our recent discovery of the first infectious *Legionella* phage (LME-1) shows this not to be the case. Instead, the apparent absence of prophages in our strains should have been a big red flag–we now know that the main feature that makes "clinical strains" a high risk to human health derives from resistance to phage attachment. In other words, encounters between *Legionella* and phages underpin the frequency and severity of Legionnaires' disease. Understanding the *Legionella*-phage arms race is essential to determining how these otherwise environmental bacteria pose such a significant risk to human health. To that end, we will discuss our expanded understanding of the diversity of LME-type phages across *Legionella* species, the many ways that isolates defend against infection, and how the hidden hand of these environmental encounters shapes human disease.

S11 Global climate gradients structure soil *Legionella* diversity, abundance and pathogen distributions

Dr. Hans Singh[1], Dr. Maggie Yuan[1]

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Legionnaires' disease has increased sixfold since 2000 in the United States, yet environmental reservoirs such as soils remain underinvestigated sources of infection. It remains unclear how the diversity and relative abundance of environmental pathogens, such as *Legionella*, will shift as the climate continues to change. Here, we utilize 4,285 publicly available global soil 16S rRNA gene amplicon datasets to analyze how the relative abundance and diversity of the genus *Legionella*, including pathogenic lineages, vary across biogeographic regions and along climatic and geochemical gradients. *Legionella* relative abundance increased across the joint precipitationtemperature gradient, with mixed-effects modeling indicating that detection of soil *Legionella* significantly increased with precipitation. *Legionella* diversity was characterized by high spatial turnover, with the majority of abundant ASVs confined to individual continents despite broad phylogenetic representation across the genus. Further, fewer than 2% of our 16S-derived *Legionella* sequences matched ASVs corresponding to characterized species, and the clinically dominant species L. pneumophila was rarely detected. In contrast, ASVs matching several other known pathogens (L. longbeachae, L. anisa, L. bozemanae, L. cincinnatiensis) were orders of magnitude more common. Moreover, pathogenic *Legionella* ASVs occurred significantly more frequently at higher precipitation and temperature ranges. Collectively, our findings suggest that *Legionella* relative abundance and diversity in soils, especially pathogenic lineages, are shaped by both dispersal and climatic filtering. As temperature and precipitation regimes shift in the future, our results indicate that soils are an important source to monitor and that *Legionella* species beyond L. pneumophila warrant increased ecological and public health attention.

S12 Modulation of L. pneumophila biofilm growth through a unique protein conformational switching mechanism

Dr. Abirlal Mukherjee[1], Dr. Leanne Cleaver[1], Dr. Anna Katarina Antonovic[2], Mr. Hunter Kuhlemeier[3], Dr. Mark Freeley[2], Dr. Saima Rehman[1], Prof. Matteo Palma[2], Dr. Arianna Fornili[2], Prof. Nicholas Cianciotto[3], Dr. James Garnett[1]

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Biofilm formation is an important survival strategy commonly employed by bacteria, which are embedded in an extracellular matrix comprising proteins, carbohydrates, lipids, and DNA. This provides protection against environmental pressures such as shear flow, host immune/inflammatory responses, and antimicrobial agents. *Legionella pneumophila* is a Gram-negative bacterium that inhabits natural/artificial freshwater systems within multispecies biofilms. It replicates within amoebae but also infects the human lung and causes Legionellosis. The *Legionella* collagen-like (Lcl) protein is an extracellular peripheral membrane protein exported by the type II secretion system (T2SS), with a fundamental role in ecology and infection of lung tissue, through mediating biofilm formation and adhesion/entry into host cells, respectively. In our recent work (doi.org/10.1038/s41467-024-49255-4) we determined the structure of the Lcl C-terminal domain (CTD) trimer and demonstrated how it recognises glycosaminoglycans and mediates host adhesion through a unique fuzzy binding mechanism. Using X-ray crystallography, molecular dynamics simulations and other biophysical approaches we now show how Mg2+ ions bind within a negatively charged internal cavity, and this results in CTD trimer rearrangement. In this state, rather than binding glycosaminoglycans, the CTD can interact with the lipopolysaccharide of *L. pneumophila,* and other Gram-negative bacteria, and this provides a role in selecting for single and multispecies biofilm growth. Furthermore, we observe that another substrate of the T2SS, the LPS deacetylase PdaA (doi.org/10.1128/mbio.01393-25), can inhibit Lcl/LPS binding. Our study establishes a novel conformational switching mechanism where local concentrations of acetylated lipopolysaccharide and divalent cations, and glycosaminoglycans, select for initiation of biofilm growth or infection of eukaryotic hosts.

M14 Structural and functional insights into natural transformation in *Legionella* pneumophila

Manuela Hospenthal

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Natural transformation enables *Legionella* pneumophila to acquire extracellular DNA and depends on a type IV pilus (T4P) for DNA capture and ComEC for transport across the inner membrane. We use structural, biochemical and genetic approaches to investigate the molecular mechanisms underlying these processes. I will present our work on the DNA-binding minor pilin FimT and the cryo-EM structure of the *Legionella* T4P filament, providing insight into the extracellular stages of DNA uptake. I will also discuss our studies of ComEC, combining structural and biochemical analysis of a tractable orthologue with functional experiments in L. pneumophila. Together, these studies provide molecular insight into distinct stages of natural transformation, from DNA capture at the cell surface to its processing and transport across the inner membrane.

M15 Co-option of a cell surface signaling system to control vacuole integrity.

Tamara O'Connor

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Abstract not received yet.

S13 Impact of amoeba hosts on *Legionella* effector horizontal gene transfer and evolution

Mx. Mische Holland[1], Dr. Tera Levin[1]

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Effector genes undergo frequent horizontal gene transfer (HGT) and recombination as they move in and out of *Legionella* genomes. Yet, it remains unclear how these processes interact with de novo mutation, selection, and neutral forces to ultimately shape the sequence and functions of effectors. Here, we present an experimental evolution study to model events that could occur and alter effector evolution in natural, environmental reservoirs. We attenuate the *Legionella* by deleting the critical effector MavN, then track the re-acquisition and inheritance of the effector gene during interactions between bacteria and *Dictyostelium* amoebae. Unlike typical experimental evolution studies that examine accumulated de novo mutations in bacterial strains, we leverage the natural competence of *L. pneumophila* to induce HGT in the lab. We find that co-culture of *Legionella* with amoeba hosts rapidly selects for the acquisition and fixation of the mavN gene in the bacterial population. Thus in this simplified system, interactions between bacteria and microeukaryotes are sufficient to cause the evolution of increased bacterial virulence. Finally, we present our future plans to uncover the withinpopulation HGT events that lead to virulence gene acquisition and spread.

S14∗/ P105∗ Modulation of intercellular tunnelling nanotubes of Dictyostelium discoideum by *Legionella* pneumophila

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Tunnelling nanotubes (TNTs) are thin, actin-dependent protrusions connecting cells and enabling intercellular communication over short to intermediate distances. Unlike classical secretion-based communication, TNTs provide a continuous route for transfer of cellular material, including cargo up to the size of whole organelles. In mammalian systems, TNTs have been implicated in tissue repair, immune regulation, inflammation, tumor progression, neurodegeneration, and host-pathogen interactions. However, despite their emerging biological relevance, the cellular mechanisms controlling TNT formation, cargo selectivity, and directional transport remain elusive. This is partly due to the lack of genetically tractable model systems. This project aims to establish *Dictyostelium discoideum* as a model organism to study TNT-mediated intercellular cargo transfer. *D. discoideum* shares important functional properties with mammalian phagocytes and is widely used as a host model for intracellular pathogens such as *Legionella pneumophila*, the causative agent of Legionnaires' disease. Using genetically encoded fluorescent reporters for mitochondria, endosomes, and lipid droplets, we show that *D. discoideum* forms TNTlike intercellular connections capable of transferring cargo between cells. Moreover, TNT formation is modulated by cellular stress and bacterial infection. Treatment with latrunculin B promotes TNT formation and enhances intercellular transfer of fluorescently labelled cargo, supporting a role for cytoskeletal remodeling in TNT induction. In contrast, infection with wild-type *L. pneumophila* reduces TNT formation, whereas mutants lacking microtubule-modulating effectors partially restore TNT formation. These findings suggest that bacterial effectors interfere with TNT-dependent intercellular communication. Together, these results identify TNTs as intercellular transport structures and establish *D. discoideum* as a powerful model to dissect the molecular mechanisms underlying TNT formation, cargo selection, and infection-dependent modulation. By combining live-cell confocal fluorescence microscopy, genetically encoded cargo reporters, and *L. pneumophila* effector mutants, this study provides new insight into how cytoskeletal remodeling controls TNT-mediated exchange of organelles and vesicular cargo during stress and infection.

∗Student Paper

M16 Beyond the Tap: *Legionella* Niche Differentiation in a Natural Freshwater Lake

Shira Ninio

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Legionella species are most often studied in the context of man-made water systems or clinical settings, yet they inhabit diverse natural aquatic environments, including freshwater lakes and streams, as well as saline systems. Experimental work has shown that the genetic makeup of *Legionella* pathogens is strongly influenced by the protozoan hosts they interact with and by the chemical composition of the growth media, but how these factors shape the *Legionella* population in natural environments remains poorly understood. Here, we used genus-specific amplicon sequencing and qPCR to examine the *Legionella* community and its associated protozoan hosts in a stratified freshwater lake. We found non-random ecological organization of the *Legionella* community, with clear temporal partitioning and niche differentiation among *Legionella* genotypes across depth, oxygen availability and host association. These patterns are consistent with environmental filtering structuring *Legionella* diversity along multiple ecological axes. Our findings raise the possibility that niche based diversification contributes to the genetic diversity observed in this system, a hypothesis that will be further explored using genomic data.

S15 Do different *Legionella* strains behave differently within Pseudomonas biofilms?

Dr. Ana Rosa[1], Ms. Francisca Ferreira[1], Prof. Sebastien P. Faucher[2]

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2. McGill University, Montreal, Québec, Canada

Biofilms are acknowledged as critical spots for *Legionella* persistence in engineered water systems. Despite efforts to advance understanding of how *Legionella* interacts with biofilms, many aspects of these interactions remain poorly understood. In the present work, we investigated whether reference and wild-type *Legionella* strains display distinct migration and spatial colonization patterns within preestablished *Pseudomonas* biofilms. To accomplish the study, a monospecies biofilm of *Pseudomonas fluorescens* (*Pf*) was established on PVC coupons placed in 12-well microplates using R2 medium under stagnant conditions. A reference (ATCC 33152) and a wild-type (WT) (ST62, disease-associated strain) strain of *Legionella pneumophila* (*Lp*), both harboring the GFP-expressing plasmid pXDC31, were selected and spiked 3 days after starting biofilm formation. *Lp* migration within the biofilm was monitored over 24 hours by confocal microscopy based on GFP fluorescence. The biofilm mesoscale structure was evaluated before and after *Lp* spiking using Optical Coherence Tomography (OCT). Five minutes after spiking, both *Lp* strains exhibited similar biovolume values (≈4 $\mu m^3/\mu m^2$), with approximately 40% and 53% of the total biovolume located in the top and middle biofilm layers, respectively. Over time, the reference strain accumulated a higher biovolume than WT ST62. However, WT ST62 penetrated the inner biofilm layers more rapidly than the reference strain. Six hours after spiking, approximately 80% of the WT ST62 biovolume was located within the middlebottom layers, whereas nearly 88% of the reference strain biovolume remained at the top-middle layers. Both *Lp* strains affected the biofilm mesoscale structure. Notably, biofilm porosity increased to a greater extent in the presence of the reference strain than in the presence of WT ST62, when compared to the control *Pf* biofilm. These findings indicate that *Lp* colonization patterns within biofilms may depend on the strain. Future work will focus on understanding the physiological determinants underlying these behaviors using targeted *Lp* mutants.

M17 Prevention and Control of *Legionella*: Emerging Perspectives from Artificial Intelligence.

Maria Luisa Ricci

Department of Infectious Diseases, National Institute of Health, Rome, Italy

Legionnaires' disease remains a public health concern, in healthcare facilities, accommodation sites and complex building water systems, where *Legionella* colonization is favoured by warm temperatures, stagnation, biofilm formation and inadequate hydraulic or disinfection control. The Water Safety Plan is a cornerstone of current *Legionella* prevention and control strategies, as recommended by WHO and reflected in European Directive 2020/2184 on drinking water. However, its effectiveness can be limited by inconsistent maintenance, delayed and variable results, and the difficulty of predicting high-risk conditions. This work explores emerging perspectives on the application of artificial intelligence (AI) to *Legionella* detection, prevention and control in building water systems. AI may improve detection by supporting the interpretation of laboratory and sensor-derived data. Computer vision and deep learning could assist with automated culture-plate reading, colony enumeration and preliminary classification, reducing operator variability. AI-enhanced biosensors may enable rapid on-site detection by improving signal processing and classification of optical, electrochemical or fluorescence-based outputs. Machine learning (ML) applied to MALDI-TOF MS spectra, qPCR or sequencing data may further improve microbial identification and interpretation, especially when combined with environmental and operational metadata. Beyond detection, ML may support a shift from reactive surveillance to predictive risk management by integrating microbiological data, temperature, disinfectant residuals, hydraulic features, environmental variables, maintenance records and water-network characteristics. Recent hospital-based evidence suggests that ML models can outperform conventional regression approaches in predicting *Legionella* contamination risk, highlighting their potential as decision-support tools within Water Safety Plans. AI should not replace culture, qPCR or established control measures, but may strengthen them through dynamic, data-driven and proactive risk management. Future research should validate models across building types, develop *Legionella*-specific image datasets, integrate real-time sensors and assess interpretability, cost-effectiveness, and public health impact.

M18 *Legionella* Story in Japan: Between Basic Science and Clinical Relevance

Kazuhiro Tateda

Toho University, Tokyo, Japan

Legionnaires'disease was first recognized in 1976 following an outbreak of pneumonia among attendees at a Veterans of Foreign Wars convention in Philadelphia, USA. Five years later in Japan (1981), Prof Atsushi Saito, who was affiliated with the Second Department of Internal Medicine at Nagasaki University, have reported the first case of *Legionella* pneumonia in Japan. Prof Saito learned *Legionella* diseases and cultivation techniques in University of Pennsylvania under the supervision of Prof Paul Edelstein. Recent epidemiological data in Japan demonstrated more than 2,000 cases of *Legionella* pneumonia annually, but it may be still underestimated. When I was 30 years old trainee (1991) in the Toho university hospital, I was shocked by the lethal case of *Legionella* pneumonia. He was 43 years of age otherwise healthy man. We obtained typical colony on BCYE- agar from his respiratory specimens, but we could not save his life even after aggressive antibiotic treatment. The Chest XP showed multiple shadows of consolidation with severe hypoxemia, finally complicated with acute respiratory failure after massive supplemental oxygen therapy. In 1999, I had a chance to visit the University of Michigan to learn mice model of *Legionella* pneumonia under the supervision of Prof Theodore Standiford (Respiratory and Critical Care Medicine) and there luckily enough, I met Prof Michele Swanson (Microbiology and Immunology). By their support, we had a chance to publish several papers which were hinted by clinical experiences and tried to clarify mechanisms of *Legionella* pneumonia pathogenesis. One repot demonstrated a role of apoptosis in alveolar epithelial cells, which were exaggerated by higher concentrations of oxygen. Also, we have reported possibility of next generation of urinary antigen detection kit, which is targeting not only lipopolysaccharide of *Legionella* organisms, but also ribosomal protein L7/L12. The new types of diagnostic kits targeting organism specific ribosomal L7/L12 protein are available in Japan for *Legionella*, *Mycoplasma* and *Pertussis* infections. In my presentation, I would like to summarize our experience of *Legionella* diseases, from the hits of clinical manifestations to insight into pathogenesis and mechanisms of the diseases. It may be important to discuss how we can draw the next 50 years of story of *Legionella* and related organisms in clinically and basic science.

M19 Session introduction and NYS Investigations using WGS

Ursula Lauper, Danielle Wroblewski

New York State Department of Health, Wadsworth Center, USA

NYSDOH is fortunate to have in-house capacity for conducting WGS for its community outbreak investigations. In this presentation we will briefly describe a series of community investigations and corresponding WGS results that range from definitive to puzzling. Questions we have encountered in recent years include interpreting exact matches between samples collected across extended time or space, and defining the SNP cut-off for declaring a “match” and how this might vary across different regions.

M20 Epidemiological and Bioinformatic aspects of WGS

Camille Jacqueline, Sophie Jarraud, Christophe Ginevra

Hospices Civils de Lyon, Université de Lyon, France

Using a newly developed cgMLST scheme (2009 genes), recent work has shown that the minimum allelic distance (AD) threshold required to cluster samples within a given sequence type (ST) varies substantially across STs, challenging the use of a single outbreak-level threshold at the species level. We will present our analytical workflow and conclusions from three investigations involving STs with different clustering thresholds. Overall, these case studies support the idea that outbreak case definitions based on WGS data require genetic resolution and thresholds tailored to the specific context of each investigation.

M21 How to consider *Legionella* diversity and dynamics for source matching

Sebastien Faucher, Michele Provost

McGill University, Polytechnique Montréal, Canada

Some engineered water systems harbor *Legionella* communities composed of multiple species, sequence types (STs), and genetic variants, whose relative abundances can be highly dynamic. Some strains are persistent residents, whereas others are transient colonizers. Are clinical isolates always the dominant strains present in the environment? Does a single clinical isolate accurately represent the *Legionella* population within the lungs? Given the extensive diversity of *Legionella*, how can source identification be improved?

M22 WGS of *Legionella*: Swiss stories and new technologies

Helena Seth-Smith, Tim Roloff

University of Zurich and Swiss Institute of Bioinformatics, Zurich, Switzerland

The Institute for Medical Microbiology performs the genomic comparisons for the Swiss Reference Centre for *Legionella*. We provide typing of all cultured isolates and are also involved in larger studies across Switzerland including outbreak investigations. As such we are continuously developing and improving our methods for high resolution typing, and to obtain genomes from clinical samples without culture using target enrichment techniques.

M23 Structural and functional analysis of the *Legionella* pneumophila Dot/ Icm Type IV secretion system

Youngki Yoo[1], Kenia Contreras[1,2], Jacquelyn R. Roberts[1,2], Arwen E. Frick-Cheng[1], and Melanie D. Ohi[1,3]

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Legionella pneumophila, the causative agent of Legionnaires' disease, manipulates host cellular processes through delivery of effectors through the Dot/Icm Type IV Secretion System (T4SS). A central component of the T4SS is the outer membrane core complex (OMCC), which consists of an outer membrane cap (OMC) and a periplasmic ring (PR). The OMCC is anchored by a stalk that extends from the PR to the bacterial inner membrane, as well as an inner membrane complex (IMC) containing cytoplasmic ATPases. Although cryo-EM structures of the OMCC have clarified the composition of several *Legionella*-specific T4SS elements, the mechanism of the secretion system remains poorly understood. This talk will discuss strategies for determining structures of the Dot/ IcmT4SS and will examine the functional contributions of two previously uncharacterized OMCC proteins to substrate delivery. Together, these studies aim to advance our understanding of how the Dot/Icm T4SS assembles and functions during *L. pneumophila* pathogenesis.

S16* / P007* The *Legionella* effector MavP targets host cell PMP70 to recruit peroxisomes to the replication vacuole

Ms. Katerina Romanov[1], Dr. Mohammad Hossain[1], Dr. Tamara O'Connor[1]

1. Johns Hopkins University School of Medicine, Baltimore, MD, USA

Legionella pneumophila translocate over 300 proteins termed effectors into their host cell. A subset of effectors is important for maintaining the integrity of the *Legionella* containing vacuole (LCV), which is critical for protection from host immune surveillance systems. Here we show that one of these effectors, MavP, decorates the surface of the LCV and interacts with the host peroxisomal membrane protein PMP70, allowing *L. pneumophila* to recruit peroxisomes to the LCV. Domain mapping and mutagenesis studies have identified a critical functional motif of MavP for interacting with PMP70 and peroxisome recruitment to the LCV. We found that MavP accumulation and peroxisome recruitment to the LCV coincides with the onset of bacterial replication and the need for LCV membrane expansion to accommodate increased bacterial numbers. Peroxisomes play a fundamental role in host cell lipid metabolism and homeostasis. Importantly, in peroxisome-deficient macrophages, LCVs exhibit a compact architecture and a higher frequency of destabilization, similar to what is observed when host lipid availability is limited. Furthermore, disruption of peroxisome lipid metabolic pathways for glycerophospholipid and ether lipid synthesis results in loss of LCV integrity and impaired bacterial growth. These observations have led to the model that *L. pneumophila* exploit host cell peroxisomes to expand and maintain the integrity of their intracellular replication compartment that is critical for pathogenesis.

**Student Paper*

S17* / P100* *Legionella* pneumophila subverts mitochondrial dynamics and mtDNA quality control to promote intracellular replication

Mr. Tobias JÄGGI[1], Ms. Sarah Anthamatten[1], Dr. Simone Vormittag[1], Mr. Tong Chen[2], Prof. Hubert Hilbi[3]

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3. Universität Zürich, Zürich, Switzerland

Intracellular bacteria manipulate and subvert cellular processes in their eukaryotic hosts in a sophisticated manner. While the facultative intracellular pathogen *Legionella pneumophila* naturally replicates in free-living amoeba, inhalation of this pathogen can lead to a severe pneumonia due to the ability of the bacterium to replicate in alveolar macrophages. During the intracellular state, *L. pneumophila* secrets over 300 "effector proteins" into their respective hosts. This enables the formation of a *Legionella*-containing vacuole (LCV) by subverting phagosome maturation and other cellular processes. While LCV formation is essential for the survival and replication of the pathogen, many other host cellular processes are targeted and subverted to further support the survival and replication of intracellular *L. pneumophila*. Mitochondrial function and dynamics are essential for virtually all cellular processes. Not surprisingly, intracellular bacteria such as *L. pneumophila* specifically target and modulate mitochondrial components and their function. In this study, we identified several *L. pneumophila* effector proteins localizing to and modulating mitochondria. Some *L. pneumophila* deletion mutant strains lacking individual mitochondria-targeting effector proteins are impaired for intracellular replication within host cells and inhibit host cell respiration less efficiently compared to the parental strain. We are currently investigating the mode of action of a novel secreted mitochondria-targeting *L. pneumophila* effector kinase. The kinase contains an N-terminal mitochondria targeting sequence, which is required and sufficient for mitochondria localization, where it targets a mitochondrial fission protein. Thus, the *L. pneumophila* kinase subverts critical steps in mitochondrial dynamics and mtDNA maintenance, revealing a novel post translational regulation of a critical host protein.

**Student Paper*

M24 *Legionella* pneumophila counteracts vacuole acidification through amino acid antiport

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Legionella pneumophila (Lp) replicates within diverse hosts, ranging from environmental freshwater amoebae to human alveolar macrophages. To survive intracellularly, *Lp* resides within a specialized replicative niche known as the *Legionella*-containing vacuole (LCV). A well-established feature of virulent *Lp*, in contrast to avirulent Type IV secretion system (T4SS)-defective mutants, is its ability to evade endo-lysosomal fusion. Therefore, we were surprised to find that even LCVs containing virulent *Lp* undergo substantial luminal acidification, but that *Lp* counteracts acidification using its amino acid antiporter VdeL. Whereas homologs of VdeL from enteric pathogens localize to the inner bacterial membrane, *Lp* incorporates VdeL into the surrounding LCV membrane where VdeL imports arginine while expelling agmatine along with a "virtual" proton, thereby restoring a neutral pH inside the LCV. VdeL delivery to the LCV membrane requires the Dot/Icm type IV secretion system, and deletion of the gene encoding VdeL or mutation of the predicted pH-sensing or substrate gating residues rendered *Lp* less virulent. Supplementation with a molar excess of exogenous arginine or treatment with lysosome alkalinization agents alleviated the intracellular growth defect of *Lp*D*vdeL*. Direct examination of the pH of LCVs during infection revealed that *Lp* experiences early vacuole acidification yet manages to recover from this transient stressor in a manner *Lp*D*vdeL* cannot. Taken together, these data present an interesting case where environmental host stressors incentivized *Lp* to repurpose an inner membrane amino acid antiporter as a vacuolar proton pump.

S18 Beyond Canonical Domains: *Legionella* Effectors Use Conserved α-Helical Folds to Target Phosphoinositides

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Legionella pneumophila remodels host membrane trafficking to build its intracellular replication niche, yet the lipid-binding logic encoded by its large Dot/Icm effector repertoire remains incompletely understood. Because phosphoinositides define eukaryotic membrane identity and serve as key landmarks for organelle dynamics, we asked how broadly *Legionella* effectors exploit these lipids during host-cell manipulation. Using an expression library of 241 *L. pneumophila* effectors, we performed a systematic discovery pipeline combining lipid bead pulldowns, mass spectrometry, phosphoinositide biosensor colocalization, and protein-lipid overlay assays. This approach identified 18 previously unrecognized phosphoinositide-binding effectors. Rather than resembling canonical eukaryotic lipid-binding domains, these proteins were predicted to adopt compact α-helical folds, suggesting that bacterial pathogens have evolved distinct structural solutions for reading host phosphoinositide signals. We then used this shared structural signature to expand the search beyond the initial screen, identifying candidate effectors in *L. pneumophila* and other intracellular bacterial pathogens, including *Coxiella burnetii* and *Burkholderia pseudomallei*. Experimental validation confirmed 15 additional phosphoinositide-binding proteins, bringing the total to 33 newly identified bacterial effectors with phosphoinositide-binding activity. Although these effectors displayed diverse lipid-binding profiles, many showed enrichment at compartments marked by phosphatidylinositol 3- phosphate, a lipid central to endosomal identity and pathogen vacuole biology. Together, these findings reveal a previously hidden repertoire of bacterial phosphoinositide-binding effectors and define conserved α-helical folds as a recurring strategy for host membrane targeting. This work expands the molecular framework for understanding how *Legionella* and related intracellular pathogens decode and manipulate host membrane identity.

M25 How to make a home in the ER: *Legionella* shows the way

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The endoplasmic reticulum (ER) is central to cellular homeostasis, coordinating protein folding, lipid metabolism, and stress signaling. Perturbations in ER function activate the Unfolded Protein Response (UPR), an adaptive signaling network that restores ER homeostasis. Activating Transcription Factor 6 (ATF6), one of the three major UPR sensors, is classically activated through trafficking from the ER to the Golgi apparatus, where regulated proteolysis releases an active transcription factor. However, emerging evidence suggests that ATF6 can also be activated through mechanisms that bypass this canonical pathway. Here, we use the intracellular bacterial pathogen *Legionella* pneumophila as a model to investigate non-canonical regulation of ATF6 during cellular stress and infection. *Legionella* establishes an intracellular replication niche by extensively remodeling host membrane trafficking and organelle function through the activity of specialized bacterial effector proteins. Our preliminary observations indicate that *Legionella* activates ATF6 through an unconventional mechanism, suggesting the existence of previously unrecognized regulatory pathways controlling ER stress responses. We are investigating the host and microbial factors that mediate this response and defining the molecular interactions underlying ATF6 activation during infection. Using complementary molecular, cellular, biochemical, and proteomic approaches, we aim to characterize signaling networks associated with non-canonical ATF6 activation and determine how pathogen-mediated remodeling of host organelles influences ER homeostasis. These studies will provide new insight into the regulation of ATF6 and the intersection between host stress signaling and microbial pathogenesis. Defining non-canonical mechanisms of ATF6 activation may reveal fundamental principles governing cellular adaptation to stress and identify previously unknown regulators of the UPR. Because ER stress pathways contribute broadly to infectious, neurodegenerative, metabolic, and malignant diseases, these findings may also establish a framework for understanding ATF6 regulation in diverse disease contexts.

S19 Post-translational modifications of the conserved Hippo pathway control host defense against pathogenic *Legionella*

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The Hippo pathway is highly conserved among hosts of the bacterial pathogen *Legionella pneumophila*. This eukaryotic pathway regulates important cellular functions, such as cell proliferation and differentiation. Intriguingly, humans with defective Hippo kinases experience recurrent infections, highlighting a novel but largely unexplored role for the Hippo pathway in immunity. We previously discovered that the *L. pneumophila* effector protein LegK7 manipulates host Hippo signaling by mimicking the host Hippo kinases, providing the first example of a bacterial effector targeting this conserved pathway. Our current research investigates the dynamic interactions between the Hippo components and *L. pneumophila*. In addition to LegK7, we found that the *L. pneumophila* kinase effector LegK6 directly phosphorylates MAP4K5, a conserved regulator of the p38 pathway and the Hippo pathway in the host cells. As a countermeasure against the hijacking of Hippo signaling by the bacterial effectors, macrophages proteolytically cleave the Hippo kinases, a unique reprogramming process that modulates host programmed cell death to restrict pathogenic *L. pneumophila*. This Hippo reprogramming can be triggered by host inflammasomes upon sensing molecular patterns of *L. pneumophila*. Specifically, when the flagellin protein from *L. pneumophila* with a functional T4SS is detected by the NLRC4 inflammasome, caspase-1 directly cleaves the Hippo kinases. The noncanonical inflammasome caspase-11 also promotes the Hippo reprogramming process in response to lipopolysaccharide from the bacterium lacking flagellin. Together, these findings reveal a fascinating host-pathogen interaction in which *L. pneumophila* exploits the conserved Hippo signaling pathway through its effectors whereas the host cells coordinate the Hippo reprogramming process with the innate immune sensors to counteract the invasion of pathogenic *L. pneumophila*.

S20∗ Caspase-8 mediates host resistance to *Legionella* longbeachae independently of inflammasome signaling

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Legionella longbeachae is an emerging and frequently overlooked etiological agent of Legionnaires' disease. Infections caused by *L. longbeachae* are likely underestimated because routine clinical diagnostics largely target SG1 *Legionella pneumophila*. In locations where species-specific diagnostic methods are implemented, *L. longbeachae* represents over 30% of *Legionella* infections. In addition, *L. longbeachae* infection has been associated with severe disease and high mortality and displays markedly increased virulence in mice compared to *L. pneumophila*. However, the innate immune pathways that mediate host protection against *L. longbeachae* remain incompletely understood. In this study, we investigated the contribution of inflammasome signaling and alternative cell death pathways during *L. longbeachae* infection. Although *L. longbeachae* triggered inflammasome activation, including GSDMD cleavage, pore formation, pyroptosis, and IL-1β secretion, these responses were considerably weaker than those induced by *flaA⁻ L. pneumophila*. Mechanistically, inflammasome responses were driven predominantly by Caspase-11-mediated non-canonical NLRP3 signaling, alongside activation of the AIM2 inflammasome, with both pathways contributing to maximal responses. Unexpectedly, disruption of inflammasome-associated components, including NLRP3, AIM2, ASC, Caspase-1, Caspase-11, and GSDMD, did not compromise bacterial control in macrophages or alter disease progression in infected mice. In contrast, Caspase-8 emerged as a key determinant of resistance to infection. Although Caspase-8 was dispensable for bacterial restriction in macrophages, its deficiency resulted in increased bacterial burden and mortality *in vivo*. Collectively, these findings identify Caspase-8 as an important pathway for protective immunity *during L. longbeachae* infection and reveal a limited contribution of inflammasome-dependent pathways to host defense.

∗Student Paper

M26 *Legionella* effectors modify the host epitranscriptome

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Many bacterial pathogens use specialised protein secretion systems to inject virulence "effector" proteins into eukaryotic host cells. In the last 10 years, it has emerged that bacterial effector proteins are typically enzymes that mediate sometimes highly novel post-translational modifications of eukaryotic proteins. Recent examples include arginine glycosylation, phosphoribosyllinked ubiquitination and ADP-riboxanation. As such, bacterial effectors are unique tools to explore fundamental aspects of cell biology and biochemistry. In addition, the identification of the targets of effector activity in host cells provides valuable insight into host mechanisms of cell intrinsic immunity, since effector proteins have evolved to block these pathways. While it is well established that bacterial effectors target host eukaryotic proteins, the possibilty that bacterial effectors modify host RNA is not commonly explored.
We recently discovered that an effector protein from *Legionella pneumophila* degrades host glycolytic mRNAs to inhibit host cell metabolism during infection. We demonstrated that the effector protein is a bone fide RNA binding protein that preferentially binds to host mRNA by recognising a guanine rich motif. Using RNA interactome capture we discovered additional Dot/Icm effector proteins that bind host mRNA, including an uncharacterised effector with NADPH oxidase activity that oxidised newly synthesized host mRNA. This finding reinforces the prospect that effector proteins not only mediate post translational modifications of host proteins but may also target the host epitranscriptome.

S21 The *Legionella* effector RidL binds the large fission GTPase Drp1 to promote mitochondrial fragmentation

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The environmental bacterium and facultative human pathogen *Legionella pneumophila* secretes ca. 330 effector proteins into host cells to establish a replicative *Legionella*-containing vacuole (LCV) and to subvert the host cell's defense mechanisms. Several *Legionella* effector proteins target mitochondrial dynamics and functions in order to modify the host cell in their favour. The 130 kDa effector protein RidL is known to interact with the retromer subunit Vps29, a component of the core complex required for initiation of retrograde vesicle trafficking. RidL inhibits retrograde trafficking in mammalian cells and promotes intracellular replication of *L. pneumophila*. Recently, we discovered that the C-terminal portion of RidL interacts with the human large fission GTPase dynamin-related protein 1 (Drp1), which plays a key role in mitochondrial fission and is involved in the formation of mitochondria-derived vesicles (MDVs), as well as with the closely related *Dictyostelium discoideum* protein dynamin A (DymA). Further investigation revealed that RidL localizes to the mitochondrial surface and promotes mitochondrial fragmentation as well as the depolarization of the mitochondrial membrane potential, resulting in an impact on mitochondrial respiration during infection. In agreement with this observation, RidL enhances an activating phosphorylation of Drp1 and leads to an accumulation of phosphorylated Drp1 on mitochondria. Moreover, RidL promotes the mitochondrial recruitment of Drp1 and Tom20, a component of a mitochondrial import receptor complex that is a well-known cargo protein of some MDV types. Taken together, we identified RidL as the first *Legionella* effector interacting with a large fission GTPase and assessed the role of RidL on mitochondrial dynamics. Further investigations will address whether RidL affects the formation and trafficking of MDVs as well as the function of the effector protein in amoeba.

M27 Towards the function of Fic enzymes in posttranslational modifications of *Legionella*

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Post-translational modifications (PTMs) covalently modify proteins to regulate diverse cellular processes, and many bacterial pathogens exploit enzyme-mediated PTMs to promote their survival, replication, and virulence during infection. A prominent example is the Fic family of enzymes, which act as PTM writers: using different nucleotide co-substrates, they for example catalyse modifications such as AMPylation (attachment of adenosine monophosphate), phosphocholination (attachment of phosphorylcholine), and phosphorylation (attachment of phosphate). Several *Legionella* strains encode Fic enzymes, yet for most of them the modified target proteins, the relevant co-substrate, and the function during infection remain unknown. Two obstacles make these enzymes hard to study: there is no general method to identify which host or bacterial proteins a given Fic enzyme modifies, and the weak, transient interaction between a Fic enzyme and its substrate makes it difficult to resolve how the enzyme recognises its target. To overcome this, we are studying the *Legionella* Fic enzymes LtpG and LppFic, aiming to identify their target proteins, nucleotide co-substrates, and regulatory partners. We are developing tools to map Fic targets and co-substrates. I will present these tools and discuss what our first results reveal about the role of these Fic enzymes.

S22 Structural Basis for Membrane Targeting and Secretion of *Legionella* SidE Ubiquitin Ligases

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SidE family effectors from *Legionella pneumophila* catalyse unconventional serine ubiquitination of host proteins via phospho-ribose (PR) linkages, disrupting host compartments, facilitating the formation of *Legionella*-containing vacuoles (LCVs) and blocking xenophagy. Upon infection, SidE proteins are injected into the cytosol and recruited to host membranes. Here, we present structural and functional insights into a previously uncharacterized C-terminal domain (CTD) of SidE effectors. The crystal structure reveals a banana shape resembling eukaryotic BAR-domain dimers, with an amphipathic helix and an electropositive patch mediating membrane association. Targeting host organellar membranes confers substrate specificity for PR-ubiquitination. We further unravel the interface between CTD and the secretion chaperone IcmS-IcmW-DotLc complex, which allows ejection through the Type IV secretion system. Our findings establish membrane localisation and secretion as critical determinants of SidE effector function during *Legionella* infection.

M28 Regulation of host mitophagy by *Legionella* pneumophila.

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Mitochondria are essential organelles responsible for cellular energy production and the regulation of diverse signaling pathways, including innate immune responses and cell death. Because of their central role in cellular physiology, mitochondria are frequent targets of several bacterial pathogens that manipulate host cellular systems. To maintain mitochondrial quality, host cells employ mitophagy, a selective form of autophagy that eliminates damaged mitochondria. In the well-characterized PINK1-Parkin pathway, mitochondrial damage stabilizes the protein kinase PINK1 on the outer mitochondrial membrane, where it promotes the recruitment and activation of the E3 ubiquitin ligase Parkin. Activated Parkin ubiquitinates numerous mitochondrial outer membrane proteins, thereby promoting recognition of damaged mitochondria by the autophagy machinery. Previous studies have shown that *Legionella* pneumophila induces the type IV secretion system (T4SS)-dependent mitochondrial dysfunction during infection. Interestingly, we found that, despite this mitochondrial damage, L. pneumophila actively suppresses the early steps of the PINK1-Parkin signaling. Our findings further suggest that multiple T4SS effectors cooperate to interfere with the initiation of PINK1-Parkin-mediated mitophagy.

M29 Genome size evolution and dynamics of horizontal gene transfers in 100 *Legionella* species

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The gammaproteobacterial order *Legionellales* represents an exceptional model to study host adaptation and genome evolution, as all its members are host-associated to varying degrees, ranging from facultative intracellular pathogens to obligate endosymbionts. Evolutionary theory predicts that host adaptation is largely irreversible and accompanied by progressive genome reduction. However, the family *Legionellaceae*, comprising the single genus *Legionella*, challenges this paradigm. Comparative genomics has revealed that the last common ancestor of *Legionellales* possessed a relatively small genome, whereas genome size expanded along the lineage leading to *Legionella*, likely driven by the acquisition of additional horizontal gene transfer (HGT) mechanisms. The genus *Legionella* currently encompasses 69 described species inhabiting aquatic environments and protozoan hosts, with roughly half associated with human disease. Their virulence and ecological success rely heavily on the Dot/Icm type IVB secretion system and a remarkably large, highly dynamic repertoire of effector proteins, many of which harbor eukaryotic-like domains acquired through HGT. Strikingly, despite thousands of effectors identified across the genus, only a handful are conserved, highlighting extreme genome plasticity. Yet, the mechanisms underlying the dissemination of these genes within the genus remain poorly understood. Here, we sequenced and analyzed 90 isolates of *Legionella*, representing 35 novel species. We explored the atypical evolution of genome size in *Legionella* by integrating comparative genomics, phylogenetics, ancestral state reconstruction, and HGT analyses. Together, our analyses shed light on how *Legionella* reconciles host adaptation with genome size expansion, revealing an evolutionary trajectory that defies established models of intracellular bacterial evolution. The 50% increase in the number of *Legionella* species also provides an opportunity to discuss whether *Legionella* should remain the single genus in the *Legionellaceae* family.

S23 From ST154 to ST574: Recombination within Norwegian L. pneumophila?

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Many sequence types (STs) of *Legionella pneumophila* have been identified through environmental surveillance and among cases of Legionnaires' disease. In Norway, ST574 has been isolated from both environmental sources and clinical cases, but to our knowledge has not been reported elsewhere. Based on seven-locus sequence-based typing (SBT), ST574 differs from ST154 by a single allele, *neuA*, raising questions about whether ST574 represents a distinct lineage or has arisen from within ST154. We therefore investigated the relationship between ST574 and ST154 using wholegenome sequencing and comparison with publicly available genomes. Norwegian isolates of ST154 (n=12) and ST574 (n=34) were sequenced and analysed together with publicly available global ST154 genomes identified from NCBI (n=34). No additional ST574 genomes were identified in public databases. Temporal phylogenetic analysis of the Norwegian and publicly available genomes (n=80, 7 countries, 1982-2025) estimated that ST154 emerged around 1867 (95% HPD: 1635-1950). The Norwegian genomes formed a distinct clade, with an estimated most recent common ancestor (MRCA) around 1984. Although assigned to a distinct ST, the ST574 isolates were phylogenetically embedded within ST154 sublineages, occurring within two separate ST154 subclades with estimated MRCAs around 1999 and 2020, respectively. Recombination analysis showed that the SBT-defining *neuA* allele was present within distinct recombination blocks in the two ST574 clades, supporting convergent acquisition rather than shared ancestry. Together, these findings indicate that ST574 has arisen through recombination within an ST154 phylogenetic background, rather than representing a single clonal lineage. This highlights the limitations of seven-locus SBT alone and the value of genome-wide approaches, such as coregenome typing or SNP-based phylogenetics, for resolving evolutionary relationships that may be obscured when inference relies on a small number of loci. Whether ST574's apparent enrichment in Norway reflects sampling bias, ecological adaptions or traits relevant to disease remains unclear and warrants further investigation.

S24 what is a cluster? the 6 km / 6 month rule and the challenge of community legionnaires' disease detection

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In England and Wales, potential Legionnaires' disease (LD) community clusters are investigated when two confirmed cases occur within 6 months and 6 km of each other (6m/km). Although similar thresholds are widely used, evidence of their performance against practitioner-confirmed outbreaks is limited. We assess whether the 6m/km window aligns with confirmed outbreaks and compare it with alternative approaches using back-testing. Ten years (2015–2024) of LD community cases (n=4,961) and exposure locations (n=11,266) from the National Enhanced Legionellosis Surveillance System were analysed. The spatial extent of practitioner-confirmed outbreaks (n=113) was assessed and alternative thresholds evaluated. Backtesting compared: (i) the 6m/km rule; (ii) population-adaptive radii; and (iii) a prospective space-time permutation scan statistic (SaTScan). All confirmed outbreaks fell within the 6 km threshold, with 90% of case pairs within 2 km. The 6- month window captured 90% of pairs, although some outbreaks extended beyond it through chaining. Backtesting the 6m/km rule produced 15,646 distinct case pairs (1,492 alerts per year) and detected 79 of 113 ratified outbreaks (sensitivity 69.9%; case-pair false alarm rate 83.4%). Scaling the rule by local population density detected 83 of 113 outbreaks (sensitivity 73.5%; false alarm rate 85.1%) but raised alert volume by 108 per year (1,675 vs 1,492; +7.3%). SaTScan, constrained to a single case postcode, returned 83 significant signals ($p \leq 0.05$; median radius 3.4 km, up to 6.0 km) and detected 27 of 120 ratified outbreaks (22.5%). The 6km threshold encompassed all ratified outbreaks and contained most case pairs. Against this benchmark, alternatives offered no clear operational advantage: population-adaptive radii modestly improved sensitivity but increased alert volume, while SaTScan under-performed, corroborating evidence that single-address geocoding is insufficient for LD. For a national programme, a riskstratified geospatial methodology, dependent on high-quality case information, is key to developing an operationally credible cluster detection method that is both sensitive and specific.

S25 Strengthening national surveillance of Legionnaires' disease: Evidence from SwissLEGIO

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Objectives: Exposure pathways for Legionnaires' disease remain uncertain. We conducted a national case-control and molecular source attribution study to investigate risk factors and infection sources for communityacquired Legionnaires' disease (CALD) in Switzerland. The study was undertaken by a multidisciplinary consortium of clinicians, epidemiologists, bioinformaticians, microbiologists, and building technology experts. Methods: We enrolled 204 CALD patients and 198 matched controls (age, sex, residential area, time of infection) between August 2022 and March 2024. Data were collected through structured interviews, geospatial assessments, and environmental sampling of households and other potential *Legionella* sources. Associations between risk factors and CALD were analysed using conditional logistic regression. Clinical and environmental *Legionella* isolates were characterised by whole genome sequencing (WGS) for source attribution. Results: Molecular typing indicated that 7.4% of CALD could be attributed to residential showers, with infections occurring in households where we measured *Legionella* spp. concentrations well below 1,000 CFU/litre. Our epidemiological analyses suggest that some infections not attributable to household water systems may have originated from large outdoor sources (e.g., wastewater treatment plants). CALD risk was elevated among individuals with comorbidities, low household income, or occupational exposures. Most clinical isolates were MAb 3/1-positive, with ST23 being the most frequent sequence type. ST23 was rarely detected in the environment, but if detected, could be linked to CALD cases. In Ticino, a single pathogenic ST23 clone linked to several infections was detected simultaneously in multiple reservoirs. Conclusion: Collectively, these findings suggest that CALD results from interacting host, social, and environmental factors rather than a single dominant source. Accordingly, surveillance and *Legionella* control strategies should increasingly reflect this multifactorial nature. We propose risk-based surveillance integrating epidemiological, environmental, and genomic data to capture the pathogen's complex ecology. Risk assessments should increasingly priorities the characteristics of detected strains over *Legionella* spp. concentration thresholds alone.

M30 The outer mitochondrial membrane as a platform for effector activity.

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A subset of *Legionella pneumophila* Dot/Icm effector proteins localise to host cell mitochondria. A bioinformatic screen of known effectors identified four proteins with features of mitochondrial tailanchored proteins that insert into the outer mitochondrial membrane via their C-terminal transmembrane domain and positively charged tail. All four effectors were shown to be targeted to the outer mitochondrial membrane and *Lp*PIP was identified as a bacterial protein phosphatase-1 (PP1) interacting protein. *Lp*PIP influences PP1 localisation and the subcellular phosphorylation profile. Examining the interactome of these effectors indicated shared interactions that sheds light on the import and stability of tail-anchored outer mitochondrial proteins of both bacterial and eukaryotic origin.

S26 The bacterial effector LpDot1 triggers a unique methylation on SFPQ to hijack paraspeckles and alternative splicing

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Nuclear functions are key in protecting cells against infections, yet *Legionella pneumophila* is a champion in the bacterial kingdom to exploit these mechanisms to survive. Here, we characterized a *L. pneumophila* effector protein, LpDot1 that shares sequence similarity with the eukaryotic catalytic domain of the histone methyltransferase DOT1. Phylogenetic analysis suggests its acquisition *via* horizontal gene transfer from protozoan hosts. We revealed that LpDot1 is not a typical histone methyltransferase, but targets and methylates non-histone nuclear proteins, notably the splicing factor SFPQ, a key component of the membranelles organelle paraspeckle. Specifically, LpDot1 modifies the previously uncharacterized residue K518 on SFPQ, leading to the disruption of its dimerization and dramatic structural rearrangements. By manipulating SFPQ, LpDot1 hijacks the splicing machinery of the host cell, leading to alternative splicing variants of infection-related genes such as *NF-kB2* and *CD45*. These modulations have immunosuppressive functions, including decreased CD45RO levels and NF-kB2 nuclear translocation during infection. Thus, LpDot1 is a key virulence factor that directly modifies paraspeckle organization, providing a fundamental mechanistic understanding of new strategies adopted by intracellular pathogens to dampen the host immune response and ensure successful replication.

M31 Mechanistic Insights into LnaB-Mediated Regulation of Ubiquitin Signaling and Immunity

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Legionella pneumophila deploys dozens of effectors to co-opt the host ubiquitin network and promote the biogenesis of a replication-permissive intracellular niche. Among these effectors, members of the SidE family (SidEs) catalyze phosphoribosyl ubiquitination via a mechanism that is chemically distinct from the canonical E1-E2-E3 cascade. SidE-mediated reactions, together with the activities of dedicated deubiquitinases, generate ADP-ribosylated ubiquitin (ADPR-Ub) and phosphoribosyl ubiquitin (PR-Ub), Neither species can participate in canonical ubiquitination, thereby perturbing host ubiquitin signaling pathways that are critical for both cellular homeostasis and bacterial virulence. LnaB was originally identified as a potent activator for NFkB, yet neither the biochemical basis of such activity nor the host signaling pathway it engages has been defined. In this talk, I will discuss the unique phosphoryl AMPylation reaction catalyzed by LnaB to convert PR-Ub into ADPR-Ub, which is subsequently processed by another effector, MavL, to generate ADP-ribose and naive ubiquitin. I will also discuss the molecular mechanism by which LnaB activates NFkB through a conserved host signaling pathway.

M32 Multifaceted strategies for *Legionella* pneumophila evasion of host autophagy.

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Legionella species have coevolved with protozoan host for hundreds of millions of years. One of the hallmarks of successful host cell infection is the ability of *Legionella* to evade fusion of the phagosome in which it resides with degradative endocytic organelles. This evasion process requires delivery of bacterial proteins into the host by the Type IVb secretion system called Dot/Icm, which leads to the establishment of a novel endoplasmic reticulum-derived organelle that supports bacterial replication. Eukaryotic cells have cell intrinsic innate immune receptors that recognize damaged or foreign organelles and target them for destruction by a pathway called autophagy. However, vacuoles containing *Legionella* have a remarkable ability to evade being targeted by the autophagy pathway. We have investigated the mechanisms by which *Legionella* can evade autophagy. These studies have identified multiple *Legionella* proteins that have diverse biochemical functions capable of preventing autophagic recognition of the vacuole containing *Legionella* and enable *Legionella* to avoid destruction by the autophagy pathway.

PF01 / P001 From source to tap: mapping the water distribution system microbiome and analyzing opportunistic pathogens at species-level resolution

Dr. Pierre Foucault[1], Ms. Sandra Manco[2], Mr. Vincent Lasserre[3], Dr. Sébastien WURTZER[2], Dr. Vincent Delafont[3], Prof. Yann Hechard[3], Dr. Laurent Moulin[2], Prof. Carmen Buchrieser[1], Dr. Laura Gomez Valero[4]

1. Biology of Intracellular Bacteria, Pasteur Institute, Paris, France
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4. Biology of Intracellular Bacteria, Pasteur Institute, Paris, France

Ensuring the microbiological safety of drinking water is a public health priority. Opportunistic waterborne pathogens—such as *Legionella*, *Pseudomonas* and non-tuberculous mycobacteria— persist in drinking water distribution systems (DWDS) by strategies such as colonizing biofilms or infecting protists. This poses severe risks to vulnerable populations mainly through inhalation of contaminated aerosols or tap water misuse. Despite the rising incidence of associated infections linked to climate change and demographic aging, our understanding of microbial communities from DWDS remains limited. Existing research often lacks the necessary spatial-temporal scale and taxonomic resolution to elucidate the drivers of their diversity, composition and dynamic from DWDS source to tap. Here, we characterized the diversity, composition and dynamics of microbial communities at multikingdom level for two independent networks of the Paris DWDS. We sampled at a high spatial resolution (from source to tap) and temporal (bi-weekly over a year) scale. Our innovative multimetabarcoding approach coupling universal prokaryotic and eukaryotic primers with primers targeting *Legionella* or *Pseudomonas* spp. allowed us to achieve species-level resolution. Selected opportunistic pathogens and amoebae were quantitatively tracked using dPCR. The multi-kingdom composition of the communities was strongly reshaped along the distribution systems. Opportunistic pathogens and amoeba persisted at lower total abundances after treatment but reached higher concentrations in tap water. Interestingly, tap water communities converged and became similar regardless of their distinct sources and the different treatment processes. Excitingly, the genus-targeting approach revealed a broad diversity of *Legionella*, much of which remains uncharacterized. While overall *Legionella* and *Pseudomonas* diversity sharply declined after treatment, *L. pneumophila*, pathogenic *Pseudomonas* clades and *P. aeruginosa* were detected in all environments. Our findings offer critical insights into how water parameters and ecological interactions could influence the composition of the DWDS microbiome, while the high taxonomic resolution achieved underscores the ecological adaptability of the studied opportunistic waterborne pathogens.

PF02∗ / P002∗ Experimental evolution of copper resistance in *Legionella* pneumophila also selects for improved recovery after stress

Ms. Gillian Cameron[1], Prof. Sebastien P. Faucher[1]

1. McGill University, Montreal, Canada

The opportunistic waterborne pathogen *Legionella pneumophila (Lp)* frequently encounters copper in engineered water systems (EWSs) in the form of copper pipes. While metal ions leached from the copper pipes may initially help reduce *Lp* populations, over time copper resistance can emerge. To date, copper resistance has primarily been observed to arise through the acquisition of resistance genes, but the emergence of copper resistance via the accumulation of point mutations may also occur. Indeed, copper-resistant *Lp* isolates have been found within copper pipes that contained only a handful of single nucleotide polymorphisms differentiating them from their copper-sensitive counterparts within the same EWS. Using an experimental evolution approach, we generated 6 copper-resistant lineages of *Lp*. Over their evolution, these copper-exposed populations became resistant to 0.32 mM CuCl2. The ancestral strain was resistant to 0.08 mM CuCl2. Control lineages, not exposed to copper, were used to identify adaptations to *in vitro* growth. Twenty unique mutations were identified in the copper-exposed lineages, none of which were present in the control lineages. Notably, 3 of the 6 copper-resistant lineages acquired a point mutation in the promoter region of *lpg0886,* a glutamate/sodium symporter, suggesting *lpg0886* is important for surviving copper stress. This was the only mutation fixed in multiple copper-resistant lineages. A Δ*lpg0886* mutant showed reduced survival in water compared to its ancestral strain. In a complementation study, we found that while *lpg0886* is not directly involved in surviving copper toxicity it is essential for recovery in liquid medium after copper stress. A merodiploid strain carrying *lpg0886* in a Δ*lpg0886* genetic background recovered significantly better than the Δ*lpg0886* mutant and the ancestral strain after copper treatment. Our research shows that repeated copper stress not only facilitates the development of resistant *Lp* strains, but the resulting resistant lineages are more easily revived after the treatment is applied.

∗Student Paper

PF03 / P003 Association between genetic lineage and antimicrobial susceptibility in clinical and environmental *Legionella* pneumophila isolates from Japan

Ms. Shoko Komatsu[1], Ms. Tomoka Iwamoto[1], Dr. Noriko Nakanishi[1]

1. Kobe Institute of Health, Kobe, Japan

Macrolides and fluoroquinolones are first-line treatments for Legionnaires' disease. However, clinical and environmental strains exhibiting resistance or reduced susceptibility to these agents have recently been reported. As antimicrobial susceptibility testing is not routinely performed, global data remain limited, with particularly few reports from Japan. In this study, we investigated the association between antimicrobial susceptibility and genetic lineage in 246 *Legionella pneumophila* isolates collected between 2003 and 2025, including clinical isolates (n=35) and environmental isolates obtained from bath water (n=100), cooling tower water (n=25), influent wastewater (n=43), and rivers (n=43) in Kobe City, Japan. A minimum spanning tree based on sequence-based typing revealed that three genetic lineages, regardless of their source, carried the genes *lpp2879* (*lpeA*) and *lpp2880* (*lpeB*), which encode components of the efflux pump LpeAB. These isolates exhibited reduced susceptibility to azithromycin, with minimum inhibitory concentrations of 0.75–1.5 µg/mL, as determined by the gradient method using Etest (bioMérieux), whereas LpeAB-negative isolates showed minimum inhibitory concentrations ≤ 0.38 µg/mL. Notably, the efflux pump genes were also detected in genetic lineages unique to Japan. No levofloxacin- or ciprofloxacin-resistant isolates were detected. Overall, this study provides valuable data on the antimicrobial susceptibility of *Legionella* isolates from Japan, contributing to future treatment strategies and surveillance efforts.

PF04 / P004 Targeted degradation of SidJ as a strategy against *Legionella* pneumophila infection

Dr. Giulio Giuliani[1], Dr. João Mello-Vieira[1], Dr. Rubina Kazi[1], Ms. Sara Garcia Torres[2], Dr. Koraljka Husnjak[1], Dr. Thorsten Mosler[1], Dr. Melissa Bernhardt[3], Dr. Chuang Li[4], Ms. Adela Vidov[1], Dr. Henry Bailey[1], Prof. Zhao-Qing Luo[4], Dr. Mohit Misra[1], Dr. Andreas Schlosser[3], Prof. Stephan Goettig[2], Prof. Ivan Dikic[1]

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Proteolysis-targeting chimeras (PROTACs) bring a target protein in proximity to an E3 ubiquitin ligase to induce its ubiquitination and proteasomal degradation. Recently, PROTACs that hijack a prokaryotic protease were proposed as anti-mycobacterial agents, raising the possibility of using small molecule degraders against infectious diseases. Nevertheless, the cell envelope of Gram-negative bacteria poses a significant challenge to this kind of approach. Therefore, here we propose an alternative approach where SidJ, a protein secreted by *Legionella pneumophila*, is degraded through the ubiquitin-proteasome system inside host cytoplasm. We demonstrated that SidJ deletion resulted in defective *L. pneumophila* proliferation inside human cells and decreased virulence. We discovered that SidJ is ubiquitinated and degraded by the proteasome when overexpressed in host cells. Therefore, we applied the dTAG technology to show that SidJ is efficiently degraded by dTAG-13 through CRBN E3 ligase. Using a *L. pneumophila* strain expressing dTAG-SidJ, we demonstrated that dTAG-13 treatment induced SidJ degradation in infected cells and it decreased its virulence. We thus propose a PROTAC-based pharmacological modality against bacterial pathogens that secrete virulence factors into the host cytoplasm.

PF05 / P005 Elucidating genetics of *Legionella* micdadei biofilm and amyloid production through transposon mutagenesis screening

Dr. Christa Chatfield[1], Mr. M. Saliou Diallo[1], Ms. Morgan Raymond[1]

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Legionella micdadei (*Lmic*) is known to cause 1.8-3% of *Legionella* infections. This project investigates surface attachment and biofilm physiology of *Lmic*. Like *Legionella pneumophila* (*Lpn)*, we hypothesize that *Lmic* is capable of surface attachment and biofilm growth within water systems, leading to human exposures. Indeed, *Lmic* cases or outbreaks (one of atypical pneumonia, and two of Pontiac fever) have been traced to *Lmic* from contaminated, built-environment water sources. While several genes are known that contribute to *Legionella pneumophila* (*Lpn)* biofilm growth and surface attachment, there are no known reports on *Lmic* biofilms or their genetic basis. Our data shows *Lmic* biofilms in a low-nutrient, biofilm-specific growth media (MSBB) have a similar structure to *Lpn* biofilms. Both biofilms contain amyloid or amyloid-like structures detected via amyloid-specific staining and fluorescence microscopy. These highly insoluble and proteinase-resistant fibrils may form resilient biofilm structures that promote *Legionella* biofilm formation on the interior surfaces of built-environment water systems. While many functional amyloid proteins are known to contribute to bacterial biofilms, no homologs of known amyloid proteins are found in the *Lmic* or *Lpn* genomes. Transposon mutagenesis is in progress to screen *Lmic* mutants for altered surface structures via changes in Congo red staining of colonies (grown on Congo red-containing media). Congo-redaltered colonies are then further screened for biofilm phenotypes before identification of the site(s) of mutations by whole genome sequencing. Mutants with increased Congo red staining have so far been the majority of the altered phenotypes (>500 screened mutants), many which also show decreased biofilm formation in BYE media (on glass tubes). Work continues to characterize and describe the mutations in these strains to uncover biofilm and surface adhesion mechanisms of *Lmic,* with the potential to reveal both novel *Lmic* virulence factors and shared factors as found in other *Legionella* species.

PF06/ P029 Lipid droplets: Emerging targets in *Legionella* pneumophila pathogenesis

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1. Institute of Medical Microbiology, University of Zürich, Zürich, Switzerland
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Legionella pneumophila is a Gram-negative intracellular pathogen that causes severe respiratory infection through exploitation of host cell machinery. Recent evidence demonstrates that lipid droplets (LDs), energy-rich cellular organelles traditionally associated with lipid storage, serve as metabolic platforms hijacked by *L. pneumophila* during infection. In addition, LDs have been implicated in various signalling pathways and processes, including innate immunity. This study investigates the molecular mechanisms by which this pathogen manipulates host lipid droplet dynamics to facilitate intracellular replication. Our lab has previously demonstrated that *Legionella*-containing vacuoles localize adjacent to lipid droplets, positioning the pathogen for direct access to lipids. This co-localization is dependent on the presence of a functional type IV Dot/Icm secretion system. Other labs have shown that *L. pneumophila* does indeed encode at least one type-IV-secreted effector that directly targets LDs. In this study, we aim to elucidate the involvement of novel effectors on LD dynamics and intracellular replication using the genetically tractable amoeba *Dictyostelium discoideum* and the macrophage cell line RAW 264.7. Using a mass spectrometry approach, we aim to identify bacterial effectors targeting host LDs and characterize their functional role during infection. The results of this study will not only broaden our understanding of how *L. pneumophila* manipulates the host cell, but also further elucidate the role LDs play for infection and immunity.

PF01 / P001 From source to tap: mapping the water distribution system microbiome and analyzing opportunistic pathogens at species-level resolution

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S06*/ P006* Capsule-mediated lethality in *Legionella* longbeachae is associated with host immune dysregulation and increased NET formation

Mr. Pedro Henrique Medeiros Câmara[1], Ms. Larissa Augusta Rodrigues de Oliveira[1], Dr. Danielle Pini Alves Mascarenhas[1], Prof. Dario Simões Zamboni[1]

1. Department of Cell and Molecular Biology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

Bacteria of the genus *Legionella* are intracellular Gram-negative pathogens that can cause Legionnaires' disease, a severe atypical pneumonia. *Legionella longbeachae* has emerged as an important pathogen responsible for outbreaks worldwide and exhibits increased lethality and higher replication rates than other *Legionella* species in murine infection models. Genomic and transcriptomic analyses identified capsule biosynthesis genes (*ctrC* and *ctrD*) in *L. longbeachae*. More recently, the capsule was shown to be required for virulence, although the mechanisms underlying its contribution to disease pathogenesis remain incompletely understood. Therefore, this study investigated the role of the capsule in *L. longbeachae* pathogenicity. By comparing mutant strains lacking *ctrCD* with capsule-producing strains, we observed that the capsule is essential for infection-associated lethality, as its absence dramatically reduced mortality in infected animals. We found that both encapsulated and non-encapsulated bacteria induced an early depletion of alveolar macrophages that persisted throughout infection. However, only the encapsulated strain induced a transient increase in pulmonary IL-6 and TNF-α production. Despite only a modest increase in bacterial replication, capsule expression impaired the recruitment of inflammatory monocytes and monocyte-derived macrophages, resulting in defective restoration of the pulmonary myeloid compartment. At later stages of infection, encapsulated bacteria induced increased NET formation in lung tissue and were associated with enhanced lung injury, as evidenced by morphometric analyses, bronchoalveolar lavage protein leakage, and elevated LDH levels. In tissue culture experiments, capsule expression increased epithelial cell infection and directly promoted NET formation in neutrophils, suggesting potential mechanisms contributing to tissue damage. Collectively, our findings indicate that capsule-mediated virulence is driven primarily by dysregulation of host immune responses rather than enhanced bacterial replication. We propose that defective myeloid replenishment impairs restoration of pulmonary homeostasis, promoting tissue damage and mortality. Increased NET formation and enhanced epithelial cell infection may further contribute to capsule-associated pathogenicity and warrant further investigation.

**Student Paper*

S16* / P007* The *Legionella* effector MavP targets host cell PMP70 to recruit peroxisomes to the replication vacuole

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1. Johns Hopkins University School of Medicine, Baltimore, MD, USA

Legionella pneumophila translocate over 300 proteins termed effectors into their host cell. A subset of effectors is important for maintaining the integrity of the *Legionella* containing vacuole (LCV), which is critical for protection from host immune surveillance systems. Here we show that one of these effectors, MavP, decorates the surface of the LCV and interacts with the host peroxisomal membrane protein PMP70, allowing *L. pneumophila* to recruit peroxisomes to the LCV. Domain mapping and mutagenesis studies have identified a critical functional motif of MavP for interacting with PMP70 and peroxisome recruitment to the LCV. We found that MavP accumulation and peroxisome recruitment to the LCV coincides with the onset of bacterial replication and the need for LCV membrane expansion to accommodate increased bacterial numbers. Peroxisomes play a fundamental role in host cell lipid metabolism and homeostasis. Importantly, in peroxisome-deficient macrophages, LCVs exhibit a compact architecture and a higher frequency of destabilization, similar to what is observed when host lipid availability is limited. Furthermore, disruption of peroxisome lipid metabolic pathways for glycerophospholipid and ether lipid synthesis results in loss of LCV integrity and impaired bacterial growth. These observations have led to the model that *L. pneumophila* exploit host cell peroxisomes to expand and maintain the integrity of their intracellular replication compartment that is critical for pathogenesis.

**Student Paper*

P008* Gelsolin promotes lung health, neutrophil function, and mitochondrial respiration in severe *Legionella* pneumophila infection

Mr. Owen Whitham[1], Dr. Amal Amer[1]

1. The Ohio State University, Columbus, OH, USA

Legionella pneumophila (*L. pneumophila*) is the causative agent of Legionnaires' disease, a severe and potentially fatal bacterial pneumonia. Due to the difficulty in diagnosing Legionnaires' disease at early stages of infection, cases are vastly underreported, and treatment is often delayed. As a result, rapid-acting, broad-spectrum therapies are needed to effectively treat the infection and pathology without contributing to antibiotic resistance. Here we show that gelsolin knockout (*gsn*-/-) mice succumb more quickly to severe *L. pneumophila* infection despite showing no difference in bacterial loads in the lung compared to wild type mice (WT). There is an increase in the neutrophil attracting chemokine CXCL1/KC in the lungs and macrophages of *gsn*-/- mice, which is accompanied by an increase of neutrophils and apoptosis in their lungs. Notably, neutrophils lacking gelsolin produce fewer NETs, and their mitochondrial capacity is diminished in response to *L. pneumophila*. Gelsolin also showed to be required for maintaining mitochondrial network morphology and respiration in *L. pneumophila* infected macrophages. When given recombinant gelsolin protein, *gsn*-/- mice survive significantly longer during severe *L. pneumophila* infection, with reduced lung pathology, and their macrophages produce fewer inflammatory cytokines. Together, gelsolin protects mice from severe *L. pneumophila* infection, dampens inflammation, promotes mitochondrial health, and maintains neutrophil metabolism and NET formation.

P009 Comprehensive analysis of a major Legionnaires' disease outbreak linked to a public bath facility in Hiroshima, Japan, employing PFGE, SBT, MLVA, and whole-genome sequencing

Dr. Noriko Nakanishi[1], Ms. Shoko Komatsu[1], Dr. Ryohei Nomoto[1], Dr. Junko Amemura-Maekawa[2], Mr. Takahiro Hiratsuka[3]

1. Kobe Institute of Health, Kobe, Japan
2. Japan Institute for Health Security, Tokyo, Japan
3. Hiroshima Prefectural Technology Research Institute, Hiroshima, Japan

In 2017, a Legionnaires' disease outbreak involving 58 patients occurred at a public bath facility in Hiroshima Prefecture, Japan. To investigate the source and genetic characteristics of the causative strains, we analyzed 94 *Legionella pneumophila* isolates obtained from both clinical and environmental samples. Multiple molecular typing methods were applied, including pulsed-field gel electrophoresis (PFGE), sequence-based typing (SBT), multi-locus variable-number tandem-repeat analysis (MLVA), and whole-genome sequencing (WGS). The results from these typing approaches showed strong concordance. Environmental samples revealed a wide diversity of genotypes, whereas only two sequence types, ST2398 and ST2399, were identified among patient isolates. These sequence types were also detected in bath water and surface swab samples and were traced to a single circulation system within the facility. Whole-genome sequencing-based single nucleotide variant (SNV) analysis demonstrated that ST2398 and ST2399 represent closely related, previously unreported genotypes forming a highly clonal lineage. Haplotype network analysis indicated that these two sequence types differed by approximately 30–42 SNVs, while some environmental isolates differed from patient isolates by as few as 0–3 SNVs. These findings indicate that the outbreak was caused by *L. pneumophila* strains belonging to the ST2398 and ST2399 clades. In addition, at least three patients were co-infected with multiple genetic clusters of *L. pneumophila* SG1. Overall, this study highlights the importance of analyzing multiple isolates from single samples to capture genetic diversity and detect co-infection when investigating Legionnaires' disease outbreaks. (Hiratsuka T., et al. Int J Med Microbiol. 2025.)

P010 Contribution of genomic capture approaches to investigate the source of contamination in legionellosis cases

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1. Centre national de référence des légionelles, Hospices Civils de Lyon, Lyon, France

A major challenge in legionellosis investigations is establishing a genetic link between environmental and clinical isolates. This is traditionally achieved through bacterial culture followed by sequencing and genomic comparison, but culture frequently fails, preventing identification of the causative strain. To overcome this limitation, the LEGIO-HOME project is developing an hybridization-based genomic capture approach enabling direct sequencing of *L. pneumophila* from clinical samples. This technique relies on specific DNA probes designed to selectively capture *L. pneumophila* genomic fragments prior to next-generation sequencing, allowing genomic analysis even in the absence of cultured isolates. Using a reference dataset comprising more than 8,000 genomes, we designed a panel of 93,228 probes targeting the *L. pneumophila* genome. The capture method was applied to a panel of 68 DNA extracts originating from respiratory clinical samples collected during routine diagnostic investigations of suspected legionellosis cases. All extracts tested positive for *L. pneumophila* by PCR with cycle threshold (Ct) values ranging from 20 to 36 and both culture-positive (n=37) and culturenegative (n=31). Sequencing was performed on an Illumina NextSeq platform and reads were mapped against the L. pneumophila reference genome to assess genome coverage and sequence type assignment. This approach allowed the recovery of up to 95% of the bacterial genome. A complete sequence type (ST) was obtained in 16 samples and genome coverage was most optimal when the method was used on samples with Ct<30. These results demonstrate that genomic capture can recover substantial genomic information in the absence of cultured isolates. Therefore, this capturebased genomic approach represents a promising tool for the molecular investigation of legionellosis cases. By enabling high-resolution genomic analysis directly from complex samples, it may significantly improve the ability to link clinical cases with environmental sources and strengthen source attribution investigations.

P011* Strain-dependent and regulatory determinants of CMIT/MIT susceptibility in *Legionella* pneumophila

Ms. Simran Kaur[1], Prof. Sebastien P. Faucher[2]

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CMIT/MIT (5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one) is a nonoxidant biocide widely used for *Legionella* control in engineered water systems. It exerts its antimicrobial activity by interacting with cellular thiols to form mixed disulfides, thus inactivating key metabolic enzymes. While the efficacy of CMIT/MIT against *Legionella* has been established decades ago, the bacterial stress-response mechanisms that modulate susceptibility remain poorly defined. The goal of this study is to identify genetic determinants of resistance to CMIT/MIT in *Legionella.* We evaluated the susceptibility of *Legionella pneumophila* strains Philadelphia-1, JR32, KS79, and various mutant strains to CMIT/MIT across a concentration range of 0.05 to 16 parts per million (ppm) in a tap water-based matrix with pH and hardness adjusted to match typical cooling tower water. Ancestral strain Philadelphia-1 showed a 3-log reduction after 6 hours of exposure to only 0.5 ppm of biocide. KS79, the *comR* mutant of JR32 with high natural competence, demonstrated increased tolerance. For instance, treatment with 8 ppm CMIT/MIT for 2 hours resulted in more than 200-fold lower killing of KS79 compared to Philadelphia-1. JR32 displayed a susceptible profile indistinguishable from Philadelphia-1. The results suggest that competence-associated physiological states, rather than laboratory domestication, contribute to tolerance under elevated biocide stress. To investigate the role of regulatory stress pathways, the susceptibility of isogenic deletion mutants of RpoS and LetA was evaluated. The deletion of *rpoS* resulted in a significant increase in CMIT/MIT susceptibility, which was restored by complementation. In contrast, *letA* deletion mutants retained susceptibility comparable to wild-type strains, suggesting a limited role of transmissive phase regulation. To summarize, the study identifies that competence and the global stress regulator RpoS are key determinants of *Legionella* resistance to CMIT/MIT. Further studies are needed to define the molecular basis of the pathways underlying these phenotypes and to evaluate their ecological relevance.

P012 LME-1 is the tip of the iceberg: a diversity of LME-type phages in L. pneumophila and other species.

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We recently reported the isolation of the first infectious *Legionella* bacteriophage, LME-1, and a link between *L. pneumophila* virulence and the acquisition of a phage defense gene. We present here on our recent findings that LME-1 is a frequent, globally distributed threat to *L. pneumophila*: it is present as a prophage in more than 100 sequenced strains and targeted by CRISPR-Cas in several hundred additional isolates from around the world. We have also identified prophages in two other *Legionella* species (*L. anisa* and *L. maceachernii*) that are distinct from LME-1 but share some of its strategies for integration into the host chromosome and regulation of replication. A previously identified prophage in *L. micdadei* also shares these characteristics, and genetic evidence suggests that the *L. micdadei* prophage can also infect *L. maceachernii*. Comparative analysis of the LME-type phage genomes suggests ongoing adaptation to specific bacterial hosts as well as the potential for genetic exchange. In addition to these observations, we will present on our recent efforts to activate these diverse LME-type phages so that we can investigate their host range, virion structures, and effects on *Legionella* viability.

P013* Proteomic analysis of the surface of *Legionella* pneumophila

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Bacterial respiratory infections are a major global public health concern, having caused approximately 4 million deaths in 2019 (K. S. Ikuta et al., 2022). A key factor in bacterial pathogenicity is the ability of bacteria to dynamically remodel their cell surface in response to host signals. In *Legionella pneumophila*, the causative agent of Legionnaires' disease, pulmonary collectins such as SP-A and SP-D can bind to the bacterial surface, thereby altering its physiology and potentially its virulence (K. Sawada et al., 2010). *L. pneumophila* counteracts this immune recognition by secreting a metalloprotease, ProA, which directly cleaves SP-D. Degradomic analyses of infected lung samples have also identified other ProA substrates, suggesting a broader role in modulating host-pathogen interactions. To explore the possibility of additional interactions between host proteins and *Legionella*, we performed an unbiased chemical screening using broncho-pulmonary samples from patients with legionellosis. This approach led to the identification of several previously unknown interactants. Exposure of *L. pneumophila* to these clinical samples caused a substantial remodeling of its surface proteome, indicating that the pulmonary environment plays an active role in bacterial adaptation. Taken together, these results reveal new host-pathogen interactions and suggest that the remodeling of *L. pneumophila's* surface in response to host signals is more dynamic and complex than previously thought. This opens new avenues for the development of therapeutic strategies that target the initial interactions between the host and the bacterium, disrupting these key interactions and limiting the pathogen's virulence.

P014 An NAD(H)-depletion-activated phospholipase promotes fluoroquinolone-mediated bacterial killing and autolysis

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Introduction: We recently discovered an unusual regulatory mechanism controlling the *Legionella pneumophila* phospholipase and virulence factor PlaB (1). Physiological NAD(H) concentrations stabilize inactive PlaB tetramers, whereas low NAD(H) levels trigger deoligomerization into highly active dimers. If not localized at the bacterial surface, these dimers threaten *Legionella* by disturbing the unprotected inner membrane. Bacterial NAD(H) can be depleted by NAD-hydrolyzing enzymes such as NADases. Recent studies showed that ciprofloxacin-induced genotoxic stress activates the GndRX NADase toxin-antitoxin module in *L. pneumophila*, which efficiently depletes cytosolic NAD(H) (2). We therefore hypothesized that efficient fluoroquinolone-mediated killing and lysis of *L. pneumophila* depends on PlaB, making it a potential drug target. **Aim:** Does activation of PlaB improve killing of *L. pneumophila* in cultures and infected host cells upon fluoroquinolone exposure? **Results:** Consistent with a central role of NAD(H) in PlaB-regulation, gene co-occurrence analyses revealed conservation of *plaB* with genes encoding NAD(H) consumers. Co-expression of an NADase toxin and PlaB in *E. coli* and *L. pneumophila* depleted cytosolic NAD(H) and accelerated bacterial death. Lipid analysis of *E. coli* expression cultures showed that cytosolic PlaB-activation causes membrane phospholipid digestion, suggesting that toxin-driven NAD(H) depletion induces selfdigestion when PlaB is present. Gene expression analysis in *L. pneumophila* further showed coregulation of *plaB*, toxin, and antitoxin genes, all of which are induced by fluoroquinolone exposure (2) and prolonged stress. Together, these findings support a model in which PlaB is functionally linked with an NADase effector module as part of its stress response. PlaB activation could therefore be exploited to sensitize *L. pneumophila* to antibiotic regimens. **Summary:** PlaB can act as a cell death effector and autolysin when cytosolic NAD(H) levels are low, making it a promising future drug target. It also co-occurs with NADase genes in *L. pneumophila* and other organisms. **References:** 1) PMID:34074754 2) PMID:40825874

P015 LEGIODOM: exploratory study on sporadic cases of legionellosis and exposition to domestic water distribution system

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In France, potential sources of contamination are investigated by genomic comparison between clinical and environmental strains in only 4% of notified cases. Domestic water distribution systems (DWDS) represent only 39% of all investigations, yet when they are investigated, they are identified as the source in 84% of cases. LEGIODOM is a nationwide prospective exploratory study involving regional health agencies for case recruitment and aiming to estimate the proportion of legionellosis cases linked to DWDS. Secondary objectives include assessing the genetic diversity of environmental *Legionella* strains in DWDS. Eligible participants are patients residing in mainland France, hospitalized with a *Legionella*-positive lower respiratory tract sample (by PCR and/or culture) between October 1, 2024, and September 30, 2026. Inclusion criteria include having spent at least one day at home within 14 days before symptom onset. For each case included in the study, 2-4 sampling points within the DWDS are collected and analyzed for *Legionella* by PCR and culture. As of May 31, 2026, a total of 650 home visits were conducted. Among the investigated DWDS, 408 (69%) tested positive for *L.* non *pneumophila* (Lnp) and 77 (13%) for *L. pneumophila* (Lp) by PCR. *Legionella* strains were isolated from 78 (15%) DWDS (9 positives for Lnp and 69 for Lp). On average, 12 (range: 1-40) colonies were sequenced per DWDS, with up to 10 colonies per sampling point. A high level of diversity was observed within DWDS, with multiple species, serogroups, and sequence types coexisting. Genetic diversity was also observed among isolates belonging to the same ST, reflected by differences in single nucleotide polymorphisms. This diversity may influence conclusions when comparing environmental and clinical strains if only a limited number of colonies are isolated and sequenced. Increasing sequencing efforts for environmental isolates may therefore improve source attribution when contamination from DWDS is suspected.

P016* Contribution of pulmonary tissue from exhumed remains to an investigation of a *Legionella* pneumophila ST16 outbreak

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Introduction An outbreak of Legionnaires' disease (LD) occurred in Paris between August 26 and September 29, 2024, involving 17 cases, including two fatalities. Whole genome sequencing data from nine *Legionella pneumophila* serogroup 1 (Lp1) ST16 environmental isolates and five Lp1 ST16 clinical isolates identified a cooling tower as the source of infection. More than three months after the outbreak, the remains of the two deceased patients were exhumed for legal investigations. This study aimed to assess whether the source of contamination could still be investigated despite poor preservation of the samples from exhumed patients**. Methods** For each patient, Lp1 PCR was performed on pulmonary tissue (n=2), pleural effusion (n=2), and cardiac blood (n=2) and detection of *Legionella* antigen in urine sample for patient A. Nested PCRbased sequence-based typing (NSBT) of seven target genes was performed on PCR-positive samples and metagenomics are planned. Pulmonary tissue and pleural effusion samples were cultured on charcoal selective media**. Results.** For patient A, Lp1 LPS was detected in urine sample. PCR was positive in pulmonary tissue samples (2/2). NSBT performed on DNA from the right lung identified ST16. Given the low prevalence of ST16, these findings are consistent with contamination from the implicated cooling tower. For patient B, PCR was positive in pulmonary tissue (2/2), pleural effusion (2/2), and cardiac blood (1/2). NSBT yielded an incomplete allelic profile (2, 10, 0, 10, 0, 1, 9), compatible with ST16, ST685, and ST1193. As ST685 and ST1193 are rarely reported in LD, contamination from the cooling tower cannot be excluded. **Conclusions** Despite negative cultures, Lp1 was detected by PCR in on 3-month-old exhumed remains from both patients, with complete and partial ST16 identification by NSBT in patients A and B, respectively. Overall, these findings highlight the potential use of exhumed remains in LD outbreak investigations.

P017 enhanced detection of *legionella* serogroups using dual-antigen clia technology: the liaison *legionella* urinary antigen assay

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Background *Legionella* infections are a major cause of severe pneumonia worldwide. Conventional urinary antigen tests (UATs) primarily detect *Legionella pneumophila* serogroup 1, leaving a diagnostic gap for non- SG1 strains and other species such as *L. longbeachae*. Broader detection is essential for accurate diagnosis and timely treatment. **Methods** The LIAISON® *Legionella* Urinary Antigen assay is a fully automated chemiluminescent immunoassay (CLIA) designed to improve inclusivity and sensitivity by using a novel design. It employs a dualantigen strategy targeting lipopolysaccharide (LPS) and peptidoglycan-associated lipoprotein (PAL). LPS ensures serogroup-specific recognition, while PAL, highly conserved across *Legionella* species, enables detection beyond SG1. Analytical performance was assessed using urine samples from patients with suspected pneumonia. **Results** The assay has been thoroughly validated to deliver sensitive and specific detection of all major clinically relevant *Legionella* serotypes. Validation across multiple reagent lots and both LIAISON® XL and XS analyzers confirmed consistent performance. The test detects *L. pneumophila* serogroups 1, 3, 4, 5, 6, 11, and additional strains including *L. longbeachae*, with detection limits below 10 ng/mL for purified antigens and 0.25 × 104 CFU/mL for whole cells. Comparative studies demonstrated superior sensitivity versus competitor assays, particularly for non-SG1 strains. A critical feature ensuring broad sensitivity is the use of two antibody pairs: one targeting LPS (monoclonal + polyclonal) and another targeting PAL (monoclonal), enhancing coverage and accuracy. Precision testing showed excellent reproducibility (%CV within acceptance criteria), high specificity with no cross-reactivity across 43 microbial species, and robustness against common urinary interferents. The test runs on the LIAISON® Analyzer family, delivering full automation, traceability, objective interpretation, and streamlined workflow, reducing error risk and ensuring certified results without repeat testing. **Conclusions** By combining LPS and PAL targets, the LIAISON® *Legionella* assay overcomes conventional UAT limitations, offering broad serotype coverage and high diagnostic accuracy for improved patient care.

P018 Expansion, restructuring and characterization of the Legionellaceae family

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Legionellaceae are a large and widespread family of facultative intracellular bacteria with high clinical relevance. While *Legionella pneumophila*, the most clinically relevant species, is relatively well studied, very limited information is available regarding the other species belonging to this family, despite its only recognized genus (i.e., *Legionella*) is included in several drinking water regulations worldwide. Here, we analyzed all publicly-available *Legionellaceae* genomes and metagenomeassembled genomes, nearly doubling the number of recognized species and highlighting the need to restructure the family's taxonomy to include multiple genera that present distinct clinical relevance. We found that secretion systems and traits linked to host invasion vary significant across genus clusters and species, likely influencing their host range and survival in their environment. Genus clusters exhibited variations in metabolic capabilities, but species closely related to *L. pneumophila* generally displayed more complete metabolic pathways and the ability to synthetize most amino acids, suggesting a less strict intracellular lifestyle. On the contrary, reduced genome size and the lack of poly-3-hydroxybutyrate biosynthetic potential strongly suggests a stronger host association of specific deep-branching clades, detect especially in genomes of marine origin. Finally, we showed that species are present in a variety of host-associated environment and that while they have distinct environmental distributions, niche overlap increases at higher metabolic similarity. Together, our results shed light on the ecology of these microorganisms, highlight the diversity of traits that can occur in closely related facultative intracellular bacteria, and call for the restructuring of this clade to align with genomic information and clinical relevance to aid the management of *Legionellaceae* bacteria.

P019 Hotspot mutations in the *Legionella* genome expose strategies for stress response

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A common strategy for studying microbial virulence factors is reverse genetics, a method whereby a gene of interest is deleted from the genome and phenotypic effects are analyzed. Clone selection involves their exposure to a series of selective pressures. We found that *Legionella pneumophila* clones respond to these stressors by acquiring unsought background mutations at an elevated rate. In fact, we found that 93 out of 146 examined clones (64 percent) had acquired additional mutations following allelic exchange, with 45 percent of mutations found in the genes encoding the LetA/LetS two-component system and another 13 percent of mutations found in the gene encoding Ceg35. The overwhelming number of mutations in *letA* or *ceg35* caused frameshifts and were predicted to result in loss of function of the encoded protein. Tracking a frequently observed frameshift mutation in *letA* throughout the allelic exchange protocol at single colony resolution revealed that this mutation arose steadily at each passage step and at a reproducible frequency. Notably, mutation of either *letA* or *ceg35* reduced the sensitivity of *L. pneumophila* to salt, suggesting that the integrity of the Dot/Icm type IV secretion system (T4SS) had been compromised. Consistent with this, translocation of the effector RalF into host cells was reduced. Remarkably, the frequency of background mutation acquisition during allelic exchange was dramatically reduced in strains with a non-functional T4SS, suggesting that unregulated leakage of metabolites through the T4SS may have triggered the mutations. Our findings tell a cautionary tale where genetic manipulation of *L. pneumophila* via allelic exchange may inadvertently affect its biological fitness by inducing unwanted mutations in select genomic hotspots.

P020* SidE family effector SdeC contains a cryptic PI(3)P-binding domain

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Phosphoinositide (PIP) lipids serve critical roles in eukaryotic membrane organization. Reversible phosphorylation of the inositol headgroup yields seven distinct PIP species, which help define organelle identity and regulate membrane trafficking. Numerous studies have shown that secreted effectors from *Legionella pneumophila* exploit host PIPs as membrane identity cues to achieve precise membrane targeting during infection. While eukaryotes possess several well characterized and highly conserved PIP-binding domains, the molecular mechanisms underlying PIP recognition by bacterial effectors remain poorly understood. We and others have demonstrated that several *Legionella* effectors engage PIPs using four-α-helix bundles. Here, we identify and characterize a previously unrecognized PI(3)P-binding domain within the SidE family effector, SdeC. Protein-lipid overlay assays revealed a C-terminal region that is sufficient for PI(3)P binding. Bio-layer interferometry (BLI) was employed to measure PIP-binding affinity, while membrane targeting was evaluated by co-transfecting fluorescently tagged SdeC with PIP and organelle biosensors in mammalian cells. The SdeC PIP-binding domain alone exhibited PIP binding ability comparable to fulllength SdeC and bound PI(3)P with micromolar affinity. Consistent with these findings, the SdeC PIPbinding module strongly co-localized with PI(3)P biosensor 2xFYVE and PI(3)P-enriched endocytic vesicles in host cells. To define the structural basis of lipid recognition, we determined the crystal structure of SdeC PIP-binding module in complex with Ins(1,3)P2. The structure revealed that residues R967 and R981 coordinate PI(3)P binding through interactions with the D3 phosphate of the inositol headgroup. Furthermore, bioinformatic analyses identified related four-α-helix bundles in the SidE family member SdeB and additional *Legionella* effectors, suggesting that this mode of PIP recognition is broadly conserved. Collectively, these findings establish a structural and biochemical framework for PI(3)P recognition by SdeC and support the existence of a conserved, bacteria-specific PIP-binding fold that contributes to selective targeting of host membranes by intracellular pathogens.

P021 Ethanol Extract of Lactobacillus rhamnosus AC1-Fermented Soymilk Suppresses LPS-TLR4-NF-κB Signaling: Potential Implications for Modulating Hyperinflammation in *Legionella* Infection

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Legionella pneumophila causes severe pneumonia by activating Toll-like receptor 4 (TLR4) and downstream NF-κB signaling, leading to excessive production of pro-inflammatory cytokines. Targeting this pathway represents a potential therapeutic strategy for Legionnaires' disease. In this study, we investigated whether the ethanol extract (EE) of Lactobacillus rhamnosus AC1-fermented soymilk, previously shown to possess anti-inflammatory properties, could suppress the LPS-TLR4- NF-κB axis using a dextran sulfate sodium (DSS)-induced colitis mouse model. Mice received 5% DSS in drinking water for 7 days, followed by oral administration of low (200 mg/kg) or high (400 mg/ kg) dose of EE, or 5-aminosalicylic acid (5-ASA, 200 mg/kg). EE treatment significantly ameliorated body weight loss, disease activity index, colonic shortening, and histopathological damage. Mechanistically, EE reduced serum LPS levels, down-regulated the expression of TLR4, MyD88, and NF-κB, and decreased NLRP3 inflammasome activation. It also restored intestinal mucosal barrier integrity (increased Occludin, ZO-1, and MUC2) and rebalanced gut microbiota dysbiosis, particularly reducing pro-inflammatory Proteobacteria (e.g., Escherichia-Shigella, Enterococcus). Notably, the suppression of the LPS-TLR4-NF-κB pathway by EE was associated with reduced production of IL-1β, IL-6, TNF-α, MCP-1, and CD68. Since *Legionella* infection triggers a similar TLR4/NF-κB-driven hyperinflammatory response, our findings suggest that EE of L. rhamnosus AC1-fermented soymilk could serve as a natural source of signaling modulators. Further studies in *Legionella* pneumonia models are warranted to evaluate its translational potential for mitigating immunopathology.

P022* Exploring potential mechanisms of programmed cell death in *Legionella* phage defense: Interplay of NAD+ depletion and the effector phospholipase PlaB

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Legionella pneumophila is an intracellular pathogen of human lungs and environmental protozoa. It is well established that protozoan hosts have shaped their lifestyle and virulence factor repertoire; similarly, coexistence with bacteriophages in aquatic environments may likewise have driven acquisition of phage defense mechanisms that also contribute to virulence. The phospholipase A PlaB is a well-characterized virulence factor that localizes to the bacterial outer membrane and a highly active phospholipase. Here, we propose an additional intrabacterial function for PlaB as an intrinsic cell death effector involved in phage defense. Like eukaryotes, bacteria possess a variety of mechanisms, such as NAD(H) depletion, to counteract viral infections, but many details of phage defense are currently not understood. We identified a regulatory mechanism in which intracellular NAD(H) controls PlaB activity. At physiological NAD(H) concentrations, PlaB forms inactive tetramers stabilized by NAD(H) binding. Depletion of NAD(H) promotes dissociation into active dimers, resulting in potent phospholipase activity. We hypothesize that activated PlaB targets bacterial membranes from within the cytosol, triggering autolysis and thereby limiting phage replication through an abortive infection strategy. Consistent with NAD(H) being central for regulation of PlaB's activity, gene co-occurrence analyzes show that PlaB can be found with genes encoding NAD(H)-consuming enzymes, such as RES-Xre toxin-antitoxin systems, SIR2- and TIR-domain proteins, suggesting a coordinated regulatory network. Supporting this observation, co-expression of PlaB with an NADase in *E. coli* leads to rapid, PlaB-dependent cell death under low NAD(H) conditions. These findings support a model in which NAD(H) serves as a molecular switch controlling PlaB activity and identify PlaB as a candidate effector linking NAD(H) depletion to altruistic cell death and abortive infection mechanism during possible phage infection. This work expands the functional repertoire of PlaB beyond virulence and suggests a previously unrecognized mechanism of autolysis and possible implication in phage defense in *L. pneumophila*.

P023 Cas2 promotes *Legionella* pneumophila thermotolerance and intracellular infection through a CspA regulatory hub

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CRISPR-Cas systems are common in *Legionella pneumophila* (*Lp*) strains, where they expectedly confer immunity to foreign genetic elements. However, through an analysis of strain 130b, which has the most common *Lp* CRISPR-Cas type (II-B), we previously documented that Cas2 (independently of other CRISPR-Cas components) also majorly promotes *Lp* infection of amoebae. Since this noncanonical role for Cas2 was linked to the protein's RNAse activity, we posited that Cas2 influences the amount or configuration of an RNA that encodes or regulates a key infectivity factor. By performing proteomic and qRT-PCR analysis of a *cas2* mutant, we had identified the small heat shock protein gene *hspC2* as being notably increased by the presence of Cas2. Mutant analysis confirmed that HspC2, along with Cas2, promotes intracellular infection as well as thermal tolerance. We have now determined that transcript levels for the *Lp cspA* gene are also increased by the presence of Cas2. In other bacteria, cold shock proteins like CspA modulate the levels and/or translation of target transcripts and enhance resistance to various stresses, including cold, heat, or acid. Thus, we hypothesized that CspA might be (part of) the link between Cas2 and *hspC2* expression. qRT-PCR analysis determined that *hspC2* expression is decreased in a *cspA* mutant, as it was for a *cas2* mutant. Based on new mutant analysis, CspA, like Cas2 and HspC2, is required for optimal survival after heat shock at 55oC, growth at 45oC, and infection of amoebae. Further analysis of a mutant lacking both *cas2* and *cspA* signaled that Cas2 and CspA operate on *hspC2* through a single pathway. Phenotyping of a double mutant lacking *hspC2* and *cspA* indicated that HspC2 is the sole effector of heat shock resilience targeted by this pathway. Together, these data indicated that Cas2 upregulates CspA which, in turn, upregulates HspC2 to promote thermotolerance.

P024 Improving *Legionella* pneumophila surveillance with a highresolution cg/wgMLST schema and harmonized nomenclature

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Genome-scale approaches, including core-genome (cg) and whole-genome (wg) multilocus sequence typing (MLST), provide high-level discriminatory power for the characterization and surveillance of bacterial pathogens. To support harmonized, high-resolution genomic surveillance, the *Legionella* International Typing (LIT) workgroup recently developed and publicly released a novel cg/wgMLST schema for *Legionella pneumophila*. Schema development and evaluation incorporated more than 9,000 genome assemblies spanning the known diversity of the species, culminating in a set of 2,009 core loci suitable for routine cgMLST-based surveillance, as well as 2,698 accessory loci that can be dynamically incorporated for expanded wgMLST analyses when greater epidemiologic resolution is required. The release of this novel cg/wgMLST typing schema represents an important milestone toward enhancing the comparability of *L. pneumophila* genomic data across laboratories and countries, while addressing some of the limitations of traditional sequence based typing. However, broader implementation of genome-based surveillance will require community-wide efforts extending beyond schema development. Key challenges include developing sustainable approaches for allele and genome database curation, promoting data-sharing practices that accommodate diverse requirements, and defining standardized approaches for genomic nomenclature and communication of strain relatedness. This presentation will provide an overview of the LIT schema and discuss current efforts and future priorities aimed at supporting harmonized genomic surveillance at both local and global scales. Topics will include practical considerations for schema implementation, opportunities for integration with public genomic platforms (e.g., BIGSdb-Pasteur) and the potential role of genomic nomenclature systems that provide a common language for describing genomic relatedness (e.g., Life Identification Number or LIN codes). Current efforts include evaluating the performance of the LIT schema across different allele callers and exploring candidate thresholds for a genomic nomenclature reflecting species population structure. By fostering international collaboration and standardization, these efforts aim to enhance the interoperability, reproducibility, and long-term scalability of *L. pneumophila* genomic surveillance worldwide.

P025* Understanding mitochondria-endoplasmic reticulum contact sites (MERCS) changes during infection of human cells with *Legionella* pneumophila

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Contacts between mitochondria and the endoplasmic reticulum (MERCS) serve as key platforms for many cellular processes, including mitochondrial fission and fusion, Krebs cycle, autophagy, and cell death. Several proteins involved in the cellular response to pathogens, such as MAVS, STING, and IRE, are also located there. Pathogens such as HIV, HCMV, and DENV have been found to target MERCS or proteins localized at MERCS. *Legionella pneumophila* is an intracellular bacterium that establishes a protected niche called the *Legionella*-containing vacuole (LCV) within its host cell. To establish this niche and replicate, *L. pneumophila* uses a type IV secretion system (T4SS) called Dot/Icm, which injects over 330 effector proteins into the host cell to modulate host cell functions. Using a SPLICS splitGFP system we quantified the MERCS area in U2OS cells during infection with *L. pneumophila*. Interestingly, we observed that the MERCS area is larger in infected cells than in noninfected cells, indicating that more contact sites are present. Moreover, cells infected with a mutant lacking a functional T4SS show a MERCS area that is larger than that in non-infected cells and cells infected with wild-type bacteria. These results suggest that *L. pneumophila* infection alone increases the MERCS area, but the bacterium seems in addition to actively regulate MERCS *via* its effectors.

P026* Scratching the surface: what phage attachment tells us about strain differences at the *Legionella* membrane

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Legionella pneumophila lipopolysaccharide (LPS) differences are key to classifying isolates and their disease-causing ability. For decades, it has been known that *L. pneumophila* serogroup 1, mAb 3/1 reactive (Pontiac) strains cause the vast majority (>80%) of observed human disease. Currently, this is thought to be the result of resistance to killing by human complement conferred by a single surface modification (LPS O-antigen acetylation, mAb 3/1 epitope). Due to no person-to-person transmission, the bacterium's virulence traits cannot be human adapted and must come from selective pressures in the environment. We recently discovered the first *L. pneumophila* bacteriophage (LME-1) and showed that O-acetylation efficiently blocks phage attachment and subsequent infection. Simply put, environmental selection for resistance to phage attachment has coincidentally led to the acquisition of surface modifications that support human disease. By extending our analysis of determinants of phage attachment, I have identified a second, *lag-1*-independent, layer of surface defense. This surface defense surprisingly prevents phage attachment only during the transmissive phase, when bacteria are normally extracellular and swimming to find a new host—suggesting this defense is activated when the bacteria are otherwise thought to be most susceptible to phage. I have tracked the presence of this defence to a single serogroup 1 clade and will provide an update on my search for the genes responsible for this growth phase-dependent surface modification. By understanding the surface features that modulate phage attachment in *L. pneumophila*, we will continue to gain unique insight into the novel surface modifications that give rise to its unique LPS types along with the environmental conditions that shape the pathogen's ability to cause human disease.

P027 Going Green, Growing *Legionella*?

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Temperature control is a key measure for preventing the proliferation of *Legionella* spp. in building water systems. Croatian regulations require domestic hot water (DHW) temperatures ≥50°C and domestic cold water (DCW) temperatures ≤25°C. In 2025, epidemiological surveillance in Split- Dalmatian County (423.407 inhabitants) identified 51 *Legionella*-positive water samples. *L. pneumophila* serogroup 1 accounted for 66.7% of isolates. Hotels accounted for the largest share of positive samples (39.2%), followed by hospitals (19.6%) and residential care facilities (15.7%). As renewable energy technologies, particularly solar heating systems (SHS), become increasingly integrated into building infrastructure, concerns have emerged regarding their ability to consistently maintain temperatures required for effective *Legionella* control. This study investigated DHW and DCW temperature profiles and compliance with regulatory thresholds across different heating technologies. Profiling was conducted in 60 buildings using oil, gas, electric (EB), central electric, heat pump, or SHS. Distal outlet points of each water system were measured (96). Non-parametric analyses were used to assess associations between previously mentioned variables. Nearly 2/3 of DHW measurements were below 50°C, indicating widespread non-compliance. SHS were significantly associated with DHW temperatures below 50°C (χ^2, $p = 0.0324$) and with larger boiler capacities ($\rho = 0.40$, $p = 0.0001$). Boiler capacity was positively correlated with DCW temperature ($\rho = 0.44$, $p < 0.001$), while EBs were associated with lower DCW temperatures ($\rho = -0.50$, $p < 0.001$). DHW temperatures represent the principal *Legionella*-related risk in the investigated buildings. SHS were associated with an increased likelihood of suboptimal DHW temperatures, highlighting the importance of appropriate design and balance of interconnected heat sources. Larger boiler capacities were also associated with higher DCW temperatures, further increasing conditions favourable for *Legionella* growth. Integrating *Legionella* risk assessment into design and management of energy-efficient water heating systems is essential to ensure that sustainability objectives do not compromise public health protection.

P028 Lipidomic Profiling of Dictyostelium discoideum–*Legionella* pneumophila Host–Pathogen Interaction

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Introduction *Legionella pneumophila* (*Lpn*) requires modulation of membrane lipids during intracellular infection, replication, including the formation of the *Legionella*-containing vacuole, as well as bacterial egress. These observations suggest that phospholipases are important contributors to these processes, although the underlying mechanisms and the functions of specific lipids, particularly those involved in bacterial release from the host, remain largely unclear. **Goals** To gain insight, we present a workflow of sample collection, lipid extraction, comprehensive lipid profiling and the associated lipidomic data of whole cell pellets from the host *Dictyostelium discoideum (Dd)*, *Lpn*-infected *Dd,* and *Lpn* cultured in isolation. **Materials and methods** We used untargeted 4D high resolution trapped ion mobility mass spectrometry, followed by deep structural manual curation and annotation of lipid species. **Results** *Dd* and *Lpn* were characterized primarily by the phospholipids (PLs) phosphatidylethanolamine (PE) and phosphatidylcholine (PC). In *Dd* higher abundance of alkyl ether lipids, including PE O-34:1, PE O-34:2, PG O-28:1, and PI O-34:1, compared with *Lpn,* were noted. *Dd* was further characterized by PC 34:2, PC 34:3, PC 36:3, and PC 36:4, mainly composed of C16:0, C18:1, and C18:2 fatty acids. In contrast, *Lpn* contained PE 31:0, PE 32:0, PC 32:0, PC 32:1, PG 31:0, and PG 32:0. Overall, *Lpn* displayed a tendency toward shorter-chain phospholipid-associated fatty acids than *Dd*. The lipid profile of *Lpn*-infected *Dd* changed progressively throughout infection. As bacterial replication increased, the relative abundance of bacterial lipids also increased, causing the overall lipid composition to shift from a profile closely resembling *Dd* at early time points toward one more similar to that of *Lpn*. **Summary** In conclusion, we demonstrated that both *Dd* and *Lpn* possess distinct lipid profiles that were largely preserved during infection. This enabled us to distinguish between host- and pathogen-derived lipids even when analyzing whole infected cells.

PF06/ P029 Lipid droplets: Emerging targets in *Legionella* pneumophila pathogenesis

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Legionella pneumophila is a Gram-negative intracellular pathogen that causes severe respiratory infection through exploitation of host cell machinery. Recent evidence demonstrates that lipid droplets (LDs), energy-rich cellular organelles traditionally associated with lipid storage, serve as metabolic platforms hijacked by *L. pneumophila* during infection. In addition, LDs have been implicated in various signalling pathways and processes, including innate immunity. This study investigates the molecular mechanisms by which this pathogen manipulates host lipid droplet dynamics to facilitate intracellular replication. Our lab has previously demonstrated that *Legionella*-containing vacuoles localize adjacent to lipid droplets, positioning the pathogen for direct access to lipids. This co-localization is dependent on the presence of a functional type IV Dot/Icm secretion system. Other labs have shown that *L. pneumophila* does indeed encode at least one type-IV-secreted effector that directly targets LDs. In this study, we aim to elucidate the involvement of novel effectors on LD dynamics and intracellular replication using the genetically tractable amoeba *Dictyostelium discoideum* and the macrophage cell line RAW 264.7. Using a mass spectrometry approach, we aim to identify bacterial effectors targeting host LDs and characterize their functional role during infection. The results of this study will not only broaden our understanding of how *L. pneumophila* manipulates the host cell, but also further elucidate the role LDs play for infection and immunity.

P030 Artificial Intelligence-Based Prediction of *Legionella* Contamination Risk in Building Water Systems

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Legionella contamination in building water systems is a dynamic phenomenon influenced by microbiological, physicochemical, structural and operational factors. In healthcare facilities and accommodation sites, prevention requires not only detection of contamination, but also identification of conditions that may precede bacterial proliferation and increase exposure risk. Conventional surveillance, mainly based on scheduled environmental sampling and laboratory confirmation, provides essential information but often offers a retrospective view of system status, limiting timely preventive actions in complex water networks. This project evaluated artificial intelligence (AI) as a predictive tool for *Legionella* risk management in building water systems. A retrospective observational study was conducted in a 1,552-bed healthcare facility. Data from samples collected between 2021 and 2026 from 17 hot-water systems across multiple buildings were analysed. A single cold-water system supplied all buildings, with local distribution to points of use. Microbiological, physicochemical, structural and temporal variables were collected from representative sampling points, including *Legionella* and *Pseudomonas aeruginosa* presence/absence, water temperature, pH, disinfectant residual, turbidity, point-of-use filters and sampling site/location. Microbiological analyses followed ISO 11731:2017. Decision Tree, Logistic Regression, Random Forest and Gradient Boosting models were considered to predict contamination probability and compared with conventional statistical approaches. Performance was assessed using ROC-AUC, PR-AUC, recall and F1-score, with attention to class imbalance. Explainable AI methods, including feature importance and SHAP values, were used to identify the main predictors. Preliminary analysis showed sufficient dataset consistency to support predictive modelling, despite imbalanced positive cases. A penalized logistic-regression classifier was selected as an interpretable approach and validated using 5-fold GroupKFold cross-validation by sampling point, avoiding leakage from repeated site measurements. The model showed good discrimination for *Legionella* noncompliance (ROC-AUC 0.82; recall 0.75), identifying filter presence, disinfectant residual and water temperature as relevant drivers. In contrast, *Pseudomonas* prediction was close to chance, suggesting the need for more flexible models.

P031 The *Legionella* effector RidL subverts the conserved, mitochondrialocalizing large GTPase Vps1 in yeast

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The causative agent of Legionnaires' disease, *Legionella pneumophila*, is a facultative intracellular bacterium, which secretes more than 300 different effector proteins into eukaryotic host cells, where they subvert a plethora of cell biological processes. The effector protein RidL binds through a 29 kDa N-terminal fragment to the retromer coat complex subunit Vps29, thereby displacing the Rab7 GTPase activating protein (GAP) TBC1D5 from the retromer. The function of the C-terminal portion of RidL is not known. In this study, we used yeast as a model to further assess the mode of action of RidL. We show that RidL interacts via a 75 kDa C-terminal fragment with the conserved large fission GTPase Vps1 of *Saccharomyces cerevisiae*. The binding affinity was enhanced by the presence of a nonhydrolysable GTP analogue, and the binding constant KD was approximately 31.5 nM. RidL and a Cterminal fragment also bound to the Vps1_GG fragments of the yeasts *S. cerevisiae* and *Chaetomium thermophilum*, which comprise the GTPase and GTPase effector domain (GED) but lack the "stalk" domain of the large GTPase. Purified RidL and a C-terminal RidL fragment but not an N-terminal fragment reduced the GTPase activity of Vps1, and full length RidL inhibited Vps1 filamentation induced by a non-hydrolysable GTP analogue. Finally, RidL and a C- but not an N-terminal fragment as well as Vps1 were enriched in subcellular mitochondrial fractions. Taken together, the *Legionella* effector RidL interacts through its C-terminus with the conserved yeast large fission GTPase Vps1 on mitochondria. Our study validates the use of yeast as a model to functionally study the mode of action of bacterial effector proteins.

P032 An unusual source of transmission causing a national travel associated outbreak of Legionnaires' disease

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Introduction Within a three-month period in 2022, four patients contracted Legionnaires' disease after staying in the same hotel in Uppsala city. Individual investigations included the hotel, patients´ homes and other possible sources, and the common investigation focused on possible sources in the hotel and its surroundings. Methods County medical officers interviewed the patients to enquire possible exposures to *legionella*. The patients were diagnosed by urinary antigen and/or PCR, and lower respiratory tract samples from three patients were typed with nested sequenced-based typing (nested-SBT). Water samples from taps and showers in the hotel were repeatedly cultured for *legionella* (n=23). The irrigation water of a close-by soccer field and a car wash were also sampled. The hotel management eventually identified an electrical fireplace in the lobby as a device containing water that could be sampled and analysed for *legionella*. Environmental isolates were typed by whole-genome sequencing (WGS)-SBT. Results All patients were diagnosed with *Legionella* pneumophila but negative by culture. One patient that was positive by L. pneumophila PCR was by nested-SBT found to be infected by ST42. For a second patient, also positive by PCR, six out of seven alleles matched the profile of ST42. No typing results were available for the other two patients. All samples from taps and showers at the hotel were culture negative for *legionella*. In water samples from the electrical fireplace the concentration of *legionella* bacteria was up to 4 400 000 cfu/l and *Legionella* pneumophila ST42 was detected. No *legionella* was found in any of the patients´ homes or in samples from other investigated sources. Conclusions It was concluded that the only common environment for the patients was the hotel. The outbreak investigation finally revealed that a previously unrecognized source of *legionella* – an electrical fireplace producing a mist – was the cause of the outbreak.

P033 BactoCluster: using pan-genomes for highly discriminatory relatedness analysis during outbreaks of Legionnaires Disease

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Successful investigations of outbreaks of Legionnaires Disease (LD) require that environmental and clinical isolates are sequenced and their genomes compared to identify the source and determine the scope of the outbreak. Mobile genetic elements (MGE), such as plasmids, integrative conjugative elements, insertion sequences, and genomic-island-like regions, play an important role in the biology of *Legionella pneumophila*, but they add complexity to the bioinformatic analysis and are therefore often omitted from the genome comparisons. Here we present the results from a new bioinformatic relatedness analysis tool, BactoCluster, that specifically includes MGE to increase the level of discriminatory power when isolates are seemingly identical. 1) Isolates are initially clustered based on their kmer-content and then refined based on phylogenetic data. 2) All isolates in a cluster contribute to a pan-genome sequence that serves as a reference for genome comparisons. The pan-genome is automatically annotated, which simplifies downstream investigations. 3) During pairwise comparisons, the pipeline calls single-nucleotide polymorphisms (SNPs), as well as small and large insertions/deletions (indels). Using over 1100 isolates from the Wadsworth Center's *Legionella pneumophila* repository, including numerous past outbreaks, we demonstrate how BactoCluster returns the highest level of discriminatory power possible from Illumina reads. A summary table allows the user to quickly identify clusters, determine the number of SNPs, short and long indels. Additional tables provide information on the location of these mutations and if they are part of the core or the accessory genome. This information not only identifies related isolates but also sheds light on the evolution of isolates within a cluster. During an outbreak of LD, timely and accurate analysis is critical to determine sources and exposures to prevent further cases. In our long experience of investigating LD clusters in New York, BactoCluster provides the discriminatory power needed to solve historic and current LD outbreaks.

P034 A decade of Travel-associated Legionnaires' disease surveillance in England and Wales: Trends, Destinations and Risk factors – 2015-2024

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Over half of Legionnaires' disease cases in England and Wales (EAW) are travel related and responsible for significant morbidity. Cases identified abroad present a unique challenge for prevention and control and there is limited evidence describing how travel patterns and risk factors have evolved over time. Strengthening this evidence base is critical to inform targeted prevention and control strategies. We aimed to describe the epidemiology and exposure characteristics of TALD cases in EAW residents reported to UK Health Security Agency (UKHSA), 2015–2024. We conducted a retrospective cohort study of laboratory-confirmed TALD cases among EAW residents notified to UKHSA via the National Enhanced Legionellosis Surveillance System (NELSS). Cases were defined as laboratory confirmed by urinary antigen test or culture. Descriptive analyses characterised temporal trends, travel destinations, accommodation type, seasonality, and deprivation (Index of Multiple Deprivation). We identified 1,587 TALD cases in EAW. Cases were predominantly male (71%) with a median age 62 years (IQR 54–71) and 51% with a smoking history. A strong seasonal pattern was observed with peaks in around June and September each year. 42% of cases resided in the 3 most deprived quintiles of deprivation in E&W. Leading travel destinations were Spain (271), United Arab Emirates (183), Italy (167), Greece (143) and Turkey (122); UAE had the highest rate per 100,000 flights (1.92), followed by Turkey (0.62) and Greece (0.51). The majority of cases (78%) stayed in non-private accommodation. TALD in EAW residents remains concentrated among older men and strongly linked to non-private accommodation, with the UAE emerging as a disproportionately high-risk destination. Distinct seasonal and geographical patterns, peaking in late summer post-school holidays, reflect interactions between travel behaviour, vulnerable populations, and temperatures favourable for *Legionella* growth. We recommend more targeted accommodation follow-up and better communication with travel providers, particularly in higher-risk destinations.

P035 Copper-doped mesoporous silica nano-additives for antimicrobial surface coatings in managed water systems

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The proliferation of *Legionella pneumophila* - the principle aetiological agent of Legionnaires' disease – within high-use, managed water systems has created a significant public-health, legal and regulatory onus on dutyholders for hazard mitigation. Systems of particular concern include evaporative cooling towers and those found within healthcare-providing facilities, due to operating characteristics and demographic risk factors, respectively. Notably, the association of *L. pneumophila* with biofilm-associated growth can render conventional treatment methods, such as maintenance of elevated temperatures and application of biocides, an ongoing and costly matter. Once established, mature biofilms can be difficult to remediate and may persist despite challenging bulk water environmental conditions. Accordingly, there exists significant scope to develop technologies that act to mitigate biofouling at the earliest stages of colonisation and at the site of microbial attachment. In this work, the delivery of the antimicrobial element, copper, incorporated within stabilised mesoporous silica nanoparticles (MSNs), to surfaces as an antibiofouling strategy is investigated. Cu-doped MSNs have been prepared using a one-pot, sol-gel synthesis across a range of copper loadings (0.5 – 20 mol%, relative to silica). The resulting nanomaterials were characterised using DLS, FTIR, SEM, SEM-EDX, TEM, ICP-MS and BET analysis by nitrogen adsorption-desorption. The antibacterial performance of produced materials was assessed in nutrient-rich and nutrient-poor media against *Pseudomonas aeruginosa*, *Legionella pneumophila* and *Enterococcus faecalis*. Under nutrient-poor conditions, the particles suppressed culturability across the tested species. The produced materials were subsequently loaded into optimised inorganic ethyl silicate coatings. Coating development centred on improving film integrity, with a particular focus on minimising surface cracking and coating detachment from the underlying stainless-steel substrate, as assessed by SEM. To establish coating stability under application-relevant conditions, the durability of coatings under aqueous immersion is under evaluation.

P036 Ultrastructural Imaging of *Legionella* pneumophila replication in the zebrafish model

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Legionella pneumophila, the causative agent of Legionnaires' disease, is a facultative intracellular pathogen that primarily replicates within amebae and alveolar macrophages. The zebrafish (*Danio rerio*) is widely used as a vertebrate model to study infections *in vivo*. Its transparency and the possibility to combine infection with different imaging techniques make it a powerful model to study host–pathogen interactions. Previously we observed that *L. pneumophila* infection of zebrafish larva is a good model that mimics at least partly human infection and demonstrated that *Legionella pneumophila* colonizes the zebrafish yolk region, but the cellular identity and ultrastructural organization of this infection niche remained unknown. To characterize these infection niches, larvae were infected at 72 hours post-fertilization and analyzed 48 hours later using a novel correlative imaging workflow combining fluorescence microscopy, X-ray tomography, and electron microscopy (EM). We compared infection of a *L. pneumophila* WT and a *L. pneumophila dotA* mutant to characterize the diverse *L. pneumophila* replication niches.

P037* Investigating the role of mmcO in *Legionella* pneumophila copper resistance

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Legionella pneumophila, the causative agent of Legionnaires' Disease, is often found in engineered water systems (EWS) where copper pipes are widely used. Due to its antimicrobial activity, copper could limit *L. pneumophila* colonization of EWS. However, some copper-resistant strains have been identified and one of them is *L. pneumophila* SPF581, in which a plasmid carries *mmcO*, a putative multicopper oxidase. It is hypothesized that *mmcO* could contribute to *L. pneumophila* copper resistance via conversion of Cu (I) ions into Cu (II) ions. To confirm the functional role of *mmcO* in *L. pneumophila* copper resistance, the gene was cloned with a hexahistidine tag on the plasmid pMMB207c under the transcriptional control of the *Ptac* promoter. The resulting plasmid was transformed in five clinical and environmental strains of *L. pneumophila* to account for possible genetic background effect. Western blots confirmed *mmcO*-His expression in all induced transformed strains. The survival of the strains, with and without IPTG, after exposure to 0.08 mM CuCl2 for 4 hours was then measured. Two of the strains showed a ~3-fold increase in copper resistance when *mmcO* expression was induced. The results suggest that *mmcO* could confer increased copper resistance to *L. pneumophila*, but the effect may be dependent on the genetic background.

P038* Coupled 16S and 18S rRNA analysis reveals complex *Legionella*-amoeba associations in non-disinfected shower hose biofilms

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Prokaryote-eukaryote interactions influence the persistence of waterborne pathogens, such as the well-documented proliferation of *Legionella* spp. in free-living amoebae. However, the interactions with other potential hosts remain poorly investigated and confined to a limited number of taxa, leaving much of the ecological picture incomplete. To address this gap, we collected 128 shower hose biofilms across Switzerland and sequenced their 16S rRNA and 18S-ITS-28S rRNA. Diversity analyses, correlations and SPIEC-EASI co-occurrence networks between the prokaryotic and eukaryotic communities were studied. *Sphingomonadaceae*, *Burkholderiaceae*, *Xanthobacteraceae*, *Caulobacteraceae* and *Pyrinomonadaceae* dominated the prokaryotic community, while the eukaryotic one showed a high presence of amoebae, mostly *Vermamoeba* and LKM74-lineage. Overall, 154 *Legionella* amplicon sequence variants (ASVs) were found across 82% of samples, but none of the ASVs dominated. *L. waltersii* was the most detected species, while *L. pneumophila*, responsible for most Swiss infections, were only present in 5% of samples. Alpha diversity was positively correlated between the prokaryotic and eukaryotic communities, and the same trend was observed between *Legionella* spp. and the eukaryotic community. However, no significant correlation was found between literature-based amoebae and *Legionella* spp. Co-occurrence analysis suggested different relevant interactions between *Legionella* spp. and other eukaryotes. Adding these taxa to the analysis, a significant positive correlation emerged, identifying potential host candidates belonging to 15 eukaryotic genera. In conclusion, non-disinfected shower hose biofilms are dominated by a complex microbial community, where the prokaryotic and eukaryotic richness is positively correlated. Relationships between *Legionella* spp. and its hosts are more complex than described in literature knowledge and other taxa detected appear to play a relevant role. These findings highlight the importance of integrating network-based approaches with literature to fully characterize *Legionella* ecology in drinking water biofilms and the necessity to focus more in-depth on the eukaryotic community.

P039 Exploring the role of cyclic di-GMP signalling and nitric oxide in *Legionella* pneumophila phenotypic heterogeneity and antibiotic persistence.

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Bacterial persisters represent a transient sub-population of non-growing, antibiotic tolerant cells associated with treatment failure and relapsing infections (1). There is increasing evidence to suggest that bacterial persisters are an adaptive response to host-derived defence mechanisms (2, 3), however the specific host cell stressors and associated bacterial signalling pathways involved in this process remain poorly characterised. Here we explored the role of host cell-derived nitric oxide (NO) in the emergence of *Legionella pneumophila* intracellular persisters. Specifically, we investigated the impact of NO stress and the role of the bacterial cyclic di-GMP signalling pathway NosP-NahK-NarR in the formation of non-growing subpopulations of *L. pneumophila* during infection. To this end, we carried out infections in murine macrophages activated by LPS and/or treated with a nitric oxide synthase inhibitor, L-NAME. We infected macrophages with *L. pneumophila* strains deleted or mutated in the NosP-NahK-NarR pathway and then analysed the formation of non-growing subpopulations by time lapse fluorescence microscopy. We demonstrated that NO stress likely promotes the formation of growing or non-growing bacteria during macrophage infection in a concentration-dependent manner. Furthermore, we determined that changes to the NosP-NahK-NarR pathway impact intracellular growth rate heterogeneity in relation to the phosphorylation state of the diguanylate cyclase-phosphodiesterase, NarR. Consequently, this work proposes a novel molecular mechanism to explain *L. pneumophila* growth rate heterogeneity and persistence in response to intracellular stress. 1. Fauvart M, et al. Role of persister cells in chronic infections: clinical relevance and perspectives on anti-persister therapies. J Med Microbiol. 2011;60(Pt 6):699-709. 2. Personnic N, et al. Quorum sensing modulates the formation of virulent *Legionella* persisters within infected cells. Nature Communications. 2019;10(1):5216. 3. Adams-Ward X, et al. Bacterial persistence in *Legionella* pneumophila clinical isolates from patients with recurring legionellosis. Front Cell Infect Microbiol. 2023;13:1219233.

P040 A Novel Role for the GTPase Rab5 in the Inhibition of *Legionella* VipA-Dependent Actin Polymerization

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Legionella pneumophila uses the Icm/Dot type IV secretion system to translocate more than 300 effector proteins into host cells, where they manipulate cellular pathways to establish and maintain the *Legionella*-containing vacuole (LCV). Among these effectors, VipA is an actin nucleator that associates with early endosomes and disrupts vesicle trafficking in *Saccharomyces cerevisiae*. This subversion of eukaryotic trafficking by VipA involves both its capacity to bind and polymerize actin through its COOH-terminal region, and its ability to associate with early endosomes via its NH2- terminal region. However, the molecular basis of its association with the endocytic pathway has remained poorly understood. Here, we identified molecular partners of VipA in early endosomes and investigated the functional consequences of these interactions. *In vitro* and *in vivo* interaction assays revealed a direct interation of VipA with two key components of early endosomes: the lipid phosphatidylinositol 3-phosphate (PI3P) and the small GTPase Rab5. Binding to Rab5 requires the NH2-terminal region of VipA and occurs independently of its COOH-terminal actin-binding domain. Furthermore, this interaction with Rab5 inhibits VipA-mediated actin polymerization by preventing *de novo* actin filament nucleation, indicating a regulatory mechanism exerted by the GTPase on the bacterial protein. These findings provide new insights into the molecular mechanisms underlying VipA function and reveal a previously unrecognized interaction between a *Legionella* effector and key components of the endocytic pathway, highlighting the complex interplay between *L. pneumophila* effectors and host cell targets.

P041* Subversion of mitochondrial homeostasis by *Legionella* pneumophila

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Legionella pneumophila is a facultative intracellular pathogen that replicates within environmental amoebae and, upon inhalation, within human alveolar macrophages, where it causes Legionnaires' disease. The bacterium uses the Icm/Dot type IV secretion system (T4SS) to translocate more than 300 effector proteins into the host cell, subverting virtually all key cellular processes to establish a replication-permissive compartment known as the *Legionella*-containing vacuole (LCV). Among this arsenal, the effector retromer interactor decorating LCVs (RidL) has previously been characterized as a bifunctional protein that inhibits retrograde trafficking by binding the retromer subunit Vps29 through its N-terminal domain. RidL also induces mitochondrial fragmentation by recruiting the fission GTPase dynamin-related protein 1 (Drp1) via its C-terminal domain. However, the potential coordination between these two distinct functions remains unexplored. Mitochondria, as descendants of an ancestral bacterial endosymbiont, have retained the capacity to shed vesicles, an evolutionarily conserved feature among bacteria and archaea. These mitochondria-derived vesicles (MDVs) are small (~70-150 nm), cargo-selective carriers whose functions range from mitochondrial quality control to immune signaling. Interestingly, both the retromer and Drp1 operate within this pathway, as the former mediates cargo selection and delivery, while the latter provides the mechanical force required for membrane scission. We aim to establish a robust imaging pipeline to resolve and quantify MDVs in mammalian cells infected with *L. pneumophila* wild-type and mutant strains. Specifically, we will use live-cell structured illumination microscopy (SIM) coupled with fluorescent mitochondrial probes to overcome the diffraction limit imposed by conventional light microscopy. This work will provide broader insights into how *L. pneumophila* subverts mitochondrial homeostasis during infection, and how bacterial effectors could serve as a tool to dissect the role of mitochondrial quality control mechanisms in health and disease.

P042 *Legionella* LME-1 like phage detection in Legionnaires' disease patient's lungs

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Background LME-1 has recently been described as a temperate phage of *Legionella* able to replicate and form viral particles *in vitro* (Nicholson B. *et. al.* 2025). **Methods** National reference centre for *Legionella* genomes database (n=4742) was screened for the presence of LME-1 phage. Eight patients were selected based on a culture-proven Legionnaires' disease (LD) with *Legionella pneumophila* strains containing lysogenic "LME-1 like" phage identified in the previous genome screening step. Virome analyses were performed by short reads sequencing on 27 respiratory samples from these 8 patients (8 inclusion-day samples and 19 follow-up samples). **Results** From the genome screening, LME-1 like phages were detected in 167 *L. pneumophila* genomes (mostly ST701 and related ST) and 20 *L. anisa genomes*. Four different variants were identified (three and one variants in *L. pneumophila* and *L. anisa*, respectively). From virome analyses, LME-1 like phage sequences were detected by read mapping approach in all the 8 inclusion-day samples of the patients. These reads also mapped correctly to the circularisation site of the LME-1 like phage whereas they didn't match to the sequences of the chromosomal phage integrated sites. These LME-1 like phage detections decreased during the course of the disease. **Conclusions** Different LME-1 like phages were detected in *L. pneumophila* and *L. anisa*. LME-1 like phages seem to be clonally distributed in *L. pneumophila*. Virome analyses highlighted the presence of the replicative form of LME-1 like phages in patients with LD. These LME-1 like phages were detected in all the 8 patients at the inclusion days and disappeared during the course of the disease.

P043 From Epidemiological Evidence to Integrated Public Health Policy: Aligning Evidence with Decision-Making for *Legionella* Prevention

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Background and challenges Despite extensive regulation of drinking water systems, Legionnaires' disease incidence – with 5.5 cases per 100'000 inhabitants in 2024 among the highest incidence reported in Europe - continues to rise in Switzerland and across Europe. Evidence from nationwide *SwissLEGIO* project indicates that current control strategies underperform because they are predominantly reactive, source-focused, and anchored in concentration-based thresholds that are poorly aligned with the sporadic, multifactorial ecology of community-acquired Legionnaires' disease. Fragmentation between public health surveillance, environmental monitoring and building operation further limits control and preventive effectiveness. **Aligning evidence to decision levels** Synthesising epidemiological, environmental, genomic, and building-system evidence, we show that different data streams serve distinct policy functions. Epidemiological surveillance identifies population-level risk patterns and vulnerable groups; whole-genome sequencing enables detection of pathogenic strains and clusters but rarely supports attribution of individual sporadic cases; research in *legionella* control in buildings informs preventive design and operation of water systems; and environmental monitoring provides situational awareness rather than causal inference. Treating these evidence types as interchangeable has contributed to misdirected interventions. **Governance logic and policy implications** We propose an integrated public health governance framework in which routine prevention, signalbased investigation, and outbreak response are clearly distinguished, and responsibilities across federal authorities, cantonal services and building operators are aligned with appropriate decision triggers. By replacing single-source attribution and fixed thresholds with risk-based, signal-driven coordination, this framework operationalises complexity to support more effective, proportionate and just *Legionella* prevention.

P044 Performance evaluation of MWY and GVPC selective agars for *Legionella* detection in healthcare water samples

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The latest edition of ISO 11731 for the isolation and enumeration of *Legionella* recommends using BCYE-α medium, together with one of the selective media (GVPC, MWY, or AB), for the analysis of non-clinical samples. The present study evaluates the performance of MWY and GVPC agars by assessing their sensitivity and ability to inhibit interfering microbiota in water samples. A total of 206 water samples were collected from 21 healthcare facilities in Piedmont. According to ISO 11731:2017, all samples were classified as Matrix A (low levels of interfering microorganisms). Each 1 L sample was processed using Procedures 8, 9, and 10 of the ISO decision matrix (Figure J.1). Concentrates were inoculated in triplicate onto BCYE-α, MWY, and GVPC agar and incubated at 36 °C for 10 days. Suspected colonies were identified by latex agglutination and PCR. Overall, 41.3% of the samples were positive on the MWY agar, whereas 38.8% were positive on the GVPC agar. Among the isolates recovered on the MWY agar, 53% were identified as *Legionella* spp. and 71% as *Legionella* pneumophila. On the GVPC agar, 47% of the isolates were identified as *Legionella* spp., while 74% were identified as *Legionella* pneumophila. The agreement between the two media was 92.7% ; moreover, Cohen's κ coefficient showed good concordance (κ = 0.848). The non-selective BCYE medium yielded positive results for 14 and 15 samples, respectively, that were negative on the MWY and GVPC media. The geometric mean of the *Legionella* count in positive samples was 1,700 CFU/L for MWY and 1,200 CFU/L for GVPC; however a comparison using the Mann–Whitney test did not show a statistically significant difference. In conclusion, the results showed similar performance for both media. Further studies will assess media selectivity for *Legionella* spp. detection, which may facilitate colony recognition and final confirmation.

P045 From Culture to Omics: Laboratory Methods for Environmental Detection of *Legionella* in Water Systems—A Scoping Review

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Legionella spp. remains a persistent public health concern in engineered water systems, where complex ecological interactions, biofilm dynamics, and disinfection pressures complicate reliable detection and interpretation. Traditional culture-based methods (ISO 11731:2017) continue to serve as regulatory benchmarks and are indispensable for compliance, yet their long turnaround times, laborintensive workflows, and inability to detect viable but non-culturable (VBNC) cells limit their usefulness in proactive or high-frequency surveillance. These constraints have stimulated the development of a broad spectrum of alternative approaches, ranging from rapid culture assays (Legiolert, ScanVIT, IMC, PVT Viable, amoebal co-culture, MICA *Legionella*) to molecular techniques (qPCR, vPCR, dPCR, LAMP) and real-time system-level tools (FCM and biosensors). Collectively, these methods promise faster results, improved sensitivity, and deeper insight into microbial ecology. This scoping review systematically evaluates these emerging techniques, detailing their analytical mechanisms, performance characteristics, and operational constraints. However, a comparative assessment reveals that single-method strategies inevitably redistribute rather than resolve core trade-offs between processing speed, taxonomic specificity, and biological relevance. To address these limitations, we propose a tiered, fit-for-purpose surveillance framework integrating complementary tools across four functional levels: rapid screening for early warning, quantitative assays for regulatory compliance, high-resolution methods for source attribution, and viabilityoriented approaches for advanced risk assessment. This multi-modal strategy shifts *Legionella* monitoring from a method-centric paradigm toward a decision-oriented process, aligning analytical performance with specific risk management needs and enabling more timely interventions. Remaining challenges concern cost, infrastructure demands, and workforce training, as well as the difficulty of integrating heterogeneous data streams—issues that become even more pronounced in biofilm-dominated systems, where spatial heterogeneity complicates both interpretation and predictive modelling.

P046 cluster of legionnaires' disease probably linked to industrial wastewater treatment plant, the netherlands, 2025

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In July 2025, routine surveillance by the Public Health Service identified a geographic cluster of Legionnaires' disease cases, defined as ≥3 cases occurring within six months among residents living within a 1-km radius. This concerned 6 patients diagnosed with *Legionella pneumophila* serogroup 1 (*L*p1), 2 of which had positive cultures available that were typed as sequence type (ST) 46 and 47. Following detection, an epidemiological and environmental investigation was conducted. Possible environmental sources were selected for sampling based on their proximity to cases: wet cooling towers (CT) within a 2-km radius and wastewater treatment plants (WWTP) within a 6-km radius. One CT, one municipal sewage WWTP (mWWTP) and one industrial WWTP (iWWTP) were sampled. No *Legionella spp*. were found in the CT and mWWTP, while a high concentration of *L*p1 (>1.000.000 Cfu/L) was found in the iWWTP (ST new). In total, 16 patients with an onset between 5 December 2024 and 17 September 2025 were identified to be part of the cluster. In these 10 other patients, 3 more isolates were available, but none matched the strain found in the iWWTP. The median age of the patients was 66, 14 were male (88%), 15 were admitted to the hospital and all survived. Due to the high concentration of *L*p1 found in the iWWTP, the aeration tank was covered and additional measures were taken in the installations to hamper *Legionella* growth in September 2025. No genotypical match was found, although source attribution can be challenging due to limited clinical typing and the difficulty of sampling and culturing from WWTP. The iWWTP remained a probable source however, based on the high concentration of *L*p1 present in the iWWTP and the epidemiological data, including that no new cases were detected following the covering of the tank while taking into account the incubation period.

P047 Construction of L. pneumophila multi-phospholipase knockdown strains

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Introduction *Legionella pneumophila* expresses 19 phospholipases (PLs), several of which contribute to virulence. For example, VipD is a Dot/Icm-translocated phospholipase that prevents fusion of endosomes with the LCV, helping *Legionella* to avoid degradation. Other PLs, including PlcABC, PlaA, and PlaC, require cofactors or activation (1). However, single PL deletions show limited virulence defects in host-cell models, likely due to functional redundancy (2). Therefore, simultaneous depletion of multiple PLs may better reveal their collective roles. To test this, we used a recently described multiplexed CRISPRinterference system for *Legionella* (3). **Aim** CRISPRi enables reversible, programmable repression of bacterial genes without altering DNA, making it well suited for simultaneous multi-gene control. We generated three *L. pneumophila* CRISPRi knockdown strains targeting 19, eight, or 15 PL genes. After validating knockdown efficiency by enzyme activity assays and RNA-seq, we analyzed intracellular growth in the environmental host *Acanthamoeba castellanii*. **Results** We constructed three knockdown strains targeting 19, 8, or 15 phospholipases. Transcriptome profiling showed efficient repression of 12, 7, or 10 target genes, corresponding to 60%, 88%, and 67% efficiency, including all characterized PLAs and PLCs responsible for the major catalytic activities of these groups. Fatty acid release assays using palmitoylphosphatidyl-glycerol and thin-layer chromatography confirmed nearly absent cell-associated PLA and PLC activities in the respective strains. All strains grew normally in broth and proliferated intracellularly in *A. castellanii*. However, real-time luciferase-based infection monitoring (3) revealed delayed host-cell exit when 12 phospholipase genes were depleted simultaneously. Thus, broad phospholipase depletion does not impair extracellular or intracellular growth but suggests a role for the phospholipasome in host-cell exit. **Summary** We demonstrate CRISPRi-mediated simultaneous knockdown of up to 12 *L. pneumophila* phospholipases. These PLs are dispensable for in vitro and intracellular growth but appear to contribute to host-cell exit.
References: (1) PMID:37891705 (2) PMID:19157961 (3) PMID: 33542442 (4) PMID:17506816

P048 Closing the loop on *Legionella*: A long-term experience in dental unit waterline management

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Dental unit waterlines (DUWLs) are recognized as critical points for microbial proliferation and aerosol-mediated transmission of *Legionella* spp. The risk is particularly relevant in hospitals, where dental procedures may involve immunocompromised or otherwise vulnerable patients. Since its opening in 2012, a large hospital in Northern Italy has implemented a comprehensive *Legionella* risk assessment involving a multidisciplinary team: within the hospital Water Safety Plan (WSP), dental units were identified as systems requiring specific preventive measures and continuous control. A dedicated ring-main water network was therefore designed and installed to supply all ten dental units, facilitating their management. After an initial period during which frequent *Legionella* contaminations were detected and managed by filtration and shock chemical treatments, starting from July 2020 the network has been continuously disinfected with monochloramine, already successfully used in the hospital for domestic hot water treatment. Although water from DUWLs is not considered intended for human consumption, residual concentrations were consistently maintained below the 3 mg/L limit recommended by WHO for drinking water. In accordance with the WSP, the system was monitored through periodic water sampling for *Legionella* detection, using an analytical approach combining culture-based methods and MALDI-TOF mass spectrometry. Routine verification of operational parameters and maintenance conditions was also performed. After the adoption of monochloramine, no *Legionella* has been found in DUWLs anymore. Moreover, monochloramine treatment proved compatible with the hydraulic and mechanical components of the dental units, including the most sensitive devices, with no relevant deterioration or operational limitations observed after six years of continuous use. This long-term experience suggests that preventive engineering and treatment solutions, when combined with systematic surveillance and effective governance, can provide durable control of *Legionella* in critical water systems while preserving equipment integrity and clinical functionality.

P049* Convergent evolution of *Legionella* pneumophila under environmentally relevant Triclosan exposure.

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Legionella pneumophila is a freshwater environmental bacterium responsible for Legionnaires' disease, a severe respiratory infection. Freshwater environments are increasingly contaminated with micropollutants, including triclosan (TCS), a widely used biocide that inhibits fatty acid synthesis. Whether chronic exposure to environmentally relevant concentrations of micropollutants can select adaptive mutations in environmental bacteria remains poorly understood. To address this gap, we employed experimental evolution to characterize adaptive responses of *L. pneumophila* to an environmentally relevant TCS concentration with the aim of evaluating the potential consequences of these mutations for pathogenicity. We propagated eight independent *L. pneumophila* Paris lineages in the presence of an environmentally relevant TCS concentration (0.3 µg/L) over 40 passages (~250 generations) in AYE medium, alongside eight parallel control lineages cultured in TCS-free medium. Whole-genome sequencing at passages 13, 26, and 40 revealed convergent evolutionary signatures. Several specific mutations in convergent genes were selected across TCS-exposed independent lineages (2 out of 8) while remaining invariant across all control populations. Importantly, these mutations rapidly increased in frequency and became dominant within their respective populations, indicating a selective advantage under TCS exposure. Notably, the mutational targets selected under environmentally relevant TCS exposure differed from those previously identified in our laboratory in *fabI* and *pmrA/B* under half-MIC triclosan selection. These results indicate that the magnitude of selective pressure strongly influences evolutionary trajectories and that low-level exposure engages distinct evolutionary responses. This divergence underscores the complementarity between high and low-exposure approaches. Overall, our results demonstrate that even weak, but chronic triclosan exposure can drive genetic adaptation in *L. pneumophila.* Such adaptive responses may accumulate in freshwater ecosystems over ecological timescales, highlighting the importance of assessing micropollutant impacts under realistic environmental conditions. Ongoing investigations will determine whether these adaptive mutations alter virulence-related phenotypes, thereby linking environmental contamination and infectious disease risk within a One Health framework.

P050* Beyond Disinfection: Sustainable Prevention of *Legionella* in Dental Unit Waterlines

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Objectives Dental unit waterlines (DUWLs) are highly susceptible to biofilm formation and serve as reservoirs for *Legionella pneumophila*. Due to aerosol generation during dental procedures, contaminated water systems represent a potential exposure source for both patients and healthcare workers. Sustainable control of *Legionella* in DUWLs remains challenging because of complex waterline architecture, intermittent water flow and persistent biofilm formation. This study evaluated the long-term effectiveness of different disinfection strategies for the control of *L. pneumophila* in a large university dental clinic. **Materials and Methods** Three hypochlorous acid (HOCl)-based disinfection protocols were evaluated under routine clinical conditions: (A) continuous application of HOCl (0.3–0.6 mg/L), (B) increased HOCl concentrations and (C) HOCl combined with hydrogen peroxide (H_2O_2). Water samples were analysed using standardized ISO methods for the detection of *L. pneumophila*. Following evaluation, the most effective protocol was implemented as part of a comprehensive water safety program incorporating continuous disinfection, automated flushing procedures, daily disinfectant monitoring and routine microbiological surveillance. **Results** Protocol A demonstrated the most favourable balance between microbiological efficacy, technical compatibility and operational feasibility. Increased HOCl concentrations did not provide additional benefit, while the combined HOCl/H_2O_2 protocol showed reduced performance. Following implementation of the standardized water safety program, sustained suppression of *L. pneumophila* was achieved throughout the dental unit network. During the three-year follow-up period (2023– 2025), only isolated positive findings were detected in a single dental unit. **Conclusions** A standardized HOCl-based water safety program combined with automated flushing procedures provided effective long-term control of *Legionella pneumophila* in dental unit waterlines. These findings highlight the importance of continuous monitoring and targeted water management strategies for the prevention of *Legionella* colonisation in dental healthcare settings and support their role as key components of comprehensive water safety programs.

P051* Elucidating the mechanism of membrane effector translocation through the *Legionella* Dot/Icm type IVB secretion system

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Legionella pneumophila encodes for >300 effector proteins which are injected by the Dot/Icm T4BSS into the host cell during infection. Besides soluble effectors, the T4BSS is also able to translocate transmembrane domain (TMD)-containing effectors (TMEs) which insert into various eukaryotic host membranes. It is still unclear how TMEs get translocated through the T4BSS. Depending on the TMD hydrophobicity two mechanisms are proposed. TMEs with a highly hydrophobic TMD are stated to use the two-step secretion pathway via the signal recognition particle and Sec-translocon, followed by extraction of the inner membrane intermediate and subsequent T4BSS translocation. TMEs with a lower TMD hydrophobicity are most likely directly targeted to the T4BSS. This study aims to determine if the mechanism of translocation of TMEs through the T4BSS only depends on the TMD hydrophobicity or if other T4BSS or non-T4BSS components are involved. In silico analysis of T4BSS effectors from different *L. pneumophila* strains revealed that around one-third are predicted to contain a TMD. Regarding their biophysical features, further analysis showed that most of them possess two TM segments, a small periplasmic loop, a C-terminal secretion signal and both termini localized in the cytosol (Nin/Cin topology). Based on these characteristics, model membrane effectors which only differ in the hydrophobicity of the TMD were designed to use as a backbone for modifications regarding the specific biophysical properties. The artificial TMEs showed stable plasmid-based expression in *L. pneumophila* and were successfully translocated into RAWLgBiT macrophages in a T4BSS-dependent manner. These constructs will be further tested for their topology and membrane localization. Overall, these results show that using the artificial membrane effectors hold great potential to assess the details of the translocation mechanism and classify the TMEs into those that get directly targeted to the T4BSS for translocation and those that have an inner membrane intermediate.

P052 Divergent building-level *Legionella* colonization phenotypes revealed by longitudinal surveillance and water microbiome profiling

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Background: *Legionella* status in complex building water systems is often inferred from intermittent culture-based assessments, yet it remains unclear whether long-term routine surveillance can identify reproducible building-level colonization phenotypes and whether these phenotypes are associated with distinct water microbiomes. **Methods:** We analysed 43,165 potable-water culture samples from 404 Spanish establishments, predominantly hotels and healthcare facilities, sampled during 2020–2025 under routine regulatory monitoring. Annual and cumulative positivity were calculated using *Legionella* spp. ≥100 CFU/L as the positivity threshold. Low and high establishment-year positivity were defined as ≤1% and ≥10% culture-positive samples, respectively; persistent or recurrent phenotypes required repeated yearly threshold fulfilment under predefined sampling criteria. Shotgun metagenomics was performed on 30 internal hot-water samples from 10 establishments representing extreme phenotypes. **Results:** Overall culture positivity was 6.6% (2.863/43.165). Cumulative establishment-level positivity was highly heterogeneous, with a zero-inflated, right-skewed distribution. Among 258 evaluable establishments with ≥4 years of data and ≥50 cumulative samples, 55 met criteria for persistent low positivity and 30 for recurrent high positivity. In the balanced 2023–2025 panel, 79/272 establishments remained ≤1% positive, with only one positive culture among 6,881 samples; by contrast, 41/272 establishments showed recurrent ≥10% positivity and accounted for 1.041 positives among 5.040 samples. Among establishments selected for microbiome profiling, historical culture positivity was 314/849 (36.9%) in the high-positivity group and 0/685 (0%) in the low-positivity group. Bray–Curtis PERMANOVA showed significant between-group separation of taxonomic and functional profiles ($p=0.009$ and $p=0.018$, respectively), while Shannon alpha diversity did not differ. **Conclusions:** Routine longitudinal surveillance identified divergent, reproducible building-level *Legionella* colonization phenotypes, characterized by persistently very low or recurrently high culture positivity. Distinct taxonomic and functional microbiome profiles support the biological relevance of these phenotypes and may inform phenotype-tailored surveillance and prevention strategies.

P053 U.S. responses to cruise-associated clusters of Legionnaires' disease — 2016–2025

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Cruise ships are a recognized site of Legionnaires' disease (LD) clusters as they have complex potable water systems and multiple hot tubs, and attract a wide range of travelers, including those at elevated risk of infection due to older age and underlying conditions. The U.S. Centers for Disease Control and Prevention (CDC) has jurisdiction over investigations of cruise clusters when ships operate out of U.S. ports. Upon identification of a cluster (defined as two or more LD cases with travel on the same ship in 12 months), CDC typically requests that cruise lines conduct environmental testing for *Legionella*, review guest and crew respiratory illness testing practices, and review water management activities. Response activities requested of cruise lines by CDC can include closure of hot tubs, remediation, and notification of guests and crew members regarding the risk of acquiring LD. We analyzed LD clusters using data from LD case reports sent to CDC with onsets between January 1, 2016 – December 31, 2025. During this period, 28 cruise-associated clusters were identified. Investigative environmental sampling was conducted for 21 (75%) of the clusters. Of those, *Legionella* was detected in 11 (52%) clusters (6 [55%] in potable water and 5 [45%] in recreational water). Based on epidemiologic data or testing results, among all 28 clusters identified, closure and remediation of hot tubs was requested in 9 (32%) and guest and crew member notifications were issued in 5 (18%). Identification and remediation of potential *Legionella* exposure sources during a cluster investigation is crucial to preventing additional cases from occurring. Among clusters where sampling was performed, more than half detected *Legionella*. This supports environmental sampling as a tool during the response to cruise-associated clusters. Jurisdictions should continue promptly reporting possibly cruise-associated LD cases to CDC to support early cluster identification and direct public health action.

P054 FT-IR Spectroscopy Enables High-Resolution Typing of *Legionella* spp. Beyond Routine Diagnostics

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Cultivation remains the gold standard for *Legionella* diagnostics. Although MALDI-TOF MS provides rapid species identification, it cannot sub-type, especially with respect to *L. pneumophila* serogroups. Supplementary methods (LAT, Dresden panel, PCR) are costly, subjective, or insufficiently discriminatory. This study therefore evaluates Fourier-transform infrared spectroscopy (FT-IR) as a fast, inexpensive, high-throughput tool for distinguishing *L. pneumophila* serogroups and non-pneumophila species. A total of 200 *Legionella* isolates were examined—120 environmental strains from the University Hospital Heidelberg and 80 clinical or environmental isolates from the Dresden reference laboratory. All cultures were grown 72 h at 37 °C on BCYE agar and identified by MALDI-TOF MS, latex agglutination test (LAT), and FT-IR spectroscopy. FT-IR analysis employed the manufacturer's "*Legionella* species + (linSVM)" classifier, trained with >3 200 reference spectra covering *L. pneumophila* serogroup 1, serogroups 2-15, and various non-pneumophila species. Spectral preprocessing used IR Biotyper and OPUS software; polysaccharide (1200–900 cm^{-1}) and lipid (3000– 2800 cm^{-1}, 1500–1400 cm^{-1}) regions served for species- and serogroup-level discrimination. Results were visualized by PCA and LDA. MALDI-TOF correctly identified 97.5 % of isolates at the species level but failed to separate serogroups. LAT distinguished *L. pneumophila* SG 1 from SG 2-15 and identified several species. FT-IR combined with LDA clearly separated SG 1 from SG 2-15 and formed species-specific clusters; only closely related pairs (*L. anisa*/*L. bozemanii*, *L. quinlivanii*/*L. birminghamensis*) overlapped. The approach remained robust for isolates with low MALDI-TOF log-scores and proved faster, cheaper, and automatable. In conclusion, FT-IR spectroscopy reliably discriminates *L. pneumophila* serogroups (SG 1 vs. SG 2-15) and non-pneumophila species, providing serogroup-specific information that complements MALDI-TOF. Refinement of the classification algorithms is expected to further improve serogroup differentiation.

P055 Exploring bioPROTACs with DARPins: A Novel Approach to Selective Protein Degradation

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Targeted protein degradation (TPD) is an innovative therapeutic strategy that goes beyond traditional protein inhibition by promoting selective protein degradation. While most research focuses on smallmolecule degraders such as molecular glues and PROTACs, bioPROTACs represent a new class of bifunctional degraders capable of targeting previously undruggable proteins. One such class of engineered degraders are Design Ankyrin Repeat Proteins (DARPins). Owing to their relatively small size, high target specificity and stability, DARPins have the potential to be used as research tools and therapeutics. In this study, we applied selection methods such as ribosome and phage display to generate a library of DARPin binders against the phosphodiesterase (PDE) domains of SdeA and SdeC, effector proteins that promote the survival of *Legionella pneumophila*. From a panel of 22 DARPins, we identified four candidates that bind the PDE domains of SdeA and SdeC in in vitro assays. Subsequent pull-down experiments in mammalian HEK293 cells revealed the most successful DARPin binding to the full-length SdeC. Our current aim is to obtain crystal structures of a DARPin hit in complex with its respective target protein to elucidate the molecular interactions involved. Previous research has demonstrated successful degradation of the transcription factor p73 using a bioPROTAC strategy that linked a DARPin to the SPOP E3 ligase. Building on this approach, we aim to first investigate the inhibitory potential of PDE-targeting DARPins and subsequently develop a bioPROTAC by coupling a PDE-specific DARPin to an E3 ligase.

P056 Cooling tower design can complicate management of *Legionella* pneumophila

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A large cooling tower system presented persistent culturable *Legionella pneumophila* with peaks reaching action thresholds despite aggressive disinfection. It was hypothesized that an isolated section of the system could be a shelter for *L. pneumophila* growth. Water was sampled periodically from June to September from 5 points: the cooling tower basin, the cold and hot reservoirs, the main pumps, and the coupon station. The latter is used for monitoring oxidation and consists of a small secondary loop located after the absorption machine and before the biocide injection loop. Total *Legionella* and *L. pneumophila* were quantified by qPCR and culturable *L. pneumophila* by liquid culture. Microbial communities were evaluated with 16S rRNA and 18S rRNA gene sequencing. Water temperature was higher at the coupon station and the hot reservoir. The total and residual chlorine equivalents were lower at the coupon station. Culturable and total *L. pneumophila* counts were higher at the coupon station, with culturable *L. pneumophila* reaching 106 MPN/L. At that time, the concentration at the other sampling points was only 104 MPN/L. Microbial communities were not significantly different from one site to the other, although the alpha diversity was somewhat higher in the coupon station. This point presented higher abundance of some biofilm-forming bacterial taxa and amoebae and ciliate taxa. The coupons station loop is located furthest from the biocide's injection loop, through which only a fraction of the water flows. As a result, a portion of the water passing through the coupon station does not receive the initial biocide treatment; it is only mixed with treated water when reaching the hot reservoir. This CT design creates a poorly disinfected zone, the coupon station loop, providing conditions where *L. pneumophila* can proliferate. Monitoring of cooling towers should include sampling points distal from the biocide injection point and isolated loops.

P057 Severe Legionnaires' Disease Presenting with Multi-Focal Vascular Pathology: An Epidemiological and Diagnostic Dilemma

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Background: Atypical presentations of Legionnaires' disease can delay diagnosis and obscure epidemiological links. We report a complex case of *L. pneumophila* infection in a healthcare worker, complicated by acute, life-threatening vascular events and challenging diagnostic serology. **Case Description:** A 62-year-old male anaesthetist (lifelong non-smoker, non-drinker) presented with a 48-hour history of severe rigors, fevers (40°C), hyponatremia (131 mmol/L), microscopic haematuria, and a C-reactive protein of 177 mg/L. CT imaging revealed bilateral atypical pneumonia. Concurrent CT angiography identified an acute left cervical internal carotid artery dissection (11 mm) and a left internal iliac artery aneurysm (28 mm) with an eccentric mural thrombus. Protocol driven *L. pneumophila* Serogroup 1 urinary antigen was negative. Early serology showed a persistently elevated Serogroup 1 IFA titre of 1:128, just below postive thresholds. All other extensive immunological and microbiological investigations were normal. **Ancillary Investigations:** Retrospectively, during the incubation window, individual's workplace announced contamination, with water from several outlets testing positive for *L. pneumophila* Serogroups 2–14. Lacking a productive cough, PCR or culture was impossible. Reference laboratory testing for pooled sera (Serogroups 1–7) returned a highly positive total IFA titre of 400. This confirmed a significant immune response matching the hospital's water testing profile. The systemic inflammatory cascade likely precipitated the acute vascular pathology. Following targeted Azithromycin, followed by Moxifloxacin therapy, this previously healthy individual survived the acute sepsis. But he remains with significant long-term vascular pathology requiring ongoing interventions and monitoring. **Conclusion:** This case highlights the need for multi-serogroup reference testing when singleserogroup assays mismatch environmental data. Severe *Legionella* spp.-induced inflammation can cause catastrophic, permanent vascular complications in healthy individuals. Healthcare workers remain an overlooked cohort at risk for hospital-acquired infections.

P058 The *Legionella* Reference Center: Strengthening US Public Health Response to Legionnaires' Disease

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The incidence of Legionnaires' disease (LD) in the United States (US) is increasing, and more outbreaks are being recognized. Laboratory support is critical to detect cases, identify compromised water systems, and link cases with likely sources for targeted prevention efforts. Because laboratory capacity for *Legionella* testing of clinical and environmental samples is limited in the US public health community, the Association of Public Health Laboratories (APHL) and the US Centers for Disease Control and Prevention (CDC) launched the *Legionella* Reference Center (LRC) in 2023 providing increased access to high-quality *Legionella* testing for governmental public health and environmental laboratories. The LRC, located at the State Hygienic Laboratory at the University of Iowa, offers culture for environmental and clinical samples, isolates' identification by Maldi-TOF mass spectrometry, detection and differentiation of *Legionella* species (L. sp), *L. pneumophila* serogroup 1 (Lp1) and *L. pneumophila* serogroups 2-15 (Lp) using multiplex qPCR, and short read whole genome sequencing for selected isolates. Since 2023, the LRC received testing requests for 49 outbreak investigations across 14 states, processing 1,221 environmental samples, 6 clinical specimens, and 13 clinical isolates. Lp1 was detected by multiplex qPCR on the 6 clinical specimens submitted. Clinical isolates were identified as Lp1 (6), Lp (5), *L. maceachernii* (1) and *L. longbeachae* (1). *Legionella* was recovered from 228 environmental samples (19%) from 33 investigations. Up to 3 isolates per sample were characterized for 262 total isolates, including 122 Lp1, 35 Lp, and 105 non-*pneumophila* species (*L. anisa* 72%, *L. bozemanae* 4%, *L. feeleii* 4%, *L. rubrilescens* 2%, *L. londiniensis* 1%, and unidentified species 17%). Without the LRC, environmental testing would have been unlikely or delayed in these investigations. Because *Legionella* testing capacity is difficult to maintain in individual laboratories, centralized testing has strengthened US public health, providing access to environmental testing for LD investigations.

P059 *Legionella* longbeachae in the north of Europe, 2022–2025: a multicountry surveillance study

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Objectives Legionnaires' disease (LD) is a severe pneumonia with a mortality rate of 9–25%. *Legionella pneumophila* is the predominant cause of LD in Europe, and current diagnostics favour its detection. Increased awareness and broader availability of diagnostic methods for *Legionella* non-*pneumophila* species, including *L. longbeachae*, have assisted detection of other species in Europe. We report a marked rise of *L. longbeachae* LD cases in Sweden in 2024, and additional cases in Finland, Denmark, Scotland, and Norway. **Methods** Data on diagnostic method, source investigation, age and gender of cases reported to national surveillance systems in Sweden, Finland, Denmark, Scotland, and Norway (2022–2025) as well as aggregated LD data from surveillance reporting to European Centre for Disease Prevention and Control by EU/EEA countries (2022–2024) were collected. Human *L. longbeachae* strains were whole genome sequenced, and genetic relatedness was compared by core genome multilocus sequence typing (cgMLST). **Results** In total, the five countries reported 191 *L. longbeachae* cases. Median age was 70 years, and 60% were male. Sweden reported the highest number (n=105), with a sharp increase from 2023 to 2024. In all countries, *L. longbeachae* accounted for 4–21% of LD cases. PCR-based methods detected most infections, and isolates were obtained in 45% of cases. Most cases, 68%, occurred between May and August. Handling growing media (e.g. compost and potting mix) was frequently reported. Whole genome sequencing identified matching isolates from growing media for three cases. An additional 87 *L. longbeachae* cases were reported in 2022–2024 by other EU/EEA countries. Epidemiological typing of human isolates from all five countries by WGS is ongoing and will be presented. **Conclusions** *Legionella longbeachae* contributes to a notable and likely underrecognised proportion of LD in Europe. Broader diagnostic testing and increased clinical awareness are essential to improve detection and guide in prevention.

P060 *Legionella* occurrence in drinking water installations: evidence from a large multicentre laboratory data set in Germany

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Background: In Germany, building water supply systems are subject to mandatory monitoring for *Legionella* spec., to assess technical conditions of the domestic drinking water installation. However, data on how sampling temperature, sampling location and sampling strategy influence *Legionella* detection in routine monitoring remain limited. This study evaluated a multi-laboratory dataset of *Legionella* spec. testing across Germany. **Methods:** We analyzed laboratory data from German drinking water testing laboratories collected within a retrospective and prospective part, 355,590 retrospective samples from 13 laboratories and 95,780 prospective samples from 9 laboratories were included. *Legionella* results were classified as negative, positive up to the German Technical Action Value (TAV) of 100 CFU/100 ml, or exceeding the TAV. Sampling temperatures were evaluated using 55 °C as threshold. Statistical and risk-modeling analyses evaluated the impact of sampling temperatures and facility usage types. **Results:** *Legionella* spec. was detected in 13.3% of retrospective and 12.6% of prospective samples. Exceedance of the TAV occurred in 6.1% and 4.2% of samples, respectively. A temperaturedependent pattern was observed across both study phases. At sampling temperatures below 55 °C, the TAV was exceeded in 5.5% and 3.7% of samples, respectively. In contrast, exceedance of the TAV decreased to 0.6% and 0.5%, respectively, when temperatures met the 55 °C benchmark. Prospective analyses showed that samples below 55 °C were about five times more likely to exceed the TAV. Non-compliance was more frequent in commercial than in public systems. Low number of samples per installation raise concerns about the validity of testing. **Conclusions:** The data demonstrate an association between insufficient warm water temperatures and *Legionella* contamination. This analysis provides validation for maintaining a temperature of 55 °C throughout hot water distribution networks to prevent *Legionella* growth. These findings support maintaining adequate temperature requirements and improving the selection and documentation of sampling points in regulatory monitoring.

P061 Implementation of water management standards has not altered *Legionella* pneumophila sequence type distribution in the United States between 2014 and 2024

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The first national standards for the prevention of legionellosis associated with building water systems in the United States were published in 2014 and 2015. These scientifically based consensus standards specified management of potable and nonpotable water to reduce the presence of *Legionella*, with efficacy most often validated by testing environmental samples for the bacteria. In the ensuing decade, several studies have demonstrated that implementation of water management programs reduced overall *Legionella* positivity in the built environment nationwide. However, reported legionellosis cases in the United States more than doubled between 2014 and 2024. One possible explanation for these observations is that implementation of standard water management approaches led to an increase in *L. pneumophila* sequence types (STs) often associated with transmission of the disease to humans from environmental sources, despite reducing the overall bacterial load. To investigate this hypothesis, we determined the STs of *L. pneumophila* isolates recovered from testing managed building water systems for validation of microbial control, as required by national standards, between June 2022 and October 2023. Results were compared to STs from clinical specimens associated with outbreaks and sporadic cases, collected between January 2022 and March 2024. Generally, the STs of environmentally derived *L. pneumophila* were distinct from the STs in clinical specimens. Only a small number of STs (ST1, ST36, ST37, ST42, ST187, ST211, ST1324, and ST1358) were identified in both environmental and clinical sources. A similar distribution was reported in a previous survey of *L. pneumophila* STs in the United States conducted between 1982-2012. Notably, the STs identified in both environmental and clinical sources in that study closely matched those observed in the present study. Together, these findings suggest that water management efforts implemented over the past decade have not substantially altered the distribution of *L. pneumophila* STs in the environment.

P062 Continuous monochloramine disinfection for *Legionella* spp. control in hospital water systems: short- and long-term effectiveness and comparison with other disinfection strategies

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Background Long-term control of *Legionella* spp. contamination in complex hospital water systems remains challenging despite multiple disinfection strategies. Monochloramine has emerged as a promising option because of its stability, persistent residual activity, and ability to penetrate biofilms. This study evaluated the short- and long-term effectiveness of continuous monochloramine disinfection and compared microbiological outcomes before and after the implementation of monochloramine, which replaced previously adopted continuous disinfection strategies in the same hospital water systems. **Methods** Between 2021 and 2025, 1,101 domestic hot water samples were collected from seven water networks in a large Italian university hospital according to a risk-based monitoring plan. Water temperature, monochloramine residual concentration, and free ammonia were measured at sampling. *Legionella* spp. was detected and quantified by culture according to ISO 11731:2017, and isolates were serotyped. **Results** Water systems continuously treated with monochloramine for more than a decade maintained low *Legionella* positivity rates (0.8–9.7%), demonstrating sustained control. Water systems recently converted to monochloramine achieved similarly low positivity rates (0–6.3%), highlighting the rapid effectiveness of monochloramine following implementation. Mean monochloramine residual concentration was significantly higher in *Legionella*-negative than in positive samples (2.11 mg/L vs. 1.43 mg/L; $F=17.54$, $p<0.001$). Direct comparison under identical infrastructural and operational conditions demonstrated the superiority of monochloramine over previous continuous treatments. Following replacement of chlorine dioxide, hydrogen peroxide, and hot water temperature control (maintaining >55°C at distal outlets), *Legionella* positivity decreased from 26.7% to 2.0%, from 32.9% to 6.3%, and from 47.1% to 0%, respectively. **Conclusions** This study provides real-world evidence that continuous monochloramine disinfection achieves durable long-term control and rapid reduction of *Legionella* spp. contamination in hospital water systems. However, sustained effectiveness depends on optimized dosing and appropriate maintenance of both the disinfection and water distribution systems, reinforcing the central role of an integrated Water Safety Plan for *Legionella* risk management.

P063* Proteomic profiling reveals macrophage bioenergetic responses during mid-to-late *Legionella* pneumophila infection

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Legionella pneumophila replicates within macrophages by establishing a specialized intracellular niche through the activity of the Dot/Icm type IV secretion system, which translocates hundreds of bacterial effector proteins into host cells. Although prior proteomic studies have provided important insights into *L. pneumophila* infection, proteomic changes driving cellular bioenergetics during midto-late stages of infection, when bacterial nutrient demands for intracellular replication peak, remain less well defined. To address this, THP-1 macrophages were infected with either wild-type *L. pneumophila* or a type IV secretion system-deficient (T4SS$^-$) mutant for 6, 10, 14, or 18 hours, allowing for the assessment of proteomic changes attributable to T4SS-dependent effectors. Host proteome remodeling was quantified using label-free, data-independent acquisition mass spectrometry. The data reveal a progressive, Dot/Icm-dependent remodeling of host bioenergetic pathways centered on mitochondrial respiration, calcium homeostasis, and intermediary metabolism. Early infection (6 hpi) was characterized by a T4SS-dependent increase in respiratory chain assembly factors CHCHD4 and COX14, alongside the mitochondrial calcium uniporter regulatory subunit SMDT1. By the intermediate stages (10–14 hpi), host responses shifted toward oxidative stress defense and respiratory chain regulation, exemplified by the sustained downregulation of the Complex I regulator DNAJC15 and upregulation of the antioxidant enzyme SOD1. Late infection (18 hpi) produced opposing abundance shifts in the calcium regulators MCUB and SMDT1, coinciding with broader alterations to host proteins tied to lipid and carbohydrate metabolism. Collectively, these findings demonstrate that *L. pneumophila* dynamically remodels macrophage proteins responsible for various aspects of cellular bioenergetics. By focusing on the transition into and progression through intracellular replication, this work provides a framework for identifying host pathways altered during later stages of infection and prioritizes candidate proteins for future functional analysis.

**Student Paper*

P064* Volatile Organic Compounds (VOCs) as potential biomarkers for *Legionella* pneumophila infections

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Volatile organic compounds (VOCs) are a diverse class of volatile molecules present in the atmosphere, released through natural processes and/or human activities. While extensively studied in the context of air pollution, VOCs are increasingly explored in medical research as potential biomarkers for disease diagnosis. Exhaled air, carries a complex mixture of VOCs whose composition may reflect underlying physiological or infectious states, offering a promising tool for rapid and noninvasive diagnostics. Using mass spectrometry, the VORTEX project aims to leverage this potential through the development of a standardized approach for VOC sampling and analysis in exhaled breath. Among the three pathogens tested, this work focuses on the bacterium *Legionella pneumophila*, the causative agent of Legionnaires' disease. This project integrates both *in vitro* experiments and *in vivo* / clinical studies, and relies on online (Vocus-PTR-TOF-MS) and offline (SPME-GC-MS, TD-GC- MS) mass spectrometry techniques to retrieve the chemical composition of the gaseous phase. In this context, we designed and optimized an *in vitro* system enabling the analysis of VOCs with three different methods simultaneously, while allowing the culture of infected and non-infected cells in dedicated reactors. Calibration experiments demonstrate the feasibility of the system for VOCs capture and analysis, with no significant impact of airflows on cell viability. While variations in VOCs profiles were observed depending on the analytical method, results were consistent across reactors, supporting good reproducibility of the system. The preliminary *in vitro* results obtained with the Vocus-PTR-TOF-MS revealed the emission of VOCs associated with the growth *L. pneumophila* in culture media as well as in infection experiments using A549 pulmonary epithelial cells. By providing candidate gaseous biomarkers of in vitro *L. pneumophila* infection, this work represents a key step toward the development of a breath-based diagnostics tools for infectious diseases, with ultimately potential applications in clinical and point-of-care settings.

P065 Epidemiological characteristics of a sudden increase in Legionnaires' Disease among Thai patients in Thailand, 2025 – 2026

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Background: Legionnaires' disease (LD) is an atypical pneumonia caused by *Legionella* bacteria. Immunocompromised individuals face the highest risk. In Thailand, most reported cases historically involved European travelers, with limited data on native Thai patients until a dramatic surge in 2025. This study describes the epidemiological characteristics and laboratory findings of LD among Thai patients. **Methods:** We analyzed event-based surveillance data and investigation reports from January 2013 to June 2026. A confirmed case was defined as pneumonia with a positive *Legionella* result by polymerase chain reaction (PCR), immunofluorescent assay (IFA) or urinary antigen test (UAT). Demographic, clinical and laboratory data were reviewed. **Results:** Out of 357 overall LD cases from 2013 to 2026, 24 were Thai patients (6.7%). Notably, 17 cases (70.83%) occurred during 2025–2026: 11 in 2025 (46%) and 6 in 2026 (25%). Only 7 cases were recorded during the preceding decade (2013–2024). The case fatality rate was 12.5%. The male-to-female ratio was 1:1 and the median age was 66 years (range: 4–88). Most patients (91.6%) had comorbidities with primarily chronic lung disease, cancer, autoimmune disorders and diabetes. Infection sources were community-acquired (63%), nosocomial (21%) and unknown (16%). Diagnostic methods included PCR (67%), IFA (21%) and UAT (12.5%). Point-of-care multiplex PCR was the primary tool in 2025–2026, accounting for 13 of the 17 cases. All cases were caused by *Legionella pneumophila* and 75% of patients resided in Bangkok. **Discussions:** A significant rise in Thai LD cases was observed in 2025–2026, likely driven by the adoption of rapid point-of-care PCR assays (turnaround time <90 minutes) in hospitals. Patient demographics and fatality rates align with Western cohorts, suggesting previous statistics reflected under-detection. This study is limited by the absence of environmental testing, as investigations were restricted to single-case reports.

P066 Breakthrough in Diagnosing Diverse *Legionella* Species and Other Intracellular Respiratory Bacteria

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Background Interstitial pneumonia, often caused by intracellular bacteria like *Legionella* species, poses significant diagnostic challenge, particularly for non-*L. pneumophila* (Lp) species for which comprehensive commercial assays are critically lacking. While reliable diagnostic methods are available for *Mycoplasma pneumoniae* (Mp) and *Chlamydia pneumoniae* (Cp), infections caused by the broad range of *Legionella species* (Lspp) frequently remain undetected. This study evaluates a novel commercial kit designed for simultaneous detection of five key respiratory bacteria: Mp, Cp, *Chlamydia psittaci* (Cpsi), and any *Ls*pp (*ssrA* gene) including Lp (*mip*). **Methods** The innovative Respiratory Bacterial Plus ELITE MGB® (RB+) kit (ElitechGroup) was evaluated on 200 clinical pneumonia samples. This cohort included 100 previously confirmed positive samples (20 Cp, 30 Mp, 32 Lp and 18 Lspp including 7 different species) and 100 confirmed negative samples. One positive sample was positive for both Lp and Lspp (*Legionella* coinfection). Cpsi detection was not evaluated in this study. **Results** The RB+ kit demonstrated remarkable accuracy, confirming all Cp and Mp positive samples. All Lp positive samples were successfully detected. For other *Legionella* species, 13 (72.2%) of the 18 positive samples tested positive ; the remaining 5 were *L. micdadei* and *L. maceachernii* samples. The *Legionella* coinfection tested positive without detecting two *Legionella* species. All 100 negative controls were accurately identified as negative. Overall sensitivity and specificity were 95% and 100%, respectively. **Conclusion** This study highlights the RB+ kit as a valuable tool for diagnosing respiratory infections by simultaneous detection of critical pathogens. It provides a crucial asset for early diagnosis of non *L. pneumophil*a infections. The non-detection of two *Legionella* species could be caused by a *ssrA* gene polymorphism and is indicated by the manufacturer instructions for *L. micdadei*. Further *in silico* analysis and prospective evaluations will enable assessment of the test's ability to detect all humanpathogenic *Legionella* species.

P067* *Legionella* in cistern-stored drinking water: a case study of water inequity in a Canadian First Nation community

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Rural First Nations communities in Canada experience persistent inequities in access to safe drinking water, a problem well documented across treatment, distribution, and storage infrastructure. Where piped distribution is incomplete, households often rely on water cisterns, which can introduce additional risk for microbial contamination. In collaboration with a Manitoba First Nation community, *Legionella* strains were isolated exclusively from concrete cistern sites, with no recovery from plastic cisterns or homes piped directly by the water treatment plant. While water was appropriately chlorinated at distribution, residual chlorine decayed rapidly once water entered cisterns regardless of cistern material, yet *Legionella* was recovered only from concrete sites. This points to surface water contamination through structurally compromised concrete as a key route of entry, with subsequent chlorine decay permitting persistence rather than driving initial introduction. Several recovered isolates demonstrated prolonged environmental survival and a capacity to infect U937-derived human macrophages, suggestive of genuine pathogenic potential rather than incidental presence. A novel *L. gormanii*-related isolate replicated to titres statistically indistinguishable from *L. pneumophila*, with immunofluorescence microscopy showing calnexin-positive vacuole formation and filamentous growth, an atypical but clearly productive infection phenotype. In contrast, an environmental *L. anisa* isolate showed significantly reduced replication compared to a genetically near-identical clinical *L. anisa* strain, despite over 99% sequence identity, underscoring that genomic similarity alone cannot predict pathogenic potential. These findings position alternative water storage infrastructure as an underrecognized risk factor for *Legionella* colonization, with implications extending beyond this community to similarly undermonitored settings elsewhere. Given global reports linking socioeconomic inequity to rising community-acquired legionellosis, and the likelihood that current case counts underrepresent true disease burden, we argue for expanded microbial surveillance in communities relying on nonstandard water storage and discuss this case as a broader example of the intersection between water infrastructure inequity and infectious disease risk.

P068 Lpg0173 and OxyR converge to regulate iron homeostasis and virulence in *Legionella* pneumophila

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The OxyR regulon is a central system for oxidative stress management in Gram-negative bacteria. Strides have been made in defining this system in *L. pneumophila*, its regulation, as well as the environmental trigger of OxyR remains ambiguous. Based on genome annotation, *lpg0173* was initially identified as a paralog of *L. pneumophila* OxyR, but structural comparison showed no meaningful homology to other OxyR proteins. Conspicuously, Lpg0173 lacks conserved cysteine residues seen in other OxyR proteins. The *lpg0173* gene sits adjacent to a biosynthetic gene cluster (*lpg0174-lpg0178*) that produces aromatic secondary metabolites; an isocyanide and N-acyl-L-histidines. This operon is homologous to the *pvc* operon of *Pseudomonas aeruginosa* where its corresponding metabolite, paerucumarin, was linked to oxidative stress management, biofilm formation, and virulence. When studied elsewhere, *lpg0174* (*pvcA*) and *lpg0175* (*pvcB*) were also hypothetically linked to iron homeostasis. Consistent with other studies, transcriptomic analyses confirmed that *lpg0173* regulates the *lpg0174- lpg0178* operon and RT-PCR confirmed it to be co-transcribed as a single unit. Biologically, single Δ*lpg0173* and double Δ*lpg0173*/Δ*oxyR* gene deletion mutant strains were unaffected *in vitro*, but hostspecific growth defects were observed in protozoa and U937-derived macrophages. Assays showed that *in trans* overexpression, rather than deletion, of *lpg0173* and *oxyR* reduced resistance to hydrogen peroxide and chlorine, with the double overexpression strain most affected. Additional transcriptomic analyses showed deletion or *in-trans* overexpression of *oxyR* dysregulated the *lpg0174-lpg0178* operon and suggested convergent influence over the cluster. Dysregulation coincided with broader disruption of iron homeostasis genes (*frgA, iroT/mavN, feoB*) and virulenceassociated effector and motility gene expression in both *lpg0173* and *oxyR* mutant strains. Together, these findings extend the functional role of the *lpg0174-lpg0178* metabolite cluster and reveal an unexpected regulatory link between Lpg0173 and OxyR despite their structural homology divergence, connecting oxidative stress response, iron homeostasis, and virulence in *L. pneumophila*. **Area:** Virulence & Pathogenicity

P069 Impact of pre-occupancy water management strategies on controlling *Legionella* growth: a comparative study of four newly constructed buildings.

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Extended vacancy periods prior to occupancy are not uncommon in newly constructed buildings, often due to commissioning delays, phased construction, or other project-related factors. During these periods, potable water systems may remain unused for several months, creating favorable conditions for microbial growth, including *Legionella*. Strategies such as system drainage and/or biocid water treatment have been proposed to reduce this risk. However, limited comparative evidence exists regarding their effectiveness during prolonged pre-occupancy periods. This study evaluates the impact of four pre-occupancy water management strategies on controlling *Legionella* growth in newly constructed buildings and during extended commissioning periods. The nearly identical design and construction timeline of the four buildings included in the study provides a rare opportunity to evaluate intervention strategies while minimizing confounding factors. The four residential buildings were assigned one management strategy each: 1) water stagnation, 2) water system drainage, 3) stagnation with copper–silver ionized water, and 4) drainage with copper–silver ionized water. The buildings' water is analyzed monthly for *Legionella* using culture-based methods, on both hot and cold water samples. General microbiological and chemical water quality parameters will also be monitored. Preliminary results demonstrate early *Legionella* colonization of the stagnant potable water system. The results of this study will provide evidence to support best-practice recommendations for managing potable water systems in newly constructed buildings before occupancy.

P070 Legionellosis prevention and control - The Cyprus experience during the past 3 decades

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Since the official advent of legionellosis 50 years ago, with the tragic events in Philadelphia USA, our country has only started experiencing outbreaks during the last 30 years. Although a number of incidents must have already passed undetected or undiagnosed, we have experienced some severe incidents which will form the basis of our presentation. The specific climatic conditions of the South-eastern Mediterranean region, in combination with the severe fluctuations in mains water composition, form a very characteristic legionellosis risk scenario. Being in a semi-arid region, Cyprus has been traditionally depending on rainwater collected in strategically located dams. However, after experiencing severe and prolonged droughts, the Government had started implementing a long-term policy to effectively address this water scarcity problem. Since 1997, when the first Seawater Reverse Osmosis (SWRO) plant was commissioned, the island is today having 6 fully operational SWRO's, providing a daily output of over 200K cubic meters. However, minimizing the legionellosis risk on the island still remains a challenging task. Issues relating to the biofouling potential of blended water supplies, condition of the mains water distribution network, water storage in roof tanks, and the absence of consistently applied water systems regulations, make it very difficult to effectively apply preventive measures and attain regulatory compliance. The presentation will focus on our experience during the past 30 years, through our involvement in numerous *legionella* risk assessment projects and in investigations of legionellosis incidents. Particular reference will be made to the December 2008 legionellosis outbreak in a private hospital neonatal ward, where three newborns died.

P071 Fate and environmental dynamics of *Legionella* spp. during soil aquifer treatment of secondary wastewater treatment plant effluent

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Background: As water reuse becomes increasingly important, Soil Aquifer Treatment (SAT) is emerging as a sustainable technology for wastewater reclamation. However, little is known about the fate and environmental behaviour of *Legionella* spp. during SAT, despite its relevance as an opportunistic waterborne pathogen. **Methods:** This study investigated the occurrence, seasonal variability and spatial distribution of *Legionella* spp. in three SAT systems (two pilot-scale and one demonstration-scale) receiving secondary wastewater effluent. *Legionella* spp., *Legionella pneumophila* and *L. pneumophila* serogroup 1 were quantified using multiplex qPCR. Water samples were analysed throughout the treatment train, including free-living (0.2–3 µm) and particle-associated (>3 µm) fractions. The performance of reactive barriers was evaluated, and culture-based analyses of *Legionella* spp. and *Pseudomonas* spp. were performed for comparison. **Results:** *Legionella* DNA was consistently detected throughout the studied systems. Concentrations in wastewater effluent increased during warmer months, indicating a seasonal pattern associated with temperature. Fractionation analysis revealed a shift in *Legionella* distribution during SAT: influent samples were dominated by the particle-associated fraction, whereas post-treatment water contained predominantly free-living cells, suggesting changes in the ecological niche of the bacterium during infiltration. SAT significantly reduced *Legionella* concentrations, although reactive barriers did not provide additional removal compared with conventional SAT systems. No *Legionella* colonies were recovered by culture despite repeated qPCR detection, confirming the limited sensitivity of cultivation for environmental samples. Although *Pseudomonas* spp. showed a positive trend with *Legionella* occurrence, no statistically significant correlation was observed. **Conclusions:** This study provides new insights into the environmental ecology of *Legionella* in SAT systems, demonstrating seasonal variability, changes in particle association and effective attenuation during treatment. The results highlight multiplex qPCR as a valuable tool for monitoring *Legionella* in reclaimed water while emphasizing the need to interpret molecular results in the context of viability.

P072 Temperature-dependent growth dynamics of 18 clinically relevant *Legionella* species

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Legionella growth in engineered water systems is strongly influenced by temperature, yet quantitative, species-resolved growth data remain scarce beyond *Legionella pneumophila*. This study evaluated the temperature-dependent growth of 18 clinically relevant pure cultures *Legionella* strains across 15-50˚C. Strains were revived on BCYE agar, transferred to BYEB broth, and then inoculated in triplicate into 96-well plates for batch growth assays. Growth was monitored by OD600 over 5-7 days and fitted with logistic models to estimate maximum growth rates, lag phases, carrying capacities, and temperature growth ranges, while assessing effect of prior cultivation temperature (30 or 37˚C). Maximum growth rates increased with temperature, peaking around 35-40˚C (µmax=0.2-0.8 h-1), followed by a sharp decline. At 43˚C, growth was already inhibited for more than half of the strains tested (10/18), whereas at 45˚C, only four still exhibited growth, including *L. londiniensis*, *L. jordanis*, and two *L. pneumophila* strains. Preliminary observations at 48˚C further indicated that *L. londiniensis* and one *L. pneumophila* strain remained capable of growth. Carrying capacities were consistently highest for *L. pneumophila*, likely reflecting the optimization of media, while non-*pneumophila* species generally reached lower final OD600 values. Lag phases decreased markedly with temperature, from 60-140h at 22˚C to 10-50h at 35-40˚C. Relative growth analyses showed considerable interspecies differences in temperature sensitivity (slopes=3.3-12.5%/˚C). Speciesspecific differences were also reflected in thermal niches: *L. anisa* exhibited the narrowest growth range, while *L. londiniensis* showed a clear shift toward higher temperatures. Previous cultivation temperature did not substantially alter them, but lag phases and thermal sensitivity rankings. Overall, temperature strongly shapes *Legionella* growth, yet marked interspecies variability indicates that thermal responses cannot be generalized. The persistence of certain strains at elevated temperatures suggests that current temperature-based control assumptions may not fully capture the species-specific physiology of *Legionella* in engineered water systems.

P073* Impact of Showerhead Design on *Legionella* Exposure Risk

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The availability of drinking water resources is declining due to climate change, necessitating reduced residential water use. Showering accounts for the largest share of water use, constituting 26% of the total water use of a Belgian household. Consequently, the adoption of water-saving showerheads has increased to reduce water use, energy use and the associated costs. Recent showerhead designs incorporate air-water mixtures to maintain user comfort while lowering flow rates. However, the differences in hydraulic conditions between these water-saving and conventional models, may influence aerosol generation. This study evaluates whether water-saving showerheads increase airborne concentrations *of Legionella pneumophila* compared to conventional models. *Legionella pneumophila* is a pathogen that causes a health risk when inhaling aerosols while showering. Airborne concentrations were quantified using a µ-Coriolis air sampler under controlled laboratory conditions with standardized contamination levels (200000cfu/l) and hydraulic settings. The concentrations were compared for the three different showerheads: Regular showerhead (8,5l/min), PowderRain showerhead (6,5l/min), and RainAir showerhead (7,7l/min) . The results show that the tested water-saving showerheads produce higher airborne concentrations of *Legionella pneumophila* relative to the source water concentration, resulting in at least a 2-fold higher air-to-water ratio compared to conventional models under similar conditions. These findings indicate that human health risk is influenced not only by bacterial concentrations in water, but also by showerhead design, highlighting the need to consider microbiological safety in the development of water-saving technologies.

P074 The diversity in *Legionella* spp. colony morphology when cultivated on conventional growth media

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Cultivation is used worldwide for the detection and quantification of *Legionella* spp., and is standardised in international norms. Nonetheless, different *Legionella* species do not grow uniformly on standard media and, in the absence of clear guidelines, accurate first-tier identification relies largely on the experience of the analyst. In this project, we documented and compared the growth of 24 diverse *Legionella* species/strains on conventional Buffered Charcoal Yeast Extract (BCYE) agar and Glycine–Vancomycin–Polymyxin B–Cycloheximide (GVPC) agar under standard cultivation conditions (37 °C, 3–9 days). Colonies were imaged and characterised by colour and morphology, and the images were deposited in an open-access digital library. The first main finding was that different *Legionella* species often showed completely different morphologies when cultivated under the same conditions. For example, an *L. pneumophila* SG1 strain on BCYE agar showed a "classic" smooth, egg-shaped colony with a dense centre and thin pink-purple iridescent perimeter. In contrast, an *L. pneumophila* SG2–14 strain grew as dense, granulated, cream-coloured colonies with scalloped perimeters. Such morphological heterogeneity may result in clinically relevant species being mischaracterised as non-*Legionella*. The second main finding was that antibiotics in GVPC agar frequently resulted in colonies that diverged from classic *Legionella* morphology. For example, *L. anisa, L. bozemanii, L. longbeachae* and *L. wadsworthii* all displayed markedly different colours and morphologies compared to their growth on BCYE agar. Consequently, analysts may fail to recognise positive colonies cultivated on GVPC agar. These results highlight (1) the remarkable diversity amongst *Legionella* spp., (2) the bias of cultivation media towards certain species, and (3) the importance of standardised analyst training. The digital library generated in this project can serve as a valuable starting point for recognising and identifying *Legionella*species.

P075 Phenotypic changes in *Legionella* pneumophila sg 1 over the years: experimental findings that coincide with a shift in the causal agent of TALD in Crete, Greece.

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Lp1 is the most common cause of TALD in Europe, although it does not predominate among the isolates from hotel water samples in Crete, Greece. A shift in the serogroup, to Lp3 and 6, as a cause of TALD in Crete has also been observed over the last years. To investigate a potential change in virulence of Lp1, we recorded the incubation period of all TALD cases caused by Lp1 reported in Crete during the last 25 years. We considered as incubation period the time between the first and/or the last day of stay at a hotel and the date of case recording. A continuous increase, at a slope of ~0.45, in the incubation period was observed over the years. We focused on 120 Lp1 isolated from the above hotel water samples following the notification of a TALD by the ECDC. Isolations were achieved following ISO 11731:1998 or 2017 or the Legiolert (IDEXX) approach. Biofilm formation was evaluated using a crystal violet microtiter plate assay across multiple conditions. Bacterial suspensions in BYE broth with and without Fe were inoculated into 96-well polystyrene flat-bottom plates and incubated at 25°C, 36°C, and 42°C for 4, 7, and 14 days. At each point, wells were stained with 0.2% crystal violet and biofilm biomass was quantified at OD542 using a microplate reader. Regardless of the conditions, the ability of the isolates to produce biofilm diminished over the years. The results of the biofilm study support the incubation period observations, enhancing the assertion that a slight change in virulence of Lp1 is being observed. The need to introduce diagnostic kits that will target other *L. pneumophila* serogroups and to isolate the pathogen from human cases is needed to better understand the changes in the epidemiology of the pathogen.

P076 Functional characterization of virulence-associated mutations selected during experimental evolution of *Legionella* pneumophila under triclosan exposure

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Legionella pneumophila, the causative agent of Legionnaires' disease, is an environmental pathogen frequently exposed to anthropogenic contaminants. Environmental pollutants such as triclosan (TCS) may exert selective pressure, drive bacterial adaptation and potentially influencing virulence. Experimental evolution of eight independent *L. pneumophila* Paris lineages, under increasing half- MIC TCS concentrations (ranging from 0.5× to 16×MIC of the ancestral strain) identified convergent mutations in the two-component system PmrAB. Here, we investigated the phenotypic consequences of these mutations on bacterial fitness and intracellular *Legionella* replication. Four isogenic mutants were reconstructed from the ancestral *L. pneumophila* Paris background: three carrying distinct *pmrA* mutations identified in three independently evolved lineages, and one carrying a *pmrB* mutation identified in one lineage. Intracellular replication was quantified by CFU enumeration following infection of amoebae and macrophages. The three *pmrA* mutants tended to display enhanced intracellular replication compared with the ancestral strain in both infection models, whereas the *pmrB* mutant showed no significant difference in intracellular replication but consistently reached lower final cell densities during axenic growth, indicating that distinct mutations within the PmrAB two-component system may have different phenotypic consequences. Given the known association of PmrAB with envelope remodeling and polymyxin resistance in Gramnegative bacteria, colistin susceptibility was also evaluated. No significant difference in colistin MIC was observed between the ancestral strain and any of the reconstructed mutants, suggesting that these mutations affect functions distinct from the canonical polymyxin-resistance pathway associated with PmrAB. Overall, our results suggest that adaptive mutations selected under chronic triclosan exposure could directly enhance the intracellular replication capacity of *L. pneumophila*. Ongoing transcriptomic analyses performed during exponential and stationary growth phases will help elucidate the molecular mechanisms linking environmental adaptation and virulence-associated phenotypes.

P077 Trials & tribulations of isolating *Legionella* Longbeachae in compost

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Introduction *Legionella longbeachae* is an emerging pathogen of increasing concern in the UK and across Europe, and a leading cause of compost-associated legionellosis in Australasia and Southeast Asia. This environmental bacterium can cause Legionnaires' disease and pneumonia. However, its detection, identification, and characterisation in both compost and clinical samples remain challenging, as most diagnostic methods are primarily tailored to identify *Legionella pneumophila*. This limitation complicates public health investigations of cases linked to *L. longbeachae* and underscores gaps in current diagnostic capabilities. Methods Acid-treated compost samples were cultured on GVPC and BCYE agar. Presumptive *Legionella* spp. isolates were confirmed using latex agglutination and identified as *L. longbeachae* by MALDI-TOF mass spectrometry (Bruker Biotyper). Clinical isolates were identified through amplification and Sanger sequencing of the macrophage infectivity potentiator (*mip*) gene. Whole-genome sequencing (WGS) of both clinical and environmental *L. longbeachae* isolates was carried out using the Illumina NextSeq 1000 platform with the UKHSA pipeline, enabling detailed genomic characterisation and assessment of genetic relatedness. Results UKHSA software identified all three isolates as *L. longbeachae* serogroup 1. Maximum likelihood phylogenetic analysis based on single nucleotide polymorphisms (SNPs) showed that the environmental isolates differed from the clinical isolate by more than 200 SNPs. Overall, SNP analysis demonstrated considerable genetic divergence both between the environmental isolates and between the environmental and clinical isolates, with no evidence of a close genetic relationship. Conclusion Whole-genome sequencing (WGS) enabled the accurate identification and serogrouping of all *L. longbeachae* isolates. SNP-based core genome analysis demonstrated considerable genetic diversity across both clinical and environmental isolates. This pronounced diversity, together with the limitations of current sampling methods in capturing the complex mixture of genotypes present in compost, hampers the ability to establish clear epidemiological links between patient isolates and environmental sources. Reliable source attribution in public health investigations remains difficult.

P078 From Compliance to Control: A Risk-Based Triage Model for Strategic *Legionella* Prevention in Large Municipal Building Portfolios

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Legionella prevention in large building portfolios is often managed through compliance-oriented sampling and isolated technical remediation. Practice shows this reactive approach rarely achieves sustainable risk reduction. In highly diverse portfolios, recurrent contamination is either the result of poor planning and construction or, in existing buildings, of technical failures. Systemic operational weaknesses distributed across multiple buildings are easy to track down by self-monitoring. Effective *Legionella* control therefore requires a transition from case-by-case intervention to portfolio-level risk governance. This poster presents a strategic *Legionella* management framework within the municipal real estate organization 'Immobilien Stadt Zürich' (IMMO), which manages approximately 1,950 buildings. Developing spanned several years and involved milestones, i.e. a) designing a standard on sampling and testing *Legionella* in drinking water plumbing systems; b) development of a feasible escalation and risk management ('EGM'); c) knowledge output for a technical standard (SVGW Guideline W3/E4) and d) additional routine testing in potable water cold. The outcome was the composition of a risk-based triage matrix enabling portfolio segmentation. Therefore, IMMO developed a decision framework combining laboratory analytics, building technology data and standardized intervention workflows. The dominant criterion for first-response prioritization is building classification based on the user group (building category, 'at high risk'). Following criteria are applied in descending order: presence of *Legionella pneumophila*; buildings with > 30 sampling points; *Legionella* concentrations > 10,000 cfu/l. Time (remediation) and cost are subordinate factors. The framework transformed *Legionella* management from isolated response to area-based preventive intervention planning. New properties added to the portfolio undergo a closer 2-year-monitoring to ensure that defects or warranty claims are reported promptly. The framework demonstrates that sustainable *Legionella* prevention at scale is primarily a question of management intelligence: only when microbiological evidence is converted into strategic asset decisions can drinking water hygiene be controlled efficiently, transparently and in long-term.

P079 The utilization of IDEXX Legiolert™ for the recovery and isolation of *Legionella* pneumophilia serogroup 1 (Lp1) in primary and environmental specimens

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Legionnaire's disease (LD) is a systemic infection caused predominantly by *Legionella pneumophila* (Lp), with. *Legionella pneumophila* serogroup 1 (Lp1) as the primary serogroup responsible for outbreaks in the United States. From 2019 to 2023, several community-associated outbreaks of Lp1 were identified. These outbreak investigations involved testing of both clinical and environmental samples. Using a combination of culture, real-time PCR, whole genome sequencing (WGS) and the IDEXX Legiolert™ methods, the Wadsworth Center laboratory (WC) was able to detect and characterize the environmental sources of each outbreak. The utilization of IDEXX in isolate recovery improved from both clinical and environmental primary samples. WC utilizes a streamlined testing algorithm combining molecular screening of environmental samples followed by culture on PCR-positive samples. Isolated colonies typical of Lp are confirmed by realtime PCR, sent for WGS, and analyzed using the in-house developed pipeline to determine isolate relatedness. In total, 85 primary clinical and environmental samples (22 clinical and 63 environmental) were tested over the course of 3 different outbreaks. Lp1 DNA was detected in 13 patient specimens but only isolated from 5/13 specimens using WC's traditional culture method. Using the IDEXX method, 7/13 were isolated out, improving recovery. Likewise, Lp1 DNA was detected in 19 environmental specimens. Of these 4/19 were isolated by traditional culture methods, while 11/19 were isolated by the IDEXX method only. Therefore, when comparing the WC conventional culture method to recovery from the IDEXX method, there was a 50% increase in isolate recovery using the latter method. The IDEXX Legiolert™ improved the recovery and isolation of Lp1 in both clinical and environmental sample, aiding in identification of exposure sources during the outbreak. The IDEXX method was essential in the isolation of Lp1, especially from environmental samples, providing a more rapid source attribution, minimizing additional cases during an outbreak.

P080 *Legionella* stuttgartensis sp. nov. and *Legionella* nigrisilvae sp. nov., two new species isolated from a re-cooling plant and from a drinking water house installation in southern Germany

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In Germany, the presence of *Legionella* in water installations is constantly monitored by the public health authorities responsible for the region administration. Following the Water quality and Sampling for microbiological analysis, ISO 19458:2006, two *Legionella* non-pneumophila strains were isolated from a re-cooling plant and from a drinking water installation and identified as two novel species within the genus *Legionella*. Both strains encoded antimicrobial resistance genes against betalactams, aminoglycosides and clusters for legioliulin biosynthesis that might confer fluorescence under UV light at 365 nm. The novel Species *Legionella nigrisilvae* encodes a unique environmental sensor domain, further to be characteised, not present in other *Legionella* species, that might shift the cell's cyclic di-GMP levels, triggering the bacterial dispersion into the environment.

P081 Severe Community-Acquired Legionnaires' Disease in a neonate: A Case Report

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Background: *Legionella pneumophila* is an environmental waterborne pathogen transmitted through contaminated aerosols. Neonatal legionellosis (LD) is rare, especially outside healthcare-associated settings and connected with high morbidity and mortality, requiring rapid microbiological diagnosis for appropriate targeted treatment. We report a severe case of community-acquired neonatal legionellosis in Greece, emphasizing the microbiological investigation and the isolate's molecular characterization.. **Case presentation:** A 10-day-old late preterm neonate was admitted to the neonatal intensive care unit with diarrhea, severe pneumonia and respiratory failure. Initial laboratory findings showed markedly elevated inflammatory markers. Empiric antimicrobial therapy was initiated, while bronchoalveolar lavage (BAL) was obtained for microbiological investigation. BAL molecular testing using the BIOFIRE FilmArray Pneumonia (PN) Panel, detected *Legionella pneumophila*. Specimen's culture on selective media **GVPC** and **BCYE,** confirmed *L. pneumophila* presence. The Oxoid *Legionella* Latex agglutination test identified the isolate as *L. pneumophila* serogroup 1. Targeted therapy with levofloxacin and azithromycin was then initiated. Further genomic characterization and detailed molecular typing was performed using the DNBSEQ-G400 Genetic Sequencer (MGI Tech Co., Ltd., Shenzhen, China). Extensive environmental investigation of both maternity unit and the home environment failed to identify a contamination source. Following targeted therapy, the neonate improved clinically and was discharged without sequelae. **Conclusions:** We report a rare case of severe community-acquired neonatal legionellosis in Greece, microbiologically confirmed by PCR, culture, serogrouping, and NGS-based typing. Isolation of the organism was particularly important, as it enabled serogrouping and advanced genomic analysis by NGS, providing valuable data for strain characterization and surveillance. This case underlines the critical role of specialized microbiological diagnostics in severe neonatal infections and emphasizes the public health importance of *Legionella* surveillance, including in community-acquired infections. Increased awareness of environmental pathogens as potential causes of neonatal pneumonia is essential, particularly in the context of changing epidemiology and potential climate-related influences on *Legionella* exposure.

P082 A Rare Case of *Legionella* feeleii Pneumonia: Diagnostic Challenges and Clinical Implications

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Background: *Legionella feeleii* can cause respiratory infections, including Pontiac fever and pneumonia in immunocompromised individuals. Diagnosis is challenging, as most commonly used urinary antigen tests only detect *L. pneumophila* serogroup 1, thus infections caused by other *L. pneumophila* serogroups and non-pneumophila *Legionella* species are often missed. **Case Presentation:** We report a case of severe pneumonia in a 40-year-old female patient presenting with gradual onset of tiredness, productive cough, and loose stools progressing to intermittent haemoptysis. Clinical samples from this patient were sent to the National *Legionella* Reference Laboratory at the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU), UKHSA, for further testing. **Methods:** Pus from right lungs and pleural fluid samples were processed at RVPBRU for *Legionella* species detection and identification using an in-house *Legionella* spp. qPCR assay and culture. *Legionella* spp. isolates were identified based on the amplification and Sanger sequencing of the macrophage infectivity potentiator (*mip*) gene. Whole genome sequencing (WGS) was performed using the Illumina NextSeq 1000 platform. UKHSA pipeline was used for species identification and single nucleotide polymorphism (SNP) analysis. Scanning electron microscopy (SEM) was performed at UKHSA. **Results:** All isolates were identified as *Legionella feeleii* by the *mip* identification method and WGS. SNP analysis showed that the *L. feeleii* isolates from different sample types were genetically almost identical (differing by only 0-2 SNP). SEM showed rod-shaped bacteria with flagella. **Conclusion:** This case highlights the diagnostic challenges associated with non-*pneumophila Legionella* infections. Reliance on urinary antigen testing alone may lead to missed diagnoses. Majority of diagnostic tests for legionellosis are based on the detection of *L. pneumophila*, hence incorporating wider urine antigens, molecular diagnostic screens and identification of isolates from lower respiratory samples will ensure timely identification and case management of rare infections caused by *L. feeleii* and other non-pneumophila species.

P083 Fifty Years After Philadelphia: New Challenges for *Legionella* Prevention in Building Water Systems

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Fifty years after the first recognized outbreak of Legionnaires' disease in Philadelphia (21 July 1976), *Legionella* remains a major public health concern. The case-fatality ratio observed during the original outbreak (34 deaths among 221 cases, ~15%) remains broadly representative today. Although naturally present at low concentrations in aquatic environments, *Legionella* proliferates in artificial water systems under conditions that are still frequently encountered. In Italy, reported cases of Legionnaires' disease continue to increase, with an annual growth exceeding 20%. This paper reviews the main factors promoting *Legionella* colonization and discusses how future building water systems should be designed to minimize microbial growth while meeting increasingly demanding energy-efficiency targets. Advanced technologies alone cannot ensure safety; neglecting fundamental hydraulic and thermal design principles may create conditions that favor bacterial proliferation, resulting in persistent contamination, costly remediation, and increased health risks. The presentation highlights the importance of integrating infection prevention with sustainable engineering. Effective risk reduction depends on appropriate temperature control, hydraulic performance, water turnover, and system maintenance, combined with the efficient use of energy and environmental resources. Representative case studies include low-temperature domestic hot water systems supplied by heat pumps, solar thermal and heat recovery installations, evaporative cooling towers, and adiabatic or mist humidification systems. While these technologies contribute to decarbonization, inadequate design or operation may increase the risk of *Legionella* growth. From both Life Cycle Cost Analysis (LCCA) and sustainability perspectives, insufficient prevention may ultimately generate higher operational costs and significant public health consequences. Finally, the paper examines the influence of climate change on *Legionella* epidemiology. Increasing ambient temperatures, prolonged stagnation, and extreme weather events are expanding the risk to both hot and cold potable water systems, particularly in Southern Europe. These emerging challenges require innovative engineering solutions that simultaneously achieve energy efficiency, sustainability, and microbiological safety.

P084 Evaluation of MICA *Legionella* as a Rapid Rapid Culture-Based and Automated Alternative to ISO 11731 for the Monitoring of *Legionella* pneumophila in Water Samples

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Background: *Legionella pneumophila* is the primary causative agent of Legionnaires' disease. Rigorous and frequent monitoring of domestic hot water systems and cooling towers is a fundamental part of prevention strategies. The current reference standard ISO 11731 has some limitations, such as relatively long turnaround times for results (up to 7-10 days), complex manual procedures, and significant variability among operators. **Objective:** This study evaluates the performance of MICA *Legionella*, a rapid culture-based method that allows to identify and count microcolonies after only 48 hours of incubation **Methods:** A total of 331 water samples, including domestic hot water and cooling tower water, were analyzed in parallel using the MICA *Legionella* system and the culture method according to ISO 11731. The MICA system uses metabolic labeling with a patented molecule (pLeg-N3) and click chemistry for the automatic enumeration of *L. pneumophila* microcolonies thanks to an AI-based analyzer. **Results:** The results demonstrate that MICA *Legionella* is statistically equivalent to the culture method. 27 samples (13 for culture and 14 for MICA) were found to be positive using only one of the two methods. MICA demonstrated high specificity (95%) and a sensitivity of 75%. Statistical agreement with the culture-based gold standard was found to be good (κ = 0.69). **Conclusions:** MICA *Legionella* provides a robust, automated, and rapid alternative for the enumeration of *L. pneumophila* by reducing the time-to-result from 7 days to 48 hours and eliminating the subjectivity of manual counting.

P085 Performance of MICA Advance *Legionella*, a rapid method for enumerating L. pneumophila, in a wide range of water types

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The detection of *Legionella pneumophila* in environmental water samples remains a key component of water safety management. Standard culture methods provide essential information on culturable bacteria but are limited by long incubation times, which can delay risk assessment and corrective actions. MICA Advance *Legionella* is a rapid culture-based method designed to detect and enumerate *L. pneumophila* microcolonies after 48 hours using specific fluorescent labelling and automated imaging and analysis. The performance of MICA Advance *Legionella* was compared to standard culture methods on samples of domestic hot water (154 samples), cooling tower water (86 samples) and wastewater (18 samples*). The new method results were correlated with the standard methods on all three types of water samples, demonstrating the versatility of MICA Advance *Legionella*. MICA Advance *Legionella* offers a reliable approach for rapid, culture-based *L. pneumophila* monitoring, with simplified workflow compared to the standard methods. Its integration into routine workflows could strengthen preventive strategies by combining speed, specificity and quantitative enumeration for a wide range of water systems. **experiments on wastewater samples were done by KWR water and CEW as part of the project "Reliable detection and control of Legionella pneumophila in wastewater", co-financed with PPP funding from the Top Consortia for Knowledge and Innovation (TKIs) of the Dutch Ministry of Economic Affairs and Climate and the results are made public.*

P086* Structure-Function analysis Dot/Icm T4SS protease effector LegA7

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Legionella species encode highly diverse arsenals of Dot/Icm type IV secretion system effectors. Many of the effectors are completely unique; however, several functional domains occur as frequently used modular building blocks. Understanding the roles of individual domains of effectors is key to dissecting their function but also to define the principles and constraints that shape the evolution of new effectors by domain shuffling. Here, we investigate the structure-function relationship of the effector LegA7. In silico, analysis showed that it combines two main domains, Ankyrin repeats, the most frequently found eukaryoticlike T4SS effector module, and a peptidase C58-like domain, a cysteine protease domain identified in T4SS and T3SS effectors from diverse pathogens. LegA7 is conserved in *L. pneumophila* and >20 other *Legionella* species. Moreover, *L. pneumophila* isolates encode variable numbers of related effectors with similar domain architecture; however, only LegA7 contains a putative N-terminal transmembrane helix. Ectopic expression experiments revealed that active LegA7 but not a cysteine protease motif mutant has profound effects on eukaryotic cells, disrupting the nucleoli and translation. LegA7 localised to the perinuclear endoplasmic reticulum (ER); however, surprisingly some signal in the nucleus was also observed. Analysis of truncation variants of LegA7 uncovered that the N-terminal helix was required for ER-localisation and mutants lacking the TM-helix showed cytoplasmic and strong nucleolar localization, revealing that the effector contains a nucleolar targeting signal. Using N- and C-terminally tagged LegA7 variants we observed that the effector underwent autoproteolytic cleavage. Mapping and mutating the auto-processing site revealed that it is of critical importance for the disruptive activity of LegA7. None of the homologous Peptidase C58::Ankyrin Repeat effectors tested self-cleaved or disrupted the nucleoli and translation. Our data suggest that, despite similar domain architecture, this effector family diversified and LegA7 evolved unique features to manipulate the nucleoli and thus ribosome biogenesis and translation.

P087 Streamlining *Legionella* clinical culture detection method: a 5-year analysis of incubation time and media optimization

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Spread-plate culture of lower respiratory secretions has been the gold standard for the diagnosis of Legionnaires' disease for decades; however, it is costly and time-consuming. Our laboratory clinical culture method has remained largely unchanged since initial implementation in the 1990s. For every specimen, we use a set of five agar plates: one non-selective BCYE plate, two plates with three selective antibiotics (PCV), and two plates with three selective antibiotics and glycine (GPCV). Analyst time per specimen is significant since plates are examined daily from days 3 to 7 post-plating, then periodically through day 14 or until *Legionella*-like organisms (LLO) are detected. We aimed to identify areas for improvement in this resource-intensive protocol by identifying the typical time to isolation and the optimal plate set. We reviewed records for all culture-positive specimens processed by our laboratory between 2021 and 2026, noting the day when LLOs were first detected and which plates were positive. We identified 86 culture-positive specimens: 7 non-*pneumophila Legionella* spp., 12 *L. pneumophila*, and 67 *L. pneumophila* serogroup 1. LLOs were first detected between days 2 and 7 post-plating, with most positive cultures (86.5%) detected between days 3-5; only one positive was detected on day 2 and one on day 7 (1.2% each). Nearly one third of these specimens (31.4%) were culture-negative on BCYE plates but culture-positive on selective plates. GPCV was the most successful medium, yielding growth for 74/86 (86%) specimens. Of the 12 specimens that failed to grow on GPCV, 10 (83.3%) grew on BCYE. Only 2/86 (2.3%) specimens grew exclusively on PCV plates. These observations suggest that the most critical time for plate observation is between days 3 and 5 post-plating, and the plate combination could be modified by excluding PCV media. After validation, the revised culture protocol should save resources without loss of sensitivity.

P088 PlaD, a novel type IVB secreted effector protein of *Legionella* pneumophila

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Introduction *Legionella pneumophila,* the causative agent of a life-threatening pneumonia, intracellularly replicates in a specialized compartment in lung macrophages, the *Legionella*-containing vacuole (LCV). During infection *L. pneumophila* secretes proteins, among others phospholipases, into the lumen of the LCV and the host cell cytoplasm via its type II (Lsp) and type IVB (Dot/Icm) secretion systems. At least 15 phospholipases A, which divide into the patatin-like proteins, the PlaB-like proteins and the GDSL hydrolases PlaA, PlaC and PlaD, are encoded in the genome. **Goals** We here focus on the characterization of the phospholipase PlaD and shows various differences to the other GDSL hydrolases PlaA and PlaC. We aim to understand the importance, secretion path, and mode of action in infection and activation mechanism of PlaD. **Materials and methods** We investigated the mode of secretion of PlaD by means of Western blotting and protein translocation assay. Additionally, we determined its binding to various lipids and its interactions with eukaryotic proteins by means of lipid-protein-overlay assays, proximity ligation, and pull down assays. Further, we analyzed the localization of PlaD during infection via immunofluorescence microscopy. **Results** We showed that, during infection, PlaD is Dot/Icm-dependently injected into the host cell cytoplasm where it localizes to distinct organelles. Moreover, we demonstrated that PlaD binds to a subset of phosphoinositide species and interacts with a class of regulatory proteins of the host cell. Additionally, our data revealed that the C-terminal half of PlaD is essential for its secretion and phosphoinositide binding but dispensable for interaction with the regulatory proteins. **Summary** Based on its Dot/Icm dependent injection into the host cell cytoplasm, we classify PlaD as a novel type IVB secreted effector protein of *L. pneumophila*. We propose that PlaD is involved in the regulation of host cell signaling cascades during infection.

P089* Heterogeneous dynamics of mobile genetic elements encode and spread antibiotic persistence

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Antibiotic persistence, the transient survival of a subpopulation of non-growing cells within otherwise susceptible bacterial populations, represents a major obstacle to effective antimicrobial treatment and is typically attributed to the inherent noise in bacterial cellular functions. Whether genome dynamics contribute to this phenomenon remains unknown. Here, we show that mobile genetic elements can actively generate and disseminate antibiotic persistence. Using an integrative approach combining stochastic simulations of a computational model with quantitative single-cell analyses of *Legionella pneumophila* and proteomics, we demonstrate that variations in the integrative and conjugative element pP36 activity generates stable population heterogeneity in growth states. This heterogeneity leads to the emergence of a distinct subpopulation of growth-arrested, persister-like cells, with elevated pP36 excision rates. We find that modulation of pP36 dynamics directly alters the proportion of non-growing cells and the level of antibiotic persistence, while acquisition of pP36 by a clinical isolate increases growth heterogeneity and enhances survival under antibiotic stress. These findings demonstrate that persistence-associated traits can be horizontally transferred between bacterial strains, indicating that antibiotic persistence can, in part, be a transmissible phenotype. At the molecular level, pP36 activity reshapes the proteome of persister-like cells pP36, a regulation consistent with the action of the pP36-encoded CsrA-like regulatory element, establishing a robust physiological state associated with tolerance. These findings identify reversible structural genome variation as a driver of bacterial phenotypic heterogeneity and antibiotic persistence. They further extend the role of mobile genetic elements beyond resistance gene transfer to include the control and dissemination of persistence, reshaping our understanding of, and potential strategies to combat, recalcitrant infections.

P090 Antimicrobial Resistance Profile of Clinical and Environmental *Legionella* pneumophila Isolates from Sicily

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Introduction *Legionella pneumophila* is the main causative agent of Legionnaires' disease, a severe form of pneumonia associated with morbidity and mortality. Although macrolides and fluoroquinolones remain the first-line treatment options, continuous surveillance of antimicrobial susceptibility is essential to detect potential resistance trends. This study aimed to evaluate the antimicrobial susceptibility profiles of clinical and environmental *L. pneumophila strains* in Sicily. **Materials and Methods** A total of 146 *L. pneumophila* strains were analysed, including 31 clinical and 115 environmental strains. Species and serogroups were identified using standard microbiological methods. Antimicrobial susceptibility testing was performed against rifampicin, levofloxacin, ciprofloxacin, moxifloxacin, azithromycin, clarithromycin, doxycycline, ceftriaxone, and tigecycline. Minimum inhibitory concentrations (MICs) were determined and compared between clinical and environmental isolates using non-parametric statistical analyses. **Results** *L. pneumophila* was the predominant species, and serogroup 1 represented the most frequently detected serogroup (63.7%). Overall, low MIC values were observed for rifampicin, fluoroquinolones, and macrolides. Median MICs were 0.0005 mg/L for rifampicin, 0.015 mg/L for levofloxacin, 0.03 mg/L for ciprofloxacin and moxifloxacin, 0.125 mg/L for azithromycin, and 0.03 mg/L for clarithromycin. Higher MIC values were recorded for doxycycline (8 mg/L), ceftriaxone (4 mg/L), and tigecycline (16 mg/L). Comparison between clinical and environmental isolates revealed largely overlapping susceptibility profiles. Clinical isolates showed significantly lower azithromycin MICs than environmental isolates (0.125 vs 0.25 mg/L; $p = 0.023$). A significant difference in MIC distribution was also observed for moxifloxacin ($p = 0.027$), whereas no significant differences were found for the remaining antimicrobial agents. **Conclusions** The antimicrobial susceptibility profiles of Sicilian *L. pneumophila* isolates indicate preserved in vitro activity of fluoroquinolones, macrolides, and rifampicin. Clinical and environmental isolates exhibited similar susceptibility patterns, suggesting limited evidence of antimicrobial resistance emergence. Continued surveillance of both environmental and clinical strains remains essential to monitor susceptibility trends and support effective management of Legionnaires' disease.

P091 Characterization of mitochondria-targeting *Legionella* pneumophila effectors

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Intracellular bacteria modify and control the behavior of eukaryotic host cells in a sophisticated and highly specific manner. The opportunistic, facultative intracellular bacterium *Legionella pneumophila* translocates more than 300 distinct "effector proteins" into the host cell during infection. These effectors have been shown to manipulate nearly every major cellular pathway of the host. One such target is the mitochondrion, where *L. pneumophila* effectors mimic, modify, or modulate mitochondrial components, such as the F_0F_1 ATPase or ATP/ADP translocases. Although the functions of most *L. pneumophila* effectors remain unknown, the sheer diversity of effectors suggests that a substantial number may target mitochondria. Indeed, the combination of bioinformatic and unbiased proteomics approaches has led to the identification of novel mitochondria-targeting effectors. This project investigates novel mitochondrial-targeting *Legionella* effectors that impair host cell respiration and promote bacterial replication. Preliminary data show that these toxins localize to mitochondria, disrupt their function, and structurally resemble known secreted bacterial toxins. This work will dissect the mode of action of these effector proteins using biochemical, genetic, and imaging approaches to evaluate their impact on host cell mitochondria. Additionally, the study will identify host interaction partners and assess their role in infection. These insights will advance our understanding of host-pathogen interactions and mitochondrial manipulation and may yield highly specific biomolecular probes with potential biotechnological and/or clinical applications.

P092 Thirteen years of molecular surveillance of clinical *Legionella* pneumophila isolates in Italy: sequence types, genetic relatedness and geographic distribution

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Identification of sequence types (STs) in *Legionella* pneumophila (Lp) clinical cases is essential to understand the pathogenic potential of circulating strains and to support prevention and control strategies. Sequence-Based Typing (SBT), together with Minimum Spanning Tree (MST) analysis, provides a standardized approach to investigate clinically relevant Lp strains. A total of 402 clinical Lp isolates, received by the National Reference Laboratory for *Legionella* from different Italian regions between 2013 and 2025 and stored at -80°C, were included. All isolates were typed using SBT to determine their ST. ST diversity was assessed using Index of Discrimination for Sequence Types (IODST). Allelic profiles were analyzed to evaluate genetic distances, an MST was generated using GrapeTree, and ST geographical distribution was explored using Microreact. Overall, 345 isolates were Lp1 and 57 Lp2-15. Among them, 387 isolates were assigned to an ST, while 15 could not be typed because *flaA* amplification failed. Among typed isolates, 332 were Lp1 and 55 Lp2-15. ST diversity was high in both groups, with IODST values of 0.942 and 0.962, respectively. Among Lp1 isolates, ST23 and ST1 were the most frequent STs, accounting for 18% and 12%, respectively. Within Lp2-15 isolates, ST1455 was the most common ST (14.5%), mainly due to its involvement in an outbreak, followed by ST1324, ST2841, and ST3364 (5.4% each). MST analysis showed genetic relationships among STs, while Microreact mapping highlighted a higher concentration of isolates from Milan, Rome, and Turin. This updated ST analysis provides a detailed overview of clinical Lp ST distribution in Italy, although the isolates were submitted only by some regions. Consistent with previous data, ST23 and ST1 were confirmed as the most frequent sequence types in LD cases. ST23 shows very limited allelic variation in cgMLST, and similar analyses should be extended to ST1 and other common STs.

P093 Genomic surveillance of *Legionella* pneumohila serogroup 1 isolates from hotel water systems in Italy (2020-2025)

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Among the countries reporting LD cases to ECDC, Italy has the highest number of travel-associated cases, most of which are part of clusters linked to accommodation sites. The Italian National Reference Laboratory annually receives isolates from hotel water systems associated with clusters, submitted by regional reference laboratories. The aim of this study was to use genomic surveillance to investigate the distribution and genetic relatedness of *Legionella pneumophila* serogroup 1 isolates from a subset of these hotels, to better understand their molecular epidemiology and support source attribution, risk assessment, and targeted prevention. A total of 111 *Legionella pneumophila* serogroup 1 (Lp1) isolates collected between 2020 and 2025 were selected to represent most Italian regions. Whole-genome sequencing was performed using Oxford Nanopore Technologies. Long reads were quality-checked with FastQC and Chopper and assembled using Flye. Sequence type (ST) and cgMLST profiles were also determined. The 111 Lp1 isolates were from twelve regions across northern, central, and southern/islands Italy, with most collected in Veneto, Lazio, and Lombardy. Overall, 25 ST were found: ST1 was the most represented (n=27), followed by ST42 (n=10) and ST23 (n=7). ST47 was detected for the first time (n=4), having not previously been reported among either clinical or environmental isolates from other sources. Three of the four ST47 isolates were found in Veneto, in the same hotel, while the fourth was detected in the Autonomous Province of Bolzano. cgMLST showed allelic differences (ADs) ranging from 0 to 273, with the lowest ADs found within the same ST from the same hotel. The numerous ST determined in these hotels is suggestive of the genetic diversity of Lp1 isolates circulating in Italian accommodation sites. The low allelic differences observed within the same ST and hotel confirm the value of cgMLST for identifying closely related strains and supporting cluster investigation.

P094 Genomic analysis of *Legionella* pneumophila serogroup 1 isolates from a hospital water system in Northern Italy, 2012–2023

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Legionella pneumophila is an important cause of hospital-acquired pneumonia, particularly in immunocompromised patients, and is associated with a higher mortality rate than community- or travel-acquired infections. Nosocomial cases may occur despite intensive control measures applied to hospital water systems, including mechanical, chemical and physical treatments, alone or in combination. In Italy, the number of reported nosocomial cases increased from 69 to 158 between 2014 and 2024, corresponding to a 129% increase. Sporadic cases are frequently associated with impaired immune status, chronic degenerative diseases, neoplasms, or other underlying conditions. For this reason, environmental surveillance programs are implemented in many hospitals to monitor and control changes in *Legionella* contamination levels. In this study, we analyzed environmental isolates collected during a monitoring period from 2012 to 2023 and accompanied by metadata, to determine the genomic characteristics of *L. pneumophila* serogroup 1 (Lp1) in a hospital in Northern Italy. The hospital was associated with two nosocomial cases, reported in 2021 and 2023. A total of 74 Lp1 isolates obtained from different sampling sites, including boilers, hot water return systems, showers in patients' rooms, staff changing rooms and other locations, were analyzed by whole-genome sequencing. Reads were quality-checked using FastQC and Chopper and assembled with Flye. Sequence types were assigned using Legsta, while cgMLST was performed with chewBBACA using the 2,009-loci scheme. Core SNP analysis was carried out using Snippy. Contamination levels ranged from 300 to 2.4×10^5CFU/L. All isolates belonged to ST1 and showed highly similar cgMLST profiles, with only one or two allelic differences, while core SNP analysis revealed genomic micro-diversity. Both suggest the long-term persistence of a closely related but not identical ST1 population within the hospital water system. These findings highlight the importance of integrating environmental surveillance, genomic analysis, and epidemiological metadata to better understand persistence and transmission dynamics in healthcare settings.

P095 SMART-COP Score and Lymphopenia as Complementary Predictors of Severe Clinical Evolution in Legionnaires' Disease

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Clinical outcomes in Legionnaires' disease (LD) are highly variable, suggesting that progression to critical illness depends not only on acute physiological severity, but also on host vulnerability and, potentially, strain-specific virulence factors. This study aimed to identify determinants of severe clinical evolution in hospitalized patients with microbiologically confirmed LD and to explore how complementary risk dimensions contribute to adverse outcomes.We conducted a retrospective observational cohort study including consecutive adults hospitalized with microbiologically confirmed LD between January 2021 and June 2025. The primary outcome was severe clinical evolution, defined as intensive care unit (ICU) admission, extracorporeal membrane oxygenation (ECMO) support, or in-hospital death. The secondary outcome was length of hospital stay. Admission demographic, clinical, laboratory, radiological, and severity-assessment variables were evaluated using receiver operating characteristic curve analysis and multivariable logistic regression.Sixty-two patients were included; 14 (22.6%) experienced severe clinical evolution. Patients with adverse outcomes had higher SMART-COP scores, lower lymphocyte counts, higher platelet-to-lymphocyte ratios, and more extensive radiological involvement. SMART-COP score, lymphocyte count, and platelet-to-lymphocyte ratio showed the strongest discriminatory performance, with AUC values of 0.774, 0.766, and 0.775, respectively. Subsequent analyses focused on SMART-COP score and lymphocyte count as clinically complementary markers of disease severity and host-response vulnerability. A combined model including both variables achieved superior discrimination (AUC 0.859) and significantly improved prediction compared with SMART-COP alone ($p=0.0056$). Patients with both SMART-COP ≥ 5 and lymphocyte count $\leq 0.75 \times 10^3/\mu L$ had a markedly increased risk of severe clinical evolution (69.2%; OR 19.8, 95% CI 4.43–88.53) and longer hospital stays than the remaining patients.Severe clinical evolution in LD appears to reflect the interaction between physiological severity and host-response impairment. The coexistence of lymphopenia and high SMART-COP score identifies a clinically meaningful high-risk phenotype associated with ICU admission, ECMO support, death, and prolonged hospitalization.

P096 metagenomic analysis of various processed water distribution systems in dhaka, bangladesh, reveals the presence of *legionella* pneumophila: a hidden threat to public health

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Legionella pneumophila, a pathogen causing Legionnaires' disease, was investigated in various urban water distribution systems in Bangladesh. A total of 128 water samples were collected from the water distribution systems of two hospitals, five food industries, and one hotel. Samples were examined for several physicochemical parameters, including TDS, pH, conductivity, and salinity. The metagenomic DNA of each water sample was analyzed by the detection of *Legionella pneumophila*-specific 16S rRNA gene using PCR, and the PCR products were sequenced and compared with sequences in the NCBI database. This investigation detected *Legionella pneumophila* in 32 (25%) water samples, including potable water, chiller water, cooling tower water from the food industry and hotel, ICU tap water, and fan coil unit water from the hospital. Sequencing confirmed 100% similarity with *Legionella pneumophila* sequences in the NCBI database.

P097 Handling practices of horticultural growing media among Swedish leisure gardeners: Implications for *Legionella* longbeachae exposure and prevention

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Legionella longbeachae infection has been associated with gardening, compost and commercial bagged growing media. Swedish outbreak investigations have linked disease to residential gardening and handling of commercial bagged soil, particularly dry and dusty material. However, exposurerelevant practices among leisure gardeners remain insufficiently described. We conducted a web-based questionnaire survey among Swedish leisure gardeners to describe gardening frequency, use and storage of horticultural growing media, compost and organic fertilisers, handling of dry or dusty material, risk awareness and self-reported preventive behaviours. This interim descriptive analysis includes 1,364 responses. Respondents represented a highly gardening-active group: 53% gardened daily and 43% several times per week during the growing season. Most reported activities involving repeated contact with organic growing media, including outdoor planting, soil amendment use, compost management, indoor plant repotting, raised-bed cultivation and greenhouse or tunnel cultivation. Among users of commercial growing media, 46% reported that material was always or sometimes dry or dusty, and 88% used material from opened bags that had dried out. Opened bags were often stored for long periods; 46% were kept for 2–12 months and 14% for more than one year. Most respondents did not moisten remaining bagged substrate before reuse. Awareness was limited: 72% had not heard that legionellosis may be linked to growing media, garden soil or compost. Preventive practices mainly concerned general hygiene, whereas fewer respondents reported measures aimed at reducing inhalation of dust or aerosols. Common leisure-gardening practices create plausible inhalational exposure situations for *L. longbeachae*. Prevention should therefore emphasise dust and aerosol reduction, targeted communication to vulnerable gardeners and clearer risk information through retail and productlabelling channels.

P098 *Legionella* isolates from different wastewater treatment plants differ in temperature-dependent growth, even within the same species

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Although mass balance analyses have suggested that *Legionella* proliferation in activated sludge systems of wastewater treatment plants (WWTPs), WWTP-associated outbreaks are rarely reported because *Legionella* may not remain long enough in the system to reach hazardous concentrations. Increasing wastewater temperatures driven by climate change may enhance *Legionella* proliferation and could shorten the time required to reach such levels. To explore the effect of medium and sampling site on temperature-dependent *Legionella* growth, we investigated the growth of 11 isolates from five industrial activated sludge systems in yeast extract medium and influent wastewater at 20, 25, 30, 35, and 40 °C using OD600 measurements. Isolates included two *L. pneumophila* SG1, four *L. pneumophila* non-SG1, one *L. dumoffii*, one *L. feeleii*, two *L. londiniensis*, and one *L. erythra/ rubrilucens*, whereby isolates belonging to the same species were recovered from different WWTPs. Only one isolate grew in influent wastewater. This *L. londiniensis* isolate, from a WWTP with wastewater temperatures of 40 °C, displayed an unusual growth pattern, growing only at 30 °C in yeast extract medium but exclusively at 40 °C in wastewater. In contrast, the second *L. londiniensis* isolate, from a WWTP with wastewater temperatures of 28 °C, grew between 20 and 35 °C in yeast extract medium but not in wastewater. Growth patterns among *L. pneumophila* isolates differed to a lesser extent. These findings demonstrate intraspecies variability and show that isolate sampling site and growth medium influences temperature-dependent growth. Both the lag phase and maximum specific growth rate varied with temperature, with changes in the lag phase being more pronounced. In contrast, amplitudes remained largely unchanged within isolates. This suggests that temperature affected physiological adaptation, whereas nutrient availability determined maximum biomass. Our findings demonstrate that temperature alone does not determine growth of wastewaterassociated *Legionella*; isolate-specific responses and environmental conditions play a critical role.

P099 A Presentation on a new book: A Guide to Emergency Flushing and Disinfection for *Legionella*

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This presentation outlines a newly published guideline titled: **"A Guide to Emergency Flushing and Disinfection for *Legionella*".** The book covers the steps a building owner should follow when notified of a health official confirmed positive case of Legionnaires disease associated with their building. This includes: a step-by-step plan for dealing with key issues. The book covers: Developing a *Water Management Team* and *Water management plan*, Epidemiology, Emergency Remediation steps, dealing with media, managing panic, assessment of building population, considerations for building evacuation or building closure. Discussion on "Prevention rather than cure", Requirements for flushing and disinfection, Analysis of plumbing/mechanical/piping system failures that have led to *Legionella* outbreaks. In Part 2 of the guideline, it covers a checklist to develop a customized emergency flushing and disinfection protocol as part of a Water Management Plan. It also covers remediation action items prior to flushing and disinfection operations. Part 3 covers the steps for an emergency response to *Legionella* bacteria suspected or confirmed in a building water system. It includes: Performing and documenting the process including pre-flush baseline tests, the pre-disinfection flush, disinfection process, post-disinfection flush operations then post operation testing. Part 4 covers resources & reference materials.

S17* / P100* *Legionella* pneumophila subverts mitochondrial dynamics and mtDNA quality control to promote intracellular replication

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Intracellular bacteria manipulate and subvert cellular processes in their eukaryotic hosts in a sophisticated manner. While the facultative intracellular pathogen *Legionella pneumophila* naturally replicates in free-living amoeba, inhalation of this pathogen can lead to a severe pneumonia due to the ability of the bacterium to replicate in alveolar macrophages. During the intracellular state, *L. pneumophila* secrets over 300 "effector proteins" into their respective hosts. This enables the formation of a *Legionella*-containing vacuole (LCV) by subverting phagosome maturation and other cellular processes. While LCV formation is essential for the survival and replication of the pathogen, many other host cellular processes are targeted and subverted to further support the survival and replication of intracellular *L. pneumophila*. Mitochondrial function and dynamics are essential for virtually all cellular processes. Not surprisingly, intracellular bacteria such as *L. pneumophila* specifically target and modulate mitochondrial components and their function. In this study, we identified several *L. pneumophila* effector proteins localizing to and modulating mitochondria. Some *L. pneumophila* deletion mutant strains lacking individual mitochondria-targeting effector proteins are impaired for intracellular replication within host cells and inhibit host cell respiration less efficiently compared to the parental strain. We are currently investigating the mode of action of a novel secreted mitochondria-targeting *L. pneumophila* effector kinase. The kinase contains an N-terminal mitochondria targeting sequence, which is required and sufficient for mitochondria localization, where it targets a mitochondrial fission protein. Thus, the *L. pneumophila* kinase subverts critical steps in mitochondrial dynamics and mtDNA maintenance, revealing a novel post translational regulation of a critical host protein.

**Student Paper*

RS01* / P101* Discovery of antiphage defence systems targeting a novel bacteriophage of *Legionella* pneumophila

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Recent work in the Ensminger lab has identified and characterized the first bacteriophage infecting *L. pneumophila*, called LME-1. Investigation of the interaction between LME-1 and *L. pneumophila* has already revealed multiple mechanisms by which *Legionella* defends itself against bacteriophages, including insight into the acquisition of a key *Legionella* virulence determinant (*lag*-1). In a subset of *L. pneumophila* strains, I have observed an additional layer of previously uncharacterized, robust antiphage defence. Using reverse genetics, I have recently localized this defence to an integrative and conjugative element (ICE). Bioinformatic analysis shows that other *L. pneumophila* ICEs consistently encode predicted defence genes, suggesting they may be rich reservoirs for a diversity of antiphage defences. Overall, this work expands the understanding of how *Legionella* interacts with phages and suggests that antiphage defence represents another advantageous trait provided by the mobile genetic elements that drive the diversity and plasticity of *Legionella* genomes. Finally, it reinforces the idea that bacteriophages are an important evolutionary force driving the adaptation of *Legionella* to its environment, despite the historical lack of isolated *Legionella* phages.

**Student Paper*

S03* / P102* Comparative genomic analysis reveals distinct population structure in *Legionella* anisa

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Legionella anisa is one of the most frequently detected non-pneumophila species in engineered water systems (EWS). Ecologically, *L. anisa* inhabits niches distinct from those of *L. pneumohila*, favouring cold-temperature environments. Despite its prevalence in the environmental systems, *L. anisa* has low virulence potential as evident by its association with few clinical cases. However, it is still a concern in healthcare facilities (HCFs), where vulnerable populations are at increased risk of infection. The genomic diversity and population structure of *L. anisa* remain poorly characterized. The goal of this study was to perform complete genome characterization for new *L. anisa* (n=4) isolates recovered from a HCF in Quebec, Canada. We further investigated the population structure of this species by performing comparative genomic analyses of the isolates from this study together with publicly available *L. anisa* genomes. Genome-wide phylogenetic analysis revealed the presence of three distinct clades separated by substantial genetic divergence (~500 SNPs). The Quebec isolates forming a tightly clustered group with isolates from Europe and North America, suggesting a clonal lineage. Comparative pangenome analysis indicated moderate core genome conservation accompanied by a highly variable accessory genome (~50%), consistent with the dynamic genome characteristics of *Legionella* species. The isolates characterized in this study harbored multiple plasmids encoding genes associated with conjugation, heavy metal resistance, and other stressrelated functions, suggesting potential roles in environmental persistence. Previous studies have shown that *L. anisa* can proliferate within protozoan host cells, although outcomes vary depending on the host species. Our isolates showed efficient proliferation within *Acanthamoeba castellanii*, but not within *Vermamoeba vermiformis*, under the conditions tested. Together, these findings underscore the genomic diversity of this previously understudied *Legionella* species and provide a framework for future investigations into its environmental persistence and potential pathogenicity.

**Student Paper*

S05∗ / P103∗ Exploring the biphasic lifecycle of *Legionella* through characterization of L. pneumophila protein Lpg1968

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Legionellae are Gram-negative intracellular pathogens of protozoa naturally found in freshwater environments, and in concentrated levels within piped water systems of which aerosols may be inhaled by humans resulting in a severe pneumonia termed Legionnaires' disease (LD). As infection occurs in the host, the bacteria initiate its biphasic lifecycle of which the switch from replicative to transmissive phase is tightly governed by numerous regulators. In particular, the transcription factor PsrA which responds to *in vitro* changes in long chain fatty acid concentrations, but the mechanism of fatty acid regulation is unknown. Interestingly, comparative transcriptomic analyses of the parental and isogenic Δ*psrA* mutant strain showed that the only gene that was significantly upregulated from 93 differentially expressed genes was *lpg1968*. Thus, a molecular and biophysical approach was taken to understand the function of Lpg1968. Using X-ray crystallography, the crystal structure of Lpg1968 was determined of which function is putatively a hydrolase enzyme. Using the structure, we perform *in silico* docking to discover putative substrates and test the contribution of Lpg1968 to the ability of *Legionella* to persist within host cells. Preliminary results using nanoDSF suggest that Lpg1968 is stabilized *in vitro* by numerous small molecules relevant to the biology of *Legionella*. Characterization of Lpg1968 will lead to a better understanding of the role of this protein during infection of host cells and therefore the regulation of *Legionella*'s biphasic lifecycle.

∗Student Paper

S08/ P104 SidL is an adenylyltransferase that modifies the host glycolytic metabolite 3-phosphoglycerate

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The large effector arsenal of *Legionella pneumophila* enables it to target multiple aspects of host cell biology and focused study of individual effectors has been a rich source of novel biochemistry and insight into host-pathogen relationships. Here, we biochemically characterize the purported translation inhibitor SidL/Ceg14 (Lpg0437) *in vitro* and redefine it as a glycolysis-targeting enzyme that modifies the metabolite 3-phosphoglycerate (3PG) with adenosine monophosphate (AMP) to produce the previously unknown molecule 2-AMP-3-phosphoglycerate (2-AMP-3PG). Reflecting its *in vitro* biochemistry, ectopic expression of SidL in mammalian cells generates 2-AMP-3PG, reducing cellular 3PG levels and downstream glycolytic intermediates. Consistent with this disruption of glycolysis, SidL inhibits the nutrient-responsive translation regulator mTORC1 and we propose that this indirectly decreases translation, explaining the initial identification of SidL as a translation-targeting effector. In an infection model, we observe SidL-dependent synthesis of 2-AMP-3PG in human macrophages infected with *L. pneumophila*, with its production peaking at early stages of the infection cycle. Current efforts are underway to understand how *L. pneumophila* uses SidL and 2- AMP-3PG to its benefit. To our knowledge, this is the first report of a bacterial effector that chemically modifies a host glycolytic intermediate, uncovering a new modality by which pathogens can interface with host metabolism.

S14∗/ P105∗ Modulation of intercellular tunnelling nanotubes of Dictyostelium discoideum by *Legionella* pneumophila

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Tunnelling nanotubes (TNTs) are thin, actin-dependent protrusions connecting cells and enabling intercellular communication over short to intermediate distances. Unlike classical secretion-based communication, TNTs provide a continuous route for transfer of cellular material, including cargo up to the size of whole organelles. In mammalian systems, TNTs have been implicated in tissue repair, immune regulation, inflammation, tumor progression, neurodegeneration, and host-pathogen interactions. However, despite their emerging biological relevance, the cellular mechanisms controlling TNT formation, cargo selectivity, and directional transport remain elusive. This is partly due to the lack of genetically tractable model systems. This project aims to establish *Dictyostelium discoideum* as a model organism to study TNT-mediated intercellular cargo transfer. *D. discoideum* shares important functional properties with mammalian phagocytes and is widely used as a host model for intracellular pathogens such as *Legionella pneumophila*, the causative agent of Legionnaires' disease. Using genetically encoded fluorescent reporters for mitochondria, endosomes, and lipid droplets, we show that *D. discoideum* forms TNTlike intercellular connections capable of transferring cargo between cells. Moreover, TNT formation is modulated by cellular stress and bacterial infection. Treatment with latrunculin B promotes TNT formation and enhances intercellular transfer of fluorescently labelled cargo, supporting a role for cytoskeletal remodeling in TNT induction. In contrast, infection with wild-type *L. pneumophila* reduces TNT formation, whereas mutants lacking microtubule-modulating effectors partially restore TNT formation. These findings suggest that bacterial effectors interfere with TNT-dependent intercellular communication. Together, these results identify TNTs as intercellular transport structures and establish *D. discoideum* as a powerful model to dissect the molecular mechanisms underlying TNT formation, cargo selection, and infection-dependent modulation. By combining live-cell confocal fluorescence microscopy, genetically encoded cargo reporters, and *L. pneumophila* effector mutants, this study provides new insight into how cytoskeletal remodeling controls TNT-mediated exchange of organelles and vesicular cargo during stress and infection.

∗Student Paper

P106 Actin and AnkJ reciprocally regulate the catalytic state of the *Legionella* effector Ceg14/SidL

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Ceg14/SidL is a *Legionella pneumophila* effector whose enzymatic activity is activated by host actin and inhibited by the cognate metaeffector AnkJ. Recent studies established that Ceg14 cleaves ATP to AMP and pyrophosphate and can transfer AMP to 3-phosphoglycerate, but how actin and AnkJ regulate these catalytic states remained unclear. Here, we show that Ceg14 is selectively activated by G-actin and determine the cryo-EM structure of the Ceg14-actin-AnkJ complex. Structure-guided mutagenesis identifies two actin-binding elements, a C-terminal anchor and an extended sensor region, that are required for Ceg14 activation and toxicity. Structural modelling and molecular dynamics simulations reveal that the catalytic center is assembled from three spatially distinct components: a nucleoside-binding region, a phosphate-coordinating region within the actin sensor, and the catalytic triad. G-actin constrains the sensor and thereby organizes the phosphate-binding component of the active site. AnkJ binds at a distinct surface and allosterically repositions the Nterminal domain, disrupting nucleoside coordination while leaving part of the catalytic machinery intact. Consistent with this mechanism, AnkJ strongly suppresses AMP production and 3- phosphoglycerate-stimulated activity, yet the ternary complex retains weak Ceg14-dependent ATPto-ADP activity. Thus, our results show that actin and AnkJ do not simply switch Ceg14 on and off, but reciprocally remodel its multipart catalytic center to determine its catalytic state and reaction output.

P107 Rising Environmental *Legionella* Loads in Cyprus: Comparative Analysis of State General Laboratory Surveillance Data, 2025–2026

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Background: *Legionella* spp. in water systems represents an important public-health concern, particularly in healthcare facilities and settings serving vulnerable populations. The State General Laboratory (SGL) of Cyprus supports national surveillance through microbiological examination of water samples using ISO 11731:2017.

Methods: We retrospectively analysed *Legionella* spp. results recorded in the SGL surveillance dataset for 2025 and for the period 1 January–16 July 2026. Sampling locations included healthcare facilities, residential and elderly-care settings, military facilities and other institutional water systems. Results were assessed with particular emphasis on samples exceeding 1,000 CFU/L. Until now, our laboratory has analysed samples from public hospitals and military camps. Under the new legislation, private hospitals and nursing homes have been designated as "priority installations." Consequently, these facilities are being included in a pilot surveillance programme this year. This has resulted in an increase in the number of samples that do not comply with the requirements of the legislation.

Results: In 2025, 936 results were recorded, of which 93 (9.9%) exceeded 1,000 CFU/L. During the first seven months of 2026, 413 results were recorded, with 77 (18.6%) exceeding 1,000 CFU/L. Although the 2026 dataset represents a partial year and contains fewer samples, the proportion exceeding 1,000 CFU/L was almost twice that observed in 2025. Very high concentrations were identified in both periods, with maximum values of 740,000 CFU/L in 2025 and 570,000 CFU/L in 2026.

Conclusions: The findings demonstrate persistent and occasionally substantial "*Legionella*" contamination across monitored water systems in Cyprus. The increased proportion of results above 1,000 CFU/L in 2026 underscores the value of targeted surveillance in priority installations and highlights the need for continued environmental surveillance, rapid investigation of elevated results and systematic follow-up after corrective interventions. Integration of laboratory, environmental and epidemiological data could further strengthen national prevention strategies.

P108 Structural and Functional Insights into Protein-DNA Complexes during Natural Transformation in L. pneumophila

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Natural transformation in *Legionella pneumophila* drives horizontal gene transfer through a tightly coordinated, sequential molecular cascade involving DNA capture, membrane translocation, and genomic integration. Here, we present structural and biophysical insights into two sequentially acting machinery components: the extracellular type IV pilus (T4P) and the periplasmic DNA-binding protein ComEA.

First, initial DNA uptake depends on assembled T4P and their distally located tip complexes, whose structure and subunit composition remain unresolved. Using a nanobody-based approach, combined with cryogenic electron microscopy (cryo-EM) and fluorescence microscopy, we aim to resolve the first high-resolution structures of native *L. pneumophila* T4P tip complexes and characterize their compositional plasticity across different environmental conditions.

Following uptake, incoming DNA enters the periplasm, where binding by ComEA drives the formation of a nucleoprotein filament. However, how ComEA interacts with DNA and is arranged within the context of this filament remains poorly understood. To address this, we combined biophysical characterization of ComEA-DNA interactions with cryo-EM analysis of the nucleoprotein filament architecture. Together, these approaches aim to define the molecular basis of ComEA-mediated DNA binding and begin to address the role of filament assembly during natural transformation.

Together, structural characterization of the extracellular T4P tip complex and the periplasmic ComEA-DNA filament provides a framework for understanding the sequential steps of DNA uptake and translocation across the *L. pneumophila* cell envelope.

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Anaísa B. MORENO	M29
Shaeri MUKHERJEE	M25
Daniel MÄUSEZAHL	P043
Hiroki NAGAI	M28
Nazmun NAHER	P096
Maria NAJEEB	S03* / P102*
Noriko NAKANISHI	P009
Chris NANCREDE	S01
Ramona NEUNUEBEL	S18
Hayley NEWTON	M30
Sonia NICCHI	S26
Beth NICHOLSON	P012
Shira NINIO	M16
Melanie D. OHI	M23
Tamara O'CONNOR	M15
Kiran PARANJAPE	M29
Fanny PASSOT	P085
Tiffany PENNER	S05* / P103*
Jack PENNY	M12
Ana Elena PEREZ-COBAS	M03
Andrea PIGHINI	P017
Sudha POTTUMARTHYBODDU	P057
Michele PROVOST	M21
Valerie REEB	P058
MARIA LUISA RICCI	P030
MARIA LUISA RICCI	P095
Maria Luisa RICCI	M17
Abigail RICHARDSON	P020*
Bibiana RIOS GALICIA	P078
Arshad RIZVI	M12
Jacquelyn R. ROBERTS	M23
Isabel RODRÍGUEZ-ESCUDERO	M12
Tim ROLOFF	M22
Katerina ROMANOV	S16*/ P007*
Craig R. ROY	M32
Marietta SANDKAMP-KASPERS	RS03
Maria SCATURRO	P093
Maria SCATURRO	P094
Daniel SCHATOR	PF06/ P029
Rebecca SCHECK	M01
Gunnar N. SCHROEDER	M12
Caroline SCHÖNNING	P032
SEBASTIEN	M03
Helena SEDLÁGKOVÁ	M29
Helena SETH-SMITH	M22
Sunny SHIN	M10
Hans SINGH	S11
Jessica SMITH	P053
Antonis SOTIRIOU	P107
Pia STAUFFER	RS04*
Michael STEINERT	M09
Janet E. STOUT	M04
Kazuhiro TATEDA	M18
Rebecca THOMBRE	P082
Serena VARILE	M12
Flavia VIANA	M12
Adela VIDOV	P055
Elizabeth VITTORI	P031
Guan-Bo WANG	M12
Matias WANNTORP	M29
Lisa WASSERSTROM	P059
Kristin WEBER	P028
Kristin WEBER	P088
Owen WHITHAM	P008*
Raissa WIBAWA	M12
Alexandra WOLF	P050*
Katie WRENN	S04
Danielle WROBLEWSKI	P079
Danielle WROBLEWSKI	M19
Tatsuhiko YOKOYAMA	M28
Youngki YOO	M23
Menyun ZHANG	M01
Xuluo ZHONG	P037*
Abdelrahim ZOUED	S07