STUDY OF INTRACELLULAR ACTIVITY OF DELAFLOXACIN COMPARED WITH LEVOFLOXACIN AGAINST LEGIONELLA

STUDY PROPOSED BY GELEG: LEGIONNAIRES' DISEASE STUDY GROUP

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Legionnaires' disease study group

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Research Institute Germans Trias i Pujol, Badalona (Barcelona, Spain)

STUDY OF INTRACELLULAR ACTIVITY OF DELAFLOXACIN COMPARED WITH LEVOFLOXACIN AGAINST LEGIONELLA

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Conflict of interest

This research was partially funded by Menarini Spain, which may be perceived as a potential conflict of interest.

Background-1

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- Mandatory reporting of LD in Catalonia since 1988
  - LUA test implemented in 1993
- In the last decade, strict legislation was implemented in most countries in order to decrease the incidence of LD
- LD continues to be a public health issue!!
  - Improvement in reporting
  - New sources of infection
  - Climate change
  - New and increasing host risk factors
- In 2021, the highest annual notification rate of Legionnaires' disease to date in the EU/EEA was observed, at 2.4 cases per 100 000 population. The rates are heterogenous across the EU/EEA region. Four countries (Italy, France, Spain, and Germany) accounted for 75% of all the notified cases. Males aged 65 years and above were the most affected group (8.9 cases per 100 000 population). The majority of the cases were considered to be community-acquired. A total of 19 outbreaks involving 137 confirmed ceported. *ECDC July 2023*

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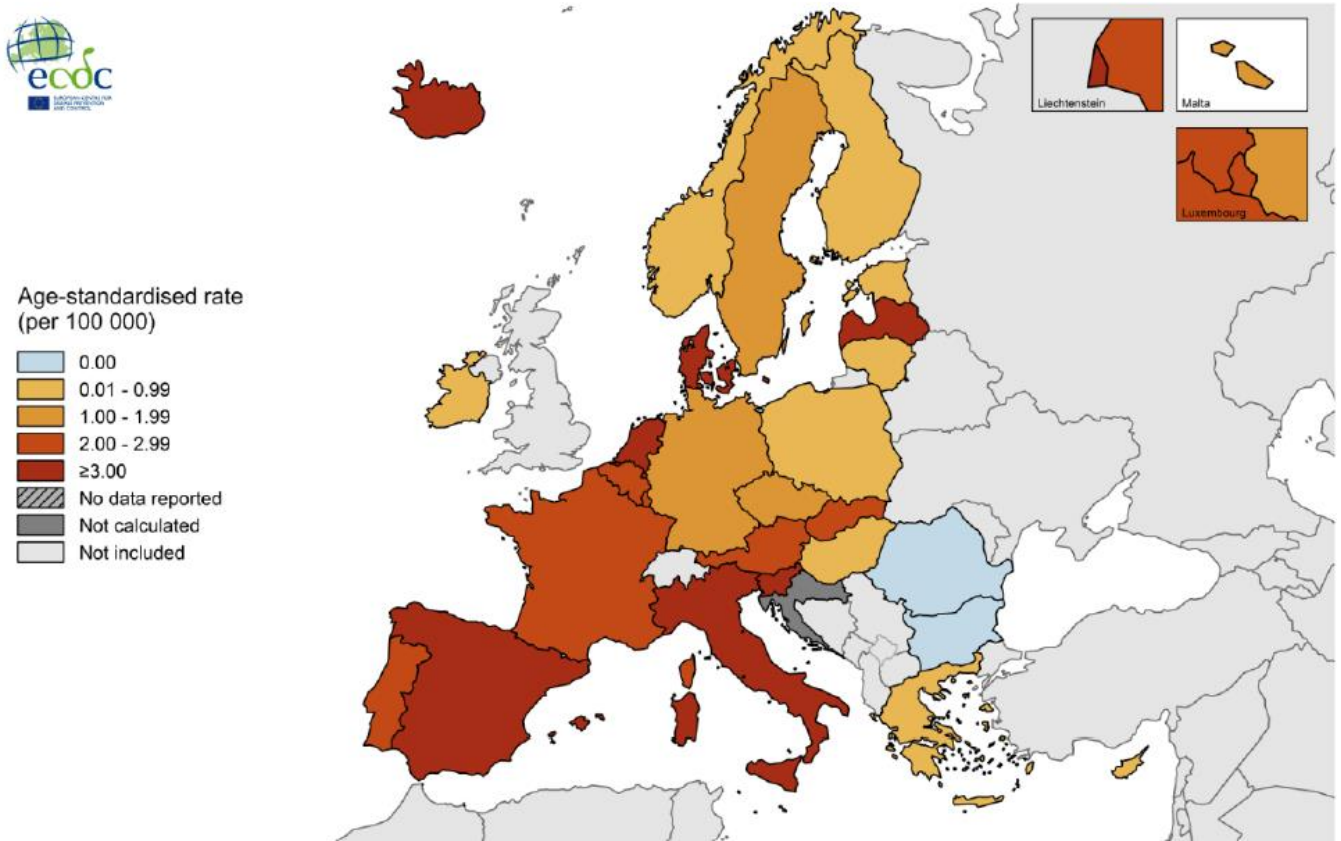
Figure 1. Distribution of cases of Legionnaires' disease per 100 000 population by country, EU/EEA, 2021



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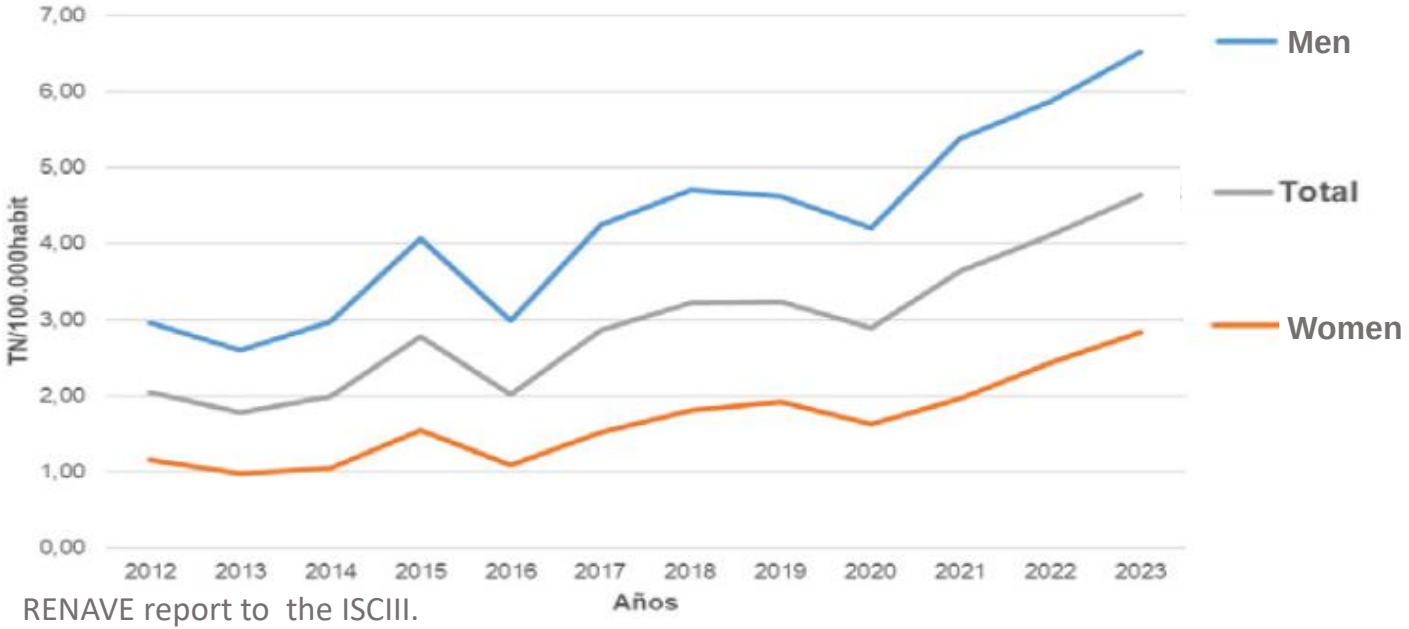
Legionelosis, tasas de notificación por 100.000 habitantes según el año de inicio síntomas y el sexo. España, años 2012 a 2023.



RENAVE report to the ISCIII.
In 2023, 2,294 cases were reported.
Notification rate of 4.66/100,000 inhabitants.
13.0% over the cases of 2022.
Cases were predominantly in the male gender (2/1).
The highest rates were in 45-64 and older than 65, for both men and women.

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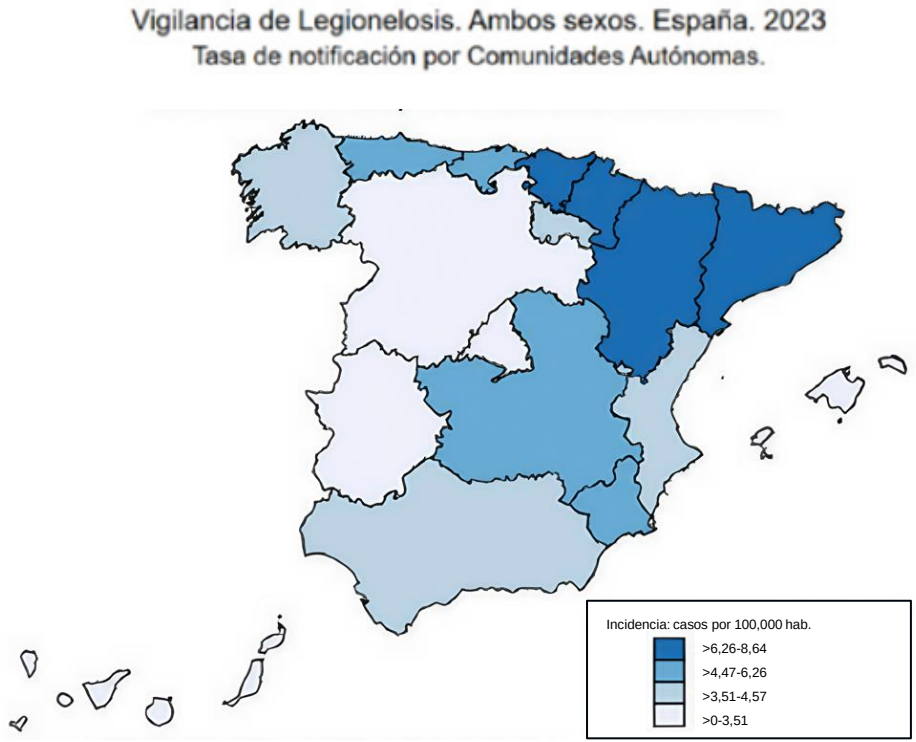
Figura 2. Legionelosis, tasas de notificación según la comunidad autónoma total y por sexo. Total. Año 2023.



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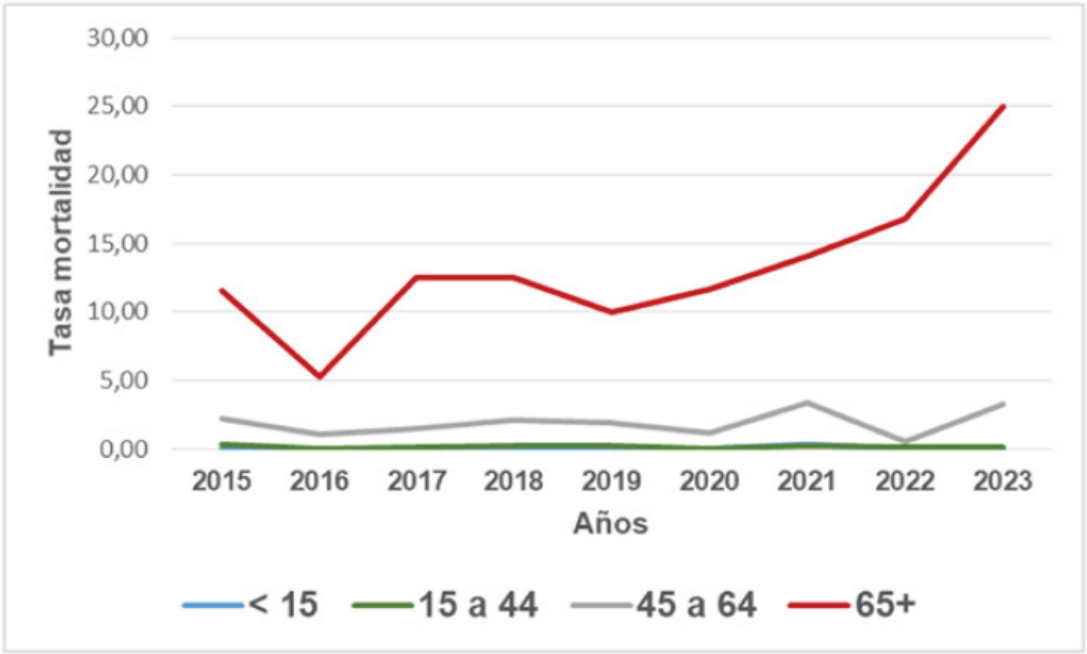

The global fatality rate was 8.4% (190/2,265) and increased with age.

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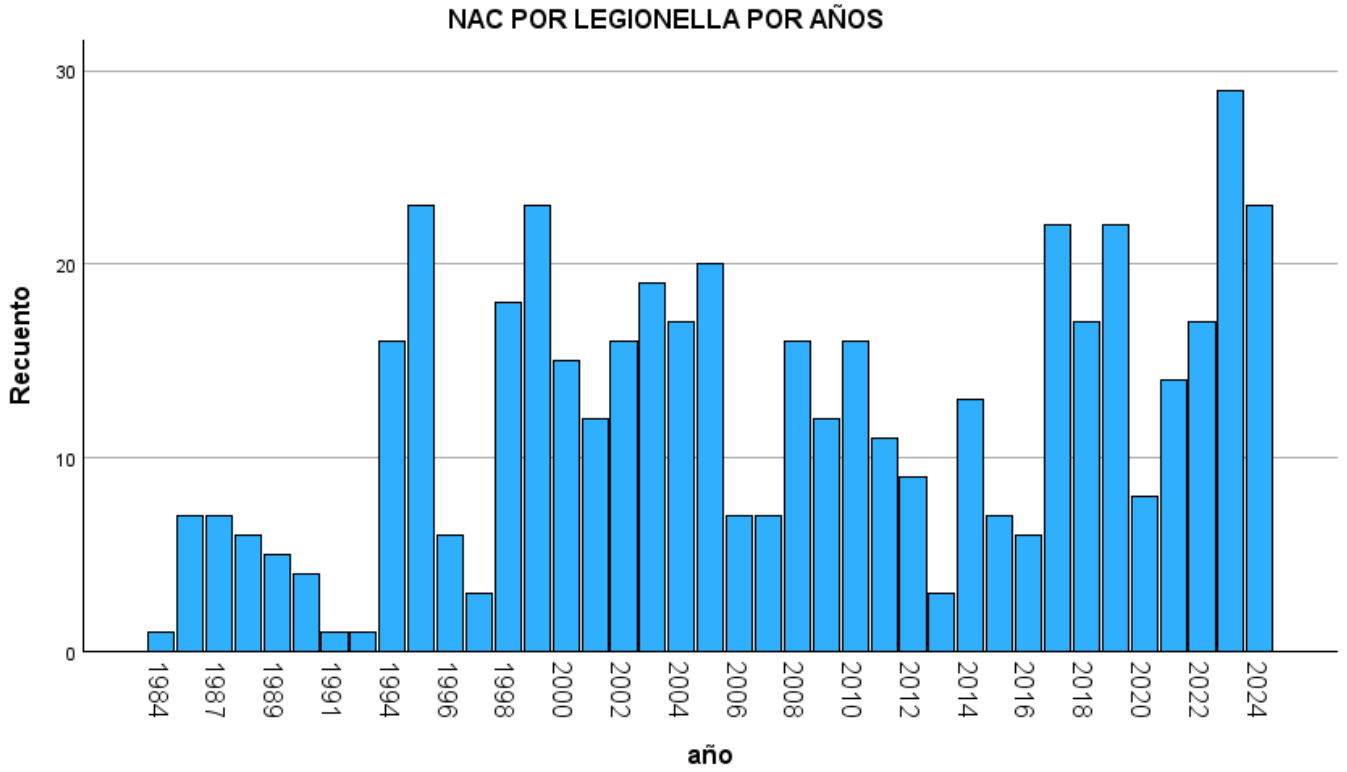
DATOS HGTiP 1984-SET/2024



Background-2

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- Legionella is more prevalent in patients with pneumonia requiring hospitalization
- *Legionella* is the 2nd cause of community-acquired pneumonia requiring admission to the ICU after *S. pneumoniae*
- *In vitro* susceptibility studies are not interpretable
- Agar plates can bind antibiotics and falsely minimize their activity against *Legionella*. In addition, numerous antibiotics with excellent *in vitro* activity against *Legionella* by conventional susceptibility methods (beta-lactams and aminoglycosides) have been shown to be relatively inactive in patients with LD.
- The intracellular character of the pathogen is relevant for the efficacy of the antibiotic. Antibiotics capable of reaching intracellular concentrations above the minimum inhibitory concentration are more effective than antibiotics with poor intracellular penetration. The antibiotics that currently meet these premises are macrolides, quinolones, tetracyclines, and rifampicin.
- There are no randomized and controlled clinical studies, however there are 4 observational based on patients with LD treated with quinolones (Levofloxacin) showing that they improve before patients treated with macrolides (erythromycin and probably clarithromycin) with no differences in the mortality.
- Finally, there are *in vitro* studies that show an additive and/or synergistic effect of quinolones (levofloxacin) in combination with macrolides (azithromycin and clarithromycin) and clinical descriptions of favorable evolution of cases of severe LD treated with these antibiotics in combination.

DELAFLOXACINO

Estructura química, acción farmacológica y mecanismo de accción

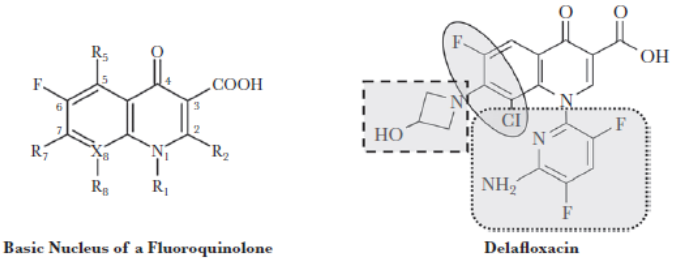

Figure 1. Structure activity relationships lead to unique features. Large and heavily substituted N1 (dotted square) and unique polarity (oval) offer photo safety regardless of presence of a halogen. Anionic nature (dashed square) and bulky molecule at N1 (dotted square) lower central nervous system toxicity.

Lodise et al. OFID 2018

Delafloxacino es una nueva fluoroquinolona aniónica con modificaciones de la estructura que mejoran su espectro de actividad antibacteriana así como su perfil farmacocinético y de seguridad. La introducción de 3 cambios concretos en la estructura base de las quinolonas, a saber, la presencia de un anillo heteroaromático sustituido en la posición N1 que incrementa la actividad anti bacteriana frente a otras fluoroquinolonas; una polaridad débil en la posición C8 la cual le confiere la mayor potencia frente a bacterias grampositivas resistentes a quinolonas, como SARM; y la ausencia de un grupo básico en la posición C7 que, al convertirla en una molécula débilmente ácida, facilita su penetración y su acumulación transmembrana potenciando su actividad antibacteriana en entornos ácidos. Por otra parte estos cambios moleculares conducen a una menor fototoxicidad y a una menor toxicidad en el SNC.

INDICACIONS CLÍNIQUES

1 ensayo clínico Fase II
3 ensayos clínicos Fase III

- IPPB x FDA en 2017 y x EMA 2019
- NAC BACTERIANA EN 2020 X FDA (Horcajada, et al 2020)
  McCurdy, et al IJID2020 (subanalisis de patógenos atípicos)
- NAC BACTERIANA X EMA 2019

Cuando se considere inapropiado el uso de otros agentes antibacterianos que se recomiendan comúnmente para el tratamiento inicial de estas infecciones

ENSAYOS FASE III

PIVOTALS	CITA BIBLIOGRAFICAA
NCT01811732	Pullman J, et al. PROCEED Study Group. Efficacy and safety of delafloxacin compared with vancomycin plus aztreonam for acute bacterial skin and skin structure infections: a Phase 3, double-blind, randomized study. J Antimicrob Chemother. 2017 Dec 1;72(12):3471-3480.
NCT01984684.	O'Riordan W, et al. PROCEED Study Group. A Comparison of the Efficacy and Safety of Intravenous Followed by Oral Delafloxacin With Vancomycin Plus Aztreonam for the Treatment of Acute Bacterial Skin and Skin Structure Infections: A Phase 3, Multinational, Double-Blind, Randomized Study. Clin Infect Dis. 2018 Aug 16;67(5):657-666.
NCT02679573	Horcajada JP, ET AL. DEFINE-CABP Study Group. A Phase 3 Study to Compare Delafloxacin With Moxifloxacin for the Treatment of Adults With Community-Acquired Bacterial Pneumonia (DEFINE-CABP). Open Forum Infect Dis. 2019 Dec 5;7(1):ofz514.

NCT02679573

- This phase 3 study compared the efficacy and safety of delafloxacin with moxifloxacin for the treatment of CABP.
- ***A randomized, double-blind, comparator-controlled, multicenter, global phase 3 study compared the efficacy and*** safety of delafloxacin 300 mg twice daily or moxifloxacin 400 mg once daily in adults with CABP.
- The primary end point was early clinical response (ECR), defined as improvement at 96 (±24) hours after the first dose of study drug. Clinical response at test of cure (TOC) and microbiologic response were also assessed.
- ***Results. N=520. In the intent-to-treat analysis population (ITT), ECR rates were 88.9% in the delafloxacin group and 89.0% in the*** **moxifloxacin group**. Noninferiority of delafloxacin compared with moxifloxacin was demonstrated. At TOC in the ITT population, the success rates were similar between groups. **Treatment-emergent adverse events that were considered at least possibly related to the study drug occurred in 65 subjects (15.2%) in the delafloxacin group and 54 (12.6%) in the moxifloxacin group.**

- **2 studies were then published regarding**:
- **A detailed analysis of the microbiology from the phase 3 study (ML-3341-306; ClinicalTrials.gov identifier: NCT02679573) (McCurdy et al., AAC 2020;64(3):e01949–19. 2020).**
- Detailed data regarding atypical pathogens (McCurdy S, et al. IJID 97 (2020) 374–379
- **Results: The microbiological intent-to-treat (MITT) population included 520 patients; 30% had an atypical bacterial pathogen identified (156/520). Overall, 13.1% (68/520) had a monomicrobial atypical infection and 2.3% (12/520) had polymicrobial all-atypical infections**. Among patients with polymicrobial infections, Streptococcus pneumoniae was the most frequently occurring co-infecting organism and Chlamydia pneumoniae was the most frequently occurring co-infecting atypical organism.
- **Delafloxacin and moxifloxacin had similar in vitro activity against M. pneumoniae and delafloxacin had greater activity against L. pneumophila**. Two macrolide-resistant M. pneumoniae isolates were recovered. No fluoroquinolone-resistant M. pneumoniae were isolated. The rates of microbiological success (documented or presumed eradication) at test-of-cure were similar between the delafloxacin and moxifloxacin groups. There was no evidence of a correlation between minimum inhibitory concentration (MIC) and outcome; a high proportion of favorable outcomes was observed across all delafloxacin baseline MICs

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RESEARCH QUESTION

1. Is the intracellular activity of Delafloxacin different between various serogroups of *Legionella pneumophila* and other species of the *Legionella* genus?
2. Is this activity bactericidal or bacteriostatic?
3. Is this activity similar or better than Levofloxacin?

HYPOTHESIS

The antibiotic Delafloxacin has intracellular bactericidal activity against *Legionella* and this activity is not inferior to Levofloxacin.

STUDY OF INTRACELLULAR ACTIVITY OF DELAFLOXACIN

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Objectives

Main objective

- Determine the intracellular activity of Delafloxacin and Levofloxacin against *Legionella.*

Secondary objectives

- To determine which minimum concentration of Delafloxacin and Levofloxacin eliminates viable cultivable bacteria.
- To determine if the effect of Delofloxacin is bactericidal or bacteriostatic in intracellular test.

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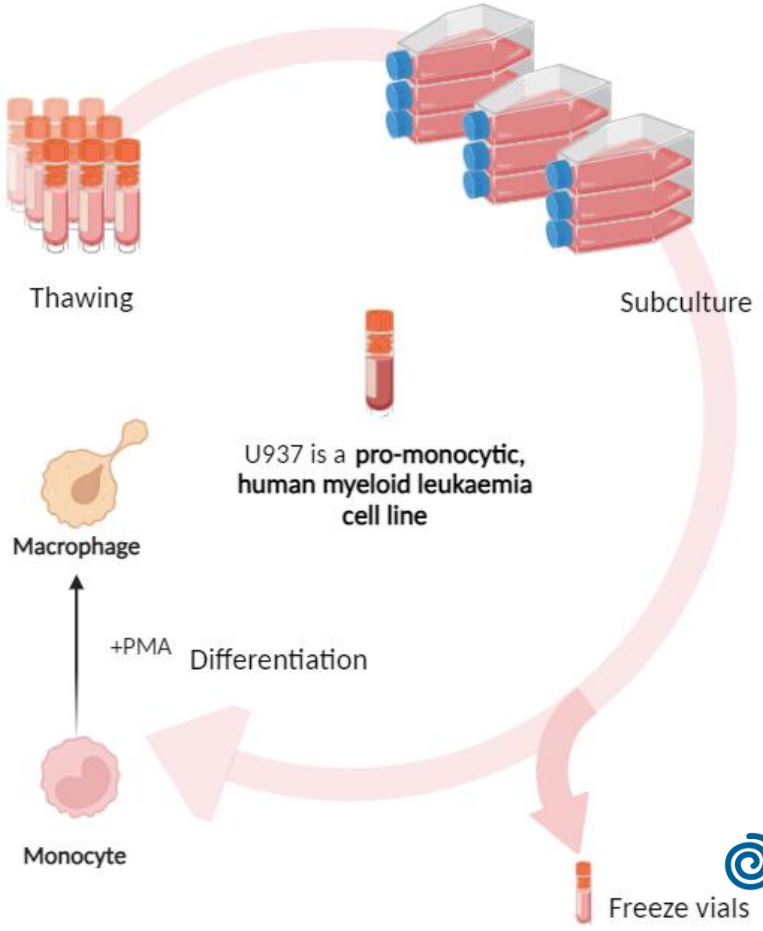
Methods – general overview

1. **Expansion. Protocol optimization.**
2. **Macrophage differentiation.**

Since *Legionella* infects macrophages and replicates inside them, it is necessary to have an antibiotic capable of penetrate into the cells.

Development of an *in vitro* model for the characterization of antibiotic activity



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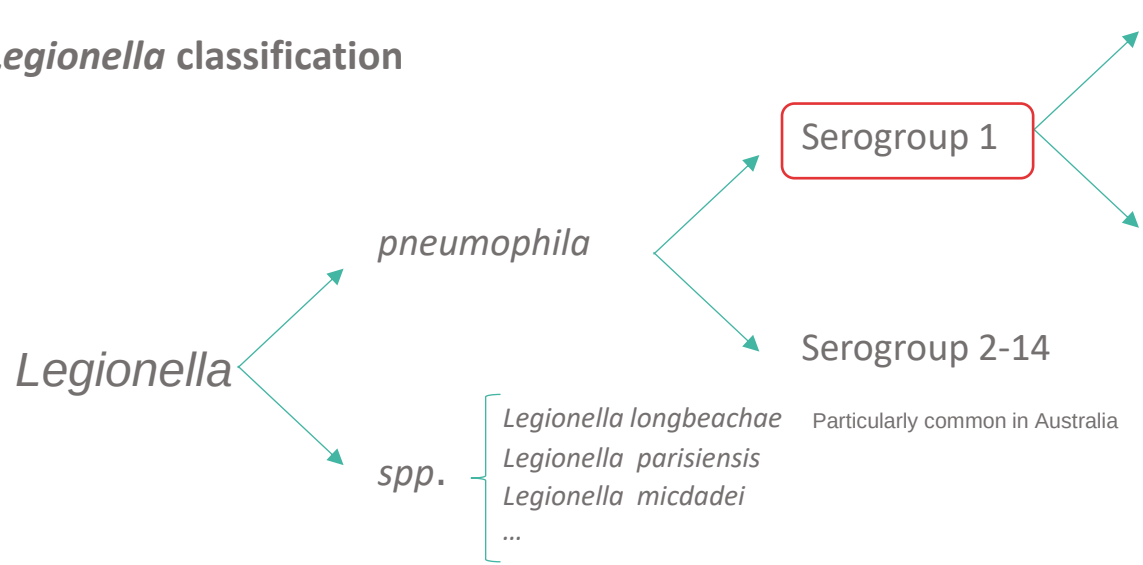
Legionella classification



Monoclonal antibody 3/1 recognizes a virulence-associated LPS from *Legionella*

Serogroups reported for culture-confirmed cases of *L. pneumophila*, EU/EEA, 2020 and 2021

Serogroup (SG)	2020		2021	
	Number	%	Number	%
1	685	83	890	82
2	5	<1	14	1
3	22	3	46	4
4	0	<1	3	<1
5	4	<1	5	<1
6	16	2	10	1
7	3	<1	6	1

Species included in the present work

- 4x *L. pneumophila* sg 1 (2x mAb3/1+ and 2x mAb 3/1-)
- 3x *L. pneumophila* sg 2-14 (sg 3 - 6 - 12)
- 3x *Legionella spp.* (*longbeachae, parisiensis, micdadei*)

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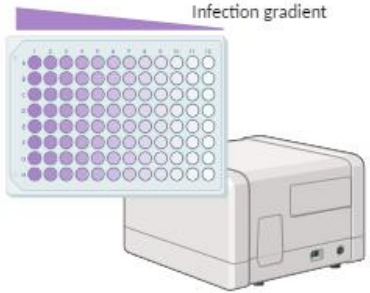
Methods – general overview

3. Determination of *Legionella* concentration for macrophage infection with Alamar Blue assay

Previous trial before antibiotics study



Assessment of inoculum pipeline

Differentiated macrophages were incubated on 96 well plate.

t = 0h

→

Infection with bacterial gradient.

t = 24h

→

Reading with Alamar Blue & assessment of 50% viability decrease.

t = 96h

	Infection inoculum (log CFU/mL)
Lp sg1	
Lp sg 2-14	
L spp	

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Methods – general overview

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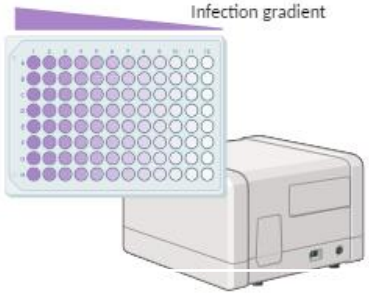
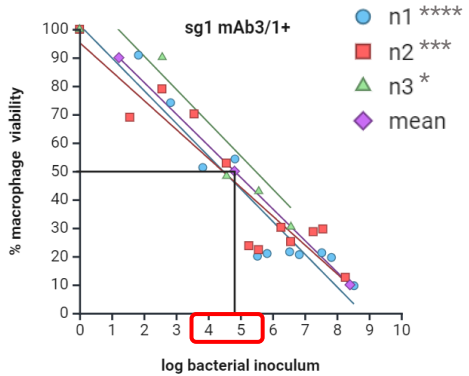
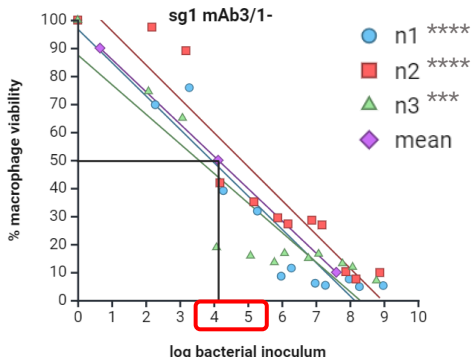
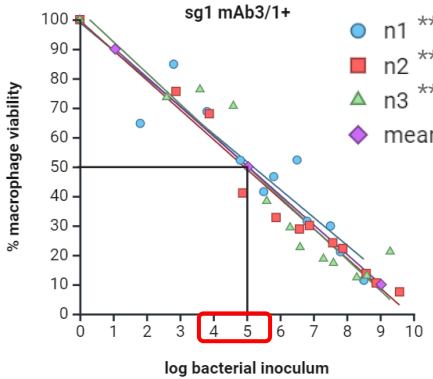


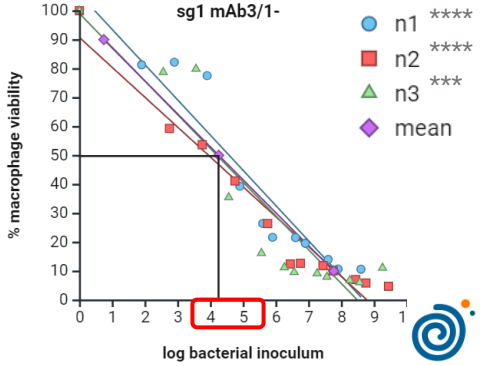
	Infection inoculum (log CFU/mL)
Lp sg1	5
Lp sg 2-14	5
L spp	6

P-value < 0.0001 ****
< 0.001***
0.03513*









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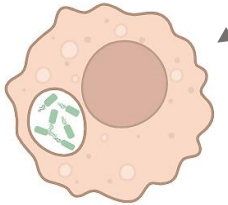
Methods – general overview

4. **Determination of the intracellular effect of Delafloxacin and Levofloxacin**

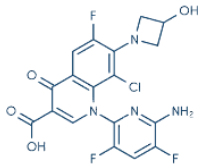
- **Assessment of <u>culturability</u>** by microbiological culture in BCYEα (bacteriostatic effect)

- **Assessment of Legionella <u>viability</u>** in a complex sample by flow cytometry (bactericide effect)

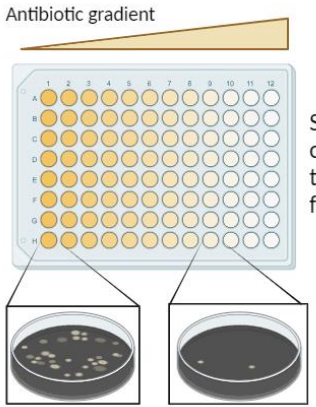
Culturability methodology



Infected macrophage



Delafloxacin / levofloxacin



Only for illustrative purposes

Study of the antibiotic concentration needed to reduce intracellular fraction culturability

Only proceed with cytometry analysis when a significant reduction in culturability (>90%) is obtained

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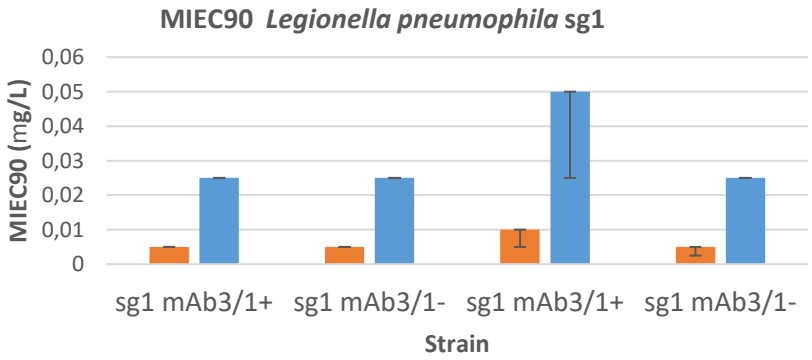
Results – culturability

Minimum Inhibitory Extracellular Concentration (MIEC90): the lowest concentration of agent which had an inhibitory effect (90% decrease) on intracellular *Legionella* multiplication.



Delafloxacin enhanced activity in culturability decrease	
Lp sg1	5 to 10 X
Lp sg2-14	
L spp	

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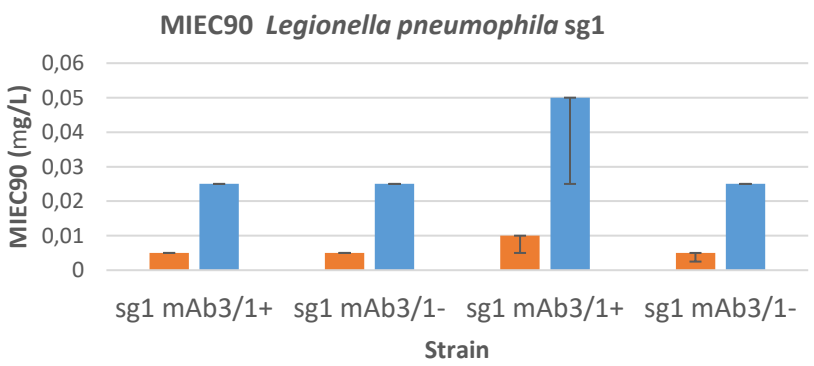

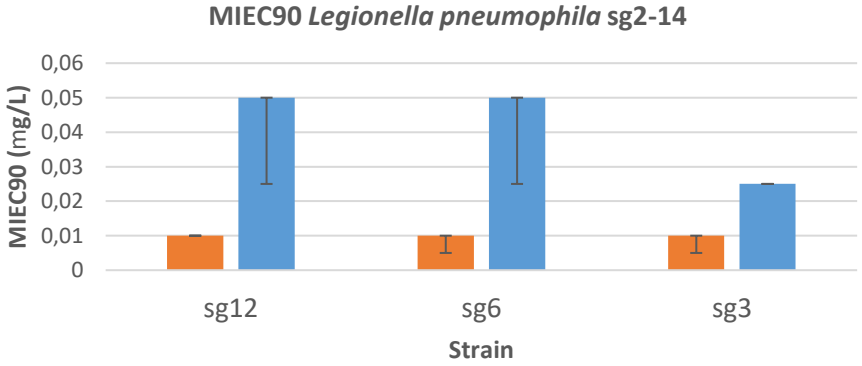

Delafloxacin

Levofloxacin

Delafloxacin enhanced activity in culturability decrease	
Lp sg1	5 to 10 X
Lp sg2-14	2,5 to 5X
L spp	

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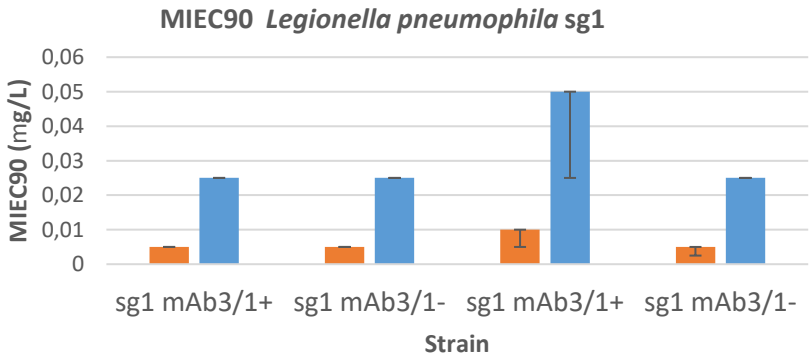
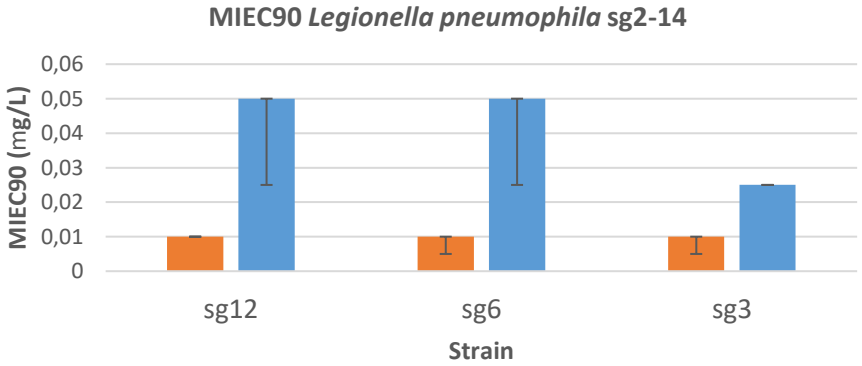
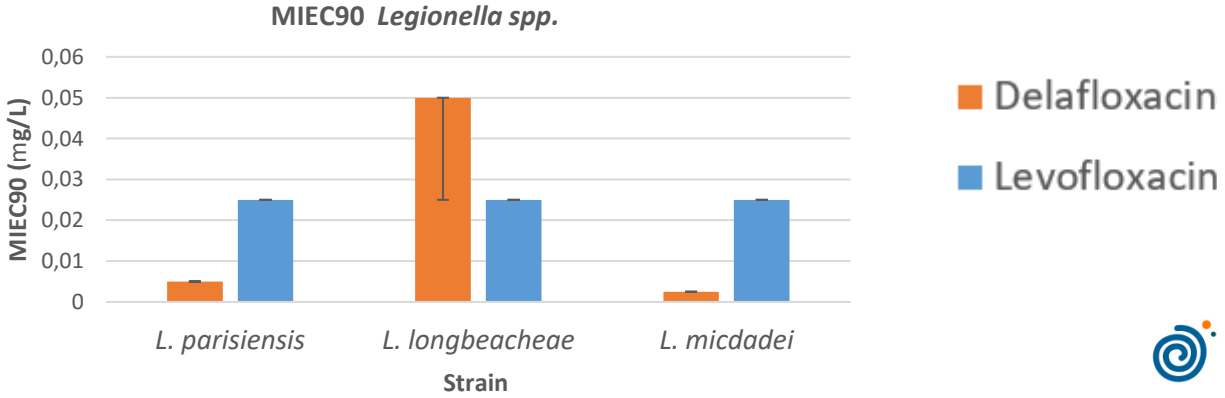
Results – culturability

Minimum Inhibitory Extracellular Concentration (MIEC90): the lowest concentration of agent which had an inhibitory effect (90% decrease) on intracellular *Legionella* multiplication.







Delafloxacin enhanced activity in culturability decrease	
Lp sg1	5 to 10 X
Lp sg2-14	2,5 to 5X
L spp	-0,5 to 10X

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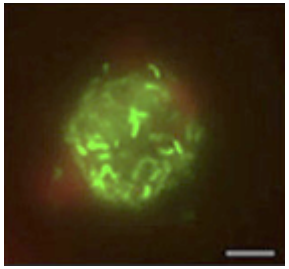
Methods – Flow cytometry

Most common CLSI (Clinical & Laboratory Standards Institute) methods are based on culturability outputs for MIC determination (broth Dilution method, agar dilution method, disk diffusion method)



Infected macrophage (3-8 days co-culture with non-culturable *Legionella*)

Dietersdorfer, E. et al. (2018).

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Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/scitotenv

Persistence of viable but nonculturable *Legionella pneumophila* state in hospital water systems: A hidden enemy?

Noemí Párraga-Niño [a,b,*], Roger Cortès-Tarragó [a], Sara Quero [a,b,c,d], Marian Garcia-Núñez [a], Elisenda Arqué [a], Sara Sabaté [e,f], Dolors Ramirez [g], Laura Gavaldà [g]

Legionella can survive for decades in drinking water systems and infect repeatedly through aerosols

Antibiotic could induce a **viable but non-culturable** state of the bacteria, in which does not growth but remains **potentially infectious.**

Analysis by flow cytometry (assessment of viability) mAbOMP28 + Viability staining

Antibody test for *Legionella pneumophila* detection

Noemí Párraga-Niño [a,b], Sara Quero [a], Naroa Uria [c], Oscar Castillo-Fernandez Francesc-Xavier Muñoz [c], Miquel Sabrià [a,b,d,*], Marian Garcia-Nuñez [a,b]

STUDY OF INTRACELLULAR ACTIVITY OF DELAFLOXACIN

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Conclusions

- In most strains studied, chemical **modifications of delafloxacin resulted in increased intracellular activity** compared to levofloxacin.
- In the studied strains of ***L. pneumophila*** , delafloxacin achieved reductions in culturability of **5x -10x** higher than levofloxacin.
- Likewise, in some strains of *Legionella spp*., delafloxacin achieved reductions 10x higher than levofloxacin. However, ***L. longbeachae*** showed the same (or less) susceptibility to delafloxacin compared to levofloxacin.
- Flow cytometry results confirming the current or higher MIEC90 will be available in the near future.
- It is important to maintain good epidemiological surveillance to detect the emergence of antibiotic resistance. If required, **resistance mechanisms need to be characterized**.

STUDY OF INTRACELLULAR ACTIVITY OF DELAFLOXACIN

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TAKE HOME MESSAGES

1. Based on the results obtained regarding culturability, Delafloxacin shows promise for **advancement to a Phase III clinical trial**, positioning it as a potential antibiotic with a new indication for patients with Legionnaires' disease.

2. Additional studies are required to investigate the mechanisms underlying **the lack of susceptibility** of certain strains with Delafloxacin.

MANY THANKS!!!

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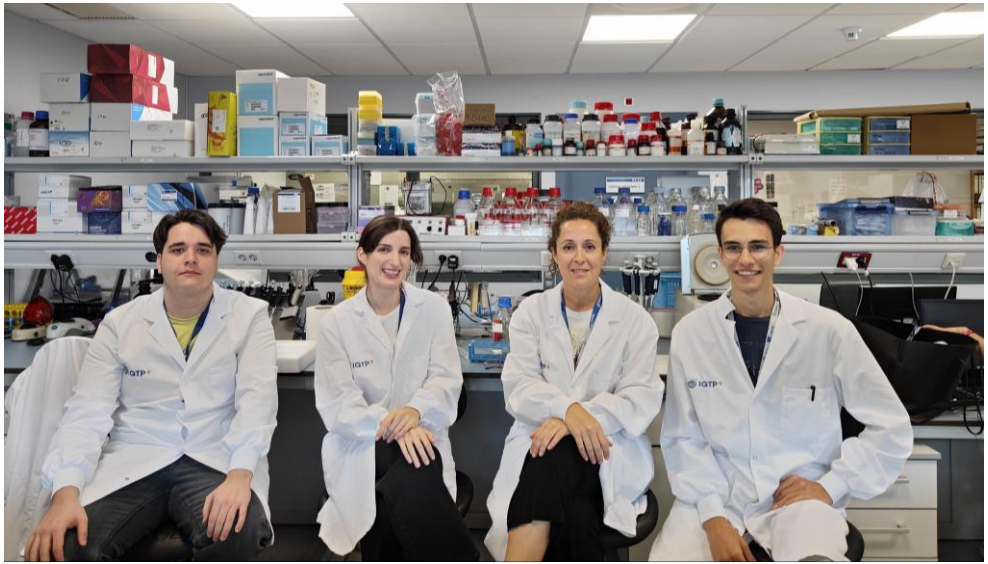

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