Mycoplasma pneumoniae infections: antibiotic resistance and therapeutic options

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USC EA 3671 Mycoplasmal and chlamydial infections in humans
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M. pneumoniae and antibiotics

- **Intrinsic resistance to ATB targeting the cell wall (β-lactams +++, fosfomycin, glycopeptides) and rifampicin**

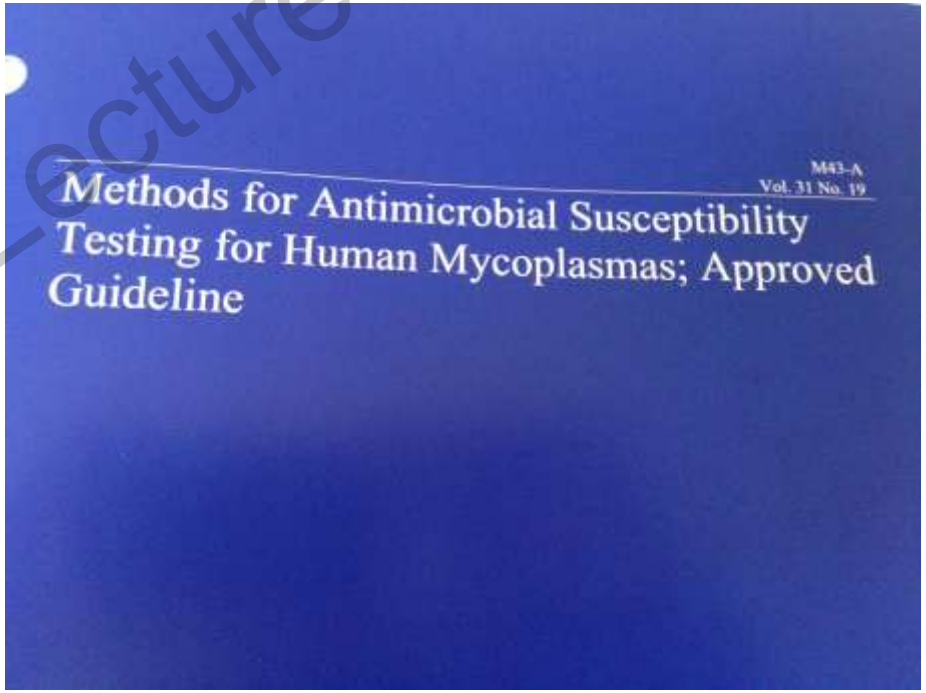
- **Active antibiotics**
  - **Macrolides and related: macrolides, lincosamides, streptogramin combinations, ketolides (MLSK)**
  - **Fluoroquinolones**
  - **Tetracyclines**

☞ **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**
  - **Broth microdilution**
  - **Agar dilution**
  - **No agar diffusion**
  - **Bactericidal tests**
    (MBCs, time-killing studies)

- **Rapid molecular based-methods to detect macrolide resistance directly from specimens**

M. pneumoniae microbroth and agar dilution
CLSI M43-A, 31(19)

- **Testing conditions**

Medium: SP4 broth or SP4 agar

Inoculum (both methods): 10^4–10^5 colony-forming units/ml

Incubation: 37˚ C; ambient air for broth microdilution; air + 5% CO_2 for agar dilution for four to six days

- **Quality control recommendation**

***M. pneumoniae* ATCC® 29342**

- **Agents to consider for primary testing**

Azithromycin or Erythromycin
Levofloxacin
Tetracycline

M. pneumoniae interpretive criteria

CLSI M43-A, 31(19)

Antimicrobial Class	Antimicrobial Agent	MIC (μg/mL) Interpretive Criteria S	I	R	Comments
Quinolones					
	Levofloxacin	≤1	–	–	
	Moxifloxacin	≤0.5	–	–	
Tetracyclines					
	Tetracycline	≤2	–	–	Organisms susceptible to tetracycline will also be susceptible to doxycycline.
Macrolides					
	Erythromycin	≤0.5	–	≥1	Macrolide-resistant strains usually have MICs ≥ 16 μg/mL.
	Azithromycin	≤0.5	–	≥1	

- **Comments**

In view of the very limited data available to designate breakpoints, no attempt was made to define “intermediate resistance.”

Quinolone, tetracycline, and macrolide MIC breakpoints were derived from evaluation of the range of MICs obtained for susceptible isolates and previously designated breakpoints for other gram-positive bacteria.

Only fluoroquinolones and ketolides bactericidal *in vitro*.

MICs of MLSK against *M. pneumoniae*

Bébéar et al., Future Microbiol 2011

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

[a] **Macrolides, Lincosamides, Streptogramins, Ketolides**

[b] **Susceptible strains**

Antibiotic	*M. pneumoniae*[a]
Tetracyclines, Glycylcylines	
Tetracycline	0.63 – 0.25
Doxycycline	0.02 – 0.5
Minocycline	0.06 – 0.25
Tigecycline	0.06 – 0.25
Fluoroquinolones	
Pefloxacin	2
Ciprofloxacin	0.5 – 2
Ofloxacin	0.05 – 2
Levofloxacin	0.5 – 1
Gatifloxacin	0.06 – 1
Moxifloxacin	0.06 – 0.3
Gemifloxacin	≤ 0.008 – 0.12
Garenoxacin	0.008 – 0.12
DC-159a	0.008 – 0.125
RD-3	0.001 – 0.016
Others	
Chloramphenicol	2 – 10
Gentamicin	4
Linezolid	64 – 256
LBM-415[b]	≤ 0.000063 – 0.008

MICs of tetracyclines and fluoroquinolones against *M. pneumoniae*

Bébéar et al., Future Microbiol 2011

[a] **Susceptible strains**
[b] **Peptide deformylase inhibitor**

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**

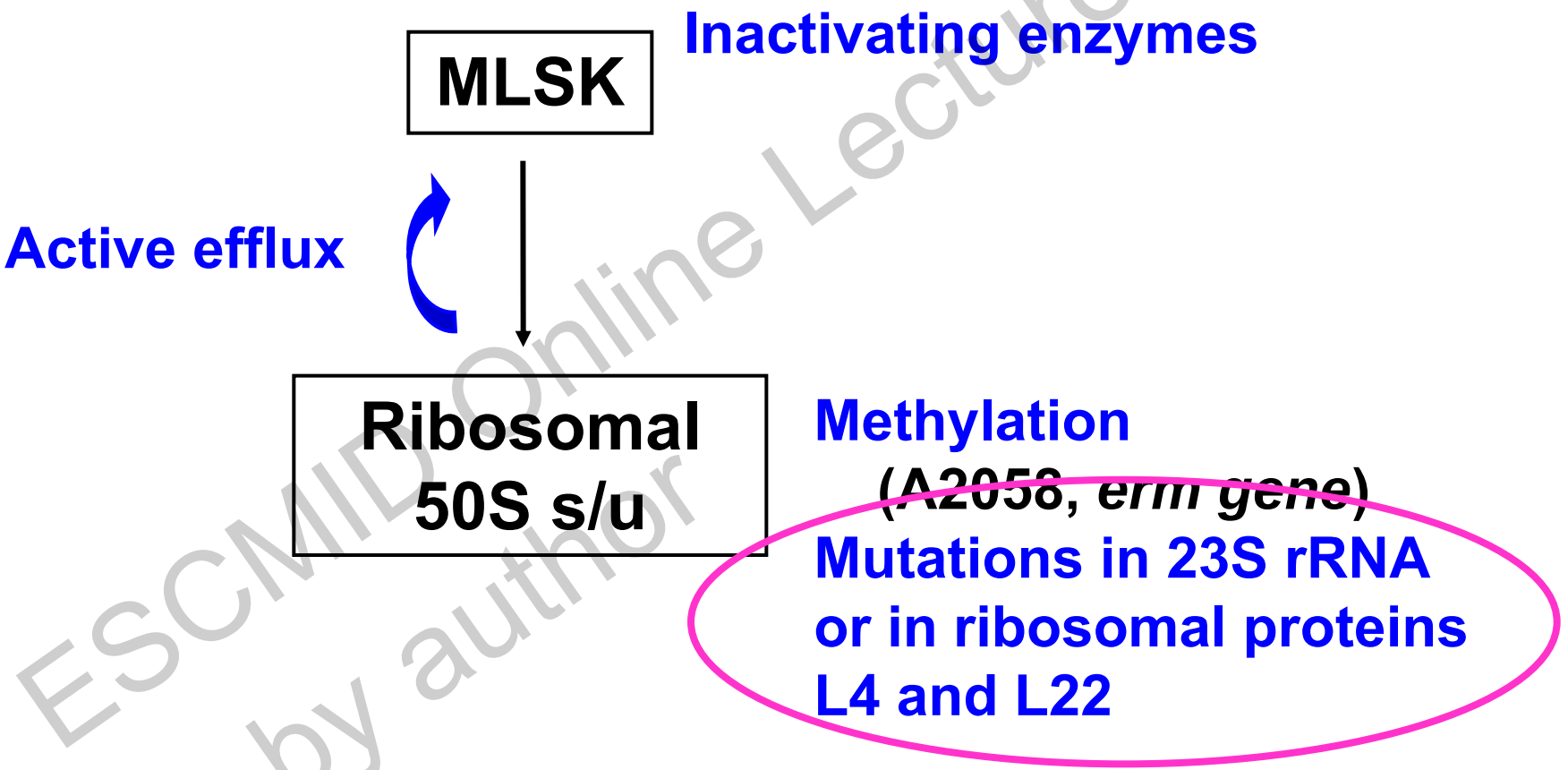
Acquired antimicrobial resistance in *M. pneumoniae*

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

Antimicrobial Class	Resistance *In vitro*	Resistance *In vivo*	Mechanism	MIC range for Resistant Isolates (µg/ml)
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 QRDRs *of gyrA, gyrB, parC* or *parE* genes	2 - 16 (levofloxacin)

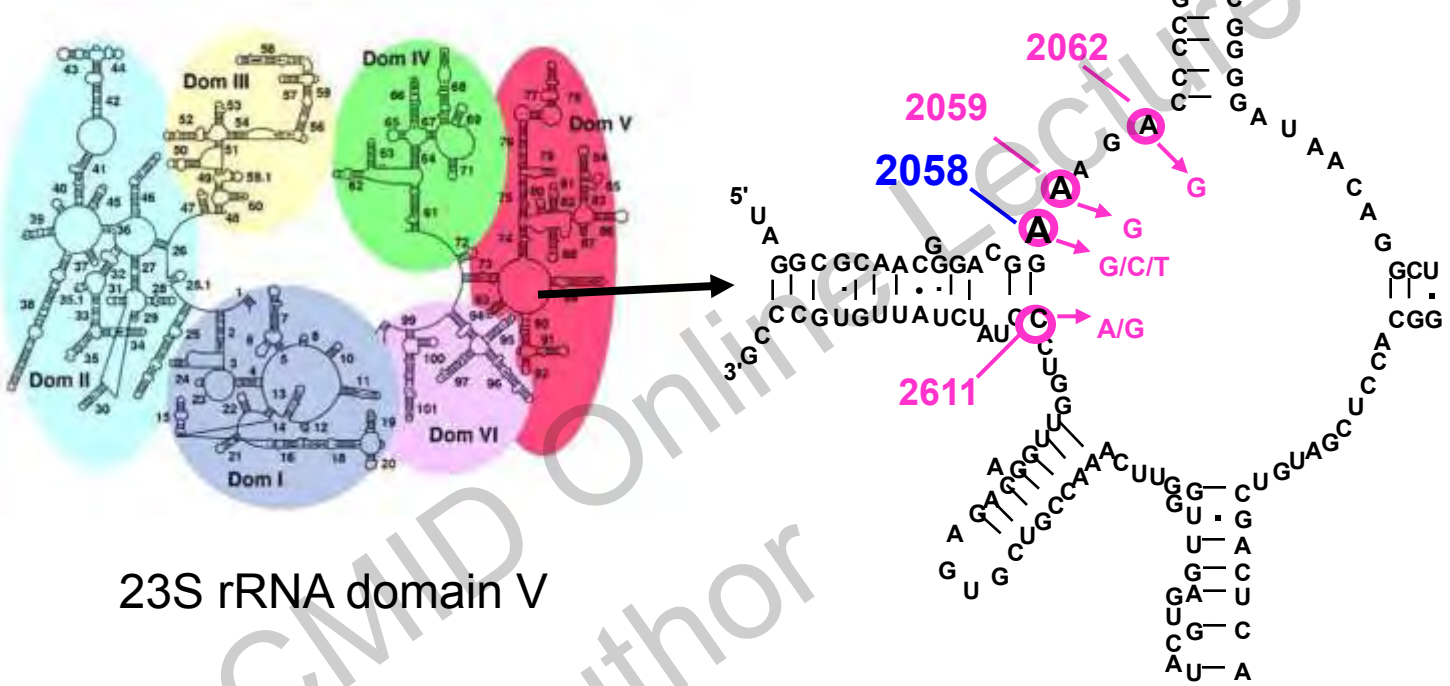
Adapted from K. B. Waites, I. Lysnyansky and C. Bébéar, in Mollicutes: molecular biology and pathogenesis, Caister Academic Press, 2014

Mechanisms of macrolide resistance in bacteria



Macrolide resistance in *M. pneumoniae* (1)

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



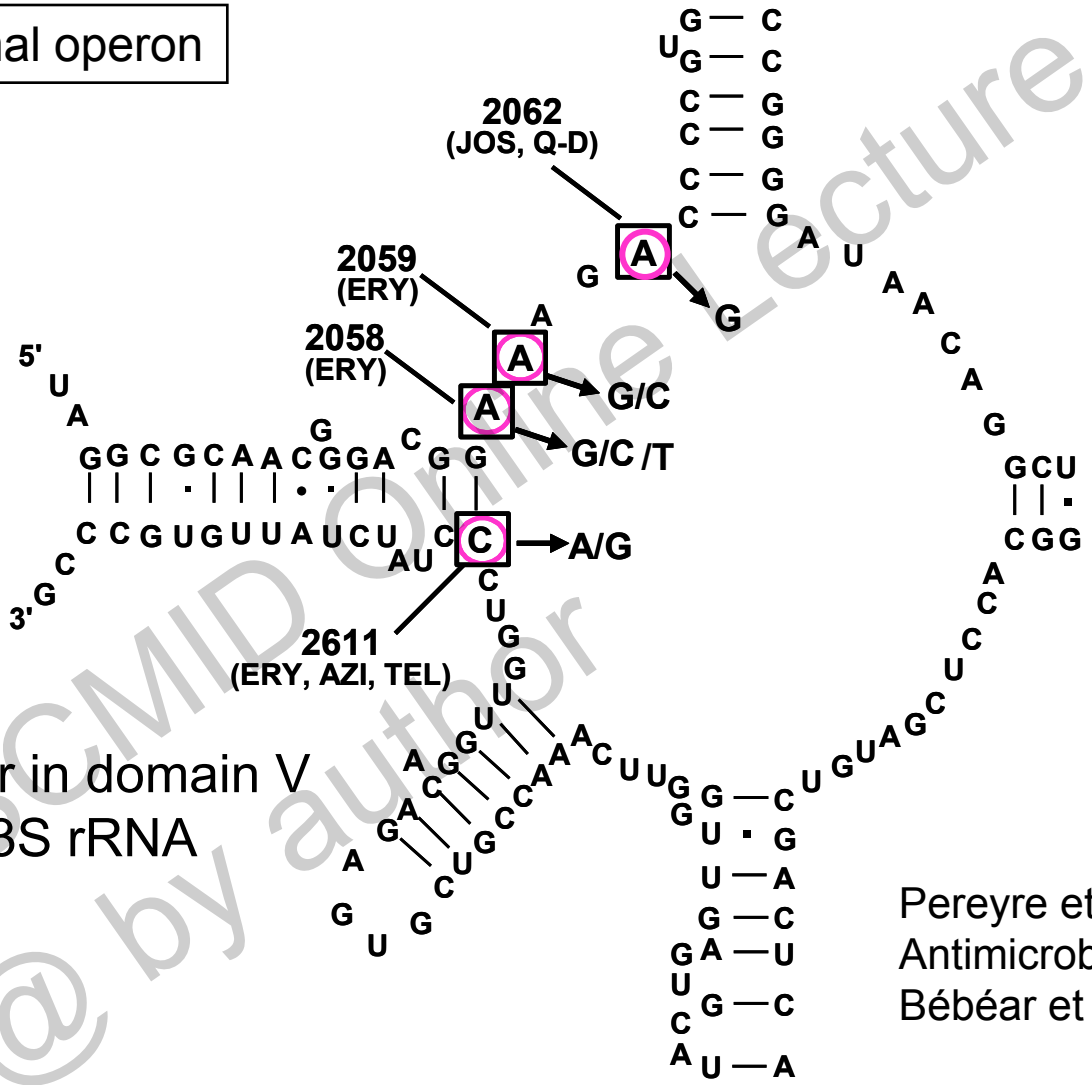
23S rRNA domain V

- **Predominance of A2058G (A2063G):** >90% of mutations

Macrolide resistance in *M. pneumoniae* (2)

23S rRNA mutations *in vivo* and *in vitro*

1 ribosomal operon



PT center in domain V of 23S rRNA

○ *in vivo*

ERY MIC 8- >256 mg/L

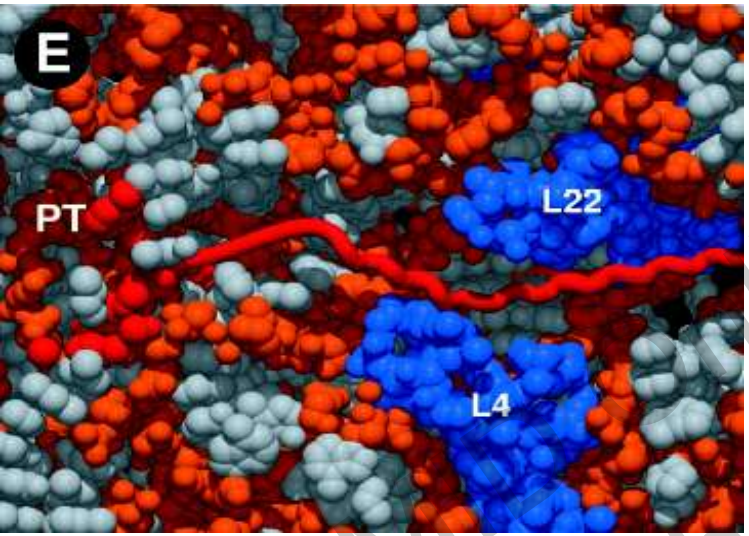
□ *in vitro*

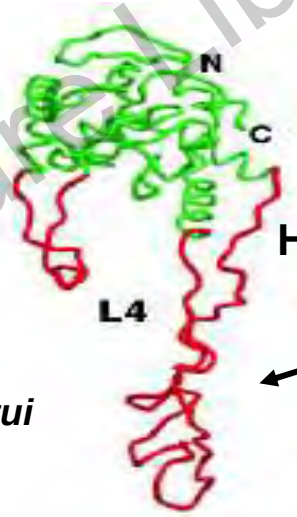
Pereyre et al.,
Antimicrob Agents Chemother, 2004
Bébéar et al., Future Microbiol, 2011

Macrolide resistance in *M. pneumoniae* (3)

Mutations in ribosomal proteins found *in vitro*

ribosomal protein L4

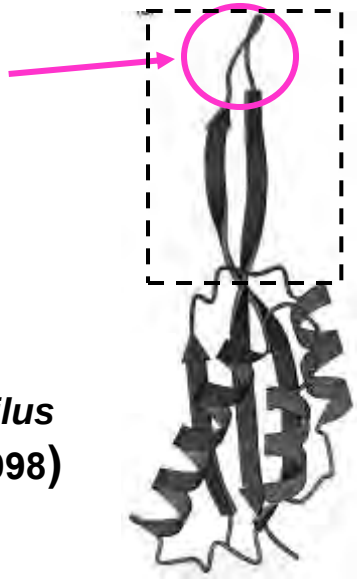




Highly conserved loop

Haloarcula marismortui
(Ban, Science, 2000)

ribosomal protein L22



Conserved β hairpin

Thermus thermophilus
(Unge, Structure, 1998)

Pereyre et al., Antimicrob Agents Chemother, 2004

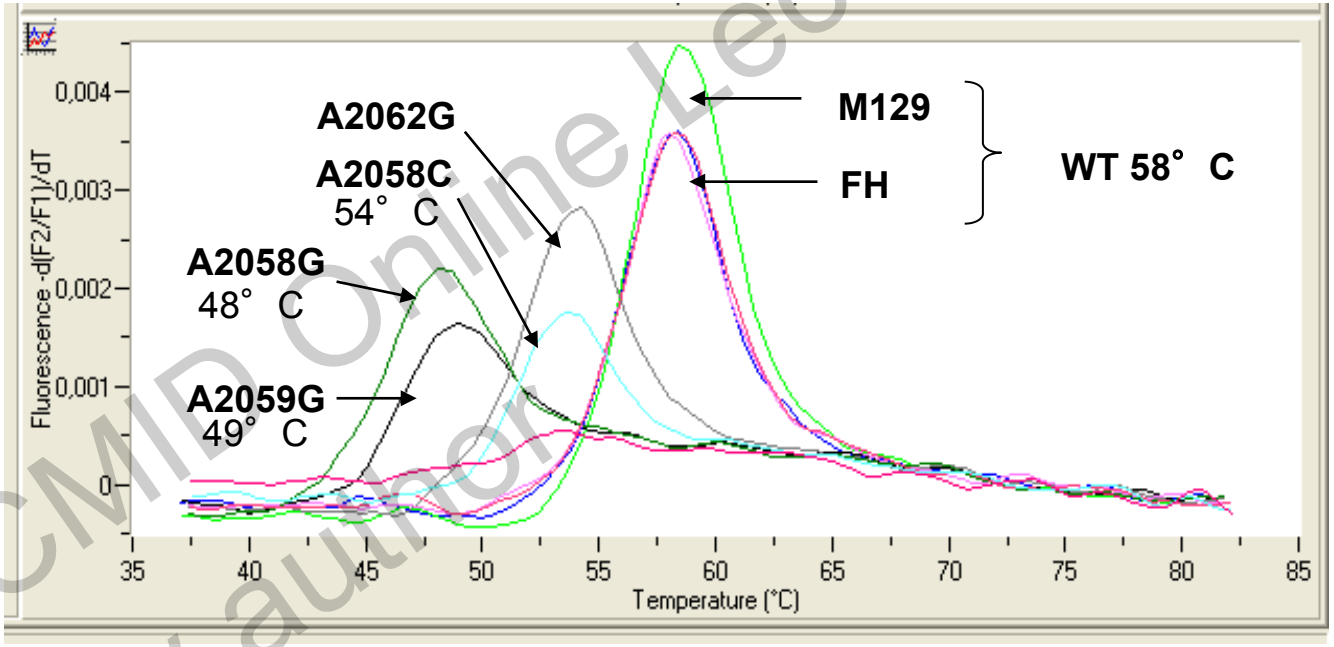
Molecular diagnostic of macrolide resistance in *M. pneumoniae* (1)

- **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

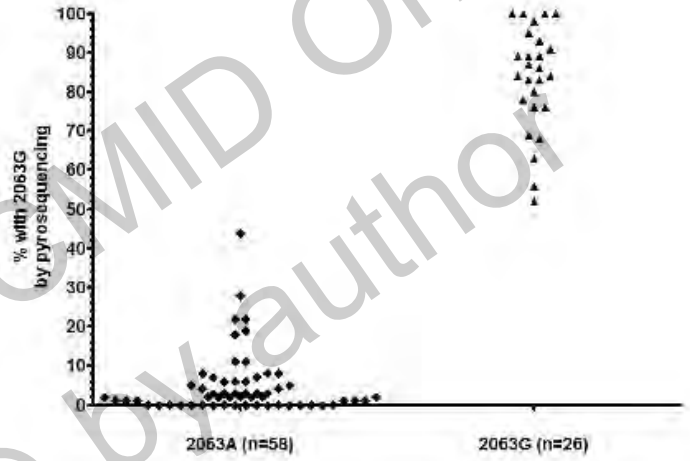
Molecular diagnostic of macrolide resistance in *M. pneumoniae* (2)

- **Comparison of 3 molecular methods to detect 23S rRNA mutations in 91 Mp (+) specimens in Hong Kong**

  → **Pyrosequencing identified 78.8% (67/91) of the specimens with A2058G**

  → **Sanger sequencing or RT-PCR and melting curve analysis identified 38.8% of the 67 mutated specimens**

  → **Mixed WT and A2058G subpopulations in specimens (quasisp.)**



58 specimens wt (Sanger or RT-PCR)
-> 40 with A2058G 1-44%

26 specimens R (Sanger or RT-PCR)
-> 26 with A2058G 52-100%

☞ **Only pyrosequencing can detect mutants present at a low frequency**

Chan et al., J Clin. Microbiol., 2013

MICs of MLSK antibiotics for *M. pneumoniae* clinical isolates resistant to macrolides (adapted from Bébéar, Future Microbiol 2011)

M. pneumoniae isolates	MIC ranges (µg/ml)							
	ERY[a]	AZM	JOS	RKI	CLI	Q-D	TEL	SOL
Reference strain M129	≤0.015	0.002	0.03-0.12	0.01-0.06	4	0.25	0.002	0.0001
Clinical strains with mutation in domain V of 23S rRNA:								
A2058G[c]	32->256	16->64	0.06-64	0.03-1	256	0.5-1	16->64	0.5
A2058C	>256	16	64	4	32	1	ND[d]	ND
A2058T	32-256	0.06-2	16	1-4	256	ND	ND	ND
A2059G	>64-256	16-64	>64-256	8-32	32	0.25	1-16	ND
C2611G	1-8	≤0.03	0.25	0.06	1-4	0.25	0.03	ND
C2611A	1	0.03	0.06	ND	ND	ND	0.06	ND

[a]ERY, erythromycin; AZM, azithromycin; JOS, josamycin, RKI, rokitamycin; CLI, clindamycin; Q-D, quinupristin-dalfopristin; TEL, telithromycin; SOL, solithromycin.

[c]*E. coli* numbering. 2058, 2059 and 2611 correspond to 2063, 2064 and 2617 in *M. pneumoniae*.

[d]ND, not determined.

Epidemiology of macrolide resistance in *M. pneumoniae* (1)

- **Emergence in 2000's (France, 1999- Japan, 2001),**
  mainly in children
  Pereyre et al., Agents Antimicrob Chemother 2007

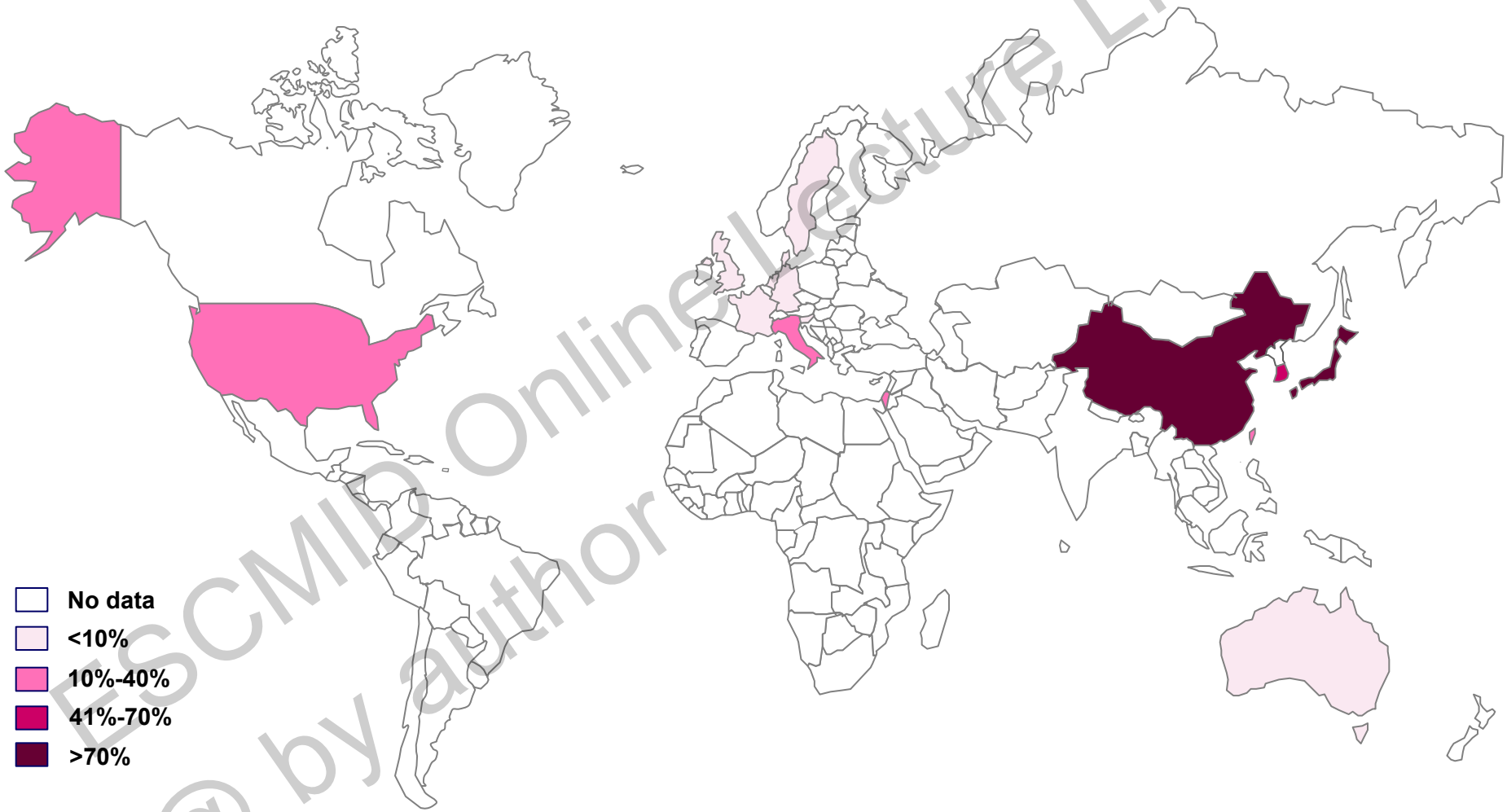
- **Prevalence variable according to the country**
  - **Europe: 0% Netherlands; 1.2-3.6% Germany; 0% England and Wales;0% the Netherlands; 1-3% Denmark; 1.7% Slovenia; 26% Italy**
  - **North America: 1.6-27% USA**
  - **Middle East: 30% Israel**
  - **Asia: 50-90% Japan; 12-23% Taiwan; 56% South Korea; >90% China**
  - **Australia: 3.3%**

- **Prevalence in France**
  - **2005-2008: 9.5%**
  - **2011 (epidemic outbreak): 8.8%**
  - **2012: 3.7%**

- **No cross-resistance to TC or FQ**

Peuchant et al., J Antimicrob Chemother, 2009
Bébéar et al., Future Microbiol, 2011
Pereyre et al., Clin Microbiol Infect, 2013

Frequency of macrolide resistance in *M. pneumoniae*



Epidemiology of macrolide resistance in *M. pneumoniae* (2)

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)

Clinical implications of macrolide resistance in *M. pneumoniae*

- **Clinical implications**

  - Therapeutic failures, children and adults, out and in patients

  - ↗ febrile days, persistent cough, worsening of chest radiographs

  - Therapeutic change for FQ or TC :

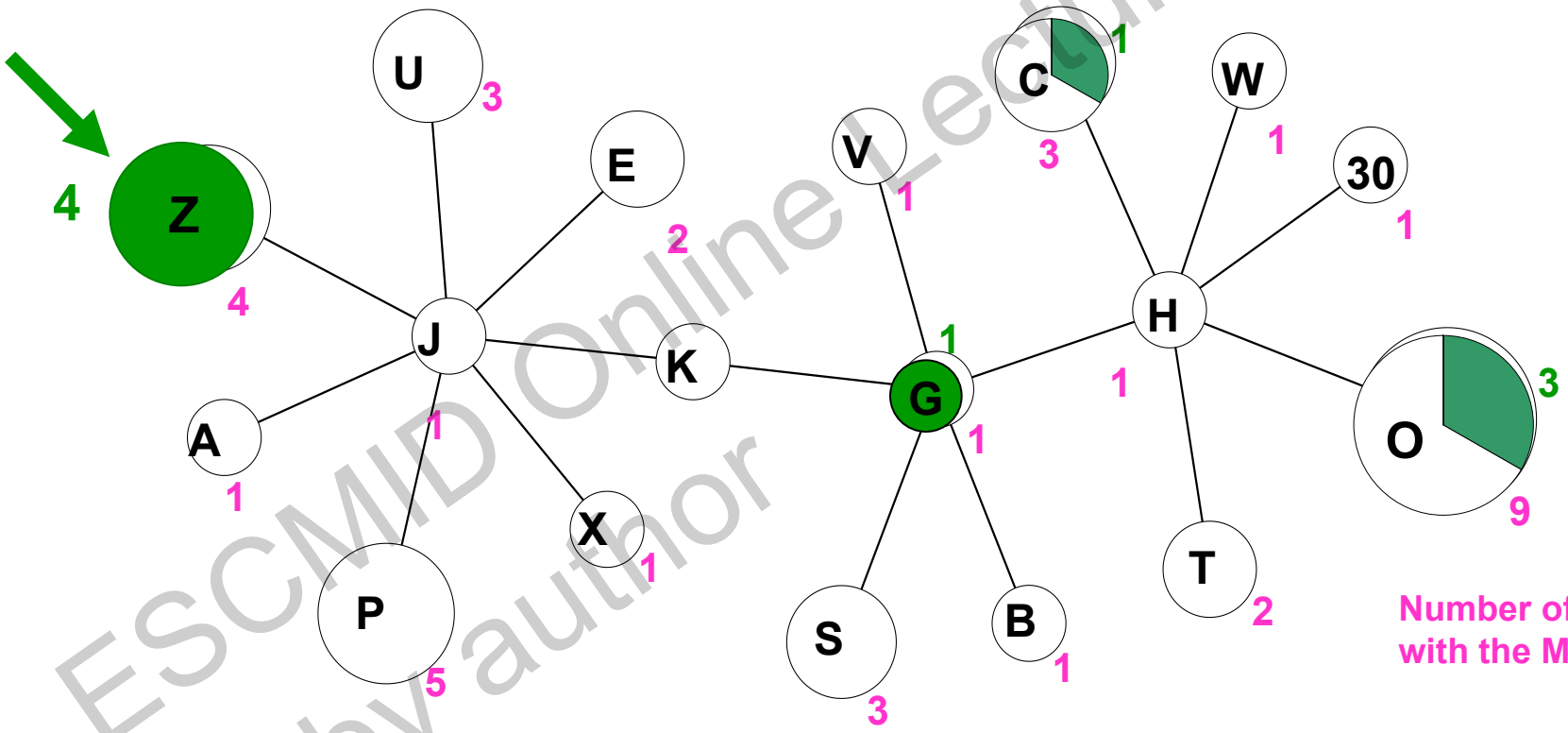
  → Minocycline (MIN) and tosufloxacin (TFX) approved for treating macrolide-resistant Mp infections in children (aged ≥8 yo for TC) in Japan

  → MIN was more effective than TFX in achieving defervescence and in decreasing numbers of Mp copies after 3 days

  Okada et al., Clin Infect Dis 2012; Kawai et al., Antimicrob Chemother, 2013

MLVA typing of a *M. pneumoniae* outbreak in Israel in 2010

A2058G mutation in 22% of patients (9)



☞ **A macrolide-R *M. pneumoniae* clone Z is spreading in Jerusalem ?**

Pereyre et al. , PloS ONE, 2012

Emergence of macrolide resistance during macrolide treatment

Boy 11 yo
Hyper IgE syndrome
Mp(+) pneumonia

→ **Josamycin** →

Tt Failure

→ **Ciprofloxacin** →

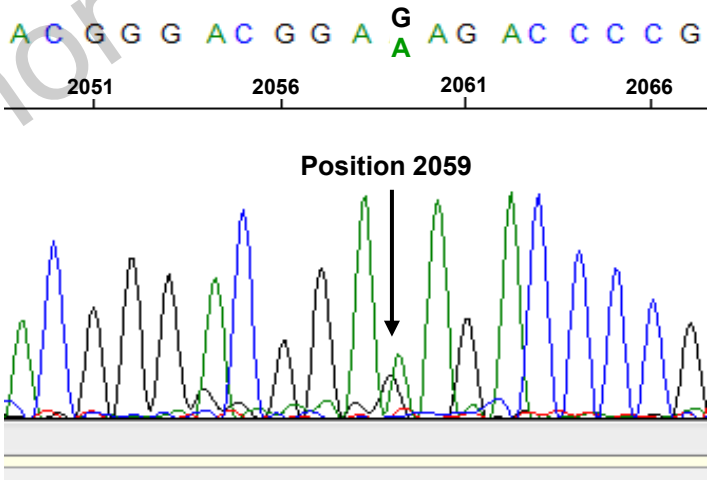
Recovering

WT *M. pneumoniae*

MLVA type I

***M. pneumoniae* with A2059G**

MLVA type I



Hantz et al., J Clin Microbiol, 2012

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
  - Streptogramin combination (pristinamycin) ? New ketolides ?

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

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