3rd clean hospitals Day

20 October 2022
International Conference
Geneva, Switzerland

Real-life experiences of controlling *Legionella* in healthcare

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French Society for Hospital Hygiene

MUSE MONTPELLIER UNIVERSITÉ D'EXCELLENCE

Pathogènes Hydriques Santé Environnements

SF2H Risque infectieux et soin

HSM

CHU Montpellier Département D'Hygiène Hospitalière

Legionella

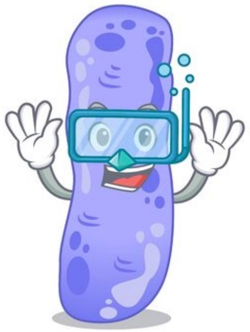
Among the 65 species

⇒ ***L. pneumophila*** responsible of >95% infections caused by *Legionella*
⇒ Agent of Legionnaire's disease which represents 0.5 to 5% of pneumopathy in the community
⇒ **serogroup 1** involved in >80% infections
⇒ 10-15% mortality

Gram-negative bacteria very common in water ecosystems but in low concentration

Colonize manmade water networks, especially **hot water** (>37°C)

Human is an **accidental host**
Inhalation of contaminated aerosols (small droplets)
Vulnerable population = immunocompromised persons, smokers, >50 yo, chronic diseases such as diabetes and chronic lung and heart disease
No direct human-to-human transmission



Water is the unique source of infection

Legionella

Opportunistic Premise Plumbing Pathogen (OPPPs)

Legionella pneumophila, *Stenotrophomonas maltophilia*, non-tuberculous mycobacteria, *Pseudomonas aeruginosa*

Common characteristics to survive and persist within plumbing systems

- **biofilm**
- **intra-amoeba survival**
- **horizontal genes transfers**, especially virulence and resistance genes

In healthcare settings, water networks constitute technologic niches

⇒ Complex reservoir for hydric pathogens

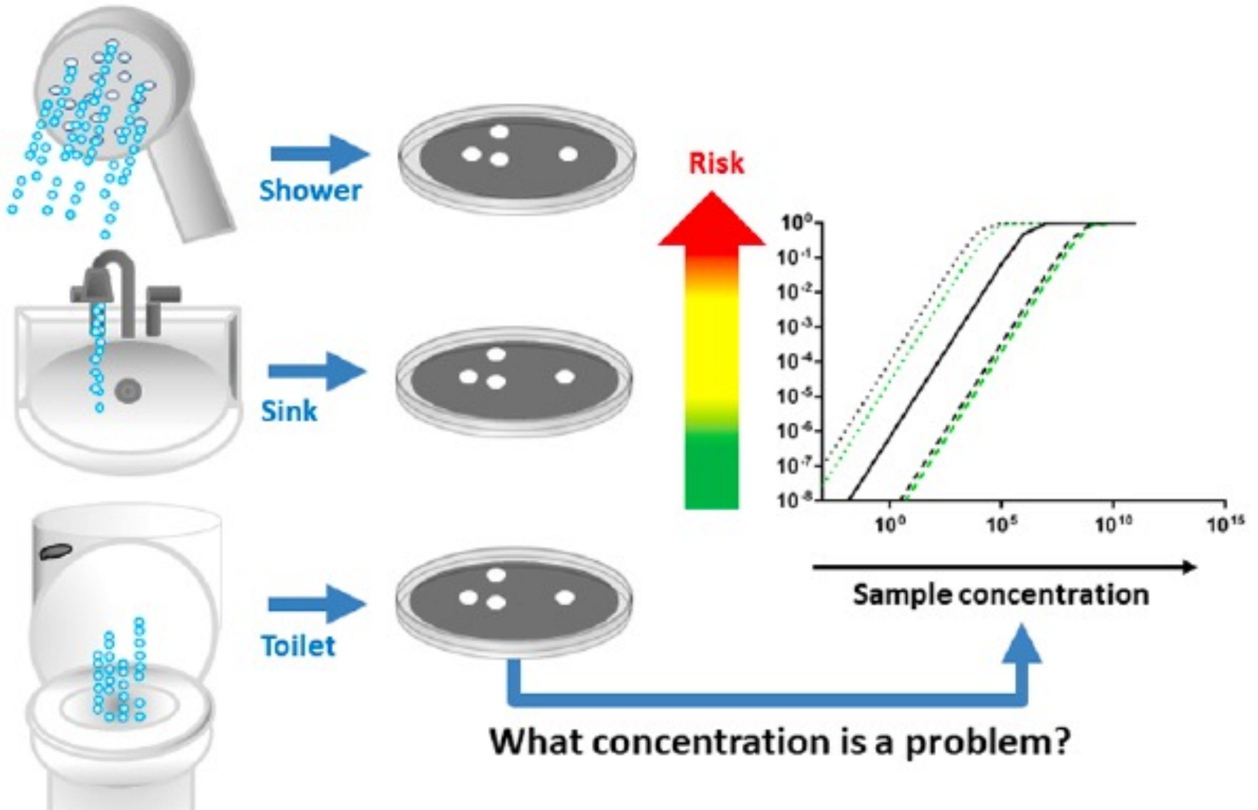
⇒ Favouring OPPPs selection and transmission to patients

"The more complex the system, the greater the likelihood of colonisation…"

Baranovsky et al. EC Microbiology. 2017 Apr ;7.6: 192-197.
Falkinham. Int J Environ Res Public Health. 2015 Apr 24;12(5):4533-45.
Naumova et al. J Public Health Policy. 2016 Dec;37(4):500-513.
Liu et al. Water Research. 2017;116:135-148.
Wingender & Flemming. Inter. J. Hygiene and Environ. Health. 2011;214:417-423.
European Technical Guidelines 2017: minimising the risk from Legionella infections in building water systems

How Legionellosis cases occur in healthcare?

Usually, from hot water showerhead, but the risk depends on the type of water source of exposure



DOI: 10.1021/acs.est.8b03000

Single sample critical concentrations considered at risk of contamination:
14.4 CFU per L in showers
1.06 x10^3 CFU per L in faucets
8.84 x 10^3 CFU per L from toilets

This is in line with current guidelines of less than 1000 CFU per L, but less than 10 CFU per L of *L. pneumophila* in healthcare or susceptible population settings.

Preventive measure = Filters on hot water points-of-use in at-risk settings

Unusual nosocomial cases occurring in a protected unit

DISPATCHES
Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 26, No. 7, July 2020

Transmission of Legionnaires' Disease through Toilet Flushing

Jeanne Couturier, Christophe Ginevra, Didier Nesa, Marine Adam, Cyril Gouot, Ghislaine Descours, Christine Campèse, Giorgia Battipaglia, Eolia Brissot, Laetitia Beraud, Anne-Gaëlle Ranc, Sophie Jarraud, Frédéric Barbut

2 cases of nosocomial Legionellosis occurring 5 months apart in a same room of haematology unit while all water points-of-use are filtered

Investigation isolating 12 strains of *L. pneumophila* within patients' environment

WGS analyses showed that clinical isolates and isolates from the room's toilet clustered together

Toilet contamination by *L. pneumophila* is usually neglected but can lead to a risk of exposure through flushing

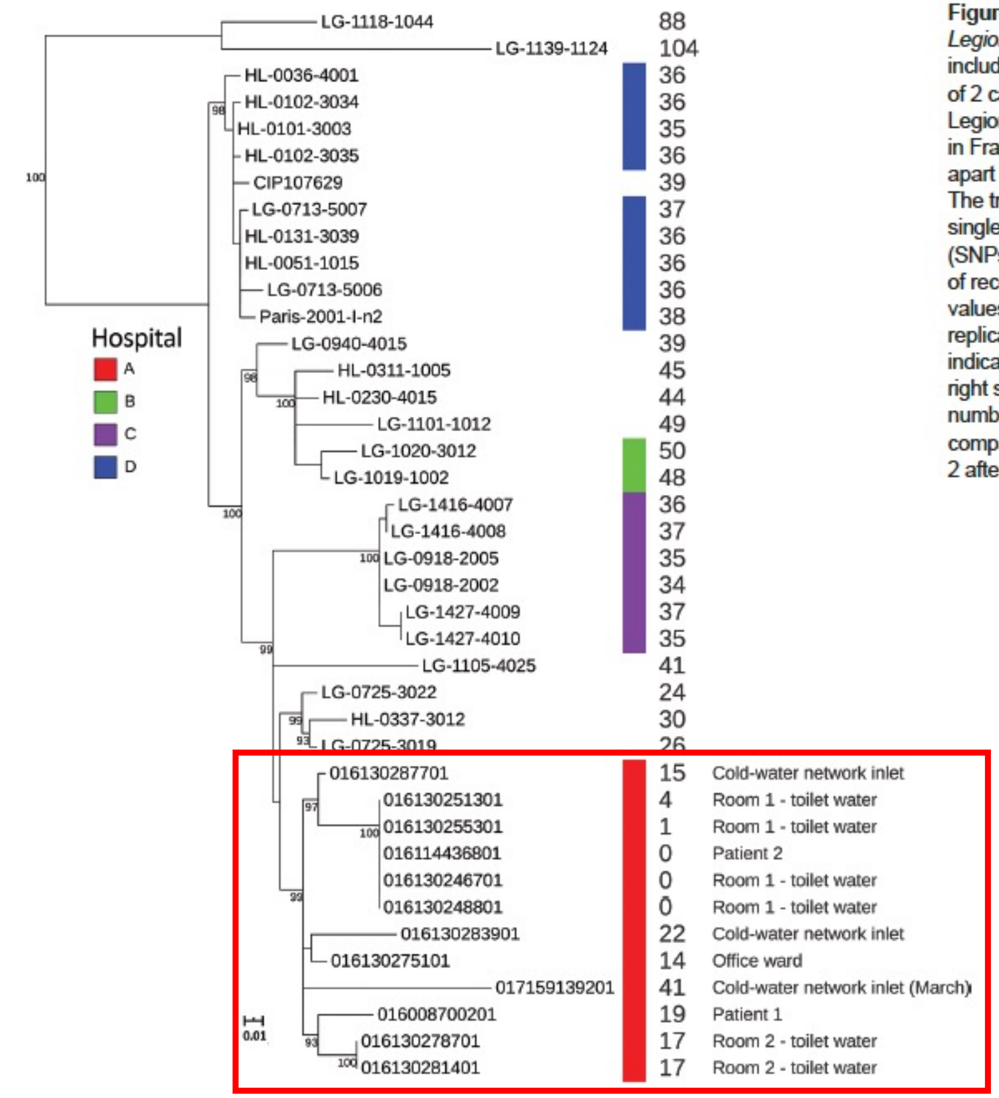
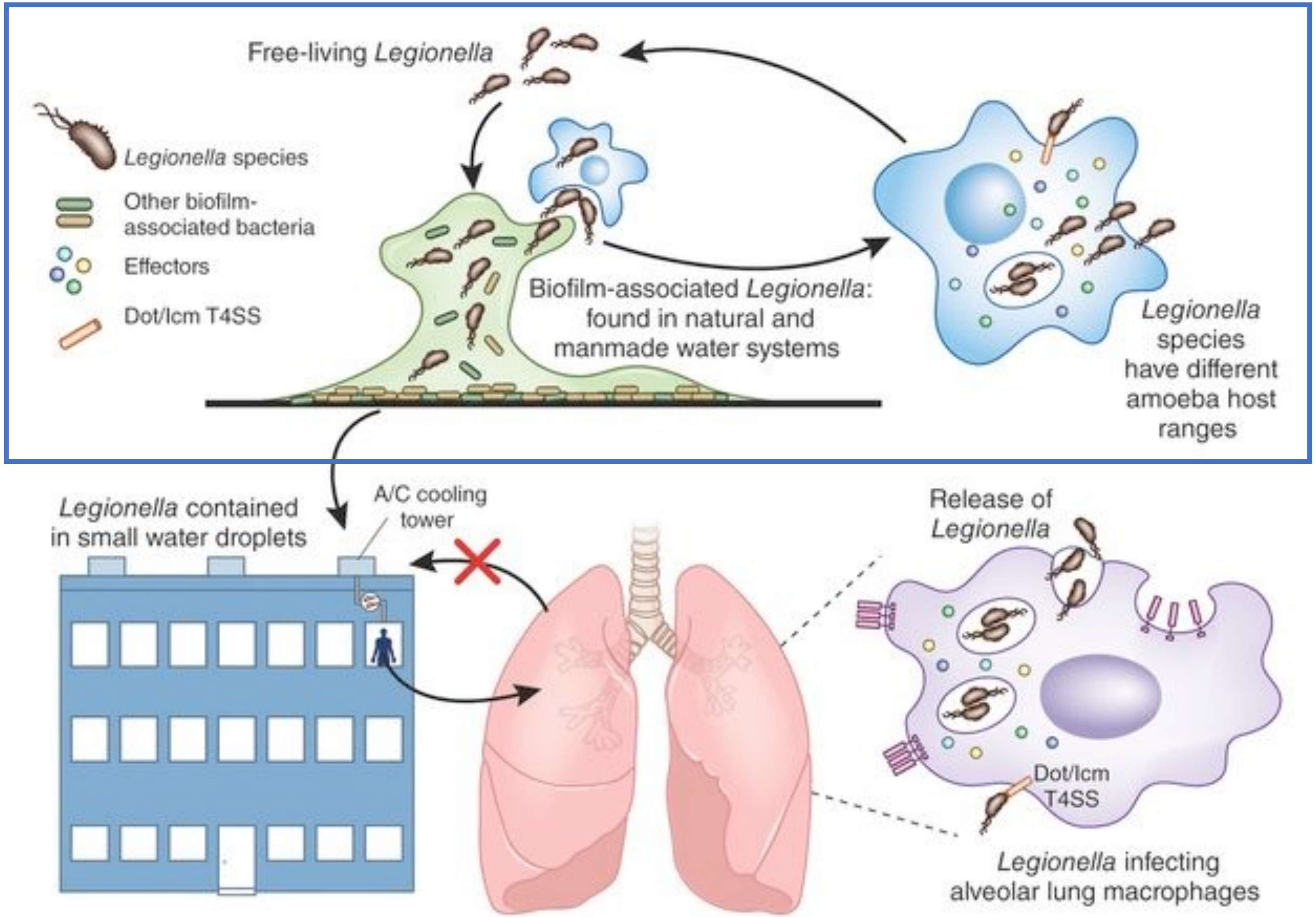


Figure. Maximum-likelihood tree of 39 *Legionella pneumophila* ST1 isolates, including isolates from investigation of 2 cases of healthcare-associated Legionnaires' disease in patients in France hospitalized 5 months apart in the same room (red bar). The tree was constructed using 258 single-nucleotide polymorphisms (SNPs) identified after the removal of recombination events. Bootstrap values were calculated from 500 replicates; only values >90 were indicated on the tree. Values at the right side of the tree represent the number of SNPs between the genome compared with the genome of patient 2 after recombination removal.

Legionella's epidemiological cycle



In water pipes

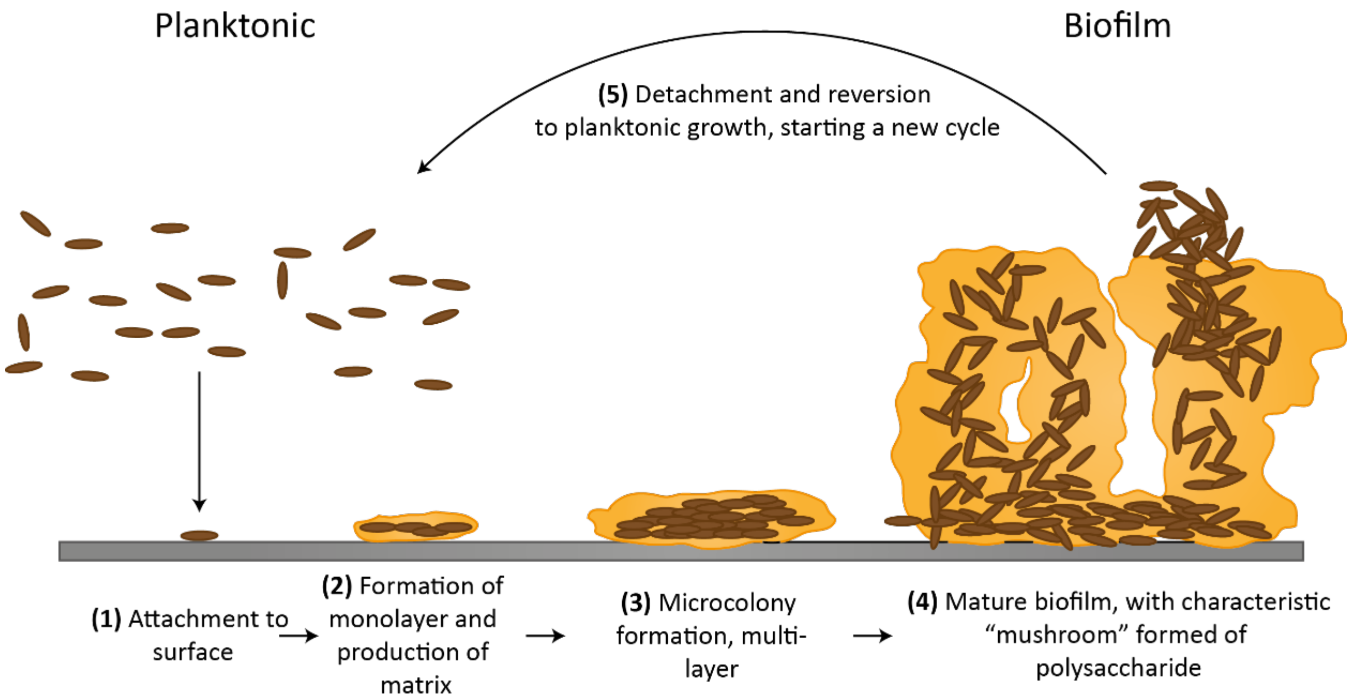
Legionella **are present in different forms**

Free-living

Biofilm-associated

Inside amoebas

Biofilms

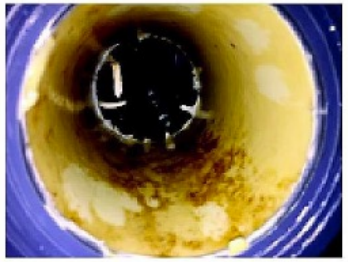


Favoured by water stagnation and scale
Up to **10^7 bacteria per cm^2** within biofilms

Transient or long-term reservoirs for
opportunistic pathogens

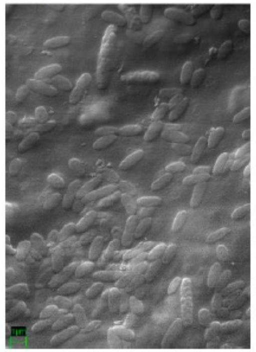
Whatever the constitutive support of wall pipes

Canalisation en PVC (D = 110 mm)



Canalisation en polyéthylène haute densité (D = 25 mm).

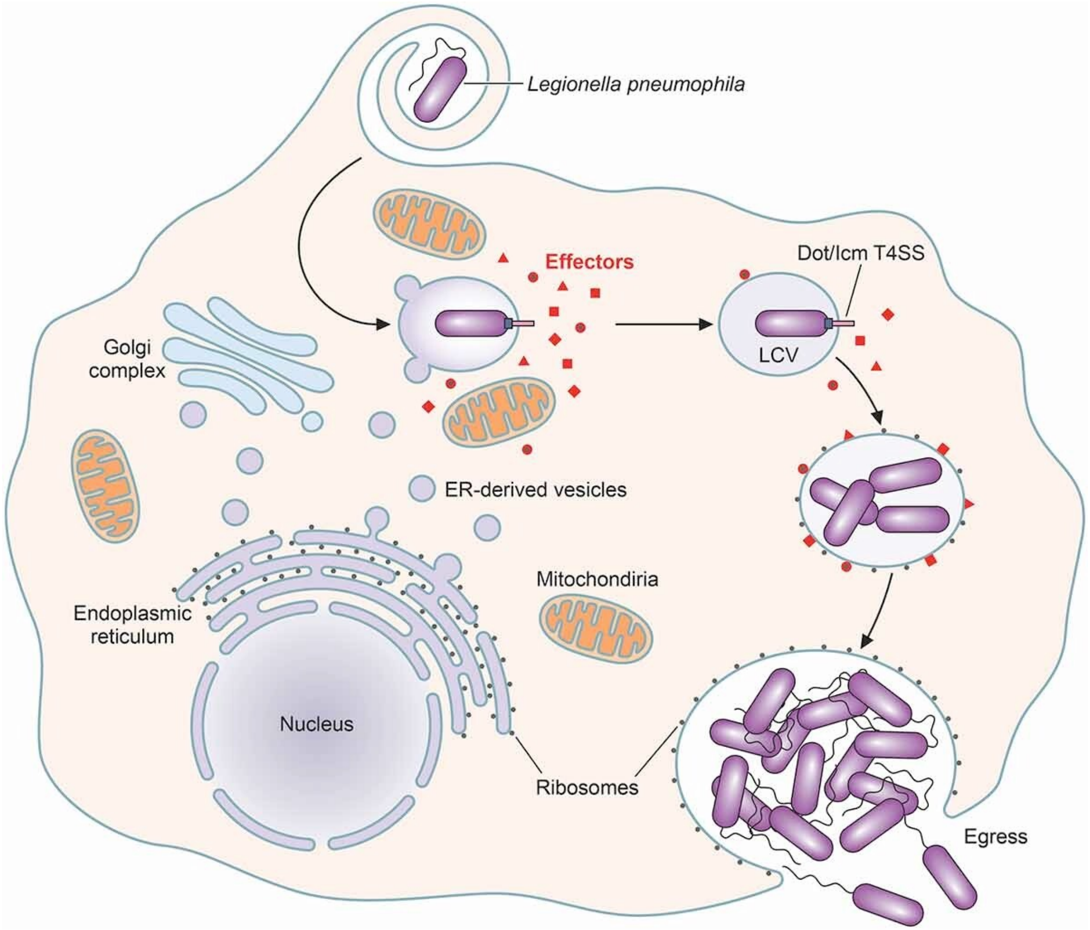
Canalisation en acier après 14 jours d'exposition à de l'eau de boisson



Canalisation en caoutchouc synthétique

Intra-amoeba life

Amoebas are phagocyting cells that feed on bacteria
During its evolution *L. pneumophila* acquired **resistance against amoeba**
Once inside the cell, *Legionella* are able to proliferate, at **temperature of 30-40°C**



Amoeba amplifies *L. pneumophila*
The predator becomes the prey

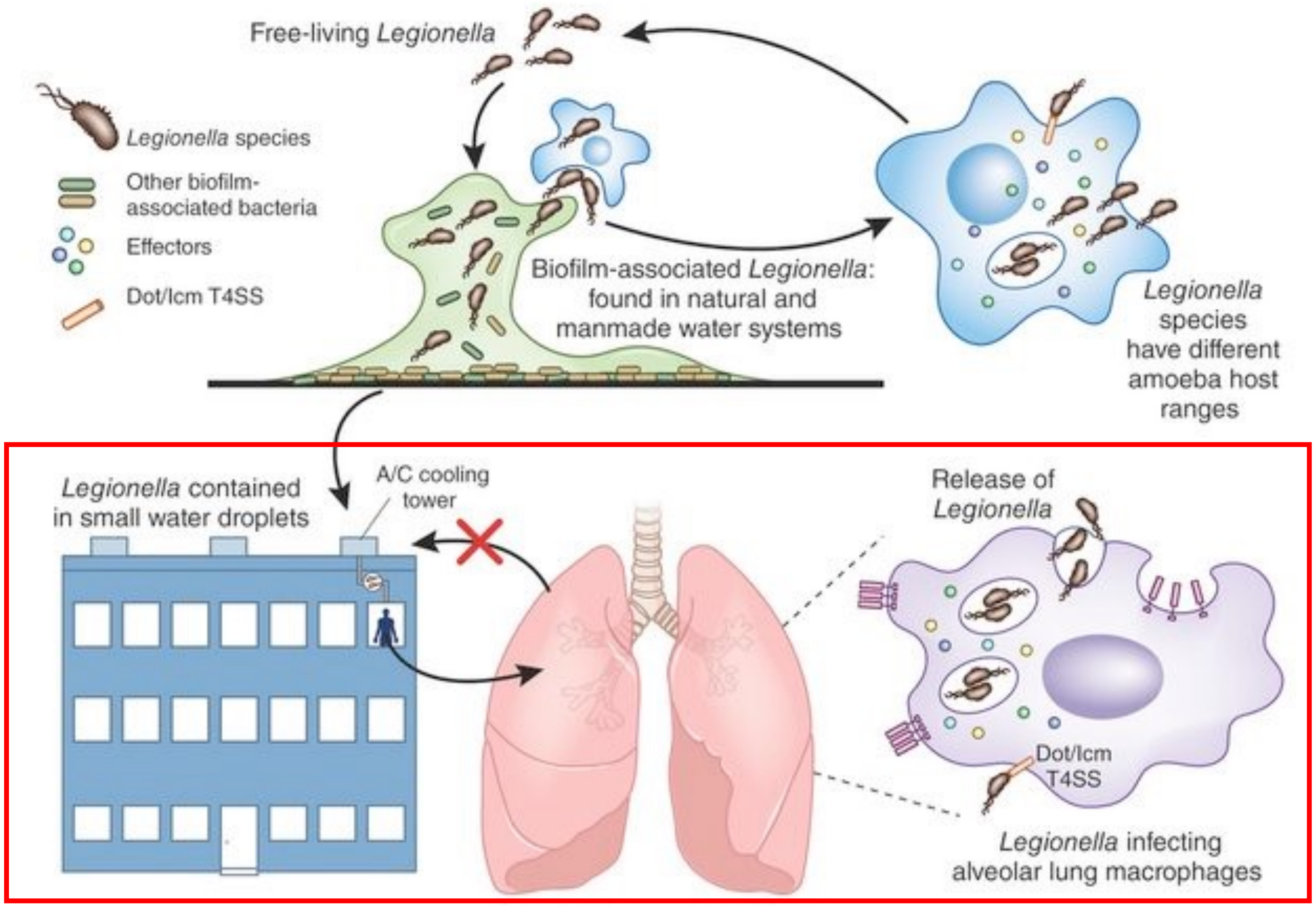
Amoeba predisposes *L. pneumophila* to human infection by adapting the bacteria to human macrophages and then to escape the immune system

L. pneumophila is « accidentally » resistant to human macrophages

Immunodepression permissive to infection

https://doi.org/10.1080/21505594.2021.1903199

Legionella's epidemiological cycle



In water pipes

***Legionella* are present in different forms**

Free-living

Biofilm-associated

Inside amoebas

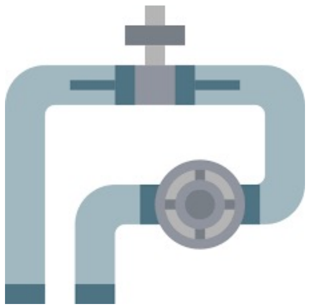
When exposed to contaminated aerosols

Legionella can contaminate the lungs, by infecting macrophages

How managing water quality and controlling *Legionella*?

In healthcare and public buildings,

it is mandatory to regularly manage and control water supplies:




ESCMID STUDY GROUP FOR LEGIONELLA INFECTIONS

ESGLI

European Society of Clinical Microbiology and Infectious Diseases

European Technical Guidelines

for the

Prevention, Control and Investigation, of Infections Caused by *Legionella* species

June 2017

- Good knowledge of the water network map
  ⇒ identification of unfavourable water points
  ⇒ suppression of backwater
- Elimination of scale from water pipes;
- Sufficient temperature: 60°C at the production, 50°C at water point-of-use;
- Sufficient chlorination: 1 mg/L at the production;
- At least once a year, microbiologic controls of water from unfavourable points
  ⇒ Conformity if *Legionella* <1000 CFU/L for all settings, except for high-risk settings hosting vulnerable patients where *Legionella* must be <10 CFU/L

https://www.escmid.org/fileadmin/src/media/PDFs/3Research_Projects/ESGLI/ESGLI_European_Technical_Guidelines_for_the_Prevention_Control_and_Investigation_of_Infections_Caused_by_Legionella_species_June_2017.pdf

How managing water quality and controlling *Legionella*?

Several measures can be used…

Received: 30 December 2019; Accepted: 21 February 2020; Published: 2 March 2020

water

MDPI

Review

Legionellosis and Recent Advances in Technologies for *Legionella* Control in Premise Plumbing Systems: A Review

Kelsie M. Carlson [1,2], Laura A. Boczek [1], Soryong Chae [2,*] and Hodon Ryu [1,*]

Preventive measures

Curative measures

Legionella Control in Premise Plumbing Systems	
Chemical treatment technologies: • Chlorine-based disinfection • Copper-silver ionization (CSI) • Ozonation	**Physical treatment technologies:** • Thermal inactivation • Filtration • Ozonation
Emerging treatment technologies: • Ultraviolet (UV) irradiation • UV light emitting diodes (LEDs) • Innovative point-of-use (POU) filters	**Other strategies:** • Superheat-and-flush disinfection • Shock hyperchlorination

Figure 2. Current treatment technologies for control of *Legionella* in premise plumbing systems.

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A Review

Kelsie M. Carlson [1,2], Laura A. Boczek [1], Soryo

At usual chlorine concentration in drinking water

Table 1. Time to 4-log reduction of various *Legionella* strains at two different concentrations of free chlorine. Adapted from [77].

Legionella Strains *	Time to 4-Log Reduction (Min.)	
	0.2 mg/L Free Chlorine	0.5 mg/L Free Chlorine
L. pneumophila serogroup 1 lab strain	Not achieved	8
L. pneumophila serogroup 1 environmental strain	Not achieved	4
L. pneumophila serogroup 7 lab strain	9	2
L. pneumophila serogroup 8 environmental strain	20	3
L. longbeachae lab strain	11	3

* Lab strains: serogroup 1 (ATCC 33152), serogroup 7 (ATCC 33823), *L. longbeachae* (ATCC 33462).

Insufficient to achieve a 4-log decrease of *L. pneumophila* serogroup 1

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Kelsie M. Carlson [1,2], Laura A. Boczek [1], Sory

Limited efficiency of heat-shock under 60°C

Table 6. Amount of time to 4-log reduction in various *Legionella* strains at different temperatures. Adapted from [77].

Legionella Strain *	Time to 4-Log Reduction (Min)				
	50 °C/R^2	55 °C/R^2	60 °C/R^2	65 °C/R^2	70 °C/R^2
L. pneumophila serogroup 1 lab strain	117/0.80	10/0.92	2/0.90	0.8/0.88	0.9/0.79
L. pneumophila serogroup 1 environmental strain	46/0.84	8/0.98	3/0.83	1.4/0.90	0.6/0.82
L. pneumophila serogroup 7 lab strain	40/0.97	25/0.96	3/0.76	0.6/0.87	1.2/0.77
L. pneumophila serogroup 8 environmental strain	68/0.97	16/0.89	4/0.94	0.8/0.90	0.7/0.99
L. longbeachae lab strain	15/0.94	2/0.88	Not achieved	Not achieved	Not achieved

* Lab strains: serogroup 1 (ATCC 33152), serogroup 7 (ATCC 33823), *L. longbeachae* (ATCC 33462).

Risk of burning to be considered

How managing water quality and controlling *Legionella*?

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Kelsie M. Carlson [1,2], Laura A. Boczek [1], Soryong Chae [2,*] and Hodon Ryu [1,*]

Continuous hyperchlorination
(0.5-1mg/L)

Table 2. Comparison of *Legionella* positivity before and after chlorination. Adapted from [80].

Condition	Number of Positive *Legionella* Sites (%)	Number of Negative *Legionella* Sites (%)	*p*-Value
Before chlorination	43 (21.1)	161 (78.9)	<0.001
With continuous hyperchlorination	23 (5.5)	393 (94.5)	

Efficient to significantly reduce the burden of *Legionella*, but 5.5% of water samples remained positive.

⇒ **The risk of infection is not completely controlled and Legionellosis cases still occur from treated drinking systems**

How managing water quality and controlling *Legionella*?

But in real-life…

PLOS | ONE

PLOS ONE | DOI:10.1371/journal.pone.0134726 August 4, 2015

RESEARCH ARTICLE

Effect of Common Drinking Water Disinfectants, Chlorine and Heat, on Free *Legionella* and Amoebae-Associated *Legionella*

Sílvia Cervero-Aragó[1,2], Sarah Rodríguez-Martínez[1,3], Antoni Puertas-Bennasar[1], Rosa M. Araujo[1]*

The problem with intra-amoeba life

Table 4. Calculated time for a 4-log reduction of *L. pneumophila* sg. 1 env. associated with *A.castellanii* CCAP 1534/2 and *Acanthamoeba* sp. 155 after the exposure to different concentrations of free chlorine and temperatures. Inactivation kinetics adjusted to first-order models. R^2 values showed the robustness of the models.

	Calculated time (min) to reduce 4 logs							
Free chlorine	**0.5 mg L^{-1}**	R^2	**1.2 mg L^{-1}**	R^2	**2.5 mg L^{-1}**	R^2		
L. pneumophila sg.1 env (Axenic)	5	0.96	—	—	—	—		
L. pneumophila sg.1 env–*A. castellanii* CCAP 1534/2	490	0.85	152	0.76	43	0.79		
L. pneumophila sg.1 env—*Acanthamoeba* sp. 155	38	0.54	17	0.64	23	0.82		
Temperature	**50°C**	R^2	**55°C**	R^2	**60°C**	R^2	**70°C**	R^2
L. pneumophila sg.1 env (Axenic)	46	0.84	8	0.98	4	0.86	0.61	0.82
L. pneumophila sg.1 env–*A. castellanii* CCAP 1534/2	825	0.56	45	0.84	5	0.99	0.45	0.82
L. pneumophila sg.1 env—*Acanthamoeba* sp. 155	664	0.95	51	0.95	5	0.73	0.50	0.92

doi:10.1371/journal.pone.0134726.t004

Usual conditions in hot drinking water

- 0.5mg/L free chlorine
- temperatures of 50°C in proximal areas of water supplies

When associated to amoeba, a same strain of *Legionella pneumophila* resists

- 7 to 98 times longer to 0.5mg/L free chlorine
- up to 18 times longer at 50°C and 5-6 times longer at 55°C

How managing water quality and controlling *Legionella*?

But in real-life…

PLOS | ONE

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doi:10.1371/journal.pone.0134726.t004

The **resistance of *Legionella*** to usual water treatment is **enhanced by its association with amoebas**

→

Amoeba survival and amoeba-associated *Legionella* should be considered when assessing disinfection processes

How managing water quality and controlling *Legionella*?

Using predictive analyses…

pathogens

Pathogens 2019, *8*, 295; doi:10.3390/pathogens8040295

MDPI

Article

Water Quality as a Predictor of *Legionella* Positivity of Building Water Systems

David Pierre [1], Julianne L. Baron [1], Xiao Ma [1], Frank P. Sidari III [1], Marilyn M. Wagener [2] and Janet E. Stout [1,3,*]

Assessment of the relationship between *Legionella* in hot water return line, water quality parameters (T°, free-chlorine residual, total bacteria…) and *Legionella* in distal site

269 samples from domestic cold and hot water in 28 buildings

⇒ **Poor correlation and low positive predictive value between the presence of *Legionella* in hot water return line and distal site**

⇒ **No correlation between *Legionella* positivity and total bacteria, pH, free chlorine, T°, incoming cold-water chlorine concentration…**

Hot water return line *Legionella* positivity and other water quality parameters are not predictive of distal site positivity and should not be used alone to determine the building's colonization rate and the effectiveness of water management programs

How managing water quality and controlling *Legionella*?

Using a temperature diagnostic of the water network…

ELSEVIER
Water Research
Volume 71, 15 March 2015, Pages 244-256

Temperature diagnostic to identify high risk areas and optimize *Legionella pneumophila* surveillance in hot water distribution systems

Emilie Bédard [a, b], Stéphanie Fey [a], Dominique Charron [a], Cindy Lalancette [b], Philippe Cantin [c], Patrick Dolcé [d], Céline Laferrière [e], Eric Déziel [b], Michèle Prévost [a]

Risk assessment based on water T° within the water network to guide effective monitoring strategies and identify high-risk areas

⇒ Monitoring of **T° and heat loss at control points** (water heater, recirculation, representative points-of-use) in hot water distribution systems of 5 healthcare facilities

Defective return valves in faucets can cause widespread T° losses because of hot and cold water mixing

Systems in which water was kept consistently above 60°C and maintained above 55°C across the network were negative for *Legionella*.

For systems not meeting these temperature criteria, risk areas for *L. pneumophila* were identified using temperature profiling and system's characterization

⇒ **Higher risk were confirmed by more frequent microbiological contamination**

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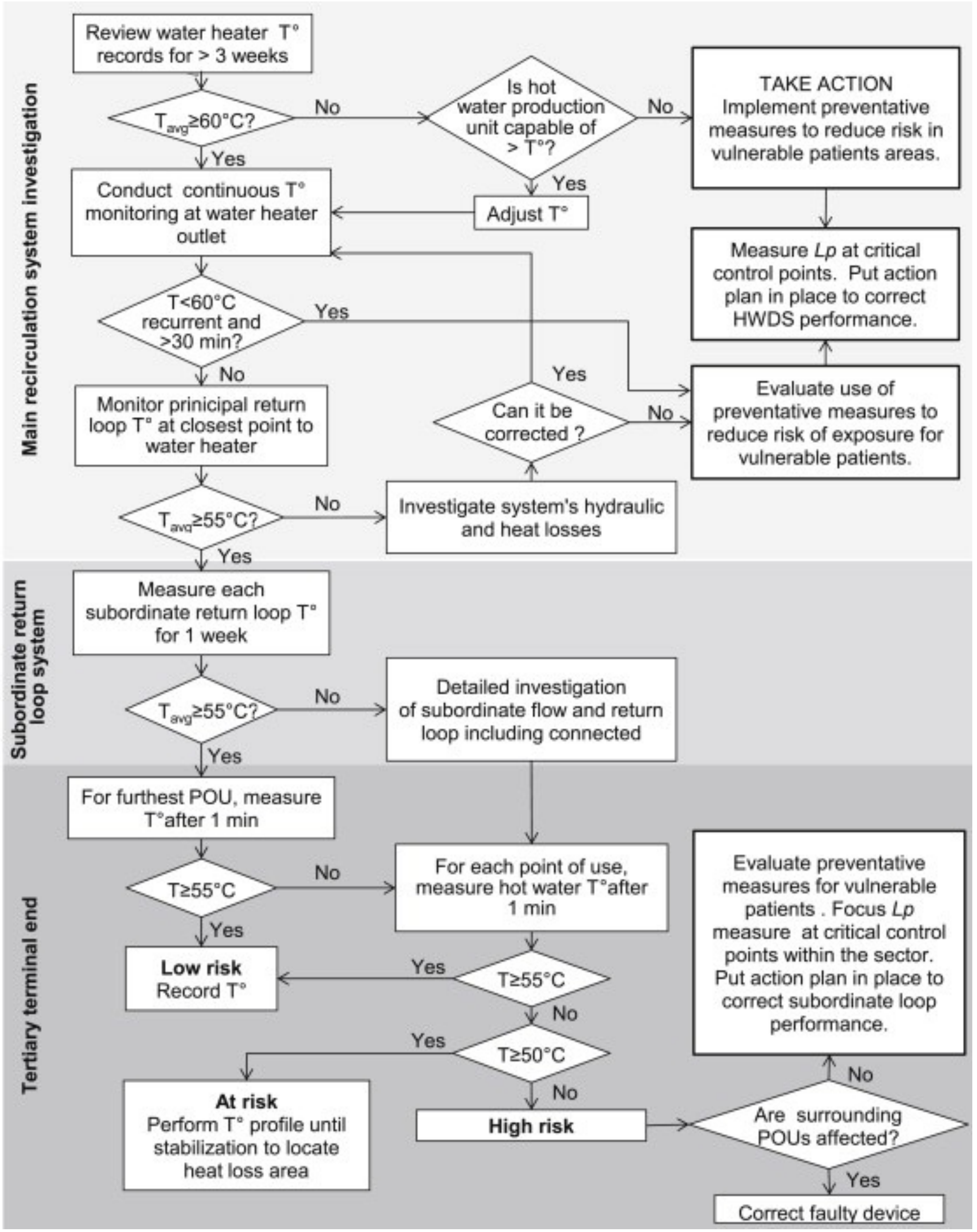
A temperature diagnosis flowchart is proposed to identify risk areas of *L. pneumophila* contamination

Useful tool for adapting measures and taking actions in case of investigation at the hot water production, on the return loop, and on unfavourable water points-of-use



How managing water quality and controlling *Legionella*?

Using ionization technologies…

American Journal of Infection Control 47 (2019) 761–766
Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major Article

Controlling *Legionella pneumophila* in water systems at reduced hot water temperatures with copper and silver ionization

Elaine Cloutman-Green PhD [a,b,*], Vera L. Barbosa PhD [c], Diego Jimenez MEng [b], Dani Helen Dunn MSc [a], Brian Needham [e], Lena Ciric PhD [b], John C. Hartley FRCPath [a]

Assessment of the efficacy of **cooper and silver ionization (CSI)** to control *L. pneumophila* at reduced hot temperatures (<43°C)

⇒ 1598 water samples during 6 years, tested for *L. pneumophila*, total viable counts, cooper and silver ion levels

100% control of *L. pneumophila* in water outlets during the entire study period with an average water temperature of 42°C

A rebalancing and a flushing regiment are needed to achieve consistent adequate levels of cooper (>0.2mg/L) and silver (0.02mg/L) at water points-of-use

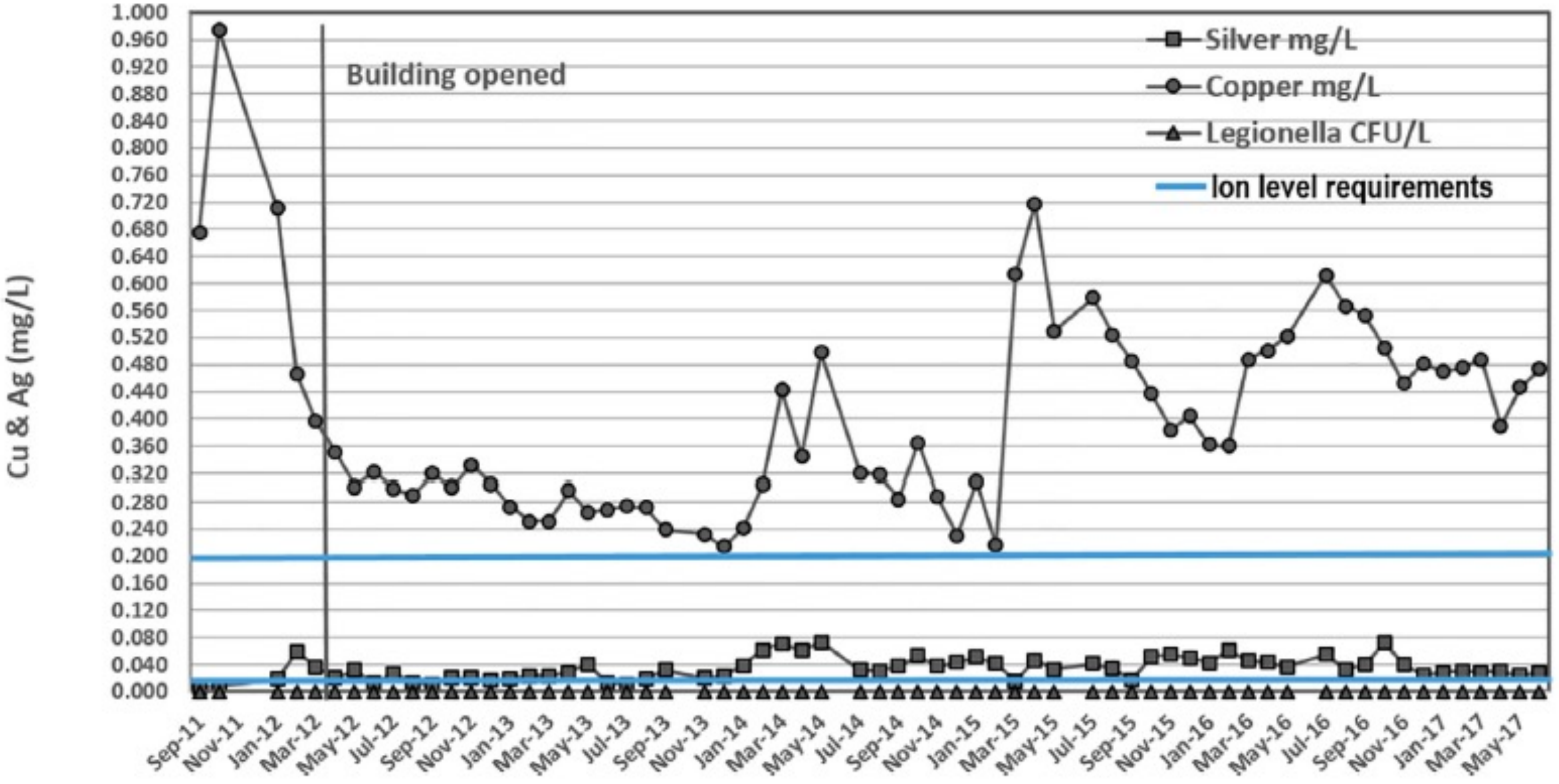


Fig 1. *Legionella pneumophila* counts and average copper and silver levels across 23 sampling points per month from September 2011 to June 2017, at the new building, United Kingdom hospital. CO2e, CO2 equivalent.

How managing water quality and controlling *Legionella*?

Using appropriate showerhead…

International Journal of *Environmental Research and Public Health*

Int. J. Environ. Res. Public Health **2022**, *19*, 3285. https://doi.org/10.3390/ijerph19063285

MDPI

Article

Risk Exposure to *Legionella pneumophila* during Showering: The Difference between a Classical and a Water Saving Shower System

Hélène Niculita-Hirzel [1,*], Audrey S. Vanhove [2], Lara Leclerc [3], Françoise Girardot [2], Jérémie Pourchez [3] and Séverine Allegra [2]

Assessing the aerosolization rate of *L. pneumophila* during showering between traditional showerhead (STA) and water-efficient showerhead (ECO) shown to emit more small particles

⇒ **Controlled experiments and determination of the emitted dose and viable airborne *Legionella* from water jets**

Table 1. Characteristics of the showerheads used in the study.

Characteristic	Continuous Flow Showerhead (STA)	Water-Atomizing Showerhead (ECO)
Number of nozzles	51	6
Diameter of nozzle (mm)	0.8	1.1
Flow rate ($L \cdot min^{-1}$)	10.2	5.5
Spray angle (°)	5	36
Water pressure (bars)	1.2	2.4
Duration of the shower (s)	15	30

How managing water quality and controlling *Legionella*?

Using appropriate showerhead…

International Journal of *Environmental Research and Public Health*

Int. J. Environ. Res. Public Health **2022**, *19*, 3285. https://doi.org/10.3390/ijerph19063285

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⇒ **Controlled experiments and determination of the emitted dose and viable airborne *Legionella* from water jets**

Bioaerosols sampled using a Coriolis® Delta air sampler. The total number of viable *Legionella* determined by flow cytometry and culture.

Similar rates of viable and cultivable Legionella aerosolized from the 2 showerheads
With a viable and cultivable fraction of only 0.0005%

⇒ The risk of exposure to *Legionella* is not expected to increase significantly with the new generation of water-efficient showerheads

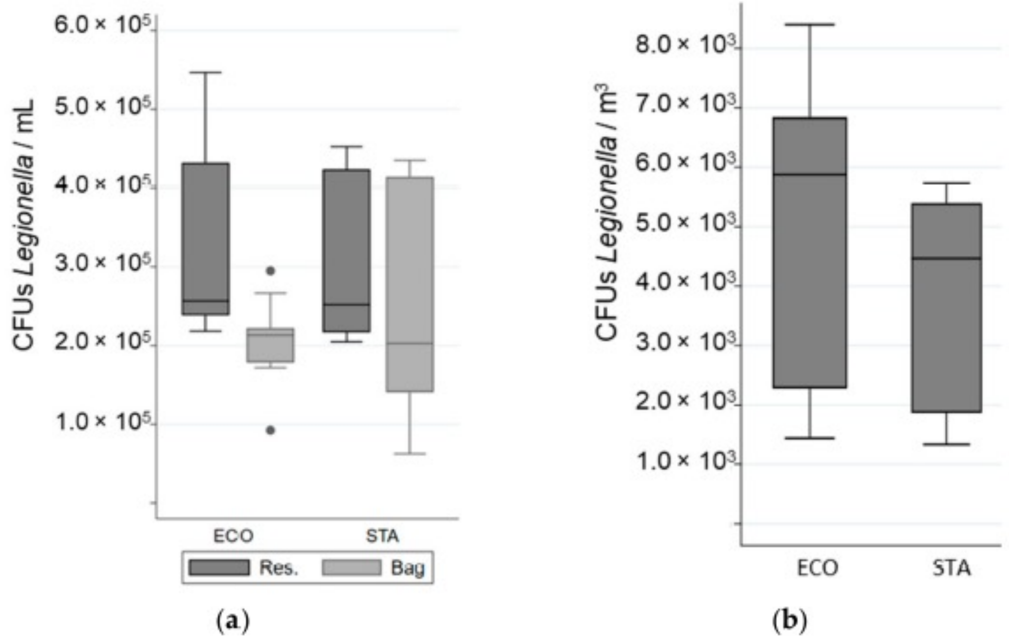


Figure 4. Variation in the number of Colony Forming Units (CFUs) of *Legionella* in different samples when a "standard" (STA) or an "economic" (ECO) showerhead was used with an equal volume of water: **(a)** in the reservoir (Res.) and in the bag; **(b)** in the aerosols. The whiskers indicate the minimum and the maximum value, the box covers the values between the first and third quartile and the line in the box marks the median value.

How managing water quality and controlling *Legionella*?

Using appropriate showerhead…

Water Research
Volume 168, 1 January 2020, 115163

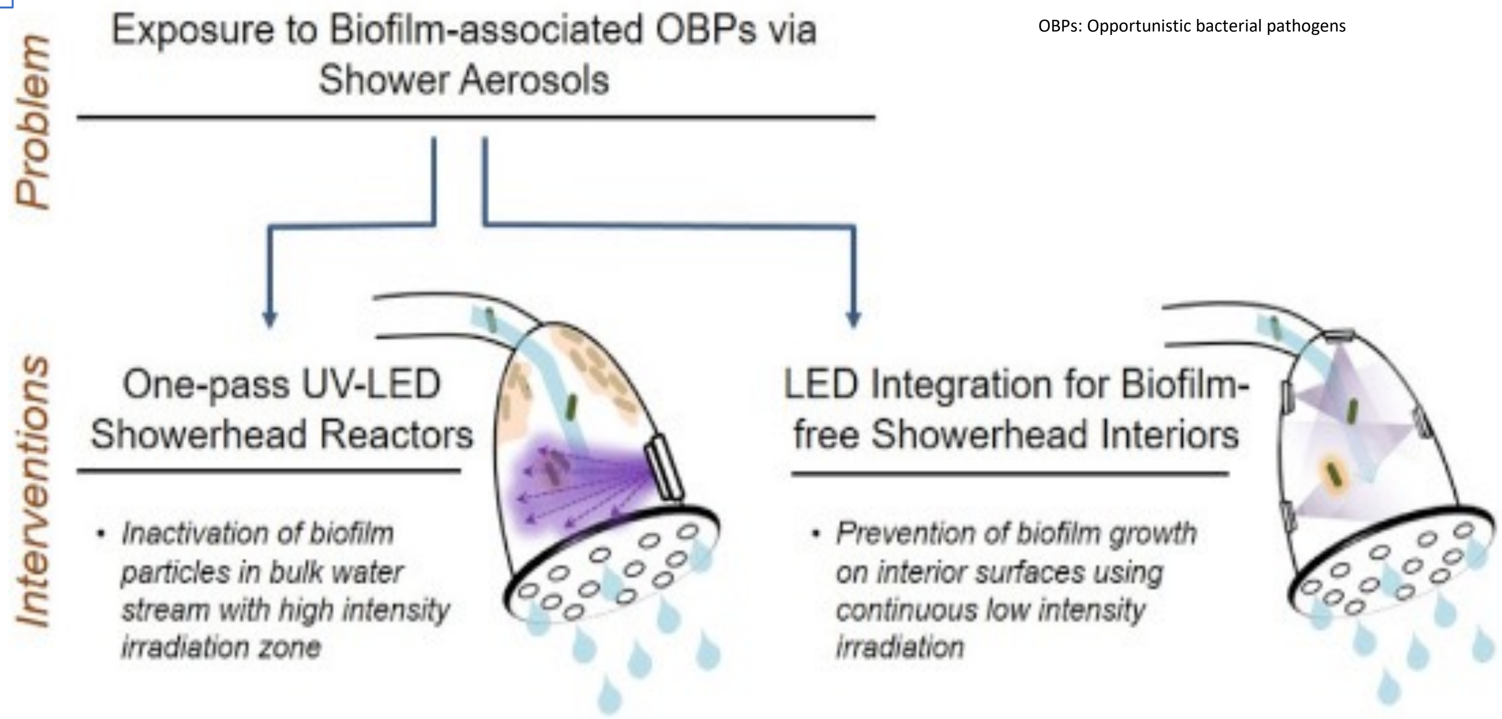
Review

Can incorporation of UVC LEDs into showerheads prevent opportunistic respiratory pathogens? – Microbial behavior and device design considerations

Ezra L. Cates, Hamed Torkzadeh

Incorporation of UVC LEDs into showerheads
⇒ significant UV irradiation in order to destroy biological matrices in which OPPPs reside including biofilm and amoebas

OBPs: Opportunistic bacterial pathogens



This disinfection engineering concept could lead to the development of showerhead devices but there is a lack of established parameters

Perspective?

Using inter-bacterial competition…

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Bacterial Long-Range Warfare: Aerial Killing of *Legionella pneumophila* by *Pseudomonas fluorescens*

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Natural and ecological way to control *L. pneumophila* using volatile compounds produced by a physically separated strain of *Pseudomonas fluorescens* (MFEO1 strain)?

Volatiles emitted by *P. fluorescens* MFE01 strain inhibit the growth of *Legionella* species.

&

The growth inhibition is irreversible.

The volatile 1-undecene, naturally produced by *P. fluorescens*, has potent activity against *Legionella*.

In small amounts, it is capable of inducing cell lysis even when the producing strain is physically separated from the target.

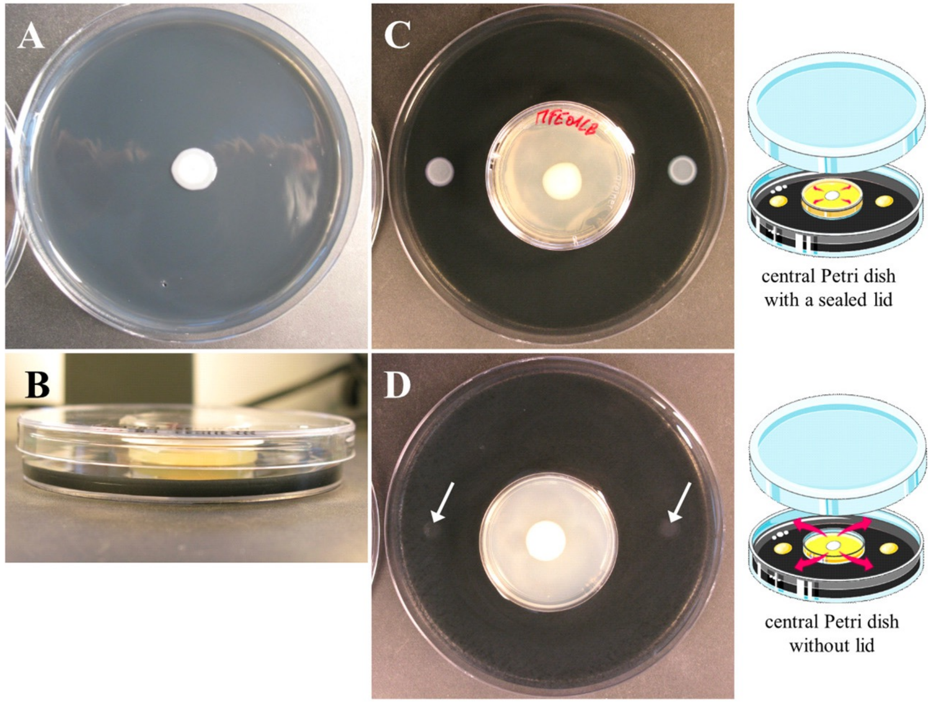
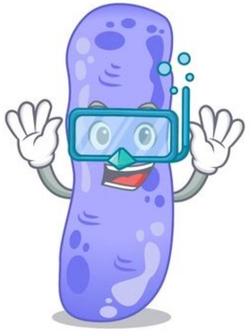


FIG 1 Antagonistic activity of P. fluorescens MFE01 toward L. pneumophila Lens. (A) Spot-on-lawn assay with P. fluorescens MFE01 (central spot). (B) Side view of the experimental two-petri-dish assay. (C) P. fluorescens MFE01 grown in a central small petri dish with a lid sealed with parafilm. (D) P. fluorescens MFE01 grown in a central small petri dish without lid. Growth of L. pneumophila Lens was monitored after 96 h of incubation at 28°C. Deposits of L. pneumophila on the agar are indicated by white arrows. The absence of bacterial growth indicates a volatile-dependent inhibitory phenotype. The pictures are representative of more than 6 experiments.

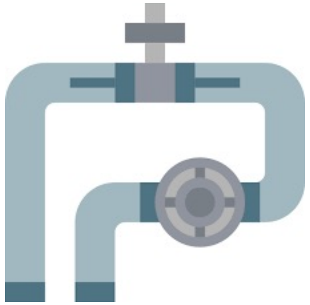
Take home messages

L. pneumophila is an OPPP => manmade opportunistic pathogen selected and amplified in water technologic niches

Human is an accidental host

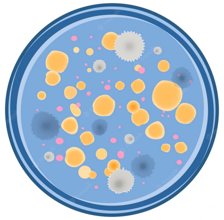


Controlling measures are based on



- Good knowledge of water network map
- Good control of physical parameters: T°, free chlorine…
- Use of appropriate materials for water pipes walls limiting biofilm formation (copper)…
- Development of devices design and technologies in water showerheads, water buses…
- Consideration of amoeba survival and amoeba-associated *Legionella* when assessing disinfection processes

- Using bacterial competition???



« L'animal qui a le plus profité de la compagnie de l'homme est le microbe »
Malcolm de Chazal

Thank you for your attention