Abstract Book

7th ESGLI meeting

ESCMID STUDY GROUP FOR LEGIONELLA INFECTIONS

23-24 October 2023 | Minoa Palace Resort, Chania, Crete, Greece

esglimeeting2023.com

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WELCOME

Dear participants,

On behalf of the ESCMID Study Group for Legionella Infections (ESGLI), we are pleased to welcome you to the ESGLI 2023 meeting in Chania, Crete, Greece.

This 7th ESGLI meeting comes after a long break due to the Covid-19 pandemic therefore we are happier than ever to meet each other again. The ESGLI meetings are precious and a unique opportunity to meet other people dealing with this important micro-organism considered by the World Health Organization as amongst pathogens present in water responsible for the greatest health burden.

This is also one of the reasons why Legionella has been included in the new European directive on water intended for human consumption 2020/2184, as it must be controlled in the water systems of priority buildings. This recent law will be one of the topics of the meeting and is a great opportunity to compare the different positions within the European member states.

The ESGLI 2023 conference will also cover a broad range of topics related to Legionella and Legionnaires' disease (LD), such as pathogenesis, but also microbial diagnosis and genomic typing as applied to detection, control and management in general and in particular to identify clusters and outbreaks.

Disinfection practices, climate change and increasingly extreme weather events will also be an important issue to address and consider in LD incidence and the occurrence of new Legionella species. In this regard, Legionella interactions with other water micro-organism that defines its ecology in artificial environments will be another topic to discuss.

The meeting will jointly host researchers from all legionella-related fields of expertise, and we will have the privilege of hosting some eminent international speakers. Therefore, we warmly welcome you to Crete, the largest and most populous of the Greek islands, and the fifth largest island in the Mediterranean, ancient site of the Minoan civilization – one of the oldest advanced civilizations in Europe, with its main centers in Knossos, Cydonia and Festo.

The local organizing committee will offer you the best hospitality in terms of location, accommodation and, last but not least, good food and the opportunity to see the most beautiful seas and beaches on the planet. On behalf of the Conference organizers, I am very pleased to welcome you all to the 7th ESGLI meeting, 2023.

Maria Luisa Ricci

Chair of the ESGLI

TOPICS

- Epidemiology & Outbreak Investigation
- Legionella: Interaction with the Host and Environment
- Legionella Genomics
- Clinical Aspects and Diagnostics for Legionella
- Legionella Typing
- Legionella Prevention and Control

COMMITTEES

Scientific Committee
(Including ESGLI EC Members)

Sebastian Crespi
Valeria Gaia
Paulo Goncalves
Lara Payne Hallstrom
Emmanuel Robesyn
Maria Scaturro
Brad Spiller
Susanne Surmann Lee

Organizing Committee

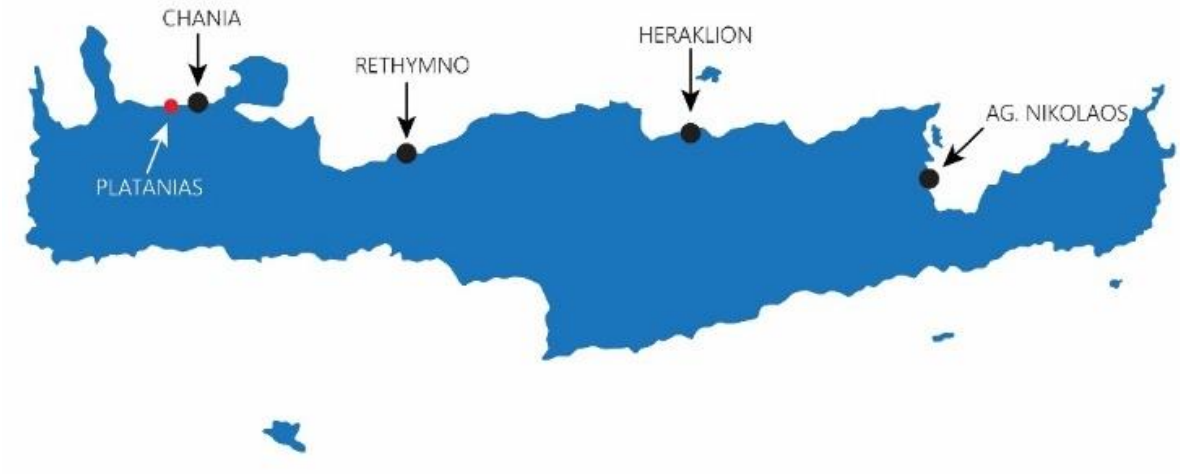
Marie Luisa Ricci
Sophie Jarraud
Diane Lindsay
Anastasia Flountzi
Markus Petzold
Elsa Mouratidou

SPONSORS

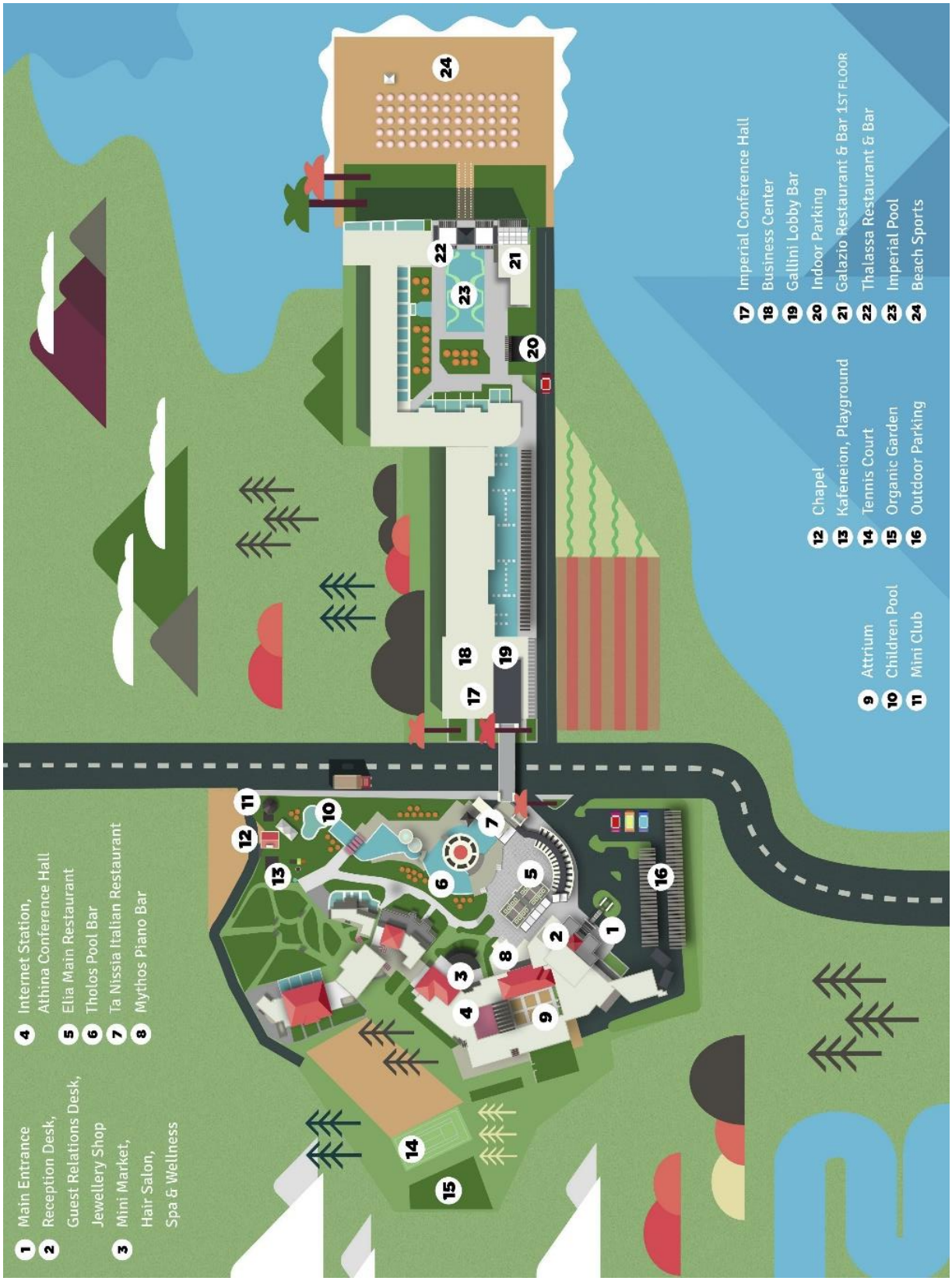
GENERAL INFORMATION

Venue

The conference will be hosted in ***Minoa Palace Resort Hotel***, a luxury 5* beach-side hotel located at the cosmopolitan area of Platanias, 12km west of the picturesque town of Chania and 30min drive from Chania International Airport. Minoa welcomes you to experience the pleasures of indulgence in the most enchanting of settings overlooking the endless azure of the Aegean. The Resort's Congress Hall is a great host for all sorts of corporate events, conferences, workshops & exhibitions, offering flexibility and functionality, as well as state of the art facilities and the latest audiovisual equipment.



Venue map



For more information about the Venue please visit the website: minoapalace.gr

Minoa Palace Resort Hotel
Platanias, Chania, Crete, Greece, 73014 Tel. +30 28210 36500
Email: info@minoapalace.gr

The closest to the conference venue airport is *Chania international airport*. (Please note that Heraklion airport, which is the largest airport in Crete, is more than two hours away from the hotel venue and without any direct and easy or cheap connection with the conference venue.)

How to get to the Venue

Arriving by plane

The **conference venue** is located at **Platanias/Chania, Crete**. During September, Chania is directly connected to several European cities by charter/seasonal flights. Information on destinations can be found in the official website of Chania Airport . Additionally, regular flights from/to Athens International Airport exist daily. You are strongly advised to choose a flight to Chania International Airport. Alternatively, one can land to Heraklion International Airport and reach Chania by bus or car. The driving distance between Heraklion and Chania is 142km.

Arriving by ship

The city of Chania is connected to Piraeus (Athens) daily. The port is in Souda, 7km away from the city center and 21 km away from the Conference Venue (about 20 min driving). You may consult the timetables or book your boat tickets here and here. Information regarding the public bus that connects Souda to Chania city center can be found here.

Bus services

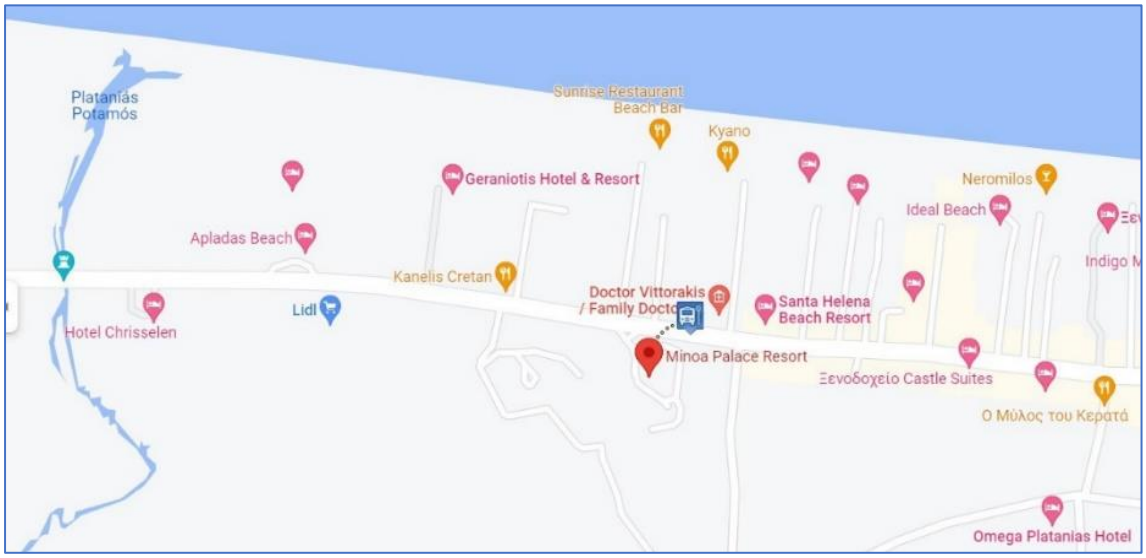
Chania airport → Chania city (Bus station)

Chania airport is located 14km from the city center, and 33.2 km away from the Conference Venue (30-40 min driving). A public bus connects the airport to the city center on a regular basis (line Chania Airport – Chania). The route lasts for 30 minutes approximately, and costs 2.30 €. You may consult the timetables or buy your tickets here

Chania city (Bus station) → Platanias (bus stop MINOA PALACE)

From Chania Bus station there are enough ***routes*** you could get to ***arrive to the Minoa Palace Resort Hotel***. For your convenience we collected here all those routes:

1.**CHANIA-KASTELI**
2.**CHANIA-KOLIMPARI**
3.**CHANIA-PLATANIAS-GERANI**
4.**CHANIA-ZYMVRAGOU**
5.**CHANIA-DELIANA**
6.**CHANIA-RODOPOU**
7.**CHANIA-VOUKOLIES**
8.**CHANIA-PALAIA ROUMATA**
9.**CHANIA-ELAFONISI**
10.**CHANIA-KASTELI-FALASARNA**
11.**CHANIA-PALAIOCHORA**



Taxi services

Moving by taxi is quite common in Crete and prior booking is not required. You may find relevant information and indicative prices in several websites (taxi4crete.gr/taxi-prices-from-chania-airport.html, www.chaniataxi.gr/en/)

The cost of transfer by taxi is approximately the following:

Chania Airport – Chania City Center	~ 25 €
Chania Airport – Conference Venue	~ 48 €
Chania City Center – Conference Venue	~ 20 €

Crete

Crete is the largest island in Greece and the fifth largest in the Mediterranean. It is endowed with an exquisite 1,000-kilometer-long coastline dotted with numerous coves, bays and peninsulas, which afford a multitude of soft, sandy beaches along the infinite blue of the Mediterranean Sea. The island is proud for its longstanding history, spanning from the Minoan civilization (3000 B.C.) until today. Crete welcomes you with its smiling Cretan sun, the sounds of the Cretan lyre, the scents of orange blossom and jasmine, a slice of cool red watermelon and a glass of iced "raki".

Some important archaeological sites of Crete:

The Palace of Knossos

According to tradition, it was the seat of King Minos and the capital of his state. The palace of Knossos is associated with the exciting myths "the Labyrinth and the Minotaur" and "Daedalus and Icarus". References to Knossos, its palace and Minos are made by Homer (the list of ships in Ilias mentions that Crete sent 80 ships under the command of the King of Knossos, Idomeneus, the Odyssey, T 178-9), Thucydides (reference to Minos), Isiodus and Herodotus, Bacchylides and Pindarus, Plutarchus and Diodorus the Sicilian. The city flourished in the Minoan Times (2000 – 1350 B.C.), when it was the most important and populated centre of Crete. It also played an important role and was particularly prosperous in later periods, like the Hellenistic Times. The city of Knossos was constantly populated from the end of the 7th millennium to the Roman Times. In the Neolithic Times there was a stage of technologically developed agricultural life (stone tools and weaving weights). The residents turned from food-collectors into producers (farmers and shepherds) and a there was a trend towards more systematic and permanent settlement. The settlement periods in Knossos succeeded each other and the population of the settlement at the end of the Late Neolithic Period is estimated at 1.000 – 2.000 residents.

The Palace of Phaistos

Phaistos is built on a low hill (altitude of about 100m from sea level), in the south of river Geropotamos (ancient river Lithaios), and dominates the fertile valley of Kato Mesara, which is surrounded by imposing mountains (Psiloritis, Asterousia, Lasithi Mountains). The Libyan Sea extends in the south. Lithaios surrounds the hill of Phaistos in the east and the north and was a source of water supply for the city. The mild and warm climate of the area made the life of its residents comfortable and pleasant. Phaistos was one of the most important centres of the Minoan civilization, and the most wealthy and powerful city of southern Crete. It is mentioned in the texts of ancient writers (Diodorus, Stravon, Pausanius) and Homer. It is one of the three important cities founded in Crete by Minos. According to mythology, the dynasty of

Rodamanthus, the son of Zeus and brother of Minos, reigned in it. Homer refers to its participation in the Trojan War and describes it as a “well populated” city. The period of prosperity in Phaistos began with the coming of the Bronze Age in Crete in the middle of the 3rd millennium B.C. when the foundations of the Minoan civilization were laid. Habitation in Phaistos started in the Neolithic period, as revealed by the foundations of Neolithic houses, tools, statuettes and potsherds discovered under the palace during the excavations. The Neolithic settlement is believed to have covered the top of the hill and its southwestern slope. In the middle of the 3rd millennium B.C. the use of metals began, which favoured the development of the city.

The main cities of Crete

The major cities of Crete (Chania, Rethymno, Heraklion, Agios Nikolaos) were once strategically placed on specific coastal locations of the island to defend against invaders. With a history that starts in prehistoric times and harbours that have always connected the island with other ports of the Mediterranean, the Cretan cities today are modern urban centres that have kept the historical identity of the island alive after countless conquerors have called it their own. In the Middle Ages, the island of Crete passed from the Byzantines to the Arabs, back to the Byzantines and then to Venetians; each one introducing different architectural and cultural elements. Every summer, Crete welcomes thousands of visitors that wish to explore the cities, charming harbours and cultural attractions that seem to be present on every corner.

Chania

In Chania city center one can enjoy the picturesque old harbour, walk around the old town alleys, and enjoy delicious local food in the numerous small restaurants.

Also, there are plenty of option for excursions to Chania region. You could enjoy exotic beaches, like the beach of Balos, which is ranked 35th among the 100 World’s best beaches. The Falassarna beach and the Elafonisi peninsula also attract millions of sea-lovers each year.

Less than 1 hour driving from Chania is the famous Samaria gorge, which is the second touristic attraction of Crete (after Knossos Minoan Palace). There are busses every day that can take you from Chania to Samaria gorge.

Discover Crete through the following websites:

incrediblecrete.gr/en/
cretanbeaches.com/en/
youtube.com/watch

USEFUL CONTACTS

Minoa Palace (VENUE)	0030-2821036500
Chania Bus Station	0030-2821093052
Taxi Chania	0030-2821098700
General Hospital Chania	0030-2821342000
Medical Center- Vittorakis Polyclinic	0030-2821060606
1st Fire Department of Chania	0030-2821079340, 0030-2821063688
Chania Police Station	0030-2821025854

Conference Secretariat and local professional organizer

"**Diazoma Conference & Events**"
https://diazoma.net

Tel: 0030-6908 215112, 0030-2810321494

Emails: info@diazoma.net, conferences@diazoma.net, meetings@diazoma.net

PROGRAM TABLE

Sunday, 22nd October 2023				
17.00-20.00	**Registration and Welcome Reception** *Lobby Athina Hall*			
Monday, 23rd October 2023				
08.00-09.00	**Registration** *Lobby Athina Hall*			
Time	**Session**		**Chairs**	
09.00-10.45	**Epidemiology and outbreak - 1**	**Oral Presentations** *Athina Hall 1*	***Elisavet MOURATIDOU & Anastasia FLOUNTZI*** **Speaker**	**A/A**
09.00-09.30		Increasing rate of Legionnaires' disease in EU/EEA continues in 2022	**Lara PAYNE** *(Guest speaker)*	1
09.30-09.45		Legionnaires' disease surveillance: comparison of clinical and environmental strains in France, 2008-2022	**Christine CAMPESE**	2
09.45-10.00		Studying the epidemiology of Legionnaires disease in Switzerland	**Daniel MÄUSEZAHL**	3
10.00-10.15		LD incidence in Scotland - pre, during and post Pandemic	**Melissa LLANO & Diane LINDSAY**	4
10.15-10.30		Environmental Legionella sources and Legionaries' disease incidence: analytical study in Catalonia (2018 and 2022)	**Elisenda ARQUE**	5
10.30-10.45		Community-acquired Legionnaires' disease (CALD) in Catalonia (Spain): Observational study (1983-2020)	**M. Luisa PEDRO-BOTET**	6
10.45-11.15	Coffee break **Poster Session** *Athina Hall 2*			
Time	**Session**		**Chairs**	
11.15-12.15	**Epidemiology and outbreak - 2**	**Oral Presentations** *Athina Hall 1*	***Lara PAYNE & Emmanuel ROBESYN*** **Speaker**	**A/A**
11.15-11.30		Cluster of Legionnaires' disease linked to newly installed residential water heaters, the Netherlands, 2023	**Daphne REUKERS**	7
11.30-11.45		First report of Legionnaires' disease (LD) fatal case in a child in Greece	**Anastasia FLOUNTZI**	8
11.45-12.00		Investigation of Legionnaire's disease cases by the Belgian National Reference Centre	**Fedoua ECHAHIDI**	9
12.00-12.15		A legionella outbreak caused by an electrical fireplace at a hotel	**Caroline SCHÖNNING**	10
12.15-12.30		LD after near drowning – a case report	**Diane LINDSAY**	11

12.30-14.00	Lunch break **Poster Session** *Athina Hall 2*			
Time	**Session**		**Chairs**	
14.00-15.15	**Clinical, diagnostics, typing and genomics - 1**	**Oral Presentations** *Athina Hall 1*	***Valeria GAIA & Brad SPILLER***	
			Speaker	**A/A**
14.00-14.30		Study of the evolution of Legionella pneumophila sg 1 by WGS: some preliminary results from 130 environmental strains isolated over a 13-year period from hotels in Crete	**Dimosthenis CHOCHLAKIS** *(Guest speaker)*	12
14.30-14.45		European evaluation of 16 assays for the detection of Legionella pneumophila antigen in urine samples from patients with pneumonia	**Sophie JARRAUD**	13
14.45-15.00		Evaluation of Sensitive 96-well plates for Legionella antimicrobial susceptibility testing	**Ghislaine DESCOURS**	14
15.00-15.15		Genome analysis of Legionella pneumophila ST901, an Italian strain causing many travel-associated Legionnaires' disease, 1987-2019	**Maria SCATURRO**	15
15.15-15.45	Coffee break **Poster Session** *Athina Hall 2*			
Time	**Session**		**Chairs**	
15.45-16.45	**Clinical, diagnostics, typing and genomics - 2**	**Oral Presentations** *Athina Hall 1*	***Sophie JARRAUD & Maria SCATURRO***	
			Speaker	**A/A**
15.45-16.00		Matching clinical and environmental isolates: complication from mixed infection and genomic diversity within engineered water systems	**Sebastien FAUCHER**	16
16.00-16.15		Microevolution and spatiotemporal dynamics of Legionella pneumophila ST1905 in Portugal, 2014-2022	**Paulo GONÇALVES**	17
16.15-16.30		An update to the Legionella pneumophila Sequence-Based Typing (SBT) online database	**Rediat TEWOLDE**	18
16.30-16.45		Comparative analysis of functional genes among Legionella species	**Marco GABRIELLI**	19
FREE TIME				
19.30-…	**Conference dinner** Restaurant Mylos "Keratas" https://www.mylosrestaurant.gr			

Tuesday, 24th October 2023				
Time	**Session**		**Chairs**	
09.30-10.45	**Legionella interaction with host and environment**	**Oral Presentations** *Athina Hall 1*	***Maria Luisa RICCI & Markus PETZOLD***	
			Speaker	**A/A**
09.30-09.45		Molecular characterization of shower hoses biofilms reveals genetic variability of Legionella spp. and specific associations with microbiome members	**Frederik HAMMES**	20
09.45.10.00		Occurrence of Legionella and Legionella pneumophila in the air and water of wastewater treatment plants	**Arthur OURADOU**	21
10.00-10.15		How L. pneumophila colonizes differently structured P. fluorescens biofilms?	**Ana Alexandra PEREIRA**	22
10.15-10.30		Susceptibility and adaptive mechanisms of L. pneumophila to triclosan	**Ghislaine DECOURS**	23
10.30-10.45		Characterization of bacterial communities and Legionella species in aquifers supplying a drinking water distribution system	**Federici FERMANNO**	24
10.45-11.15	Coffee break **Poster Session** *Athina Hall 2*			
Time	**Session**		**Chairs**	
11.15-12.45	**Legionella prevention and control - 1**	**Oral Presentations** *Athina Hall 1*	***Sebastian CRESPI & Susanne SURMANN LEE***	
			Speaker	**A/A**
11.15-11.45		German regulatory strategies for the control of Legionella and other biofilm associated waterborne pathogens in health care facilities	**Martin EXNER** *(Guest Speaker)*	25
11.45-12.00		The transposition in Italy of the New European Directive (DWD) 2020/2184	**Maria Luisa RICCI**	26
12.00-12.15		Quantification of Legionella pneumophila in drinking water: a meta-analysis comparing qPCR and culture-based detection methods	**Émile SYLVESTRE**	27
12.15-12.30		Hunting for probiotics against Legionella in building plumbing systems: potential antagonists and their natural compounds	**Alessio CAVALLARO**	28
12.30-12.45		Impact of climate change on Legionella and other waterborne microorganisms in water systems - is it too late to do anything?	**Jimmy WALKER**	29
12.45-14.00	Lunch break **Poster Session** *Athina Hall 2*			

Time	Session		Chairs	
14.00-15.45	**Legionella prevention and control - 2**	**Oral Presentations** *Athina Hall 1*	***Diane LINDSAY & Paulo GONCALVES***	
			Speaker	**A/A**
14.00-14.30		Managing Legionella in engineered systems: recent developments in monitoring and treatment to guide efficient water safety plans	**Michele PREVOST** *(Guest Speaker)*	30
14.30-14.45		Occurrence of Legionella pneumophila in US Drinking Water Distribution Systems	**Mark LECHEVALLIER**	31
14.45-15.00		Comparison of Legionella colonisation in 20 buildings before and after the 2020 UK COVID-19 lockdown, and the effect of chlorine dioxide dosing on long-term positivity	**Maddy JOWITT**	32
15.00-15.15		In situ monochloramine disinfection in a large hospital to control Legionella, nontuberculous mycobacteria and Pseudomonas aeruginosa	**Michele PREVOST**	33
15.15-15.30		Chlorine vs bromine for Legionella control in spa pools: unmasking the winner	**Sebastian CRESPI**	34
15.30-15.45		An Update of the ESGLI European Technical Guidelines for the Prevention, Control and Investigation, of Infections Caused by Legionella species	**Susanne SURMAN LEE**	35
15.45-16.15	Coffee break **Poster Session** *Athina Hall 2*			
16.15-16.45	**Discussion session/workshop**		**1.** DWD and guidelines **2.** cgMLST **3.** Legionella pneumophila vs Legionella species	
16.45-17.00	**Closing remarks/thanks**			

POSTER LIST

1. Clinical Aspects and Diagnostics for Legionella

P1: Legionella longbeachae wound infection: case report and review of reported Legionella wound infections

Wasserstrom Lisa[1], Frostadottir Drifa[2],Lundén Karolin[2], Dahlin Lars B[2]
[1] Clinical Microbiology, Laboratory Medicine Skåne, Lund, Sweden
[2] Department of Translational Medicine - Hand Surgery, Lund University, Malmö, Sweden

P2: Antimicrobial susceptibility testing reveals reduced susceptibility to azithromycin and other antibiotics in Legionella pneumophila serogroup 1 isolates from Portugal

Gonçalves Paulo[1],Minetti Corrado[1],Rodrigues Raquel[1],Barton Rachael[2],Farley Caitlin[2],Owen B. Spiller[2]
[1] National Institute of Health, Portugal
[2] Cardiff University, UK

P3: In-house Legionella PCR as easy as commercial kit

Beraud Laetitia, Chastang Joelle, Ibranosyan Marine, Ginevra Christophe, Descours Ghislaine, Jarraud Sophie
French National Reference Center

P4: Antibiotic susceptibility in Portuguese clinical isolates of Legionella pneumophila recovered between 1987 and 2016

Chasqueira Maria Jesus[1], Rodrigues Lúcia[1], Paixão Paulo[1], Pereira Bernardo Beirão[2], Araújo Ibéria[2], Cruz Carolina[2], Santos Ricardo[3]
[1] CHRC, Nova Medical School, Universidade Nova de Lisboa, Lisboa, Portugal
[2] Laboratory of Microbiology, Nova Medical School, Universidade Nova de Lisboa, Lisboa, Portugal,
[3] CERIS, Laboratório de Análises de Água, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

P5: LIAISON® Legionella urinary assay: a novel, high-throughput, fully automated assay based on dual-antigen detection with improved sensitivity

Ingallinella Paolo, Dal Corso Andrea, Brusasca PierNatale, Panizzo Massimo, Ferrante Deboarah, Ghezzi Elisa, Querin Lorenzo, Bou Nasser Eddine Farah, Cappellini Daniele, Ferraiuolo Serena, Mauro Chiara, Mento Alfredo, Rigamonti Marco, Rossini Clara, Savinelli Antonio, Zytko Karolina, Pighini Andrea, Zanin Davide, Pallavicini Luca, Bonelli Fabrizio
DIASORIN ITALIA SPA – Biotech

P6: Identification of potential novel antigenic proteins in Legionella pneumophila

Flountzi Anastasia (Nancy)[1], Myrto Koutantou[2], Dimosthenis Chochlakis[2], Georgios Tsiotis[2], Anna Psaroulaki[2]
[1]Hellenic Public Health Organization
[2]University of Crete

2. Epidemiology & Outbreak Investigation

P7: Environmental Monitoring of Legionella spp. in a Regional Hospital of Crete (Greece) during the Covid-19 Pandemic

Flountzi Anastasia (Nancy)[1], Antonios Papadakis[2],Dimosthenis Chochlakis[2], Anna Psaroulaki[2],
[1] Hellenic Public Health Organization
[2] University of Crete

P8: Increase in Legionnaires Disease among elderly Danish population, 2002-2023

Kjelsø, Charlotte, Eves, Caroline
Statens Serum Institut

P9: Trends in legionella-related mortality in Denmark, 2002-2022

Eves Caroline, Kjelsø Charlotte
Statens Serum Institut

P10: Professional Legionellosis of ice resurfacer driver on the ice hockey stadium. Is skating health risk?

Sedlackova Helena[1], Drasar Vladimir[1], Polcar Radomir[2],
[1] National Legionella Reference Laboratory, Public Health Institute Ostrava,
[2] Factor.E, Brno

P11: Sources of infection and settings in outbreaks of legionellosis --- Japan, 2018-2022

Kura Fumiaki, Amemura-Maekawa Junko, Izumiyama Shinji
National Institute of Infectious Diseases, Tokyo

P12: SwissLEGIO - Legionnaires' disease in Switzerland: A national case-control and molecular source attribution study

Mäusezahl Daniel[1], Bigler Melina[1], Fischer Fabienne B.[1], Egli Adrian[2], Gaia Valeria[3], Hammes Frederik[4], LeCo Consortium[5], SwissLEGIO Hospital Network[6]
[1] Swiss Tropical and Public Health Institute (associated institute of the University of Basel)
[2] Institute for Medical Microbiology, University of Zurich
[3] National Reference Centre for Legionella, Service of microbiology, Institute of laboratory medicine, EOC
[4] Eawag, Swiss Federal Institute of Aquatic Science and Technology
[5] LeCo Consortium
[6] SwissLEGIO Hospital Network

3. Legionella Genomics

P13: Assessment and Implementation of a standardized Whole Genome Sequencing scheme for rapid resolution of Legionella pneumophila outbreaks within Canada

Tanner Jennifer, Pratt Molly, Duggan Ana, Burdz Tamara, Lyubashenko Dmytro, Joanisse Kalissa, Unger Mark, Antonation Kym
Public Health Agency of Canada

P14: Historical Genomic Epidemiological Analysis of Legionnaires' disease in Scotland Uncovers Long-term Endemic Clones of Public Health Importance

Lindsay Diane[1], Ure Roisin[1], Llano Melissa[2], Cameron Ross[2], Mcmenamin Jim[2], Fitzgerald Ross[3], Alves Joana[3], Gorzynski Jamie[4], Smith Andrew[5]
[1] SMIRL, Glasgow
[2] PHS
[3] Roslin Institute
[4] University of Edinburgh
[5] University of Glasgow

P15: ONTmompS - a tool for in silico sequence-based typing (SBT) of Legionella pneumophila completed genomes

Krøvel Anne Vatland, Hetland Marit A.K., Bjørheim Anna Steensen, Bernhoff Eva, Löhr Iren H., National Reference Laboratory for Legionella, Stavanger University Hospital

4. Legionella Prevention and Control

P16: Digital PCR versus Real Time PCR: looking for a more sensitive method for Legionnaires' disease diagnosis.

Scaturro Maria, Mancini Fabiola, Girolamo Antonietta, Ricci Maria Luisa
Istituto Superiore di Sanita', Roma, Italia

P17: Activity of Algerian essential oils againt Legionella species

Bouanane-Darenfed Amel, Boilattabi Nesrine
Laboratory of cellular and Molecular Biology, Microbiology team/FSB/USTHB, Beb Ezzouar/Algeria

P18: Comparison of Legiolert and traditional plate culture, according to Italian guidelines, from hospitals water samples

Arrigo Ignazio[1], Fasciana Teresa[1], Giammanco Anna[1], Galia Elena[2], Tricoli Maria Rita[2], Cannova Lucia[2], Gallina Giuseppe[2], Palermo Mario[3], Mariyam Laiba[4], Serra Nicola[5]
[1] Regional Reference Laboratory for Clinical Environmental Surveillance and Control of Legionellosis, Palermo
[2] A.O.U.P. P.GIACCONE
[3] ASP PALERMO
[4] University of Palermo
[5] University Federico II Naples

P19: Improved Prevention and Control of Legionella Pneumophila using a Rapid On-Site Testing Method

Mark Dawson, Connolly Michael, Greg Rankin
Hydrosense Ltd

P20: Pre-post study on the microbiome profile of a hospital water network treated with hydrogen peroxide for Legionella contamination control

Paduano Stefania[1], Marchesi Isabella[1], Bargellini Annalisa[1], Frezza Giuseppina[1], Valeriani Federica[2], Romano Spica Vincenzo [2], Carluccio Eugenia [3], Bray Antonio[4], Maiorano Osvaldo[5]
[1] Department of Biomedical, Metabolic and Neural Sciences, Section of Public Health, University of Modena and Reggio Emilia

[2] Department of Movement, Human and Health Sciences, Public Health Unit, University of Rome 'Foro Italico'
[3] Medical Direction, Hospital "Vito Fazzi", Lecce
[4] Health Direction, ASL Lecce
[5] Medical Directions, ASL Lecce

P21: Identification of Legionella contamination in the water installation of a grammar school in Germany

Katsemi Vicky[1], Gollnisch Carsten[2]
[1] Dr. Vicky Katsemi Water Hygiene
[2] Hygiene Inspection Body for Drinking Water Systems

P22: Identification of Legionella contamination in decentralised water systems of a German kindergarten

Katsemi Vicky[1], Gollnisch Carsten[2]
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[2] Hygiene Inspection Body for Drinking Water Systems

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Audit Process

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Keše Darja, Celar Šturm Andraž, Obreza Aljoša, Strašek Smrdel Katja, Triglav Tina
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ORAL ABSTRACTS

Oral Presentation 1

Increasing rate of Legionnaires' disease in EU/EEA continues in 2022

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Increasing rates of Legionellosis is reported in several countries worldwide. Legionnaires' disease is a notifiable disease under European Union (EU) surveillance. Comparable data are collated at the European Centre for Disease Prevention and Control (ECDC) through the European Legionnaires' disease Surveillance Network (ELDSNet), concerning cases of Legionnaires' disease (LD) reported in EU Member States, and EEA countries of Iceland, Liechtenstein, and Norway.

While notification rates of LD were stable during 2010-2016, varying within 1.1-1.5 per 100 000 population, in recent years notification rates have been increasing. This has been particularly notable 2017-2019 where rates reached 1.9-2.4 per 100 000 population.

In 2022, the highest level of notification rates of Legionnaires' disease observed to date in the EU/EEA was reached at 2.6 per 100 000 population and 11 452 cases. This is an increase on 2021, where a rate of 2.4 per 100 000 was observed. A notable decrease of 22% in annual reported cases in the EU/EEA was observed in 2020, under the occurrence of the COVID-19 pandemic.

As seen in previous years, the distribution of the disease in 2022 across the EU/EEA remains heterogenous, with age-standardised rates by country varying between <1 case per 100 000 population to 6.1 cases per 100 000 population.

Legionnaires' disease remains an important cause of potentially preventable morbidity and mortality in Europe.

Legionnaires' disease surveillance: comparison of clinical and environmental strains in France, 2008-2022

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In France, between 2008 and 2022, an average of 1 500 cases of Legionnaires' disease (LD) were notified each year. However, since 2017, an increase of LD cases has been observed. Systematic investigations are undertaken to identify the probable source of contamination and when possible to allow for comparison of clinical and environmental strains. Clinical strains were available for about 24% of cases and were routinely typed at the National Reference Centre by Sequence-Based Typing (SBT) or/and Whole Genome Sequencing (WGS).

We describe the characteristics of clinical strains and their comparisons with environmental strains for LD cases notified from 2008 to 2022.

Among the 22 542 notified LD cases, the vast majority (92%) of the 5 302 clinical strains available was Legionella pneumophila serogroup 1. Among them, 47% belonged to 5 sequence types (ST1, ST23, ST47, ST62, ST259). Environmental strains were available for 15% (790/5 302) of cases with clinical strains. Identical characteristics (ST and/or WGS) were found in 64% (520/817) of the comparisons. The profile of the environmental strains was more often identical with clinical strains when isolated from water networks rather than the cooling tower systems (CTS): in 88% (37/41) of elderly settings, 74% (136/183) of hospitals, 74% (86/117) of tourist accommodations, 74% (165/222) of residences, 65% (89/136) of other sites and only in 6% (7/117) of CTS.

Since the 1990s, many regulations have been implemented in France to limit the risk associated with Legionella in CTS systems, collective water misting system, spa, water-networks in hospitals, in elderly settings and in leisure or tourist facilities. However, domestic water networks appear to pose a risk of Legionella infection and are not yet regulated in France. In the context of the new European Drinking Water Directive 2020/2184, further systematic investigations are needed to document the proportion of LD and the risk factors that can be linked to domestic contamination via water distribution networks.

Studying the epidemiology of Legionnaires disease in Switzerland

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Introduction: Notification rates for Legionnaires' disease (LD) in Switzerland are continuously rising. In 2022, Switzerland recorded 6.7 cases/100,000 population, one of the highest annual LD notification rates in Europe. To better understand the epidemiology of the disease in Switzerland, our research group investigates the trajectory from exposure to Legionella spp. to final and mandatory reporting of LD cases to the Swiss notification system for infectious diseases since 2015.

Methods: We conducted several studies investigating trends in disease notification, diagnostic testing behaviour, physicians' disease perception and treatment strategies. In a case-crossover study, we further studied the impacts of weather and climate on the regional and seasonal distribution of LD cases. Finally, to understand small-scale determinants of LD, we currently conduct a nationwide case-control and molecular source attribution study (SwissLEGIO) jointly with the LeCo consortium.

Results: The positivity of diagnostic LD tests in Switzerland remained stable over the last decades. Awareness of the disease is high amongst physicians in the secondary and tertiary settings. Therefore, surveillance artefacts alone are unlikely to account for the strong increase in cases observed in Switzerland. Furthermore, we observed a strong effect of large-scale determinants (weather, temperature and humidity) and suspected effects of air pollution on notification rates. The main sources of infections, however, remain unknown and are currently investigated in the SwissLEGIO study.

Conclusion: In the presentation, we summarise the main findings from the various epidemiological studies conducted in Switzerland in the last five years. We present first insights and lessons from the source investigation within the current SwissLEGIO study. We provide an outlook on further research in the framework of SwissLEGIO and nationally.

Keywords: Legionella surveillance, Switzerland, epidemiology

Publications: Impacts of weather and air pollution on Legionnaire's disease in Switzerland FB.Fischer, A.Saucy, D.Vienneau, et al. 2023
Legionnaires' disease in Switzerland: A prospective national case-control and molecular source attribution study (SwissLEGIO) F.B.Fischer, M.Bigler, D.Mäusezahl, et al, 2023
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Legionnaires' disease on the rise in Switzerland: A denominator-based analysis FB.Fischer, C. Schmutz, V.Gaia, D.Mäusezahl, 2020

LD incidence in Scotland - pre, during and post Pandemic

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Introduction: Legionnaires' disease cases were affected by country wide lockdowns and travel restrictions during the COVID-19 Pandemic. In Scotland, LD is a notifiable disease under the Public Health Act (Scotland) 2008 and enhanced surveillance continued even though health protection teams were overwhelmed with the SARS-CoV-2 response.

Methods: The LD data for the last 6 years (2017-2022) that encompassed the pre and post Pandemic era was collated. A key diagnostic change in 2017 was the addition of Legionella spp PCR positive to the confirmed case definition along with urinary antigen and culture. Serology and DFA was no longer performed and removed from our case definition.

Results: Case numbers and incidence were stable from 2017 to 2019, ranging from 6.5 to 6.8 cases per million population annually. Lower case numbers and incidence were observed during the COVID-19 pandemic years in 2020 (n=19) and 2021 (n=16) compared to 2017 (n=35), 2018 (n=37) and 2019 (n=36). Travel-associated LD accounted for the majority of cases (n=111, 59.4%) during the period 2017 to 2022, which is consistent with previous years. During 2020 and 2021, the proportion of travel-associated cases was less than community-acquired cases and reflected reduced travel due to lockdown restrictions. Case numbers and incidence increased in 2022 (n=44), exceeding pre-pandemic levels with no common sources, exposures or links identified

Conclusion: The COVID-19 pandemic caused a marked reduction in LD cases in Scotland in 2020 and 2021 compared to the previous three years (2017-19) as a result of less travel associated cases. There was however an increase in CALD in 2022 and the possible reasons will be discussed including increased testing/ascertainment following the Pandemic

Key words – Legionnaires' disease, surveillance, Scotland

Environmental Legionella sources and Legionaries' disease incidence: analytical study in Catalonia (2018 and 2022)

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Introduction

Reporting of Legionaries' disease (LD) is mandatory in Catalonia since the 80's. Accordingly, both potable and non-potable water facilities are currently required to carry out regular analyses to control Legionella colonisation. Between 2018 and 2022, LD incidence increased an 8.6% in Catalonia.

Cooling towers and recreational water systems are historically thought to represent a higher risk source for LD, although other facilities considered low risk have caused outbreaks recently.

The aim of this study was to evaluate the rate of Legionella positive environmental samples, the proportion detected in each type of facility, seasonality, inoculum and Legionella phenotyping and to compare it with LD incidence over the study period (2018-2022).

Materials/Methods

All the Legionella environmental data from 2018 and 2022 were obtained from an accredited environmental laboratory in Sabadell (Catalonia, Spain). The data corresponded to routine analyses of water samples and no modification was done in the analytical procedure. 11,380 samples were analysed in 2018 and 2022, and were classified based on the type of facility: hot and cold water systems, cooling towers, recreational water facilities (jacuzzis, baths and swimming pools), road sweepers, high aerosol producing systems (irrigation systems, car washers and decorative fountains) and firefighting systems.

Results

An increase in Legionella positive environmental samples between 2018 and 2022 was noted, 6.5% and 10.8% respectively ($p<0.0001$), with the highest increment in the season september-november. The inoculum found in positive samples also increased in the year 2022 ($p<0.0001$). Legionella phenotyping between both years remained constant ($p<0.066$). A relative increase in the rate of positive samples was detected for: recreational water facilities (160.7%), cooling towers (110.7%), cold water systems (96.5%), high aerosol producing systems (74.8%), hot water systems (56.1%) and road sweepers (38.8%).

Conclusions

Coinciding with an increment in LD incidence in Catalonia, we have observed an increase in Legionella positive environmental samples and their inoculums. The increase in "low risk" facilities highlights the importance of strengthening their surveillance, especially cold water systems and high aerosol producers. Our study emphasises that preventive strategies should not be limited to "high risk" facilities.

Keywords: Legionella, Legionaries' disease, environmental microbiology, public health

Community-acquired Legionnaires' disease (CALD) in Catalonia (Spain): Observational study (1983-2020)

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Background: Mandatory reporting of Legionnaires' disease (LD) in Catalonia since 1988 prompted the use of specific techniques to diagnose LD with Legionella urinary antigen (LUA) test being implemented in 1993. Community-acquired (CALD) and hospital-acquired LD database (1983-2020) has been fulfilled since 1983 in our tertiary 750 bed-reference hospital. The aim of this study was to determine demographic and clinical data, individual risk factors, outcome and microbiologic diagnosis over time.

Methods: We selected patients with CALD from our Legionella database (1983-2020). Clinical manifestations and trends in case numbers before and after LUA test was implemented in 1993, mean age, and rate of males, smokers, underlying diseases, extrarespiratory symptoms and outcome per year were calculated by linear regression.

Results: 398 patients were identified. Mean age was 59.5 years (19-95yrs) with 62.3%< 65 yrs, males 80.7% (306/379), underlying diseases 50.8% (202/398) with chronic lung disease, diabetes and solid organ transplant being the most prevalent, smokers 46.5% (185/398), fever 92.6%, expectoration 37.1%, extrarespiratory symptoms 43.5 % (173/398), Fine score≥III 64.3%, Na<130 mmol/L 22%, CK>145U/L 24%, AST>35U/L 47.1%, PO2<60mmHg 43.3%, unilobar infiltrate 73.7%, complications on evolution 51.3% (194/378) with respiratory failure(43.7%) being the most prevalent and mortality 4.8% (18/398). 57.3% received quinolones, 34.5% macrolides and 8.2% combination therapy. 50.3% did not receive appropriate antibiotic in the first 36 h since the diagnosis of CALD was done. 32 cases of CALD occurred in 1983-1993 with 9 (28.1%) being culture-based diagnosed and 366 in the period 1994-2020 with 19 (5.2%) being culture-based diagnosed. Mean age, male gender, extrarespiratory symptoms and complications on evolution tended to increase over the study period while underlying diseases, smoke habit and mortality tended to decrease.

Conclusions: Since 1983, the incidence of CALD has remarkably increased and some interesting epidemiological and clinical data have changed over time. Based on the results of our study, LUA and Legionella spp. sputum investigation should be strongly recommended when facing community-acquired pneumonia. Focusing LD surveillance just by LUA test limits the recognition of non L. pneumophila serogroup 1 disease and the insignificant proportion of culture-based confirmed cases impairs further epidemiological studies.

Key Words: Legionella/Legionnaires' disease/community-acquired pneumonia/Urinary antigen test

Cluster of Legionnaires' disease linked to newly installed residential water heaters, the Netherlands, 2023

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Introduction: At the beginning of 2023, two small clusters (two and four cases) of Legionnaires' disease (LD) emerged within residential apartment buildings in the Netherlands. Most possible sources were excluded, except for the fact that all cases had recently installed a new water heater (Ferroli brand). Sampling revealed Legionella pneumophila sg1, ST37 in the hot water systems in both buildings, the same as a clinical isolate. A case-control study was initiated to confirm the link to newly installed Ferroli heaters.

Methods: Cases were identified as LD cases in the Netherlands in 2022 or 2023 where ST37 was either found in the clinical isolate or in the water system of the patients home. As controls, notified LD cases with an unknown Legionella pneumophila strain were selected whose water system was sampled negative for ST37, or who had reported a technical problem or low water temperature in that system. For these cases and controls, inquiries were made about the water heaters in the 6 months before onset of illness.

Results: Until 6 September 2023, 20 cases were identified of which 18 had a Ferroli heater installed within 6 months prior to onset of illness. Three patients died. Five cases had a genomic match between the clinical and environmental isolate. A total of 57 controls were identified. For 26 controls information about the heater was unavailable. None of the remaining 31 controls had a Ferroli heater or had recently installed a new water heater from a different brand. Analysis showed that cases had a strong association with newly installed Ferroli heaters (OR: 466, 95%CI: 21.21 – 10,247.95).

Discussion: Our hypothesis is that some (test)water containing ST37 remained in the heater after production. After installation the (test)water got introduced in the plumbing system and was able to multiply and subsequently cause infection. Control measures have been implemented in the Netherlands, which includes the advice to thermally or chemically disinfect the residential water system and replace components which are difficult to disinfect if a Ferroli heater (production date from January 1, 2022) was recently installed.

Keywords: Legionella pneumophila, cluster, water system, private homes

First report of Legionnaires' disease (LD) fatal case in a child in Greece

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Objectives: LD is an increasing public health problem with overall mortality rate within 5-10%. We aim to describe the clinical and microbiological investigation of a possible travel-related fatal LD case in a seven-year-old child from Northern Greece.

Methods: On May 2022, the patient was admitted seriously ill to the outpatient paediatric department of a Thessaloniki tertiary hospital with generalized seizures, upper limb tonic spasms, dyspnoea, and tachypnoea. Within 2 hours of admission, the patient deteriorated despite the efforts, presenting profuse pulmonary haemorrhage and cardiac arrest. Although cardiopulmonary resuscitation and tracheal intubation were initiated, the patient died about 4 hours later. Bronchial secretions were sent to the hospital microbiology laboratory for film-array, and the CPHL in Athens for culture and RT-PCR. Sequence-Based-Typing (SBT) was performed for further characterization. Environmental investigation was performed by regional public health authorities and included water samples from patient's residence and the hotel related to travel.

Results: Regional PH authorities issued a risk assessment report, mentioning travel to Northern Greece, one week prior to child's infection and death. The patient had no known underlying conditions/risk factors. Film-array test was positive for Legionella pneumophila, RT-PCR confirmed it, identifying Legionella pneumophila sg2-15 with CT at 29 cycles. Standard culture confirmed the identification and SBT results attributed the ST 93 to the isolate. Environmental investigation resulted negative for Legionella spp.

Conclusions: To our knowledge, this is the first fatal LD case in a child in Greece. LD can be fatal, when not diagnosed and treated promptly, leading to life-threatening complications. Approximately 10–15% of LD reported cases occur in travelers during their exposure period. Clinicians should be aware of updated disease specifics based on patients' medical history and risk factors. Timely reporting and management of all LD cases, collaboration with well-trained laboratory personnel and public informative campaigns, are important steps to be taken for susceptible populations protection, public health promotion and better understanding of factors/conditions leading to severe/fatal LD cases.

Key words: Legionnaires' Disease (LD), fatal, children, travel-related (TALD)

Investigation of Legionnaire's disease cases by the Belgian National Reference Centre

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The Belgian National Reference Centre (NRC) for Legionella gathered data for 622 infections from 2011 to 2022. The number of Legionnaires' disease (LD) cases increased over the years, in line with the data from the European centre for disease prevention and control. A decrease of cases was observed in 2020 (minus 43%), probably related to the COVID-19 pandemic and the related containment measures and/or a lower reporting. The number of cases rose again after the pandemic reaching the number recorded in 2019 by 2022.

L. pneumophila (Lp) was responsible of 97% of infections, 81% of which were Lp serogroup 1 (SG-1). Other serogroups were not documented in 12% of cases. Other Legionella species accounted for 16 cases, of which 10 were unidentified, 4 Legionella bozemanii, 1 Legionella wadsworthii and 1 Legionella longbeachae. Among Lp cases, ST1 and ST47 were the most frequent.

Cultures were positive in 49% of cases, urinary antigen tests in 30%, PCR in 19% and serology in 2%.

Infections were community-acquired in 45% of cases, nosocomial in 8% and associated to foreign travel in 11%. Only 1% were associated to healthcare and 1% to domestic travel. The source of infection was not documented in 34% of cases. The NRC was not able to assess the mortality rate, as this information is often not known when samples reach the laboratory.
Some Belgian provinces are less well represented than others, probably due to differences in the habits of the laboratories, some of which may not systematically send samples to the NRC.

Three epidemics were investigated during the studied period: in 2016, in Dendermonde (East Flanders), with 17 cases (ST48); in 2017, in a potato factory at Nieuwkerke (West Flanders), with 127 cases; and in 2019, in a paper factory near Ghent (East Flanders), with 32 cases, associated with a cooling tower (ST921).
Only 54% of the nosocomial and healthcare-associated cases were investigated for environmental contamination. A matching isolate was found in 71% of these investigations. In 38% of total infections, there was no information on the possible source of infection, due to insufficient completion of the NRC forms.

A legionella outbreak caused by an electrical fire place at a hotel

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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. The patients were residing in various regions in Sweden and had no other common connection. Individual investigations, including the hotel, patients´ homes and other possible sources, were conducted. The common investigation lead by the municipal environmental health office in Uppsala, 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 resipiratory tract samples from three patients were typed with nested sequenced-based typing (nested-SBT) at the Public Health Agency of Sweden. Water samples from taps and showers in the hotel were repeatedly cultured for legionella (n=23). Environmental health officers also sampled the irrigation water of a close-by soccer field and a car wash. 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, indicating the same source of transmission. 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: All agencies involved in the outbreak investigation 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.

Keywords: legionella outbreak, electrical fireplace, hotel, rapidly evolving cluster

LD after near drowning – a case report

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Introduction: Legionnaires' disease is normally acquired by inhalation of contaminated water droplets. However aspiration is also a known route of entry and is the mode of entry in near drowning incidents when the lungs fill with water.

Methods: Urinary antigen, PCR and/or culture was used to diagnose two cases of LD following near drowning incidents.

Results: Two cases of LD following near drowning incidents in the same estuarine river water but over 20 years apart are discussed. The first has been previously reported (1) in a 27 year old female and the causative organism was identified as L. pneumophila Sg 13 from a tracheal aspirate. The most recent case was also female and under 50 years old who was urinary antigen and Legionella pneumophila PCR positive and L. pneumophila Sg 1 Philadelphia ST54 and L. pneumophila Sg 10 ST 3191 were isolated from the sputum. The clinical details of the cases will be discussed.

Conclusion: LD following near drowning, although rarely reported, should be considered as a possible cause of any subsequent pneumonia.

Keywords – Legionnaires' disease, near drowning, river water

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Study of the evolution of Legionella pneumophila sg 1 by WGS: some preliminary results from 130 environmental strains isolated over a 13-year period from hotels in Crete.

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Introduction: Legionella is known for having a high genome diversity because of recombination and Horizontal Gene Transfer between different bacteria and eukaryotes.
While sequence-based typing (SBT) has been used for outbreak investigations because of its historically lower cost, WGS has revealed how genetic exchange plays a role in shaping the virulence potential of the species.
Genes encoding features such as drug resistance islands, secretion systems and a large repertoire of secreted effector proteins are part of the mobile accessory portion of the pan-genome.
The aim of the current study was to study the potential evolution of *L. pneumophila* sg 1 strains isolated from various hotel units all over Crete.

Materials/methods: The sampling period ranged from 2010 – 2023. The sampling sites covered hotel units from all four (4) prefectures of the island of Crete. The sampling took place following notification of a TALD case from the ECDC. Isolates were characterized by MALDI-TOF analysis. 130 *L. pneumophila* 1 isolates were selected for WGS based. Attention was paid to process multiple isolates from a hotel and if possible, in consecutive years. WGS was performed following the Illumina technology at a NextSeq 2000.
Genome editing was completed using the Minion technology.
All isolates were characterized based on SBT. Their core-genome was determined, followed by core-genome phylogeny. The average nucleotide identity was recorded. Virulence factors and antibiotic resistance genes were identified.

Results: ST 1 seems to prevail in Crete, especially, but not exclusively, in hotels where a human case was recorded. STs seem to appear for just one year and then they seem to vanish. The genome of *L. pneumophila* sg 1 seems to have evolved over the years. Several isolates carried genes that may enhance virulence and/or may give some resistance to antibiotics.

Conclusions: Studies tracking the population structure over time of *L. pneumophila* growing in water distribution systems have repeatedly confirmed that persistence and micro-evolution are more common than recontamination from the incoming water supply.
Repeated exposure to high temperatures through bursts of hot water demand or failed cycles of superheat-and-flush is liable to favor heat resistance in the surviving bacteria.

Keywords: *Legionella pneumophila* sg 1; evolution; whole genome sequencing

European evaluation of 16 assays for the detection of Legionella pneumophila antigen in urine samples from patients with pneumonia

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Introduction: This study aimed to compare the performance of 16 urinary antigen tests (UAT) for the detection of Legionella pneumophila (Lp) antigen in urine samples (US), including 12 immunochromatographic tests (ICT), 2 fluorescent immunoassays and 2 Enzyme immunoassays, in 9 European National Reference Centers for Legionella.

Materials / methods: A total of 479 US from four panels were tested with the same protocol: proficiency panel (PP) (n=10 including 7 core samples (6 pos/1 neg) and 3 challenging samples), sensitivity panel (SP) (n=46 samples from LD patients with positive culture (n=39), or Lp1 PCR (n=7)), routine panel (RP) (n=388 from LD investigation) and urinary tract infection (UTI) panel (n=35). All ICT tests (without reader) were read separately by two persons; for tests with an automated reader, a visual reading was also performed if possible. All positive US were retested after heat treatment (HT).

Results: For the PP, the intended results were reported by all laboratories for 3 UATs. For the seven core samples, the correct results were reported by all laboratories for 3 further UATs. Using the SP, the variability in sensitivity between UATs was ranging from 51.1% to 98.7%. Using the RP, all participating laboratories gave congruent results for four UATs with a total of 14/388 positive samples. One additional sample was considered true positive (US positive in 7 other kits and infection confirmed by Lp1 PCR), giving a high LD prevalence rate of 3.9% (15/388). HT was essential to confirm positive results, except one kit showing no false positives prior treatment. The 35 UTI specimens were negative in all assays after HT, some samples were positive with 5 kits prior to HT, highlighting the importance of HT.

Conclusion: All laboratories and all kits could detect samples with a high antigen concentration, the sensitivity of some UAT decreased when samples had a low antigen concentration. However, this was not systematic and differed between laboratories. Even if the global false positive proportion is less than 1%, HT has an important added value due to the high number of false positive results.

Evaluation of Sensititre 96-well plates for Legionella antimicrobial susceptibility testing

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Background and objectives: In 2021, the ESGLI community and the EUCAST issued guidance documents for the standardization of Legionella antimicrobial susceptibility testing (AST) methods. The broth microdilution (BMD) was encouraged as a gold-standard methodology that provides unbiased MICs results and allows global surveillance for the emergence of resistance. Yet BMD is not widely adopted in laboratories as it is time-consuming. To overcome this limitation, we had custom-made 96-well ready-to-use plates (Sensititre, ThermoScientific) that allow a 10-min AST implementation.

This study aimed to (i) compare performance of the Sensititre plates with that of in-house plates, (ii) describe the MICs distribution for a large panel of Legionella strains (n=300).

Materials and Methods: The following antibiotics and ranges (2-fold dilutions) were tested: azithromycin (AZI, 0.004-256 mg/L), erythromycin (ERY, 0.008-512 mg/L), moxifloxacin (MXF, 0.004-32), ciprofloxacin (CIP, 0.004-0.25), rifampicin (RIF, 0.0005-64 mg/L), doxycycline (DOX, 0.06-16 mg/L). MICs were first determined using both Sensititre and in-house 96-well plates according to our BMD protocol (Vandewalle-Capo et al., IJAA, 2017) for 109 Lp1 clinical strains and 19 fluroquinolone-, macrolide- or rifampicin-resistant strains (gyrA/gyrB/parC, rrl/rplD/rplV, rpoB mutations, respectively). The results were expressed by essential agreement (EA), defined as MIC ± one-fold dilution of the in-house MIC. The presence/absence of LpeAB macrolide efflux pump was determined from WGS data. Then, 98 additional clinical Lp1, 14 clinical Lp2-14, and 79 clinical or environmental Legionella non pneumophila (Lnp, of which 34 L. longbeachae and 10 L. bozemanii) were tested using the Sensititre plates.

Results: The EA between MICs were 84.4% for AZI, 85.3% for ERY, 95.4% for MFX, 89.0% for CIP, 67.0% for RIF, 26.6% for DOX; 15 of the 109 Lp1 strains harbored the LpeAB pump and showed the higher MICs for AZI (1-2 mg/L) and ERY (0.5-1 mg/L) among susceptible strains. All resistant strains showed MICs distinct from that of susceptible ones, except for macrolide-resistant Lp1 with rplD/rplV mutations that showed macrolide MICs close to that of LpeAB-positive strains. For the 221 Lp strains, MIC (mg/L) distributions were: 0.016-2 (AZI), 0.03-1 (ERY), 0.008-0.06 (MFX), 0.004-0.06 (CIP), 0.0005-0.002 (RIF), 1-16 (DOX). Among Lnp strains, 31/34 L. longbeachae and 9/10 L. bozemanii showed RIF MICs ± 0.002 mg/L.

Conclusion: Although the EA% were not excellent for all antibiotics, the Sensititre plates showed reliable performance and seem like a rapid and valuable tool for Legionella AST and detection of antimicrobial resistance responsible for therapeutic failures. A multicentric evaluation could be relevant.

Genome analysis of Legionella pneumophila ST901, an Italian strain causing many travel-associated Legionnaires' disease, 1987-2019

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Travel-associated Legionnaires' Disease (TALD) cases are frequently reported in Italy. Legionella pneumophila (Lp) Sequence Type (ST) 901 have been causing numerous TALD cases over a 32-years and have so far been identified only in a town of the northern Italy.

In this study, in order to understand if ST901 had specific characteristics of virulence, 41 of these strains were analyzed by whole genome sequencing (WGS) and compared with other unrelated Lp strains. To this aim core genome multi locus sequence type, single nucleotide polymorphisms and pangenome analyses were performed.

ST901 resulted highly similar each other, characterized by I-C CRISPR-cas system located on the chromosome, probably making bacteria able to resist to phages or viruses. Additionally, they contain a putative plasmid highly similar to that found in Lp strain Lens, characterized by the type I-F CRISPR-cas system. Accessory genomic islands, either already described or specifically found in ST901, were also shown, revealing similarity with other Lp strains known to be quite virulent.

Genomic analysis was used in this study to determine which virulence factors characterize those strains found in recurrent TALD cases, demonstrating how microbiological surveillance of TALD infections could be improved. Additionally, the building of a cgMLST database for Legionella would be of great help to quickly identify strains more virulent than others that have to be considered more cautiously in Legionella risk assessment of engineered water systems.

Matching clinical and environmental isolates: complication from mixed infection and genomic diversity within engineered water systems.

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Introduction: Sequence-based typing (ST) has been used to confirm source of outbreak by matching clinical and environmental isolates of Legionella pneumophila. Whole genome sequencing (WGS) may also be useful for this purpose, but a clear threshold of similarity is needed. A cluster of Legionnaires' disease was detected in 2021 in Montreal, Quebec, Canada. ST2858 was detected in 3 of the cases. One nosocomial case presented with a mixed infection, and ST378 was isolated in addition to ST2858. Despite substantial environmental sampling, ST2858 was not identified in environmental isolates, while ST378 was isolated from the healthcare facility's hot water distribution system. ST378 seems to be endemic in Montréal as it was also isolated from 3 other healthcare facilities over the last 15 years. Five more cases caused by ST2858 were detected in previous years and associated with the same geographical location. The goal of this study was to determine the diversity of both STs and determine appropriate threshold for matching clinical to environmental isolates.

Methods: Short- and long-read sequencing was used to produce high quality closed genomes of ST2858 and ST378 isolates. KSNP4 was used for phylogenetic analysis using single nucleotide polymorphisms (SNPs). Pangenome analysis was performed with Roary.

Results: Genomic analysis of the clinical ST2858 isolates, one from 2018, 4 from 2019 and the 3 from 2021 revealed almost identical genomes, with less than 1 SNP in pairwise comparison (whole genome) and perfectly conserved genome synteny, suggesting a common source in which the diversity of ST2858 is very low. In contrast, ST378 isolates differs by ~35 SNPs. The isolates are grouped according to their source; however, in pairwise comparison, the number of SNPs is not significantly different between strains from the same facility than between strains from different facilities.

Conclusion: This study shows that the diversity of L. pneumophila vary amongst STs and that threshold for matching environmental and clinical isolates should be adjusted based on the measured diversity within and across environmental sources. Environmental surveillance program should include WGS to estimate local diversity of L. pneumophila and build database that can inform outbreak investigation.

Keywords: Whole genome sequencing, hybrid assembly, genomic diversity, water distribution system.

Microevolution and spatiotemporal dynamics of Legionella pneumophila ST1905 in Portugal, 2014-2022

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Introduction: The second largest-to-date outbreak of Legionnaires' Disease (LD) occurred in Lisboa, Portugal, in 2014, associated with the new ST1905 genotype of Legionella pneumophila subspecies fraseri serogroup 1. This strain was characterised by a particular genetic profile that included elements associated with increased pathogenicity and virulence, and was strongly associated with person-to-person transmission of LD. Since 2014 this strain has been sporadically identified in the region in the context of clinical and related environmental investigations. The aim of this study was to explore the 2014-2022 molecular evolution and spatiotemporal dynamics of the ST1905 strain.

Methods: Twelve ST1905 strains, isolated between 2014 and 2022, were analysed. DNA libraries and sequencing were performed using Illumina's Nextera XT and MiSeq. Genomes were de novo assembled using the INNUca v4.2.2. Snippy v4.5.0 was used to reference-based mapping and SNP/indel analysis, using the 2014 outbreak strain PtVFX/2014 as reference. Core genome SNPs were identified using ReporTree 1.1.2, manually inspected and used to build a maximum likelihood phylogenetic tree with MEGA 11.0.

Results: All ST1905 clinical and environmental isolates analysed were genetically similar to PtVFX/2014, with the observed microevolution being marked by ten SNPs, four indels and one recombination event. All strains presented a recombination event in the T4ASS region. Two deletions were detected in one strain, on a region corresponding to a genomic island harboring a lvh/lvr T4ASS cluster and on the CRISPR-associated intergenic region. In-depth phylogenetic analysis showed that the isolates did not cluster by year of isolation, and both clinical and environmental isolates associated to a particular location clustered apart, supporting the persistence of the strain in that location and the linkage between different events. Clinical and related epidemiological isolates differed by three or less SNPs, further supporting their epidemiological linkage.

Conclusions: Our study suggests that ST1905 has been conserved overtime and seems well adapted and potentially persistent in diverse and geographically dispersed environments. The intrinsic pathogenic and virulence characteristics of this strain and the potential for spreading to other locations not previously affected by the 2014 outbreak may result in serious implications to public health, highlighting the importance for continuous monitoring and control.

Keywords: L. pneumophila; ST1905; Microevolution; Portugal

An update to the Legionella pneumophila Sequence-Based Typing (SBT) online database

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Introduction: The internationally accepted method for typing Legionella pneumophila is sequence-based typing (SBT), a rapid and discriminatory method based on the DNA sequences of seven loci (flaA, pilE, asd, mip, mompS, proA and neuA/neuAh). Using the SBT protocol Public Health England (PHE), the predecessor organisation to UK Health Security Agency (UKHSA), developed an online database in 2004. The tool assigns Sequence Types (STs) and new allele sequences/ST into the SBT schema after a quality control check on submitted ab1 Sanger sequence files. The database currently stores 3,166 STs submitted by expert health professionals from more than 72 countries that have a remit for Legionella surveillance and laboratory typing. In addition, the online interface allows national and international users to upload epidemiological data (e.g., location, isolation date, source) and laboratory findings (e.g., allelic profile, ST, Serogroup) to the database. UKHSA users of the L. pneumophila SBT tool upload every sample to the database. The database currently holds a total of 14,422 entries: 4,910 (34.05%) Environmental, 9,415 (62.28%) Clinical and 97 (0.67%) of unknown origin. This online database and its associated tools assist in the epidemiological investigation of L. pneumophila infections, especially travel-associated cases, by which it will allow a rapid comparison of isolates obtained in more than one country.

In 2018, PHE withdrew external access to the database due to outdated infrastructure and underlying code. The UKHSA plans to release a fully updated version of the online database in 2023.

Materials and methods: UKHSA rebuilt the system using up-to-date software and infrastructure to meet the security standards required for a public-facing web site for a global audience. The site is now written in Python 3, using the Flask web framework. The system is deployed on Red Hat OpenShift, a platform build around the Kubernetes container management system. The industry standard OpenID Connect protocol is used for user authentication, connected to UKHSA's identity and access management platform, Azure Active Directory.

Results: The updated technology used to rebuild the system improves functionality and quality, reduces system vulnerabilities and inefficiencies, and overall uplifts security and stability of the SBT online database.

Conclusions: UKHSA has developed an updated secure version of the Legionella pneumophila Sequence-Based Typing (SBT) online database. The tool can now carry out Legionella pneumophila typing in real-time by UKHSA colleagues as well as international users. The database is currently undergoing final internal review. A link will be made available as soon as possible.

Comparative analysis of functional genes among Legionella species

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Keywords: Legionellaceae, diversity, pangenome

Introduction: Legionella are opportunistic pathogenic bacteria responsible for legionellosis, a disease of which the incidence drastically increased in recent years. While most cases have been attributed to L. pneumophila, the genus contains over 60 named species. In fact, a notable discrepancy exists both between clinical and environmental isolates, as well as the prevalence of species across different environments. However, currently only little evidence is available to explain the discrepancies in prevalence observed experimentally. For this reason, we investigated the differences in the genomes of Legionellaceae strains which could affect and explain their different ecology.

Material and methods: Over 4900 genomes belonging to the family Legionellaceae were collected from both culture- and metagenomic-based studies targeting both environmental and clinical settings. After quality control, genomes were clustered based on their nucleotide identity into species-level and strain-level clusters from which representative genomes were chosen. We evaluated the presence/absence and difference of genes related to their metabolism, iron acquisition, cellular membrane and resistance to stresses (e.g., heat, antimicrobial resistance) using several methods and we compared the results across strain belonging to the same species and across different species.

Results: Genomes similarity analysis highlighted how the family Legionellaceae is composed of 126 species, divided in 402 strains, most of which identified as belonging to L. pneumophila due to much greater number of genomes derived from this organism. As expected, differences across closely-related strains resulted lower than the ones among species, but, in any case, the analysis highlighted a great diversity among the different species and strains considered. For example, regarding iron-related genes the number of iron transport (i.e., acquisition) genes detected was commonly equal to two, but this increased to more than three genes in L. jordanis, L. tunisiensis, L. lytica and one unnamed species, characteristic possibly related to the isolation environments (i.e., surface waters and soils).

Conclusions: Our analysis suggests that, given the genetic diversity across different Legionellaceae species, the microorganisms belonging to this family are likely to be adapted to different environmental niches. For this reason, it not possible to extend what is known for L. pneumophila to other species.

Molecular characterization of shower hoses biofilms reveals genetic variability of Legionella spp. and specific associations with microbiome members

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Introduction: Legionella are natural inhabitants of building plumbing biofilms (e.g. shower hoses), where interactions with other microorganisms influence their survival, proliferation, and death. Their interactions with their surrounding microbiome remain poorly understood.

Methods: Here, we investigated the associations of Legionella with prokaryotic and eukaryotic microbiomes in biofilm samples extracted from 85 shower hoses of a single residential building. We used specific ddPCR quantification as well as general 16S rRNA and 18S rRNA amplicon sequencing to characterize the shower hose microbiomes

Results: Sequencing data revealed a complex prokaryotic and eukaryotic microbiome in all samples. Legionella spp. relative abundance in the biofilms ranged between 0 - 7.8%, of which only 0 - 0.46% was L. pneumophila. Our data suggests that some microbiome members were associated with high (e.g., Chthonomonas, Vrihiamoeba) or low (e.g., Aquabacterium, Vannella) Legionella relative abundance. Interestingly, the data analysis also revealed high genetic variability in the 16S rRNA sequences assigned to the genus Legionella (30 unique Amplicon Sequence Variances detected). The correlations of the different Legionella variants with microbiome members showed distinct patterns, suggesting separate ecological niches potentially occupied by different Legionella species.

Conclusions: This study provides insights into the ecology of Legionella with respect to: 1) the colonization of a high number of real shower hoses biofilm samples; 2) the ecological meaning of associations between Legionella and co-occurring prokaryotic/eukaryotic organisms; 3) the presence of multiple species of Legionella in building plumbing systems, and the potential of 16S rRNA based detection of Legionella diversity in the environment.

Occurrence of Legionella and Legionella pneumophila in the air and water of wastewater treatment plants

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Wastewater treatment plants (WWTPs) are recognized as important sources of bioaerosols which can be contaminated with pathogens such as Legionella pneumophila (Lp). A significant correlation between exposure to bioaerosols and the incidence of respiratory or enteric diseases in WWTPs has been documented. Objectives: 1) Evaluate the level of exposure of WWTPS workers to Legionella pneumophila according to the treatment step and operating factors 2) Describe the aerosolization mechanism of Legionella from wastewater into the air. Methodology: Fifteen WWTPs in the province of Quebec were selected according to treatment type and location in relation to previously identified legionellosis clusters. Processes with the high bioaerosol-generating potential and high risk activities for workers' health were targeted according to literature and workers input. For each WWTP, 5 to 7 sampling points were investigated, collecting 1L of wastewater and 18m3 of air at each point. DNA was extracted using the PowerSoil PowerLyser kit (Qiagen). Quantitative polymerase chain reaction (qPCR) was performed for Legionella spp. and Lp using the Microproof® Legionella Quantification LyoKit from Hygiena®. Results: All 84 water samples were positive for Legionella spp (from 5.03E+03 to 4.62E+09 Genome Copies (GC)/L) and 12% were also positive for Lp (maximum at 8.89E+06 GC/L). Of the 83 air samples taken for Legionella spp, 28% were positive and quantifiable (from 2.33E+01 GC/m3 to 1.74E+03 GC/m3) and 34% were positive but below the limit of quantification. Only 5 air samples were positive but non-quantifiable for Lp (< 1.5E+02 GC/m3). The processes with the highest Legionella loads are in line with the literature. Of the 17 different processes analyzed, those generating high turbulence (mixing tank, grit removal, Archimedes screw), processing highly contaminated material (screening removal, waste and sludge bin) or being biological in nature (activated sludge, biofiltration, RBS) were responsible for the greatest Legionella spp. aerosolization. Conclusion: Lp was not detected in most air samples from WWTPs. Its impact on workers' health might therefore limit and more sampling is required. Other potential pathogenic species such as non-tuberculous mycobacteria and viruses may also be present in higher concentrations, justifying containment or protective measures to improve workers' health.

How L. pneumophila colonizes differently structured P. fluorescens biofilms?

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Several operational and environmental factors enable Legionella persistence in water systems. For instance, those include temperature, corrosion, hydrodynamic conditions, protozoa and biofilms. Biofilms - complex communities of microorganisms embedded in self-produced extracellular polymers – can trigger Legionella colonization and proliferation. Studying biofilm-Legionella interactions is very challenging since biofilms are affected, to a greater extent, by the operational factors previously listed. In the present work, we aimed to answer the following questions: how do the hydrodynamic conditions (stagnation vs dynamic) affect the mesoscale biofilm structure?; Is the Legionella colonization/persistence pattern dependent on those different biofilm structures?

To increase reproducibility among the biofilm structures a monospecies biofilm of Pseudomonas fluorescens was considered. The biofilm was formed in PVC surfaces placed inside 12-well microplates, using R2 media, under stagnation and 80 rpm for 14 days. Legionella was spiked 3 days after starting biofilm formation. Biofilm was sampled and characterized at days 3, 4, 7, 9, 11 and 14, by Optical Coherence Tomography (OCT) to assess the mesoscale structure and P. fluorescens and L. pneumophila were enumerated by Colony-Forming Units (CFU).

Preliminary results showed that hydrodynamic conditions, besides affecting the biofilm structure, also had an impact on how Legionella colonized such biofilms. Stagnation biofilms became thicker after being spiked with Legionella and were colonized over a longer period by bacteria. L. pneumophila was able to colonize all tested biofilms, although Legionella was recovered in lower concentrations from the thinner biofilms found in the dynamic conditions. This work provides new insights into the need to study the biofilm structure as it plays a crucial role in Legionella persistence.

Keywords: Biofilm structure; Hydrodynamic conditions; Legionella colonization.

Susceptibility and adaptive mechanisms of L. pneumophila to triclosan

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Introduction and objectives: Legionella pneumophila serogroup 1 (Lp1) is the causative agent of Legionnaires' disease. Legionella hydric environment is contaminated by biocides such as triclosan (TCS). TCS inhibits fatty acids synthesis of bacteria (such as E. coli or Salmonella) by targeting the FabI enoyl-ACP-reductase. Adaptive mechanisms under TCS exposure are reported in E. coli and involve TCS tolerance and antibimicrobial resistance. However, its exposure on L. pneumophila has never been described. Therefore, this study aimed to i) define TCS Minimal Inhibitory Concentrations (MICs) for Legionella; ii) evaluate the impact of TCS on antibiotic susceptibility of L. pneumophila; iii) characterize the adaptive mechanisms of L. pneumophila to TCS environmental-level exposure.

Methods: TCS MICs were determined by microdilution on a panel of 111 clinical and environmental strains of various serogroups and Sequence Types (ST). The role of TCS on antibiotic susceptibility was assessed on Lp1 Paris using the checkerboard method between TCS and azithromycin (AZI), erythromycin (ERY), levofloxacin (LVX) or rifampicin (RIF), and evaluated using the Fractional Inhibitory Concentration Index (FICI): FICI ≤ 0.5: synergy; 0.5 < FICI ≤ 4: indifference; FICI > 4: antagonism. An experimental evolution was set up on 8 independent lineages of Lp1 Paris under TCS sub-inhibitory concentrations for 20 passages over 3 months. Illumina® sequencing paired-end 2x100 bp was achieved on each population at different steps of the evolution procedure.

Results, discussion, and conclusion: TCS MICs ranged from 0.016 to 0.125 mg/L corresponding to environmental levels, with a median MIC of 0.031 mg/L. No difference between serogroups or STs was observed. No synergy or antagonism between TCS and AZI, ERY, LVX or RIF was observed. Experimental evolution showed adaptive mechanisms in all 8 lineages from passage 10 by missense mutations of fabI. Variants were mainly found at the active site (I213T, K212T, F210S), but not exclusively (G118E, G118R). FabI mutations were associated with a 4 to 64-fold increase in TCS MICs, respectively K212T and F210S. Interestingly, K212T mutant also exhibited a slight tolerance to levofloxacin with a 2-fold increase in LVX MICs. Isogenic mutants reconstruction and assessment of TCS/antibiotics tolerance is ongoing.

Keywords: Legionella - Triclosan - One Health – FabI

Characterization of bacterial communities and Legionella species in aquifers supplying a drinking water distribution system

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Introduction: The new EU Drinking Water Directive includes Legionella monitoring for quality analysis of drinking water distribution systems. Indeed, even if Legionella shows limited proliferation in the natural environment, it may be present in aquifers used as drinking water reservoirs.

We have investigated the abundance and diversity of bacterial communities and Legionella species in non-treated waters supplying a public distribution network in Umbria (Italy).

Methods: Abundance and diversity of total bacteria and Legionella species was assessed in 20 different aquifers, including natural springs, wells, and surfaces waters, by culture-independent molecular methods, namely universal and genus-specific qPCR and Next Generation Sequencing (NGS).

Results: Legionella was detected in most of the samples (14/20) with gene copy numbers positively correlating with total bacteria abundance, indicating that Legionella is part of the normal environmental bacterial community in natural waters.

Highly diverse bacterial communities were observed by NGS analysis, including more than 500 known genera across all samples and 142 present, on average, in each one. By applying genus-specific NGS, a high number of different Legionella populations were found. Among the most abundant populations (i.e. those with a relative abundance higher than 3% in at least one sample), which represented, on average, 73% of the total reads for each sample, several known Legionella species were found (i.e. L. pneumophila, L. rubrilucens, L. dumoffi, L. lytica, L. cincinnatiensis, L. cherri, L. drozanskii, L. waltersii, L. fallonii and L. rowbothamii). Nevertheless, most of these populations (on average more than 60% of the reads for each sample) could not be affiliated to an acknowledged Legionella species, indicating how this genus encompasses a greater variety of species in natural water environments than is currently known.

Conclusions: Abundant and highly diverse Legionella populations, including potentially novel species, are naturally present as part of complex microbial communities in aquifers supplying non-treated water used for public distribution systems. Consequently, the absence of Legionella, as other water-borne pathogens, relies on the sanitation actions implemented in the drinking water distribution network.

Keywords: Bacteria diversity; Legionella diversity; water sources; drinking water.

The transposition in Italy of the New European Directive (DWD) 2020/2184

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The New European Directive 2020/2184 (DWD) concerning the quality of water intended for human consumption includes for the first time Legionella among the pathogens to be controlled in domestic water systems (DWS). It was implemented in Italy by a legislative decree and by two guidelines published in 2022 and 2023 (1-3).

Premises where the level of Legionella in plumbing systems must be maintained at <1000 CFU/L, were established based on the incidence of Legionnaires' disease (LD) in our country and microbiological data. As required by DWD, the Water Safety Plan (WSP) approach has been considered the milestone for all actions aimed at maintaining the healthiness of DWS.

The premises have been divided into five classes (A, B, C D and E) with decreasing order of Legionella risk. The first class considered priority (hospitals, health facilities, etc.) must carry out the Legionella check and apply a detailed WSP. The other three classes of priority buildings (i.e. accommodation facilities, medical clinics, dental centers, etc.) adopt a self-monitoring plan with a different level of severity compared to class A buildings, obligatorily checking both Legionella and L. pneumophila. The last one, class E, does not include priority buildings (condominiums, houses, offices, etc.) and site-specific risk assessment and management is generally not required, except for the recommendation of a periodic check on Legionella and L pneumophila.

An important aspect introduced by the Italian legislation on DWD is the sanction envisaged for those who do not apply the indications contained in the decree. Besides, based on the new opportunities offered by the DWD to use molecular methods and other culture-based methods for Legionella detection in water samples, supported also by a large study carried out at national level, we have confirmed the reliability of Real Time PCR and Legiolert.

Therefore, the existence for the first time of a law regulating the assessment and management of Legionella risk in priority buildings, the possibility of using faster methods to detect Legionella will certainly contribute to the reduction of LD cases in our country, which will be important to monitor in the future.

Quantification of Legionella pneumophila in drinking water: a meta-analysis comparing qPCR and culture-based detection methods

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Introduction: Accurate quantification of Legionella pneumophila (L. pneumophila) is essential for managing building water quality. Quantitative Polymerase Chain Reaction (qPCR) is a valuable tool to augment traditional culture-based methods, offering fast, automated, and on-site quantification of L. pneumophila. However, qPCR detects not only viable bacteria but also free DNA and DNA derived from dead or non-culturable forms of the bacterium, posing challenges in interpreting results and potentially leading to an overestimation of infection risks.

Aim: Our study aims to:

1.Evaluate the relationship between qPCR and culture-based measurements of L. pneumophila in studies published to date.

2.Identify factors influencing this relationship.

3.Promote qPCR's role in health risk assessment.

Materials/Methods: We developed a literature database incorporating sample- and site-specific data. Parametric models were used to analyze the variability in the qPCR:culture concentration ratios, and we employed meta-analysis models to synthesize findings across studies.

Results/Discussion: The findings revealed variable qPCR:culture ratios, spanning up to 10,000 times (4-log difference) within a single site. Geometric mean ratios varied from 1:1 to 100:1 across sites. From a safety perspective, we argue that a qPCR:culture adjustment factor of 1:1, representing equal weights for qPCR and culture results, should be used to integrate qPCR data into risk assessment. However, this may lead to overly conservative risk estimates in certain settings. Subgroup analyses suggest that maintaining a stable temperature of 55°C in hot-water systems results in a lower ratio, approaching unity. We also found that sampling methods can influence the relationship, with first-draw samples resulting in lower ratios than flushed samples, suggesting regrowth in the evaluated systems.

Conclusions: qPCR has the potential to enhance risk assessment, but wide variability relative to culture highlights the need to further understand influencing factors. Site-specific factors, such as system operation and the vulnerability of the exposed population, should be considered when using qPCR data to inform risk assessment. Whenever feasible, qPCR data should be coupled with targeted culture or viability-qPCR data collection. Moving forward, the standardization of study design and rigorous data reporting are essential for adequate accounting of the environmental and methodological factors that influence the qPCR:culture relationship.

Keywords: Quantitative Polymerase Chain Reaction (qPCR), Monitoring, Risk Assessment, Culture-based Methods

Hunting for probiotics against Legionella in building plumbing systems: potential antagonists and their natural compounds

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Introduction: Legionella are ubiquitous in freshwater and are often found in building plumbing systems, where they inhabit biofilms and increase the risk of infection for humans. Conventional management and control of Legionella includes water treatment with high temperature boiler settings and disinfectants (e.g. chlorine), but these treatments are sometimes not effective. Here, we explored the inhibitory potential of indigenous waterborne bacteria towards multiple Legionella species.

Methods: We collected a large number of isolates from which we finally selected, characterized and identified 10 strains that were antagonistic towards L. pneumophila in so-called spot-on-lawn agar assays showed. We then tested the antagonists against 10 pathogenic strains of Legionella, and mined their genomes for biosynthetic clusters. We then co-cultured selected antagonists with Legionella, confirmed inhibition in the cell-free spent media, and then proceeded to identify the compounds responsible for Legionella inhibition.

Results: Spot-on-lawn agar assays showed differential inhibition patterns of the Legionella species (e.g., only two antagonists were able to inhibit all the Legionella species), suggesting different attack/defense mechanisms involved in the specific interactions. Genome mining of the antagonists revealed a range of interesting biosynthetic gene clusters encoding for natural products that could be involved in the inhibition, including pathways for lipodepsipeptides and siderophores. We further co-cultured the Legionella species and the antagonists and confirmed the biological activity of the extracellular compounds towards Legionella. Preliminary LC-MS data detected the presence of the lipodepsipeptide viscosin suggesting its potential role in the inhibition of Legionella.

Conclusions: This data informs some potential ecological interactions between Legionella and other microbiome members. It furthermore offers the opportunity to explore the potential of alternative probiotic strategies to control Legionella in plumbing systems.

Impact of climate change on Legionella and other waterborne microorganisms in water systems - is it too late to do anything?

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Introduction: There is no doubt that the earth is warming at an unprecedented rate and the Intergovernmental Panel on Climate Change (IPCC) has stated that there is a "very high confidence" of adverse impacts on public health. Anthropogenic climate change is increasing the number of warm days and nights, the frequency and intensity of droughts and heavy rainfall events. Increasing temperatures are resulting in warmer water in the drinking water supply, and consequently the proliferation of waterborne pathogens that will subsequently compromise water systems and their control strategies. In terms of microbial growth, cold water temperatures entering buildings is increasingly above 20°C, often above 25°C, and for longer periods of time, resulting in the proliferation of adverse microorganisms including Legionella in our water systems and increasing risks to public health. It is incumbent upon those who produce guidance and those in charge of buildings to act and prioritise strategies to prevent increasing numbers of waterborne disease outbreaks. However, focus and activities from LEED (Leadership in Energy and Environmental Design) and BREEAM (Building Research Establishment Environmental Assessment Methodology) which are designed to reduce climate change impact often increase water temperature and reduce water flow and therefore create the unintended consequences of conditions conducive for Legionella growth. There are a number of strategies that need to be improved if we are to combat the impact of climate change on Legionnaires' disease.

Methods: This review of published and unpublished data will assess the impact of climate change on waterborne pathogens to identify inherent risks that can be reduced using technologies such as remote monitoring of water temperatures and improved microbiological testing. Results: Results from studies on i) remote monitoring and ii) microbiological testing will be presented to demonstrate that online reporting improves water quality following appropriate remediation, and that improving microbiological testing can result in greater counts that are more specific for L. pneumophila. The implementation of intelligent real time remote monitoring systems to assess water temperatures and improve how we assess for the presence of L. pneumophila can lead to more effective best practice, future guidance and better public health outcomes.

Managing Legionella in engineered systems: recent developments in monitoring and treatment to guide efficient water safety plans

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Keywords: *Legionella*, engineered water systems, treatment, monitoring

Introduction: Water safety plans (WSP) rely on a multi-barrier approach including the implementation of control strategies and environmental monitoring to manage the risks associated with *Legionella* in engineered water systems. Recent findings in surveillance monitoring, preventative and curative controls regimes provide valuable insight to define more effective water safety plans to minimize risks associated with *Legionella*.

Monitoring and treatment of cooling towers

The ability of current sampling approaches to monitor *Legionella* and adjust treatment in cooling towers (CTs) will be reviewed. Quick response methods are needed to monitor levels of *L. pneumophila* (Lp) in CTs allowing timely adjustments to prevent outbreaks. Online, laboratory qPCR, plate and liquid culture were used to monitor spatial and temporal variations of *Legionella* in complex CTs circuits. Multiple location sampling showed that current monitoring plans have significant limitations such as: low frequency, single sampling locations, and the reliance on plate culture methods. A combination of liquid culture for compliance and frequent qPCR provides a more agile and robust monitoring scheme, while online qPCR enables online retroactive process adjustment, preventing non-compliance response and shutdowns. The efficacy of mist eliminators, the main barrier to aerosol dissemination from CTs, to retain inhalable size aerosols contaminated with Lp, varied especially during high demand critical periods of production. Finally, a statistical analysis of temporal variations in Lp concentrations coupled with QMRA from a regulatory database (2852 CTs) revealed that a minority of CTs drove the risk analysis, supporting the modulation of monitoring requirement to estimated risk levels.

Monitoring and treatment of building hot water systems

Two recent drivers affect the occurrence of *Legionella* and the perceived performance of control strategies. Firstly, multiple opportunistic drinking water pathogens (ODWPs), including *Legionella (Lspp, Lp, LpSG1)*, nontuberculous mycobacteria (NTM) and *Pseudomonas aeruginosa* (*Pa*) should be considered. A holistic approach to evaluate the efficacity of control strategies is warranted to minimize waterborne infections in building water systems. The efficacy of common *Legionella* control strategies such as thermal control, on site disinfection and shock chlorination will be presented considering the risks of potential microbial shifts towards NTM, *Pa* or other ODWPs. Secondly, the impact of implementing water and energy conservation needs to be quantified and managed. Lower water demand and extended stagnation impact the residence time of water in these systems, lowering the ability to prevent the growth of *Legionella* and other ODWPs. Recent COVID-19 shutdowns drastically increased the frequency and duration of water stagnation events in building water systems. Several studies have documented the extent of water quality losses especially the impact on *Legionella*. Also demonstrated were the limited performance of subsequent remedial methods such as flushing and shock chlorination. Finally, the risk associated with the introduction of energy conservation measures such as preheating will be discussed.

Key findings that are most relevant to risk mitigation in hot water systems and cooling towers will be summarized to improve guidance and regulations.

Occurrence of *Legionella pneumophila* in US Drinking Water Distribution Systems

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Introduction: Little information is available on L. pneumophila in drinking water distribution systems. Adequate management of risk requires collaboration between buildings and the water utility.

Materials/Methods: WRF project 5156 objectives are to determine the relationship between disinfectant concentrations and other system features and the occurrence and concentration of L. pneumophila. The project will collect 1800 samples from 48 utilities over the summer of 2023 and add the data to the 3,875 samples collected in 2022 and 1,149 samples from a prior study of 12 water utilities. Samples were analyzed by the utility using the Legiolert method collected after flushing taps for 3-5 minutes. The US utilities are geographically dispersed and include a mix of surface and groundwater sources and systems that use free chlorine or chloramine disinfection. Most samples were collected when water temperatures were >18oC.

A workshop reviewed protocols for responding to and communicating the results of L. pneumophila detection in drinking water systems and was valuable given the lack of formal guidance from US regulatory agencies.

Results: For results collected to date, L. pneumophila was detected in 54 of 5,024 drinking water samples (1.075%). A total of 44 of 3,606 samples from free chlorinated systems were positive (1.22%), while L. pneumophila were detected in 10 of 844 samples from chloraminated systems (1.18%). L. pneumophila concentrations greater than 1.0/mL generally occurred when disinfectant residuals were low (<0.2 mg/L). All positive Legiolert samples were sent to an independent laboratory for serotyping and identification using MALDI-TOF-MS. A separate sampling effort in 2023 will also analyze distribution system samples using the conventional culture (BCYE) assays and a commercially available viability qPCR assay.

Conclusions: Beyond the L. pneumophila results, a key learning was the experience of water utilities in setting up the monitoring program, conducting the analyses, and responding to positive results. The results show that L. pneumophila can occur in drinking water distribution systems, but that best practices including maintaining a disinfectant residual and flushing and cleaning of the system can control Legionella risk.

Keywords: L. pneumophila, distribution system, disinfectant residuals.

Comparison of Legionella colonisation in 20 buildings before and after the 2020 UK COVID-19 lockdown, and the effect of chlorine dioxide dosing on long-term positivity

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Legionella bacteria are the causative agent of Legionnaires' disease, a major type of severe pneumonia. Legionellae are naturally occurring parasites of protozoa and can colonise man-made water systems, favouring stagnant conditions and causing disease when inhaled via aerosols. Legionella colonisation in water systems can be controlled by temperature, flushing, and use of disinfectants such as chlorine dioxide. The COVID-19 pandemic resulted in widespread building closure, especially in the hospitality sector, leading to increased stagnation. It is therefore important to investigate what effect the disuse of water systems during the pandemic has had on Legionella colonisation, as increased levels may lead to higher incidence of disease. The routine microbiological monitoring results of 20 buildings throughout the UK which had partial or full closure during 2020 were analysed to establish whether average Legionella levels isolated in cfu/250ml were significantly higher after the pandemic. The date range for sample selection was 26 March 2019 – 26 March 2021, giving one year either side of the start of lockdown measures in the UK. Percentage positivity rates were also used to establish whether positive samples were more common after the lockdown. Buildings with chlorine dioxide dosing were compared to those without, and specific focus was put onto hotels as they are a known reservoir of Legionella due to intermittent use. The average cfu/250ml of Legionella isolated in all buildings which had at least one Legionella positive result (n=20) was 25.37 before lockdown compared to 53.15 afterwards; hotels (n=7) had 29.86cfu/250ml before and 115.28 after lockdown. Percentage positivity rates for all buildings increased from 6.85% before lockdown to 13.21% after, and in hotels increased from 3.39% before to 24.38% after. Although there was an apparent trend towards increased Legionella colonisation, no statistically significant difference was found between any of the groups tested, although this may be due to small sample size, inherent variation in the Legionella isolation method, or issues comparing relative colonisation in differently managed water systems.

In situ monochloramine disinfection in a large hospital to control Legionella, nontuberculous mycobacteria and Pseudomonas aeruginosa

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Introduction: In-situ monochloramine disinfection has risen in popularity due to its ability to rapidly reduce Legionella-positive sites in healthcare facilities (HCFs). However, holistic longitudinal studies at representative building-wide sites with variable stagnation periods are lacking to clarify: (1) factors contributing to its efficacy across large hot water systems; (2) the impact on other opportunistic pathogens (OPs) such as nontuberculous mycobacteria (NTM) and Pseudomonas aeruginosa (Pa).

Methods: Culturable L. pneumophila (Lp) and gene copies (gc) of Legionella species (Lspp), Lp, Lp sg 1, NTM and Pa were measured at 22 distal sites (faucets, showers, hand washing stations) with low or normal water demand and 10 hot water control points (main sectorial pipes) in a large 440 bed hospital. Sampling was conducted 3X before the introduction of monochloramine, 24h after, and on a weekly then monthly basis for one year, including periods where the dosage was interrupted (2d and 2 weeks).

Results: Introduction of monochloramine resulted in immediate (24h) Lp cultivability suppression (up to 4-log), except at one low use site where concentrations of 101-103 MPN/L were still measurable despite 0.1-1.7 mg/L of monochloramine. Undetectable levels of Lp gc were found in 91% of samples by the third week (up to 3-log decrease) at all water points except that same low use site for the remainder of the study (14 months). A temporary shift from Lp sg 2-14 to sg 1 was observed at several distal sites 24h after disinfection. A more gradual 2-log decrease in mean Lspp gc was observed over the first month, followed by stable concentrations ranging 102-104 gc/L. Mean NTM gc decreased by 2-log in the first two weeks of dosage, and then remained steady at 104-105 gc/L, while Pa qPCR positivity was reduced from 26% to 15%. Low use and stagnant sites showed significantly higher concentrations (1-4-log) of Lspp and NTM and than sites with higher water demand. Interruption of disinfection resulted in rapid increases in Lspp (> 2-log) and NTM (> 3-log) but not of Lp at all sampling sites.

Conclusion: Monochloramine was strikingly effective in nearly eliminating culturable and qPCR Lp and significantly lowering Lspp, confirming the high potential of in-situ chloramination to control growth of Legionella in HCFs when optimization of thermal regimes and heat-and-flush measures were not sufficient. A more modest but systematic decrease was however observed for NTM and Pa. Higher distal prevalence supports preventative flushing low use distal sites to ensure control of Legionella. HCF managers can use this evidence to guide preventative flushing and identify long-lasting engineering controls and monitoring strategies.

Chlorine vs bromine for Legionella control in spa pools: unmasking the winner

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Keywords: spa pools, chlorine, bromine, Legionella

Introduction: Chlorine and bromine are both currently used for Legionella control in spa pools. Although it is assumed that the effectiveness of both biocides is equivalent under the respective conditions of use, the comparative effectiveness of the two treatments remains uncertain.

Methods: We have compared the monthly analytical results of Legionella spp. over a year in two groups of spa pools treated with chlorine and bromine respectively. Both groups of pools were operated following Spanish regulations for pools (parametric values: Free chlorine: 0,5-2,0 mg/L; Total bromine: 2-5 mg/L; pH: 7,2-8,0). Differences between the two groups, in terms of Legionella spp. positive results, concentrations of the bacterium and presence of L. pneumophila sg1 were analyzed.

Results: A total of 211 samples were included in the study, 96 from 21 chlorinated pools and 115 from 24 brominated pools. In the chlorinated pools, average chlorine and pH levels were 1,80 ± 1,53 mg/L and 7,40 ± 0,18 respectively. In the brominated pools the average total bromine level was 4,90 ± 3,35 and the pH was 7,36 ± 0,13. In the chlorine group, 6,2% of the samples were positive compared to 33% positive in the bromine-treated group. The average concentration of Legionella spp. was 206 ± 214 CFU/L in the first group and 6284 ± 15506 in the second. In both cases the differences were statistically significant ($p < 0,05$). L. pneumophila sg 1 was present in 50% of the positive samples of the chlorine group and in 60% of the samples from the bromine group ($p > 0,05$).

Conclusions: Chlorine was superior to bromine for the control of Legionella spp. in spa pools under the studied conditions. Larger studies are necessary to confirm these results.

An Update of the ESGLI European Technical Guidelines for the Prevention, Control and Investigation, of Infections Caused by Legionella species

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The ESGLI European Technical Guidelines for the Prevention, Control and Investigation of Infections Caused by Legionella species were developed by a multidisciplinary group of experts and last published in 2017. Whilst these guidelines are still valid and useful both for hoteliers and those investigating cases and outbreaks, much has changed in recent years due to a range of factors including:

- the innovations in water management measures such as the use of artificial intelligence for monitoring
- additional risks associated with climate change, such as rising incoming water supply temperatures and
- evidence of previously unidentified sources of exposure to *Legionella*.

There have also been developments in the detection and identification of *Legionella* from environmental sources in recent years. The guidelines are currently being updated by the authors to bring the guidelines up to date and provide further background information to assist those using them in understanding the reasons behind the advice for example an appendix on the different water treatment methods. Theis version is more inclusive and includes not just land based accommodation sites but also cruise ships, ferries etc.

The main text of the guidelines is kept clear and succinct for easier consultation by the users. At the same time, additional background information is added in appendices to help those investigating cases and managing the risk from legionellae in accommodation premises and public buildings. An overview of the changes in the updated guidelines to date will be presented.

1. Clinical Aspects and Diagnostics for Legionella

P1: Legionella longbeachae wound infection: case report and review of reported Legionella wound infections

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Introduction: Extrapulmonary manifestations of Legionella species infection are exceedingly rare. We present a case involving a 61-year-old immunocompetent woman who developed pain and swelling in her index finger after sustaining rose thorn pricks during gardening. Clinical examination revealed fusiform finger swelling, mild redness, warmth, and fever. While her blood cell count was normal, there was a slight elevation in CRP levels. Intraoperative findings unveiled extensive infectious destruction of the tendon sheath but sparing the flexor tendons. Conventional cultures were negative, but Legionella longbeachae was identified via 16S rRNA PCR analysis and cultivation on BMPA-agar. A 13-day course of oral levofloxacin led to rapid resolution of the infection.

Literature Review

A comprehensive literature review of cutaneous Legionella infections was performed, excluding postoperative infections, septic arthritis, blood-borne infections, and pulmonary involvement.

Results: Eighteen confirmed cases of cutaneous Legionella infections were included: five L. longbeachae, six L. pneumophila, three L. micdadei, and one each of L. bozemanii, L. maceachernii, L. feeleii, and L. cincinnatiensis. Females accounted for the majority (14/18, 78%) of cases, with a median age of 66. Most infections originated in the United States (9/18). Seventy-eight percent of patients had underlying conditions necessitating immunosuppressive medication. Garden-related activities, particularly involving flowers or flowerpots, were implicated in all L. longbeachae and L. maceachernii infections. 16S rRNA PCR and Sanger sequencing (44%) followed by culturing on BCYE agar were the most common diagnostic methods. All patients received fluoroquinolones and/or macrolides as monotherapy or in combination.

Conclusion: Our case and literature review underscore the potential underdiagnosis of Legionella spp. wound infections due to the need for specific diagnostic methods. Clinicians should maintain heightened awareness of Legionella when evaluating patients with cutaneous infections, particularly those engaged in garden work. Moreover, in diagnostic laboratories it is essential to remain vigilant for the presence of Legionella in locations outside the lungs. However, when relying solely on 16S rRNA PCR for detection, one must exercise caution in interpretation. This is because Legionella DNA may potentially contaminate the sample and PCR reagents. Therefore, it is advisable to confirm the presence of Legionella through specific Legionella-PCR and cultivation methods.

P2: Antimicrobial susceptibility testing reveals reduced susceptibility to azithromycin and other antibiotics in Legionella pneumophila serogroup 1 isolates from Portugal

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Background: Antibiotic resistance in Legionella pneumophila has not yet been a subject for major concern. However, studies show that these bacteria can develop resistance, and failure to detect it may lead to serious consequences for patients and promote its spread. As there is limited data available for Portugal, we determined the antibiotic susceptibility profile of Portuguese clinical and environmental L. pneumophila serogroup 1 (sg1) isolates against 10 antibiotics used in the clinical practice.

Methods: Minimum inhibitory concentrations (MICs) for L. pneumophila sg1 clinical (n=100) and related environmental (n=7) isolates, collected between 2006 and 2022 in the context of the National Legionnaire´s Disease (LD) Surveillance Programme, were determined for azithromycin, clarithromycin, erythromycin, levofloxacin, ciprofloxacin, moxifloxacin, rifampicin, doxycycline, tigecycline, and amoxicillin/clavulanic acid, using gradient strip (E-test) and broth microdilution (BMD) assays, following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Isolates were also PCR-screened for the presence of the lpeAB gene, previously associated with decreased susceptibility to azithromycin and erythromycin.

Results: Nine (8.4%) isolates were lpeAB +ve and had an azithromycin MIC within the BMD distribution of non-susceptible lpeAB-carrying L. pneumophila sg1, but nine (8.4%) without the gene had azithromycin MIC values two- to four-fold higher than the EUCAST breakpoint. MICs for clarithromycin and erythromycin were within the breakpoints. MICs were higher than the EUCAST breakpoints for seven (6.5%) isolates against ciprofloxacin, 17 (15.9%) against levofloxacin, eight (7.5%) against moxifloxacin, 82 (76.6%) against doxycycline, four (3.7%) against tigecycline, and 11 (10.3%) against rifampicin. For amoxicillin/clavulanic acid, for which EUCAST breakpoints are not available, we estimated the ECOFFs on our sample and one (0.9%) isolate had a MIC eight-fold higher than the E-test ECOFF. Additionally, a clinical isolate generated three colonies growing on the E-test inhibition zone that, when re-tested separately, resulted in MICs 4-fold higher (E-test) for this antibiotic than for the parental isolate.

Conclusions: We report, for the first time, elevated MICs against first-line (azithromycin, fluoroquinolones) and other antibiotics (including amoxicillin with clavulanic acid, commonly used to treat pneumonia patients in Portugal) in Portuguese L. pneumophila strains. Results point towards possible decreased susceptibility or resistance in circulating strains, justifying further investigation.

Keywords: Legionnaire´s Disease, Legionella pneumophila, Portugal, antibiotic susceptibility

P3: In-house Legionella PCR as easy as commercial kit

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Legionnaires' disease (LD) is mainly diagnosed by urinary antigen test in Europe. Due to the lack of commercial kits capable of diagnosing Legionella non-pneumophila species and the difficulty of performing daily in-house PCR, LD due to Legionella other than Legionella pneumophila (Lp) serogroup 1 may be underdiagnosed.

During 2022, French National Reference Center (NRC) for Legionella evaluated a new in-house real-time PCR adapted from the Templeton 2003 publication. The PCR was composed of 6 primers and 3 probes to target Legionella non-pneumophila species, Lp and an internal control. Over 300 (328 for Legionella species PCR and 331 for Lp PCR) DNA extracts known to be negative (n = 220 for Legionella species PCR and 248 for Lp PCR), positive with Legionella non-pneumophila DNA or positive with Lp DNA were analyzed and compared to the previous PCR used. Then the PCR was simplified by using commercial strips (Eurogentec) filled with our primers and probes. Only the master mix and the DNA sample should be added. Commercial strips were compared to in-house strips with positive DNA extracts and a standard DNA range.

The overall agreement between the new PCR and the previous one was 90% for Legionella species and 92% for Lp. The discrepancies were mainly due to weak DNA extracts. For the Lp PCR, some discrepancies were observed with DNA extracts known to be positive with Legionella non-pneumophila species (6 with the previous PCR due to L.longbeachae and L.macaerchenii and 1 with the new one due to L.longbeachae). The performances of commercial strips were similar to those of in-house strips with less than 1 Ct (Cycle threshold) between the 2 PCRs carried out on the same extracts.

The new in-house PCR evaluated by the French NRC show good performance and the use of commercial strips make it easier to perform the PCR daily. In 2024, the French NRC also wishes to create with Eurogentec commercial strips for ESGLI PCR.

P4: Antibiotic susceptibility in Portuguese clinical isolates of Legionella pneumophila recovered between 1987 and 2016

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Legionnaires' disease is a severe pneumonia caused by Legionella, for which Legionella pneumophila is responsible for most cases. Currently, there is no gold standard for antimicrobial susceptibility tests for this genus. In this study, we evaluated the MICs, ECOFFS and, the presence of resistance-associated gene lpeAB and mutations in the gyrA gene in clinical isolates from our collection.

The susceptibility patterns were determined in 42 clinical isolates, one for each allelic profile recovered in Portugal between 1987 and 2016, for azithromycin, levofloxacin, and ciprofloxacin. MICs were determined with the gradient test (GT) and microbroth dilution method (MDM), according to EUCAST guidelines. Charcoal interference was analyzed with GT in BCYE-α medium with 80, 60, 40 and 20% activated charcoal. Isolates lpeAB positive were investigated for the presence of plasmid DNA.

MDM presented lower MIC values compared to the GT, particularly for levofloxacin and ciprofloxacin achieving a mean of MIC values 16-fold, ranged from, 4 to 32-fold and 16 to 32-fold, respectively. For azithromycin, the mean was 2-fold, ranged 0 to 32-fold. ECOFFs for the GT and MDM were, respectively, 0,5 and 0,25 mg/L for azithromycin, 0,5 and 0,064 mg/L for levofloxacin, and 1 and 0,064 mg/L for ciprofloxacin. Considering these ECOFFs, azithromycin displayed 7 non-wild type isolates in GT, while for MDM showed 4. Levofloxacin detected one non-wild type isolate in GT and none in MDM. Ciprofloxacin detected the same one non-wild type isolate in both methods. The charcoal interference test showed, for levofloxacin and ciprofloxacin, equivalent MIC values when 40% charcoal was used. Azithromycin showed no real difference between concentrations of charcoal. The lpeAB gene was identified in 24% (10/42) isolates analyzed, where 8 are considered non-wild type for azithromycin, according to the EUCAST guidelines. No plasmid DNA and no mutations in the QRDR of the gyrA gene were found.

These results highlight the importance of standardization of antibiotic susceptibility testing and resistance-associated gene search in Legionella clinical isolates, to define more robust ECOFFs and to identify the occurrence of resistance mechanisms. This data will contribute to establish clinical breakpoints and to standardize a gold method for antibiotic susceptibility testing.

Key words: Legionella; Antimicrobial susceptibility testing; Resistance mechanisms; clinical isolates.

P5: LIAISON® Legionella urinary assay: a novel, high-throughput, fully automated assay based on dual-antigen detection with improved sensitivity

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Legionella bacterium includes more than 50 species and 70 serogroups. Almost half of the species are pathogenic, with Legionella pneumophila serogroup 1 (Lp1) being the most common cause of Legionnaires' disease (LD), an atypical pneumonia with a high mortality rate [1-4]. One of the most important determinants of outcome is a rapid diagnosis to start accurate antibiotic treatment [4, 6, 7]. Although culture of respiratory samples is still considered the gold standard for diagnosis of legionellosis, it is a very demanding test requiring incubation for several days and expertise [4]. Because of the ease of specimen collection, simplicity of analysis, rapid result, and relatively low cost, diagnosis mostly relies on commercial urinary antigen tests (UAT) [8, 9]. UAT is estimated to represent up to more than 90% of diagnostic tools used for LD confirmation in Europe [1, 4]. The currently available rapid UATs are based on detection of LPS (Lipopolysaccharide) antigen of Lp1 in urine [8, 9]. However, Lp1 causes only 79% of culture-confirmed cases in Europe according to the Epidemiological Report for 2017 of the ECDC (European Centre for Disease Prevention and Control) [4, 10]; so theoretically, at least as many as 21% of cases remain undiagnosed if relying solely on UAT. Other serogroups of L. pneumophila and non-pneumophila species are increasingly recognized as causes of severe lower respiratory tract infection [2, 3, 5, 9]. Due to overreliance on UAT testing, a diagnostic gap for LD caused by non-serogroup 1 L. pneumophila and other species has been created [2, 4, 9].

Here, we describe the development of a novel UAT, designed to run on the fully automated chemiluminescent immunoassay (CLIA) analyzer LIAISON®. This assay, based on the innovative "dual-antigen" detection, can detect two Legionella's antigens (LPS and PAL) improving sensitivity and specificity. Like LPS, the PAL (Peptidoglycan-Associated Lipoprotein) antigen is excreted and detectable in infected urines [11], but, differently from LPS, is highly conserved among the Legionella species and serogroups, thus allowing the detection of the non-Lp1 species. Using contrived samples, this assay has been shown to detect all the Lp serogroups as well as the other main pathogenic Legionella species (longbeachae, bozemanii, micdadei). This assay, the first high-throughput CLIA for qualitative detection of Legionella in urine with improved sensitivity, has been recently launched in all countries accepting the CE mark.

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P6: Identification of potential novel antigenic proteins in Legionella pneumophila

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Introduction: Several methods have been developed for the rapid detection of L. pneumophila. The immunochromatographic assay is the easiest, quickest and widely used among them. The aim of the study was to identify potential novel antigenic proteins against L. pneumophila, for diagnostic tests development using patients' sera to confirm their ability or not to act as efficient and sensitive diagnostic agents.

Materials and Methods: 23 sera from hospitalized patients at Heraklion University Hospital during 2018-2019, were tested by IFA for IgM/IgG antibodies against L. pneumophila. Ten more sera samples from blood donors were used, tested negative in a previous study and ten sera from patients infected by other pathogens (Coxiella burnetii, Mycoplasma pneumoniae) were used as controls. L. pneumophila sg1 isolates from laboratory's library were used for antigen production. 46 environmental L. pneumophila sg1 isolates were used, taken from hotels of all four Cretan prefectures during the past ten years (2013-2023). Two L. pneumophila sg1 clinical isolates and the ATCC 33152 L. pneumophila sg1 isolate were included. Bacteria were osmotically shocked, protein concentrations were measured by the BCA assay kit and proteins recovered were submitted to ELISA methodology, 2D electrophoresis (2D IEF/SDS PAGE), immunoblotting, nLC-ESI-MS/MS identification, cloning into plasmid vectors (pET vectors) and purified using affinity and size exclusion chromatography. Statistical analysis was performed by IBM SPSS Statistics v.25.

Results: Four recovered proteins, elongation factor G, elongation factor T2, dihydrolipoamide succinyl-transferase and 27kDa outer membrane protein were identified as proteins with potential antigenic activity against L. pneumophila. Discussion: It is the first time that such a study is conducted in Greece. The identification of novel antigenic proteins in patients with Legionnaires 'disease (LD), could support an update on the existing antigenic tests and/or development of novel kits with increased sensitivity and specificity, conferring to an early and more accurate diagnosis of LD cases. Further studies are needed to evaluate the implementation and usefulness of these findings and its role in LD diagnosis.

keywords: Legionella pneumophila, antigenic proteins, diagnostic tests

2. Epidemiology & Outbreak Investigation

P7: Environmental Monitoring of Legionella spp. in a Regional Hospital of Crete (Greece) during the Covid-19 Pandemic

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Introduction: On May 2020, the Hellenic Ministry of Health issued a circular on the prevention of Legionnaires' disease in healthcare facility water systems in the context of the Sars-CoV-2 pandemic. We surveyed the compliance with these guidelines by recording the water network colonization with Legionella spp. of a Cretan regional hospital, during 2021-2023.

Materials and Methods: 137 water samples were collected during 2021-2023. Temperature, disinfectant, pH, and other parameters were recorded according to a specific checklist designed for the hospital. All samples were tested following the ISO 11731:2017. The detection limit was set at 50 cfu/L. Isolates were typed by MALDI-TOF MS and serotyped using individual anti-sera for each serotype.
Results: All 9 samples collected before COVID-19 pandemic were positive for L. pneumophila sg 1 (2,250-6,250 cfu/L). The samples' positivity was 73.68% in 2021 (14/19 positive samples; 350 cfu/L to 14.000 cfu/L), 12.72% in 2022 (7/55; 50-2400 cfu/L) and 12.69% in 2023 (8/63; 50-5600 cfu/L). Legionella pneumophila sg1 was detected in 58/137 samples, L.pn.sg2 in 1/137, L.pn.sg3 in 5/137, L.pn.sg7 in 6/137 and L.pn.sg8 in 4/137 samples. The hot water temperature at the boiler's exit, was not compliant to the national guidelines in 93 out of 112 measurements, the hot water temperature in the drinking water system was not in 101/111 measurements, the residual chlorine was found >0.2 mg/L in 6/121 measurements and >0.2 mg/L in 12/121 measurements regarding the most far sampling points to the water system entrance of the buildings. A relative risk (RR) of 1,44 was calculated for the out-ranged residual chlorine with an odds ratio of 3,02 and a p-value of 0,03. A RR of 3 was calculated for the out-ranged hot-water temperatures with a p-value of 0,003.

Conclusions: The additional measures taken due to the pandemic, the monitoring of hot water temperatures, and the use of chlorine dioxide as a disinfectant, played a significant role on enhancing the protection from Legionella colonization of the potable water system in this Cretan region. Risk assessments implementation, systematical control efforts and compliance with all preventive procedures, are needed to minimize and keep the risk to acceptable levels.

Keywords: hospitals, legionella spp., covid-19

P8: Increase in Legionnaires Disease among elderly Danish population, 2002-2023

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Introduction: With an overall increasing life expectancy in developed countries, the incidence and demographic trends for certain diseases might be changing . Legionnaires Disease (LD) is associated with unclear clinical features and severe disease outcomes, especially among elderly and/or already impaired persons . Over the past two decades Denmark has registered an increase in the elderly population over 65, and an increase in LD. Here we describe trends in Danish clinically notified LD with probable or confirmed diagnoses, by age, sex and country of infection, over two decades.

Materials/methods: We used national clinical disease surveillance data from 2002-2022 to calculate and describe number of notified LD with a probable or confirmed Legionella pneumophila diagnosis by year, age group, sex and domestic- versus travel- acquired infections.

Results: During the last 20 years, the total number of clinically notified cases with probable or confirmed LD increased from approximately 100 to approximately 300 annually. We saw varying gender distributions from 1:3 to 1:1 depending on age groups and year. Incidence rose primarily in age groups 75-79 and 80+ with a resent predominance among 80+ year old women. Further, when comparing country of infection, we saw an increase in domestic infections, particularly among older age groups 70+, with 779 of 1345 domestic infections during the last six years.

Conclusions: With regards to aging LD populations preparedness and prevention has become even more important in order to minimize rising incidence, fatal outcomes and/or prolonged severe hospitalizations. Implementation of drinking water directives together with citizen information might help to reduce the overall rising incidence, including among the more vulnerable elderly age groups . Additionally, awareness of LD especially in elderly age groups, can help point clinicians in the right direction with regards to early detection and correct medical care.

Keywords: Legionnaires disease, elderly burden, domestic infections, prevention

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26 Apr 2022https://doi.org/10.2144/btn-2022-0047. https://www.future-science.com/doi/10.2144/btn-2022-0047

P9: Trends in legionella-related mortality in Denmark, 2002-2022

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Introduction: Legionnaires Disease (LD) is caused by the bacteria Legionella pneumophila and is transmitted to humans via contaminated water droplets that are inhaled (1). LD can cause severe pneumonia and death, and the elderly and immunosuppressed are particularly vulnerable to infection and severe disease (2). Across Europe, an increase in legionella-related mortality has been observed over the past decade (2), a trend also found in Denmark.

Materials/Methods: Routine national surveillance data comprising of clinical disease notifications for legionella in Denmark as well as mortality data from The Danish Civil Registration System were analyzed using R Studio (3).

Results: Out of 3552 patients clinically notified to Statens Serum Institut who diagnosed with probable or confirmed LD between 2002 and 2022 in Denmark, there were 351 deaths within 30 days of date of symptom debut or date of first positive test, whichever came first. Among the 351 deaths, 81% (286) were Danish, 56% (195) were 75 years of age or older, and 62% (219) were men. Deaths per year ranged from 8 in 2008 to 45 in 2022 with a steady increase in mortality over the study period. 93% (327) of all cases reported country of infection as Denmark. 42% (149) of deaths were among individuals who were not employed or connected to an institution and 9% (30) were residents of a nursing home. The majority of deaths were community acquired LD of unknown origin (38%, 133), 67 (19%) were hospital acquired, 40 (11%) were acquired from other public institutions, and 18 (5%) were travel-related infections.

Conclusion: 30-day mortality among clinically notified LD has been steadily increasing over the past two decades in Denmark, despite travel-related lockdowns due to the Covid-19 pandemic during 2020-2021. This trend of increased domestically-acquired infections, in tandem with an aging population, are of particular public health concern.

Keywords (3-4): Legionella, Mortality, Surveillance

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P10: Professional Legionellosis of ice resurfacer driver on the ice hockey stadium. Is skating health risk?

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Objectives: Source tracking has proved a professional LD case in a man, who operated an ice resurfacer at the ice hockey stadium (Prague). Virulent legionella was found in high numbers in clinical isolates, water supply, ice making water tank and in ice samples. Possible risk for the employees and visitors prompted us to focus our attention on other stadiums. In the study samples were taken at 11 stadiums and analysed. The types of ice resurfacers used and the handling scraped ice and ice making water were also monitored.

Methods: Samples were examined by two laboratories. Accredited procedures were utilised for identification of colonies, MIP gene sequencing, Mab subtyping and sequence-based typing (SBT).

Results:
Legionellas were found in 83% of all water supplies and 75% ice making water tanks respectively (both 10e1 – 10e5 CFU/ 100 ml). High numbers of viable legionellas were present in ice scraped from ice rink, or in snow pit (10e1 – 10e5 CFU/ 100 ml). All pozitive findings contained L. pneumophila (sg. 1,3,5,6,12). L. sp. (L. spiritensis a L. quateriensis) were present in 6/11 monitored stadiums. In two cases were proven highly virulent Legionella pneumophila sg.1 ST 664 Benidorm, in one case L.p.sg.1 ST novel 2924 Philadelphia.
Only one stadium had all collected samples negative.
Risk of legionellosis is related to aerosols production during use of ice resurfacer (12 machines and 5 basic types were examined) and increases significantly with the use of recirculated hot water (scraped ice – snow pit – filtration and heating – repeated use). The cleaning and disinfection of the tanks is not taken into account(!)

Conclusion: The study has confirmed that ice treatment with an ice resurfacer could be risky in presence of virulent legionella. The risk is also increased by using recirculated water. Disinfection/sanitization of ice making water tanks and storage tanks is not systematically addressed.
Health risk appears low for common visitors of ice hockey stadium.

P11: Sources of infection and settings in outbreaks of legionellosis --- Japan, 2018-2022

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Background: In Japan, legionellosis is one of infectious diseases which requires mandatory notification to the National Epidemiological Surveillance of Infectious Diseases under the Disease Control Law since April 1999. The number of reported cases of legionellosis stayed high during 2018-2022, with the rates of reported cases of about 1.7 per 100,000 persons.

Materials/Methods: Data from the National Epidemiological Surveillance of Infectious Diseases were used. Academic papers, reports and news on outbreaks of legionellosis in Japan were collected and analysed.

Results: Eight outbreaks, at least, were found during 2018-2022. In total, 34 outbreak-related cases were reported. The median number of cases was 3 (range = 2-13) and median outbreak case fatality rate was 0% (range = 0%-50%). Community-acquired infection (CAI) and healthcare-associated infection (HAI) were 7(88%) and 1(13%), respectively. Sources of infection were baths (5, 63%), humidifiers (2, 25%), and a car wash installation (1, 13%). Causative agents were L. pneumophila serogroup 1 in all the outbreaks.

Conclusions: The distribution of sources of infection in legionellosis outbreaks is different from those of Europe and USA, in which major sources of infection were hot and cold water supply systems. This difference in distribution may depend on the differences in lifestyle habits of people and, possibly, in the genotype distribution of Legionella pneumophila between Japan and Western countries.

Acknowledgement: This work was supported by Health and Labour Sciences Research Grant (JPMH22LA1008 to S.I.) from the Ministry of Health, Labour and Welfare, Japan.

Keywords. Outbreaks, Legionellosis, Sources of infection, Baths

P12: SwissLEGIO - Legionnaires' disease in Switzerland: A national case-control and molecular source attribution study

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Introduction: Switzerland has one of the highest annual Legionnaires' disease (LD) notification rates in Europe (7.6 cases/ 100,000 population in 2022). The main sources of infection and the cause for this high rate remain largely unknown. This hampers the implementation of targeted Legionella spp. control efforts. The SwissLEGIO national case-control and molecular source attribution study investigates risk factors and infection sources for community-acquired LD in Switzerland. The study is conducted in an inter- and transdisciplinary approach involving various national governmental and national research stakeholders.

Methods: Over one year, the study is recruiting 205 newly diagnosed LD patients through a network of 20 secondary- and tertiary hospitals across Switzerland. Healthy controls matched for age, sex, and residence at the district level are recruited from the general population. Risk factors for LD are assessed in questionnaire-based interviews. Clinical and environmental Legionella spp. isolates are compared using whole genome sequencing (WGS). Direct comparison of sero- and sequence types (ST), core genome multilocus sequencing types (cgMLST), and single nucleotide polymorphisms (SNPs) between clinical and environmental isolates are used to investigate infection sources and the prevalence and virulence of different Legionella spp. strains detected across Switzerland.

Results: Data collection for the study started in autumn 2022 and is expected to be completed in November 2023. To date, as of July 2023, we have enrolled 125 patients, collected 255 standardised household samples from case- and control households, and 60 samples from other potential infection sources of cases. A first batch of 21 clinical and 68 environmental isolates are further sequenced and analysed. The sequencing of this first batch revealed matches of clinical and environmental isolates from the Southern part of Switzerland.

Conclusion: With the proposed poster, we present the SwissLEGIO study design and some first preliminary results. We will describe the patient population enrolled in the study and show first results from the sequencing of clinical and environmental Legionella spp. isolates.

Keywords: case-control study, whole genome sequencing, Switzerland

3. Legionella Genomics

P13: Assessment and Implementation of a standardized Whole Genome Sequencing scheme for rapid resolution of Legionella pneumophila outbreaks within Canada

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Introduction: The National Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC) is the reference centre for Legionella pneumophila (Lp) and is the centre of expertise in Canada for Sequence Based Typing (SBT) to support conclusive Sequence Type (ST) identification and outbreak investigations. The standard SBT scheme has proven effective in typing Lp in Canada however, with the increasing availability and affordability of whole-genome sequencing (WGS), it has become apparent SBT alone does not provide sufficient resolution to discern outbreak and non-outbreak isolates or to attribute source. With WGS increasingly being employed by public health laboratories in Canada, a nationally standardized approach for WGS-based Lp typing is required. Herein outlines the approach to developing and implementing a nationally standardized WGS-typing method in partnership with provincial health collaborators to improve Lp cluster detection and provide higher resolution to epidemiological investigations in Canada.

Methods: We have selected 250 isolates based on ST to represent unique isolates and known clusters of both clinical and environmental origin, in order to have representation of population diversity across clinical and environmental settings within Canada. All isolates will be sequenced via an Illumina platform and a subset sequenced with Oxford Nanopore Technologies for assessment of long-read data utility. Computational tools and pipelines that employ Single nucleotide polymorphism (SNP)- and core genome Multi-locus Sequence Typing (cgMLST)-based approaches will be evaluated for speed, discriminatory power, agreement with epidemiological based clustering, ease of implementation and use, and phylogenomic visualizations. As well, in silico typing platforms will be evaluated for incorporation to the standardized workflow to maintain backwards compatibility with established international ST nomenclature. A workshop to overview methodologies with corresponding test and blinded panels will be distributed to end-users for implementation and end-to-end evaluation of the workflow.

Results: The outcomes of this work will be: the generation of a WGS dataset from the Canadian Lp repository of isolates; development and assessment of computational pipelines for the rapid discrimination of STs and clusters across the Canadian Lp dataset; assessment of nanopore technology for generation of rapid sequence data and utility of hybrid or independent long read assemblies for cluster detection; technology and knowledge transfer to relevant stakeholders, and multicentre proficiency panel evaluation for end users.
Conclusions: The nationally standardized workflow will improve Lp cluster detection and provide higher resolution epidemiological investigations within a timeframe that allows for rapid public health response within Canada.

Key words: Genomics, Outbreak, Clustering, Standardized

P14: Historical Genomic Epidemiological Analysis of Legionnaires' disease in Scotland Uncovers Long-term Endemic Clones of Public Health Importance

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Introduction: Legionella pneumophila is an opportunistic human pathogen and the main cause of Legionnaires' disease (LD) worldwide. Whole Genome Sequencing (WGS) can help trace environmental origins of L. pneumophila infections and identify epidemiological links between cases.

Methods: The relationship within and between community, hospital and travel associated clinical and environmental L. pneumophila isolates in Scotland (1984-2020) was analysed along with all available global data. A stratified clustering approach was used to capture short-term epidemiological connections in context and the long-term evolution of causative strains.

Results: Our analysis revealed the existence of previously undetected endemic clones of L. pneumophila that have existed for many years in hospital, community, and travel-associated environments. Our analysis supports six previously recognised epidemiological links related to travel and identified additional, previously undiscovered travel links between closely related isolates connected to the same geographical regions. Phylogenetic analysis revealed cryptic geographical clusters of TALD isolates and other clinical and environmental isolates from travel destination regions. Multiple Scottish hospitals were colonized with long-term, persistent clones of L. pneumophila. A single, widely disseminated endemic clone was responsible for numerous infections in Scotland over many years.

Conclusion: Based on these data, we propose the importance of WGS-based surveillance as a crucial public health tool for real time identification and mitigation of endemic clones that represent a threat to public health.

Keywords – L.pneumophila, WGS, epidemiology

P15: ONTmompS - a tool for in silico sequence-based typing (SBT) of Legionella pneumophila completed genomes

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Sequence-based typing (SBT) of Legionella pneumophila is valuable for studying epidemiology and outbreaks of Legionnaires' disease. Although SBT performed by Sanger-sequencing has long been considered the gold standard, whole-genome sequencing is emerging as the method of choice as it allows for in silico SBT as well as comparative genomic analysis of isolates. Because there are two copies of the mompS gene in the L. pneumophila genome, of which only one (mompS2) is used for SBT, short-read sequencing is sometimes unable to resolve the correct sequence type (ST), as the reads are too short to distinguish between the two genes. We recently showed that long-read sequencing using Oxford Nanopore Technology resolves this severe issue and allows for reliable in silico SBT (Krøvel, AV., et al (2023); PMID37256104). We also developed ONTmompS, an in silico approach for assigning L. pneumophila STs, which works on completed genomes created with either long-read or hybrid assemblies. Here, we report on the reliability of the tool in both an in-house dataset and on publicly available genomes.

ONTmompS v2.0.0 uses BLASTn and Smith-Waterman sequence alignments to distinguish the two mompS genes, by identifying the mompS copy that aligns to the 1116R sequence, the mompS2-specific primer used in Sanger Sequencing. It then determines the alleles and subsequent ST using the same logic as that in Kleborate v2.3.1 (Lam, MMC., et al. (2021); PMID34234121).

We used ONTmompS to identify the STs of 35 in-house L. pneumophila long-read sequenced isolates, which we also had Sanger-sequencing results available for. The long-reads were assembled with Trycycler v0.5.3 and polished with Medaka v1.7.2. We also ran ONTmompS on all available closed RefSeq L. pneumophila genomes (n=118).
ONTmompS resolved the mompS alleles and the ST in accordance with Sanger-sequencing for all 35 isolates in our in-house dataset. Of the 118 RefSeq sequences, 93% (n=110) were assigned an ST and both mompS genes were identified. The remaining 8 had either one mompS gene (n=7) or three (n=1) and were deposited before 2017 with no sequencing/assembly information provided.

Here we showed that ONTmompS can reliably identify L. pneumophila ST from completed genomes. The tool is available at https://github.com/marithetland/ONTmompS.

Key words: SBT, mompS, long-reads

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4. Legionella Prevention and Control

P16: Digital PCR versus Real Time PCR: looking for a more sensitive method for Legionnaires' disease diagnosis

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The constantly increasing number of Legionnaires' disease cases requires diagnostic methods that are as rapid and accurate as possible. Real Time PCR has been shown to be able to detect all species identified so far and results are obtained very quickly. As Legionella pneumophila serogroup 1 (Lp1) is responsible for the majority of the infections, a multiplex Taqman Real Time PCR assay able to simultaneously detect wzm gene targeting Lp1 and mip gene targeting Lp non-serogroup 1 has been developed and evaluated, proving to be 100% specific for both targets.

Digital PCR (dPCR) has recently been demonstrated to improve both end-point PCR and Real Time PCR with large-scale technological modification. Since the dPCR sensitivity depends only on the number of amplifiable molecules for the intended target and is independent of PCR inhibitory factors, the dPCR test is an excellent diagnostic tool in clinical microbiology, especially when the target concentration is very low. Another important advantage of dPCR is the absolute DNA quantification that allows to count only the truly positive molecules, without the need of a standard curve.

In this pilot study we analyzed 34 DNAs extracted from respiratory samples by the above-mentioned multiplex Taqman Real Time PCR assay and the same primers and probes were used for a dPCR assay on QuantStudio Absolute Q Digital PCR System (Thermo fisher). The obtained results were compared.

For the wzm gene target, good concordance (K Cohen= 0,65) was found between dPCR and Real Time PCR data. One sample was negative only by dPCR while 11 were negative by Real Time PCR and positive by dPCR (p=0.012).

Considering the mip gene target, an excellent concordance (K Cohen=0,91) was calculated, being 30 out of the 34 samples positive for both methods (p=0.65).

These preliminary results suggest that primers and probes targeting the wzm and mip genes can also be successfully used by dPCR. Furthermore, dPCR seems more reliable in detecting the Legionella gene wzm and mip targets.

P17: Activity of Algerian essential oils againt Legionella species

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Legionella is a pathogenic bacterium growing in high temperature. Thermal spas must be regularly controlled. Physical and chemical methods used to reduce the risk of Legionella have been described, however they cause harmful effects for the environment. Biological method using essential oils could be an alternative to reduce the risk of Legionella growth and spread.

Essential oils of Mentha pulegium and Rosmarinus officinalis were extracted by water distillation and their chemical constituents were quantified by gas chromatography-mass spectroscopy (GC-MS). Legionella pneumophila, Legionella longbeachae and Legionella gormanii isolated from thermal springs were used as model to test antimicrobial activity of essential oils.

The antibacterial activity of essential oils against Legionella was demonstrated by agar diffusion and microdilution methods. The results showed that the essential oils of Mentha pulegium and Rosmarinus officinalis have a high anti- Legionella activity against all strains of Legionella isolates tested.

Essential oils can be used as biocides to prevent the Legionella risk in hot springs.

Key words: Legionella, essentials oils, antimicrobial activity

P18: Comparison of Legiolert and traditional plate culture, according to Italian guidelines, from hospitals water samples

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Introduction: Monitoring of Legionella pneumophila (Lp) populations in environmental water is very important for public health. In Italy, the plate culture method is considered the gold standard for detection and enumeration of Legionella in water samples. However, is highly complicated and requires large volume of sample, Legiolert is a new culture method to enumerate L. pneumophila and is based on quantification by MPN. The enterion of this method is based on a bacterial enzyme detection technology that light on the presence of L. pneumophila using a substrate present in the Legiolert reagent.

Matherials and method: 80 potable water samples were collected from hospitals located in Palermo (Sicily - Italy), they were collected according to the protocol contained in the Italian guidelines for Legionella, 1L of each sample was analyzed by the culture method, only 100 mL of potable water using the Legiolert method. After filtration, bacteria collected on the membranes were resuspended in 10 ml of the original water sample and 0,1 mL was spread on BCYE agar. Finally, a Legiolert blister reagent was added to the 100 mL of sample and poured into a Quanti-tray/Legiolert tray and incubated at 37°C.

Results: Overall, 80 potable water samples were analyzed, 35 of them were positives by plate culture. Among these, all except one sample were positives for L. pneumophila, 45 were negative. In three case, the isolate were recovered by the BCYE method while the Legiolert result was negatives. 6 samples were negatives by BCYE but positives by Legiolert. The mean concentration of L. pneumophila as detected by Legiolert was similar to the mean concentration determined by plate culture.

Discussion and conclusion: The Legiolert method was highly specific and easy to use, representing a significant advancement in the quantification of L. pneumophila from potable water. Legiolert was more likely than BCYE to detect L. pneumophila when it was present in low numbers. The present study demonstrated that both Legiolert and plate culture present comparable results. These advantages include greater sensitivity with reduced interference from other species, an unequivocal positive signal that is easy to read and quantify.

P19: Improved Prevention and Control of Legionella Pneumophila Using a Rapid On-Site Testing Method

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Introduction: Legionella pneumophila serogroup 1 (LpSG1), is the cause of most fatal outbreaks of Legionnaires' disease recorded globally. Outbreaks from other serogroups (LpSG2-15) are known to occur and in some geographies LpSG2-15 bacteria can be prevalent. Monitoring for Legionella presence in different water samples is widely performed by laboratory culture testing methods. This method lacks accuracy and speed to properly manage disease risk. Factors such as transportation time, lack of biocide neutralization, exposure to variable temperature and presence of background bacteria can affect the performance of lab culture results. The development of a test that can rapidly detect all serogroups of Legionella pneumophila on-site would therefore be of value for reducing global Legionella risk. This presentation discusses the advancements made by the Hydrosense lab team in developing a rapid on-site testing method for Legionella pneumophila serogroups 1-15 (LpSG1-15) in water.

Materials/ Methods: Antibody Generation, Purification and Testing

Legionella serogroups 2-15 were grown from NCTC and ATCC strains, resuspended in solution and heat killed. A mixture of the LpSG2-15 was used as an immunogen to immunise the host animal. Bleeds were assessed for reactivity by direct ELISA to each Legionella serogroup (1-15), Legionella species and a mixture of common water bacteria. Protein G and Affinity purification was used to isolate antibodies.

Lateral Flow Development

Anti-LpSG1 and anti-LpSG2-15 polyclonal antibodies were conjugated to different colour and type of nanoparticles (red and green respectively). Matched partner antibodies for the anti-LpSG1 and anti-LpSG2-15, was dispensed on to nitrocellulose membrane. Test cards were laminated with sample and absorbent pads, cut into strips and placed in the lateral flow plastic housings.

Results: The test can achieve a high sensitivity level when combined with a filtration/ concentration step. Both the LpSG1 test line and the LpSG2-15 test line have a limit of detection of 100 cfu/ L. Validation of the HydrosensePRO® was performed after development/ optimisation to ensure it would perform as expected in the field.

Conclusions: Hydrosense have developed a lateral flow test which detects all Legionella pneumophila serogroups 1-15. Its use as an onsite testing method alongside lab culture and/or other methods could eliminate the risk of inaccurate culture results arising from transportation, temperature, time and biocide action.

Keywords: Legionella Testing, Public Health, Rapid Testing, Water Management.

P20: Pre-post study on the microbiome profile of a hospital water network treated with hydrogen peroxide for Legionella contamination control

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Introduction: On-site physical and chemical treatments for secondary disinfection can be adopted for controlling opportunistic pathogens such as Legionella in hospital pipelines in order to reduce infection risk for immunocompromised patients. Each method has benefits and disadvantages, differing in effectiveness, mode of application (in continuous and shock), cost and management. Disinfection can modify the composition of the whole bacterial community of a water network in terms of abundance and diversity. Nowadays, Bioinformatics and Next Generation Sequencing technologies offer high-throughput, rapid and accurate methods for detecting all cultivable and non-cultivable bacteria in an ecological niche.
Our study aims to characterise the bacterial community pre-post installation of a device that continuously distributes hydrogen peroxide in the hot water network of an Italian hospital for controlling Legionella contamination.

Materials/Methods: The main hospital building includes 10 blocks fed by the same water plant. Municipal cold water, cold and hot hospital water before treatment and after 1, 6 and 12 months are analysed for microbiome characterization by 16S amplicon sequencing. Additionally, Legionella is quantified with culture method ISO 11731:2017. Here, we present the pre-treatment and post-one month results.

Results: A total of 32 samples was collected: 14 cold water and 18 hot water samples (9 before treatment and 9 after 1 month). The cold water bacterial profiles at genus level did not vary significantly between the two sampling sessions. On the contrary, the hot water profiles differed between pre-treatment and post-one-month treatment samples. Before treatment the two most abundant genera were Sphingorhabdus and Thermus, while after one month were Porphyrobacter and Blastomonas. To date, the analysis of post-6 months' samples is ongoing.

Conclusions: Our findings highlight the presence of bacteria more resistant to disinfection such as Porphyrobacter and Blastomonas after one-month continuous hydrogen peroxide treatment. Determining the microbial profiles of hospital water networks by metagenomics is an innovative approach for Public Health. Important strength of our study is the pre-post treatment approach to investigate under real-life conditions the possible modifications of bacterial community associated with continuous hydrogen peroxide disinfection. Moreover, over time monitoring allows to evaluate microbiome stability in the treated water.

P21: Identification of Legionella contamination in the water installation of a grammar school in Germany

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Introduction: According to the German Drinking Water ordinance, public buildings have to be tested for Legionella spp. at least once a year. When Legionella concentration exceeds 99 CFU / 100 ml in one of the samples analysed, a Risk Assessment has to be completed immediately, followed by extended resampling and implementation of remedial measures. During the energy crisis of the last two years, many building managers have applied energy saving measures and reduced hot water temperatures, thus creating a risk for microbial contamination in their water systems.

Materials & methods: Hygiene inspection of a grammar school including extensive sampling of cold and hot water for Legionella spp. according to the German Code of Practice, assessment of compliance with the national and European Code of Practice as well as the WHO guidance on Water Safety in Buildings have been carried out to confirm the elimination of Legionella contamination of the grammar school.

Results: Switching off the gas heating system for energy saving purposes in combination with a defect in the solar energy system has led to a decrease of hot water temperature in the grammar school, resulting in a significant Legionella contamination. The building owner had identified both defects and completed the appropriate corrective measures, thus re-establishing compliant hot water temperatures. The hygiene inspection and water sampling completed by a DIN EN ISO 17025 accredited laboratory confirmed compliance with the Code of Practice and absence of Legionella spp. after completion of corrective measures allowing compliant hot water temperatures.

Conclusions: The present case shows that compliant water temperatures are a simple way to suppress Legionella contamination in a grammar school water system. Compliance with the Code of Practice including correct hydraulic balance, correct hot and cold water temperatures as well as normal operation are the main prerequisites to minimise Legionella spp. contamination risk and to avoid possible infections. Recognising technical defects of a water system requires high level of technical knowledge, which is officially confirmed for the DIN EN ISO/IEC 17020 Type A accredited Hygiene Inspection Body since 2004.

Keywords: Legionella spp., energy saving, hot water temperature, Code of Practice

P22: Identification of Legionella contamination in decentralised water systems of a German kindergarten

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Introduction: The German Drinking Water ordinance requires regular sampling of public and commercially used buildings for Legionella spp. One way to avoid mandatory sampling is the installation of local heating devices. In the present case we show that local heating devices can be contaminated with Legionella spp. and can therefore pose an infection risk to water installation users.

Materials & methods: Hygiene inspection of the kindergarten including extensive sampling of cold and hot water for Legionella spp. according to the German Code of Practice, assessment of compliance with the national and European Code of Practice as well as the WHO guidance on Water Safety in Buildings to identify the contamination sources and make suggestions of their elimination.

Results: Inspection of the kindergarten's water system revealed incomplete technical documentation, cold water temperatures > 25 °C and too low hot water temperatures at the local heating systems for hot water provision, leading to high contamination of both hot and cold water system.

Conclusions: Local heating devices with a content of up to 3 L can be highly contaminated with Legionella spp. and pose a risk to the user. Hot water pipes of up to 3 L of volume should be considered as a possible contamination source as well. A disadvantage of local heating devices compared to water installations with boilers is that in such installations the existing temperature gradient changes continuously, thus creating better conditions for Legionella growth. This practical example shows that Legionella spp. can grow in cold water as well, when temperature exceeds 25 °C. Microorganisms grow in any environment under appropriate conditions, independently of whether it is a hot or cold-water system.

Keywords: Legionella spp., local heating devices, cold water contamination

P23: Interest of a continuous improvement approach for Legionellosis prevention

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Keywords: continuous improvement approach, prevention

Introduction: French legislation regarding legionellosis prevention in social and medical establishments requires annual analysis campaigns since 2010. Establishments must organize corrective or curative actions if they exceed regulatory limits. The number of curative actions has therefore significantly increased since 2010. This observation brought one of the main French groups in the medical and social-medical fields to establish, since 2017, action flowcharts defined as depending on the results of the analysis, and an improvement policy based on the reinforcement of preventive measures and improving constructions. The aim of the study is to assess the statistical evolution of the actions undertook for this purpose.

Materials: The actions' types' statistics performed according to the defined flowcharts were used between 2017 and 2023 to assess the impact of the continuous improving procedure depending on the different institutions concerned: care homes, psychiatric and rehabilitation clinics. The number of analysis campaigns led varies between 421 to 816 depending on the corresponding period.

The types of presented actions are the following:

- Protocol 1: control of hot water temperatures and maintenance operations, study of the factors.
- Protocol 2: in case of a contamination of the hot water tanks: control of the equipment maintenance and the collecting conditions.
- Disinfection of water networks.
- Other interventions : diagnosis, technical assistance to perform the works.

Methods: Dispatching of actions led after each analysis campaign is recorded the same way as the previous year in the form of a percentage. The results are compared between years: number of disinfections, number of campaigns requiring an intervention, number of protocols 1 and 2, and other types of intervention.

Results:

One can notice a decrease in the percentage of campaigns requiring an intervention, in particular a continuous decrease of urgent curative disinfections during the given period, and a decrease in percentage of protocols of actions transferred after each campaign for all types of establisments.

Conclusion: The results show the necessity of the continuous improvement protocol requested by the sanitary authorities in order to reduce the networks contaminations and the number of urgent curative actions.

P24: Impact of temperature and pipe material on Legionella sp.

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Question: The presence of pathogenic bacteria in potable drinking water pipelines, like Legionella pneumophila, can pose significant health risks. Legionellae can be aerosolized by e.g. showers, cooling towers and dental chairs. Once inhaled they can cause severe pneumonia. To prevent growth of legionellae it is recommended to maintain the temperature of warm water (PWH) plumbing systems above 55°C.

We investigated how different PWH-temperatures in the circulation in combination with different pipe materials (PE, SST [stainless steel], CU) affect the amount of legionellae in a test rig.

Material/Methods: We constructed a test rig consisting of a circulation ring line and distal pipes, representing the outlets for end users. The module was filled with potable water and flushed automatically. To mimic highest risks for hypothetical users, low water use was simulated. The experiment consisted of 4 temperature periods (45,50,55,60°C), lasting 28 days each. Samples were taken every 7th day.

The presence of legionellae was culturally determined according to DIN EN ISO 11731:2019-03 and investigated via a life-dead qPCR considering only intact legionellae for the interpretation.

Results: Water temperatures >45°C reduced cultivatable legionellae within the circulation, PE- and CU-pipes, while they remained stable within the SST-pipe. Water temperatures of 55°C and 60°C further reduced the CFUs (colony forming units) within the circulation, until legionellae were no more provable. Within the distal pipes, the CFUs fluctuated around the technical action value (100 CFU/100 ml).

Via qPCR more intact Legionella sp. than L. pneumophila were detected within all pipes. The GUs (genomic units) of Legionella sp. were alike for the distal pipes during all tested temperatures. Within the circulation, the GUs of Legionella sp. decreased between 45°C - 55°C. The intact L. pneumophila decreased within the circulation, CU- and PE-pipes with increasing temperatures. In contrast, they increased between 50°C-60°C within the SST-pipe.

Discussion: Water temperatures of ≥ 50°C significantly reduced legionellae within the circulation. The raised temperatures were less effective in the distal pipes, where the system did not always reach the desired temperature. At 60°C the amount of legionellae was reduced by 1 log10 unit, except for the SST-pipe.

The trends were alike for PE- and CU-pipes. Lowest temperature effects were observed for the SST-pipes.

P25: Can ozonation be a better treatment than chlorination to control Legionella in man-made water systems?

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Background: Waterborne pathogens are a global concern for public health worldwide. Legionella pneumophila colonizes man-made water systems and can infect humans causing Legionnaire's disease (LD). LD prevention is a public health issue and requires specific systems to detect and control Legionella. Chlorination is the most commonly used treatment but it has demonstrated some drawbacks due to its lack of stability in hot water systems and its generation of carcinogenic byproducts.

Objective: To compare the effectiveness of ozone disinfection system and chlorination (gold standard method) against planktonic Legionella (culturable and viable but non-cuclturable, VBNC), Legionella biofilm formation and amoeba.

Methods: The biocide effect of chlorine and ozone on planktonic L. pneumophila was quantified using bacterial suspension of 104 cfu/ml (107 cfu/L), in water at 37 °C 150 rpm in different incubation times. The effect was quantified by culture plate (culturability) and by viable qPCR (viability). The effect against biofilm was quantified by diacetate fluorescein while the effect against amoeba viability was quantified by flow cytometry.

Results: Ozone was able to eliminate the viable and cultivable L. pneumophila in 5 min while the chlorine treatment only reduced the 18%. Furthermore, we demonstrated that chlorine treatments at 0.6 – 0.8 ppm (standard treatment) induce the viable but not culturable state in this bacterium, state not detectable by culture plate but with infection capability. On the contrary, ozone was able to reduce the concentration of VBNC L. pneumophila under the qPCR detection limit after 5 min of exposure.

Neither chlorine nor ozone showed effect against Legionella pneumophila biofilm or Acanthamoeba castellanii, main environmental host of Legionella.

Ozonation cannot eradicate Legionella pneumophila colonization as it is not effective against biofilm and amoeba. Nevertheless, this treatment has shown better results in vitro to control planktonic Legionella pneumophila. Only planktonic Legionella pneumophila produces bacterial containing aerosols in man-made water facilities being the way to be infected. Therefore, the complete eradication of biofilms and amoeba is not as crucial as the removal of planktonic bacteria.

P26: Antibacterial Surface Coatings containing Cu-doped Mesoporous Silica Nanoparticles

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Introduction: Bacterial colonisation of large-scale water distribution systems, as found in hospitals, is associated with the spread of disease.[1] Once bacteria colonise these systems, they form biofilms which can be difficult to eradicate through commonly used disinfection protocols, such as chlorination.[1] Pathogenic and opportunistic bacteria that reside in these biofilms can be deleterious to human health. Therefore, prevention of microbial colonisation and subsequent biofilm formation is vital.

Aims and objectives: In this study, we report on the use of copper-doped mesoporous silica (CuMSN) paints as a viable solution for infection control in water distribution systems.

Methods, Results, and Conclusions: CuMSNs were prepared according to a modified-Stöber method at varying concentrations of copper, from 5 – 20 mol%.[2] Particles were analysed using transmission electron microscopy (TEM), scanning electron microscopy (SEM), DLS, SEM-EDX and BET analysis. Produced CuMSNs were in the size range 130 – 300 nm. 10 mol% CuMSNs were deposited into three types of paint; a polyurethane based paint, a self-polishing paint, and a fouling-release paint. Paint formulations were deposited by spin coating onto glass-reinforced plastic constructs. The constructs were analysed for their homogeneity by FTIR, SEM, SEM-EDX, water contact angle, and surface profiling. Results of antibacterial activity of painted constructs against gram positive (*Enterococcus faecalis*) and gram negative (*Pseudomonas aeruginosa* and *Legionella pneumophila*) bacteria showed broad spectrum efficacy at 4- and 24-hour time points in nutrient-rich and nutrient-poor media for self-polishing and fouling-release coated constructs. CuMSN self-polishing paints were also effective at preventing biofilm adhesion.

As such, there is scope for CuMSN embedded paint formulations to be used for prevention of bacterial colonisation and biofilm formation in water distribution systems.

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P27: An integrated monitoring model to improve Legionella control in water systems

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Legionellosis incidence and its associated health risks is increasing worldwide, due to aging populations, urbanization, climatic changes and circular economy. Addressing global water challenges, in particular linked to water reuse, while extremely relevant, is increasing the number and complexity of the water systems, the water age, and is changing water consumption patterns. This is expected to increase Legionella prevalence in water systems, as well as legionellosis risk.

Legionnaire's disease is known to be preventable when proper preventive measures are implemented at the water systems. However, one of the main drawbacks in current Legionellosis prevention practices is the over-reliance on single, infrequent water sampling, disregarding Legionella interactions with the vast community of microrganisms (which change over time), and its association to protozoa and biofilms. Too often, the application of counter-measures is grounded on those unrealistic pictures of Legionella amount in the water systems, implementing reactive practices rather than preventive ones.

The present work aims to strengthen Legionella surveillance approaches, by establishing an integrated monitoring model, that combines discrete sampling with online, real-time monitoring about the water characteristics (including protozoa), Legionella and the surface (biofilms). This monitoring model is being applied in laboratorial studies concerning water-biofilms-Legionella interaction and will then be implemented in hospital hot water networks. Specifically the model encompasses: a) the Surface Sensor technology (based on how the vibration properties shifts when biofilm is builded-up/detached from the probe) for online, real-time biofilm monitoring; b) pH, temperature and conductivity continuous measurement; c) periodical sampling of water and biofilm to screen for Legionella, protozoa, and biofilm characterization (among others); d) combining culture based techniques with molecular tools for Legionella screening.

Discrete sampling allows the gain of specific and detailed understanding about the characteristics and interactions of Legionella within the ecosystem, while continuous monitoring provides early-warning information towards a more pro-active and knowledge-based Legionella and water management control.

Bridging areas of knowledge is essential to fulfill the tremendous Legionella control challenge. The successful definition and establishment of the proposed monitoring platform requires the broad involvement of all stakeholders involved in legionellosis prevention.

5. Legionella Typing

P28: First Report of Legionella pneumophila and Bordetella bronchiseptica coinfection in immunocompromised patient: possible identification of the source of infection by core genome MLST

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The first case of Legionnaires' disease (LD) in an immunocompromised patient who simultaneously contracted a Bordetella bronchiseptica (Bp) infection is described. The possible source of infection was associated to a travel in Paris the 10 days before the onset of symptoms, while the Bp infection was probably transmitted by his dog.

A 69-year-old man, was admitted to the hospital with high temperature and worsening dyspnea. Blood cultures, serum and urine were immediately collected as well as the bronchoalveolar lavage (BAL) that was tested both by culture and by the BioFire® FilmArray® Pneumonia plus panel that rapidly detected Legionella pneumophila (Lp) infection confirmed by Lp positive urinary antigen. In order to trace the origin of infection, water samples from the patient's home were collected while those from the hotel where the patient stayed during a travel in France were not available.

Lp serogroup 1 was isolated from patient BAL culture and typed using monoclonal antibodies and sequence-based typing, resulting the subgroup Philadelphia ST1.

Whole genome sequencing was also performed and the core genome Multi-locus Sequence Typing (cgMLST)was used to compare the ST1 isolated from the patient with some other ST1 isolated in Paris and present in the public database.

Interestingly, BAL microbiological culture was also positive for Bb. Urinary antigen, immediately positive after hospital admission, remained positive for up to 7 months. IgG and IgM titers for Lp were positive, blood culture negative. Water samples collected from the patient's home bathroom faucet and shower provided Lp serogroup 2-15 contamination in the range of 600-3500 CFU/L, while Lp1 was not isolated.

The allelic profiles obtained by the cgMLST analysis showed only from one to seven difference loci between the patient's ST1 and those isolated in Paris.

The quick detection of this rare co-infection, made it possible to start a specific antibiotic therapy, which is essential for improving the patient's conditions while avoiding a unfortunate outcome.

Furthermore, this case of LD demonstrated that using cgMLST it is possible to attempt to trace the origin of the infection even in the absence of both human and environmental strains of Lp.

P29: Core genome MLST analysis of Legionella pneumophila isolated from clinical samples in Slovenia

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Introduction: The aim of this study was to use whole-genome sequencing to analyze genome variability and phylogenetic relatedness of Legionella pneumophila isolates collected from patients between 2007 and 2022.

Materials and Methods: DNA was extracted from 54 L. pneumophila clinical isolates using the DNeasy Blood and Tissue kit (Qiagen, Germany) according to the manufacturer's instructions. Whole genome sequencing was then performed using the NextSeq 2000 system (Illumina). Raw reads were trimmed using fastp and assembled into contigs using SPAdes. Sequence-based typing (SBT) was performed according to the protocol of EWGLI. Further phylogenetic relatedness of isolates was determined by cgMLST analysis using the L. pneumophila typing scheme available in Seqsphere+ software. A minimum spanning tree (MST) was constructed based on the cgMLST allele differences, ignoring pairwise missing values and using only samples with more than 95 % of the available alleles. Isolates were clustered together with the maximum difference of four alleles between samples.

Results: The most common SBT sequence types were ST62 (6/11,1 %), ST1 and ST23 (each 5/9,3 %) with a total of 28 different STs determined. cgMLST analysis of 1521 alleles revealed that 26 of the 54 isolates belonged to an existing cgMLST sequence type. The most common being 90 (3/ 5,7 %) and 187 (3/ 5,7 %), corresponding to ST23 and ST37, respectively, while the others were still unknown. Phylogenetic visualization revealed 11 clusters consisting of either two or three isolates (total clustered n = 25/47,1 %). Five pairs of isolates showed no allelic differences. Some of the clusters included isolates collected in different years, possibly indicating long-term persistence of the bacterial strain in the aquatic environment.

Conclusion: Using cgMLST, we were able to achieve higher phylogenetic resolution of L. pneumophila strains collected in Slovenia than using SBT alone. Applying this method to more isolates in the future will allow us to detect possible outbreaks and determine sources of infection. Further recombination and virulence factor analyses among isolates could help elucidate genetic changes in strains over the years and identify possible horizontal gene transfers.

Key words: whole-genome sequencing, cgMLST

P30: Comparison between different sample preparation for MALDI Biotyper System to improve the Legionella spp. identification performance

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Bacteria identification is often very expensive and time-consuming for the long growth time and technical expertise required.
MALDI-TOF MS represents an easy-to-use, fast and reliable technique, which results are superimposable with the more sensitive molecular approaches. For Legionella, MALDI-TOF MS is primarily used in the clinical field, but it is hardly used in environmental surveillance.
Legionella is often underestimated and under-identified at species level, considering the use of time-consuming techniques, with low specificity and sensitivity for all 64 species recognized. Although the MALDI-TOF MS performance on Legionella identification has already demonstrated, database requires an update.
Legionella isolates (n=94) belonging to different species, collected during environmental surveillance, were analyzed by MALDI Biotyper System (Bruker Daltonics), comparing three sample preparation methods: direct smear (DS), extended direct smear (EDS) and extraction (E).
To evaluate the best method for Legionella species identification, the obtained data were compared with mip gene sequencing.
Moreover, the isolates relationship was investigated by comparing the dendrogram developed by MALDI-Biotyper software with phylogenetic tree returned by sequencing results.
Comparing the three different methods, the MALDI Biotyper System accurately identified at the species level 62.77% (59/94) of the isolates for DS, 63.83% (60/94) for EDS, and 62.77% (59/94) for E with a high confidence score. In addition, identification at genera level was returned in 34.04% (32/94) of the isolates for DS, 34.04% (32/94) for EDS, and 29.79% (28/94) for E with low confidence score. Instead, only 3.19% (3/94) of the isolates for DS, 2.13% (2/94) for EDS while 7.44% (7/94) for E, resulted not identified.
Considering the methods applied, on the basis of the different species present in our dataset, a decrease or improvement in identification score value has been observed. In detail, regarding L. nautarum the best method was BS, while for L. rubrilucens was E. Concerning the other species, no differences were found.
The results support the MALDI–TOF MS introduction in environmental Legionella surveillance as fast and cheap technique, able to increase the sensitivity of species identification, already using the DS method only. Moreover, it is important to better investigate different performance among the methods, in relation to species characteristics.

Keywords: Legionella identification, MALDI-TOF MS, mip gene sequencing

P31: qPCR based detection of virulence associated Legionella pneumophila by lag-1 gene amplification

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Question: Legionella are ubiquitous environmental bacteria. By inhaling legionella-containing aerosols, the pathogens are able to trigger atypical pneumonia called Legionnaires´ diseases (LD). L. pneumophila serogroup 1 mAb 3/1+ is described as particularly virulent and reported in the majority of clinical samples. MAb 3/1+ Legionella harbour the lag-1 gene as a part of the lipopolysaccharide (LPS) biosynthesis locus and are related to a significantly increased virulence. The finding of mAb 3/1+ underrepresented strains in environmental isolates but their responsibility for the majority of community-acquired outbreaks underlines the increased virulence and thus the importance to detect and prevent potential infection sources in time. However, this contradicts to the time consuming and less sensitive culture-based methods for Legionella diagnostic and prevention. Therefore, we developed and validated a culture independent qPCR method to detect the lag-1 gene in environmental and clinical samples.

Material/Methods: Two primer-probe sets for lag-1 gene amplification were designed. Both assays were validated according to international standards. The primer specificity was evaluated in silico and in vitro by testing environmental and respiratory microorganisms other than Legionella (n=114). Efficiency, limit of detection and quantification were defined using a plasmid carrying the lag-1 gene (standard). The evaluation was carried out by comparing the currently used gold-standard 'mAb typing' and our investigations by lag-1 qPCR in 100 patient and 50 water samples. Due to the complexity of the various sample matrices we designed an internal control to detect inhibition factors.

Results: We successfully designed and validated two primer-probe sets. Following the ISO/TS 12869:2019 the limit of detection (10 GU/reaction) and quantification (50 GU/reaction) were defined and no unspecific amplification of the 114 validation strains could be detected. The evaluation of clinical and environmental samples showed 100 % correlation with both methods and confirmed that the protocols are able to amplify all genetic variations of the lag-1 gene.

Conclusion: The lag-1 qPCR is a new culture-independent tool to detect mAb 3/1+ legionella strains and has the potential to improve the legionella risk management by detecting virulence-associated markers. Unlike the current technical alert value of 1000 cfu of legionellae in 1000 ml by cultivation the described qPCR enables a straight forward risk management and control of water-bearing installations.

6. Legionella: Interaction with the Host and Environment

P32: Modeling of exposure to Legionella spp. during showering events with experimental measured aerosol data

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Presence of Legionella spp. in engineered water systems such as building plumbing is a major contributor to the waterborne disease burden. Legionella spp. exposures indoors are mainly via inhalation of aerosols. Water fixtures, such as showers, are perceived as the predominant sources. Quantitative microbial risk assessment (QMRA) frameworks have previously been applied to evaluate population level risk associated with Legionella spp. in aerosols during showering. However, a limitation of current QMRA frameworks is the difficulty of generalizing model outputs to other scenarios. This is because published frameworks usually apply a simple volumetric estimation and adopt aerosol concentration data from other studies. This can lead to large uncertainty of model output and poor generalizability since aerosol concentration data are usually site-specific. To address such limitations, we developed a mechanistic model to describe fate and transport of Legionella spp.-containing aerosols. The model was calibrated with experiments measuring aerosol concentrations and generation rates in showers using an aerodynamic particle sizer. The impact of water flow rate (highest vs lowest), water temperature (hot water vs cold water), showerhead types (conventional showerhead vs rain showerhead) and shower stall types (glass wall vs curtain) on aerosol generation rates were evaluated. Dynamic change of ventilation rates were measured and included for model calibration. The calibrated model was capable of predicting size resolved temporal variation of aerosol concentration for a shower event. Water temperature compared to water flow rate have larger impact on aerosol concentration. For both rain showerhead and conventional showerhead, 40℃ hot water with a flow rate of 8 L resulted in the highest respirable aerosol concentration (6×10-5 kg/m3 and 3×10-5 kg/m3 respectively). Under such condition, the highest aerosol generation rates (1.1×10-7~1.5×10-7 kg/min) were obtained 1-2 minutes after shower was turned on. Considering highest concentration of Legionella spp. could also be obtained for the first 1 minutes during flushing, precautions should be taken to reduce exposure to aerosols for this period. Through integration with dose-response relationships and sensitivity analysis, our model can be further applied to inform risk mitigation strategies.

P33: Inhibitory effects of Bacillus against Legionella

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Legionella are ubiquitous bacteria, they colonize warm waters and can cause fatal pneumonia. Interaction between Legionella and host is very complicated. As an intracellular bacterium, Legionella has a viable form which cannot grow on media.
In this study, 10 thermal spas were investigated and revealed the presence of Legionella in all samples. Legionella sp strains were isolated by co-culture using amoebae. This method was very effective compared to the direct culture using media. It detects the viable form of Legionella species. The different strains were identified by serological, proteomic and molecular tests by gene amplification and sequencing mip gene.
During incubation at 32°C on BCYE media, characteristic colonies of Legionella were invaded by Bacillus. No Legionella species grown when Bacillus is identified. This antagonism by inhibitory action of Bacillus against Legionella was detected in samples from thermal hot springs.
This result demonstrates that some strains of Bacillus have the potential to decrease or prevent the number of viable Legionella in water, or to prevent its increase. This is an indication that a biological abatement of Legionella could be possible.

Key words: Legionella, antagonism, Bacillus, co-culture.

P34: Presence of resistance-associated genes lpeAB and tet56 in Portuguese environmental Legionella spp. isolates: a three-year study.

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Presence of resistance-associated genes lpeAB and tet56 in Portuguese environmental Legionella spp. isolates: a three-year study.

Due to its ubiquity in water ecosystems, Legionella species are susceptible to human contamination with antibiotics and other pollutants which might induce selection of modified strains and lead to untreatable or unresponsive outcomes of Legionnaires Disease. In this study, we investigated the presence of common resistance mechanisms genes in environmental Legionella samples.

Resistance-associated genes tet56 and lpeAB, which encode the tetracycline destructase Tet56 and the efflux pump LpeAB, were screened using PCR, in Legionella samples (n=263) recovered from potential sources of Legionnaires' disease in the regions of Algarve (n=14), Alentejo (n=115), Lisbon metropolitan area (n=69), Center (n=6), North (n=8), and the remaining unknown (n=51) in the last three years. Samples, isolates (n=140) or extracts (n=123), were provided by Laboratório de Análises de Água, Instituto Superior Técnico, (Lisboa), Laboratório de Saúde Pública do Alentejo (Évora), Laboratório de Saúde Pública do Norte (Braga), and Laboratório da Empresa Portuguesa de Águas Livres (Lisboa).

The majority of the samples was collected in 2022 (173/263). Most samples 67% (175/263) belong to Legionella pneumophila. The regions of Alentejo and Lisbon metropolitan area contributed with the highest number of samples, corresponding to respectively, 44% (115/263) and 26% (69/263). The lpeAB gene was identified in 22% (57/263) of the samples, of which all but one are L. pneumophila. Of all L. pneumophila samples, 32% (56/175) presented this gene. L. pneumophila sg.1 samples presented a frequency of lpeAB detection of 61% (20/33). Regarding the tetracycline destrutase gene, this was found in only two isolates, one L. longbeacheae and one L. spp.

This study allowed us to know the distribution of the resistance-associated genes tet56 and lpeAB in Portuguese isolates from different regions. Our findings highlight the wide presence of resistance-associated genes in Legionella, mainly in L. pneumophila sg1, that colonizes potential sources of dissemination of infection, emphasizing the importance of monitoring the environmental populations of the bacterium to identify potential risks to public health.

Keywords: Legionella; Resistance mechanisms; lpeAB; tet56

P35: Functional diversity of environmental Legionella strains isolated from academic buildings

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Introduction: Legionella genus comprises pathogenic species and strains that can colonize anthropogenic water environments that represent a contamination source. Since these pathogens are diverse from a genetic point of view, they can differ in the proliferation ability in water systems and virulence potential. Thus, assessing the functional diversity of different environmental Legionella strains is fundamental to improving the evaluation of the public health risk of Legionella in drinking water distribution systems.

Methods: This work aims to determine the functional diversity of Legionella environmental strains isolated from water systems of academic buildings and characterized at species and strain levels by sequencing Legionella-specific genes. The ability of the isolates to grow in BYE at different temperatures was determined by measuring the optical density at 600 nm. Moreover, the capability of the strains to kill and replicate towards and within THP-1-like macrophages at distinct times and multiplicity of infections (MOI) was assessed with viability assay and CFU plate counting after the host lysis, respectively.

Results: The Legionella strains tested revealed a temperature-specific growth trend. Temperature-growth values of the isolates are consistent with those observed in their environmental niche, suggesting that those strains can be considered as different ecotypes.

The cytotoxic activity of the strains revealed significant differences after 24 hours of infection at MOI 1 and 10, while no significant differences among the strains were observed after 48 hours. Furthermore, the Legionella strains tested showed a diverse ability to replicate into macrophages between 24h and 48h of infection, indicating that different phylotypes can involve distinct strategies to infect human alveolar macrophages.

Conclusions: These data suggest that Legionella contaminations are characterized by populations having specific temperature-dependent growth patterns and virulence traits.

Understanding the environmental factors (e.g. temperature) improving the colonization of drinking-water systems by different genotypes and their virulence potential is important for health risk assessment in the presence of specific strains within drinking-water distribution systems.

Keywords: environmental strains; temperature-dependent growth; virulence.

P36: Interactions between Legionella, free-living amoeba, and trace elements in domestic internal water supply systems

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Introduction: Local ecosystems of drinking water supply network can change water quality and pose risks to human health. The aim of this study was to evaluate interactions between Legionella, free-living amoeba, and trace elements in water supply system of apartment buildings.

Materials and Methods: Two five-storey apartment buildings of similar characteristics (incl. polypropylene communal pipelines) were selected for the study. Both buildings received treated municipal drinking water from the same source and had previously confirmed Legionella spp. contamination. Before the study period, both buildings were chemically disinfected.

In total, 26 inlet water samples, 80 hot water samples from showerheads and 26 hot water samples from circulation return were collected. Samples were tested for Legionella spp. (ISO 11731; MALDI - TOF), free-living amoeba (FLA) (Schuster, 2002; Thomas, 2006; Microscopy) and levels of Ca, Mg, Mn, Cu, Zn, Fe and Pb (ICP-MS). For further studies, new shower hoses were installed in apartments for biofilm growth.

Results: First L. pneumophila positive samples were detected in both buildings eight days after disinfection, with levels of colonization from 1.0×10^2CFU/L to 9.0×10^2 CFU/L. Overall, 83 of 132 (63%) samples where L. pneumophila positive, with an average level of colonization 1.9×10^3 CFU/L (min 50 CFU/L, max 1.0×104 CFU/L). Observed colonization levels and occurrence of serogroup 1 (SG1) were different between buildings ($p<0.05$), while SG2 and SG3 showed no differences ($p>0.05$). Significant correlations were observed between L. pneumophila colonization and levels of Mg ($p<0.01$), Mn and Cu ($p<0.05$). Significant changes of Cu, Zn and Fe levels were observed in internal water supply systems, with Cu and Zn levels increasing at distal points ($p<0.05$). Three genus of FLA - Acanthamoeba spp., Vermamoeba spp., Vahlkampfia spp. and their combinations were identified in 64 of 132 samples (48%), with overall and genus occurrence similar in both buildings ($p>0.05$). Significant correlation was observed between presence of FLA and L. pneumophila colonization ($p<0.05$).

Conclusions: Changes in chemical parameters, interactions between Legionella, FLA and trace elements in the internal water supply system may affect Legionella control activities. Distal sites of the system can play significant role and requires particular attention in disinfection procedures.

Key words: Legionella, trace elements, free-living amoeba, internal water supply.


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