

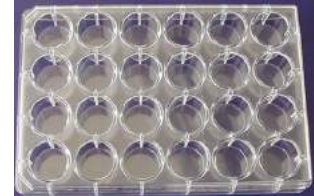
Role of two genes of putative efflux pump in macrolide resistance of *L. pneumophila*

Clémence Massip

Massip C., Gilbert C., Ginevra C., Doublet P., Jarraud S., Descours G.

Macrolide resistance mechanisms in *L. pneumophila*

Previous work:



Resistance

6 different lineages

Reference
strain Paris



Resistance ↗



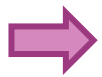
Resistance ↗↗



Resistance ↗↗↗

Sequencing a final
resistant clone from
all 6 lineages

Increasing **azithromycin**
concentrations



Mutations in:

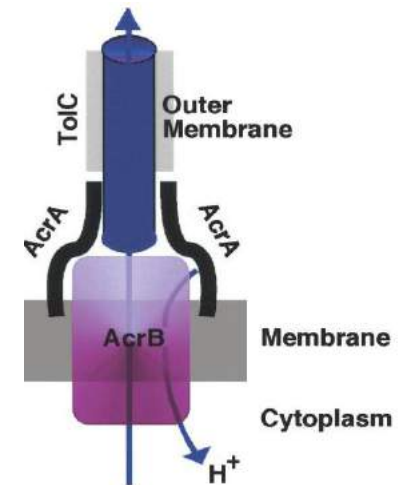
- genes encoding **23S rRNA** and **L4 / L22** proteins in all lineages
- upstream region of ***lpp2879-2880*** in **2 lineages**

Operon *lpp2879-2880*, an homolog to *Escherichia coli* *acrAB*

AcrAB: 2 proteins of a **tripartite efflux pump** (AcrAB-TolC)

AcrAB-TolC-like efflux pumps:

- **Widely** distributed among **Gram-negative** bacteria
- Strongly involved in **resistance to toxic** components:
 - o dyes (e.g. methylen blue)
 - o biliary salts
 - o antimicrobial peptides
 - o antibiotics (e.g. fluoroquinolones, **macrolides**)
 - o ...

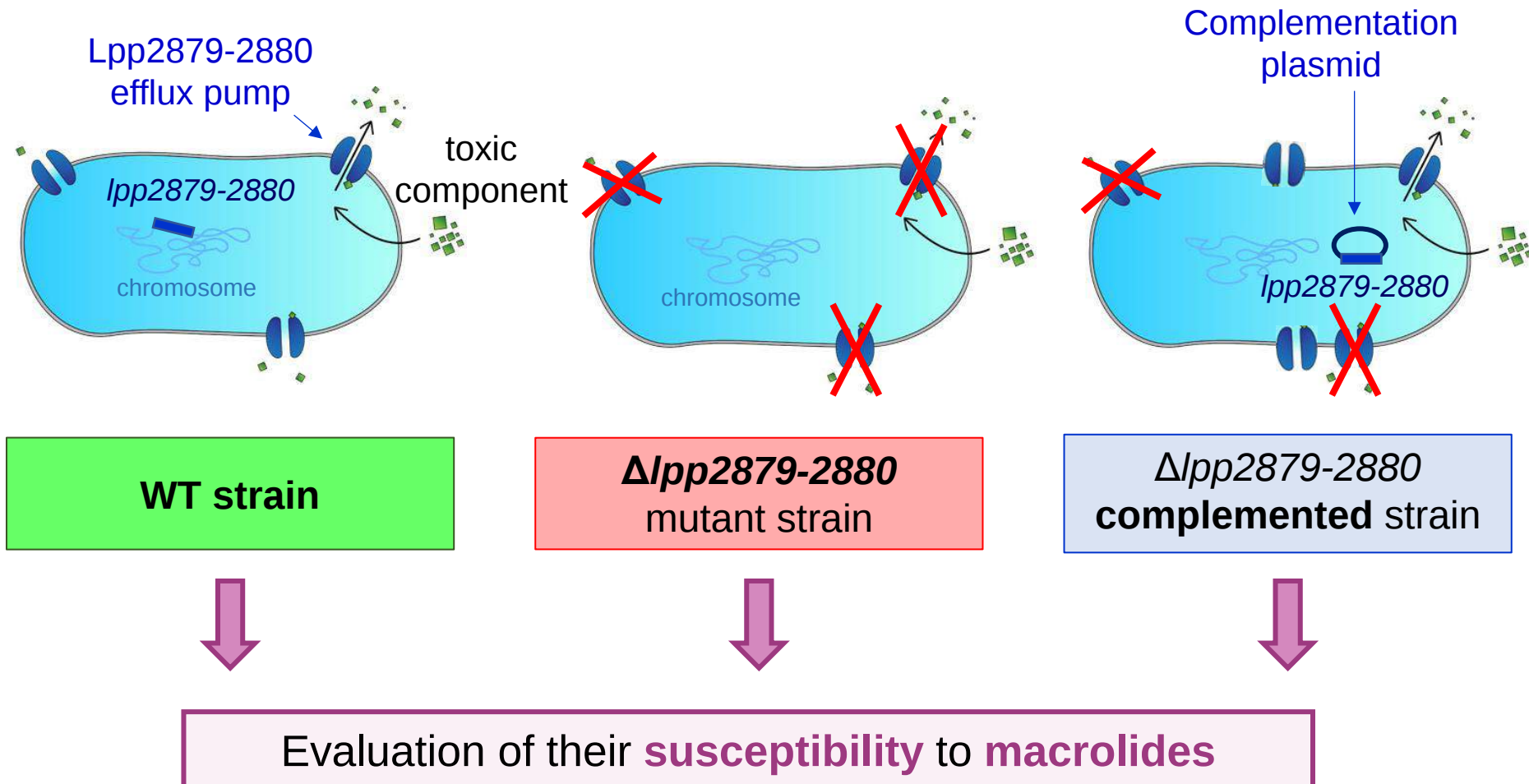


Piddock et al., 2006

Role of *lpp2879-2880* in *L. pneumophila* **resistance to macrolides** ?

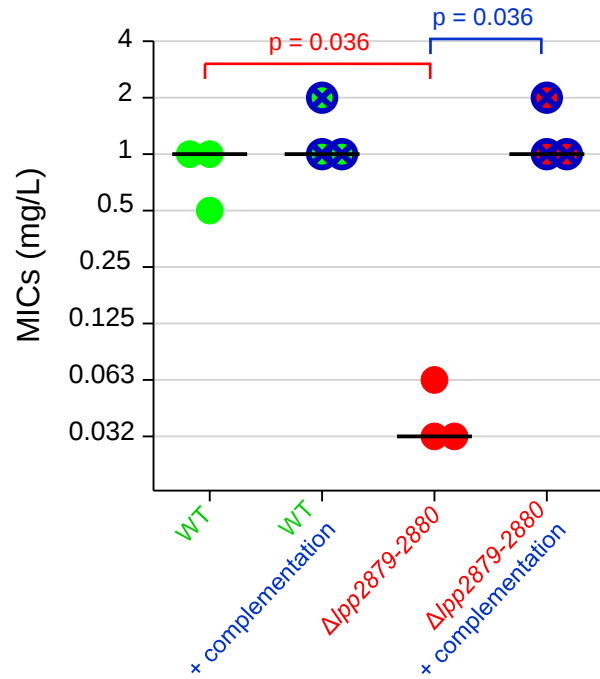
Role of Lpp2879-2880 in *L. pneumophila* macrolide resistance

Construction of *L. pneumophila* $\Delta lpp2879-2880$ mutants
+ **complementation**

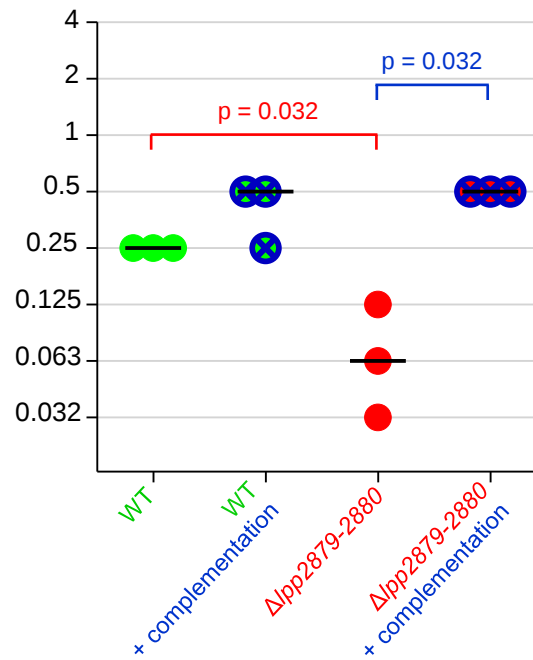


Role of Lpp2879-2880 in *L. pneumophila* macrolide resistance

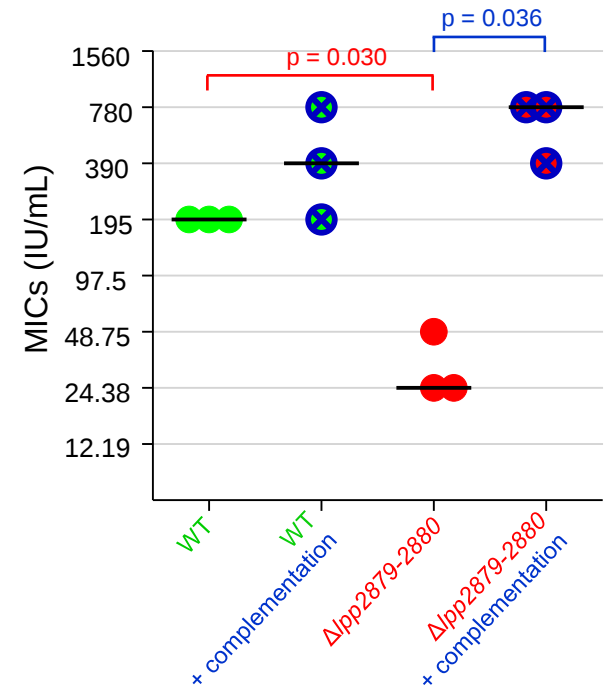
Minimum inhibitory concentrations (MICs) in liquid medium (Kruskall-Wallis test)



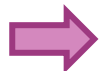
Azithromycin



Erythromycin



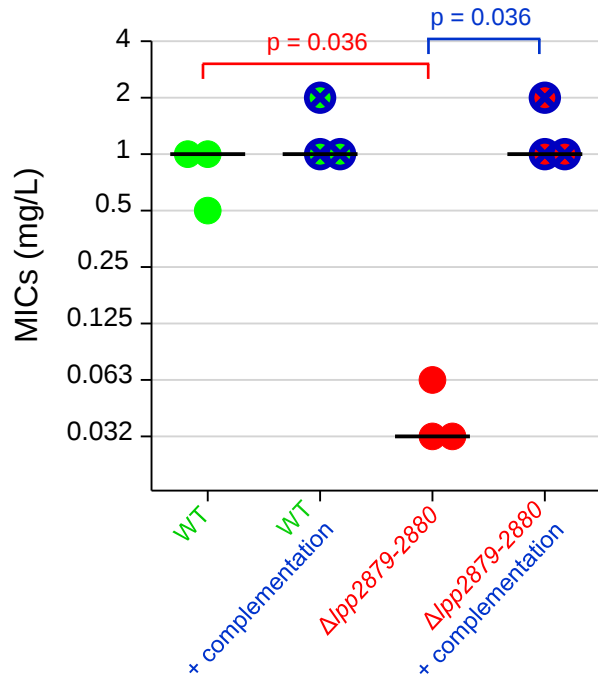
Spiramycin



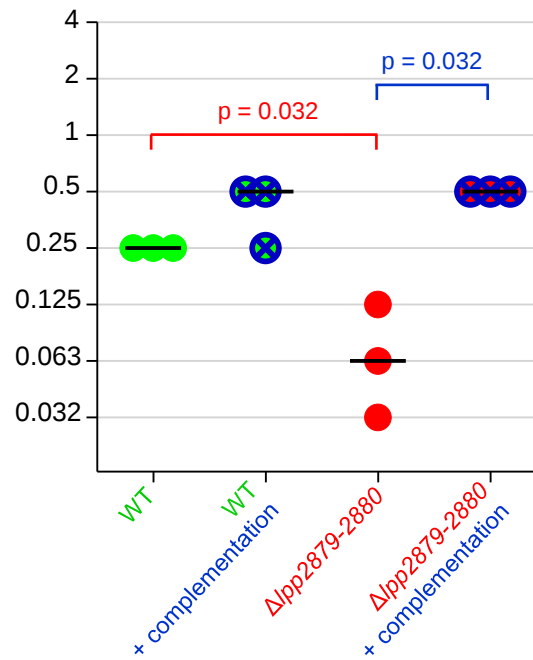
Role of *lpp2879-2880* in *L. pneumophila* resistance to **macrolides**

Role of Lpp2879-2880 in *L. pneumophila* macrolide resistance

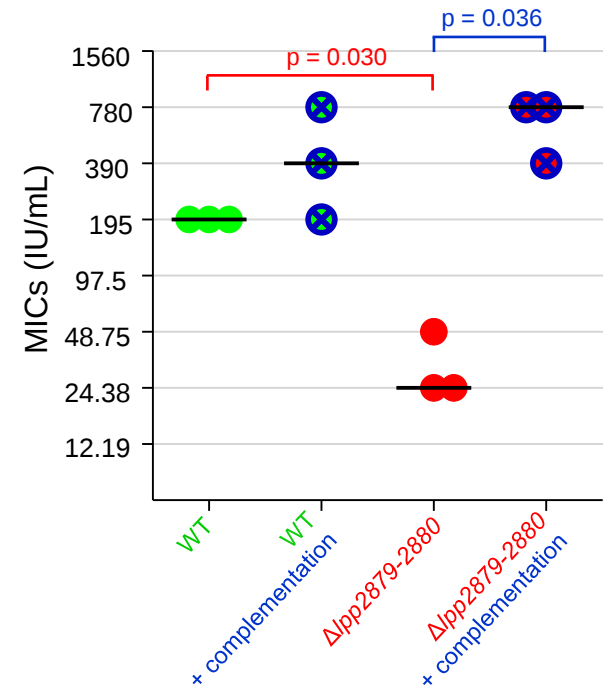
Minimum inhibitory concentrations (MICs) in liquid medium (Kruskall-Wallis test)



Azithromycin



Erythromycin



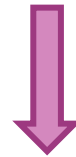
Spiramycin



Role of *lpp2879-2880* in *L. pneumophila*
specific resistance to macrolides

Distribution of *lpp2879-2880* in *L. pneumophila* strains

Screening for *lpp2879-2880*



in the genome of:

280 *L. pneumophila* strains
of **different sequence types (ST)**

+

=

ST1

ST6 (close to ST1)
ST701

Other tested ST



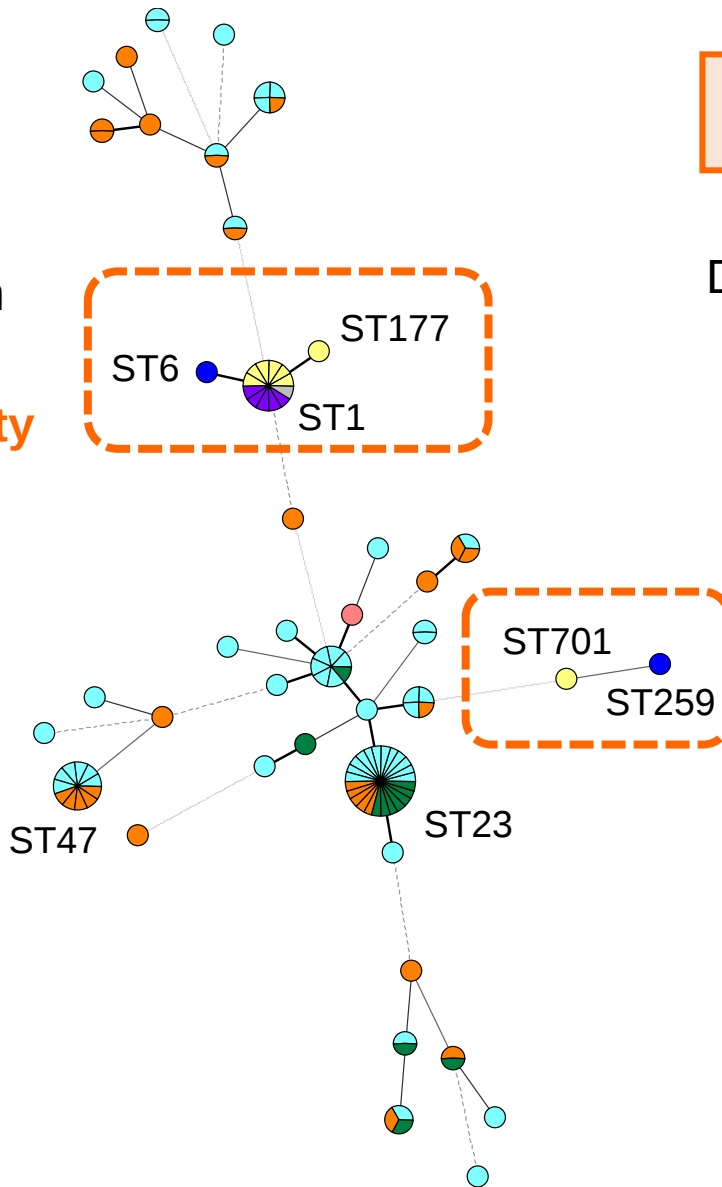
Lpp2879-2880 **specific** of clones (ST1)

Role of *lpp2879-2880* in azithromycin decreased susceptibility

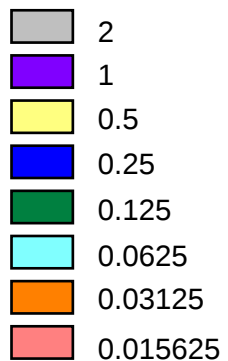
Back to our **1st** presentation

Distribution of the 109 clinical strains
sequence types (ST)

Strains with
**decreased
susceptibility**

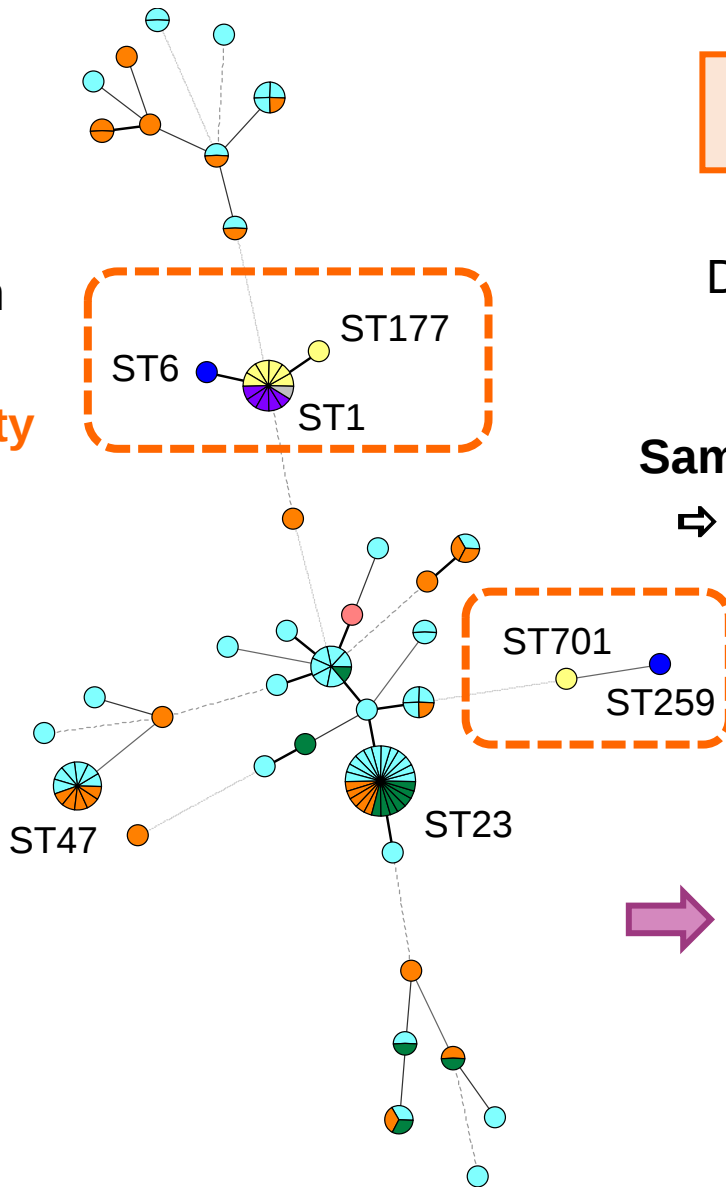


Azithromycin
MICs (mg/L)



Role of *lpp2879-2880* in azithromycin decreased susceptibility

Strains with
**decreased
susceptibility**



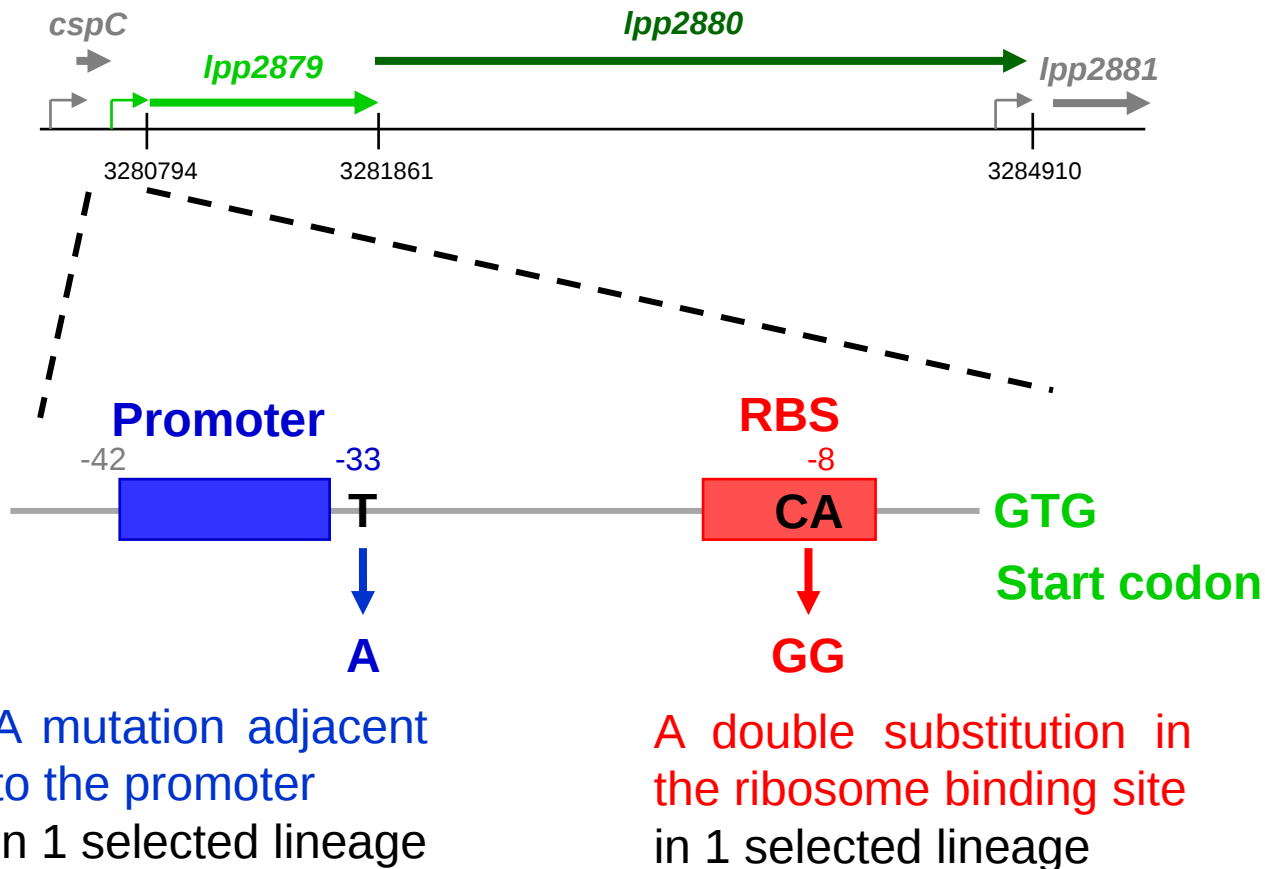
Back to our **1st presentation**

Distribution of the 109 clinical strains
sequence types (ST)

Same observation by Mallegol *et al.*, 2014
⇒ no resistance mechanism identified

↘ azithromycin **susceptibility**
due to *lpp2879-2880*

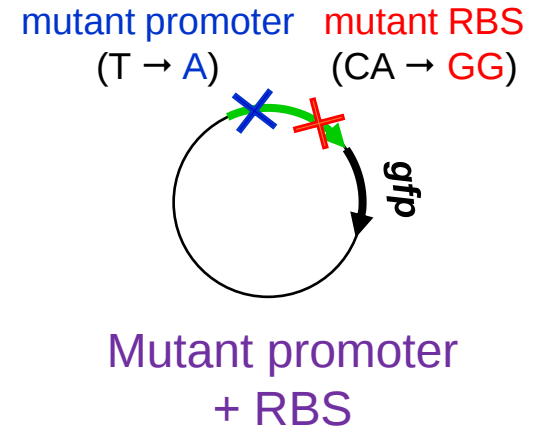
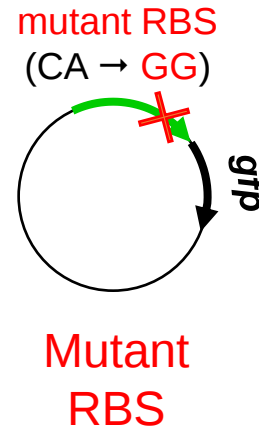
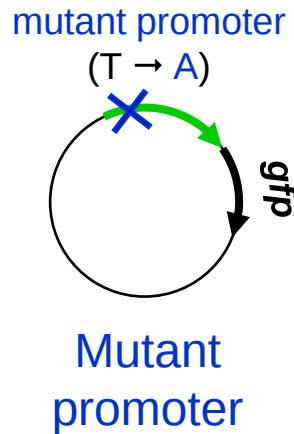
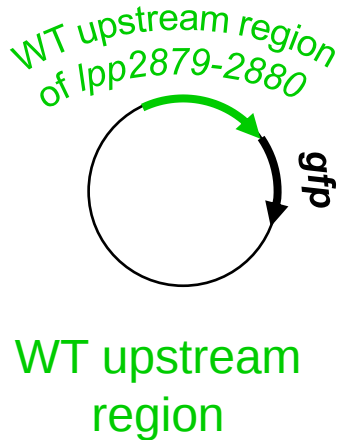
Impact of the mutations in the upstream region of *lpp2879-2880*



Impact of these **mutations** on the putative efflux pump expression ?

Reporter: gene encoding green fluorescent protein (GFP)

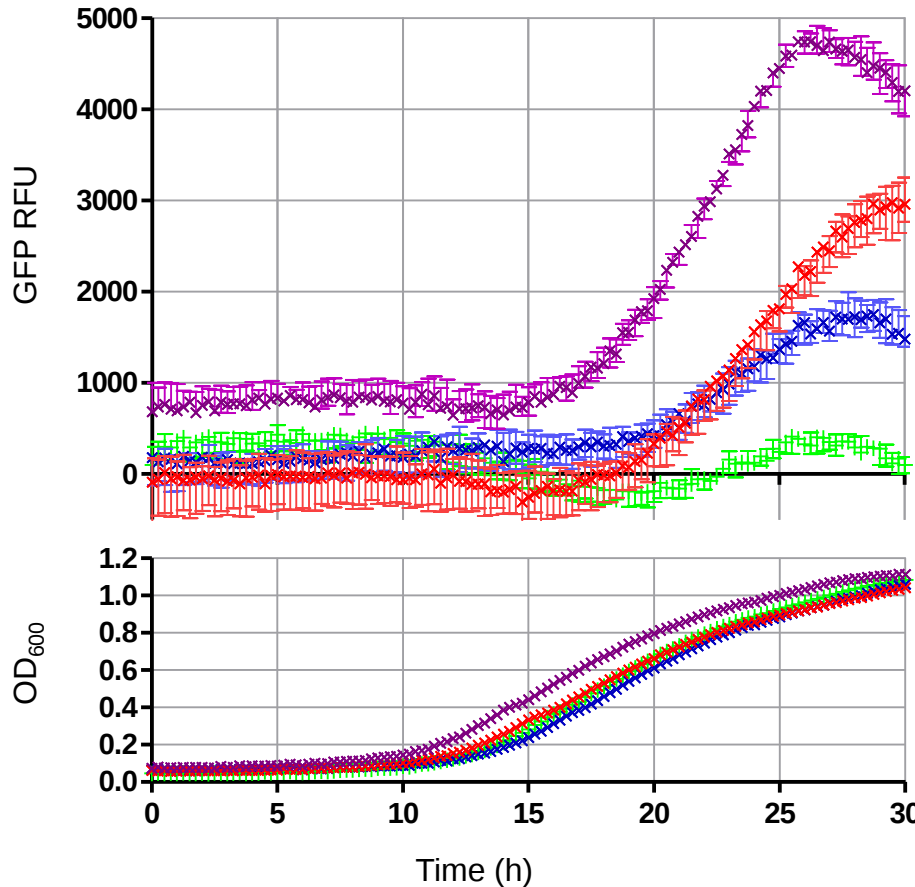
- Construction of 4 plasmids:



- Transformation of *L. pneumophila* reference strain Paris
- Evaluation of GFP expression (fluorescence)

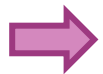
Impact of the mutations on GFP expression

GFP expression



- × Mutant promoter + RBS
- × Mutant RBS
- × Mutant promoter
- + WT upstream region

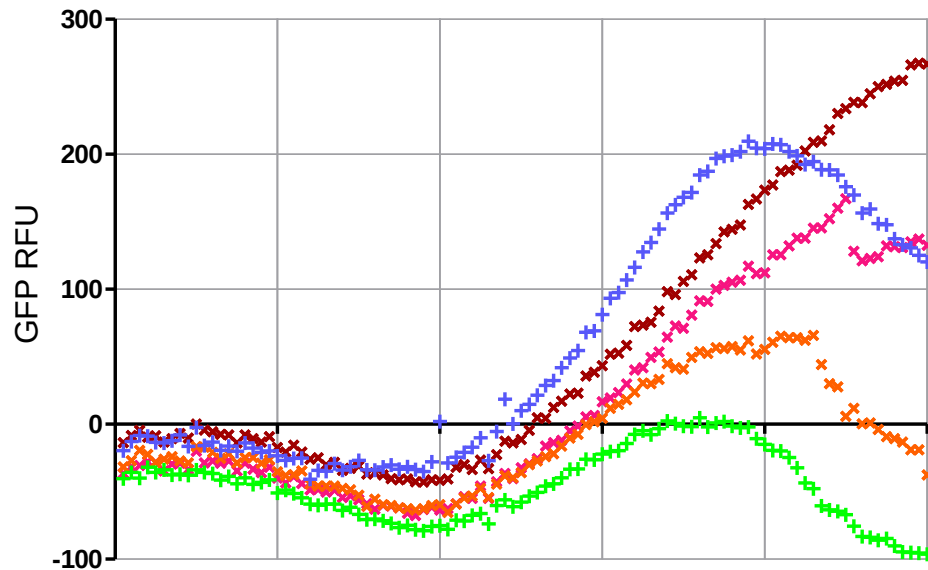
Bacterial growth



Mutations in *lpp2879-2880* promoter and/or RBS
 ⇒ ↗ in **protein expression**

Impact of sub-inhibitory concentrations of erythromycin on GFP expression

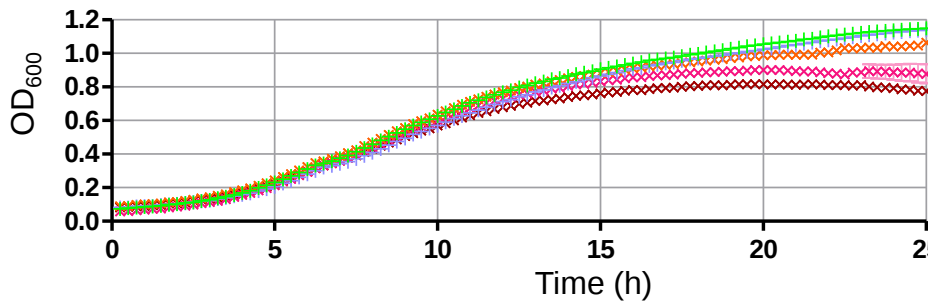
GFP expression



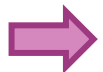
WT upstream region of *lpp2879-2880* with erythromycin concentrations of:

- × MIC / 4
- × MIC / 8
- × MIC / 16
- + no antibiotic
- + Mutant RBS without erythromycin

Bacterial growth



Same observations with **azithromycin** with the **mutations** in the upstream region of *lpp2879-2880*



Induction of protein expression by macrolide

Role of *lpp2879-2880* in *L. pneumophila* resistance to macrolides



In *L. pneumophila* endemic strain ST1:

- Lpp2879-2880 $\Rightarrow$ $\searrow$ azithromycin susceptibility
- Exposition to macrolides $\Rightarrow$ $\nearrow$ Lpp2879-2880 synthesis
 $\Rightarrow$ protection mechanism ?



In *L. pneumophila* selected *in vitro*, highly resistant to macrolides:

- Mutations in *lpp2879-2880* promoter or RBS
 $\Rightarrow$ $\nearrow$ Lpp2879-2880 synthesis
 $\Rightarrow$ $\nearrow$ macrolide resistance in addition to mechanisms of macrolide high-level resistance (mutations in 23S rRNA or L4 / L22)

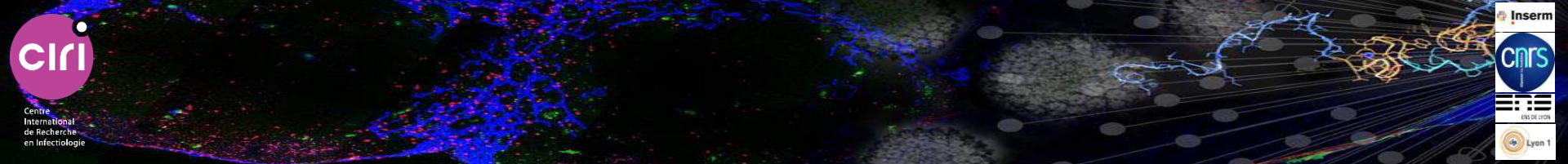
National Reference Centre of *Legionella*, Lyon

Team “Pathogenesis of *Legionella*”, International Research Centre in Infectiology, Lyon

Gilbert C., Ginevra C., Doublet P., Jarraud S., Descours G.



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des Internes et des Anciens Internes
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Thank you for your attention

Any question ?



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