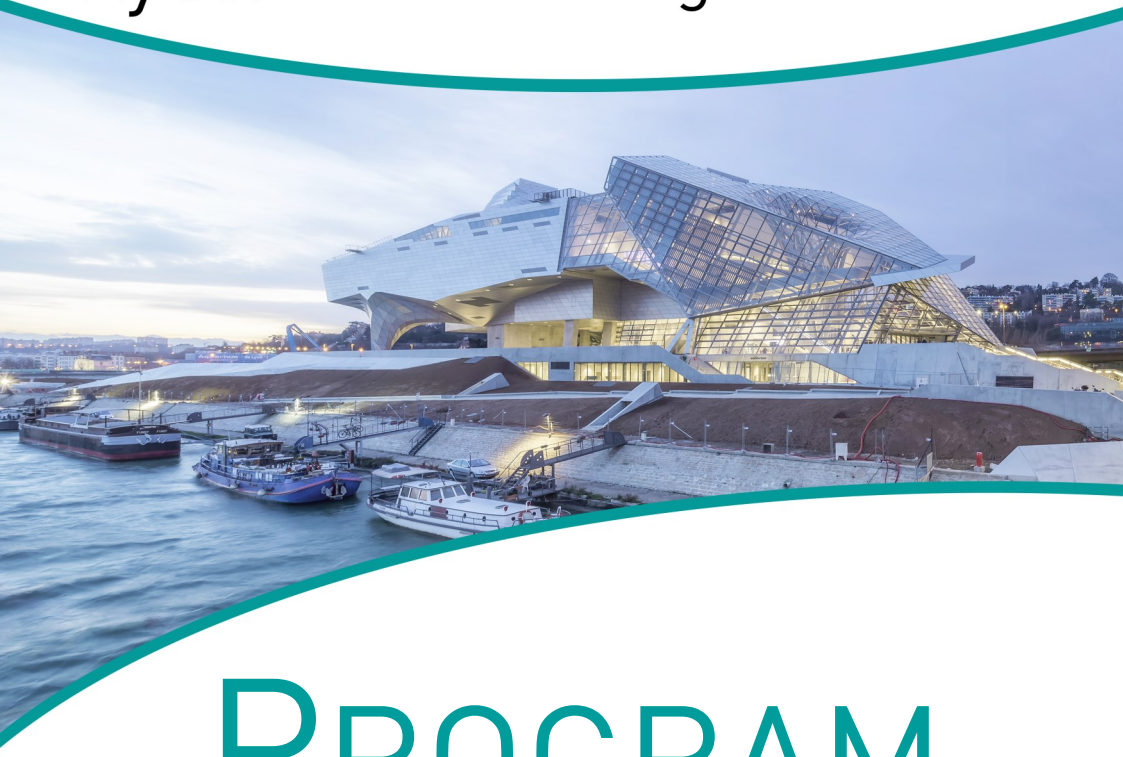
ESCMID STUDY GROUP FOR LEGIONELLA INFECTIONS

ESGLI CONFERENCE 2018

Lyon France August 28-30



PROGRAM

http://**esgli2018.univ-lyon1.fr** esgli2018@univ-lyon1.fr

SOMMAIRE

Dear Colleagues,

The 5th meeting of the ESCMID Study Group for *Legionella* Infections (ESGLI) will be held in Lyon, France 28 - 30 August, 2018. The ESGLI meetings named until 2011 'European Working Group for *Legionella* Infections' (EWGLI) have been organized every year all over the Europe since 1986. This conference is a valuable and unique opportunity to meet other members of the community of European experts on *Legionella*.

The ESGLI 2018 conference will cover a wide range of topics related to *Legionella* and Legionnaires' disease from basic microbial genetics and pathogenesis to applied aspects of detection, control and management. The goal of the meeting is to bring together researchers and professionals with more applied issues. The conference will present the latest scientific developments and knowledge about epidemiology and surveillance; clinical aspects and diagnosis; Host - Microbe interactions; Microbe - Environment interactions; outbreaks and case reports; genetics and genomics; prevention and control strategies.

We hope you will find during these two days matter to enrich you, exchange and initiate new collaborations during the social program, and coffee breaks and lunches regularly proposed. We greatly encourage participation by students and scientists in the early stages of their careers by a low cost of registration.

We look forward to welcoming you in Lyon, classified as a UNESCO World Heritage site, a city of charm with many assets for you to discover by velo'V, by vaporetto or by metro, by tram or by funicular railway, but also by foot…

Sincerely,

Sophie Jarraud
Chair ESGLI 2018 Organising Committee

COMMITTEES

Scientific Committee

Petra Brandsema, *Netherland*
Carmen Buchrieser, *France*
Christine Campese, *France*
Vicki Chalker, *UK*
Xavier Charpentier, *France*
Valeria Gaia, *Switzerland*
Sophie Jarraud, *France*
Christian Lück, *Germany*
Jacob Moran-Gilad, *Israël*
Laura Payne Halistrom, *Sweden*
Carmen Pelaz, *Spain*
Maria Luisa Ricci, *Italy*
Soren A. Uldum, *Denmark*

Organising Committee

Sophie Jarraud
Patricia Doublet
Gerard Lina
Ghislaine Descours
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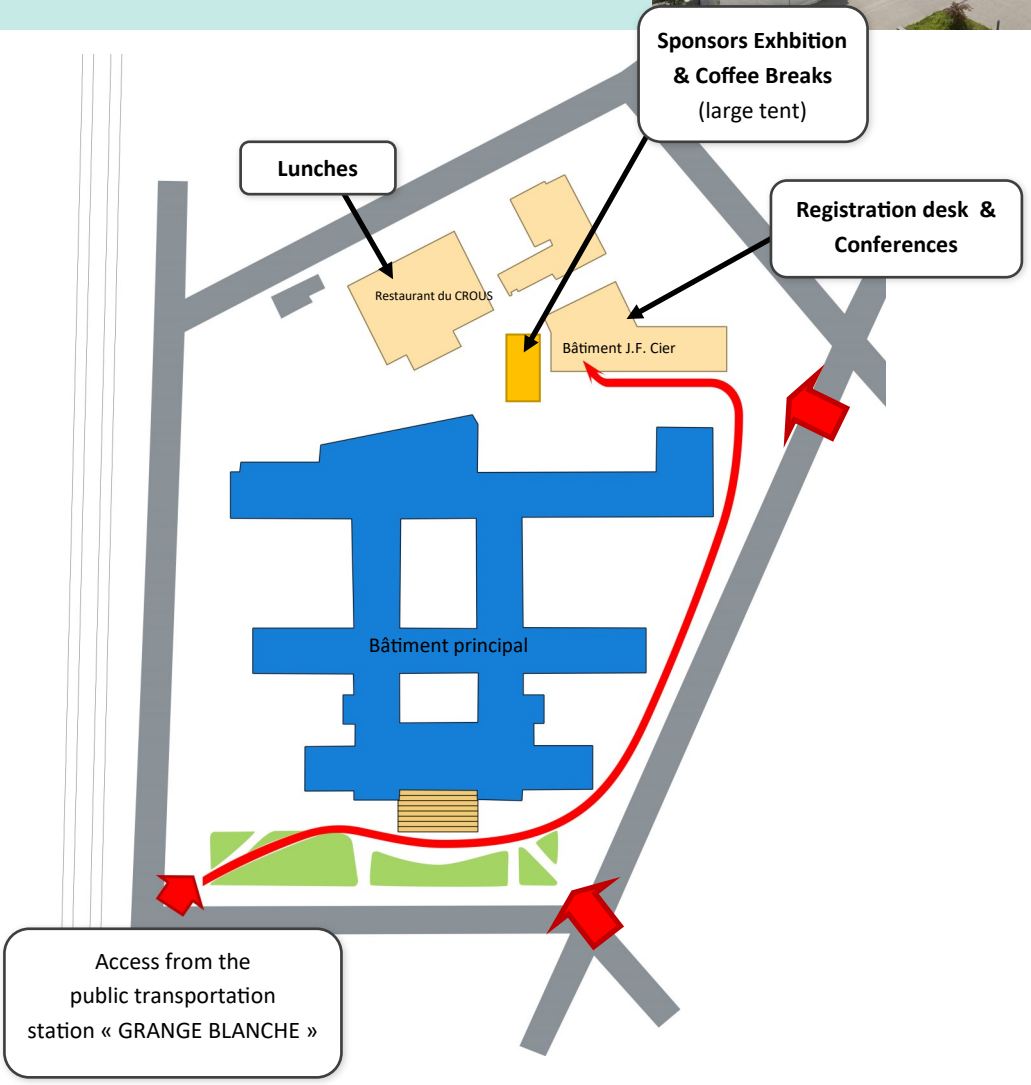
Anne Gaëlle Ranc

CONFERENCE VENUE

Université Claude Bernard Lyon 1
Domaine de Rockefeller - Bâtiment CIER
8 Avenue Rockefeller
69008 Lyon, France



SOCIAL PROGRAM

WELCOME COCKTAIL

Tuesday 28th August

From 6pm to 8pm

CAMPUS de ROCKFELLER

8 Avenue Rockefeller

69008 LYON

The Welcome cocktail is included for all attendees.

GALA DINNER

at L'ABBAYE **DE COLLONGES**
Paul Bocuse

Wednesday 29th August

Meeting point for boat pick up 7pm
13 bis quai Rambaud, 69002 Lyon

The Gala Dinner is not included.
Extra fees apply.

From the conference venue to the meeting point (40 min) :

> Take tramway T2, direction "Perrache"

> Stop at "Perrache"

> Change for tramway T1, direction "Debourd"

> Stop at "Sainte Blandine"

> Walk 500 meters (10 min)

Return by bus around midnight

3 stops:

- Place Bellecour
- Gare Part Dieu
- Grange Blanche (Conference Venue)

TUESDAY, AUGUST 28TH, 2018

5:00 pm-8:00 pm		Registration
6:00 pm-8:00 pm		Welcome reception at the medical faculty **« Wine and cheese » party** (Clos Saint Marc – Wine maker)

WEDNESDAY, AUGUST 29TH, 2018

8:00 am-8:30 am		Coffee welcome – registration
8:30 am-10:15 am		**Session 1: Epidemiology and surveillance** ***Chair: Rosa CANO PORTERO & Christine CAMPESE***
8:30 am-9:00 am	I.01	Invited speaker - Lara PAYNE HALLSTROM: Epidemiology of Legionnaires' disease in Europe 2017
9:00 am-9:15 am	O.01	Soren ULDUM: Legionnaires´ disease in Denmark 2017 - a large increase in number of cases.
9:15 am-9:30 am	O.02	Petra BRANDSEMA: Two Biological Wastewater Treatment Plants linked to multiple outbreaks of Legionnaires' disease with long distance transmission
9:30 am-9:45 am	O.03	Madeleine KAIS: WGS contributing to the investigation of *Legionella* outbreak associated with a cooling tower in Stockholm County, Sweden 2017
9:45 am-10:00 am	O.04	Vladimir DRASAR: Domestically acquired *Legionella* infections. Seek and you shall find
10:00 am-10:15 am	O.05	Karine WYNDELS: An outbreak of Pontiac fever among workers at a potato-processing factory in the north of France
10:15 am-10:45 am		Coffee break
10:45 am-12:30 am		**Session 2: Microbe - Environment interactions – environmental detection** ***Chair: Christian LÜCK & Valeria GAIA***
10:45 am-11:00 am	O.06	Rafik DEY: Imagestream flowcytometry: Accurate and rapid method for the detection of *Legionella pneumophila* within free-living amoeba hosts
11:00 am-11:15 am	O.07	Ahlem LARIBI: Immunodetection of *Legionella pneumophila* in environmental samples by a novel microfluidic electrochemical biosensor
11:15 am-11:30 am	O.08	Séverine ALLEGRA: Characterization of Legionella aerosols from shower
11:30 am-11:45 am	O.09	Maria Luisa RICCI: Microbiome analysis of drinking water systems: evaluation of new approaches for prevention and control of Legionnaires' disease
11:45 am-12:00 am	O.10	Marie Hélène CORRE: Exploring the potential of environmental waterborne bacterial species to find new natural anti-Legionella active biomolecules
12:00 am-12:15 am	O.11	Stephen CHAMBERS: *Legionella longbeachae* in potting mix and pine bark in New Zealand
12:15 am-2:00 pm		Lunch

1:15 pm-2:15 pm		Poster session

2:15 pm-3:45 pm		**Session 3: genetics – genomics – part 1** ***Chair: Alex ENSMINGER & Jacob MORAN-GILAD***
2:15 pm-2:45 pm	I.02	Invited speaker - Ross FITZGERALD: Population genomics of *Legionella spp*: insights into epidemiology and virulence
2:45 pm-3:15 pm	I.03	Invited speaker - Carmen BUCHRIESER: *Legionella pneumophila* serogroups: genomics, genetics and infection
3:15 pm-3:30 pm	O.12	Diane LINDSAY: Longitudinal study of the genomic diversity and epidemiology of *Legionella pneumophila* in Scotland
3:30 pm-3:45 pm	O.13	Diane LINDSAY: *Legionella harrisonii* sp. nov., isolated from composted material in the UK and a clinical isolate from New Zealand

3:45 pm-4:15 pm		Coffee break

4:15 pm-5:45 pm		**Session 4: Host - Microbe interactions / Immunology** **Chair: Carmen BUCHRIESER & Ross FITZGERALD**
4:15 pm-4 :45 pm	I.04	Invited speaker - Patricia DOUBLET: Evolution and secretion for the best stewardship of Dot/icm effectors repertoire
4:45 pm-5:15 pm	I.05	Invited speaker - Alex ENSMINGER: Effect the unexpected: metaeffectors in *L. pneumophila* and beyond
5:15 pm-5:30 pm	O.14	Corentin JABOULAY: Orchestration of Dot/Icm bacterial effector secretion by cyclic-di-GMP metabolizing enzymes during *Legionella* infectious cycle
5:30 pm-5:45 pm	O.15	Christophe GILBERT: Is *Legionella pneumophila* able to target its host cells?

7:00 pm-11:30 pm		Gala Dinner Abbaye de Collonges – Paul Bocuse

THURSDAY, AUGUST 30TH, 2018

8:30 am-10:15 am		**Session 5: Session from NGS ESGLI study group - Legionella typing** ***Chair: Soren ULDUM & Vicki CHALKER***
8:30 am-10:00 am	I.06 I.07 I.08	Invited speaker - Jacob MORAN-GILAD - A cgMLST scheme for the ESGLI community: we're almost there Invited speaker - Brian RAPHAEL (CDC) - Evaluation of a cgMLST scheme for subtyping *L. pneumophila* and comparison with wgMLST Invited speaker - João André CARRICO - Evaluating cgMLST schemes with chewBBACA and all available *Legionella* SRA data Discussion
10:00 am-10:15 am	O.16	Christophe GINEVRA: Genotyping of French isolates: moving from SBT to cgMLST
10:15 am-10:30 am	O.17	Baharak AFSHAR: An evaluation of the international *Legionella pneumophila* sequence based typing (SBT) database
10:30 am-11:00 am		Coffee break – Poster session
11:00am-12:15 am		**Session 6: Genetics and genomics – part 2** ***Chair: Gérard LINA & Brian RAPHAEL***
11:00a m-11:30 am	I.09	Invited speaker - Xavier CHARPENTIER: Widespread natural transformation in *Legionella pneumophila* clinical isolates
11:30 am-11:45 am	O.18	Leo HARDY: Querying the genomes of *Legionella pneumophila* clinical isolates using transposition sequencing
11:45 am-12:00 am	O.19	Guillaume CARRILLO: Evolution of virulence traits during experimental evolution in *Legionella pneumophila*
12:00am-12:15 am	O.20	Vicki CHALKER: Low genomic diversity of *Legionella pneumophila* within clinical specimens
12:15 am-2:00 pm		Lunch
1:15 pm-2:30 pm		Poster session & ESGLI business meeting
2:30 pm-4:30 pm		**Session 7: *Legionella* prevention and control strategies** ***Chair: Maria Luisa RICCI & Ghislaine DESCOURS***
2:30 pm-2 :45 pm	O.21	Edward PORTAL: New high-throughput agar-based *Legionella pneumophila* antibiotic sensitivity testing method for large scale screening
2:45 pm-3:00 pm	O.22	Noemí Párrag-Niño: New biocides for the eradication of Legionella
3 :00 pm-3:15 pm	O.23	Regina NOGUEIRA: Monitoring of *Legionella pneumophila* in an industrial wastewater treatment plant
3 :15 pm-3:30 pm	O.24	Pau GALLES: Emerging sources of *Legionella* in the city of Barcelona
3:30 pm-3:45 pm	O.25	Wilco VAN DER LUGT: An overview of 5 years sampling for *Legionella spp*. in drinking water in 206 buildings in The Netherlands from 2011 to 2015
3:45 pm-4:00 pm	O.26	Emilie BEDARD: *Legionella pneumophila* population in a hospital premise plumbing
4:00 pm-4:15 pm	O.27	Susanne LEE: Interventions to reduce colonisation of a hospital water distribution system
4:15 pm-4:30 pm	O.28	Maria Anna CONIGLIO: How to keep hospitals safe with monochloramine and a Water Safety Plan. Long-term experience for Legionella prevention in Sicily
4:30 pm-4:40 pm		Closing of the conference - Valeria GAIA & Sophie JARRAUD


SANDWICHES


TABAC
PRESSE
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Abstracts of Invited Speakers

Epidemiology of Legionnaires' diseases in Europe 2017

Lara PAYNE HALLSTROM

Population genomics of *Legionella spp*: insights into epidemiology and virulence

Ross Fitzgerald

Legionnaires' disease is a severe form of pneumonia caused by the environmental bacterium *Legionella pneumophila*. Outbreaks commonly affect people with known risk factors, but the genetic and pathogenic complexity of *L. pneumophila* is not well understood. In this presentation, I will summarise genomic epidemiology, population genomic and genome-wide association studies that have been carried out recently in my laboratory. The results are providing new insights into the heterogeneity of *L. pneumophila* isolates during an outbreak, the complexities of source attribution in Legionellosis infections, and genes associated with human clinical infection.

Genomic and virulence differences within the species Legionella pneumophila

Christophe Rusniok[1,2], Monica Rolando[1,2], Christophe Ginevra[3,4], Sophie Jarraud[3,4], Carmen Buchrieser[1,2]

[1] Institut Pasteur, Paris, France; [2] CNRS, UMR 3525, Paris France; [3]CIRI, International Center for Infectiology Research, Inserm, U1111, CNRS, UMR5308, Université Lyon 1, École Normale Supérieure de Lyon, Lyon, F-69008 France; [4]National Reference Centre of Legionella, Hospices Civils de Lyon, France,

Keywords. *Legionella pneumophila*, serogroup, LPS

Background.

Among the over 60 *Legionella* species described to date, *Legionella pneumophila* and *Legionella longbeachae* are the ones most frequently isolated from Legionnaires' disease cases. Interestingly, *L. pneumophila* is associated with 90% of human disease and within the 15 serogroups (Sg), *L. pneumophila* Sg1 causes over 84% of Legionnaires' disease worldwide. Why *L. pneumophila* Sg1 is so predominant is unknown.

Materials & Methods.

We have used high throughput sequencing and comparative genomics of 48 *L. pneumophila* isolates belonging to the 15 different Sgs to get insight in the genomic diversity of the *L. pneumophila Sgs*. In addition, the infection and replication capacity of one reference strain of each Sg was analysed compared to the epidemic strain Paris, Sg1.

Results.

The only specific region of Sg1 strains was, as we previously reported, the LPS encoding gene cluster. We thus further compared and analysed this region in over 200 Sg 1 strains and in three strains of each Sg.

Conclusions.

The LPS of Sg 1 is highly conserved but different groups can be identified. In *Acanthamoeba castellani* infection, all other Sgs are equally or less replicating compared to Sg1 Paris

Evolution and secretion for the best stewardship of Dot/Icm effectors repertoire

Virginie Lelogeais [1-3], Fabrice Vavre [2-3], Julie Allombert [1], Corentin Jaboulay [1], Anne Vianney[1], Patricia Doublet [1-3]

[1] *CIRI, Centre International de Recherche en Infectiologie, Pathogenèse des légionelles, Inserm U1111, Université Lyon 1, CNRS UMR5308, École Normale Supérieure de Lyon, Univ Lyon, F-69007, LYON, France*
[2] *Laboratoire de Biométrie et Biologie Evolutive, UMR 5558, Université de Lyon, Université Lyon 1, CNRS, F-69622 Villeurbanne, France*
[3] *Labex Ecofect, Université de Lyon, F-69622 Villeurbanne, France*

Background.

Legionella pneumophila, the etiological agent of the severe pneumonia legionellosis, is a paradigm of highly adapted "intravacuolar" pathogens that acquired the ability to replicate within phagocytic cells, such as environmental protozoans, in particular amoeba, and alveolar macrophages of its accidental human host. In these cells, L. pneumophila evades endocytic degradation and triggers the biogenesis of a Legionella-containing vacuole (LCV) permissive for its intracellular replication. LCV biogenesis mobilizes complex molecular mechanisms that are strictly dependent of the Type 4 Secretion System (T4SS) Dot/Icm and its exceptionally high number of 300 effectors (1). Recent genomics studies have shown that the T4SS effectors repertoire was poorly conserved and could result from the selective pressure demanded by various natural hosts infected by the different strains (2). Moreover, the secretion of this repertoire has to be fine-tuned to ensure succesfull infection (3).

Results.

Through the example of the interaction between L. pneumophila and host cell autophagy, we highlight here that specific evolution could result in diverse and fine-tuned relationship between L. pneumophila strains and their incidental hosts that are the human cells. Indeed, we observed that the effector RavZ described in Philadelphia strain to irreversibly deconjugate LC3-phosphatidylethanolamine and thus to inhibit the autophagic process (4) was often lost in L. pneumophila genomes. We demonstrated that (i) by contrast with L. pneumophila Philadelphia-1, L. pneumophila Paris induces host cell autophagy, (ii) this phenotypic difference is due to the absence of ravZ gene in the Paris strain genome, (iii) RavZ is detrimental to L. pneumophila Paris intracellular replication in human cells. Together, these results suggest that each L. pneumophila strain evolved independently to maintain a specific effectors' repertoire that optimizes the fine-tuning of host-cell functions such as autophagy for efficient replication. Otherwise, we demonstrated that the effectors repertoire translocation is tightly controlled by the second messenger c-di-GMP. More precisely, one among the 22 c-di-GMP metabolizing enzymes post-transcriptionally controls the delivery of the effectors repertoire into the host cell. Overall, our work highlights that both evolution and control of secretion contribute to the best stewardship of Dot/Icm effectors repertoire for infection by L. pneumophila.

(1) Allombert J, et al.. Microbes Infect. 2013 Dec;15(14-15):981-8. (2) Burstein D et al. Nat. Genet., 2016; 48(2): 167-75. (3) Allombert et al., Infect Immun. 2014 Mar;82(3):1222-33. (4) Choy et al. Science. 2012 Nov 23; 338(6110): 1072–1076.

Effect the unexpected: metaeffectors in *Legionella pneumophila* and beyond

Malene Urbanus[1], Harley Mount [1,2], Dylan Valleau [3], Alexei Savchenko [3,4], Alexander Ensminger [1,2]

[1] University of Toronto, Department of Biochemistry, Toronto, Canada; [2] University of Toronto, Department of Molecular Genetics, Toronto, Canada; [3] University of Toronto, Department of Chemical Engineering and Applied Chemistry, Toronto, Canada; [4] University of Calgary, Department of Microbiology, Immunology and Infectious Diseases, Calgary, Canada.

Keywords: Microbial systems biology; effectors; metaeffectors; genetic interactions; physical interactions

A central pillar of molecular pathogenesis is that translocated bacterial effectors modulate host proteins to remodel the host cell and escape immunological defenses. We have recently uncovered a functionally heretical class of translocated proteins in *Legionella pneumophila* that adds a new dimension to our understanding of host-pathogen interactions. Through a systems biology approach, we have identified several "metaeffectors" (or “effectors of effectors”) that target other effectors rather than the host. This discovery reveals a critical gap in our understanding of how effector function is regulated during infection and opens up several exciting new avenues of study. Several recent advances in experimental throughput and scalability, pioneered by ourselves and our colleagues at the University of Toronto, provide a framework to systematically identify metaeffectors and other instances of effector-effector modulation. We propose that, as built-in effector killswitches, metaeffectors will betray critical nodes of effector activity while also exposing ways to perturb them. Identifying metaeffectors is also of immediate practical importance as these proteins are a potential trap to the unsuspecting researcher using standard biochemical and molecular approaches in an attempt to identify host-pathgen interactions. (Indeed, the first metaeffector, LubX, was initially characterized as a ubiquitin ligase targeting a host protein before it was shown to instead target another effector.) In addition to our findings on *Legionella pneumophila* metaeffectors, we will describe how this pathogen, with its unmatched arsenal of over 300 Dot/Icm translocated proteins, serves as an excellent training ground for metaeffector discovery across a diverse set of intracellular pathogens.

A cgMLST scheme for the ESGLI community: Almost there

Jacob Moran-Gilad[1] on behalf of the ESGLI International Working Group for Whole Genome Sequencing of *Legionella* (ESGLI IWG-WGS)*

[1] Ben Gurion University of the Negev, School of Public Health, Beer Sheva, Israel; [2] Ministry of Health, Public Health Services, Jerusalem, Israel; [3] ESGLI, Basel, Switzerland;

Keywords. cgMLST, Typing, Whole Genome Sequencing, Cluster, Investigation

Abstract:
Sequence-based typing (SBT) has dramatically improved Legionnaires' disease (LD) molecular typing. Microbial whole genome sequencing (WGS) is a promising modality for cluster investigations but analysis methods are neither standardised, nor agreed. Core Genome Multilocus Sequence Typing (cgMLST) has recently emerged as a phylogenomic typing approach that is portable, scalable and reproducible and could allow standardised and nomenclature-based typing for pathogens of public health importance. A cgMLST scheme for *Legionella* has first been proposed in 2015 (Moran-Gilad et al, Eurosurveillance). That scheme utilises 1,521 core gene loci for typing and has successfully been implemented in several cluster investigations worldwide. WGS has also been applied for LD investigation using modified cgMLST, whole genome (wg) MLST as well as calling of variants (single nucleotide polymorphisms, SNPs). On its annual meeting of 2015, the ESGLI Board has formed an International Working Group (IWG) tasked with examining a range of issues concerning the application of WGS for the Legionella community. One of the key decisions of the ESGLI IWG-WGS was that cgMLST should be the method of choice for typing of Legionella and will succeed SBT in due course. Over the last two years, the IWG-WGS has made significant progress towards achieving its goals. The group has created a draft 'slim' cgMLST scheme that is based on 50 loci aiming to simplify typing and information sharing without compromising typing resolution. The IWG-WGS has collated and analysed an unprecedented number of raw sequence data of Legionella genomes, creating a global repository that allows an ongoing validation of the new typing scheme, which so far looks promising. Importantly, a computational tool that allows maintaining reverse compatibility with SBT has been developed and implemented. Lastly, the IWG-WGS is setting up the bioinformatics infrastructure that is required for maintaining and operating the new scheme. While there remain significant challenges ahead of full implementation for routine use, the ESGLI IWG-WGS envisages that its new 'extended SBT' solution will gradually be adopted by the ESGLI community in the near future. In session #5 of the Lyon meeting, the ESGLI IWG-WGS will share these exciting developments, particularly, evaluation data for the new scheme that will inform its anticipated launch and adoption.

*Current members of the ESGLI IWG-WGS: K. Bernard (CA), JA Carriço (PT), V. Chalker (UK), S. David (UK), A. Ensminger (CA), C. Ginevra (FR), S. Jarraud (FR), C. Lück (DE), J. Moran-Gilad (IL) – Chair, B. Raphael (US), D. Ready (UK), SA Uldum (DK).

Evaluation of a cgMLST scheme for subtyping *Legionella pneumophila* and comparison with wgMLST

Brian H. Raphael[1], Subin Park[2], Jason Caravas[1], Jonas Winchell[1]

[1] Centers for Disease Control and Prevention, Respiratory Diseases Branch, Atlanta, Georgia, USA; [2] Association of Public Health Laboratories, Silver Spring, Maryland, USA

Keywords

bioinformatics, bacterial subtyping, allelic profile

Background

Next generation sequencing (NGS) of *Legionella pneumophila* has been used to investigate outbreaks of Legionnaires' disease and to characterize the environmental distribution of this organism. In some cases, NGS has been useful for resolving differences among strains which were indistinguishable using other methods. Although there are numerous bioinformatic approaches to comparing bacterial genomes, a standardized approach using a defined set of genes (loci) is needed for analysis of sequences across laboratories. Variations (alleles) among these loci can be catalogued and used in future comparisons. As part of the EGSLI NGS working group efforts, we evaluated a 50-gene core genome (cgMLST) scheme using publicly available data and sequences provided by several international laboratories. We also examined the ability of a whole genome (wgMLST) scheme containing over 5700 loci to compare outbreak-associated *L. pneumophila*.

Materials & Methods

cgMLST alleles were extracted from assembled genomes using custom python scripts. Raw Illumina MiSeq sequences were analyzed using wgMLST as implemented in BioNumerics v.7.5.

Results

cgMLST analysis of nearly 800 genomes representing sequences available from NCBI and >250 ST1 sequences from the US revealed that >20% of the genomes had a unique allelic profile (subtype). For this dataset, between 21 and 42 unique alleles were identified per locus. Analysis of >1100 genomes including those submitted by international laboratories revealed several examples where not all 50 loci were detected; often this was due to truncation of one or more alleles in the assembly. Using a set of 30 outbreak-associated genome sequences, cgMLST was able to cluster strains similarly to wgMLST.

Conclusions

Overall, evaluation of the cgMLST scheme using multiple datasets demonstrated allelic diversity among the selected loci. This evaluation supports the potential usefulness of the scheme for outbreak investigations. A strain nomenclature will depend on a centralized, publicly accessible allele database.

The findings and conclusions in this presentation are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Evaluating cgMLST schemes with chewBBCA and all available *Legionella* SRA data

João André CARRIÇO

Widespread natural transformation in *Legionella pneumophila* clinical isolates

Xavier Charpentier[1]

[1] *CIRI, Centre International de Recherche en Infectiologie, Team "Horizontal gene transfer in bacterial pathogens", Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, École Normale Supérieure de Lyon, Univ Lyon, 69100, Villeurbanne, France*

Keywords. Natural transformation, genome, mobile genetic element, horizontal gene transfer

Background.

Legionella pneumophila belongs to the growing list of pathogens capable of undergoing natural genetic transformation. Natural transformation results from the capture, import and integration of exogenous DNA and is a common mode of horizontal gene transfer (HGT). Natural transformation is considered the sole mechanism of HGT inherent to the species. It is under control of the recipient cell that actively acquires genetic material from its direct environment using a dedicated DNA uptake system. Natural transformation plays an important role in genome evolution and may constitute the underlying mechanism of genome recombination and genetic exchange in *L. pneumophila*. Also, *L. pneumophila*'s ability to infect cells is dependent upon a large repertoire of genes of foreign origin and natural transformation offers a plausible route for acquisition of foreign genes. Thus, natural transformability is expected to be a conserved trait, yet intraspecific distribution of this trait is generaly poorly documented and had never been investigated in *L. pneumophila*.

Materials & Methods.

We investigated the conservation of natural transformability in one hundred *L. pneumophila* clinical isolates using a semi-quantitative assay. We found that natural transformability is widespread in *L. pneumophila* clinical isolates but we observed large variations incongruent with the phylogeny. We conducted a genome-wide association study (GWAS) to identify the genetic factor associated with low levels of natural transformability.

Results.

Low levels of transformability associate with a mobile genetic element (MGE) present in ~15% of clinical isolates. We experimentally demonstrate that MGE-dependent inhibition of natural transformation result from a genetic interference with the regulatory mechanism of expression of the DNA uptake system. Paralogs of the gene responsible for inhibition of transformation are present in species other than *L. pneumophila*, providing evidence that this phenomenon is occuring at the level of the *Legionella* genus.

Conclusions.

This work reveals a genetic conflict between MGE and natural transformation that may impact genome evolution in the *Legionella* genus.

Abstracts of Oral communications

Legionnaires´ disease in Denmark 2017 – a large increase in number of cases

Søren A. Uldum[1], Karsten D. Bjerre[2], Emmanuel Robesyn[3,4], Charlotte Kjelsø[5].

[1,2,5] *Statens Serum Institut.*

[1] *Dept. of Bacteria, Parasites and Fungi.*

[2] *Secretariat of Data Integration and Analysis.*

[5] *Dept. of Infectious Disease Epidemiology and Prevention.*

[1,2,5] *Copenhagen, Denmark.*

[3] *European Centre for Disease Prevention and Control, Epidemic Intelligence and Response, Solna, Sweden.*

[4] *Karolinska Institutet, Department of Public Health Sciences, Solna, Sweden.*

Keywords

Legionnaires´ disease, Epidemiology, Diagnostic tests, Increase.

Background

In 2017, Denmark saw a 59% increase in notified cases of Legionnaires' disease (LD), from an average of 175 cases in 2015-2016 (31 cases per million) to 278 cases in 2017 (48 cases per million). Since 2015, an enhanced surveillance system is in place, where positive laboratory results from the national microbiological database (MiBa) are used to request missing clinical notifications. The aim of this study was to compare the situation in 2017 with previous years in terms of use of diagnostic methods, distribution of *L. pneumophila* sero- and DNA types, and epidemiology.

Materials & Methods

The surveillance of LD is based on notifications by physicians and voluntary submission of clinical isolates and positive samples to the national *Legionella* reference laboratory. We described the surveillance results and further investigated whether the yearly national number of patients tested by *L. pneumophila* PCR and/or urinary antigen test (UAg) was associated with the number of notified cases. For this, we extracted the number of patients tested each year from 2012-2017 from MiBa.

Results

Of the 278 cases in 2017, 218 (78%) were diagnosed by PCR, 130 (47%) were positive by UAg and 130 (47%) were confirmed by culture of *L. pneumophila*. The most prevalent serogroups (SGs) were SG1 (n=74; 57%), SG3 (n=28; 22%) and SG6 (n=12; 9%). Of the SG1 isolates, 52 belonged to the Mab 3/1+ve group (70%). Fifty-one different Sequence Types (STs) were identified. The most prevalent ST was ST1 (n=26; 20%), ST87 (n=19; 15%), and ST42 (n=10; 8%), with the other STs representing less than five cases each. Hundred sixty-six cases were community-acquired (60%), 68 had travelled abroad (24%), 28 were healthcare-associated (10%), and 16 had unknown setting.

From 2012 to 2017, the yearly number of patients tested (by test) rose from 15,000 to 25,000 (67%): by UAg from 7,000 to 11,000 and by PCR from 8,000 to 14,000. However, from 2015-2016 (average) to 2017, the increase was only 6%.

Conclusions

The use of diagnostic methods, sero-/subgroup and ST distribution and the proportions according to settings were in 2017 not different from 2015-2016 (data not shown in this abstract). The surveillance system was the same in both periods and the increase in number of patient tested could not explain the large increase in notified cases. In 2017, no LD outbreaks were registered and all Danish cases were considered as sporadic. The increase requires further investigations.

Two Biological Wastewater Treatment Plants with identical ST-typing linked to multiple outbreaks of Legionnaires' disease with long distance transmission

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Keywords

Legionnaires'disease, Outbreak, Biological wastewater treatment.

Background

Since 2013 an increasing incidence of Legionnaires 'disease (LD) was observed in the region of Eindhoven, The Netherlands. The annual number increased from 1-3 to 8-14 domestic cases per year, with a sporadic temporal pattern. Typing of clinical isolates showed *Legionella pneumophila* sg 1 ST1646 in 1-2 patients each year. This rare sequence type (ST) had not been found in The Netherlands before 2013. A common source was suspected, but source finding investigations were unsuccessful. In 2016 and 2017, the same ST-type was detected in two consecutive community clusters in Boxtel, a the small town 15 km North of Eindhoven. An outbreak investigation in Boxtel was started.

Material and Methods

Cases were interviewed using a standardized questionnaire. Potential sources were sampled according ISO11731:2017. Sequenced based typing of isolates was done using the ESGLI SBT scheme. A model for detection of environmental sources of airborne pathogens was applied to the Boxtel LD data, estimating areas (hotspots) most likely to contain the actual infection source.

Results

In total 14 LD cases were linked to Boxtel: 6 cases in autumn 2016 and 8 cases in the second half of 2017, with 5 clinical isolates typed as ST1646. No common source of exposure was identified based on patient interviews and sampling of potential sources in 2016. After continued search for possible sources in 2017 a biological wastewater treatment plant (BWTP) in Boxtel was found and sampled. High concentration levels (up to 10^9 CFU/L)) of *L. pneumophila* sg 1 ST1646 were found in the aeration ponds and effluent. The model calculated a hotspot near the BWTP. Subsequently the area of Eindhoven was searched for a BWTP. A similar BWTP (biogas and anammox installation) was found and sampled, and ST1646 was also found in this installation.

Discussion

Based on residential addresses and movements of cases, and the sporadic nature of the outbreak we assume direct transmission from the aeration ponds over a distance of 1.6 km in Boxtel and about 3 km in Eindhoven. The investigations highlight the importance of BWTPs as sources for LD outbreaks and suggest they may also be a relevant source for sporadic LD. Both wastewater installations were recently adapted to a BWTP with biogas production. Modern wastewater treatment is increasingly using energy producing BWTP's. With the increasing trend in number of LD, the contribution of BWTP needs to be further evaluated at the national level.

Whole Genome Sequencing contributing to the investigation of *Legionella* outbreak associated with a cooling tower in Stockholm County, Sweden 2017

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Keywords

Legionella, Outbreak investigation, Cooling tower, Whole Genome Sequencing

Background

Legionnaire's disease (LD) are notifiable in Sweden by law. Notified cases are investigated thoroughly, including environmental sampling. A geographic cluster of LD cases was noted in August 2017, and an outbreak investigation was initiated. As LD outbreaks, previously have been associated with cooling towers (CT), an increased environmental sampling in the area was performed. To investigate an association between clinical and environmental isolates, both phenotypic and genotypic methods was used. To confirm the source of infection, we used whole genome sequencing (WGS).

Material & Methods

Diagnosis of LD was performed by urine antigen test (U-ag) detecting *Legionella pneumophila* sg 1 (Lp1) and/or detection of *Legionella* spp., in lower respiratory tract samples. Isolates of Lp1 were further subjected to serosubtyping and multilocus Sequence-based typing (SBT). SBT was also performed on extracted DNA. All cases were interviewed on potential sources of infection, followed by environmental sampling. Clinical and environmental isolates with the same sequence type (ST) were characterized with WGS (Ion Torrent), and analysis of single nucleotide polymorphisms (SNPs).

Results

Between July-September 2017, 13 cases of community-acquired LD were notified in Stockholm County (population 2.3 million). Eight of these resided in two neighbouring municipalities (population 78 000). Median age was 76, 7/8 were male, and 4/8 died. Seven had Lp1 by U-Ag. Lower respiratory samples were available for six cases; in four of these Lp1 could be isolated, all belonging to ST1 and serosubtype Philadelphia.

No common exposure was found. Water from the cases' homes were negative for Lp1.

Four properties with a range of 2-18 CT each were identified within the area, 13 samples were taken. From two properties, five water samples were positive for Lp1 and ST1. Isolates from four clinical samples and two water samples from one property, were within one SNP difference in a common core representing > 99 % of the reference. This tower was located within 3 km from were all cases resided.

Conclusions

Water from the CT at one property was the source of infection. WGS supported the association between clinical and environmental isolates. No further cases were notified after closing the CT. Eight cases were confirmed; however milder cases might have gone unrecognized.

Adequate control of cooling towers can contribute to the prevention of community outbreaks of LD.

Domestically acquired Legionella infections. Seek and you shall find

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Background.

Legionella colonization of residential premises is well known. However, confirmed domestically acquired infections are rarely reported in the literature. Seventy-nine such incidents are presented. Samples were taken in various housing estates across the Czech Republic and also from single family houses connected to private wells.

Methods.

Accredited laboratory procedures were used for PCR detection, culture, serological urine testing and sequence based typing (SBT).

Results.

The first group comprised 39 culture confirmed cases and the second 40 urinary antigen (UAg) positive cases with associated environmental isolates. *Legionella pneumophila* sg.1 dominated among the clinical strains (67%); a majority (77%) possessed the virulence–associated epitope recognised by the monoclonal antibody Mab 3/1 and. ST62 was the predominant sequencing type (38.5%). The isolation of *L. pneumophila* sg.3,4,6,9 and12 indicated that susceptible persons, especially those receiving immunosuppresive therapy, could contract non-serogroup 1 *Legionella* which have been missed by the UAg test. The group of 40 UAg positive cases showed a similar picture with ST62 making up 33% of all sg.1 The remainder consists of ST641, ST1, ST42, ST23 and others. A large cluster of *L. pneumophila*sg.1. Mab Knoxville, ST641 was identified in a Bohemian city. Between 2012-2018, 14 cases were recognized; 7 persons had predisposing factors (IMS, COPD, Diabetes) and one death. There was a good congruence among clinical and environmental isolates. A subsequent risk assessment in the city confirmed that some blocks of flats received the virulent *L.pneumophila sg.1* strains directly from local plant facilities of hot water providers. Currently, the Czech legionella legislation does not cover water systems in residential premises. Consequently, public health officers have no powers of authority to make owners introduce remedial measures.

Conclusions.

The data presented confirm that domestically acquired infections are underreported. The presence of known virulent sequence types in water appears a much higher risk than the artificial *Legionella* species risk levels“derived from the legislation. Prevention of domestically acquired Leginonnaires Disease cases is difficult and routine disinfections of large housing complexes cannot eliminate legionella from their pipeworks. Preventative measures should include a clinical notification of patients who are at risk.

An outbreak of Pontiac fever among workers at a potato-processing factory in the north of France

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Keywords

Pontiac Fever, *Legionella bozemanii*, Outbreak, Workers, Flu-like syndrome

Background

In July 2017, local public health authorities in France were notified of an outbreak of a flu-like syndrome among workers at a potato-processing factory in Belgium, near the French borders. We investigated to identify the causative agent.

Materials & Methods

A case was defined as a worker at the Belgian potato-processing factory who presented in an emergency department, with fever and one of the following symptoms: asthenia, headache, myalgia, cough, shortness of breath, since 20 July 2017. In France, medical records were reviewed and cases were interviewed about their professional exposures, using a standardized questionnaire. Belgian authorities investigated cases and conducted environmental investigations. Blood and respiratory samples were analyzed in France using 3 methods: serology, culture, PCR.

Results

127 cases were identified between 21 July and 3 August 2017: 76 in France and 51 in Belgium. In France, 23 (30%) cases were hospitalized in a short stay unit; symptoms included fever, asthenia, myalgia or headache (≥78%) and cough (30%). No case had pneumonia. One (1%) case was culture positive for *Legionella bozemanii* (in sputum), 2 (3%) cases were *L.* non-*pneumophila* PCR positive, and 11 (15%) were positive in serology (including *L. bozemanii*).

Of the 51 interviewed cases, 41%-61% of present cases per day were present in triage/cleaning area, 23%-45% in production area (and 12%-16% in purifying/retention area). 9% (n=12) and 43% (n=6) of cases were exposed to splashes, 42% (n=11) and 86% (n=12) to saturated-water in the environment and 31% (n=8) and 64% (n=9) to aerosols in triage/cleaning and production area, respectively. In production area, those were 43% (n=6), 86% (n=12) and 64% (n=9), respectively. In triage/cleaning area, *Legionella* was identified in water samples up to 340,000 CFU/L in culture and air concentrations of endotoxin increased up to 118 ng/mm^3.

Conclusions

Epidemiological and microbiological evidence pointed towards *L. bozemanii*, as the causative agent of this Pontiac fever outbreak in the potato-processing factory. Obtaining of sputum in absence of pneumonia was very useful for identification of the species *L. bozemanii* and for carring out the specific serology. However, *L. bozemanii* rarely caused *Legionella* infection in the past and toxic syndrome linked to organic dust cannot be excluded. The Belgian health authorities recommended public health measures in the factory for both possible sources of contamination.

Imagestream flowcytometry: Accurate and rapid method for the detection of *Legionella pneumophila* within free-living amoeba hosts

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Keywords

Legionella pneumophila, Imagestream flowcytometry, free-living amoebae, detection method.

Abstract

Legionella pneumophila is a water-based pathogen responsible for Legionnaires' disease, which long ago evolved to utilize free-living protozoa for its main site for environmental growth and more recently, colonization of engineered water systems. Culture in selective media and molecular techniques represents the standard methods to confirm *Legionella pneumophila* presence; however, these traditional methods suffer from low sensitivity due to poor cultivability of environmental forms that result in time consuming and inaccurate results. Image stream flow cytometry (ISFCM) is a new technology that incorporates aspects of both microscopy and flow cytometry that performs multi-color spectral fluorescence and bright field imaging simultaneously through a laminar flow stream. Here we describe a protocol for using imaging flow cytometer to quantify the attached and phagocytized GFP-*legionella pneumophila* that are associated with different free living amoebae, in combination with propidium iodide allowed us to distinguish between viable, dead, and damaged- cells**.** Imaging flow cytometry identified infected and non-infected amoebae. For each cell, a spot count algorithm was employed to quantify the number of intracellular GFP-*L.pneu*mophila per trophozoite and the percent of internalized bacteria was determined. Compared with conventional methods (cultivation, qPCR and electron microscope imaging) ISFCM provided very similar data, suggesting that ISFCM is suitable for targeting and obtaining reliable counts for *L. pneumophila* in environmental water system. Our results showed that ISFCM approach is a rapid, easy, and convenient tool for detecting and assessing *L. pneumophila* and its environmental hosts (FLA).

Immunodetection of *Legionella pneumophila* in environmental samples by a novel microfluidic electrochemical biosensor

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Keywords

Microfluidic biosensor, electrochemical techniques, flow cytometry.

Legionella pneumophila is responsible of Legionnaires' disease, a pneumonia which may be fatal for humans. The bacteria are present in natural and man-made aquatic ecosystems. While standardized methods for detection in water samples (such as ISO for culture and PCR) do exist, they are either hampered by their low sensitivity (culture) or their inability to discriminate between different physiological forms of the bacterium (PCR which detects DNA from dead cells). Here, we investigated the application of a new simple immunosensor version which may be of practical value in the field.

This work presents a comparison between static and dynamic modes of biosensing using a novel microfluidic assay for continuous and quantitative detection of *L. pneumophila* in artificial water samples.

A self-assembled monolayer of 16-amino- 1-hexadecanethiol (16-AHT) has been covalently immobilized onto a gold substrate to co-immobilize an anti-*L.pneumophila* monoclonal antibody (MAb) for which we benefit from a world exclusive use. When using dynamic mode of biosensing, each artificially contaminated samples (total volumes of 2 mL) were re-circulated 5-times on the sensor at a 100 $\mu L.min^{-1}$ maximum speed.

The screen-printed sensor was characterized using Square Wave Voltammetry (SWV), Cyclic Voltammetry (CV), Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), and Confocal Laser Scanning Microscopy.

All methods confirmed the immobilization of both MAb and targeted bacteria. The electrochemical response obtained under a hydrodynamic regime showed a significantly enhanced response when compared to a static detection on the same biosensor for the detection of *L. pneumophila* cells in artificial samples. The biosensing performance (data were treated based on current peak height using SWV) showed that low concentrated samples generated signals in the range of 0 to 0.16 ΔI (µA) under static conditions vs signals in the range of 0.34 to 0.44 ΔI (µA) under dynamic conditions.

For the lowest concentrations of bacteria in artificial samples (10 - 10^3 CFU/mL range), we were able to deplete almost all the bacterial stock in the suspensions tested using the sensor functionalized as explained. Using a flow cytometry assay (FCA), we were able to determine: (i) the percentage of cells immobilized on the sensor (for instance, 78% and 34% for artificial samples contaminated with 10^3 and 10^5 CFU/mL respectively) and; (ii) the viability of the cells in the artificial samples before and after recirculation. We found that all viable cells of the bacterium stock in the artificial samples were captured on the sensor surface for 10^3 CFU/mL concentrations (recirculating the samples on the sensor is not decreasing bacterial cells viability as shown, once again, using FCA). Within this viable cells population, viable but not culturable cells are also detected/captured using the sensor (as confirmed by FCA). For highly concentrated samples, the signal of the sensor is exhibiting a saturation effect when reaching 10^5 CFU/mL of *L. pneumophila*. This concentration could very much be the highest detectable using the antibody concentration selected in this study. Whether an increase of antibody concentration on the surface of the sensor would be more efficient for highly contaminated samples remains to be tested. Overall, the sensor is able to detect *L. pneumophila* cells in artificial samples within a 10 to 10^8 CFU/mL concentration range with an absence of correlation when considering ΔI (µA) and CFU/mL concentrations for those higher than 10^5.

This version of *L. pneumophila* biosensor is highly sensitive, simple and rapid to use. This method could be potentially expanded for use with other types of probes or pathogenic agents and could provide continuous analysis and monitoring of water samples at the point of need. It remains to be tested on real artificial samples for complete validation.

Characterization of *Legionella* aerosols from shower

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Keywords

Legionella, aerosols, nebulization systems, showers.

Background

How *Legionella* are aerosolized and enter the respiratory tract remains poorly documented. In previous studies [1–3], we characterize the *Legionella* aerosols dispersed by a vibrating-mesh nebulizer and their deposition in thoracic region and in an *ex vivo* porcine lung.

The aim of this study, in a context close to the human anatomy and its physiological respiratory functions, is to develop experimentations to assess the infectivity of *Legionella's* aerosols generated by shower systems.

Materials/Methods

As previously done for mesh-nebulizer, dispersed *Legionella* aerosols by a Bio-Aerosol Nebulizing Generator (BANG) were characterized using a 13-stage cascade low-pressure impactor. The physiological state and concentration of *Legionella* were assessed by qPCR for total cells, culture for viable and cultivable *Legionella* (VC), and flow cytometry for viable but non-cultivable *Legionella* (VBNC). Dispersed bacteria aerosols from a "classical" shower were quantified using a Coriolis air sampler and the quantity of bacteria reaching the thoracic region was determined using our experimental human-like model [2] by qPCR and culture. The infectivity of *Legionella's* aerosols will be done on *A. polyphaga* and U937 cells using VITROCELL® Cloud 6 technology. The quantity of aerosols (generated by mesh and BANG nebulizers) deposited in each well of VITROCELL was determined by qPCR and culture.

Results

The compilation of all these results makes it possible to establish an experimental protocol approaching the human exposure to *Legionella* during a "classical" shower of about 8 minutes.

Conclusion

To our knowledge, it is the first time that experiments mimicking so closely a real human exposure were performed. New insights on aerosols dispersion are provided about a "classical" shower system. Depending on the concentration of *Legionella* in the hot water system we will can determined the human dose-response for LD using VITROCELL technology

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Microbiome analysis of drinking water systems: evaluation of new approaches for prevention and control of Legionnaires' disease

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Background

Legionella has developed many strategies to survive and multiply in Drinking Water Systems (DWSs), making control measures often ineffective. A large number of chemical-physical parameters and microorganisms affecting *Legionella* growth in DWSs have been revealed. Next generation sequencing (NGS) for environmental metagenomics, revealing the presence and proportion of whole microbial population in a DWS, could give new perspective in the management of Legionnaires' disease (LD). Main aims of this study are: 1.to investigate DWSs microbiome in selected buildings, in four European countries with different LD incidence: 2.to understand if the incidence correlated with different microbiomes; 3.to assess the presence of seasonal variability of microbial community; 4. to find "marker microorganisms" of *Legionella* presence.

Materials and methods

Four operative units (OUs) located in Italy (U1), Denmark (U2), Greece (U3) and Poland (U4) participated in the study. Three hospitals and/or accommodation sites, public buildings were selected in each country. Over the four seasons samplings in 3 points of hot DWSs including the entrance of municipal water of each building was carried out. Microbial cells were collected by filtering 1 L of water. Each OU monitored temperature and chemical parameters. The OU extracted DNA using PowerWater DNA isolation Kit and sent DNA samples to U1.

Miseq2 instrument (ILLUMINA) was utilized to obtain the sequences. For analysis of 16S rRNA data from Illumina Miseq paired-end reads the Amplicon Analysis Pipeline software by Galaxy ARIES for the Istituto Superiore di Sanità platform was used.

Results

Preliminary data showed a prevalence of Proteobacteria, Bacteriodetes and Acidobacteria Phyla in each country. Nitrospirae were detected quite exclusively in U2, while Actinobacteria were shown more abundant in U2 and U1. In U4 a discrete presence of Nitrospirae and Thermi bacteria was observed, while in U3 there were a representatives of Gemmatimodates and Actinobacteria. A deeper analysis provided a more specific differentiation as follow: Commamonadaceae, Nitrospirae and Betaproteobacteria in U2; a major diffusion of Rizobiales and Rhodocyclaceae in U3; Acidobacteria and Gammaproteobacteria and in some sites Methilophilales and Legionellales in U1; Betaproteobacteria, Alcaligenaceae and Rhodocyclaceae in U4.

Conclusions

These data suggest that the composition of microbiome has different characteristics in each country. More comprehensive analysis will clarify these preliminary findings.

Keywords: Legionella, NGS, microbiome, drinking water systems.

Exploring the potential of environmental waterborne bacterial species to find new natural anti-*Legionella* active biomolecules

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Keywords

anti-*Legionella* producers, waterborne bacteria, 16S rRNA sequencing, purification of molecules, biocontrol.

Abstract

L. pneumophila is a Gram-negative natural inhabitant of freshwater environments. This bacterium can colonize manmade water settings from natural water sources, being now considered as an opportunistic plumbing pathogen. Multiplication of *Legionella* in those artificial water systems is highly facilitated by temperatures around 35°C and factors such as water stagnation, poor maintenance, no or reduced water disinfection and the presence of free-living protozoa feeding on biofilms. Its survival in water environments, aside from protozoa, is also driven by interactions with other bacterial species. In this microenvironment the competition for nutrients is raging. Thus, it is tempting to hypothesize that active molecules are locally produced by biological challengers. As more efforts are needed to control disinfection by-products and minimize people exposure to potentially hazardous chemicals while maintaining adequate disinfection and control of targeted pathogens, the aim of the present study was to investigate the potential of those unexplored water bacteria to inhibit *L. pneumophila*.

Environmental aquatic bacteria were sampled in various water environments in order to create a large bacterial collection. To investigate taxonomic affiliations of isolated bacteria, a systematic sequencing of a part of the 16S rRNA coding gene was performed. Moreover, the antagonistic activity towards *L. pneumophila* was assayed for each environmental isolate.

Around 280 bacterial isolates were screened and more than 60% were found to produce anti-*Legionella* molecules. Those molecules were purified from four selected strains (*R. aquatilis, A. bestiarum, Flavobacterium sp, Pseudomonas sp*) using various biochemical procedures. *R. aquatilis* produces one or several siderophores that were isolated by IMAC-Fe^{3+}. By using ammonium sulfate precipitation and RP-HPLC, we purified a protein aqueous compound from the supernatant of *A. bestiarum.* Moreover, flavolipids, a class of biosurfactants produced by *Flavobacterium* species, were shown to inhibit the growth of *L. pneumophila*. Finally, we highlighted a long-range aerial interference between some *Pseudomonas* and physically separated *L. pneumophila*

Those molecules could represent a potent tool for the biological control of *L. pneumophila* in water treatment industry although experimental data are needed to evaluate how effective they would be in real conditions.

Legionella longbeachae in potting mix and pine bark in New Zealand

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Keywords

Legionella longbeachae, Potting mix, pine bark.

Background

Legionella longbeachae is the commonest cause of Legionnaires' Disease (LD) in New Zealand (NZ) but is uncommonly in Europe. In NZ most cases occur in spring and summer (November – January) and are associated with the use commercial potting mix. The primary reservoir of *L. longbeachae* is unknown but the differences in global incidence may relate to the content of potting mix. In Europe this is peat based but in NZ often contains pine bark.

Materials & Methods

Identification of L. longbeachae. 5 g potting mix and bark samples were mixed with sterile DNA free water and DNA from the aqueous phase extracted. *L. longbeachae* DNA was by identified by TaqMan qPCR. PCR inhibitor controls were used to validate results.

Stored potting mix. Bags were purchased commercially and one manufacturer donated product from stores.

Potting mix feedstock. Samples of feed stock were donated by a commercial supplier from three sites in New Zealand.

Culture. Selective *GVPC* media after decontamination.

Bark from living trees. During summer bark samples at chest height (north and south) from mature *Pinus radiata* trees, and adjacent soil were collected.

Results

Quantitative PCR. 3 types of potting mix from 4 independent manufactures' were all negative.

Feed stock, Mixing area and stored samples on manufacturing site.

	Site one	**Site 2**	**Site 3**
Pine bark	30/100	3/150	0/100
Pine sawdust	17/95		
Peat	0/100	0/5	0/100
Composted Green waste	Not available	Not available	0/100
Feed stock Mixing area	3/53	2/10	
Stored bagged compost and potting mix.			10/180

	Central city	**North**	**South**
Bark	0/44 (dry)	4/24 (wet)	0/20 (dry)
Soil and litter	0/44 (dry)	0/24 (wet)	0/22 (dry)

Cultures

All cultures of PCR positive samples were negative.

Conclusions

One reservoir of *L. longbeachae* may be the bark of living pine trees and it enters potting mixes during manufacture. Wetting by rain may allow identification. Other reservoirs need to be identified.

Longitudinal study of the genomic diversity and epidemiology of *Legionella pneumophila* in Scotland

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Keywords
Epidemiology, Legionella, WGS.

Background
Whole genome sequencing and comparative analyses were performed on four hundred historical isolates of *Legionella pneumophila* isolates from Scotland including all clinical isolates and a representative selection of environmental origin. These data were used in conjunction with epidemiological data to determine the relationships between sporadic and outbreak isolates.

Materials & Methods
All patient and a selection of environmental isolates were whole genome sequenced in the Illumina HiSeq. The collection covered the years from 1984 to 2015 and were collected as part of national surveillance of Legionnaires' disease and environmental monitoring.

Results
Phylogenetic analysis of the whole genome sequences showed that the *L. pneumophila* subsp. *pneumophila* population could be divided into seven major and distinct phylogroups. Clinical isolates from this dataset have emerged from all seven major phylogroups, indicating that the potential for human infection is present irrespective of the clonal origin of the isolate. Whole genome sequencing enabled the retrospective identification of 30 infection clusters that were previously classified as sporadic cases due to the separation of the cases either by long periods of time and large geographic distances or both. For example, we found 15 clusters in the dataset where multiple isolates in a cluster were spread over time scales between two to twenty years apart. Further investigation of the patient metadata held by Health Protection Scotland often identified an overlap in either residential or travel histories of the infected individuals but some clusters were spread throughout the globe with no obvious connection from the metadata. This suggests the presence of unrecognized but persistently colonized sources of infection in the environment that may present an ongoing threat to public health.

Conclusions
The application of genomic epidemiology to the retrospective investigation into the aetiology of all identified legionellosis infections in Scotland has revealed epidemiological relationships that were not apparent using the tools that were available previously. In particular, we identified clusters of infections that were separated by years that had travel or geographical relatedness. These data highlight the potential role for prospective sequencing of all clinical isolates in conjunction with surveillance sequencing of environmental samples to identify the emergence of outbreaks or new clinically-relevant strains.

Legionella harrisonii sp. nov., isolated from composted material in the UK and a clinical isolate from New Zealand

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Keywords

New species, Legionella, WGS.

Background

As part of an outbreak investigation of L. longbeachae in Edinburgh in 2013, Bacigalupe et al (2017) found that two isolates previously identified as L. longbeachae might represent a novel Legionella species. The strains were isolated from an environmental source and a clinical case in two very distant geographical locations. The environmental strain was isolated from a compost sample in the spring of 2012 in Scotland. The clinical isolate was recovered one year later from a patient in New Zealand.

Materials & Methods

The bacterial isolates were cultured in a microaerophilic and humid environment at 37ºC on buffered charcoal yeast extract (BCYE) agar plates for 48 h. Whole-genomic DNA of isolates was extracted using the QIAGEN DNeasy Blood and Tissue Kit and sequenced using the MiSeq and HiSeq. Phylogenetic relationships of these two strains within the Legionella sp. genus were inferred by constructing NJ and ML phylogenetic trees based on the 16S rRNA genes and core genome sequences. In addition, various genomic analysis proposed for the taxonomical delineation of prokaryotes were computed.

Results

The new species is a Gram-negative filamentous bacillus that produces a bright yellow fluorescent pigment after 48-72 h at 37oC on buffered charcoal yeast extract. This is an unusual fluorescence only documented once previously related to clinical strains of Legionella steelei. The phylogenetic trees and similarity comparisons of species based on the 16S rRNA genes were not discriminatory enough to resolve the taxonomy of these two isolates. However, phylogenetic trees based on the core genome sequences located the strains in an independent clade sister to L. sainthelensi. Genomic comparisons of the isolates with other Legionella species, including average nucleotide identity comparisons, in silico DNA-DNA hybridization and orthoANI calculations revealed that these two isolates are taxonomically different to all the other Legionella species described so far.

Conclusions

According to the biochemical tests, physiological properties and genomic analyis, the novel isolates can be recognized from their closest phylogenetically related strains. Based on the features studied, these isolates belong to a novel species of the genus Legionella, for which Legionella harrisonii, in honour of Tim Harrison, is proposed.

Orchestration of Dot/Icm bacterial effector secretion by cyclic-di-GMP metabolizing enzymes during *Legionella* infectious cycle

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Keywords

c-di-GMP signaling, intracellular multiplication, Dot/Icm secretion.

Background

Successful *Legionella* infection requires a functional Icm/Dot type IV secretion system (T4SS) which translocates a large repertoire of effectors into the host cytosol. The kinetic of translocation of these effectors have to be tightly coordinated to ensure their delivery in correct amount and at the precise timing at each step of the infection process. Our objective aims at deciphering the regulatory networks involved in this orchestration.

We previously shown that among the 22 GGDEF/EAL proteins, which are responsible for the synthesis and hydrolysis of the c-di-GMP second messenger in *L. pneumophila* Lens strain, 3 of them are specifically required for bacteria survival at the early stages of infection and for adequate Icm/Dot effector delivery inside host cytosol (Allombert et al. 2014).

Materials & Methods

The role these 3 c-di-GMP metabolizing enzymes were further investigated in Lens and Paris strains using assessment of intracellular replication, transcriptomic approaches, protein localization, and protein interaction analysis.

Results

We showed that 1 of the 3 c-di-GMP metabolizing enzymes identified in the Lens strain is crucial for virulence of the Paris strain both in macrophages and amoeba cells. Transcriptomic approaches revealed that there are few differences concerning the gene expression in the corresponding deleted strain compared to the wild type strain suggesting that this protein acts at a post-transcriptional level. Finally, localization assays during infection showed that this c-di-GMP metabolizing enzyme is localized to the bacterial poles, which is consistent with a direct or indirect interaction with the polar Dot/Icm T4SS. Moreover, cross-linking assays pointed out potential partners.

Conclusion

Our results strengthen the role of c-di-GMP signaling in the fine-tune regulation of effectors translocation at the early steps of the infectious cycle of *Legionella pneumophila*.

Is *Legionella pneumophila* able to target its host cells?

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Keywords

Secretion system, pathogen/host interaction, infection.

Background

Previous work in our team reported that 3 proteins, LssB, LssD and TolC, are the components of a type 1 secretion system (T1SS) involved in *Legionella pneumophila* virulence. This T1SS enables the secretion of a unique substrate, RtxA (800 kDa) from the RTX protein family (1). The presence of a functional T1SS enabling RtxA secretion is associated with a higher capacity of *Legionella pneumophila* to infect amoeba and macrophages. Moreover, two locations of RtxA have been identified, embedded in outer membrane or released in medium via a LapG/LapD complex dependent manner, similarly to LapA adhesin system in *Pseudomonas* depending on local c-di-GMP concentration (2). The goal of this work is to elucidate the role of RtxA protein during the first steps of infection depending of targeted host cells.

Methods

Expression and purification of tagged RtxA fragments in *E. coli* was used to produce polyclonal rabbit antibodies. Cross-linking and co-immunoprecipitation techniques followed by Mass spectrometry characterization were performed to identify potential host proteins interacting with *L. pneumophila* RtxA. Complementary experiments were done to assess the relevance of identified proteins during infection process.

Results

Our work suggests that RtxA may be involved in the first step of infection by recruiting specific proteins of host cells to facilitate this process. The specificity of this mechanism towards different host cells in relation to the evolution of T1SS/RtxA system in *pneumophila* and non-*pneumophila* species may also be relevant in understanding Legionnaires' disease.

Conclusion

RtxA may play different roles in *Legionella* depending on its location, therefore during its life cycle, and depending of targeted host cells. Even if *Legionella pneumophila* T1SS/RtxA system does not appear to be essential in virulence, it may play a role in increasing its capacity to initiate infection in Human.

1) Fuche et al. (2015) Appl. Environ. Microbiol. 74, 753-761.
2) Kanaan et al. submitted.

Legionella pneumophila genotyping of French isolates: moving from SBT to cgMLST

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Keywords

Legionella pneumophila typing, Sequence type, WGS, cgMLST.

Background

Molecular typing of bacteria is evolving since NGS technologies have been developed which are going to become the new gold standard. David *et al.* have recently described a cgMLST approach for molecular typing of *Legionella pneumophila*. An ESGLI NGS working group has been created, it is currently working on the optimization of a cgMLST typing scheme.

Materials & Methods

A set of 50 genes was selected by the ESGLI NGS working group. ChewBBACA was one of the bioinformatics tools that was selected by this working group to perform the analyses. We evaluate the typing efficiency of these tools compared to standard SBT on 497 genomes representing 98 different STs, including those of the major clonal STs isolated in France.

Results

All but one genes were detected in more than 95% of the genomes. One gene was detected in only 58% of the genomes. Regarding the cgMLST types of the 473 isolates for which a correct allele call was obtained for the 49 well detected genes; 96 STs were subtyped into 156 cgMLST types. The 3 majors STs detected in France: ST1, ST23 and ST47 were divided into 15, 11 and 2 subtypes, respectively.

Conclusions

cgMLST improve significantly the molecular typing of *Legionella pneumophila*. It allows the discrimination into subtypes of the 3 major clonal STs responsible for a large part of LD cases in France.

An evaluation of the international *Legionella pneumophila* sequence based typing (SBT) database

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Keywords
Legionella pneumophila, sequence based typing (SBT), database.

Background
The seven locus ESCMID Study Group for Legionella Infections (ESGLI) DNA Sequence-Based Typing (SBT) scheme for *Legionella pneumophila* is well-established internationally. The SBT database was first established in 2004 and since then this "gold standard" method has yielded useful and comparable data and has shown to be applicable in the investigation of a number of large Legionnaires' disease (LD) outbreaks. This typing method is applied not only for LD epidemiological investigations to determine the source of infection, but can also investigate the distribution of *L. pneumophila* sequence types (STs) in specific regions within a country or across continents. The aim of this study was to evaluate and describe the current SBT database in order to identify the most common STs and to determine any associations between STs and serogroups or country.

Materials & Methods
The SBT database was queried using standard Structured Query Language (SQL) to produce summary values and an initial data export. These data were then manipulated using R software to produce graphical representations and statistical analysis.

Results
The current SBT database consists of 2,595 STs designated to 12,395 *Legionella pneumophila* (Serogroups 1-16) isolates, submitted by 198 users from 41 countries globally, as of 3rd May 2018. Of 12,395 *L. pneumophila* isolates, 8,238 (66.5%) were clinical, 4,061 (32.8%) environmental and 95 (0.7%) were unknown. The most common STs included; ST1 (12.8%), 23 (6.9%), 47 (5.4%), 62 (3.7%) and 42 (2.9%), respectively. Almost half of the isolates (6,102; 49%) submitted to the database have 'unknown' investigation context. Of the remaining isolates, the majority were from community acquired cases (2,552; 20.6%), followed by 2,064 (16.7%) sporadic routine cases, 846 (6.9%) nosocomial cases and 831 (6.7%) were travel associated. Preliminary analysis revealed that certain STs were unique to certain geographical locations and specific STs were associated with serogroups; STs 23, 47, 42 and 37 were unique to Serogroup 1.

Conclusions
Despite requiring improved data entry and interrogation aspects, the ESGLI SBT scheme remains a rapid, portable and robust method for typing *L. pneumophila* isolates internationally. Used by 41 counties this resource is important for outbreak investigation and geographical interpretation of results. Further interrogation of serogroup specific STs, geographical distribution and genomic phylogeny will increase understanding of *L. pneumophila* diversity and transmission dynamics in humans and water systems.

Querying the genomes of *Legionella pneumophila* clinical isolates using transpositionsequencing

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Keywords

Transposition mutagenesis, genome, sequencing, gene function.

Background

Transposition-sequencing (Tn-seq) has recently emerged as powerful technique to query bacterial genomes. Tn-seq can be used to query the bacterial genome with unprecedented resolution, allowing the identification of small genes (e.g., non-coding RNA) that may be missed in conventional screening approaches. Tn-seq can be used to predict genes essential for *in vitro* growth and to directly identify genetic requirements for survival under multiple conditions. For instance, Tn-seq can be applied to determine the genes, and cellular processes, required to resist an antibacterial treatment or to acquire new resistance genes, to adapt to intracellular life or to compete with other bacteria. Virtually any assay that involves a selection pressure can be used to identify the associated genetic determinants. So far, genomewide Tn-seq has not been applied to *Legionella* species.

Materials & Methods

A random insertional mutagenesis assay was applied to multiple *L. pneumophila* clinical isolates to identify strains permissive to a Tn-seq approach. We report the successful construction of high-density Tn-seq libraries in two disctint *L. pneumophila* clinical isolates.

Results

We identified several hundreds genes essential for growth in rich media and validated the method by identifying genes required for infection of macrophages and amoebas.

Conclusions

A Tn-seq approach is thus now available to identify new therapeutic targets and to reveal the genetic determinants of the *L. pneumophila* biology under environmental or clinical settings.

Evolution of virulence traits during experimental evolution in *Legionella pneumophila*

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Keywords
Virulence, experimental evolution, *Legionella pneumophila*.

Background
In natural environment, *Legionella* replicates within many different eukaryotic hosts and this coevolution would allow this bacterium to acquire the adequate virulence tools to become a broad host-range pathogen. Here, we address the question of the mutational robustness of virulence and host spectrum. Notably, we wondered whether bacteria evolving in the absence or the presence of hosts for hundreds of generations lose or retain their ability to infect various hosts.

Materials/Methods
To answer this question, we conducted a mutation accumulation (MA) evolution experiment in which several replicate populations, founded from the common ancestor *L. pneumophila* Paris, were propagated on standard agar medium for hundreds generations. Such MA experiment allows the accumulation of non-lethal mutations regardless their impact on fitness and virulence. Then, we compared the evolved clones to the ancestor at the phenotypic, genetic and transcriptomic levels.

Results
After several hundred generations of genetic drift in the MA experiment, major phenotypic changes have been detected. Evolved clones showed a strong fitness decrease compared to the ancestor and a reduced ability to infect both amoeba and human macrophages. This intracellular growth defect is correlated to a reduced capacity to establish the replication-permissive vacuole, although the translocation of effectors *via* the Dot/Icm secretion system into the host cells was not affected in evolved clones. This suggests that mutations affect other virulence genes or regulators. In particular, we have identified one genomic modification that confers a strong selective advantage in this condition as it is shared by all the independently evolved lineages and causes the main phenotypic changes observed. Finally, we demonstrated that this mutation and the associated-phenotypes can be reversed when the attenuated evolved clone was re-evolve in a constrained environment (amoebae) for several hundred generations.

Conclusion
Overall, these experimental evolution approaches led us to make connections between genetic mutations and phenotypic outcomes and to identify novel targets of the virulence/host spectrum evolution.

Low genomic diversity of *Legionella pneumophila* within clinical specimens

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Keywords
WGS, diversity Sequence based typing

Background
Legionella pneumophila causes pneumonia of individuals and multiple patients in outbreak scenarios. Investigations of both sporadic cases and outbreaks mostly rely on analysis of a single to a few colony pick(s) isolated from each patient. However, due to the lack of data describing diversity within single patients, the optimal number of picks is unknown. This study investigated diversity within individual patients using sequence based typing (SBT) and whole genome sequencing (WGS).

Materials & Methods
By routine culture ten isolates were obtained *L. pneumophila* from each of ten epidemiologically unrelated patients. SBT and WGS were undertaken, and single nucleotide polymorphisms (SNPs) identified between isolates from the same patient. The same sequence type (ST) was obtained for each set of ten isolates.

Results
Using genomic analysis, zero SNPs were identified between isolates from seven patients, a maximum of one SNP was found between isolates from two patients, and a maximum of two SNPs was found amongst isolates from one patient. Assuming the full within-host diversity has been captured with ten isolates, statistical analyses showed that, on average, analysis of one isolate would yield a 70% chance of capturing all observed genotypes and seven isolates would provide a 90% chance.

Conclusions
This study showed that SBT and WGS analyses of multiple colony picks obtained from ten patients indicated no, or very low, within-host genomic diversity of *L. pneumophila*, suggesting that analysis of one colony pick per patient will often be sufficient to obtain reliable typing data to aid investigation of Legionnaires' disease cases. This study was limited to a sample set of ten patients and additional in depth study of multiple patients with infection from different defined environmental sources may yield greater diversity.

New high-throughput agar-based Legionella pneumophila antibiotic sensitivity testing method for large scale screening

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Keywords

Legionella, Antibiotic resistance, novel method, screening

Legionella pneumophila susceptibility testing to antibiotics and biocides is not routinely undertaken due to the lack of defined internationally agreed standardised methodology and MIC levels, the length of time it takes to carry out a micro broth dilution method and poor visualisation of growth on charcoal agar. Here we present data on Minimum Inhibitory Concentrations (MICs) for 8 antibiotics against 360 *L. pneumophila* isolates from our archive using a novel method enabling rapid screening of antibiotics at multiple concentrations in a high throughput system.

Legionella Antibiotic Susceptibility And Resistance Universal Screening (LASARUS) is a media that sustains high density Legionella growth. It was used to test 360 strains of *Legionella pneumophila*; combining LASARUS medium with automated multipoint inoculator enables testing 80 strains simultaneously against ranges of 8 antibiotics per experiment.

Results in µg/ml for 360 clinical and environmental Public Health England isolates:

- Levofloxacin MIC_{50}=0.03 MIC_{90}=0.03
- Ciprofloxacin MIC_{50}=0.03 MIC_{90}=>0.03
- Tetracycline MIC_{50}=128 MIC_{90}=128
- Azithromycin MIC_{50}=0.03 MIC_{90}=≥0.125
- Rifampicin MIC_{50}=0.004 MIC_{90}=0.008
- Ampicillin MIC_{50}=8 MIC_{90}=≥32
- Gentamycin MIC_{50}=0.125 MIC_{90}=0.25
- Chloramphenicol MIC_{50}=1 MIC_{90}=1

The ranges for levofloxacin, gentamicin, chloramphenicol and rifampicin sensitivity were narrow (universal growth to complete inhibition within 4x doubling concentrations) and consistent with previously published data. Ampicillin sensitivity was much more variable (1 to 32 µg/ml) and only 1 strain was susceptible to tetracycline <32 µg/ml.

Preliminary comparison of 22 environmental isolates found they tended to be more susceptible to chloramphenicol (MIC_{50}=≤0.05) and more resistant to ampicillin (MIC_{50}=≥16) compared to the clinical isolates.

AST determination on LASARUS was compared to Muller Hinton (MH) for ATCC strains *Escherichia coli* 25922, *Staphylococcus aureus* 29213 and *Pseudomonas aeruginosa* 27853 and MICs were within the expected EUCAST version 7 values for MH, while LASARUS MICs were 2-8 fold higher as expected with the high nutrient content of LASARUS medium.

Our system allows high-throughput inoculation of 80 isolates per day, with no additional growth observed after 5-day incubation. We are adapting our method for biocide sensitivity and will have results for 2000 clinical and environmental isolates (1978-2016) by August 2018.

New biocides for the eradication of *Legionella*

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Keywords

Biocides, eradication, Legionella, amoeba, biofilm.

Background

The most commonly used treatments for the elimination of *Legionella* in the water systems are chlorination, heat shock and copper-silver among others, and the presence of the bacteria in a water sample is tested mainly by culture plate. Due to the successive cases of legionellosis observed, both sporadic and in outbreaks, it makes us ask ourselves if some aspects of the strategies used are not efficient enough for the control of this microorganism.

Materials & Methods

Strains used: 5 strains of *Legionella pneumophila* and 3 species of *Legionella* genus.

Biocides tested: biocide A, B, C and D, in 24 hours treatments.

Tests:

1) Planktonic *Legionella* culture: effect on 10^7 cells/ml *Legionella* suspensions in water, quantification by culture plate and by viability qPCR (vPCR);
2) Effect on *Acanthamoeba castellanii* (environmental host): the effect of biocides on amoebas has been tested by trypan blue staining;
3) Biofilm elimination of all *Legionella* strains: biofilm formation by 10^7 cells/ml inoculums of *Legionella* during 96 hours in culture medium. Biofilm elimination has been quantified by fluorescein diacetate staining (FDA).

Results

1) Planktonic culture: all 4 biocides eliminated 107 cells/ml inoculums of Legionella present in water testing by culture plate. When these samples were analyzed by vPCR, we observed that only biocide A killed Legionella, while the other 3 caused the VBNC state in these cells (cells were viable but not culturable).
2) Effect on Acanthamoeba castellanii: all 4 biocides produced amoeba cell death (trypan blue staining) at lower concentrations than those used with planktonic Legionella.
3) Biofilm elimination: biocide A removes 100% of the biofilm at 24 hours post-treatment. Biocides B and C reached 90% elimination rates and biocide D did not show favorable results.

Conclusions

The conclusions of this work are, although preliminary, that biocide A is postulated as an effective and cheap alternative for the elimination of Legionella in all possible environmental states. This new biocide comes from plant extracts so it has no harmful effects on humans and could be used in water for human consumption.

A great advantage of this biocide against chlorination is that it does not induce the VBNC state in Legionella. Chlorination produces this state and is one of the biggest reasons why is not totally effective since it does not kill Legionella but leaves it in a dormant state, which can be reverted and be infective.

Monitoring of *Legionella pneumophila* in an industrial wastewater treatment plant

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L. pneumophila; industrial wastewater; activated sludge; growth kinetics

Wastewater treatment plants are an environmental niche for *Legionella*. However, little is known about the growth kinetics of *L. pneumophila* in these biological systems and their dependence on temperature. A monitoring study was done at an industrial wastewater treatment plant from the food industry. Samples were taken from anaerobic and aerobic reactors for the quantification of *L. pneumophila* by the qPCR method.

L. pneumophila was detected in a concentration range of $3 \cdot 10^1$ to $2.6 \cdot 10^4$ GU/ml in the aerobic activated sludge tanks. Interestingly, it was also present in the anaerobic reactor, despite the lack of dissolved oxygen, suggesting that this bacterium can survive anaerobic periods. Fluorescence *in situ* hybridization of activated sludge samples with specific probes for *Legionella* and eukaryotes showed very few protozoa infected with *Legionella*. Mainly, *Legionella* was found outside protozoa as individual cells and small clusters. Environmental isolates from *L. pneumophila* SG1 obtained from the activated sludge tanks were characterized concerning their growth kinetics in yeast extract broth medium at three different temperatures: 30 °C, 35 °C and 42 °C. The maximum specific growth rate was observed at 35 °C for all isolates; however, growth was also observed at higher (42 °C) and lower (30 °C) temperatures.

Many industries from the food and beverage sector produce wastewater with a temperature range of 25 °C to 35 °C, which is suited for the growth of *L. pneumophila*. Thus, new innovative strategies to prevent proliferation of this bacterium are in demand, since lowering the wastewater temperature is very costly. Most literature studies state that *Legionella* in engineered water systems multiply intracellularly within protozoa. However, our monitoring of activated sludge systems showed mainly *Legionella* outside protozoa and the reported amoeba supporting *Legionella* growth, namely *Naegleria* spp., *Hartmannella* spp. and *Acanthamoeba* spp., were rare. To overcome this knowledge gap, fundamental studies with environmental

Emerging sources of *Legionella* in the city of Barcelona

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Keywords

Legionella, exposure source, street cleaning trucks, nebulizer fan, car wash installation

Background

Legionella bacteria are ubiquitous in natural and man-made water systems. Cooling towers, spas and hot water systems have been related with most of Legionnaire's disease (LD) outbreaks and sporadic cases. Current regulations and guidelines in Spain are mainly focused to these sources, whereas there are not properly established prevention and control guidelines related to other potential sources not commonly associated to LD.

Materials & Methods

Environmental investigations of sporadic LD cases notified between 2015 and 2017 in Barcelona city have been selected. Specific locations of potential exposures during the incubation period (IP) for each case were obtained from the epidemiologic survey. For each facility, water samples were collected and *Legionella* counting, and serogroup were analysed in accordance with the ISO 11731.

A description of the water system and critical points identified, main results of *Legionella* analysis and measures implemented were described in each case.

Results

Car wash installation

It had a hermetic hot water tank with high-pressure water hoses and no maintenance. Temperatures around 32ºC and lack of disinfectant was observed.

Street cleaning trucks

Trucks had 2 m^3 water tanks and high-pressure water hoses. Tanks had an internal foam lining which could act as a reservoir for *L. pneumophila,* lack of disinfectant was observed in the water and there weren't enough taps for a complete drainage of the water in the tanks.

Fan nebulizers

They were outdoors in a bar terrace. They had water tanks of 5 litres and fresh air was nebulized through fans. Lack of maintenance and stagnation of water were the main problems detected.

Three investigations were conducted on summer when cold water temperature raised over 25ºC. Counts over 1,000 CFU/L of *L. pneumophila* serogroup 1 were found in water samples of all installations. Immediate cleaning of disinfection of the water systems, specific control measures and the implementation of a *Legionella* prevention program were required in each facility.

Conclusions

Lack of maintenance and an incorrect design and water temperature were the key factors for *Legionella* growing in all the facilities. Although it wasn't possible to confirm the exposure source for these 3 LD cases, we could consider car wash installations, street cleaning trucks and fan nebulizers as potential sources for LD, so it is necessary to develop prevention and control guidelines and programs.

An overview of 5 years sampling for Legionella spp. in drinking water in 206 buildings in The Netherlands from 2011 – 2015

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Keywords
Legionella, Water management plan, Drinking water quality, Risk analyses

Background
In 2015, de Boer et al. published a paper in which they analysed the results of the National Legionella Outbreak Detection Program between 2002-2012 in The Netherlands. Of the samples taken from buildings/sites with an obligation for maintaining a Legionella control plan (water management plan, WMP) for the drinking water installation, 38% was positive for Legionella spp. While buildings without a WMP had 22% positive samples. This raises several questions: why is this percentage in buildings with a WMP plan higher? A WMP is based on risk factors, do we use the right risk factors?

Materials & Methods
To answer this question we gathered the risk analyses from 206 buildings in The Netherlands together with the sample results from the mandatory sample procedure of these buildings (6171 samples), taken from 2011 until 2015.

Results
Out of the 6171 analysed samples, 16.2% samples exceeded the Dutch action level of 100CFU/litre Legionella spp. The average proportion of samples containing ≥ 100CFU/litre from buildings with ≤ 50 tap points was 28.2% and from buildings with > 50 tap points 12.2%. Every 6 months 33.2% of all buildings tested positive with at least one sample containing ≥100CFU/litre, with an overall increase of 4.4% year. In 1.0% of the samples L. pneumophila serogroup 1 was detectable, in 2.1% L. pneumophila serogroup 2-14 and in 96.9% L. non-pneumophila. There was a large difference in the proportion of samples that exceeded the action level per geographical location.

Conclusions
Based on our data, it seems that the major risk factors for samples with ≥ 100CFU/litre Legionella spp. are the geographical location and the number of tap points, where more tap points mean less risk. This is in contradiction to the risk factors given in the Dutch risk analysis. Extending this database to other areas of the Netherlands will give further knowledge about the risk of the geographical location. The reason why the geographical location is a risk factor and why more tap points means less risk can be interesting in controlling the proliferation of Legionella spp.

Legionella pneumophila population in a hospital premise plumbing

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Keywords
Sequence-based typing, large building, hot water system, *Legionella pneumophila*, distal amplification

Background
Opportunistic microbial pathogens are present and amplified in large building premise plumbing, posing a health risk to vulnerable individuals. Recent studies report distal amplification of *Legionella pneumophila* (*Lp*) of up to 100-fold compared to levels in the building water system. However, the actual cause and source of the bacterial amplification has not been established. Furthermore, it is unclear whether the same *Lp* sequence types (ST) are present throughout the system or if different ST are recovered in distal vs. flushed samples or from one faucet to another. The objectives are to 1) quantify the presence of *Lp* in successive volumes from tap to pipe and 2) evaluate *Lp* population in a single faucet and its connection piping and between different sites.

Materials & Methods
Water sampling was performed in hot water systems using first flush at 3 designated taps. Two successive volumes of 500 mL and a volume of 1L were collected, followed by flushed samples after 2 min and 5 min. In addition, first flush was collected for 3 showers and in the recirculation loop. *Lp* load was evaluated by culture, in parallel with heterotrophic plate counts (HPC) and viable and total bacterial counts. Temperature, pH, turbidity and chlorine were measured after 2L and after 5 min for each sampling event. Isolates were analysed by sequence-based typing (SBT). Infectivity toward macrophage cells and *Acanthamoeba castellanii* was tested for dominant ST recovered from the system.

Results
Overall, *Lp* levels were comparable between first draw (distal) and flushed samples, whereas a decline was observed for HPCs (1 log) and for viable cells (<0.5 log). Two clonal complex were identified throughout the system. The clonal complex A (non-sg1) was present in 85% of collected samples, while clonal complex B (sg1) was present in the 9%. Both clonal complexes were present in 33% of positive samples. Infectivity assay revealed that both clones are infectious toward THP1 cells and *A. castellanii*, similar to the Quebec strain responsible for a large outbreak associated with a cooling tower in 2014.

Conclusions
Understanding the relative importance of the faucet in promoting high levels of *Lp* in hot water systems is important to help define corrective actions. Furthermore, understanding if environmental conditions present within the distal selects for specific sequence type will help determine factors influencing strain dominance within a system.

Interventions to reduce colonisation of a hospital water distribution system

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Keywords

Legionella , Healthcare, Thermostatic Mixing Valves,

Background

Many studies have shown that *legionellae* are common within complex water systems with no panacea to minimising the risk. Experience has shown that Thermostatic Mixing Valves (TMVs) become colonised and *Legionella* can be readily recovered from surfaces of their internal components. However; the impact of TMVs on water quality supplied to outlets has not been fully assessed. A healthcare building with a history of *Legionella* positive results was investigated to determine the impact of TMVs and outlet type on water quality supplied to clinical and kitchen sinks; sluices and patient wash hand basins and to determine if engineering solutions alone would result in a sustained improvement.

Materials & Methods

The system was intensively sampled both before and after the remedial work. A total of 1337 samples were taken from 170 outlets over 17 months. Samples for *Legionella* analysis were taken on four successive occasions prior to any intervention. In outlets with levels of *Legionella* >1000 cfu/L TMVs were removed where the risk assessment allowed, and tap fittings changed. The tap replacement was with one of four types of outlet based on intended use:- a Single Flow Nozzle,:a Monobloc H&C, a basin mounted mixer tap, or a sensor tap with a TMV. Further samples were taken post remedial work. No other interventions on the water systems were carried out during this time to minimise confounding factors for interpretation. Statistical analyses were undertaken to assess the data prior to and following remedial work. Variables representing both the tap and TMV interventions were assessed in regression analyses.

Results

The increased rate of sampling of outlets revealed that colonisation was much more widespread than expected from prior routine sampling: 70/170 taps had persistently high levels of Legionella (>1000 cfu/L). Post remedial work; the level of Legionella present in outlets where taps had been replaced was significantly lower. Compared to the levels of contamination in taps without remedial work the estimated odds ratio for the replaced taps was 0.33 (95% CI 0.14 to 0.74, p=0.008). There was a significant difference between the colonisation of different types of tap. The odds of Legionella in pre-existing TMVs compared to outlets with no TMV was greatly increased with an estimated odds ratio of 36.74 (95% CI 16.1 to 84.1, p<0.001).

Conclusions

Routine sampling may underestimate the risk of *Legionella* colonisation and outlets with TMVs significantly increase the risk of *Legionella* colonisation and hence microbial load within the water distribution systems. The type of tap used also has an impact on the risk of colonisation. For safe management of healthcare water systems TMVs should be removed where possible based on risk assessment and where they are required to manage scalding risk they need active management.

How to keep hospitals safe with monochloramine and a Water Safety Plan. Long-term experience for *Legionella* prevention in Sicily

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Keywords

Legionella, Water Safety Plan, monochloramine, risk assessment, hospital.

Background

The WHO suggests the Water Safety Plan (WSP) together with an effective disinfection of water systems (WS) as the best approach to prevent hospital-acquired legionellosis.

Materials & Methods

Hospital 'Santa Marta e Santa Venera' is a 120-beds hospital located in Acireale (Eastern Sicily). In total, 481 water samples were collected during 2014 and a systemic colonization of the WS with *Lp* sg5 was demonstrated (100% positivity of sampling points). The WSP team was assembled at the beginning of 2015. An *in situ* monochloramine (MC) generator for domestic hot water (DHW) disinfection was started in April 2015.

Results

The WSP consisted of: system assessment, monitoring, management/communication, surveillance. In the choice of the disinfecting method, attention was focused on piping materials, presence of dead legs and water quality. The output of the WSP suggested two preliminary remediation phases of the DHW. Firstly, a chlorine hyper-dosage + flushing, secondly, an MC hyper-dosage at the hot water production station. The temperature of the DHW boiler was also lowered from 65°C to 60°C, with a saving on energy costs of 2.219,00 €/year. After the start up of the continuous disinfection with MC, from April 2015 to April 2018, all the previously sampled outlets were re-sampled every 4 months and specific WSP maintenance and monitoring protocols have been applied. All the samples were negative for *Legionella* for the entire period. The levels of ammonium, nitrites and nitrates ions never exceeded their limits. No corrective action have been applied.

Conclusions

Our study is the first report on the application of a WSP together with the disinfection of the DHW with MC in controlling *Legionella* growth. Results of water samplings carried out during a 3-years period show that all the samples were negative for *Legionella*. Thanks to the saving in energy costs, MC demonstrated to be also a cost-effective solution. In our experience, the application of the WSP together with the disinfection of the DHW with MC was effective in controlling *Legionella* growth and in preventing nosocomial legionellosis.

POSTERS SESSIONS

POSTER SESSION I

Wednesday, August 29th

1:30am - 2:30am

P.I - 01	Maria Rosa	SALA	Legionella community-acquired pneumonia in the Vallès county, Catalonia, 2010-2017
P.I - 02	Frances	GRAHAM	Global Seroprevalence of Legionellosis: a Systematic Review and Meta-analysis
P.I - 03	Junko	ISOBE	Evaluation of an immunomagnetic separation method to detect Legionellla pneumohila serogroup 1 from environmental specimens
P.I - 04	Heiko J.	JAHN	Evidence for faucet water as infectious source for community acquired Legionnaires' disease; Berlin, Germany 2016-2018
P.I - 05	Udo	BUCHHOLZ	Risk factors for community acquired cases of Legionnaires' disease; Berlin, Germany 2016-2018
P.I - 06	Sari	JAAKOLA	Legionnaires' disease in Finland, 2014-2017
P.I - 07	Anna Maria	ROSSI	Activity of Legionella Reference Laboratory: report of the year 2017
P.I - 08	Christine	CAMPESE	Spatiotemporal disparities of Legionnaires' disease incidence in France: what part does climate play?
P.I - 09	Christine	CAMPESE	Legionnaires' disease surveillance in France, 2008-2018: should we improve the surveillance of domestic water systems?
P.I - 10	Cyril	ROUSSEAU	Unusual long-lasting community outbreak of legionnaires' disease linked to an aquatic therapy center, Montpellier, France, 2017
P.I - 11	Beatriz	BELLIDO	Travel associated Legionnaires' Disease clusters in Spain (2009-2017)
P.I - 12	M. Luisa	PEDRO-BOTET	Community-Acquired Legionella Pneumonia (CALP) in HIV-Infected Adult Patients: A Matched Case-Control Study
P.I - 13	Kahina	SOUAMI	Legionella pneumophila at Mitidja area (Algiers and neighbourhood)-Algeria
P.I - 14	Fumiaki	KURA	Sources of infection and settings in outbreaks of legionellosis - Japan, 2000-2017
P.I - 15	Sylvie	HALLIER-SOULIER	Detection, quantification and identification of Legionella, L. pneumophila and in L. pneumophila serogroup 1 in water samples
P.I - 16	Darja	KESE	Comparison of Alere BinaxNow Legionella Urinary Antigen Card with Alere Reader Interpretation and Binax Legionella EIA
P.I - 17	Nesrine	BOILATTABI	Detection of Legionella using MALDI-TOF
P.I - 18	Anna	GIAMMANCO	Validation of a LAMP-based kit for the detection of Legionella pneumophila in environmental samples.
P.I - 19	Stephen	CHAMBERS	Enhancing the culturability and qPCR efficiency for Legionella longbeachae in sputum specimens using Immunomagnetic Separation
P.I - 20	Diane	LINDSAY	Emergence of Legionella maceachernii infection in the United Kingdom.
P.I - 21	Christian	LÜCK	Detection of non-serogroup 1 urinary antigen in two patients
P.I - 22	Christophe	GINEVRA	Legionella pneumophila subspecies raphaeli prevalence in France: the far side of the moon
P.I - 23	Sophie	JARRAUD	Persistent Legionnaires' Disease: series of 12 cases and review of the literature
P.I - 24	Vladimir	DRASAR	Domestically acquired Legionella infections. Seek and you shall find
P.I - 25	Florian	PRILLER	A harmonized protocol for Legionella enumeration via qPCR and ISO 11731 from the same filter
P.I - 26	Inmaculada	SOLIS-ANDRES	ULISENS: Automatic early warning system to detect and quantify Legionella species in water determination

POSTER SESSION II

Thursday, August 30th

1:30am - 2:30am

P.II - 01	Mona	SCHOUSBOE	Legionella pneumophila sg.1 in hospital DH and CW systems: influence of engineering controls and major earthquakes over 15 years
P.II - 02	Pasqualina	LAGANA	Presence of Legionella in water distribution systems of prisons and schools in Sicily (Italy).
P.II - 03	Udo	BUCHHOLZ	Algorithm to increase yield of identified infectious sources among sporadic community-acquired cases of Legionnaires' disease
P.II - 04	El Mostafa	MLIJI	Prevalence of Legionella pneumophila in hot water systems in Morocco and risk factors associated with contamination
P.II - 05	Daina	PULE	Legionella contamination in water supply systems' a five-year survey in Latvia
P.II - 06	Olga	VALCINA	Diversity of Legionella in hot water distribution systems in Latvian hotels
P.II - 07	Christophe	GINEVRA	Legionella direct detection and identification using 16S MinION sequencing
P.II - 08	Kalpy Julien	COULIBALY	Monitoring of Legionella in the waters flowing in establishments and dwellings of the city of Abidjan-Cote d'Ivoire
P.II - 09	Teresa	FASCIANA	Biofilm production and typing of Legionella pneumophila serogroup 1 in Sicily.
P.II - 10	Chiara	MASCARELLA	Molecular typing of Legionella pneumophila: cluster in two Sicilian prisoners.
P.II - 11	Laura	FRANZIN	Whole-genome sequencing in the investigation of a Legionnaires' disease case.
P.II - 12	Corinna	GAGELL	PCR-based subtyping of Legionella pneumophila serogroup 1 from respiratory material
P.II - 13	Alaeddine	MEGHRAOUI	Subtyping of Legionella pneumophila Sequence Type 1 Belgian strains with Pulsed Field Gel Electrophoresis
P.II - 14	Ricardo	SANTOS	LegiolertTM, the next generation enzymatic test for Legionella pneumophila
P.II - 15	Pia	RÄSÄNEN	Occurrence and viability of Legionella bacteria in industrial cooling waters
P.II - 16	Brian	RAPHAEL	Distribution of US Clinical Legionella pneumophila Strains Using Whole Genome MLST
P.II - 17	Toshitsugu	TAGURI	On-site inspection method for Legionella pneumophila in bath water
P.II - 18	Sandra	LAI	A study to investigate the impact of neutralising agents in water sampling bottles on legionella & pseudomonas bacteria recovery
P.II - 19	Laura	FRANZIN	Efficacy of continuous disinfection by monochloramine for Legionella and Amoeba control in hospital water system.
P.II - 20	Christine	LAWRENCE	Network of Hospital Laboratories accredited for Legionella analysis in water: a four years feedback
P.II - 21	Maud	BAUME	Quantification of Legionella DNA Certified Reference Material (CRM) by digital droplet PCR (ddPCR)
P.II - 22	Laetitia	BERAUD	Evaluation of Legiolert, a most probable number method for the enumeration of Legionella pneumophila from potable water samples
P.II - 23	Sharmin	JAMSHID BAIG	Whole-genome sequencing of clinical and associated environmental isolates of Legionella pneumophila, Denmark 2017.
P.II - 24	Joana	ALVES	Legionella pneumophila in Scotland: from genome-wide association analysis to immune evasion

Legionella community-acquired pneumonia in the Vallès county, Catalonia, 2010-2017

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Keywords

Legionellosis; Legionnaires' Disease; Surveillance; Epidemiology.

Background

In Catalonia (7.555,830 inhabitants), Legionellosis is an individualized notifiable disease since 1988. The Vallès county is the Catalan area with the highest incidence of Legionella community-acquired pneumonia (CAP). In this county reside 17% of the 7.5M inhabitants of Catalonia, and is divisided in the Western and Eastern Vallès. The aim of this study was to analyse the incidence of Legionella CAP in the Vallès county during 2010-2017.

Materials & Methods

We analysed all the reported and laboratory-confirmed cases of Legionella CAP from Vallès county between 2010 and 2017. Cases associated to hotels, spas, residences, gymnasiums, nosocomial cases and Pontiac Fever were excluded. We calculated the annual incidence rate (IR) and mortality rate (MR) (number of cases/100,000 inhabitants) with their 95% confidence interval. The relative risk (RR) was calculated using the lowest IR as reference.

Results

During 2010-2017, 579 cases of Legionella CAP were reported in the Vallès county (1.313,290 inhabitants). Median age was 65 years (IQR 53-79 years). Legionella CAP was more common in males among all age-groups. The disease was more frequent between August and October, when 281 cases (48.78%) were notified. The annual IR of Legionella CAP remained stable during 2010-2017, and it was higher on the Eastern compared to the Western Valles except for the year 2014, when there were important epidemic outbreaks. The overall RR for Legionella CAP in Valles was higher for the Vallès than for the rest of Catalonia (RR 2.55; 95%CI 2.30-2.82). The overall MR was 0.38 cases per 100.000 inhabitants, without significant differences between Western and Eastern Vallès. The annual MR was similar for the Vallès and for the rest of Catalonia, except during 2014 when a higher mortality was observed in the Vallès (MR 1.23; 95%CI 0.63-1.83 vs. 0.08; 95%CI 0.01-0.15, respectively). The RR was higher in the Vallès compared to the rest of Catalonia for the whole period of 2010-2017 (RR 3.31; 95%IC 2.18-4.98).

Conclusions

The incidence of Legionella CAP is greater in the Vallès county than in the rest of Catalonia, especially in the Eastern Vallès. These results should be considered to decide priorities in public health protection. Population density, industrialization and environmental factors are aspects that can influence the higher incidence of Legionella CAP in the Vallès.

Global Seroprevalence of Legionellosis: a Systematic Review and Meta-analysis

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Keywords

Legionellosis; *Legionella*; seroprevalence; systematic review; meta-analysis.

Background

Legionella is a ubiquitous pathogen yet the global prevalence of legionellosis is poorly understood. To address this, we conducted a systematic literature review of published population-based studies on the prevalence of *Legionella* antibodies and explore factors that may influence prevalence estimates.

Materials & Methods

Five electronic databases were searched for articles published after 1 January 1990. A total 3979 studies were critically appraised; 100 seroprevalence estimates from 58 eligible studies, which met the inclusion criteria, were identified. These estimates were drawn from 30 countries.

Results

The median seroprevalence was 12.1 per 1,000 people (25% to 75% quantiles: 5.2 to 28). Seroprevalence estimates in children ranged from 6.7% (Taiwan) to 21.7% (Iceland) and in adults estimations varied from 0.3% (Italy) to 45.1% (Denmark). Prevalence estimates also varied by occupation.

Conclusions

Legionellosis has a global distribution. Although seroprevalence estimates appear highest in high income countries in temperate regions, there are insufficient studies from tropical countries to make conclusions about rates in these regions. Studies on the seroprevalence of the pathogen have contributed to a better understanding of the burden of disease. However, further research is necessary to address these gaps in understanding the epidemiology of legionellosis and trends in seroprevalence over time.

Evaluation of an immunomagnetic separation method to detect *Legionella pneumohila* serogroup 1 from environmental specimens

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Keywords

Legionella pneumophila, immunomagnetic separation, immunomagnetic beads, Recovery rate of Lp1.

Background

In Japan, *Legionella pneumophila* serogroup 1 (*Lp*1) is the major causative agent isolated from the patients with legionellosis. Therefore, isolation of *Lp*1 by culture is an important step to determine the sources of infection. However, the sources of infection have been confirmed hardly, because the isolation of *Legionella* by culture from environmental specimens is often disturbed by heavy contamination. Since immunomagnetic separation (IMS) has been shown to enrich the targeted bacteria effectively, IMS for *Lp*1 detections was evaluated.

Materials & Methods

Preparing immunomagnetic beads (IMB)

Polystyrene beads were coated with antibodies against *Lp*1 (*Lp*1-IMB)

Spike testing

The specimens containing *Lp*1, *L. pneumophila* non-serogroup 1, or other *Legionella* spp. alone or containing *Lp*1 with *L. pneumophila* non-serogroup 1 or other *Legionella* spp. were prepared. Then the suspension was treated with *Lp*1-IMB, and recovery rate of *Lp*1 was calculated. In brief, 25 µl of *Lp*1-IMB was added dropwise to 1 mL of the suspension, allowed to adsorb the bacteria for 30 minutes by mixing. The beads were collected with a magnet, washed and suspended finally in 100 or 200 µl of saline as IMB specimens.

Field tests with bath water samples

*Lp*1-IMB suspension was added dropwise to 1 mL of concentrated bath water and the recovery rate was calculated as described in the above procedures. Eighty-nine bath waters were examined.

Results

In the spike testing, the recovery rates of *Lp*1 ranged from 25.0% to 37.6%. In contrast, those of *Lp* serogroups 5 and 6 were 7.1% and 9.6%, respectively. As for *Legionella bozemanii*, *Legionella cherrii*, and *Legionella anisa*, the recovery rates were 0.1% or below. When *Lp*1 was mixed with other *Legionella* spp., the recovery rates of *Lp*1 were 40% or more. On the other hand, the average of other *Legionella* spp. were 8.9%. In the separation of *Lp*1 from bath water samples, *Lp*1 was detected from 4 samples using *Lp*1- IMB, but not detected with culture method without Lp1-IMB. Adversely, in 5 samples, *Lp*1 was only detected using direct culture, but not detected using *Lp*1 IMB. The bacterial count of *Lp*1 in those specimens was as small as 10 cfu/100 mL by direct culture.

Conclusions

Concentration by *Lp*1-IMB is revealed to be useful alternative method for isolating *Lp*1 in bath water which is a most potential source of infectous agents of legionellosis.

Evidence for faucet water as infectious source for community acquired Legionnaires' disease; Berlin, Germany 2016-2018

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Keywords

MAb type 3/1, shower, sink, faucet, Legionnaires' disease

Background

Community acquired Legionnaires disease (CALD) is thought to be mainly transmitted through aerosol contaminated with pathogenic Legionella, in Germany mostly *L. pneumophila* SG1 of the monoclonal antibody (MAb) type 3/1. Showers have been implicated as sources for LD, however, the role of water from the faucet is still unclear, for example because most cases are exposed to both shower AND faucet water. We investigated cases of CALD for evidence for possible infection through faucet water.

Materials & Methods

As part of a wider study (the LeTriWa study) which is underway since December 2016, we have interviewed cases of LD in Berlin about possible exposures in the two weeks before symptom onset. We have taken water and biofilm samples from the faucet and shower of the patients' bathrooms as well as from other potential infectious sources outside the patients' residence and have attempted to type the Legionella strain of the patient. We defined faucet water that the patient had used as the possible/probable/very probable infectious source if (1) the water or biofilm sample from the faucet was positive for a strain with MAb type 3/1 AND (2) the water or biofilm sample of the shower of the same water system was positive for Legionella but was not MAb type 3/1 (possible), or did not contain Legionella (probable) or was irrelevant (because the patient did not take a shower; very probable), AND (3) no other water system that the patient was exposed to contained Legionella of the MAb type 3/1.

Results

Of 54 study patients we identified two cases with probable and two with very probable infection through faucet water. The first of the two classified as "very probable" was a patient where no Legionella was identified at his home, but strains with MAb type 3/1 (Knoxville) were identified from two sinks where he worked. He did not take showers at work. The second case classified as "very probable" was a patient who took no showers at home and washed his hair always under the faucet. The shower did not contain Legionella, but a strain with MAb type 3/1 (Knoxville) was identified from the faucet. In urine of both patients we found Legionella antigen typed as MAb type 3/1 (Knoxville).

Conclusions

We found evidence that not only showers but also water from sinks may transmit Legionella suggesting that the spectrum of water sources that are capable of transmitting Legionella effectively should be generally widened.

Risk factors for community acquired cases of Legionnaires' disease; Berlin, Germany 2016-2018

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Keywords
Legionnaires' disease, MAb-type, Knoxville, case-control.

Background
While older age, male sex, smoking and certain underlying diseases are well known to be associated with Legionnaires' disease (LD) many risk factors relating to patients' behaviour or the causative type of strains still need to be further investigated. We were particularly interested in the risk factors for those cases of LD who acquired their infection likely through residential drinking water (RDW).

Materials & Methods
As part of a larger, ongoing study which is underway since December 2016 (the LeTriWa study) we investigated cases of community acquired LD who had been ruled out as having been infected through a non-RDW-source, i.e. where we assumed infection through RDW. Each case was matched with two controls by hospital and age group (<50, 50-74, 75+). We interviewed cases and controls and took water and biofilm samples from the faucet and shower of the bathroom. In addition all water samples were analysed for Legionella concentration (any species) and were subtyped for monoclonal antibody (Mab) type. We analysed risk factors in bivariate analyses and included significant risk factors as well as previously known risk factors in a backward stepwise multivariable analyses.

Results
So far (April 2018) we recruited 49 cases of LD and 54 controls. In bivariate analysis we identified "wearing dentures", riding in a truck, living in a short distance (≤ 100m) to a large street, taking short showers (≤ 7 minutes), rare alcohol consumption (≤ once per month), living in a building with at least 3 parties, identification of a strain Mab type Knoxville in household water or biofilm samples, identification of any Legionella in household water or biofilm samples, and smoking as significant variables for LD. In a multivariable analysis we also included sex, age group and education in the model. The factors "wearing dentures", taking short showers and smoking remained in the multivariable model.

Conclusions
Preliminary analysis suggests that the presence of a strain Mab type Knoxville in a bathroom water sample may be a risk factor for LD. Taking short showers was an unexpected risk factor, however, it is possible that taking short showers is a proxy for stagnant (and then aerosolized) water. Similarly, wearing dentures was a surprise finding, perhaps explained through the inhalation of biofilm that might be present on dentures and is inhaled through deep breathing, e.g. at night. If confirmed it might open new ways to prevent LD.

Legionnaires' disease in Finland, 2014-2017

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Keywords
Legionnaires' disease, domestic, travel, cluster, source

We analysed data on Legionnaires' disease (LD) cases notified to the National Infectious Disease Register during 2014-2017.

Samples from the potential infection sources of the LD cases not related to travel abroad were cultured by standard method, and the strain comparison was performed by whole genome sequencing and sequence-based typing.

A total of 69 LD cases (37 related to travel abroad and 32 domestic) were identified (0.31/100000); 80% was diagnosed by urine antigen test, 22% by culture, 13% by PCR, and 6% by serology. Case fatality was 8.7% (6/69). With the domestic cases, the median age was higher (62 vs. 59 years) and the proportion of females bigger (25% vs. 22%) than with the travellers. The month of notifying showed no seasonality.

Among the 32 domestic cases, the infection source was confirmed for 18 cases (56%). Hot and cold water at home was the source in nine cases and hospital water in five cases. From these water systems, the isolated strains were *L. pneumophila (L.p.)* sgs 1, 3, 5, *L. anisa and L.* sp.. In addition to, an 87-year old man got infected at home from a small humidifier (*L.p.* sg 1, 3300000 cfu/l) and a 75-year old man during mould spreading at home courtyard (*L. longbeachae* and *L. bozemanii,* 1100000 cfu/g). Two cases of 40- and 41-year old men were associated with wastewater scrubber in industry. From the 32 registered domestic cases, the source remained unknown with the 14 cases (44%).

Four clusters were detected. One cluster with two LD cases in 2015 was related to hot and cold water of a terrace house (*L.p.*1, 15000 cfu/l and *L.* sp., 230000 cfu/l). Three clusters occurred in 2017: the first cluster with two LD cases was nosocomial (hot and cold water *L.p.*1, 5000 and 1700 cfu/l) and the second with two employees was related to cleaning of a wastewater scrubber (*L.p.*1, 510 cfu/l and *L.*sp., 10000 cfu/l). The third, not registered cluster in 2017 with seven persons (three pneumonias and four Pontiac fevers, two with positive serology), was among group of domestic travellers in a hotel with a whirlpool bath (*L.p.* 6, 1200000 cfu/l).

The infection sources of the domestic LD cases were homes, hospitals, a workplace and a hotel. The mean annual incidence of LD in Finland was much lower than the mean European incidence (0.31 vs. 1.4), the proportion of domestic cases smaller (46% vs. 87%), suggesting that LD is likely underdiagnosed in Finland, especially among elderly persons who are not travelling abroad.

Activity of Legionella Reference Laboratory: report of the year 2017

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Background

Since 2001 Regional Agency Environmental Protection (ARPAC) is responsible for environmental surveillance for *Legionnaire's disease* (L.D.) in Campania, by means the Regional Reference Laboratory for Legionella (LRL). Its role is to find out the environmental source of contamination of L.D. In these years we observed an improvement of prevention with a reduction building contamination in terms of Legionella concentration

Methods

In 2017, 116 cases notified of L.D. with 5 death, occurred in Campania: 71 were travel-associated, of which 62 notified by ELDSNet, 32 were community-acquired, and 13 nosocomial. For 38 total cases we registered 17 clusters regarding 16 hotels and 1 thermal station. We made a first control to investigate the presence of Legionella in 87 sites: 51 hotels, 14 health-care facilities, 18 private houses, 4 work-places. Furthermore, in positive sites we did others checks after disinfection treatments. A total of 1902 samples (air, water, biofilm) were collected from several points (tap, shower, swimming-pools, thermal springs, air conditioning system and other). Legionella was detected by culture methods (UNI EN ISO 11731:2017).

Results

27/51 hotels resulted contaminated by Legionella spp. with 10% of positive samples showing a concentration > 10^4 CFU/l, 50% ranging from 10^3 CFU/l to 10^4 CFU/l and 40% < 10^3 CFU/l. In 68% of positive samples we isolated Lp1 and in lower percentage others strains (Lp3, Lp5, Lp6 and Lp8). In 7/14 health-care facilities were found Legionella and 75% of positive samples presented values ranging from 10^3 CFU/l to 10^4 CFU/l, mostly with Lp1 and only in one structure we found Lp8.

There was no Legionella in the workplaces.

3/18 private house resulted contaminated by Legionella spp. with concentration ranging from 100 to 30000 CFU/l of Lp1. It's had to be stressed that in two of this houses were recorded the fatal cases.

Conclusion

In our experience, in Campania the Italian 2015 guidelines seems to be largely applicated in hospitals and receptive accommodations, in fact, despite the last years, we have not noticed a sensitive reduction of positive samples with concentration > 10^4 CFU/l, however, awareness should be implemented in prevention and control of legionella contamination of building water system even in private house in order to control the occurrence of LD cases and most of all to avoid fatal cases.

Spatiotemporal disparities of Legionnaires' disease incidence in France: what part does climate play?

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Keywords

Legionnaires' disease, weather, spatiotemporal model, ecological analysis

Background

In France, around 1300 confirmed cases of Legionnaires' disease (LD) are notified annually. Each year, we observe a west-east gradient in incidences, which cannot be explained by differences in the distribution of notification rates, populations at risk or diagnosis methods.

We investigated if meteorological factors play a role in the occurrence of LD cases and could explain this gradient.

Materials & Methods

We analyzed LD cases notified from 2008 to 2015 in metropolitan France. We restricted the analysis to sporadic, non-nosocomial and non-travel related cases.

Daily mean temperatures, precipitation amounts and duration of relative humidity rates above 80% were obtained from one reference meteorological station by county. Multivariate negative-binomial regression models were constructed to explain the log of the weekly number of new cases in each county, stratified by age class and sex, by the weekly mean of the meteorological variables, lagged by 1 to 10 weeks. Non-linear relationships were allowed. The residual spatial variation was modelled with a conditional autoregressive model, the temporal one with a random walk model of order 2.

Results

Of 10,178 cases, 7,447 were included in the study. The best model comprised the 3 meteorological variables lagged by 1 week. LD incidences significantly increased with the duration of relative humidity rates > 80% and with the amount of precipitations. Temperature had a nonlinear bell-shaped effect, with maximal incidences at 17°C. The inclusion of the meteorological covariates decreased the temporal variation by 19%, but not the spatial variation.

Conclusions

We showed on an unprecedented spatiotemporal scale that mean temperature, precipitations amounts and duration of high levels of relative humidity were significantly associated with LD incidence. Nevertheless, these variables did not explain the observed spatial gradient. This may indicate that their action mechanism is more complex and require a finer modelling, or that other factors, such as the density of sources of contamination are involved in this spatial disparity.

Legionnaires' disease surveillance in France, 2008-2018: should we improve the surveillance of domestic water systems?

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Keywords

Legionnaires' disease, source of contamination, isolates comparisons, water systems

Background

In France, around 1300 confirmed cases of Legionnaires' disease (LD) are notified annually and clinical isolates are available for about 22% of them. Most of LD cases are sporadic. Systematic investigations are undertaken to identify the probable source of contamination.

We describe the characteristics of the clinical isolates and present the results of the comparisons with environmental isolates for LD cases notified in France from 2008 through 2017.

Materials & Methods

Clinical isolates are routinely typed by the National Reference Center (NRC) using several methods: monoclonal antibodies (mAbs) subtyping; Pulsed-Field Gel Electrophoresis (PFGE), Sequence-Based Typing" (SBT) and more recently Whole Genome Sequencing (WGS). When both environmental and clinical isolates, related to a suspected source of contamination, are available, their characteristics are compared by the NRC.

Results

13 304 cases were confirmed and notified during the period and 2 971 isolates were available. The vast majority (95%) of clinical isolates was Legionella pneumophila serogroup 1(Lp1) and 49% belonged to 6 Sequence Type (ST1, ST23, ST47, ST62, ST146, ST259). Environmental isolates were available for 17% (474/2815) of Lp1 cases. Identical characteristics (NRC methods) were found for 281 (57%) of the 494 comparisons. The similarity of isolate's profiles was more frequent when environmental isolates are sampled from the domestic water systems (70%) than from cooling tower systems (8%); $p < 10^{-6}$. The similarity was 84% (16/19) for elderly settings, 73% (80/110) for hospitals, 68% (47/69) for tourist facilities, 67% (80/119) for homes and 65% (51/78) for other accommodations.

Conclusions

Our results suggest that domestic water system could be an important source of contamination for LD cases. In France, LD surveillance of water systems has been already implemented in high risks settings (hospitals in 2002, elderly settings in 2005, touristic facilities and public facilities in 2010). There is no such surveillance for the home water systems and before implementing such surveillance, studies are necessary to estimate its potential efficiency. Nevertheless, awareness of the population about the LD risk must be raised, aiming at reducing the exposure at home.

Unusual long-lasting community outbreak of legionnaires' disease linked to an aquatic therapy center, Montpellier, France, 2017

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Keywords

Legionnaires' disease, outbreak, whole genome sequencing.

Background

In the first week of January 2017, 4 cases of legionnaires' disease (LD) were notified to the local health authority of Occitanie region. The 4 cases lived or stayed in the same area in Montpellier. Therefore, an investigation was immediately carried out in order to identify a common source of contamination and to implement control measures.

Materials & Methods

A case was defined as a person with a pneumonia and laboratory evidence of Legionella infection, living in or, visiting the area in Montpellier during the 14 days prior date of onset. Interviews were conducted using a standardized questionnaire. A descriptive analysis was performed in order to guide environmental investigations. Clinical and environmental isolates were typed by Whole Genome Sequencing (WGS).

Results

A total of 18 cases were identified with onset of illness from December 2016 to July 2017. Cases were aged from 41 to 93 years. All cases were diagnosed by urinary antigen test and for 5 (18%), Lp1 isolates were available. No death was reported. Environmental investigations identified several possible sources of contamination: cooling tower systems, fountain and streets cleaning trucks. None environmental samples were positive in *L. pneumophila*. Nevertheless, one case diagnosed in April, declared having been exposed to an aquatic therapy center. Investigations in this center identified several failures of maintenance such as leaks, insufficiently hot water production, ventilation failures. Finally, epidemiological investigations showed that 9 cases visited this center (mostly in July) and 9 cases stayed close. Environmental samples in the center, taken in July, were positive in Lp1. Phylogenetic tree based on WGS identified 2 distinct clusters, one cluster comprising 4 clinical and 8 environmental ST2471 isolates and the second one including 1 clinical and 1 environmental ST2470 isolates close to 3 ST23 environmental isolates. All the ST2471 isolates showed 3 to 46 SNP differences and ST2470 isolates showed 163 SNP differences. Isolates belonging to the two clusters showed more than 50 000 SNPs differences.

Conclusions

The investigations of this long lasting community outbreak showed that the aquatic therapy center was the source of contamination with 2 different Lp1 strains. People staying inside and outside of the center were affected. This outbreak emphasizes the importance of investigations and the need for exploring the multiple probable sources of contamination.

Travel associated Legionnaire´s Disease clusters in Spain (2009-2017)

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Keywords

Travel associated Legionnaires' disease (TALD); Cluster; Epidemiological concordance; *Legionella* persistence.

Background

Legionella colonizes water systems of buildings; hotels are frequent hosts of the bacteria. Spain is among the five EU countries with the highest incidence rates in 2017. The aim of this work is to describe the magnitude of the disease associated to Spanish hotels and to identify the hotels that had clusters repeatedly despite the control measures applied after a cluster notification.

Materials and methods

Travel associated Legionnaires' disease (TALD) clusters notified through ELDSNet were selected for the period 2009-2017. Information on the microbiological results, both in cases and environmental samples studied in the NRL, was added to the information in the database of the selected TALD cases and associated hotels. We studied the persistence of *Legionella* in hotels. Persistence was defined as *Legionella* identified (same or different species or serogroups) in several occasions in water samples taken at the same hotel during the study period. We studied epidemiological concordance between human and environmental isolates.

Results

122 hotels associated with TALD clusters were notified (annual average 14 hotels) and 394 cases were involved in those clusters (annual average 44 cases). 95 hotels (80%) were in four Spanish Regions. 17 clinical isolates from the reported cases involved and 50 isolates in water samples from the environmental investigation were identified. Both clinical and water isolates were available only for 13 hotels (11%), in 10 of them (77%) clinical and water isolates matched. In 25 hotels out of 50 with water isolates, we found *Legionella* repeatedly during the study period and in 8 of them clinical and water isolates matched. In 18 hotels the same *Legionella* was found during the study period and in 7 hotels was different.

Conclusions

Microbiological results of patients are scarcely available. This deserves an effort in order to improve the results, what would be as easy as to update the information on the microbiological results in TESSy. This information allows surveillance teams (epi and micro in each country) to establish the association and matching of the cases with the accommodation site. We obtained a high percentage (77%) of matching but in a reduced number of hotels. A high percentage of the accommodation sites associated with TALD clusters on which environmental isolates were present for several years showed *Legionella* persistence (50%). Most of them had identified the same type of *Legionella* (18/25 = 72%).

Legionella, HIV, Pneumonia, Acquired immune deficiency syndrome, Community-acquired pneumonia

Background

Community-acquired pneumonia (CAP) continues to be a major complication in HIV-infected patients. Legionella pneumonia (LP) has a poor prognosis in severely immunossuppresed patients. Although publications on LP in the HIV population are scarce, some studies suggest that LP tends to present with more severe clinical features in HIV infected patients.

The aim of this study was to investigate whether the clinical presentation and outcomes of LP in HIV-infected patients were comparable to those seen in non-HIV infected patients.

Materials & Methods

We performed a multicenter observational case-control study in three Spanish hospitals; two in Barcelona and one in Badalona. Case patients were defined as HIV-infected adults with a diagnosis of CALP between 1994 and 2016. Three control cases of LP without HIV infection were selected for each case patient. Patients were matched based on age, center, sex, severity index (PSI) score and date of the episode.

Results

CALP was diagnosed in 32 consecutive HIV infected patients and 96 controls. 124 (98%) were diagnosed by urinary antigen test and 8 patients (16%) by sputum culture. Case patients were younger than controls and had a higher rate of HCV co-infection and neurological disease. According to the HIV infected patients, 23 (74%) were on ART at the time of diagnosis, with 13 (54%) having an undetectable HIV RNA viral load in plasma. The median CD4+ T cell count before diagnosis was 335/mm3 and 2 cases received PCP prophylaxis (22%). Most patients (84%) were classified as low PSI risk class (I-III). Three patients (9%) were admitted to the ICU and 2 required mechanical ventilation. The median LOS was 7·0 (4·0; 11·0) days. The overall 30-day mortality was 3%. No differences were found on empirical antimicrobial treatment or on the delay of diagnosis. On evolution, no differences were found on ICU admission, mechanical ventilation, LOS or 30-day mortality. In a multivariate logistic regression analysis, previous antibiotic treatment, PSI risk class IV-V, PaO2/FiO2 <250, the presence of pleural effusion, and multilobar involvement were associated with ICU admission. HIV infection was not associated with ICU admission or increased LOS.

Conclusions

This is the first case-control study performed on HIV infected patients with CALP, and our main conclusion is that HIV-infected individuals presented neither a more severe disease nor a worse clinical outcome than matched HIV-negative control patients.

Legionella pneumophila at Mitidja area (Algiers and neighbourhood) – Algeria –

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Keywords

Algiers, Legionella pneumophila, diagnosis, epidemiology,GIS.

Background

We have few data on Legionnaires´ disease (LD) from Algiers, Algeria, where lower respiratory infection is the first cause of death. The aim of this study was to evaluate the importance of Legionella pneumophila (Lp), and its characteristics at Algiers and neighbourhood.

Materials & Methods

A prospective study targeting adult pneumonia patient was run from February 2016 to April 2018. The patients were from intensive care units, kidney, transplantation, haematology, emergency and pneumology departments of 14 hospitals at Algiers and neighbourhood. PCR following the ESGLI recommendations was run for 358 patients of the cohort. For each patient, urinary antigen (UAG) by immunochromatography (Binax Now, Alere) and enzyme immunoassay (Binax, Alere) was run, as well as culture (BMPA/MWY–oxoid) and *L. pneumophila* (Lp). A standardized questionnaire was completed to collect demographic, clinical, radiological, biomarkers and treatment information. A map (Arc Gis) was drawn, regarding the residency of the LD cases and the areas they have visited up to 14 days before the onset. The National Reference Centre of Legionella in Lyon and the Legionella reference laboratory in Copenhagen have re-tested the positive and equivocal samples, in double blinded way and have provided support along the time.

Results

The prevalence of LD due to Lp (UAG and/or PCR and/or Culture) was 8,94% (32/358). The mean of age was 54,56 years and the median was 55,5 years. Almost all of them were males (27/32).Two patients died. Thirty cases were caused by Lp serogroup (sg) 1 and one by sg 3. UAG was positive for 29 cases, culture for two cases and PCR for nine cases, 8 cases were UAG and PCR positive. Only two patients had all three tests positive. Some discrepancies were observed: one patient who was infected by Lp sg 3, discovered thanks to Nested-PCR, was also urinary antigen positive for Lp sg1. Another patient infected by Lp sg1, who had more than 30 colonies grown by culture and was PCR positive, was urinary antigen negative. A novel SBT profile was found (2,2,9,10,2,1,6) from direct samples (no isolate). Some areas were identified as high-risk areas, because many Lp cases were found there.

Conclusions

Legionnaire's disease is a public health problem at Algiers and neighbourhood. Further studies are needed to identify the source/sources by matching clinical types with environmental types by SBT or whole genome sequencing.

Sources of infection and settings in outbreaks of legionellosis – Japan, 2000-2017

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Keywords
Outbreaks, Legionellosis, Sources of infection, Baths

Background
In Japan, legionellosis is an 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 is increasing. During 2000-2016, the rate of reported cases increased from 0.12 to 1.26 per 100,000 persons; 2% of reported cases were outbreak-associated.

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
Twenty-five outbreaks, at least, were found during 2000-2017. The median number of cases was 3 (range = 2-58) and median outbreak case fatality rate was 0% (range = 0%-33%). Community-acquired infection (CAI) and healthcare-associated infection (HAI) were 21(84%) and 4(16%), respectively. Sources of infection were baths (21, 84%), cooling towers (1, 4%), and humidifiers (1, 4%), and the remaining was unknown.

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 systems. This difference in distribution may depend on the differences in lifestyle of people and genotypes of *Legionella pneumophila* between Japan and Western countries.

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Detection, quantification and identification of *Legionella, L. pneumophila* and in *L. pneumophila* serogroup 1 in water samples

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Keywords

Legionella pneumophila serogroup 1, Real-time PCR.

Background

Legionella, ubiquitous bacterium in aquatic environments, is an opportunistic pathogen of significant public health concern, especially the species *Legionella pneumophila* among which *L. pneumophila* serogroup 1 (Lp1) accounts for about 84 % of human infections worldwide. Hence, identification and quantification from environmental sources is crucial for tracking outbreak, risk assessment and disease prevention. The two most widely used and accepted methods are culture and real-time polymerase chain reaction (qPCR). In order to circumvent limits of the culture method enabling to detect culturable bacteria only, Pall® offers a range of qPCR-based methods for the detection, the quantification and the identification of *Legionella* in water samples.

Materials & Methods

Sample preparation relies on bacterial concentration by filtration of the water samples (100 mL – 1 L) through 0.45 µm polycarbonate membrane, mechanical bacterial lysis by sonication and heating, and optional DNA purification onto silica column. PCR analyses are performed with the GeneDisc Cycler, by using 3 different GeneDisc® Plates: GeneDisc *Legionella pneumophila* for the quantification of *L. pneumophila*, GeneDisc *Legionella* DUO for the simultaneous quantification of *Legionella* spp. and *L. pneumophila*, and GeneDisc Legionella ID for the detection/identification of the genus, the species and *L. pneumophila* serogroup 1. Development of the GeneDisc Plates was realized according to requirements of the NF T90-471 (2015) standard and the MIQE (minimum information for publication of quantitative real-time PCR experiments) guidelines.

Results

Specificity of the PCR assays was demonstrated by exclusivity and inclusivity tests from 45 and 35 pure cultures, respectively.

The limit of detection and the limit of quantification were validated at 5 Genomic Units (GU)/PCR well and 25 GU/PCR wells, respectively.

Accuracy of the linearity was determined for each PCR assay under reproducibility conditions (independent DNA ranges, different cyclers…) using standard DNA ranges from 250,000 to 25 GU/PCR well. For each DNA quantity, results were inferior to 0.15 Log, accordingly to the NF T 90-471 requirements.

Finally, robustness of the entire method, including the sample preparation was verified with 3 types of water samples: mineral, sanitary and cooling tower. The global recovery was at least 75 % whatever the sample type.

Conclusions

The GeneDisc technology provides a range of flexible methods enabling to monitor *Legionella* contamination in water samples, either by detection or quantification of as low as 80 GU/L and 400 GU/L, respectively. This quick method enabling to get results in less than 3 hours and its very friendly use, can be easily implemented in quality control laboratory for routine analysis.

Comparison of Alere BinaxNow *Legionella* Urinary Antigen Card with Alere Reader Interpretation and Binax *Legionella* EIA

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Keywords

Diagnostic, urine antigen, reader interpretation.

Background

The preferred diagnostic tests for *Legionella* infections are still culture combining with urinary antigen tests (UAg) or with molecular assay. Urinary antigen detection tests are highly specific and represent an established and valuable tool in diagnostic of *L. pneumophila* sg.1 infection. Most of commercially available UAg tests are prepared as rapid lateral flow immunochromatographic assay (ICH) with visual interpretation of the result. Recently, some automated readers of ICH tests have been introduced to increase sensitivity and to avoid operator subjectivity. In this study we evaluate the Alere BinaxNow *Legionella* Urinary Antigen Card test with automated interpretation by Alere Reader (Alere Scarborough, USA) in comparison with Binax™ *Legionella* Urinary Antigen EIA test which is routinely used in our laboratory.

Materials & Methods

For evaluation, a total of 120 urine samples from adult pneumonia patients were tested: 42 frozen urine samples (on -80°C) which were identified as *L. pneumophila* positive by Binax EIA and collected from January 2017 till December 2017; in addition, 78 fresh native urine samples were included in the study. All samples were analysed nonconcentrated side by side with both tests. The results of BinaxNow ICH were interpreted visually and by Alere Binax Reader. Weak EIA positive urine samples (OD ≤ 0.400) were boiled and retested again with EIA and ICH test. All testing was performed according to the manufactures instructions.

Results

Among a panel of 42 frozen samples and 17 fresh urine samples tested positive by EIA, 37 and 13 found positive by automated interpretation of BinaxNow respectively. Nine urine samples found negative, but after boiling and retested they were still found weak positive by EIA. It was noted that the sensitivity of automated interpretation increased in comparison with visual reading, namely 13 weak EIA positive samples were visually negative by ICH, but automated reader gave positive result. Among positive tested urine samples, we did not have such one with negative result after boiling. All 61 EIA negative urines were also negative by BinaxNow Reader.

Conclusion

The results obtained by Alere reader interpretation of BinaxNow tests were comparable with results obtained by Binax EIA. Furthermore, it was found higher sensitivity of automated reader in comparison with visual reading.

Detection of *Legionella* using MALDI-TOF

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Keywords
Legionella, Diagnosis, MALDI-TOF

Abstract
Legionella is an environment'bacterium, we can find it both in soil or aquatic area. It is very dangerous to be contaminated by these bacteria wich cause legionellosys disease and can be mortal. They proliferate into organism and cause pneumonia. Official methods for *Legionella* detection are based on the growth of the microorganism in selective medium (ISO 11731). However this selective medium cannot confirm that it is *Legionella*. In this context, we used the MALDI-TOF to be sure about the diagnosis.

The deposition of the bacteria on MALDI plate crystallizes with a chemical compound called matrix. The laser shots on the crystallized deposits of peptides form peptide ions in the source part of the apparatus. These ions will be accelerated and driven into the part of the TOF analyzer of the device to be separated according to the mass / charge ratio. All of this mass information measured for each laser shot is recorded as a spectrum. Each spectrum tells us about a bacterial profile listed in the device database and provides us with a reliable result in minutes.

At least, we obtained a confirmation about the presence or not of *Legionella*.

Validation of a LAMP-based kit for the detection of Legionella pneumophila in environmental samples

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Keywords

Legionella pneumophila, water, LAMP, screening, method validation.

Background

L. pneumophila, the leading cause of a severe pneumonia known as the Legionnaires' disease, is a freshwater ubiquitous opportunistic pathogen. It can be found in all water systems, including air conditioning, showers and cooling towers in a wide variety of facilities, as hospitals, hotels and spas, where it is transmitted to humans through inhalation of contaminated droplets. The available cultural method allows a slow detection and it does not detect viable but non-culturable bacteria, consequently increasing the risk of infection. Therefore the innovative LAMP (Loop-mediated isothermal amplification) method was used to develop a new kit allowing a rapid and labour-saving detection of *L. pneumophila*.

Materials & Methods

After water filtration, the kit "Legionella pneumophila Glow" provides for rapid DNA extraction from membranes with the specific ready-to-use extraction buffer, and the genetic amplification through LAMP technology using the dedicated device ICGENE mini (Enbiotech Cat.N. EBT 801). This kit is under validation in compliance with AFNOR NF148 (2015), so accordingly, sensitivity, inclusivity and exclusivity tests were performed and different contaminated matrices were tested: hot water, mineral water and cooling tower water. In addition, 45 real water samples were evaluated both with LAMP and cultural method to compare the performance of the two methods.

Results

Firstly, sensitivity showed that "Legionella pneumophila Glow" kit can detect up to 28 plasmid copies/µl. As for exclusivity and inclusivity, all the other species resulted negative, while all the *L. pneumophila* serogroups tested showed positive results. Moreover, the contamination experiments showed consistent results, with both contamination levels and the 3 matrices giving reproducible results. Of the 45 real water samples, 29 were negative and 16 were positive for L. pneumophila both with LAMP genomic amplification and the traditional culture method, with 100% consistency between the two methods.

Conclusions

The above-mentioned LAMP kit offers a rapid, cost-effective and sensitive option for the effective screening of *L. pneumophila* in various types of water samples. In this study, all the criteria and parameters requested by AFNOR validation were met, thus suggesting that this kit is suitable for official controls, helping to prevent the spread of the disease.

Enhancing the culturability and qPCR efficiency for *Legionella longbeachae* in sputum specimens using Immunomagnetic Separation

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Keywords
Legionella longbeachae, culture, qPCR, Immunomagnetic separation.

Background
Detecting Legionella bacteria by culture of clinical samples is difficult because contamination may interfere with the culture results and specimens with low bacterial load may be reported as negative. Application of molecular methods such a quantitative PCR has improved detection rates. The protocol at our diagnostic laboratory is to screen respiratory samples for legionella DNA by genus specific qPCR, identify the species by a specific PCR, and culture the positive samples on MWY selective agar following acid wash (AW). Immunomagnetic separation (IMS) could help capture organisms and concentrate the microbial load prior to both culture and DNA extraction for PCR testing. In this study, we examined impact of IMS on stored respiratory specimens which were PCR positive for *Legionella longbeachae*.

Materials and Methods
Residual material from 55 sputolysin treated respiratory specimens that had been submitted to the diagnostic laboratory for testing that were qPCR for *L longbeachae* were retrieved from storage at -80^{o}C. The samples were divided in two and half processed by AW and half treated M280 Tosylactivated Dynabeads coupled to polyclonal antibodies raised against *L. longbeachae sg1*. The treated samples were resuspended and inoculated onto MWY selective media. DNA was also extracted from the IMS treated samples using GenElute bacterial genomic DNA Kit. TaqMan qPCR for *ssrA* gene target was conducted and the results of culture and qPCR were compared with the initial findings. Results from the diagnostic laboratory and retesting were compared using the Mid-p exact statistical test.

Results
The diagnostic laboratory found 17/55 qPCR positive samples were culture positive for *L. longbeachae*. After storage 10/55 were positive on culture after AW (p=0.13) and 27/55 positive on culture after IMS concentration (p=0.05). The CT values on PCR were similar in the diagnostic laboratory (mean 33.95, SD 4.8) and after IMS treatment (mean 33.66, SD 6.1).

Conclusion
Immunomagnetic separation is a promising method to enhance the recovery of the legionella isolates from the clinical specimens and also for the improvement of qPCR crossing threshold (Ct) values in microbiologically complex matrices such as sputum.

Emergence of Legionella maceachernii infection in the United Kingdom

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Keywords

Case studies, *Legionella maceachernii.*

Background

Legionella maceachernii is a rarely identified cause of Legionnaires' disease (LD). There have been only six previously reported cases worldwide. This report relates to two cases from Scotland.

The first was a 70 year old male who presented in September 2014 with a five day history of shortness of breath. He worked as a painter and decorator. Of note, he had a recent chicken pox which had resolved. He was diagnosed with community acquired pneumonia. Additionally, he had an acute kidney injury and raised total white cell count (WCC) with neutrophils. Blood film suggested a diagnosis of a lymphoproliferative disorder, most likely chronic lymphocytic leukaemia.

The second case was a 60 year old female who presented in November 2015 with a dry cough, loss of appetite and chronic diarrhoea. Her complicated past medical history included two renal transplants (2000 and 2015) for polycystic kidney disease, previous thrombus and ulcerative colitis. She kept exotic pets was a non-smoker and had no recent travel history. She had no occupational exposures. Both patients died from the infection.

Materials & Methods

In both cases, *Legionella spp*. DNA was detected by in-house quantitative PCR as part of an initial respiratory screen on respiratory samples and were subsequently culture positive. The first isolate was slow growing and was only culture positive after 14 days and the second sample needed an acid pre-treatment before it was able to be cultured from a heavily contaminated sample containing commensal micro-organisms. Environmental screening samples taken from the patients homes were only incubated for 10 days.

Results

Both cases were confirmed as *L. maceachernii* by *mip* gene sequencing. No clear link was established between the two despite both living within the same geographical region and no clear environmental exposure was identified.

Conclusions

L. maceachernii is a rare cause of LD but could be an emerging disease as it is likely to be under diagnosed by current laboratory techniques. The previous case reports of *L. maceachernii* infection showed that the six patients (5 of which died) all had underlying disorders including: HIV infection, multiple myeloma, pulmonary fibrosis, systemic lupus erythematosus, autoimmune haemolytic anaemia and liver transplantation. These two cases, along with previous case reports, suggest that *L. maceachernii* is associated with a high mortality, causing fatal infection in the immunosuppressed.

Detection of non-serogroup 1 urinary antigen in two patients by using a flow based chemiluminescence sandwich microarray immunoassays (Legiotyper)

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Keywords

Urine antigen, serogroup 3, serogroup 6, Legiotyper, mAb microarray.

Background

Currently most of the legionellosis cases are diagnosed using the urine antigen test. However, this test can reliably detect only *L. pneumophila* serogroup (sg) 1. The “Dresden Panel” of monoclonal antibodies (mAbs) includes mAbs against all sgs. Therefore, we investigated whether specific mAbs of this panel can be used to detect specifically *L. pneumophila* urinary antigen from serogroups other than sg 1.

Materials & Methods

An automated flow-based chemiluminescence sandwich microarray immunoassays (CL-SMIA) platform, MCR 3 (GWK Präzisionstechnik GmbH, Munich, Germany), was used to analyse urine samples of culture-confirmed (non-sg1) legionellosis cases. Monoclonal antibodies against *L. pneumophila* non-sg1 (sg3 and sg6) were immobilized on a microarray chip as capture antibodies. Patient’s urine samples, stored at -40 °C, were thawed and concentrated 10-fold by micro concentration columns (5-kDa separators, Vivascience, Sartorius). A biotine labelled detection antibody specifically recognizes the sg 2-14 of *L. pneumophila*. Bound detection antibodies were visualized using Poly-Strep-HRP followed by the addition of the substrates luminol and hydrogen peroxide. The catalyzed reaction was recorded by a CCD camera.

Results

Here, we report on two cases of culture-confirmed Legionella pneumonias caused by *L. pneumophila* sg3 and sg6, respectively. In both cases we were able to detect *L. pneumophila* antigen by using either sg3 or sg6 specific monoclonal antibodies as capture antibodies and a biotinylated broad-spectrum mAb in the automated flow-based microarray. In both cases, the heterologous system was negative in these two urine samples.

Conclusions

By using mAbs against *L. pneumophila* non-sg1 it seems to be possible to detect urinary antigen from these sgs, which broadens our spectrum of diagnostic methods.

Legionella pneumophila subspecies *raphaeli* prevalence in France: the far side of the moon

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Keywords

Legionella pneumophila subspecies, Sequence type

Background

Legionella pneumophila (*Lp*) is the most common *Legionella* species causing Legionnaires' disease (LD). In the 80s, it has been divided into 3 subspecies based on DNA-DNA hybridization experiments: *Lp* subspecies *pneumophila*, *fraseri* and *pascullei*. More recently, based on Whole genome sequencing data, Kozak-Muiznieks *et al*. have described a 4th subspecies: *Lp* subspecies *raphaeli*.

Materials & Methods

Phylogenetic and ortho ANI analyses were conduct on 267 genomes of *L. pneumophila* to characterize the corresponding strains at the subspecies level. Based on the corresponding STs the prevalence in France of these subspecies were characterised over the last 10 years.

Results

Phylogenetic analyses and ortho ANI analyses identified 3 subspecies: *Lp* subspecies *pneumophila*, *fraseri* and *raphaeli*. From 2007 to 2017, *Lp* subspecies *pneumophila* represent 3739 isolates (95.7%) characterized by 266 STs, *Lp* subspecies *fraseri* represent 2 isolates (0.1%) of 1 ST and and *Lp* subspecies *raphaeli* represent 170 isolates (4.3%) characterized by 4 STs. *Lp* subspecies *raphaeli* prevalence was 4.3% of all clinical isolates from 2007 to 2017 with a minimum of 0% 2007 and a maximum of 8% in 2014.

Conclusion

Whole genome analyses highlighted an unexpected high prevalence of *Lp* subspecies *raphaeli* in French clinical isolates.

Persistent Legionnaires' Disease: series of 12 cases and review of the literature

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Keywords

Legionella, Legionnaires' disease, persistent Legionella infection, recurrent community-acquired pneumonia, Legionella lung abscess.

Background

Legionella is the causative agent of Legionnaires' Disease (LD), characterized by an acute pneumonia. Immunosuppression could favour slow bacterial clearance and result to persistent or recurrent disease.

Materials & Methods

A nation-wide retrospective study was leaded by the National Reference Center for *Legionella* from 2013 to 2017 including cases of persistent or recurrent LD with positive respiratory sample for *Legionella* using PCR or culture over a period of 2 months to 1 year with or without period of clinical improvement or cure during this period.

Results

Twelve cases of persistent or recurrent LD were identified, with a median age of 63 (IQR, 29-82) year-old. Ten (83.3%) patients had ≥ 1 immunosuppression factor. Six patients (50%) had haematological malignancy, of whom 2 (16.6%) underwent hematopoietic stem cell transplant. There were 7 (58.3%) patients under immunosuppressive therapy, among whom 6 had glucocorticoids. 4 (33,3%) patients had hypogammaglobulinemia. First LD episode was a pneumonia with extra pulmonary manifestation(s) in 4 cases (33.3%) and 2 (16.6%) without prior medical record had extremely severe cases with acute respiratory distress syndrome (ARDS). All patients received an appropriate treatment with macrolides and/or fluoroquinolones associated with rifampicin for 14 to 28 days. Three patients had a persistence of disease for a median time of 48 days under appropriate treatment. In the other 9 cases, recurrence of LD occurred after a median symptom-free period of 30 days (IQR 18, 55). There was a third episode of LD in 3 cases. Microbiological diagnosis of recurrence was made with positive culture or *Legionella* positive PCR in a respiratory sample. No *Legionella* resistance to antibiotic nor genetic evolution of strains have been demonstrated. Imagery showed lung abscess in 5 cases (41, 6%). Only 1 required surgical resection. The mechanism for other cases was suspected to be a reinfection or a clearance defect of *Legionella* that would favor relapse. One patient died of LD and 2 of another cause.

Conclusions.

Persistent LD can be explained by cavitation, relapse of infection with the same strain by clearance defect and reinfection with another strain. Immunocompromised heamatological and hypogammaglobulinemia patients are predominantly affected. Treatment prolongation may be discussed in selected immunocompromised patients and end treatment CT scan may be performed.

Domestically acquired Legionella infections. Seek and you shall find.

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Background. Legionella colonization of residential premises is well known. However, confirmed domestically acquired infections are rarely reported in the literature. Seventy-nine such incidents are presented. Samples were taken in various housing estates across the Czech Republic and also from single family houses connected to private wells.

Methods. Accredited laboratory procedures were used for PCR detection, culture, serological urine testing and sequence based typing (SBT).

Results. The first group comprised 39 culture confirmed cases and the second 40 urinary antigen (UAg) positive cases with associated environmental isolates. *Legionella pneumophila* sg.1 dominated among the clinical strains (67%); a majority (77%) possessed the virulence–associated epitope recognised by the monoclonal antibody Mab 3/1 and . ST62 was the predominant sequencing type (38.5%). The isolation of *L. pneumophila* sg.3,4,6,9 and12 indicated that susceptible persons, especially those receiving immunosuppresive therapy, could contract non-serogroup 1 *Legionella* which have been missed by the UAg test. The group of 40 UAg positive cases showed a similar picture with ST62 making up 33% of all sg.1 The remainder consists of ST641, ST1, ST42, ST23 and others. A large cluster of *L. pneumophila*sg.1. Mab Knoxville, ST641 was identified in a Bohemian city. Between 2012-2018, 14 cases were recognized; 7 persons had predisposing factors (IMS, COPD, Diabetes) and one death. There was a good congruence among clinical and environmental isolates. A subsequent risk assessment in the city confirmed that some blocks of flats received the virulent *L.pneumophila sg.1* strains directly from local plant facilities of hot water providers. Currently, the Czech legionella legislation does not cover water systems in residential premises. Consequently, public health officers have no powers of authority to make owners introduce remedial measures.

Conclusions. The data presented confirm that domestically acquired infections are underreported. The presence of known virulent sequence types in water appears a much higher risk than the „artificial *Legionella* species risk levels“ derived from the legislation. Prevention of domestically acquired Leginonnaires Disease cases is difficult and routine disinfections of large housing complexes cannot eliminate legionella from their pipeworks.. Preventative measures should include a clinical notification of patients who are at risk.

A harmonized protocol for *Legionella* enumeration via qPCR and ISO 11731 from the same filter

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Keywords
Real-time PCR, viability PCR, ISO 11731.

Background
Determination and control of *Legionella* spp. - especially *Legionella pneumophila* - in environmental water samples is pivotal for public health. As some sample types like water from pools, fountains or cooling towers prove challenging for culture due to residual disinfectants or high accompanying flora, quantitative real-time PCR is gaining popularity as a robust alternative method. However, regulations worldwide define action levels solely by cultural cfu counts, which are sometimes erratic and cannot adequately be reproduced by qPCR. To confirm the safety of an installation both methods should therefore be used, ideally on the same filter to avoid variation introduced by sampling. This not been possible with existing PCR methods as DNA extraction requires on-filter lysis of cells.

Materials & Methods
Rinse Buffer reagent developed by BIOTECON Diagnostics was used to allow for efficient recovery of *Legionella* spp. from filters for viability-qPCR applications. Bacteria stay viable in Rinse Buffer and can also be plated on agar. The recovery of this method was compared to the washing procedure set forth in ISO 11731:2017. qPCR analysis was carried out with the Reagent D viability reagent and the **micro**proof® *Legionella* Quantification LyoKit. Culture was conducted according to ISO 11731 on BCYE agar.

Results
Recovery from filters was significantly better with BIOTECON's method compared to the ISO protocol. *Legionella* enumeration by culture benefited mostly from modifications in the flushing procedure. Use of Rinse Buffer was essential for viability-qPCR as it apparently protects viable cells from intercalating agents such as Reagent D. Despite ISO 11731:2017 allows only a very limited set of methods and buffers a protocol could be developed combining an ISO-compliant washing procedure with Rinse Buffer treatment of one aliquot for qPCR analysis while the other aliquot can be plated.

Conclusions
An ISO-compliant protocol has been devised which conveniently allows to conduct both viability-PCR and ISO 11731:2017 on the same filter, thus reducing hands-on-time and eliminating sample-to-sample variation. Using this protocol it is also possible to first conduct a screening by PCR and later plate only positive samples for cultural enumeration. Enhanced recovery resulting from our processing method and use of Rinse Buffer also indicates that current procedures in ISO 11731 are not optimal and may be improved in the course of future revisions.

ULISENS: Automatic early warning system to detect and quantify *Legionella* species in water determination.

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Keywords

Legionella, automation, immunosensing, on-site, prevention.

Background

Early detection of *Legionella* sp concentrations is essential to avoid inadequate or unnecessary disinfection treatments. Culture confirmation takes 10 to 12 days, delaying the identification of potential infection sources. Faster, but accurate alternatives are needed. Use of immunomagnetic separation-based method for *Legionella* sp quantification as a fully-automated system could provide dramatic improvement in time-to-result to shortening decision-making process. We describe an automated immunosensor, ULISENS, including filtering module and a disposable reagents cartridge that allows for rapid determining of *Legionella* levels (< 2 hours).

Materials & Methods

Magnetic immuno-beads provide the separation of the whole cell target from the rest of the sample and their concentration. This attachment is mediated by antibodies immobilized onto the surface of the beads and the antigens expressed on the surface of *Legionella* cells. The ULISENS immunosensor is based on the principles of an immunomagnetic separation (IMS) based method combined with colorimetric enzyme-immunoassay. Incorporated automated filtering module allow for rapid and efficient water samples concentration. An optical reader provides easily accessible digital readouts of *Legionella* concentration measurements.

Results

The present study prospectively evaluated the ULISENS device as a reasonable approach for *Legionella* quantification and could produce results in as few as 2 hours with no downstream workup. The performance of the completely automated immunosensor approaches laboratory-based test (Legipid®) and allows quantitative and automated analysis of *Legionella* levels with reporting times compatible with on site applications without hands-on steps prone to human error. Performance parameter was also comparable (sensitivity 100%; specificity 91.7%; Accuracy 96%).

Conclusions

On site measurement of the *Legionella* sp concentration will greatly facilitate the timely steps and management of potentially risk sources. We developed a rapid method based on the immuno-magnetic separation combined with enzyme-immunoassay for the quantitative determination of *Legionella* sp in water samples. The aim of this work was to adapt this method to develop a completely automated device able to perform on site.

Legionella pneumophila sg.1 in hospital DH and CW systems: influence of engineering controls and major earthquakes over 15 years

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Keywords
Legionella controls; Hospital Hot and Cold-water supply; Maintenance and Engineering; major earthquakes.

Reports on *Legionella* surveillance in Christchurch Hospital's domestic hot (DHW) and cold water (DCW) were presented at the International *Legionella* Conference 2005 and 2013, and ICPIC 2013. Major earthquakes struck Christchurch 2010 &11. This review compares *Legionella* surveillance results and *Legionella* engineering controls in both DHW & DCW "pre" (2003-10) and "post" earthquakes (2011-17). The hospital has its own water supply colonized with a specific *L. pneumophila (*(L.pn) sequence type.

Materials & Methods

- Water: Cold (CW) and DHW samples were filtered using a 0.2µ Nitrocellulose filter. The resuspended filtrate was cultured onto BCYE and MWY media. (10^2cfu/L limit of detection).
- Swabs (interior surfaces of showerhead) cultured onto BCYE and MWY.
- Engineering controls
  Well-water storage: post: 5 additional tanks to the 3 pre; DCW: recorded failure of the UV light treatment pre-twice, post 9 times.
  DHW: Calorifiers temperature 80°C, DHW circulation 60°C. Return 57-58.7°C.
  Water sampling: CW supplying Calorifiers and DHW after tempering (2 sites) 12 each /year.
  Shower roses sampled twice to 4 times/year depending on patient risk. The mixers were disinfected or changed after positive results.
- Summary of nosocomial infections and related water sampling
- Statistical significance: p-value calculation.

Results

Positive *Legionella* isolates**:** Number isolates/total samples

Water samples CW for Calorifiers: Pre: 7/96; Post: 11/84. P-value 0.1.

DHW sampled near tempering sites with UV exposed CW: Pre. 3/192; post 32/168

P-value 1.56E-08 Colonisation level 1-4 10^2cfu/L all samples

Swab samples positive "Pre" (2003-2010) 12/4490; "Post" (2011-2017) 43/5572. P-value 0.00016

Nosocomial infection Pre:1, post 2.Positive water samples (CW): Water coolers: 1 pre and 1 post period; CW from hand basins: 1 pre and 1 post period.

Conclusions

The increase in positive shower roses "post" and negative DHW (60°C) *L.pn* culture questions the contribution of the DCW in colonising the patient side of shower roses. Positive cultures from CW storage tanks and UV exposed DCW from basin taps and water coolers have been obtained. Positive DHW samples taken close to tempering sites increased post-earthquake. Two of three bedbound nosocomial infected patients had received oral care with tap water. This question the contribution of DCW in nosocomial *L.pn* infection and contribution of the earthquakes to the increase in *Legionella* isolation. Limit of detection needs to be 10^2 cfu/L.

Presence of Legionella in water distribution systems of prisons and schools in Sicily (Italy)

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Keywords

Legionella, water contamination, prison, school.

Background

Legionella spp. is widely present in soil and freshwater but that can also contaminate water systems; it replicates between 25 °C and 42 °C surviving at higher temperatures. Infection is acquired by inhalation, aspiration, or micro-aspiration of *Legionella*-carrying aerosols. Droplets carrying the pathogen can originate by water spraying or by gurgling air through contaminated water. Cases of Legionnaires' disease have been reported in the last years worldwide. Most reports come from high-density community housing, such as prisons or schools. Water Distribution Systems (WDS) are often involved as were the ultimate sources of the infection. This study evaluates *Legionella* contamination in the WDS of the 22 prisons and 13 schools located in Sicily (Italy).

Materials/Methods

The project was proposed and funded by the Regional Health Department. Samples were collected by the three Sicilian Legionella Reference Laboratories in collaboration with the local Public Health Departments. Both prisons and schools, samples were from central lines (water storage tanks and boilers) and from distal sites (faucets and showers selected on the basis of distance from boilers). The water samples, both those carried out in schools and those from the prisons, were taken from the water circuit both hot and cold. Isolation and enumeration of *Legionella* was performed by cultural method (ISO 11731). The isolates were identified on the basis of cultural and serological features.

Results

149 samples of water were collected from 22 prisons and 61 from 13 schools. *Legionella* was isolated from 19 prisons for a total of 86% and from 5 schools (38%).

From the 19 positive prisons, 4 strains of Legionella pneumophila sgr 1 and 44 of Legionella pneumophila sgr 2-14 were identified; from schools 1 of Legionella pneumophila sgr 1; 8 strain of Legionella pneumophila sgr 2-14 and 2 of Legionella gormanii.

Conclusion

Data show a higher contamination of the whole WDS, both cold and hot water and suggest that not only hot water supply may be a source of infection. Anyway, *Legionella* monitoring of cold and hot WDS should be recommended in all those facilities that host vulnerable populations. Prison and school health services should work closely with national and local health services, in order to provide the same standard of care as local hospitals and communities.

Algorithm to increase yield of identified infectious sources among sporadic community-acquired cases of Legionnaires' disease

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Keywords

Legionnaires' disease, infectious source, drinking water, case-control study

Background

There is little sound evidence about the sources of infection of community-acquired Legionnaires' disease (CALD). Reasons are that usually a genotypic match is required to consider an infectious source as confirmed. However, even under study conditions it is difficult to obtain both typeable patient AND water samples usually not yielding more than 5 % of patients with a confirmed infectious source. We have attempted to introduce a more "sensitive" methodology with the goal to still provide a reasonable rationale to determine if a potential source was causative or not.

Materials & Methods

As part of a study conducted in Berlin since December 2016 (the LeTriWa study), we have collected the following material for patients with CALD: (1) detailed questionnaire, (2) urine and/or respiratory samples of patients, (3) warm water (and biofilm) samples from the patient's household and (if possible) of the entire residential drinking water (RDW) system, (4) water (and biofilm) samples from potential other sources outside the patients' residence (external source). Patient and water samples have been cultured and typed for monoclonal antibody type and – if appropriate – sequence type. Because (a) cases were almost always caused by *L. pneumophila* SG1 with monoclonal antibody (MAb) type 3/1, (b) in the past such strains have been rarely seen in water samples unrelated to cases, and (c) MAb type Knoxville (one type of MAb type 3/1) has been shown to be associated with the occurrence of CALD in this study (see other abstract), we considered a water source as the infectious source if it contained at least one strain of MAb type 3/1.

Results

We recruited 54 patients of CALD. We identified a strain with MAb type 3/1 in samples of an external source in 6 (11 %) patients and in samples of RDW of 12 (22 %) patients. Low pathogenic Legionella were found in RDW samples of 18 (33 %) patients and no Legionella was identified in RDW samples of 18 (33 %) patients.

Conclusions

With a source identification rate of 33 % we have substantially raised the proportion of patients with attributed infectious sources. However, two thirds of patients remain with an unidentified source. In one third of patients no Legionella could be identified in RDW samples. Limitations are that potentially attributable water systems may have undergone changes since the transmission event. Nevertheless, results suggest that there may be other sources or types of samples that need to be explored.

Prevalence of *Legionella pneumophila* in hot water systems in Morocco and risk factors associated with contamination

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Keywords

Legionella pneumophila, Infectious disease, Environmental surveillance, Water-related infection, Hot water distribution system.

Background

Legionella pneumophila is a Gram-negative waterborne pathogen causing a severe form of pneumonia known as Legionnaires' disease, which is commonly acquired by inhalation of aerosol particles originating from contaminated man-made water systems. Sources of infection were indeed demonstrated to be hot water systems, cooling towers, spa pools, etc.

Materials & Methods

The prevalence of *Legionella pneumophila* in hot water systems of residential facilities in Morocco was performed during the period from January 2015 to April 2018. A total of 132 water simple from 98 different water supply systems were analyzed. Possible risk factors were prospectively recorded. The minimum inhibitory concentrations (MICs) of environmental strains were tested using broth dilution and the susceptibility test was performed for thirteen antimicrobial drugs from Macrolide, ketolide, Fluoroquinolone and authors antibiotics family.

Results

Out of the 132 samples, 66 (50%) were positive for *L. pneumophila*. Serological typing of 66 *L. pneumophila* isolate revealed that 47 (71.2%) are *L. pneumophila* serogroup2-15 and 19 (28.7 %) are *L. pneumophila* serogroup1. Contamination was strongly correlated with temperature in the circulation, but not with the age and size of the building. All tested strains of *L. pneumophila* were inhibited by low concentrations of antibiotic family. Rifampicin was the most effective antibiotic against the isolates in vitro.

Conclusions

This finding shown a relevant exposure to *L. pneumophila* in the community. Water systems compliance to current technical standard are a major concern.

Legionella contamination in water supply systems – a five-year survey in Latvia

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Keywords

Legionella, legislation, criteria, apartment buildings

Background

In Latvia, almost 90% of residents live in multi floor apartment buildings, which were built before year 1990. At least in 55% of apartment buildings internal water distribution networks have never been renovated. Moreover, there is no national legislation regarding permitted levels of *Legionella pneumophila* contamination in internal water distribution networks and regular control of *L.pneumophila* is not carried out. Therefore aim of this study was to obtain comprehensive data for assessment of occurrence of *L.pneumophila* in apartment buildings in Latvia.

Materials & Methods

Overall 1043 samples were tested for presence of *Legionella* spp. according to ISO 11731. Sampling was performed in 273 randomly selected multi floor apartment buildings in all regions of Latvia during years 2013 – 2017. In total, 19 sediment samples, 315 cold water and 709 hot water samples were taken. Temperature measurements were performed for water samples.

Results

During five – year survey period, at least once *L.pneumophila* was observed in 160 of 273 (59%) apartment buildings. In capital of Latvia, prevalence was even higher and reached in 66% (119 of 180 buildings). Occurrence of *L.pneumophila* differed significantly ($p<0,05$) for different types of samples. The highest occurrence was observed in hot water samples, where 52% of samples (372 of 709) tested positive. 23% (73 of 315) of cold water samples were *L.pneumophila* positive as well, while occurrence in sediment samples reached 11% (2 of 19 samples). In addition, it was observed that in 76% of cases hot water temperature did not exceed 50°C at the point of water consumption.

Conclusions

Evaluation of the survey results indicates that prevalence of *L.pneumophila* in water supply systems of apartment buildings in Latvia is very high. Conditions in internal water distribution networks are suitable for multiplication of *Legionella* and may pose serious risks for public health. On basis of this survey, first changes in national legislation have been proposed. Since the end of year 2017, minimum temperature threshold for hot water at the exit of water heating unit is defined and criteria for assessment of disinfection efficiency are set.

Diversity of *Legionella* in hot water distribution systems in Latvian hotels

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Keywords

Legionella, hotels, hot water, sequence type diversity.

Background

Legionnaires disease is often associated with staying in hotels. Different guidelines and control measures have been recommended to control proliferation of *Legionella* in man-made water systems, however they have not been widely introduced in Latvia. Also, there is no data on the prevalence of *Legionella* in water supply systems in Latvian hotels. The aim of this study was to investigate the sequence type diversity and occurrence of *Legionella* in hot water distribution systems.

Materials & Methods

Overall, 240 hot water samples from taps and showerheads were collected from 40 hotels in all regions of Latvia. In each hotel 3 water samples were taken before flushing and 3 samples after 3 min flushing. Isolation and identification of *Legionella pneumophila* was carried out according to ISO 11731. Genotyping was conducted according to the standard Sequence-Based typing method of the EWGLI using 7 genes ().

Results

At least one *Legionella* positive sample was detected in 26 of 40 hotels (65%). Overall, 108 of 240 samples (45%) contained *Legionella* up to 1,1 x 10^4 cfu/l. 99 isolates were identified by MALDI-TOF MS as *L.pneumophila* and 9 isolates as *L.rubrilucens*. The most common sg was sg 3 (68 isolates), followed by sg1 (13 isolates), sg 2 (10 isolates) and sg 6 and 9 (four isolates each). In this study, 26 different sequence types were detected, including four new sequence types. According to EWGLI database, 20 of detected sequence types were associated with human disease. Despite the broad overall diversity of sequence types, high diversity was not observed within a single hotel and no more than two different sequence types were detected.

Conclusions

Better understanding of molecular diversity and better identification of strains would provide a basis for more targeted intervention measures.

Legionella direct detection and identification using 16S MinION sequencing

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Keywords

Legionella diagnosis, Identification, 16S rRNA, MinION sequencer.

Background

Legionella molecular diagnosis is fast but usually allows only the differentiation of 2 groups: *Legionella pneumophila* and the other species. Amplicon sequencing by NGS technology offer the possibility of identifying at the species level the *Legionella* detected in the sample.

Materials & Methods

DNA from 31 clinical samples (sputa, BALs, bone join fluid, sera…) from 15 patients infected by *Legionella* (20 *L. pneumophila* and 11 *Legionella* non *pneumophila*) were submitted to 16S rRNA PCR and amplicon sequencing by MinION sequencer.

Results

Legionella sequences were detected in 19 samples from 8 patients. *L. pneumophila* were identified in 14 samples from 3 patients including one serum. *Legionella* non *pneumophila* were identified in 5 samples of 5 different patients including one bone join fluid.

Conclusions

Despite a lack of sensitivity, this method allows the rapid simultaneous detection and identification of at the species level *Legionella* present in various clinical samples.

Monitoring of Legionella in the waters flowing in establishments and dwellings of the city of Abidjan-Cote d'Ivoire

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Keywords

Legionella, sanitary risk, PCR, Hotel, Residential.

Background

In Côte d'Ivoire, legionellosis is under diagnosed or even unknow because the diagnosis of pulmonary infections is more oriented towards tuberculosis. Today, health safety requirements require public facilities to conduct microbiological water quality monitoring to monitor Legionella. Thus, to prevent this risk, certain hotel, hospital, thermal power plant and other structures request the services of specialized laboratories. It is within this framework that this laboratory has had to analyze circuit water from the city of Abidjan. The objective of this work was to highlight the health risk related to the presence of legionella in waters flowing in public institutions and homes from 2015 to 2017.

Materials & Methods

Water samples were collected from hotels, health centers, thermal power stations and dwellings in Abidjan from 2015 to 2017. These analyzes were carried out by conventional PCR at the Institut Pasteur in Côte d'Ivoire. DNA extraction was done with the Quiagen kit. The data was collected from the Laboratory's archives. The collection and exploitation of the data was made from the Microsoft Excel software

Results

A total of 169 water samples were analyzed. This was 88 (53.99%) samples taken from hotels, 45 (27.60%) from a thermal power station, 27 samples (16.56%) of dwellings and 3 samples (1.84%) taken from an health facility.

59.51% of all the samples were negative vs 40.49% of the samples that contained Legionella DNA. The home samples were 81.48% contaminated. The hotels, on the other hand, recorded the least positivity with 23 positive samples that is 26.14% against 65 negatives or 73.86%.

Conclusions

This study evaluated the microbiological health quality linked to the presence of Legionella in 169 water samples between 2015 and 2017 in Abidjan. All sites studied are affected by contaminations in Legionella.

So the risk to have legionelosis exist. It is therefore necessary and urgent to proceed with the treatment of infected circuits and adapt diagnosis protocol in hospitals

Biofilm production and typing of *Legionella pneumophila* serogroup 1 in Sicily

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Keywords

Legionella pneumophila, Sgr1, typing, Biofilm.

Background

The present typing method of *Legionella pneumophila* suggested by the European EWGLI Consortium is a sequence-based typing (SBT).This method is very manageable and extensively used to perform epidemiological surveys and out-break analysis. Recent studies have established the values of using the multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA) and the diversity of clustered regularly interspaced short palindromic repeat (CRISPR)-based typing technique (Spoligotyping) as genotyping markers.

The persistence of microorganism in natural environment are mediated by biofilm formation and recent reports suggest that the growth of Legionella in biofilms may lead to enhanced the virulence.

In this study we compared the three methods for typing of *L. pneumpophila* and their ability to biofilm formation.

Materials & Methods

For the study, 40 strains of *L. pneumophila*, belonged to serogroup 1, were analysed. The strains evaluated were isolated from 2 clinical samples and 38 environmental samples.

SBT alleles were coded following the EWGLI protocols and minimum spanning tree was made using BioNumerics 6.5. For VNTRs typing 12 VNTR loci and MLVA clustering was performed by Hamming's distance and UPGMA. The Spoligotyping was performed by Luminex devices.

The quantization of biofilm production was conducted according to Donelli et al. 2014.

Results

SBT analysis discriminated ten different genotypes (STs). In particular, only one clinical isolate showed the same ST of environmental strains obtained from the structure in which the subject was hospitalized.

Moreover through the MLVA analysis four different clonal complex (VACCs) were identified.

Regarding to biofilm formation the 40% was non producers, the 15% was medium producers and the 35% was low producers while none strain was high producers.

Conclusions

Phylogenetic analysis in *L. pneumophila* is a difficult task considering the enormous genetic differences observed in the accessory genome of this species. However, by using VNTR polymorphism and Spoligotyping is possible to group isolates within complex which are highly congruent with SBT clustering results and to provide further insight into relationships among these groups.

In this study we demonstrate that typing by the three methods provides valuable information for epidemiological studies and for identification of clonal complex in L. pneumophila.

However none correlation was found between clonal complex and ability to produce biofilm.

Molecular typing of *Legionella pneumophila*: cluster in two Sicilian prisoners.

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Keywords

Legionella, Typing, Cluster, Prison.

Background

Legionella is an intracellular microorganism living in natural and artificial aquatic environments, in particular *Legionella pneumophila* serogroup 1 (Lp1) is the most common etiological agent causing Legionnaires' disease. Water systems provide optimal growth conditions for Lp and help its transmission by generating aerosols. Then, the microbiological aspect of an investigation is to seek evidence linking the source of the outbreak to the cases, by comparing Legionella isolates from environmental and clinical samples. Accurate discrimination among Legionella isolates is important in order to identify cases with a common source of infection and the transmission routes of the microorganism. The current typing method of Lp recommended by European Working Group for Legionella Infections (EWGLI) is a Sequence-Based Typing (SBT). High-resolution genotyping of Lp isolates can be achieved also by Multiple-Locus Variable-number tandem-repeat Analysis (MLVA).

Materials & Methods

In this study, we analyzed ten different colonies suspected to Legionella strains isolated from sputum of two patients admitted with severe pneumonia, imprisoned in Palermo. However, ten different colonies from water samples were considered. The serological typing of Lp was assessed using polyvalent and monovalent antisera. Subsequently, the isolates were typing by SBT and MLVA.

Results

Colonies of presumptive Legionella organisms were confirmed by serotyping and all were identified as Lp 1. The molecular investigation showed that Lp strains from sputum and water samples had the same SBT and MLVA profile and we identified a new strain, identified as ST 2451.

Conclusions

Accurate discrimination among Legionella isolates is important in order to identify cases with a common source of infection and the transmission routes of the microorganism. Phylogenetic analysis in Lp is a problematic task considering the vast genetic differences detected in the genome of this specie. Cases of Legionnaires' disease at prisons have been reported in the last years worldwide. In order to limit the scale and recurrence of outbreaks, Legionella monitoring of water distribution systems should be recommended in prisons, because penitentiary populations could contain vulnerable people. We confirmed that, in our cases, typing by two methods provides valuable information for epidemiological correlation between clinical and environmental isolates.

Whole-genome sequencing in the investigation of a Legionnaires' disease case

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Keywords

Legionella, culture, WGS, SBT, genotyping

Background

A nosocomial Legionnaires' disease (LD) case was suspected last summer. Culture of *Legionella*, promptly performed from hospital hot water samples collected in the ward, was negative. However *Legionella* were previously found in few samples and at low level, and colonies were stored. The aim of the study was to use whole-genome sequencing (WGS) in the investigation of the LD case. The draft genome of two isolates from the patient with suspected nosocomial LD was compared with genomes of isolates from other patients and water sources in the hospital in order to identify the source of infection.

Materials & Methods

WGS was performed for 14 isolates of *L. pneumophila* serogroup 1 (Lp1) subgroup Philadelphia ST1. Isolates from BAL and from blood of the patient, isolates from seven subjects epidemiological unrelated to this case in Piemonte and five hospital related environmental isolates were included in the study to create a basis for comparison. The Lp1 Philadelphia ST1 environmental isolates examined were detected sporadically at very low level in the hospital hot water system during the last two years, while prevalent and historically hospital environmental isolates were Lp1 Bellingham ST59. Hot water temperature is normally high in the hospital and after a thermal shock no other cases were observed. The 14 Italian isolates were compared to 19 Danish clinical and environmental Lp1 Philadelphia ST1 isolates.

Results

The single nucleotide polymorphism (SNP) analysis showed that the Italian isolates form a separate cluster distinct from the Danish ST1 Philadelphia. The patient isolates and one water isolate showed no SNP differences and can be considered as highly related. The other hospital environmental isolates show only few SNP differences (≤ 4). Three clinical isolates considered epidemiologically unrelated were near in the cluster with only 3 to 6 SNP differences to the isolates from the suspected nosocomial case. The other isolates did not cluster together with these isolates and belonged to other linages. WGS results must be viewed however in context with epidemiology. Lp1 ST1 isolates can show very little genetic variability, despite the fact that they are unrelated epidemiologically.

Conclusions

Phylogenetic analysis showed close relatedness between one patient isolate and a strain found in hospital water. WGS can be a useful tool in the investigation of LD cases and provide genotyping resolution to distinguish genetically distinct linages.

PCR-based subtyping of Legionella pneumophila serogroup 1 from respiratory material

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Keywords

mAb type 3/1, lag-1 gene, PCR, Legionnaires' disease.

Background

Most cases of legionellosis are caused by Legionella pneumophila serogroup 1 strains carrying the virulence-associated lipopolysaccharide (LPS) epitope, the so-called Pontiac group. However, commercial PCR kits only allow differentiation up to the serogroup. So far, the Pontiac group is identified by its reactivity with the monoclonal antibody (mAb) 3/1. This subtyping usually requires a positive legionella culture in order to have enough antigen. Therefore, the PCR of the lag-1 gene encoding the O-acetyltransferase and associated with the presence of the LPS epitope should be established for the direct subtyping of respiratory samples.

Materials & Methods

Based on 67 published Legionella pneumophila lag-1 gene sequences, a real-time PCR approach with a primer pair and a TaqMan probe was developed. The performance of the lag-1 PCR was compared with commercial Legionella pneumophila PCR (AnDiaTec GmbH, Kornwestheim, Germany). Lag-1 positive and negative stains were used for the specificity tests. Furthermore, the DNA of twenty-four respiratory samples, mainly sputa from community-acquired cases collected in a large-scale study (LeTriWa project) and previously classified as positive by commercial PCR was examined for the presence of the lag-1 gene.

Results

PCR analysis of nine lag-1 positive strains (mainly ATCC type) of the Philadelphia, Knoxville, Benidorm and Bellingham subtypes showed successful amplification of the lag-1 gene region, confirming that all lag-1 gene variants were detected. No signals were obtained for lag-1 negative strains indicating that the PCR is specific. Based on a standard curve analysis, lag-1 PCR performed similarly to the commercial PCR. The lag-1 gene was detected for seventeen of the twenty-four respiratory samples from the LeTriWa project. Fifteen cases with lag-1 positive results were also mAb 3/1 positive by direct subtyping of urine with our in-house ELISA. For four mAb 3/1 positive cases, the lag-1 gene could not be detected, which is most likely due to the limited amount of legionella DNA, as very high Ct values were obtained with commercial PCR.

Conclusions

A lag-1 PCR was established which has good performance characteristics compared to a species-specific PCR and enables direct subtyping of respiratory samples. Lag-1 PCR results for samples from community-acquired cases are consistent with previous mAb 3/1 reactivity data. However, further samples need to be analysed to confirm our preliminary results.

Subtyping of *Legionella pneumophila* Sequence Type 1 Belgian strains with Pulsed Field Gel Electrophoresis

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Background

Legionella pneumophila (*Lp*) is an aquatic bacterium responsible of Legionnaires' disease. To discriminate between *Lp* strains, Sequence Based Typing (SBT) is the gold standard typing method. In Belgium, the world-wide distributed Sequence Type (ST) 1 and the northwest-European ST47 constitute 44% of clinical cases. Due to the wide distribution and presence in the environment of *Lp* ST1, further discrimination of such isolates is needed. Therefore, we applied the Pulsed Field Gel Electrophoresis (PFGE). The PFGE was also performed in order to determine the pulsotypes distributed in Belgian's clinical cases and to help in the interpretation of environmental investigations.

Materials & Methods

Agarose plugs of *Lp* ST1 strains isolated from clinical samples of the Belgian collection (2000-2017 / N=42) and water samples related to 3 patients were subjected to *SfiI* digestion and run on PFGE. The profiles obtained were classified in comparison to the Paris reference strain CIP107629 as follows: pulsotypes with 1-3 bands of difference were considered as Paris-related and pulsotypes with >3 bands of difference were considered not related.

Results

The PFGE profiles obtained for the ST1 clinical strains were classified into three groups: pulsotype A1 (n=25, 59.5 %) similar to the Paris strain, pulsotype A2 to A7 (n=9, 21.4%) related to the Paris strain, and pulsotypes B to G (n=8, 19%) unrelated to the Paris strain. For 3 environmental investigations, water and clinical ST1 strains were compared: a match was showed for 2 cases (similar A6 and E pulsotypes respectively), confirming thereby the infection source. For the third case, different pulsotypes were obtained discarding the presumed infection source.

Conclusions

The *Lp* ST1 clinical strains in Belgium could be differentiated into 16 PFGE profiles, with the Paris pulsotype being the most frequent one, as in France. PFGE results were useful in environmental investigations related to ST1, especially for pulsotypes unrelated to the prevalent Paris clone. In 2 cases (A6 and E pulsotypes), the source of infection was confirmed. In one case, the profile of the clinical strain was totally different from the environmental one, showing that SBT is not enough to confirm the infection source. These examples underline the importance of combining the 2 typing methods. Nevertheless, the core genome Multi Locus Sequence Typing is under development and shows higher discrimination than these classical typing methods.

Legiolert™, the next generation enzymatic test for *Legionella pneumophila*

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Keywords
Legiolert™, *Legionella*, specificity, sensitivity.

Background

Legionella pneumophila (LP) is an opportunistic pathogen of great concern worldwide as a result of the large number of outbreaks associated not only with cooling towers but also with other sources including potable and shower water. In Portugal alone, two large LP outbreaks occurred with close to 20 deaths and accounting to more than 400 people infected. Currently, there is no legislation in Portugal for the detection of LP in water other than thermal waters. A technical standard value of 0 CFU per L was established and ISO 11731:1998 was the chosen method. Real time quantitative PCR (qPCR) is another method widely used globally for the detection and quantification of LP. A novel alternative was developed based on the Most Probable Number (MPN) for the determination of LP. Legiolert™ is introduced in a blister pack format as a powdered reagent for the testing of 100 mL of potable water and 1 mL of nonpotable water. Legiolert™ employs a selective formulation developed by IDEXX for the detection of LP following incubation at 39 ºC and 37 ºC during 7 days with humidity, for potable and nonpotable water sources, respectively. If LP is present, the test will produce any combination of brown pigment and turbidity, rendering a confirmed positive result. Quantification is obtained by the MPN technique. In the present study we report the results of a comparison between both ISO 11731:1998 and qPCR for the detection and quantification of LP in potable and nonpotable water samples.

Materials & Methods

Legiolert is a commercially available test consisting of two supplement solutions in conjugation with the Legiolert™ powder. For potable water, the total hardness was measured and depending on the results, 0.33 mL or 1 mL of hardness supplement was added to 100 mL of sample. To the sample, the content of a Legiolert™ blister was added and the mixture was agitated. Samples of nonpotable water were mixed in a ratio of 1:1 with a pretreatment used to remove background flora and incubated for 60 s at r.t.. Two mL of the mixture was added to 100 mL of diluent (sterile distilled water) and the content of a Legiolert™ blister was added to the sample. The mixture (potable or nonpotable) was poured into the Legiolert™/Quanty-Tray® samples were incubated with the wells facing upwards at 39 ± 0.5ºC for potable waters and 37 ± 0.5ºC for nonpotable water in a humidified environment. Legiolert™/Quanty-Tray® samples were visualized after 7 days for the presence of brown color and/or turbidity. For both ISO 11731:1998 and qPCR, samples were filtered and resuspended in 5 mL of water from the sample and processed accordingly.

Results

Samples from the entire Portugal were analysed over the period September 2017 to February 2018 and the samples were from nonpotable origin (cooling towers, and air conditioning) and potable water (cold and hot tap water, shower water, and thermal waters) from different origins (hospitals, industry, domestic, among others). Enumeration with Legiolert™ ranged from <1 to 4.28log MPN L-1 (mean 3.42log), from 0 to 5.38log GC L-1 for qPCR (mean 4.03log) and from 0 to 2.81log CFU L-1 (mean 2.34log). Legiolert™ presented the highest percentage of positive samples for LP (22%) followed by qPCR (17%) and ISO 11731:1998 (6%). The paired results between the different techniques varied between 78 % for ISO 11731:1998 vs. Legiolert™ and 90% for qPCR vs ISO 11731:1998. Only 28% of the samples analysed were positive for the three methods, 44% were positive for Legiolert™ and qPCR, 28% for Legiolert™ and ISO 11731:1998 and finally 28% were positive for qPCR and ISO 11731:1998. McNemar test was conducted to determine quantitative differences between methods. The results have shown that quantification using Legiolert and qPCR were not statistically significant ($\rho = 0.188$), whereas for the remaining pairs the difference was statistically significant ($\rho < 0.05$). Correlation analysis was used to investigate the quantitative relation between the different methods. The concentrations of Legiolert™ and qPCR did not show any correlation (Spearman rank order correlation $r = -0.19$). The correlation between Legiolert™ and ISO 11731:1998 levels showed a higher association ($r = 0.38$). qPCR and ISO 11731:1998 were strongly correlated in the samples ($r = 0.62$).

Conclusions

Legiolert™ is a valid alternative to the commonly used methods for the detection of *Legionella pneumophila* in water samples with the advantages of being easy to perform, avoiding the need for highly trained personnel, and without the need for very specialized equipment to obtain a confirmed result.

Occurrence and viability of *Legionella* bacteria in industrial cooling waters

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Keywords
Legionella, culture, qPCR, viability, cooling water.

Abstract
According to European Technical Guidelines, cooling systems should be treated with biocides if *Legionellae* are present in numbers exceeding 1000 CFU/L. Next to culture, quantitative PCR (qPCR) techniques offer possibility to estimate occurrence and viability of *Legionellae*. Herein, we studied *Legionella* from metal industry cooling water samples (N=42) during one year period with the standardized culture (ISO 11731:1998) and various qPCR techniques. A combination of propidium monoazide (PMA) treatment with qPCR and the ratio of reverse transcription (RT)-qPCR copy numbers of ribosomal RNA (rRNA) to copy numbers of rRNA genes (rDNA) were used for evaluation of bacterial activity. 120-600 ml of cooling waters were concentrated on nylon filter (0.2µm) for culture, qPCR (gDNA) and PMA-qPCR, and 50-100 ml on polycarbonate filter (0.2µm) for qPCR (rDNA) and RT-qPCR (rRNA). The qPCR assays were targeted for *Legionella* species (*L*.spp.), *L. pneumophila* (*L.p.*) and *L.p.* sg 1. From 11 processes, *L*.spp. were detected from 73% (8/11) with both culture and molecular methods, 18% (2/11) only with qPCR technique, and 9% (1/11) was negative. *L*.spp. were isolated from 79% (33/42) of all water samples by culture ($8.5x10^1$-$1.0x10^5$ CFU/L/l) and were detected from 29-50% (12-21/42) of samples by qPCR ($1.3x10^3$-$5.7x10^5$ GC/L), depending on the nucleic acid extraction method. *L.p.* was detected 64% (27/42) of samples with culture ($4.3x10^1$-$3.2x10^5$ CFU/L) and from 21 to 31% (9-13/42) by qPCR ($7.4x10^2$-$9.3x10^4$ GC/L). *L.p.* 1 was detected 17% (7/42) of samples with culture ($4.3x10^2$-$4.6x10^4$ CFU/L) and none by qPCR. 50% (21/42) from samples was found active by rRNA:rDNA ratio and 95% (40/42) by RT-qPCR ($7.0x10^4$-$1.8x10^7$ GC/L). With PMA-qPCR, a viability of *L*.spp. was detected from 29% ($1.6x10^3$-$4.3x10^5$ GC/L) and *L.p.* from 36% of samples ($1.2x10^2$-$1.6x10^4$ GC/L). Cooling waters contained high amount inorganic compounds (e.g. at least 8.7 mg Fe/L, 110 mg Al/L) and other microbes (HPC up to $1.5x10^{10}$ CFU/L), which all may cause inhibition in qPCR analyzes. Detection of *Legionella* was significantly more sensitive by culture than qPCR, showing culture to have remarkable tolerance against to inhibitors. RT-qPCR of rRNA offered better sensitivity for detection of *L*.spp. compared to when rDNA was used as a qPCR template. However, using DNA as template for detection *L.p.* was necessary, because qPCR assay's target was located in virulence gene (DNA), when RT-qPCR target for the *L*.spp. exists in 16S rRNA.

Distribution of US Clinical *Legionella pneumophila* Strains Using Whole Genome MLST

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Keywords

Genome sequencing, wgMLST, molecular epidemiology, bioinformatics

Background

In 2017, the US Centers for Disease Control and Prevention (CDC) initiated a collaboration in partnership with the Association of Public Health Laboratories and 6 public health laboratories located in Arizona, Massachusetts, Michigan, Minnesota, New York State, and New York City. Partner laboratories conducted genome sequencing of archived clinical *Legionella pneumophila* (Lp) isolates. The study objectives were to evaluate the performance of whole genome MLST (wgMLST) using a standardized scheme, examine the distribution of Lp strains causing disease in different locations, and to identify Lp strain clusters of potential regional significance.

Materials & Methods

Laboratories selected up to 100 Lp isolates per site for genome sequencing and provided associated metadata including year of isolation, Lp serogroup (if known), and geographical origin (e.g. county/city of residence, location of treating hospital, etc.). Sites were encouraged to sequence non-Lp1 isolates where available. All sites conducted sequencing using the Illumina MiSeq instrument and uploaded raw sequences directly to CDC. Raw sequences were processed using wgMLST as implemented within BioNumerics version 7.5.

Results

Lp1 isolates accounted for more than half of the approximately 500 isolates analyzed. The second most common serogroup sequenced was Lp6; approximately half of these isolates were from patients in Arizona. Serogroup was generally not predictive of phylogenetic clustering. We identified potential locally endemic strains such as those belonging to ST731 that caused an outbreak in New York City in 2015. ST213 and ST222 strains were distributed in the Northeast and North Central US. Using a threshold of 99% allele identity, wgMLST identified more than 60 clusters of 2 or more isolates. About one-third of these possible clusters contained isolates recovered in different states.

Conclusions

wgMLST allowed large scale analysis of the strain distribution of Lp using data generated from multiple sites. We show that the distribution of Lp strains in the US in not uniform and that various regions appear to have different circulating strains of Lp. Development of a national wgMLST database may help identify clusters linked to a common source through travel.

The findings and conclusions in this presentation are those of the authors and do not necessary represent the official position of the Centers for Disease Control and Prevention.

On-site inspection method for *Legionella pneumophila* in bath water

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Keywords

On-site inspection method, *Legionella pneumophila*, Rapid detection method, Flow cytometry

Background

Legionella is repeatedly occurring in artificial warm water, avoiding bactericidal effect of chlorine, mainly by hiding in biofilms. Mature biofilms are costly to remove, requiring intensive and iterant sanitation management in bathing facilities. A rapid detection method (RDM) using flow cytometry has been developed to make facility managers recognize the risk of *Legionella*, but it has not yet been conducted as an on-site inspection, because of the unwieldy machine and the non-specificity of bacteria detected by the method. In this study, we examined and improved the RDM by applying a portable flow cytometer together with nucleic acid staining and Legionella-specific antibody staining.

Materials/Methods

The flow cytometer was a device with a 532 nm green laser and weighed 6.5 kg. After confirming the efficiency of the machine as the RDM, first, we examined the specific and quantitative potency of the method on spike testing in comparison with a culture method. Antibodies specific for *Legionella pneumophila* SG2~SG15 or *L. pneumophila* SG1 were used. Second, we evaluated the two methods using 30 water samples collected from 3 different hot tubs with water originating from 2 wells or a hot spring, at a bathing facility, before and after hyperchlorination under strict disinfection condition for 5 weeks. After concentrating the samples about 1000 times, the disinfection effect was examined by counting *Legionella* cells using RDM.

Results

The RDM showed a high correlation ($y = 409.26 \times ^{0.8689}$, $R^2 = 0.95113$) with the culture method in the range of about 10^2 to 10^5 CFU/mL in *L. pneumophila* SG1 spike testing, and *L. pneumophila* SG1, SG3, SG4, SG5, SG6, SG9, SG10 and untypable strains could be detected. The detection limit and the quantification limit of this test method were determined to be 5 cells/100 mL and 229 cells/mL, respectively, corresponding to 32 CFU/100 mL and 235 CFU/mL in culture. In the field study, three *Legionella* positive samples averaging 10 CFU/100 mL were found to be equivalent to an average of 17 cells/100 mL of *L. pneumophila* SG1 by the RDM in about an hour.

Conclusions

The RDM would be available as a monitoring tool for the risk of *Legionella* on-site, especially that of *L. pneumophila*, while discriminating between SG1 or non-SG1. The practical utility in various facilities remains to be clarified.

Acknowledgement

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A study to investigate the impact of neutralising agents in water sampling bottles on legionella & pseudomonas bacteria recovery

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Keywords

Copper, silver, neutralisation, EDTA, thiosulphate.

Background

Microbiological analysis of water and environmental samples in healthcare environments are an important component of the Water Safety Plan (WSP) framework for safe water quality; to validate the effectiveness of controls and surveillance. Microbiological monitoring may be indicated by the local risk assessment of hospital water systems. The quality of the microbiological analysis of water samples is dependent on the sample being representative of the condition of that sample at the actual time of collection. It is imperative that the activity of any residual biocide is completely neutralised. In the absence of an appropriate effective biocide neutralising agent, false negative results could lead to serious public health impact. Ethylenediaminetetraacetic acid dihydrate ($Na_2EDTA.2H_2O$) concentrations of 10 and 50mg/L are recommended in various sampling standards for neutralising biocidal processes based on silver and copper ionisation treatment systems.

This study aims to investigate the efficacy of $Na_2EDTA.2H_2O$ as a neutralising agent compared with sodium thiosulphate ($NA_2S_2O_35H_2O$) on water samples treated with electrolytically generated copper and silver ions and to assess the biocidal activity in water samples.

Materials & Methods

An interlaboratory investigation was carried out using simulated water samples spiked with *Legionella pneumophila* or *Pseudomonas aeruginosa.* Bacterial recovery was assessed with and without silver and copper ions to indicate biocidal efficacy. These were also tested in sample bottles dosed with either 180mg/L $Na_2S_2O_3 \cdot 5H_2O$ or 50mg/L $Na_2EDTA.2H_2O$ to assess the neutralising efficacy on copper and silver ions.

Results

Based on this study 180 mg/L of $NA_2S_2O_35H_2O$ effectively complexed both copper and silver ions at concentrations that are typical of copper silver ionisation devices and inhibit their biocidal activity. The 50mg/L of $Na_2EDTA.2H_2O$ did not neutralise the Ag ions in solution which continued to demonstrate significant biocidal activity in the spiked samples.

Conclusions

This study has demonstrated that sample bottles dosed with 180mg/L $Na_2S_2O_3.5H_2O$ is recommended for water that has been treated by typical copper/silver ionisation systems to ensure rapid neutralisation of both silver and copper ions generated by such systems.

Efficacy of continuous disinfection by monochloramine for *Legionella* and Amoeba control in hospital water system

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Keywords

Monochloramine, *Legionella*, culture, qPCR, Amoeba.

Background

The prevention of *Legionella* colonization of water distribution system is a critical issue in healthcare settings. An effective disinfection of water systems combined with appropriate environmental surveillance strategies can prevent nosocomial legionellosis. The aim of the study is to evaluate the efficacy of the continuous disinfection by monochloramine (MC) for *Legionella* and Amoeba control in a hospital water system.

Materials & Methods

A device continuously injecting monochloramine in the hot water loop was installed in a 3-floors hospital building in October 2017. Water samples were taken from cold water, heater, income and return loop hot water and from 8 hot water distant outlets (7 tap water and 1 shower head). Samplings were performed twice before installation, one week after installation and then monthly for six months. *Legionella*, *Pseudomonas*, heterotrophic bacteria (HB) at 37°C and 22°C on PCA and R2A, Amoeba were detected at 37°C and 25°C by culture. Water samples were concentrated by filtration for *Legionella* culture (1 L) and Amoeba coculture. Aliquots of untreated, heat-treated and acid-washed suspensions were plated on BCYE, BMPA and MWY, incubated at 37°C for 15 days and *Legionella* colonies were typed. *Legionella* spp. and *Legionella pneumophila* DNA was detected from 1 liter water by qPCR with and without Free DNA removal (Biorad). MC, free ammonia, nitrate, nitrite and free chlorine were determined.

Results

A week after disinfection with MC, the load of *L. pneumophila* serogroup 3 ST728 decreased from 10^2-10^4 cfu/L at undetectable levels (<25 cfu/L) in 100% of samples. VBNC *Legionella* was not detected. *Legionella* DNA was however detected after 1 week and 1 month of disinfection, the treatment by DNA removal reduced DNA by 1 log. HB plate count was <1 cfu/mL. *Pseudomonas* and Amoeba were not detected respectively in 250 mL and in 100 mL. MC was maintained at mean dosage of 3 mg/L, free ammonia was <0.5 mg/L, nitrate was < 50 mg/L, nitrite was < 0.5 mg/L, free chlorine 0.35-0.9 ppm.

Conclusions

The continuous disinfection of hot water by MC can fully control *L. pneumophila* and HB in contaminated hospital hot water system. As free-living Amoeba (FLA) may favour the multiplication, dissemination and virulence of *Legionella*, FLA presence in water increases the risk of *Legionella* colonization. Amoeba was not detected after disinfection. The results show that monochloramine is also effective for the control of FLA within the biofilm.

Network of Hospital Laboratories accredited for *Legionella* analysis in water: a four years feedback

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Keywords

Legionella analysis, standard, accreditation, network laboratories

Background

Sample and analysis for the search and enumeration of *Legionella* in water must be carried out under accreditation by COFRAC (French Committee for Accreditation) in French health facilities. They must meet the requirements of NF EN ISO / IEC 17025 for the quality management system, NF EN ISO 19458 for sampling, and NF T90-431 for analysis. Their complexity, such as the cycles monitoring by COFRAC, led to the creation of a network of accredited hospital laboratories for *Legionella* in November 2013. Its purpose was to encourage feedback from experience to facilitate accreditation procedures of his members.

Materials & Methods

Specifications included sharing data and experiences on deviation from standards, metrology and technical records, forming a group of internal auditors and discussing costs. The representatives of each establishment were the hygiene practitioners and biologists responsible for management, and the technicians authorized for sampling and / or analysis.

Results

The number of accredited laboratories increased from 8 in November 2013 to 11 in December 2016. Eleven meetings addressed the points of the specifications and the methodology to be followed for the verification of method of the new versions of the NF T90-431 standard. A database constituted by compiling the deviation from conformity opened during each initial assessment, monitoring or renewal visit, was available for each participant. Out of 303 recorded deviations only 31 were critical. 142 concerned the management part of the NF EN ISO / IEC 17025 and 160 concerned its technical part (21 on sampling, 38 on analysis, and 44 on metrology). No decision to withdraw accreditation has been pronounced. An average of 13 deviations from conformity per facility was opened out of the 11 initial visits, versus 6 out of the 7 renewal visits. On average, five annual cross-audits were organized among six network members. Three mutual subcontracting agreements were signed between 4 institutions.

Conclusions

Sharing of experience of the technical manager as well as that of the technicians allowed everyone to apprehend the visits of the first accreditation cycle, and to integrate the requirements of the new versions of the standard texts. Database and cross-audits have also made it possible to anticipate potential deviation from conformity and reduce their number.

Quantification of *Legionella* DNA Certified Reference Material (CRM) by digital droplet PCR (ddPCR)

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Keywords

Legionella, digital droplet PCR, environment, quantification

Background

The monitoring of *Legionella* levels in waters can be done by quantifying *Legionella* DNA using PCR based methods. To ensure calibration of these methods, a CRM has been available since 2009. Here we show the possibility to use a novel method of absolute quantification of DNA, digital droplet PCR (ddPCR), to provide absolute quantification and stability monitoring of the CRM.

Materials & Methods

In order to quantify the *Legionella* CRM by ddPCR, the method linearity, limit of detection (LOD) and limit of quantification (LOQ) were first qualified. Then, to estimate the CRM value by ddPCR, 10 randomly chosen samples of the CRM were tested. Each sample was diluted independently into 8 replicates and 5 µL of each replicate were analysed by ddPCR. For each sample tested, the mean, median, standard deviation of the 8 replicates was calculated. A linear mixed model was used to estimate the within and between replicate variability, and to estimate the overall mean value.

Results

The estimated concentration obtained by ddPCR measurements is found not significantly different from the certified value determined in 2009 through inter-laboratory assay using PCR-based limiting dilution method.

Conclusions

The ddPCR method for quantifying *Legionella* DNA could be useful for future reference materials, as it can be used to assign a value to a new batch with reduced uncertainty and less time-consuming protocol. The additional advantage is its independence from pre-existing standards as well as lesser number of samples to be tested, which make it the preferred method for stability monitoring.

Evaluation of Legiolert™, a most probable number method for the enumeration of *Legionella pneumophila* from potable water samples

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Keywords

NF T90-431:2017, *Legionella pneumophila*, potable water samples, IDEXX Legiolert™

Background

The aim of this study was to compared the performance of Legiolert™, a test developed by IDEXX Laboratories for the detection and enumeration of *Legionella pneumophila* (*Lp*) from potable water samples, to the French standard NF T90-431:2017 method (close to ISO 11731-2).

Materials & Methods

A total of 125 potable water samples from Lyon's hospital were tested with both the Legiolert™ kit and the French standard NF T90-431:2017 method. Theses samples were collected from water taps, showers, water supplies, water gates or dental chairs. After 7 days, 10% of positive wells per Legiolert™ test were plated on *Legionella* growth media to determine specificity.

Results

72 samples were negative by both methods, 10 were positive for *L.anisa* (by the standard method only), 43 were positive for *Lp* (35 by both methods, 5 by standard method only and 3 by Legiolert™ only). All wells plated on agar media grew *Lp* only, yielding a specificity of 100%. The overall agreement between the two methods was 93.6%. The enumeration of *Lp* obtained by Legiolert™ was compared to that obtained by the standard method (uncertainty of measurements: [-0.540; 0.540]) on the 35 samples positive by both methods. 26 samples were included in the zone of uncertainty. Among the few samples with *Lp* other than serogroup 1 (3 samples with *Lp* serogroup 3, 4 samples with *Lp* serogroup 3-6 and 1 positive sample with *Lp* serogroup 4), Legiolert™ method gives lower result for 6 samples. More often, the difference of values between the two methods was significant when the amount of *Lp* was under 1000 UFC/L (for 2 samples with significant difference, the Legiolert result was above 1000 UFC/L and the standard result below 1000 UFC/L).

Conclusions

Legiolert™ proposes a method for *Lp* identification and quantification in the potable water which is relatively simple to perform, quick, easy to interpret and relatively reliable (good specificity and good overall agreement with standard method). However, some differences in the enumeration were observed but samples analyzed are insufficient to draw a conclusion. More positive samples would be analyzed to conclude. To conclude, Legiolert™ could help to perform regular controls of sanitary hot waters equipment from a 7-day culture, for set up corrective actions as early as possible.

Whole-genome sequencing of all clinical and associated environmental isolates of *Legionella pneumophila*, Denmark 2017

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Keywords
L. pneumophila, WGS, SBT, SNP analysis

Background
In 2017, Denmark saw its highest number of legionnaires´ disease (LD) cases (n=278), giving an incidence of 4.8/100.000 inhabitants. *Legionella pneumophila* was isolated from 131 cases. Traditionally, all clinical isolates were typed by serogrouping and SBT. From 2017, all clinical and selected environmental isolates associated with cases were subjected to whole-genome sequencing (WGS).

Materials & Methods
All included isolates were serogrouped. Single nucleotide polymorphisms (SNPs) were analysed with *L. pneumophila* Philadelphia-1 (ST36) as reference and sequence types (STs) were extracted from the whole-genome reads. One or two isolates for each of 131 culture positive patients (n=143) and environmental samples (n=66) were included. Each patient and environmental isolates were categorised according to setting (community-acquired, healthcare-associated, travelling abroad or unknown setting). Presumed place of infection was recorded according to zip code and region for Danish cases or country for travel-associated cases.

Results
The most prevalent serogroups (SGs) among the clinical isolates were SG1 (n=76; 58%), SG3 (n=28; 21%) and SG6 (n=12; 9%). Altogether 51 different STs were identified, including 7 novel STs. ST1 was most prevalent (n=26; 20%) followed by ST87 (n=19; 15%), and ST42 (n=11; 8%). The phylogenetic analysis revealed nine major phylogenetic groups, corroborating the SBT/ST results. One major group harboured exclusively all ST1 isolates and was characterised by low diversity (≤31 SNPs). The largest group was dominated by ST87 (SG3) and SG 6 strains, but also harboured SG1. Two groups were dominated by ST42 and ST23/62, respectively, and were both strongly associated with travelling abroad (n=14). Related environmental isolates were in most cases confirmed by the SNP analysis.

Conclusions
The genomic diversity among *L. pneumophila* isolates from Danish patients was high, with several phylogenetic groups spread across the country. Conventional SBT would in many cases be sufficient for establishing links between patients and suspected source. However, some lineages were widespread and involved several cases with only little genomic diversity. WGS-based typing is useful in many of these cases, but it is also evident that several unrelated epidemiological isolates, even from different countries, may only differ by 1-6 SNPs. Interpretation of molecular typing results must always be done in the context of an epidemiological investigation.

Legionella pneumophila in Scotland: from genome-wide association analysis to immune evasion

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Background

Since the first reported Legionnaires' disease outbreak in the United Kingdom in 1984, the Scottish microbiology reference laboratory has maintained a comprehensive collection of both clinical and environmental isolates of *Legionella* spp. Whole genome sequencing of the extensive Scottish *Legionella pneumophila* collection, combined with the publicly available genomes, represents a powerful tool for investigating Scottish outbreaks and also the relationships between clinical and environmental isolates. Genome-wide association analyses (GWAS) from this large and diverse population allow us to identified genes that were enriched in *L. pneumophila* isolates from human infection. The main aim of the current study is to investigate the role of these genes in human disease pathogenesis.

Materials & Methods

More than twenty *L. pneumophila* Scottish strains were selected to represent the breadth of diversity of the *L. pneumophila* species as well as the presence/absence of genes identified by GWAS as significantly associated with clinical isolates. The interaction of these strains with components of the innate immune response was evaluated and cloning strategies were used to validate the identified associations.

Results

Our data indicate that *Legionella* infections in Scotland over the last 30 years originated from diverse lineages indicating that the potential for human infection is present in diverse genetic backgrounds. GWAS on this dataset highlighted genes that are over-represented in clinical isolates, in particular, subsets of genes involved in lipopolysaccharide (LPS) biosynthesis. We observe that strains that differ in LPS gene content could be phenotypically distinguished based on their interaction with the innate immune system.

Conclusions

A GWAS analysis of a large and diverse *L. pneumophila* genome dataset allowed us to identify genes that were enriched in clinical isolates relative to environmental isolates. These genes are widely distributed across the species and show differential ability to interact and modulate important steps of the protective immune response to this bacterium.

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