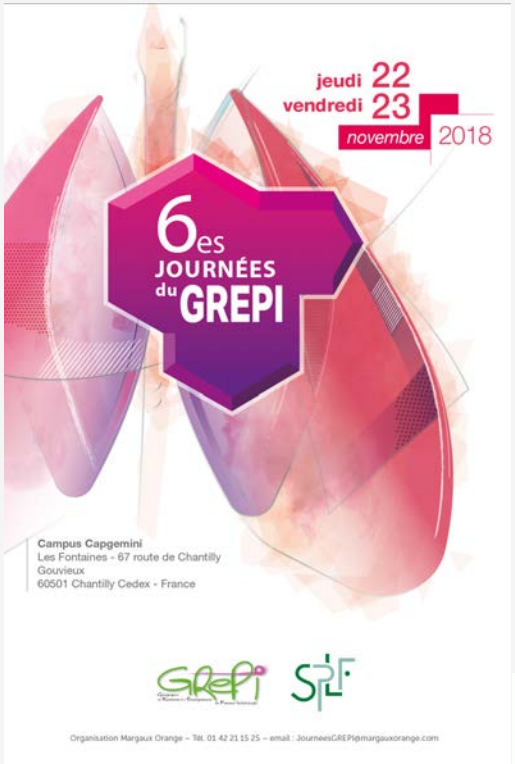

L'élargissement des indications des macrolides au long cours doit-il faire craindre l'émergence de *Mycoplasma pneumoniae* résistant aux macrolides ?

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INRA SCIENCE & IMPACT

Conflit d'intérêt

- Pas de conflit d'intérêt en rapport avec cette présentation dans les 5 dernières années

Characteristics of mycoplasmas

- **Smallest free-living eubacteria (500 to 2200 kbp)**
- **Lack of cell wall (« *Mollis cutis* » or Mollicutes)**
- **17 human species: respiratory or urogenital tract**
- **5 pathogenic species:**

- *M. pneumoniae* → **respiratory tract infections (RTI)**

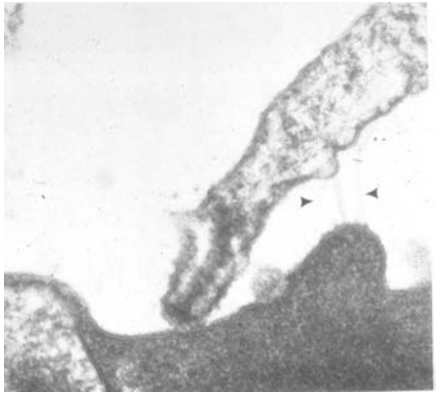
- *M. genitalium*
- *M. hominis*
- *U. urealyticum*
- *U. parvum*

} **urogenital tract infections**

Mycoplasma pneumoniae infections

- **Children** +++ and young adults

- **RTI** +++
  - Atypical pneumonia (20%)
    2nd cause of CAP after *S. pneumoniae*
  - Tracheo-bronchitis (80%)
  - Asthma ?



M. pneumoniae **with respiratory epithelial cells**

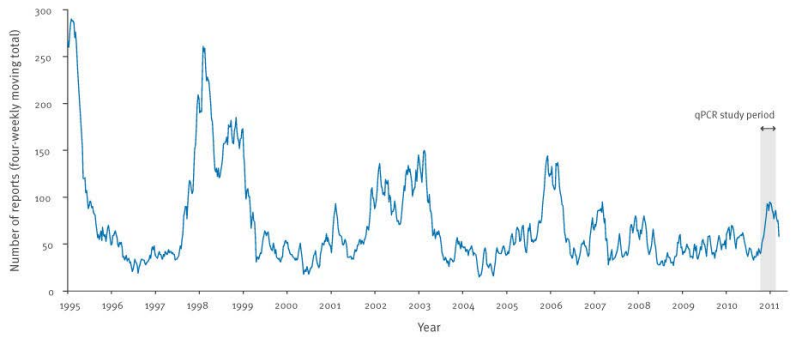
- **Extrarespiratory diseases**
  - Cutaneous
    Skin rash, exanthema, Stevens Johnson syndrome
  - Neurologic
    Meningoencephalitis, peripheral neuropathy, Guillain Barré syndrome

M. pneumoniae epidemiology

- **Endemic with surges**
  - Every 4 to 7 years, lasting ≈18 months
  - Short-course immunity
    (antigenic variations of the P1 adhesin)

- **2010-2012 increase in the incidence of Mp infections worldwide**

FIGURE 1
Laboratory reports of *Mycoplasma pneumoniae* infection by date of report, England and Wales, January 1995–March 2011



qPCR: quantitative real-time polymerase chain reaction.
Four-weekly moving total of the number of reports collated by the Health Protection Agency Centre for Infections, including serological, molecular and culture test results. Report numbers per year are: 1,687 in 1995, 490 in 1996, 990 in 1997, 2,278 in 1998, 727 in 1999, 483 in 2000, 804 in 2001, 1,409 in 2002, 819 in 2003, 472 in 2004, 991 in 2005, 818 in 2006, 737 in 2007, 573 in 2008, 624 in 2009, 743 in 2010, 162 in 2011 (up to the first week of March), giving a total of 14,807 for all years), giving a total of 14,807 for all years. The arrowed line indicates the qPCR study period, October (week 40) 2010 to January (week 3) 2011, during which time 322 reports were received.
Source: [9].

Chalker Euro Surv 2011

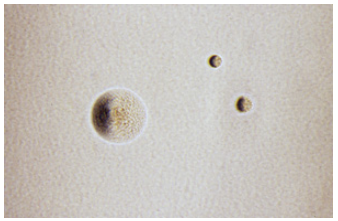
RESEARCH NOTE MYCOLOGY

The increased incidence of *Mycoplasma pneumoniae* in France in 2011 was polyclonal, mainly involving *M. pneumoniae* type 1 strains.

S. Pereyre[1,2,3], A. Touati[1,2], J. Petitjean-Lecherbonnier[4], A. Charron[1,2], A. Vabret[4] and C. Bébéar[1,2,3]

Keywords: Macrolide resistance, multilocus variable number tandem repeat analysis, *Mycoplasma pneumoniae*, PCR-RFLP, typing
Original Submission: 3 October 2012; **Revised Submission:** 15 November 2012; **Accepted:** 18 November 2012
Editor: G. Greub
Article published online: 28 November 2012
Clin Microbiol Infect 2013; **19:** E212–E217
10.1111/1469-0691.12107

Diagnosis of *M. pneumoniae* infections



- **Direct diagnosis**
  - Rarely culture, fastidious
  - Nucleic acid amplification tests (NAATs) +++
    more sensitive than serology at the early stage of infection
    New « gold standard »

- **Serological diagnosis ++**
  - Specific antibodies, ELISA++ (variable performances)
  - Widely used but retrospective diagnosis
  - 2 serums collected 2 weeks apart
  - No POC but qualitative serological tests in ambulatory care (single IgM or IgM/G)

Diagnosis of *M. pneumoniae* infections

- **Molecular techniques definitively more sensitive than culture and serology in the early phase of infection.**

- **Still a great emphasis on serology despite limited value in diagnosis of acute infections.**

- **Serology is useful in epidemiological studies.**

- **Most cases detected by a combination of IgM antibody detection and PCR.**

M. pneumoniae and antibiotics

- **Intrinsic resistance to ATB targeting the cell wall (β-lactams +++)**

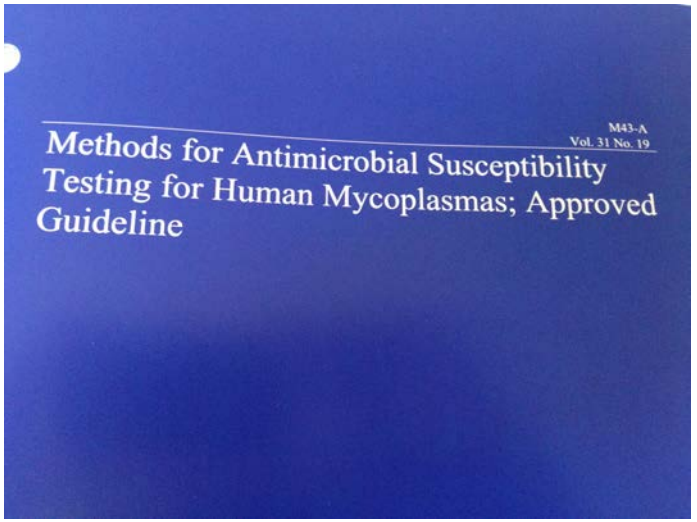
- **Active antibiotics**
  - **Macrolides and related: macrolides, lincosamides, streptogramin combinations, ketolides (MLSK)**
  - **Fluoroquinolones**
  - **Tetracyclines**

☞ **Macrolides and related antibiotics = 1st line treatment for *M. pneumoniae* infections**

Antibiotic susceptibility testing in *M. pneumoniae*

- **Not in routine (fastidious growth)**
  No commercialized kit or test

- **CLSI recommendations**
  M43-A 31(19), 2011



- **2 phenotypic techniques**
  No agar diffusion

- **Molecular techniques to detect macrolide resistance directly from specimens**

MLSK[a]	*M. pneumoniae*[b]
14-membered M	
Erythromycin	≤ 0.004 – 0.06
Roxithromycin	≤ 0.01 – 0.03
Dirithromycin	≤ 0.015 – 0.5
Clarithromycin	≤ 0.004 – 0.125
15-membered M	
Azithromycin	≤ 0.004 – 0.01
16-membered M	
Josamycin	≤ 0.01 – 0.03
Spiramycin	≤ 0.01 – 0.25
Midecamycin	≤ 0.015
Rokitamycin	≤ 0.06
Lincosamides	
Clindamycin	≤ 0.008 – 2
Lincomycin	4 – 8
Streptogramins	
Pristinamycin	0.02 – 0.5
Quinupristin/ Dalfopristin	0.008 – 0.25
Ketolides	
Telithromycin	≤ 0.001 – 0.06
Cethromycin	≤ 0.001 – 0.016
Solithromycin	≤ 0.000000063 – 0.000125
Pleuromutilins	
Lemafulin	≤0.00025-0.001 (MS) 0.0005-0.004 (MR)

[a] MLSK, macrolides, lincosamides, streptogramins, ketolides.
[b] Susceptible strains.

MICs of MLSK against *M. pneumoniae*

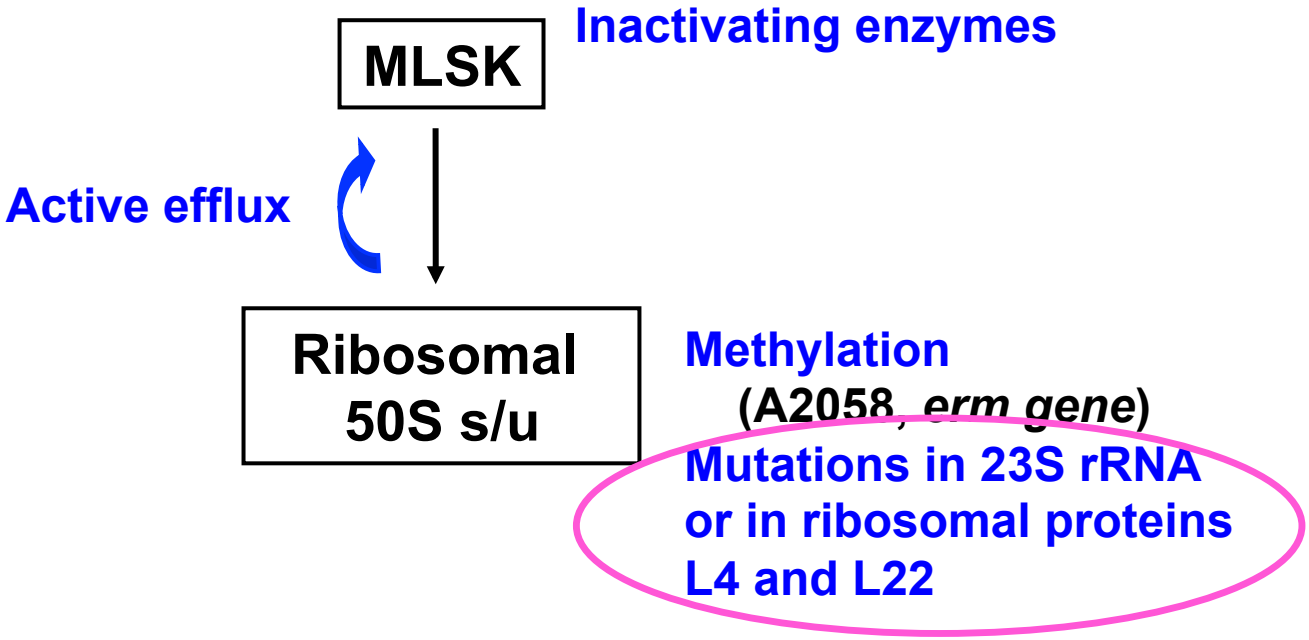
Adapted from *Bébéar et al., Future Microbiol 2011*

Acquired resistance of human mycoplasmas

- **Genetic support**
  - **Chromosomal mutations ++**
    (lack of DNA repair systems -> high mutation rates)
  - **Transposons +**
  - **No extrachromosal element**

- **Biochemical mechanisms**
  - **Target modification or protection +++**
  - **Efflux + (*in vitro*)**
  - **No enzymatic inactivation**

Mechanisms of macrolide resistance in bacteria

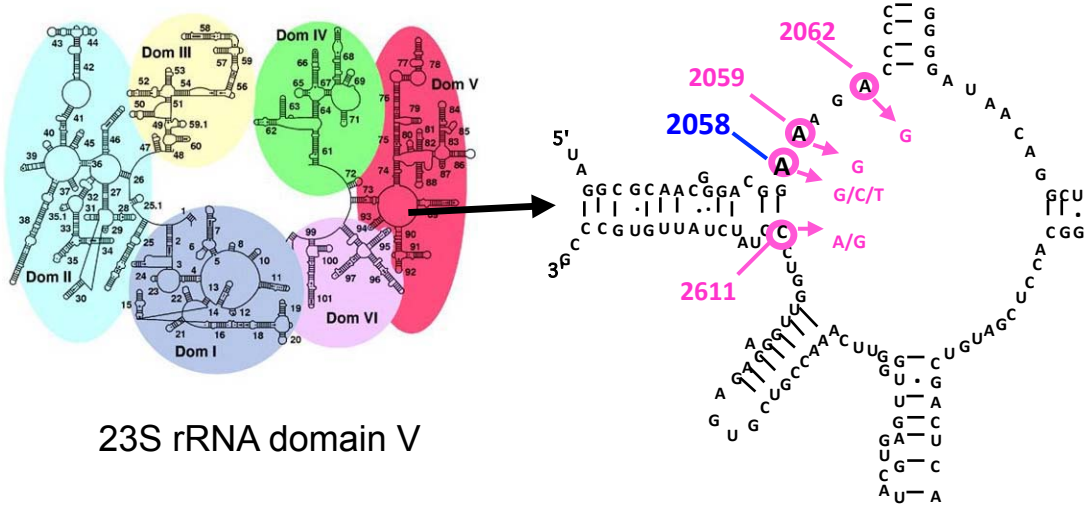


Acquired antimicrobial resistance in *M. pneumoniae*

Antimicrobial Class	Resistance		Mechanism	MIC range for Resistant Isolates (µg/ml)
	In vitro	*In vivo*		
MLSK	Yes	Yes	23S rRNA mutations at positions 2611, 2058, 2059, and 2062 Mutations, insertions or deletions in L4 and L22 ribosomal proteins (*in vitro* only)	64 - >256 (erythromycin)
Tetracycline	Yes	No	16S rRNA mutations at position 968 and 1193 (*in vitro* only)	2
Fluoroquinolones	Yes	No	Mutations in *gyrA, gyrB, parC* or *parE* genes of QRDRs [c]	2 - 16 (levofloxacin)

Macrolide resistance in *M. pneumoniae*

- **Mutations in 23S rRNA** (1 copy in *M. pneumoniae*)



23S rRNA domain V

- **Predominance of A2058G**

Emergence of macrolide resistance during macrolide treatment

Boy 11 yo
Hyper IgE syndrome
Mp(+) pneumonia

→ **Josamycin** →

Tt Failure

→ **Ciprofloxacin** →

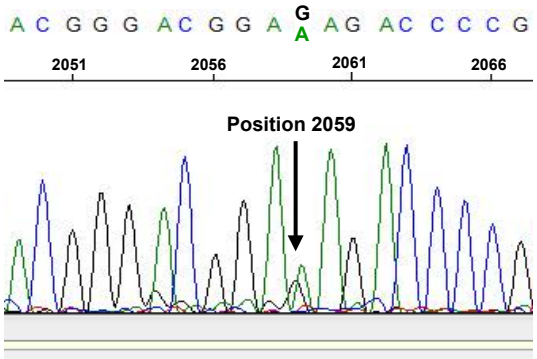
Recovering

Wt *M. pneumoniae*

MLVA type I

***M. pneumoniae* with A2059G**

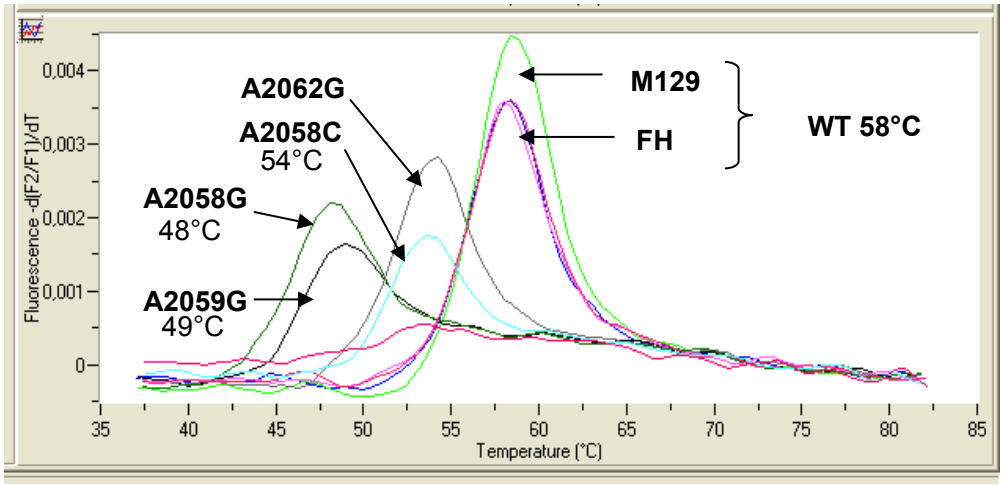
MLVA type I



Hantz et al., J Clin Microbiol, 2012

Molecular diagnostic of macrolide resistance in *M. pneumoniae*

- **Real-time (RT)-PCR (FRET) with melting curve analysis**
  - → mutated strains : lower Tm
  - → directly from clinical specimens
  - → used routinely in our diagnostic lab

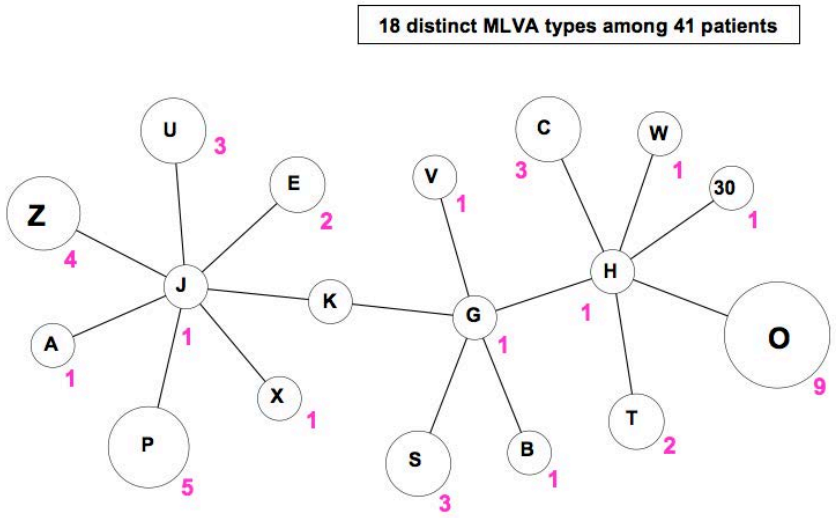


- **Other molecular methods:** **HRM RT-PCR, Sanger sequencing, pyrosequencing…**

Peuchant et al., J Antimicrob Chemother, 2009
Bébéar et al., Future Microbiol, 2011

Molecular typing to explore macrolide resistance?

- **P1 adhesin gene and MLVA typing**
  - **No correlation between subtypes and antimicrobial resistance in most studies worlwide**
  - **Polyclonal origin of macrolide resistance -> emergence of resistance de novo during tt**



The Spread of *Mycoplasma pneumoniae* Is Polyclonal in Both an Endemic Setting in France and in an Epidemic Setting in Israel
Pereyre et al PLoS One, 2012

Consequences of macrolide resistance in *M. pneumoniae* clinical isolates

- **Need for macrolide resistance detection?**
  - **Depends on macrolide resistance rates**
  - **If rate >10% : molecular detection on all Mp-positive specimens**
    - ✓ Non-macrolide treatment promptly started if macrolide-resistant genotype identified
  - **If rate <10% : molecular detection in case of treatment failure**

Pereyre et al. Frontiers Microbiol. 2016

Evolution of macrolide resistance in *M. pneumoniae* in Japan

Year	% macrolide resistance	Reference
2000	**1st clinical isolate described**	Okazaki et al. Microbiol. Immunol. 2001
2000-2004	**6 to 17% pediatric isolates**	Matsuoka Antimicrob. Agents Chemother. 2004; Morozumi Antimicrob. Agents Chemother. 2005
2002-2006	**30%**	Morozumi et al. Antimicrob. Agents Chemother. 2008
2008-2012 (national surv) 2120 children with RTI	**73%**	Kawai et al., Antimicrob. Agents Chemother 2013
2012	**81.6%**	Tanaka et al., Emerg. Infect. Dis. 2017
2013 and 2014	**65.8% and 59.3%**	Tanaka et al., Emerg. Infect. Dis. 2017
2015	**43.6%**	Tanaka et al., Emerg. Infect. Dis. 2017

Most frequent substitutions : A2058G (95.8%)
and A2058T (3.1%), A2059G (0.6%), C2611G (0.2%%), C2611T (0.1%)

➔ **Resistance high during 2008-2012 but gradually decreased throughout Japan during 2013-2015**

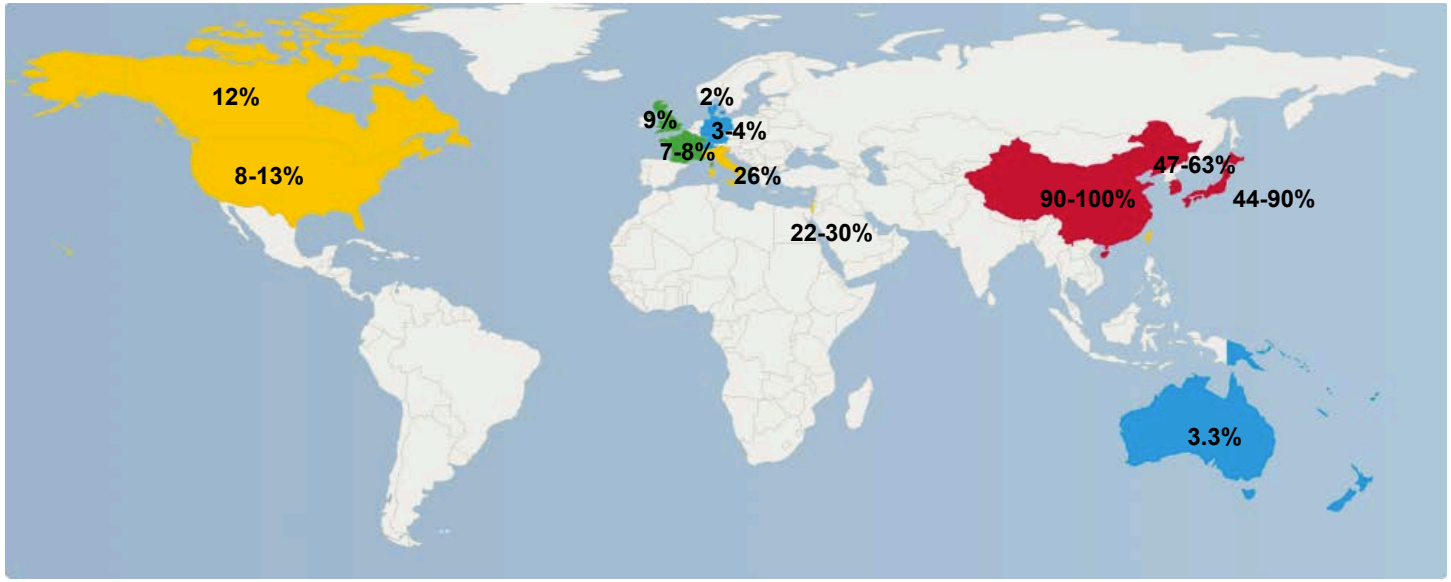
Evolution of macrolide resistance in *M. pneumoniae* in Bordeaux, France

	% macrolide resistance	Reference
Before 2005	**0%**	Peuchant et al. J. Antimicrob. Chemother. 2009
2005-2007	**9.8%**	Peuchant et al. J. Antimicrob. Chemother. 2009
2007-2010	**3.4%**	Pereyre et al. PLoS One 2012
2011	**8.3%**	Pereyre et al. Clin. Microbiol. Infect. 2012
2015	**6.7%**	Personal data
2016	**4.2%**	Personal data
2017	**0%**	Personal data (a few *M. pneumoniae*(+) samples)

Most frequent substitutions : A2058G (54%)
and A2059G (23%), C2611G (15%), A2062G (8%)

➔ **Resistance remains below 10% in France**

Macrolide resistance in *M. pneumoniae* worldwide 2010-2015



Data collated from the literature between 2010-2015

☞ **High macrolide resistance rates certainly associated with antibiotic selective pressure because of extensive macrolide use**

Adapted from *Pereyre et al. Frontiers Microbiol. 2016*

Epidemiology of macrolide resistance in *M. pneumoniae*

Country	Years	Macrolide-resistant strains (%)	1st-line macrolide used
France	2005-2012	3.7-9.5	Azithromycin, clarithromycin
England/Wales	2005-2012	0	Azithromycin, clarithromycin
Germany	2003-2012	1.2-3.6	Azithromycin, clarithromycin
Netherlands	1997-2008	0	NA
Denmark	2010-2011	0.9-2.9	Azithromycin
Sweden	2011	ND	Erythromycin
Norway	2010-2012	ND	Erythromycin, clarithromycin
Slovenia	2007-2011	1.7	NA
Italy	2010	26	Azithromycin
Israel	2010	30	Roxithromycin, Azithromycin
USA	2006-2010	1.6-27	Azithromycin, clarithromycin
Japan	2008-2012	50-93	Azithromycin, clarithromycin
China	2008-2012	>90	NA

☞ **Increase in resistance: result of antibiotic selective pressure in patients during a period of extensive macrolide use in countries (whatever the macrolide prescribed)**

Clinica stance

- **Clinical**
  - **- Therape atients**
  - **- ↗ febril**
  - **- Therape**
    - **→ Mino reating**
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    - **→ MIN w ence and in**
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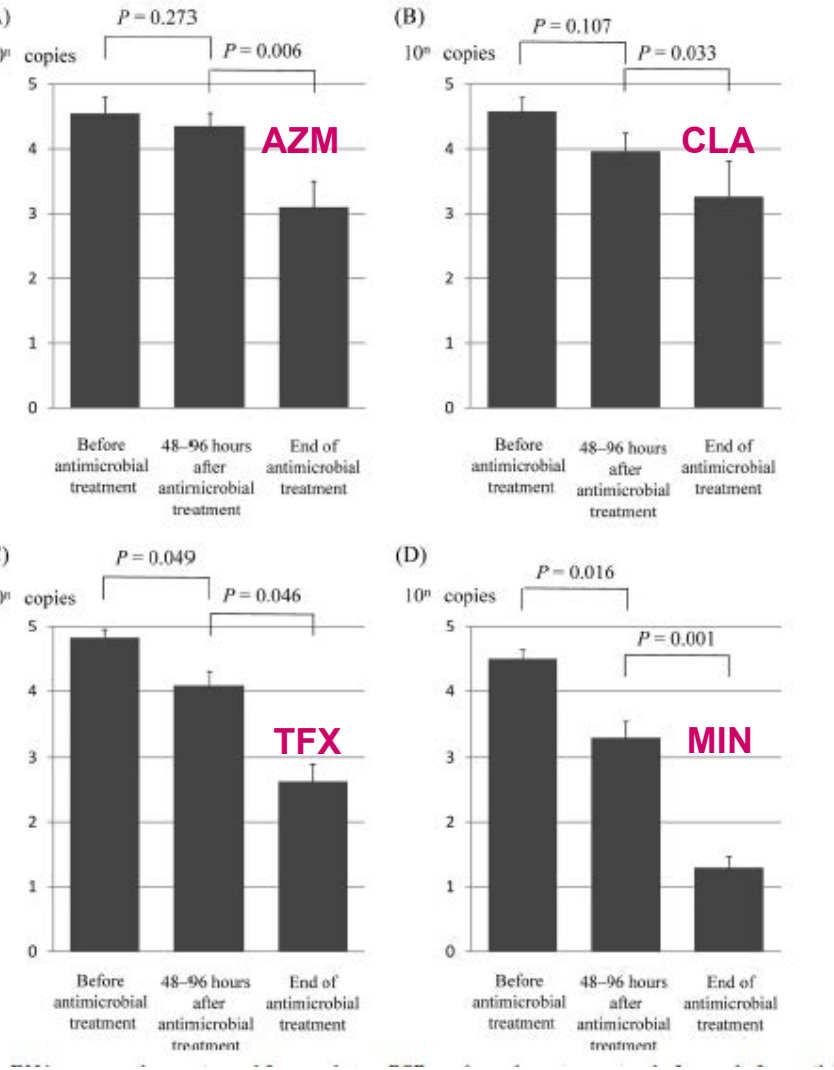

Kawai et al, AAC 2013

Treatment regimens for *M. pneumoniae* RTI

- **Macrolides : 1st line treatment**
  - Azithromycin 5 days, clarithromycin 7-14 days
  - Erythromycin 10-14 days
- **Tetracyclines (not before 8 yo)**
  - Tetracycline, doxycycline, minocycline 10-14 days
- **Fluoroquinolones (not before 18 yo)**
  - Levofloxacin, 5 days
  - Moxifloxacin, 7-14 days
- **Macrolide-R strains**
  - Japan: minocycline or tosufloxacin , 7-14 days
  - Streptogramin combination (pristinamycin) ? New ketolides ?

Conclusion

Mp macrolide resistance, a matter of concern!

- **In Asia**

  - Resistance rates extremely high,

  - Consider using an alternative to macrolides as initial treatment of Mp infection

- **In Europe and USA**

  - Resistance still much less common,

  - Macrolides still be considered the first-line treatment, but with consideration for change to another drug class if unsatisfactory clinical response

Waites et al. Clin Microbiol Rev 2017

Conclusion

What measures might be taken to prevent an increase in the prevalence of resistance to macrolides in *M. pneumoniae*?

- **RT-PCR and pyrosequencing assays for the rapid detection of macrolide-R mutants directly from clinical specimens:**
  - **→To adjust ATB treatment**
  - **→ To control and prevent macrolide-R outbreaks by decreasing the numbers of pharyngeal Mp and the dissemination of the infection.**
- **Macrolides used carefully especially for patients with mild symptoms**
- **Randomized trails to establish clinical guidelines for treatment of macrolide-R strains of *M. pneumoniae***
- **Vaccine? No identified project …**

Bébéar et al., Clin Infect Dis 2012
Blyth and Gerber JPIDS 2018

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IOM The International Organization for Mycoplasmology
Dedicated to the study of the Mollicutes

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