ESGLI 2015: 16th-17th September

The broth microdilution method allows for the detection of macrolides, fluoroquinolones and rifampicin resistant *L. pneumophila*

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Hôpitaux de Lyon

ciri Centre International de Recherche en Infectiologie

LEGIONELLES Centre National de Référence

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Antibiotic resistance: a growing issue in *L. pneumophila*

Classically described as "**constantly susceptible**" to:

Rifampicin

Fluoroquinolones

Macrolides

Antibiotic resistance: a growing issue in *L. pneumophila*

Classically described as "**constantly susceptible**" to:

Rifampicin | **Fluoroquinolones** | **Macrolides**

However,

In vitro, easy selection of resistant mutants

Nielsen *et al*., 2000

1st ciprofloxacin-resistant clinical strain

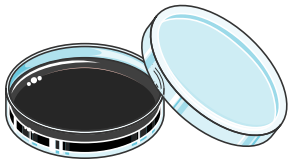
Bruin *et al*., 2014

Need for a **standardized method** to detect *Legionella* **resistant** strains

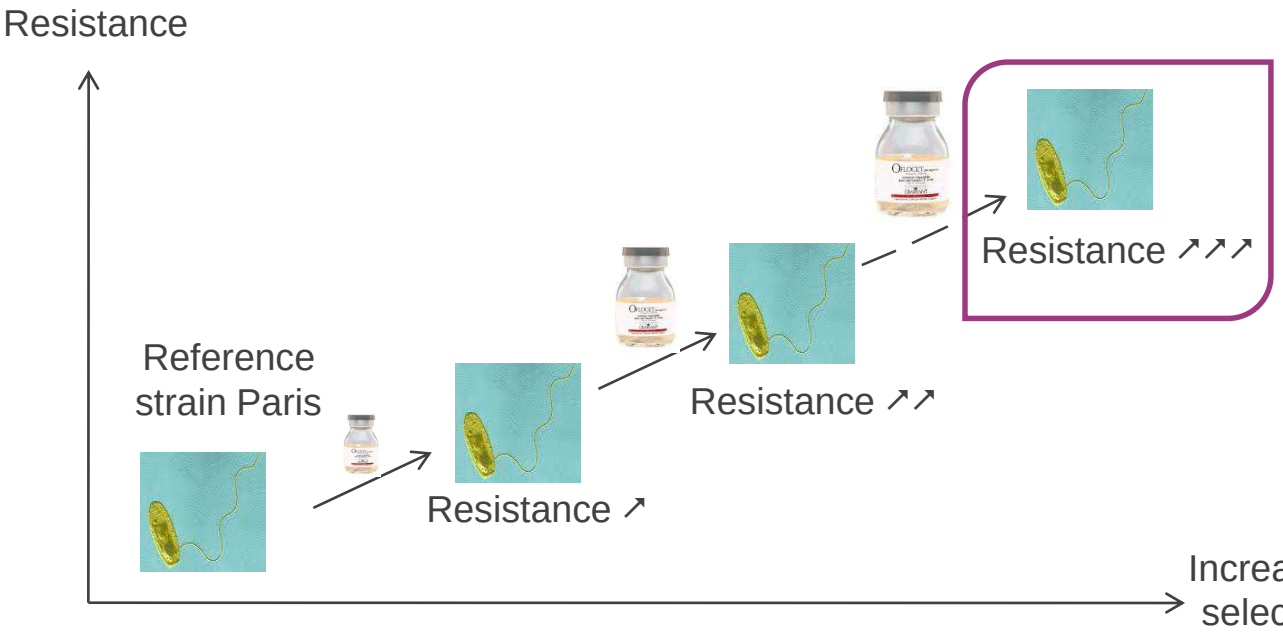
Selection of *L. pneumophila* resistant strains

From the reference strain ***L. pneumophila* Paris** (ST1), selection of **resistance** to:

Rifampicin



By serial passages with ↗ sub-inhibitory concentrations of rifampicin on BCYE



10 independent resistant lineages

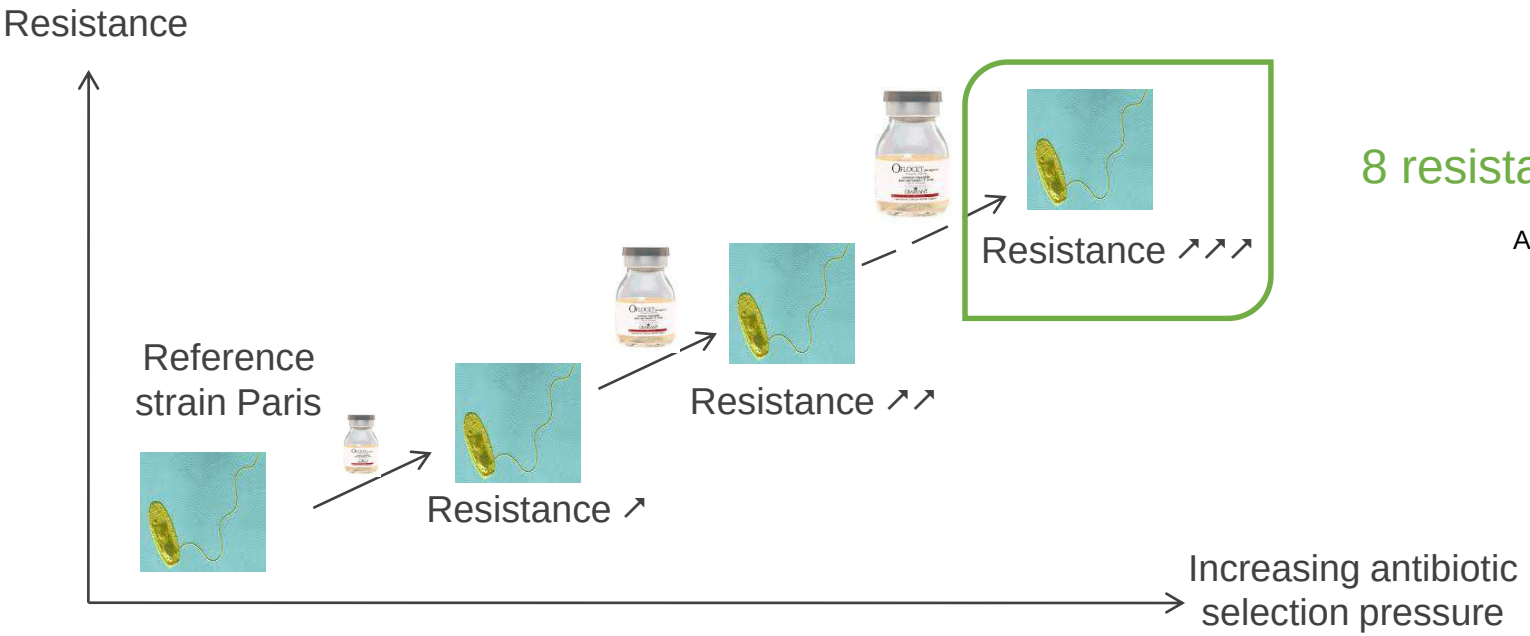
Unpublished work, French National Reference Centre of *Legionella*

Selection of *L. pneumophila* resistant strains

From the reference strain ***L. pneumophila* Paris** (ST1), selection of **resistance** to:

Fluoroquinolones

By serial passages with ↗ sub-inhibitory concentrations of moxifloxacin in liquid medium



8 resistant lineages

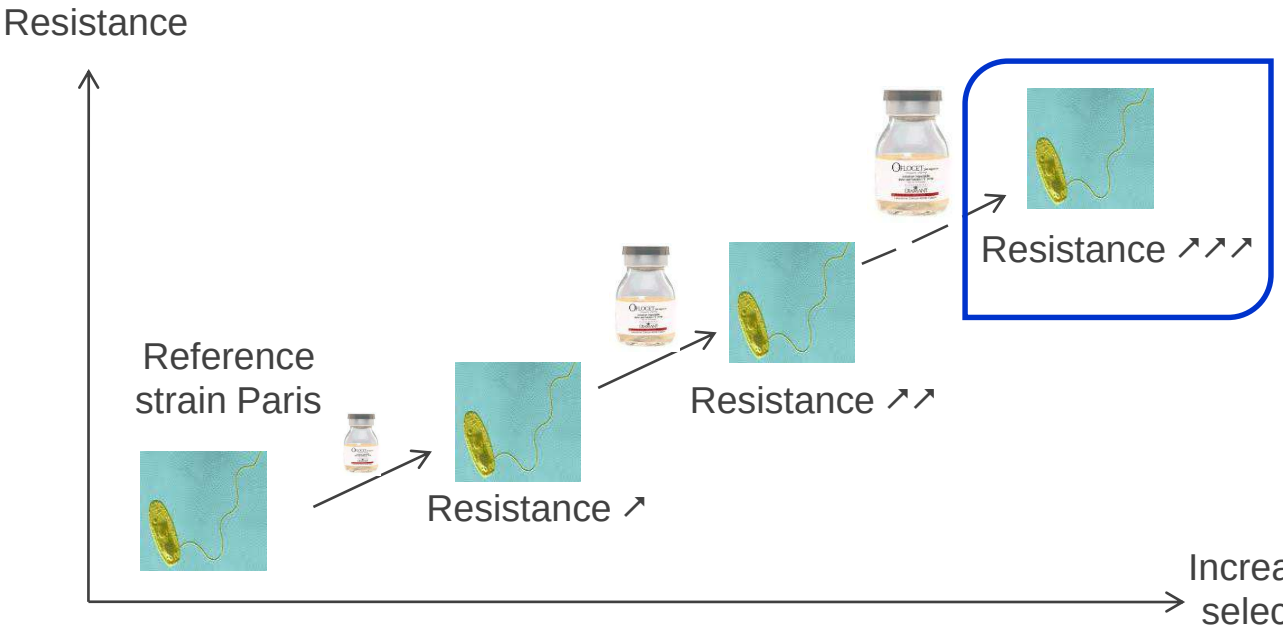
Almahmoud *et al.*, 2009

Selection of *L. pneumophila* resistant strains

From the reference strain ***L. pneumophila* Paris** (ST1), selection of **resistance** to:

Macrolides

By serial passages with ↗ sub-inhibitory concentrations of erythromycin or azithromycin in liquid medium



12 resistant lineages:
6 selected with erythromycin
6 selected with azithromycin

Unpublished work, French National Reference Centre of *Legionella*

Objectives of our study

Determine the **minimum inhibitory concentrations** (MICs) of **rifampicin, fluoroquinolones and macrolides** by **broth microdilution method** for:

2 reference strains

109 clinical strains:
L. pneumophila serogroup 1,
(variable STs, severity levels)

Chidiac *et al.*, 2012

10 rifampicin resistant strains

8 fluoroquinolone resistant strains

12 macrolide resistant strains

Comparison of MICs distributions

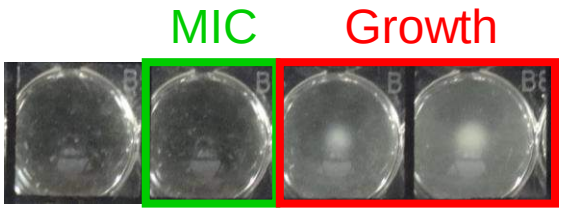
Distinct distributions ?

Susceptibility testing by broth microdilution method

Bacterial suspension of each strain

Decreasing 2-fold concentrations of antibiotics

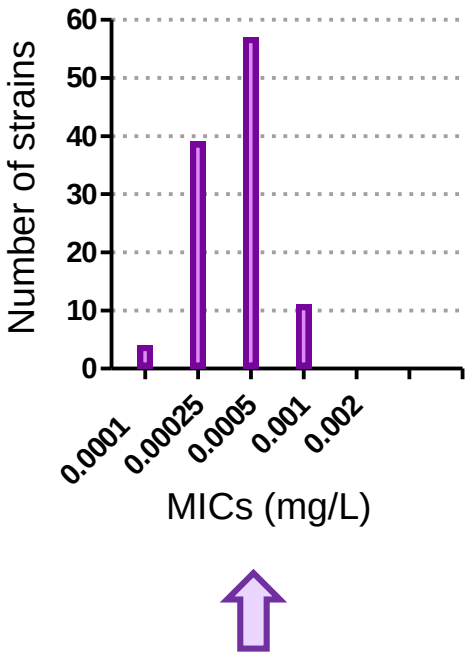
Incubation 48 hours at 35°C



Minimum inhibitory concentrations (MICs)
= lowest antibiotic concentrations inhibiting visible bacterial growth

Rifampicin susceptibility

Clinical strains MICs:
0.00012 – 0.001 mg/L



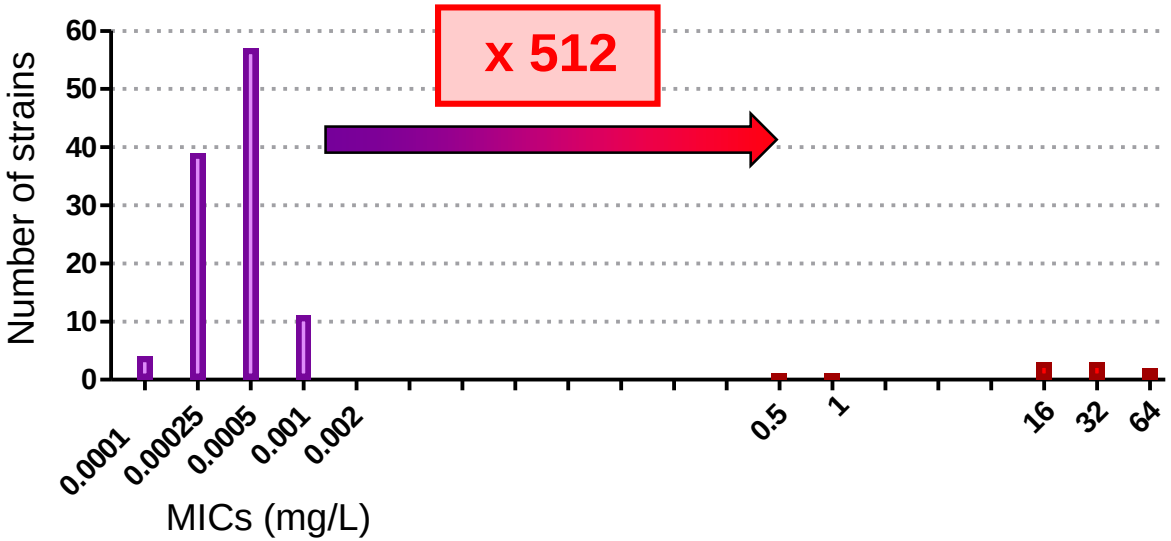
Median: 0.0005 mg/L

Rifampicin susceptibility

Resistance mechanisms: mutations in ***rpoB*** gene encoding **RNA polymerase**

Clinical strains MICs:
0.00012 – 0.001 mg/L

Resistant strains MICs:
0.5 - 64 mg/L



Mutation in *rpoB*

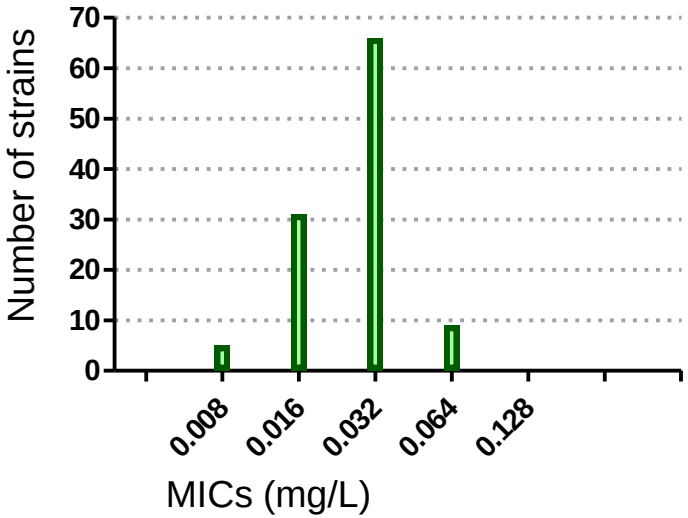
Median: 0.0005 mg/L

Median: 24 mg/L

Fluoroquinolones susceptibility

Moxifloxacin

Clinical strains MICs:
0.008 – 0.064 mg/L



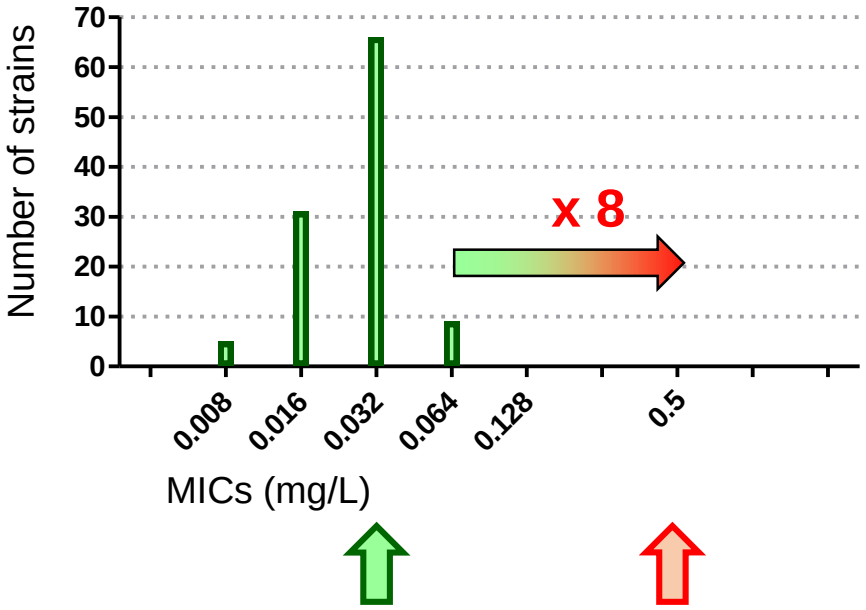
Median:
0.032 mg/L

Fluoroquinolones susceptibility

Resistance mechanisms: mutations in **DNA gyrase** (*gyrA*)

Moxifloxacin

Clinical strains MICs:
0.008 – 0.064 mg/L



Median:
0.032 mg/L

1st mutation in *gyrA*
⇨ MIC: 0.5 mg/L

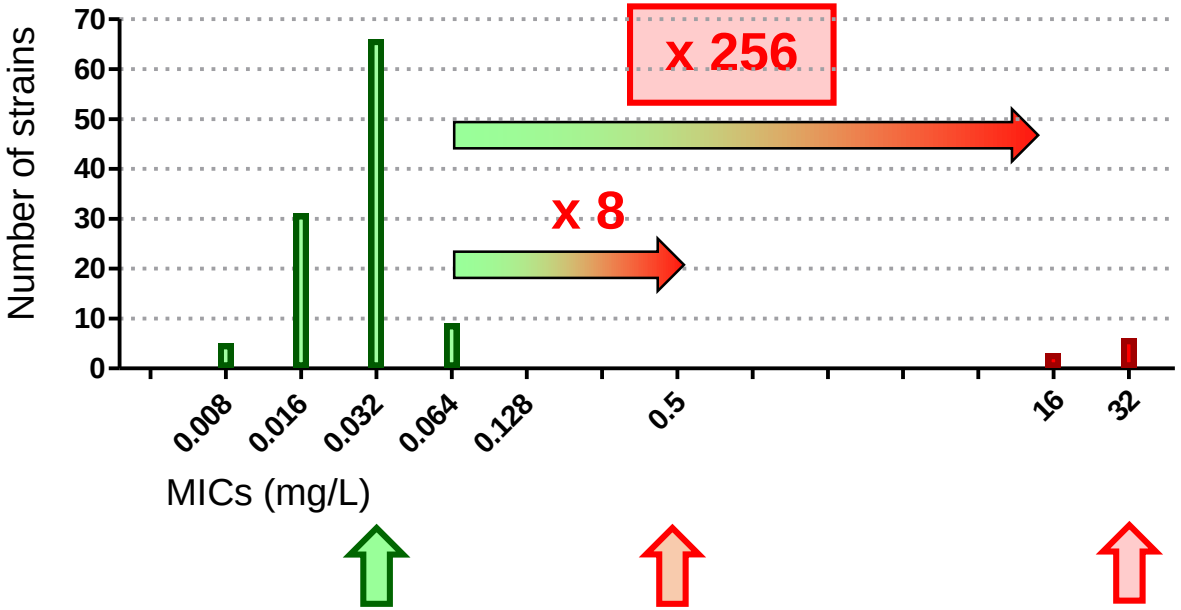
Fluoroquinolones susceptibility

Resistance mechanisms: mutations in **DNA gyrase** (*gyrA*, *gyrB*) and **topoisomerase IV** (*parC*)

Moxifloxacin

Clinical strains MICs:
0.008 – 0.064 mg/L

Resistant strains MICs:
16 – 32 mg/L



Mutations in *gyrA*, *gyrB* and *parC*

Median:
0.032 mg/L

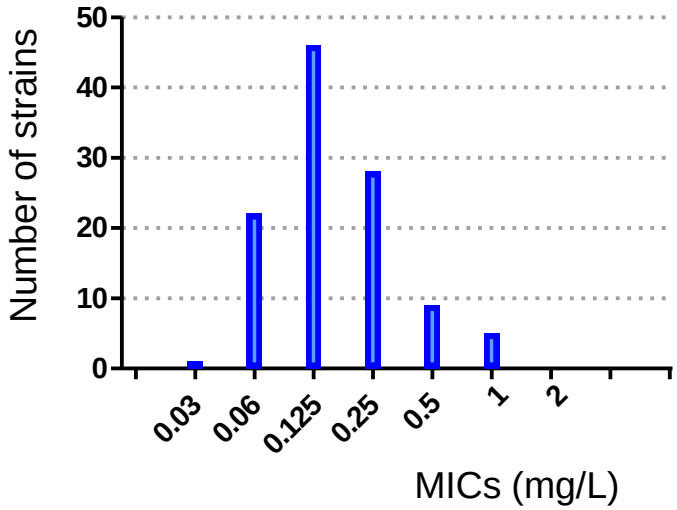
1st mutation in *gyrA*
⇨ MIC: 0.5 mg/L

Median: 32 mg/L

Macrolides susceptibility

Erythromycin

Clinical strains MICs:
0.003 – 1 mg/L



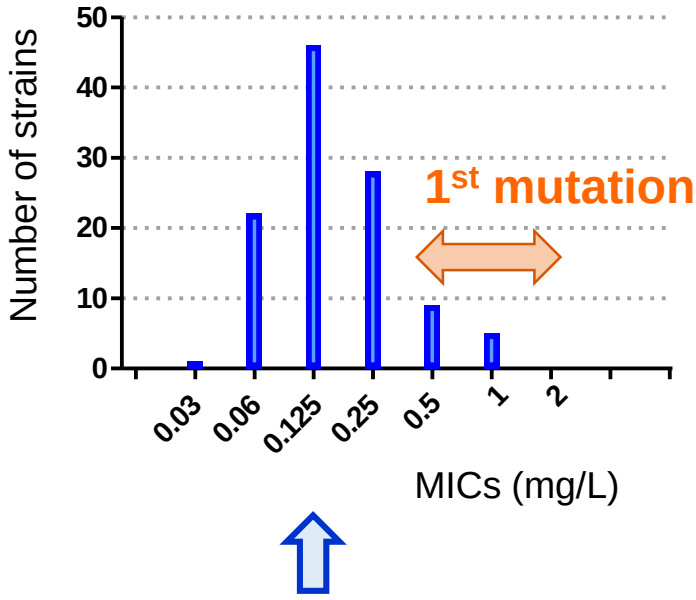
Median: 0.125 mg/L

Macrolides susceptibility

Resistance mechanisms:
mutations in genes encoding **ribosomal proteins L4 / L22**

Erythromycin

Clinical strains MICs:
0.003 – 1 mg/L



1st mutations: L4 / L22 (MICs: 0.5 – 2 mg/L)

Median: 0.125 mg/L

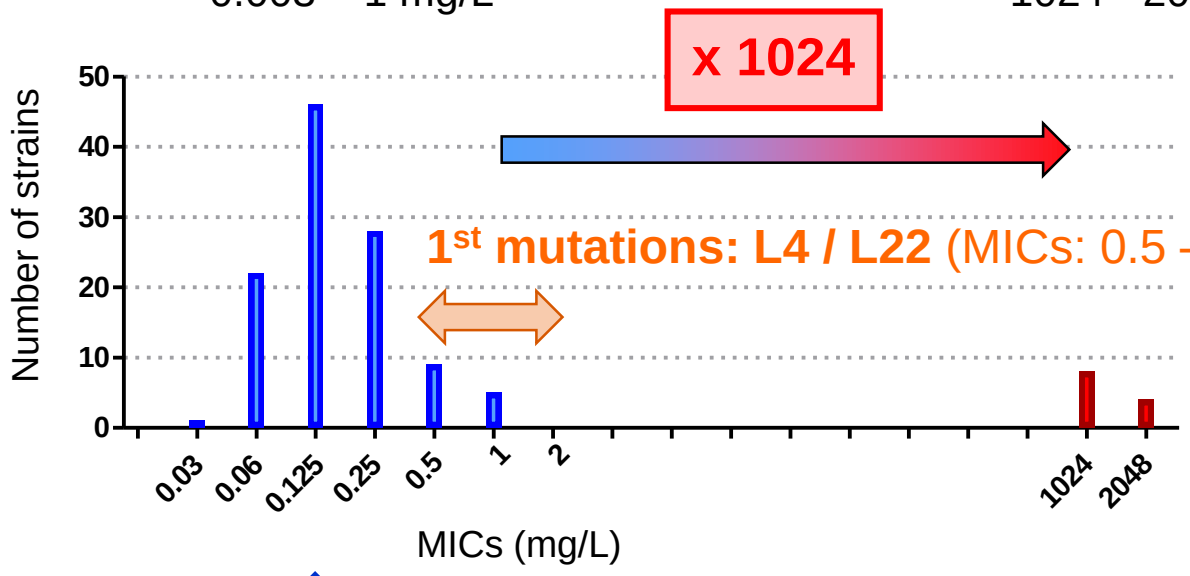
Macrolides susceptibility

Resistance mechanisms:
mutations in genes encoding **ribosomal proteins L4 / L22** and **23S rRNA**

Erythromycin

Clinical strains MICs:
0.003 – 1 mg/L

Resistant strains MICs:
1024 - 2048 mg/L



Mutation in genes encoding L4 / L22 and 23S rRNA

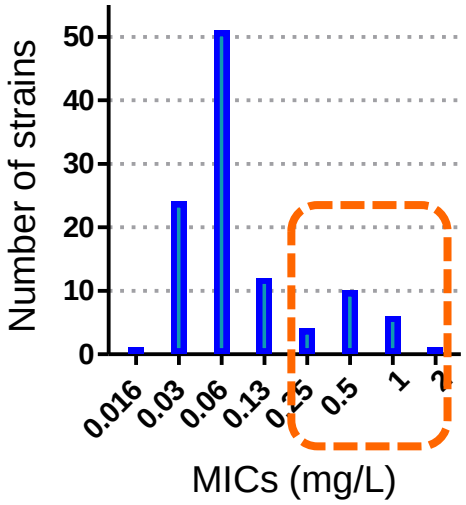
Median: 0.125 mg/L

Median: 1024 mg/L

Azithromycin specificity

Strains with **decreased susceptibility ?**

Azithromycin clinical strains MICs:
0.016 – 2 mg/L



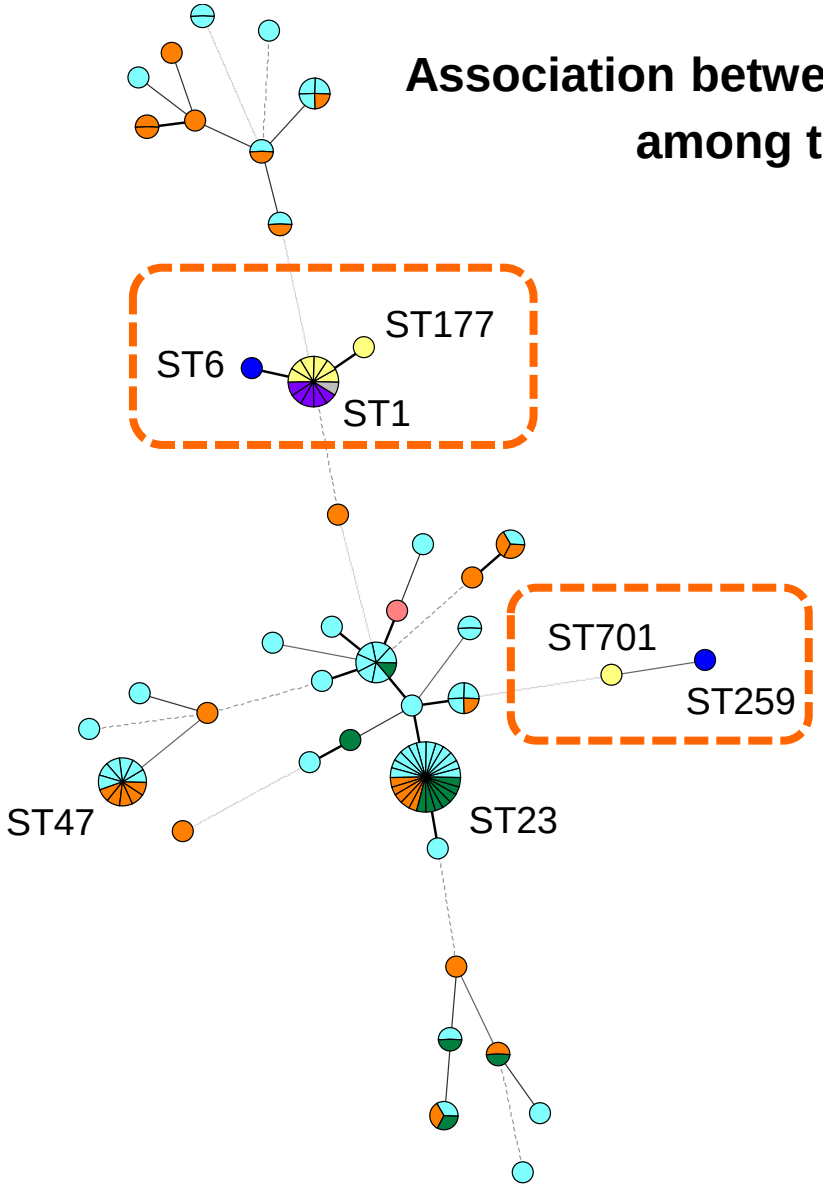
Median: 0.06 mg/L

Common features ?
(a single clone ?)

Azithromycin specificity

Association between STs (sequence types) **and MICs among the 109 clinical strains**

Strains with **decreased susceptibility**



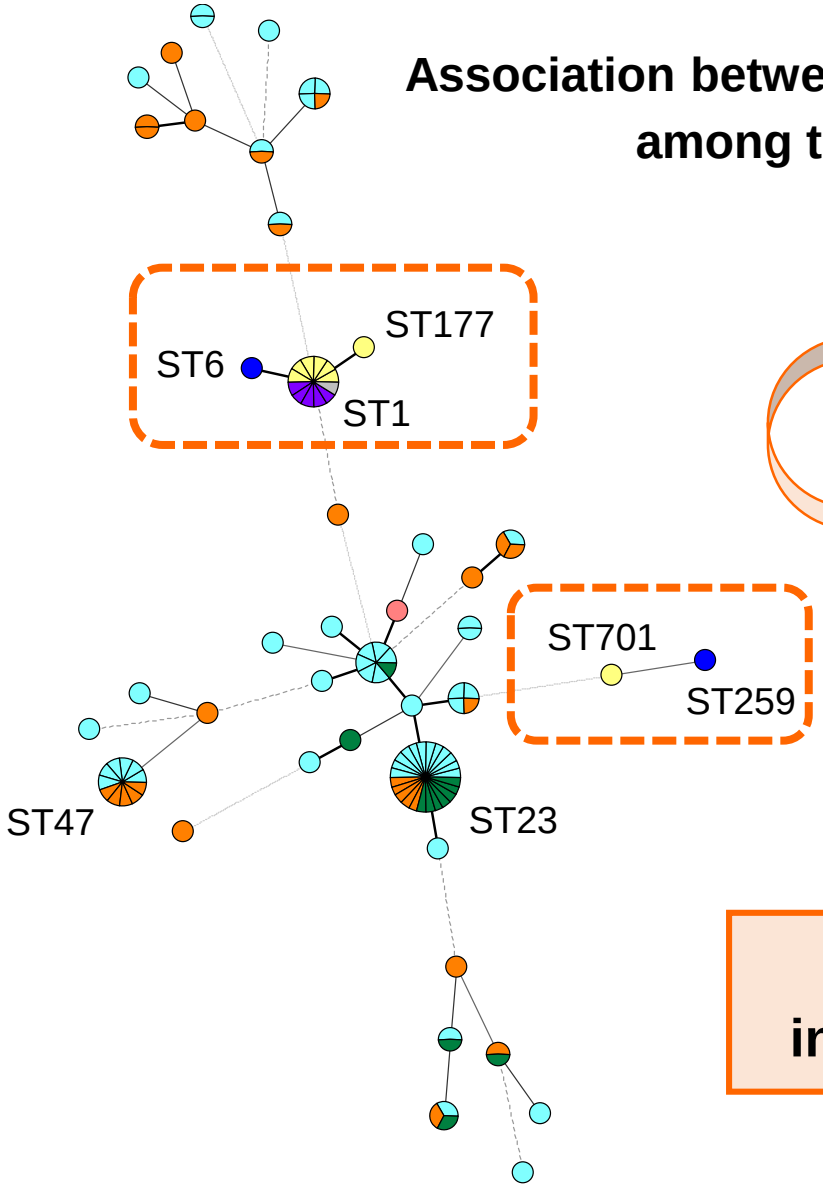
Azithromycin MICs (mg/L)

- 2
- 1
- 0.5
- 0.25
- 0.125
- 0.0625
- 0.03125
- 0.015625

Azithromycin specificity

Association between STs (sequence types) **and MICs among the 109 clinical strains**

Strains with **decreased susceptibility**



Azithromycin MICs (mg/L)

- 2
- 1
- 0.5
- 0.25
- 0.125
- 0.0625
- 0.03125
- 0.015625

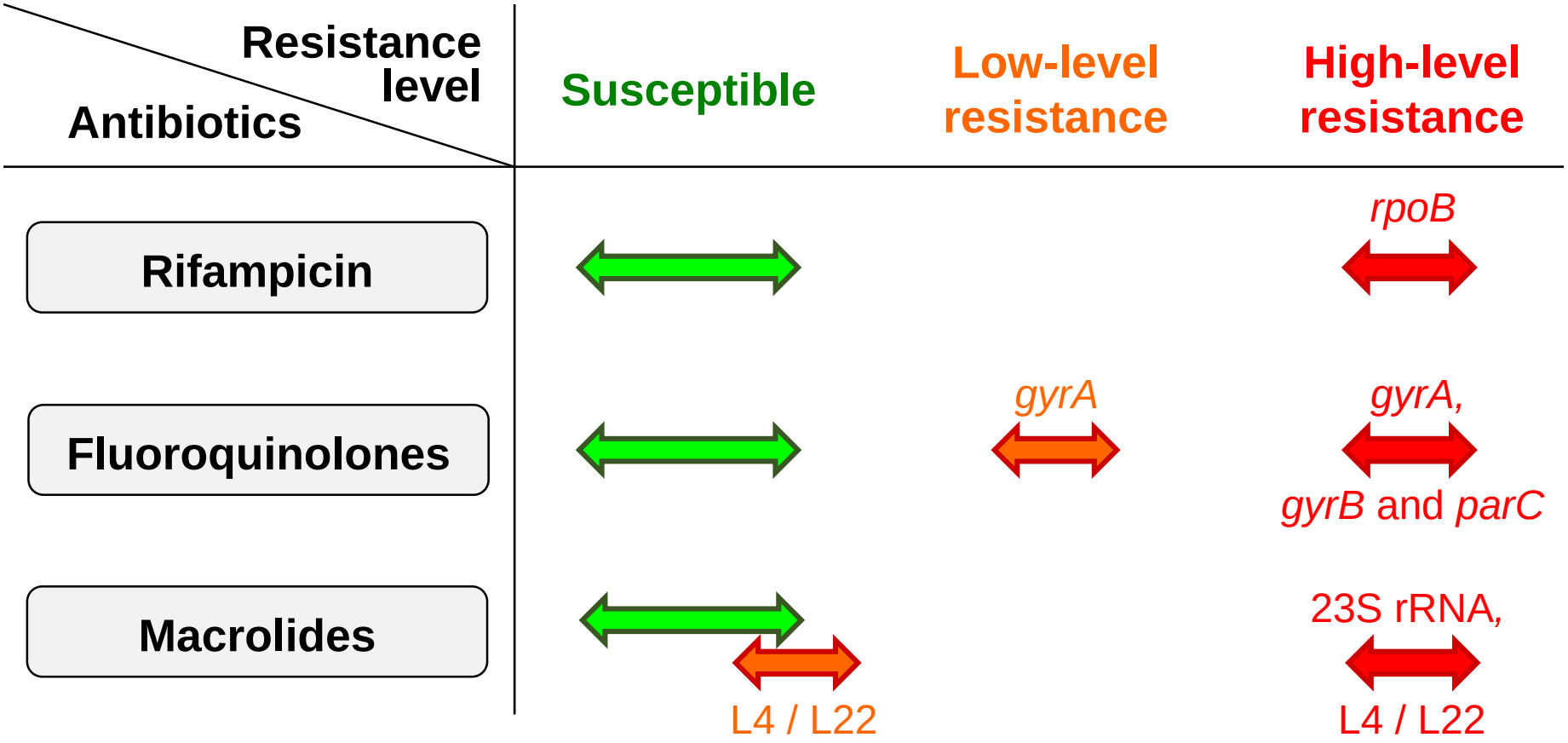
No mutation in genes encoding **L4 or L22**

Another common resistance mechanism ?

To be discussed in our next presentation …

Conclusions and take home messages

Schematic MICs distributions of *L. pneumophila* strains:



Acknowledgements

Bacteriology Laboratory, Grenoble Hospital

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Thank you for your attention

Any question ?

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