Macrolides-lincosamides-streptogramins

14-membered ring macrolides

-erythromycin, roxithromycin, clarithromycin, dirithromycin

-ketolides

15-membered ring macrolides

azithromycin

16-membered ring macrolides

spiramycin, josamycin, myokamycin, midecamycin

Lincosamides

clindamycin, lincomycin

Streptogramins

A-type: dalfopristin, pristinamycin IIA

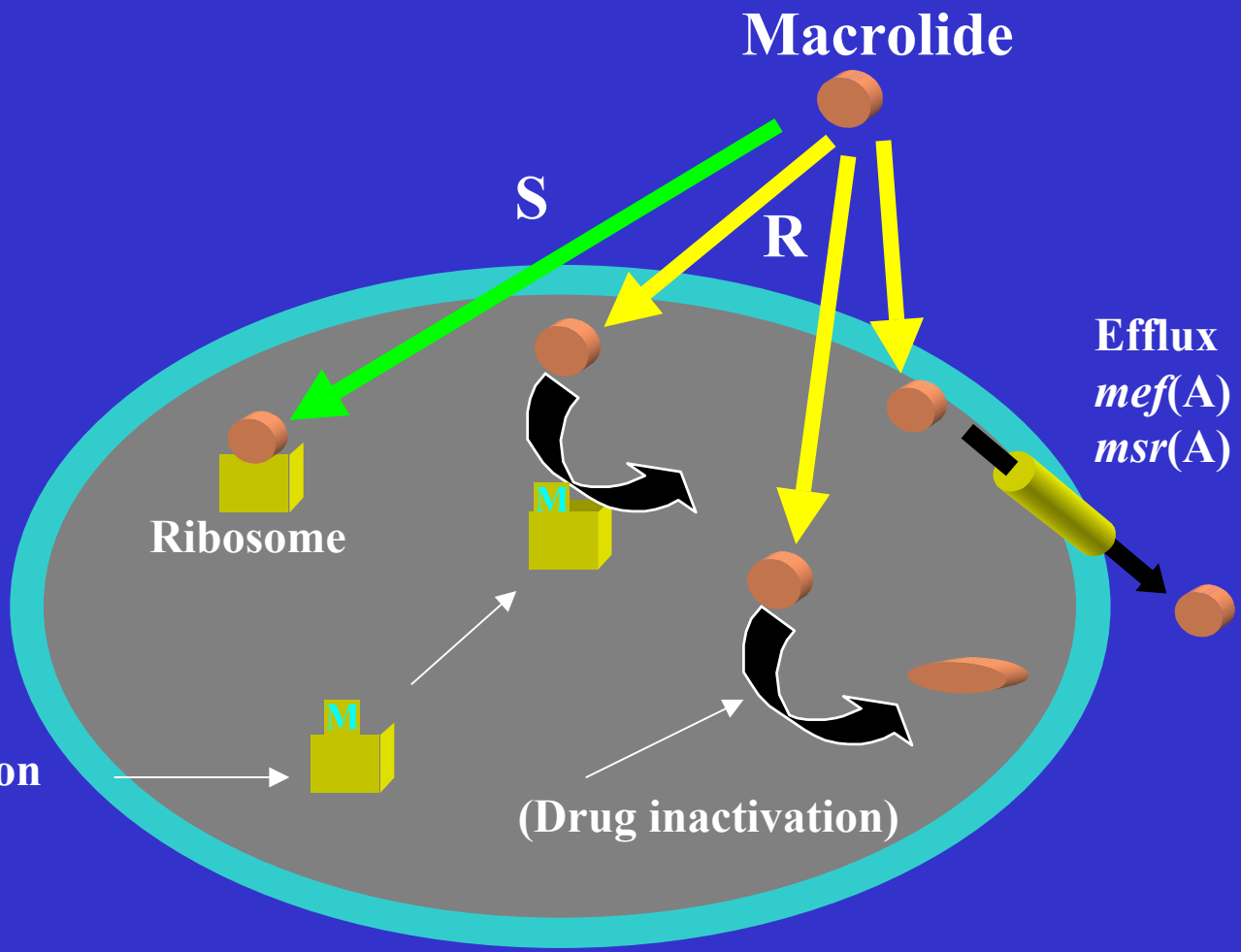
B-type: quinupristin, pristinamycin IA

Macrolide evolution

- 1985-1990: macrolides with improved pharmacokinetic properties
  - derived from erythromycin A (azithromycin, clarithromycin, dirithromycin, roxithromycin)

- 1985-1995: emergence of macrolide resistance in *S. pneumoniae* and *S. pyogenes* (certain countries)

Mechanisms of resistance to macrolides in Gram-positive organisms



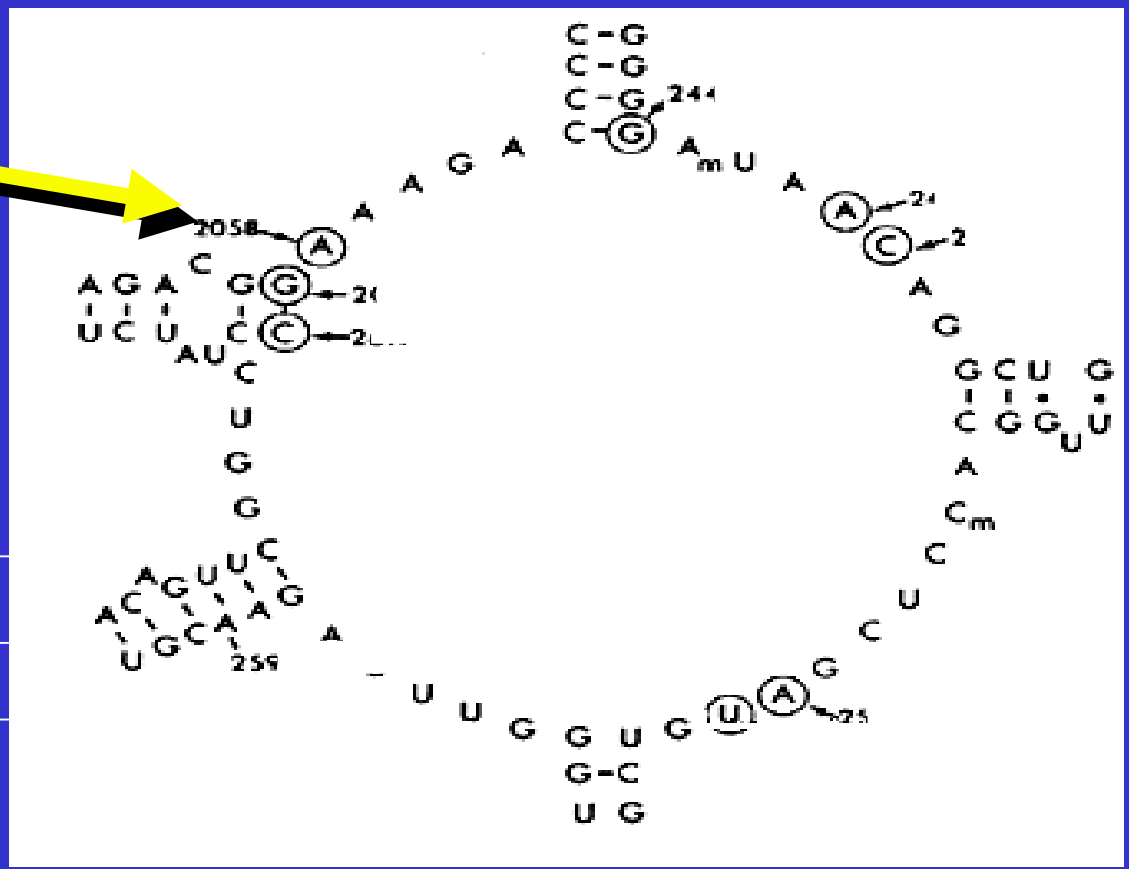
Efflux
mef(A) in streptococci
msr(A) in staphylococci

Target modification
erm

23S RNA secondary structure (domain V)

Methylation

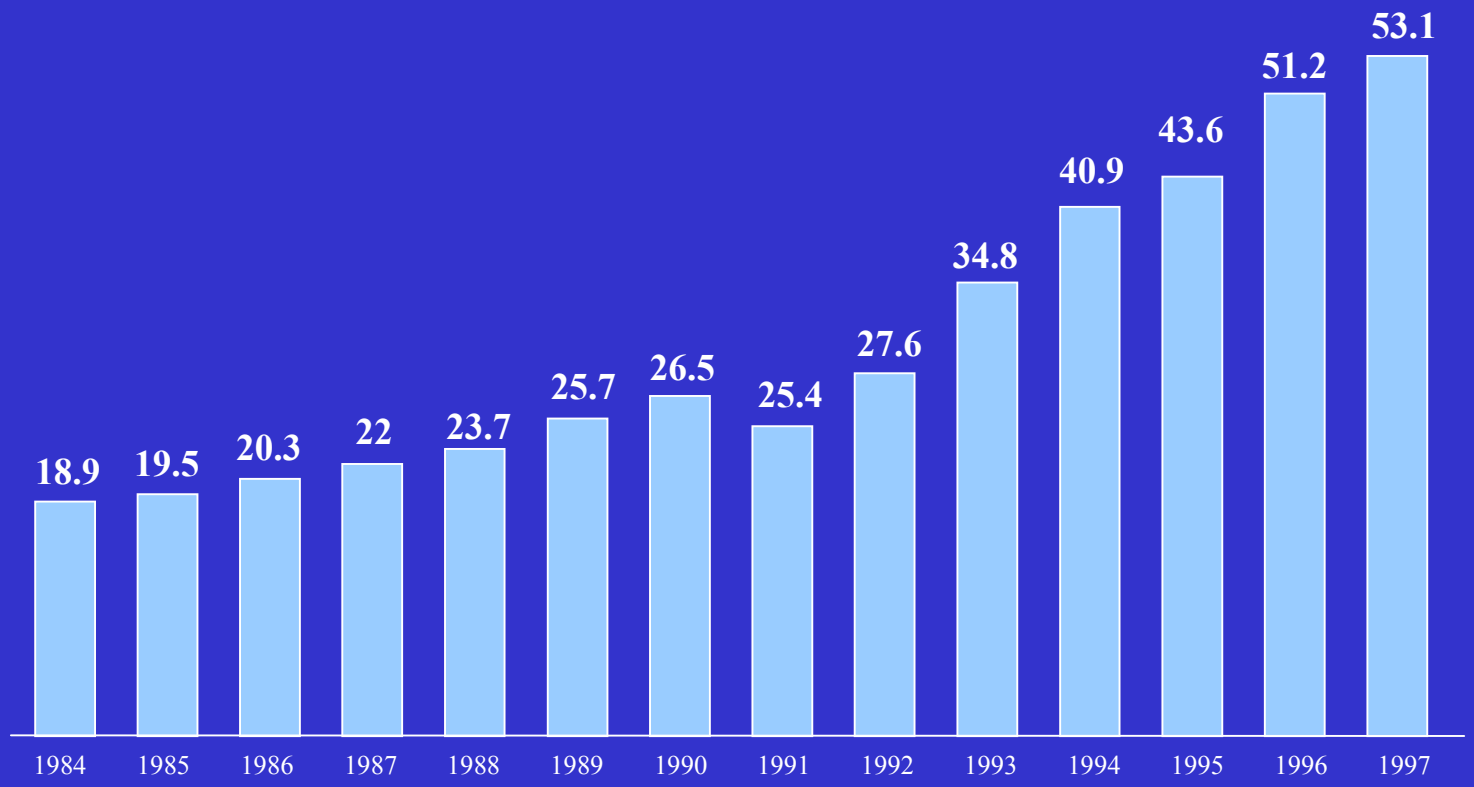
(*erm* genes; MLS_B resistance)



	Increase in MIC (x)	
	Ery	Lin
Dimeth.	>10,000	>20,000

Pernodet et al., 1996

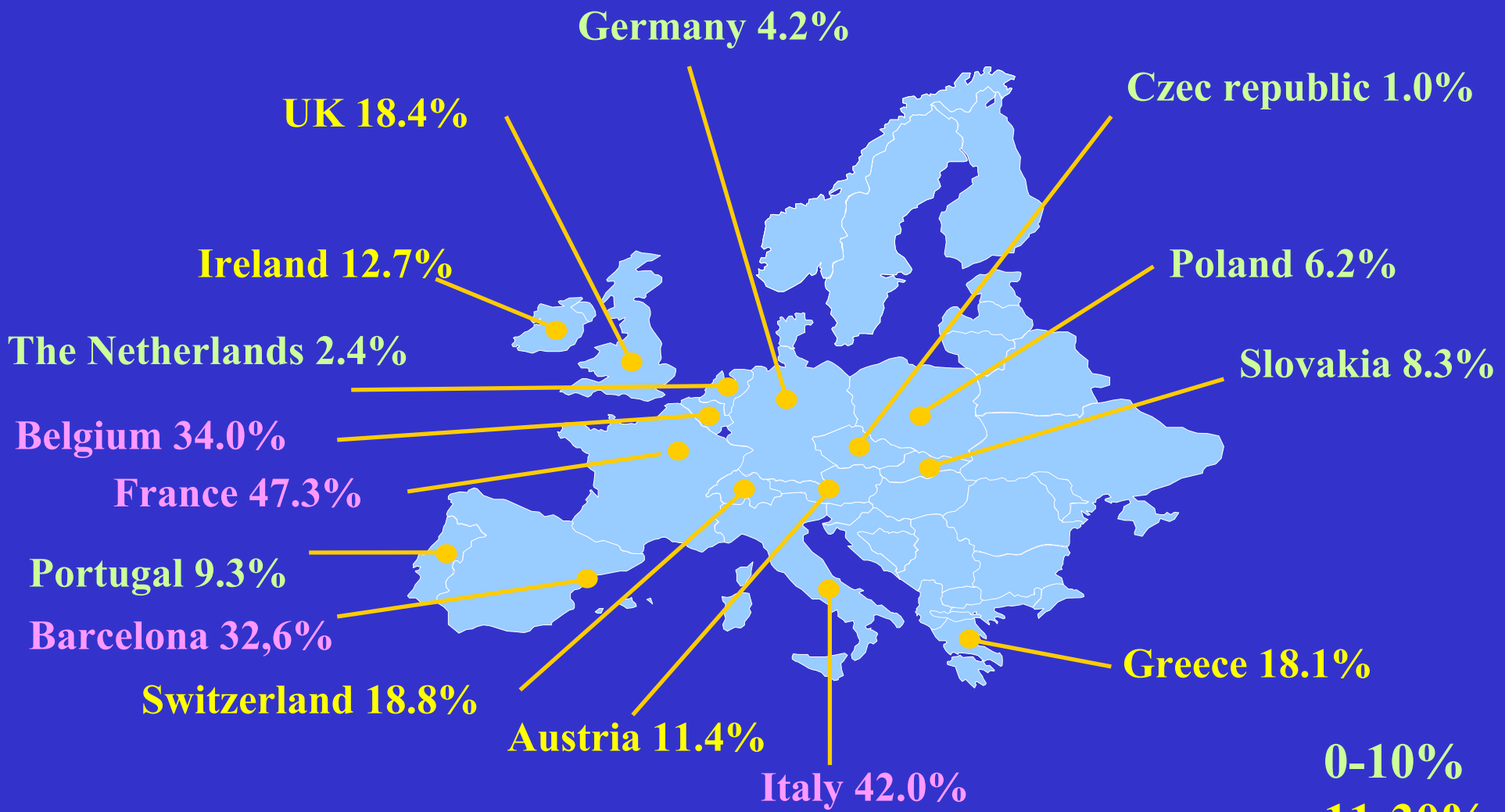
Evolution of erythromycin resistance in *S. pneumoniae* in France



41195 strains isolated in France

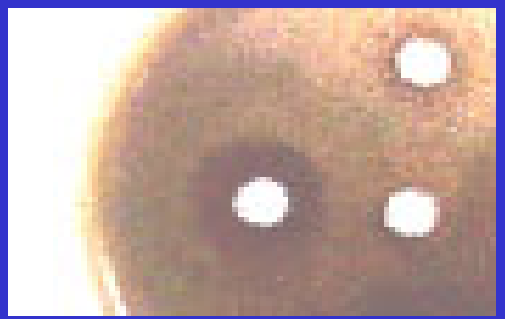
Geslin ***et al, Rapport d 'activité du Centre National de Référence des pneumocoques***

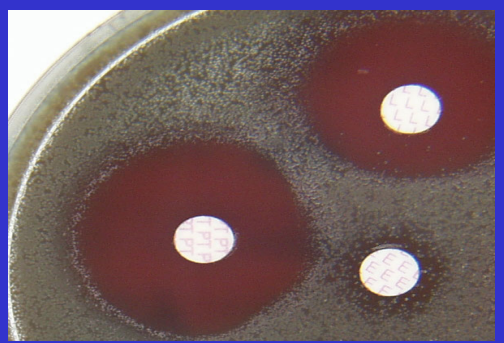
Macrolide resistance in pneumococci in Europe (1997–1998)



Schito *et al*, JAC 2000
Felmingham *et al*, JAC 2000

MLS_B resistance due to *erm*(B)

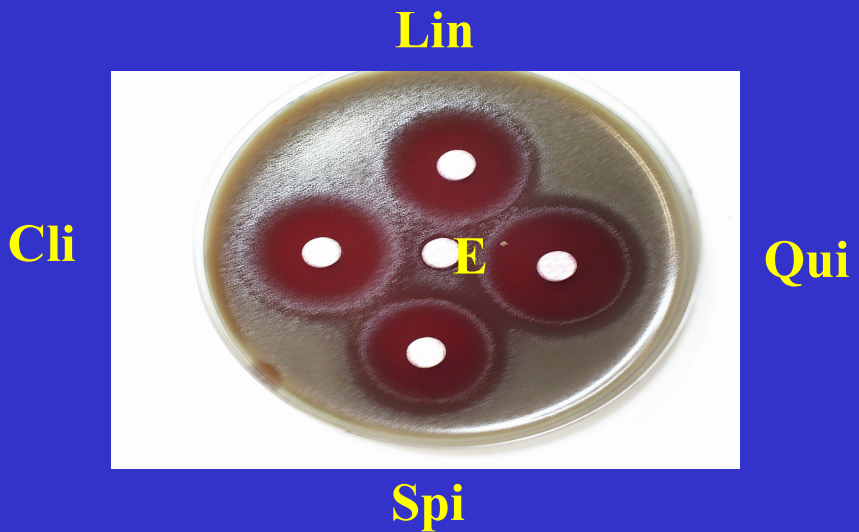




Expression of MLSB resistance
Inducible or constitutive
Inducible
Inducible

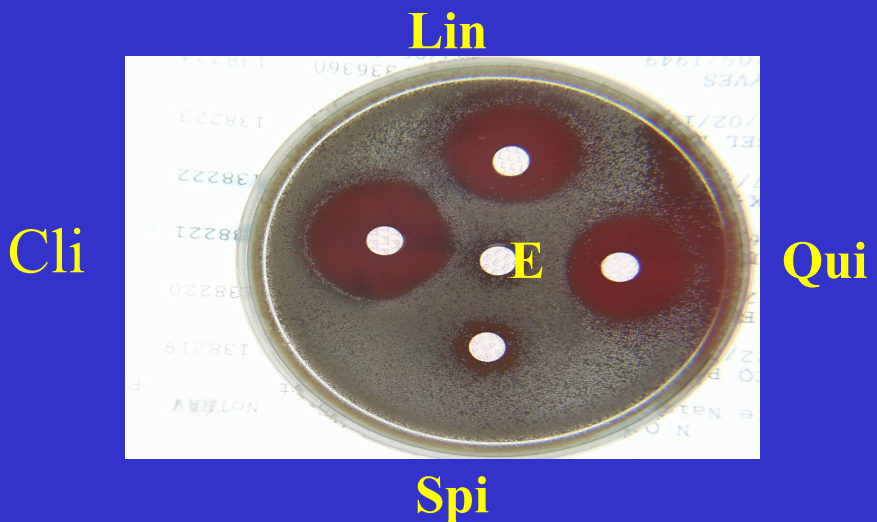
S. pneumoniae, other streptococci, (*S. aureus*), (enterobacteria)

Erythromycin resistance by efflux in *S. pneumoniae*



Drug efflux *mef*(A)

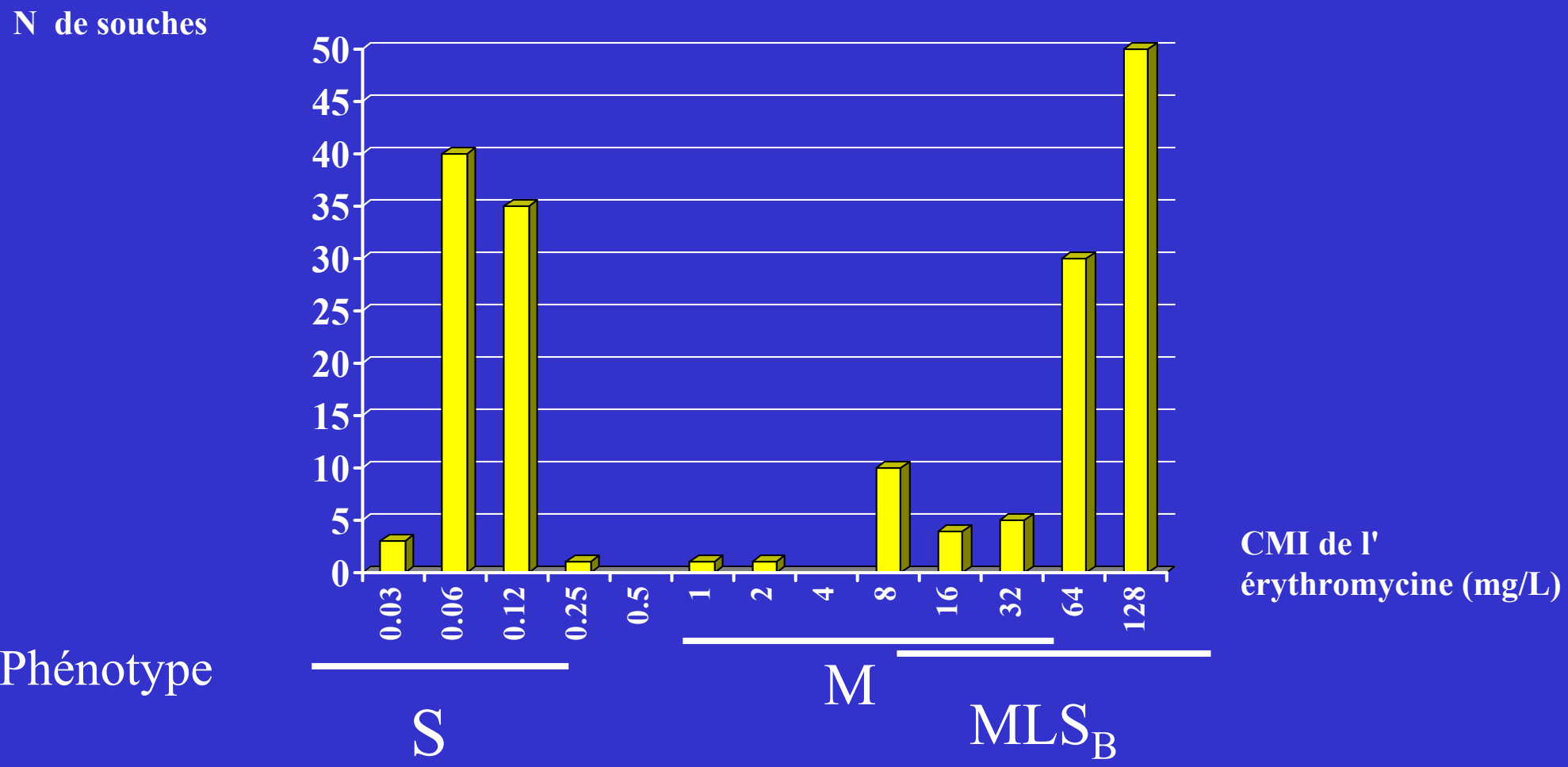
14-,15-Md	16-Md	Lin
R	S	S



Target modification *erm*(B)

14-,15-Md	16-Md	Lin
R	R	"S"

Analyse de population pour les souches de *S. pneumoniae* sensibles ou résistantes à l'érythromycine

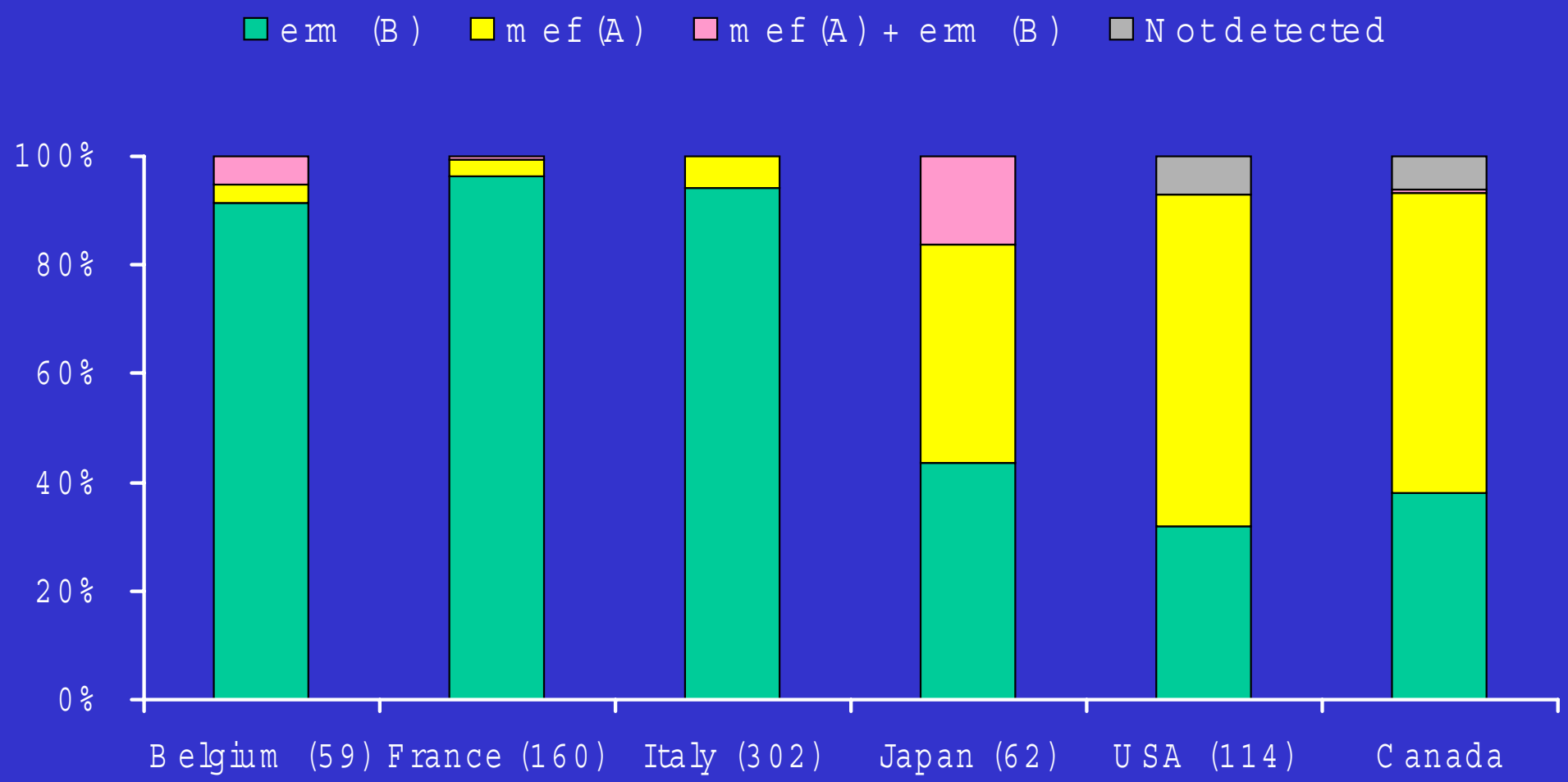


MICs of macrolides against *S. pneumoniae* according to resistance phenotypes/genotypes

	MIC (mg/L)					
	MLS_B or *erm*(B)			M or *mef*(A)		
	range	MIC50	MIC90	range	MIC50	MIC90
Azithromycin	8->128	>128	>128	2-32	16	16
Clarithromycin	2->128	>128	>128	1-64	8	8
Erythromycin	8->128	>128	>128	1-64	8	8
Roxithromycin	2->128	>128	>128	1-32	8	8
Josamycin	0.5->128	16	>128	0.03-0.025	0.06	0.12
Miocamycin	4->16	2-16	>128	0.25-0.5	-	-
Spiramycin	16->128	8-128	>128	0.06-1	0.25	0.5

Descheemaker (agar CO2),Fitoussi (agar), Ip (agar),
Montanari (NCCLS broth microdil), Nishijima (agar)

Diversity of macrolide resistance genotypes in pneumococci



Controversy with clinical relevance of macrolide resistance

- Difficulties to establish correlation: poor mortality rate, spontaneous favourable evolution.

- Intracellular antibiotics for extracellular pathogens

- Impact of resistance on bacterial eradication in AOM
  - MIC of azi <0.12 mg/L: pneumococcal eradication at D4 or D5 in 12 patients
  - MIC >16 mg/L persistence in all 5 patients, acquisition of a R strain in 1 patient.

    (Dagan et al. Antimicrob Agents Chemother 2000;44:43)

More controversy with the efflux mechanism

- 41 patients with pneumococcal bacteremia: 7 had previously received antibiotics. Four previously treated with azithromycin or clarithromycin for 3-5 days had true failures. The 4 blood isolates had a M phenotype (MIC of ery=8 or 16 mg/L)

  (Kelley et al. Clin Infect Dis 2000;31:1008)

Incidence of macrolide resistance in *S. pyogenes*

	% R ery	% M phenotype
Belgium (1994-1998)	6.9	87.5
Finland (1996)	8.6	-
France (1996-1999)	6.2	45.2
Italy (1995)	26.8	-
Spain (1998)	23.5	95.6
USA (1997)	2.6	-
USA (San Francisco, 1995)	32	-
Korea (1998)	41.3	-
Taiwan (1998)	63.2	64.3

Descheemaker, Seppälä, Bingen, Cornaglia, Alos, Granizo, Kaplan, York, Cha, Yan

MICs of macrolides against *S. pyogenes* with *erm* genes

Genotype	Antimicrobial	MIC (mg/L)		
		Range	MIC50	MIC90
erm(B)	Erythromycin	1->128	>128	>128
	Azithromycin	2->128	>128	>128
	Clarithromycin	0.5->128	>128	>128
	Roxithromycin	2->128	>128	>128
	Josamycin	2->128	>128	>128
	Miocamycin	16->16	>16	>16
	Spiramycin	64->128	>128	>128
erm(A)	Erythromycin	1->128	2->128	4->128
	Azithromycin	2->128	8->128	32->128
	Clarithromycin	0.5->128	1->128	2->128
	Roxithromycin	2->128	16->128	16->128
	Josamycin	0.06-1	0.03-0.25	0.06- 0.25
	Miocamycin	0.06-0.5	0.25	0.25
	Spiramycin	0.03-1	0.06-0.25	0.12-0.5

Bingen (NCCLS), Descheemaker (agar), Giovanetti (broth microdil.), Kataja (agar NCCLS)

MICs of macrolides against *S. pyogenes* with *mef* genes

Genotype	Antimicrobial	MIC (mg/L)		
		Range	MIC50	MIC90
mef(A)	Erythromycin	1-32	8	8-16
	Azithromycin	2-32	2-8	2-8
	Clarithromycin	0.5-16	2-8	4-8
	Roxithromycin	2-64	16	16
	Josamycin	0.01-1	0.03-0.25	0.12-0.5
	Miocamycin	0.06-0.5	0.25	0.25
	Spiramycin	0.03-1	0.06-0.25	0.12-0.5

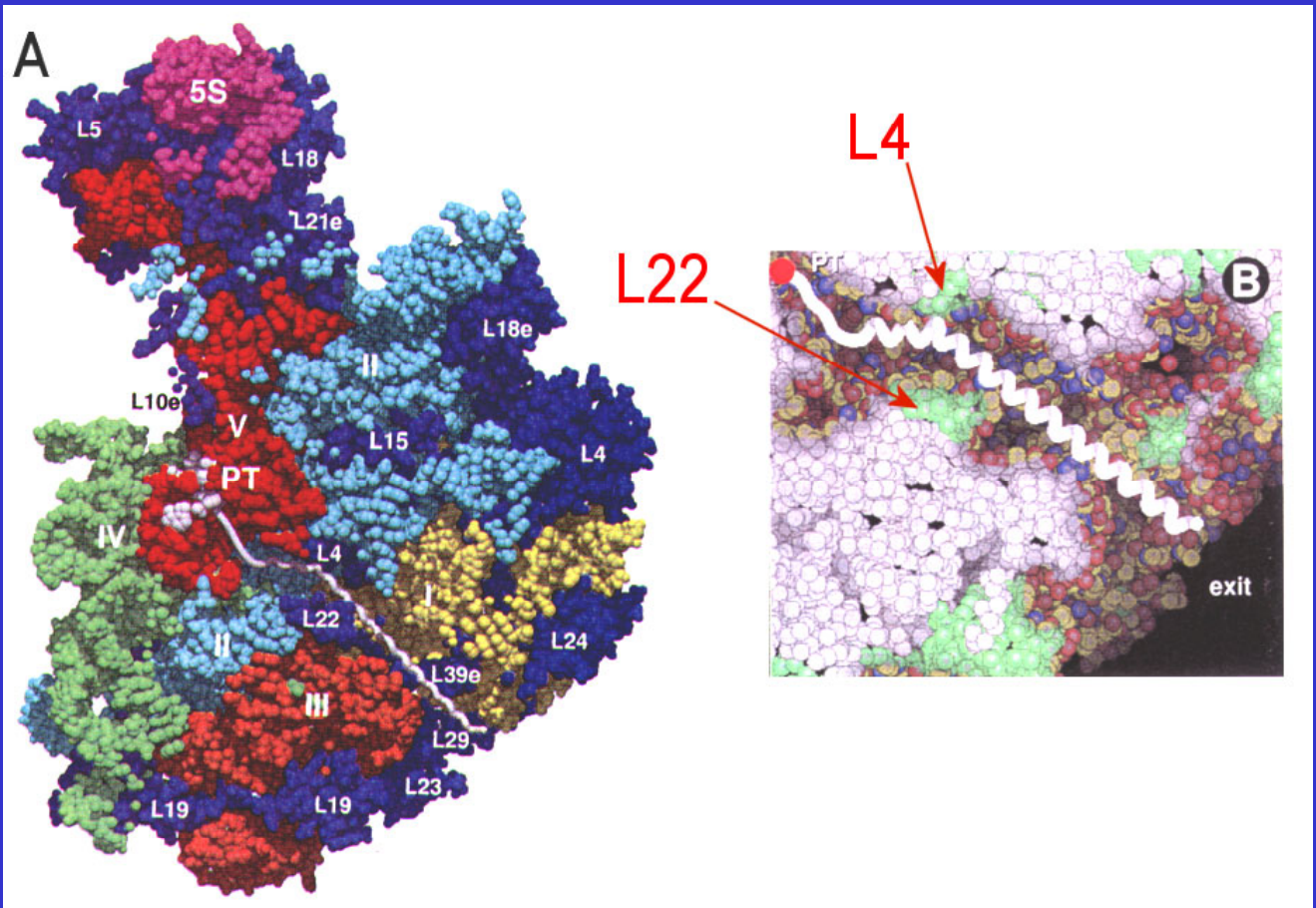
Descheemaker (agar), Giovanetti (broth microdil.), Kataja (agar NCCLS)

Phenotypes and genotypes of MLS_B resistance in *Staphylococcus aureus*

Phenotype	Genotype	Susceptibility to				
		14- 15-Md	Lin	SgB	SgA	Sg
Constitutive	*erm*(A, B,C)	**R**	**R**	**R**	**R**	S[1]
Inducible	*erm*(A), *erm*(C)	**R**	S	S	S	S

[1]Altered bactericidal activity

50S ribosomal subunit



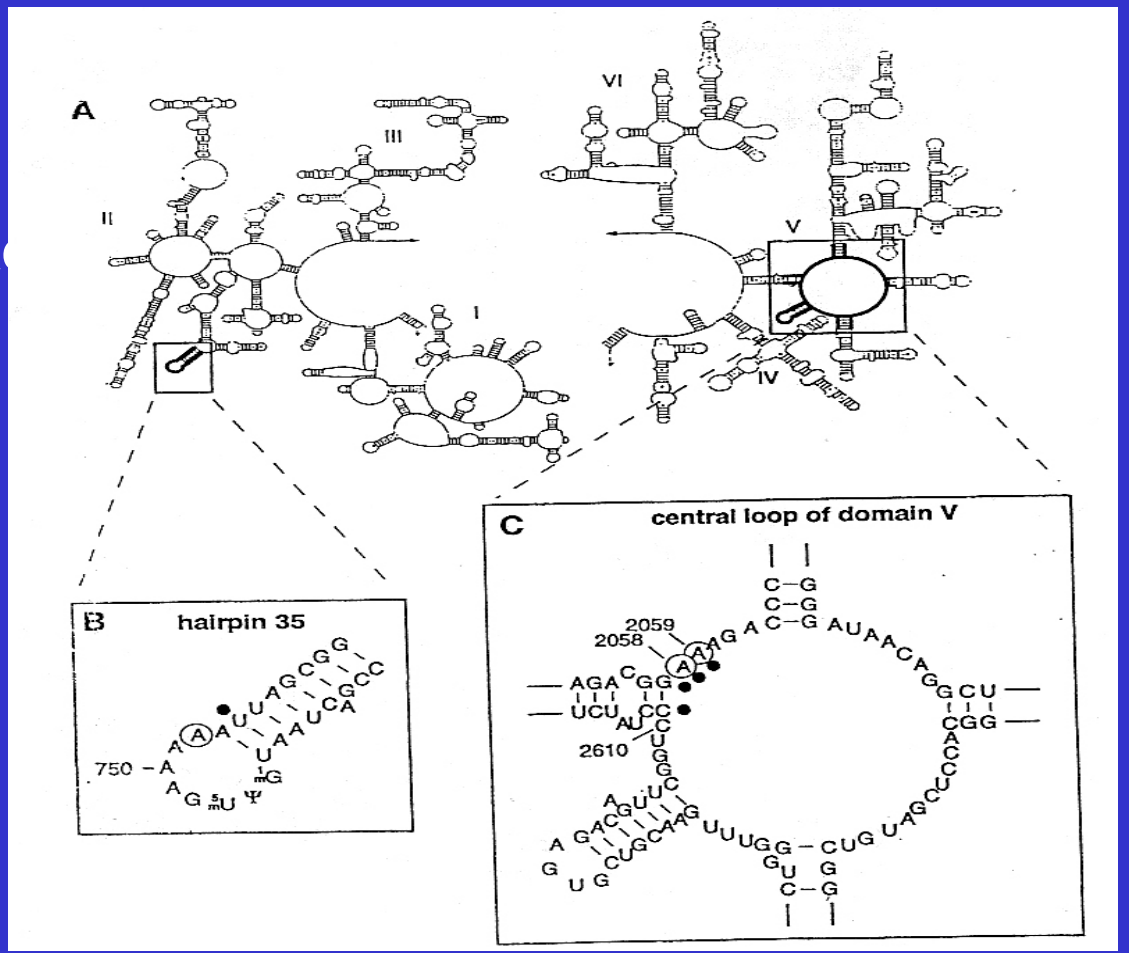
Nissen, P et al. 2000. Science. 289:920-930

Mutational resistance to macrolides: *rrl* genes

- **23S rRNA :**
  - **Domain V : A2058-2059**
    in organisms with 1 or 2 copi
    of *rrl* gene

    Mycobacterium
    Helicobacter
    Propionibacterium….

  - **Domain II : 752**



In vitro and in vivo mutants of 23S rRNA in *S. pneumoniae*

M, K, L, S_B

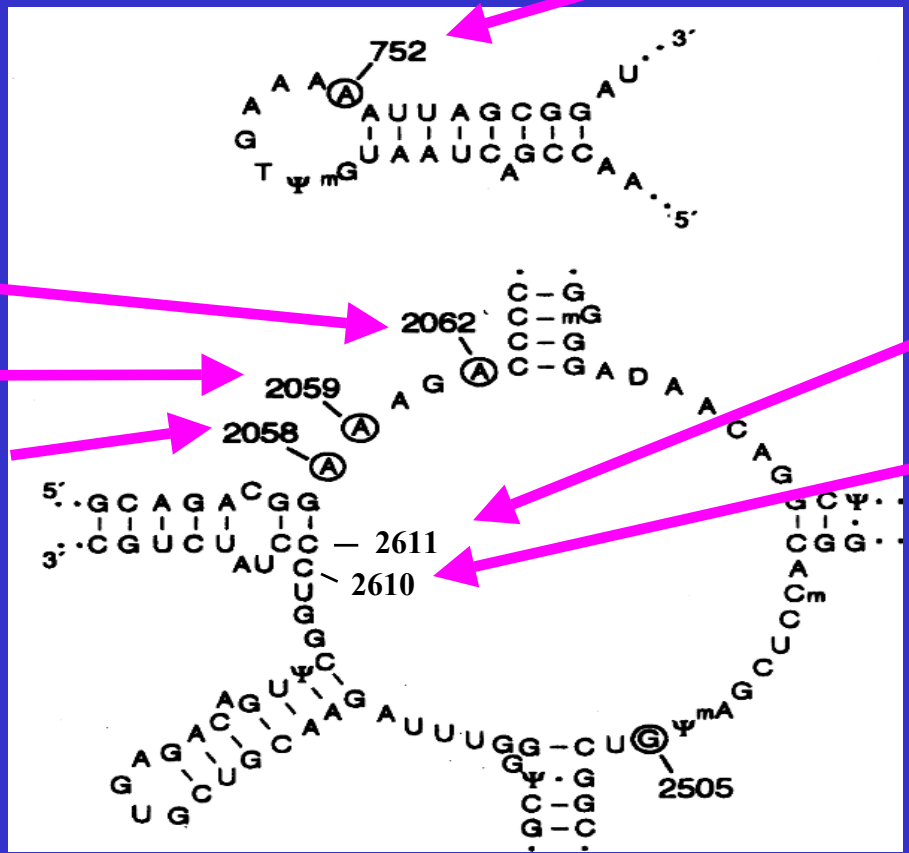
M16, S_B, S

M, L

M, K, L, S_B

M/M, K, L, S_B

M, L



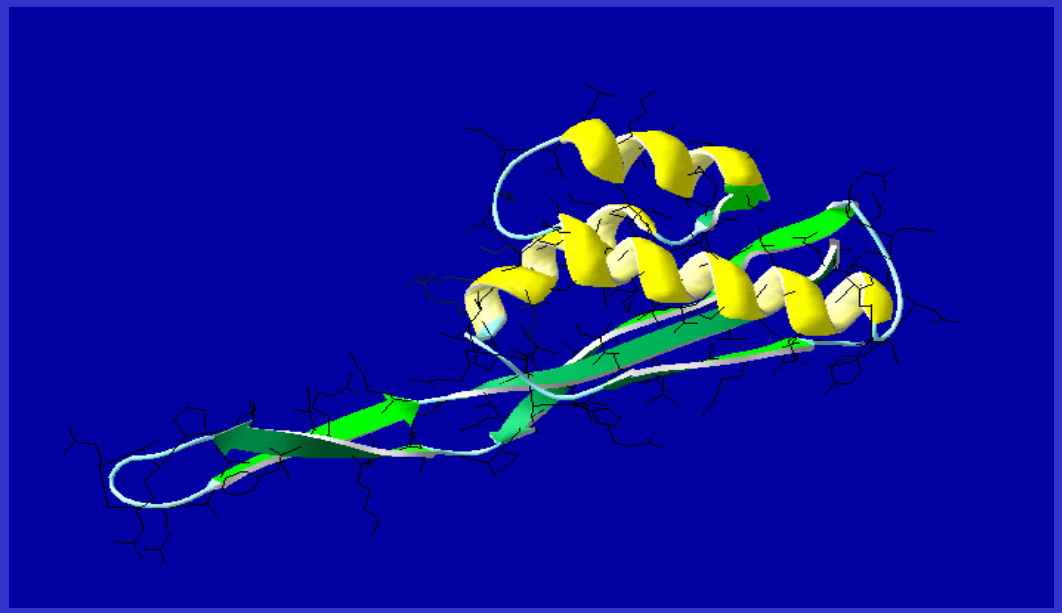
Tait-Kamradt et al., Antimicrob. Agents Chemother, 2000;
Depardieu and Courvalin, Antimicrob. Agents Chemother, 2001
Canu et al., Antimicrob. Agents Chemother 2002;
Pihlajamaki, M., et al. Antimicrob. Agents Chemother 2002

Gene dosage effect

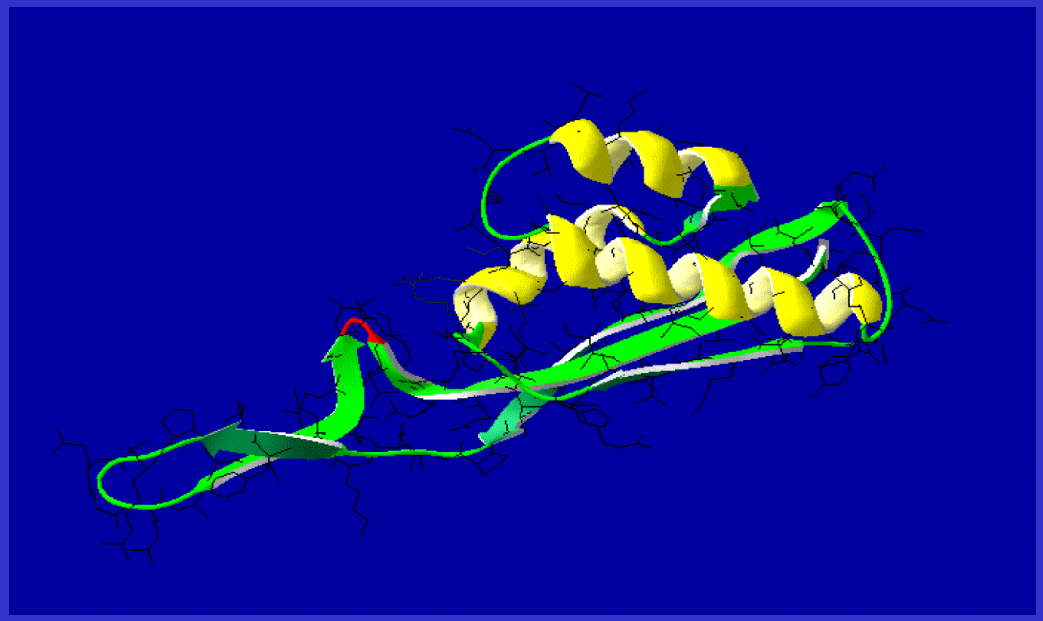
	MIC (µg/ml)				
A2059 / G2059	Ery	Azi	Jos	Tel	Lin
4/0	0.02	0.1	0.1	0.006	0.39
2/2	3.12	400	1.56	0.006	6.25
1/3	12.5	>400	200	0.006	12.5
0/4	25	>400	200	0.006	12.5

Tait-Kamradt et al. Antimicrob Agents Chemother, 2000

Mutations of the *rplV* gene (L22)



Wild-type L22



Mutation in L22

M, k, SgB, SgA+B phenotype

S. pneumoniae

Canu et al., Antimicrobial Agents Chemother 2002
Farrell et al., ICAAC 2001
Musher DM, et al. N Engl J Med. 2002

S. aureus

Malbruny et al., Antimicrobial Agents Chemother 2002

Mutation in L4 ribosomal protein

	63	74
S. pneumoniae Ery-S	---KPWRQ--KG--TG-------RAR-	
S. pyogenes Ery-S	---KPWRQ--KG--TG-------RAR-	
S. pneumoniae BM4419	---KPWRQ--KG--TGRQKGTG--RAR-[a]	
S. pneumoniae 4Az	---KPWRQ--KC--TG-------RAR-[b]	
S. pneumoniae 5Az	---KPWRQSQKG--TG-------RAR-[b]	
Group G str. UCN10R	---KPWRQ--KG--TGREGTG--RAR-[c]	
Oral strept. 4596R	---KPWRQ--KG--TGRREKGTGRAR-[c]	
S pyogenes 237	---KPWRQ--KGKGTG-------RAR—[d]	

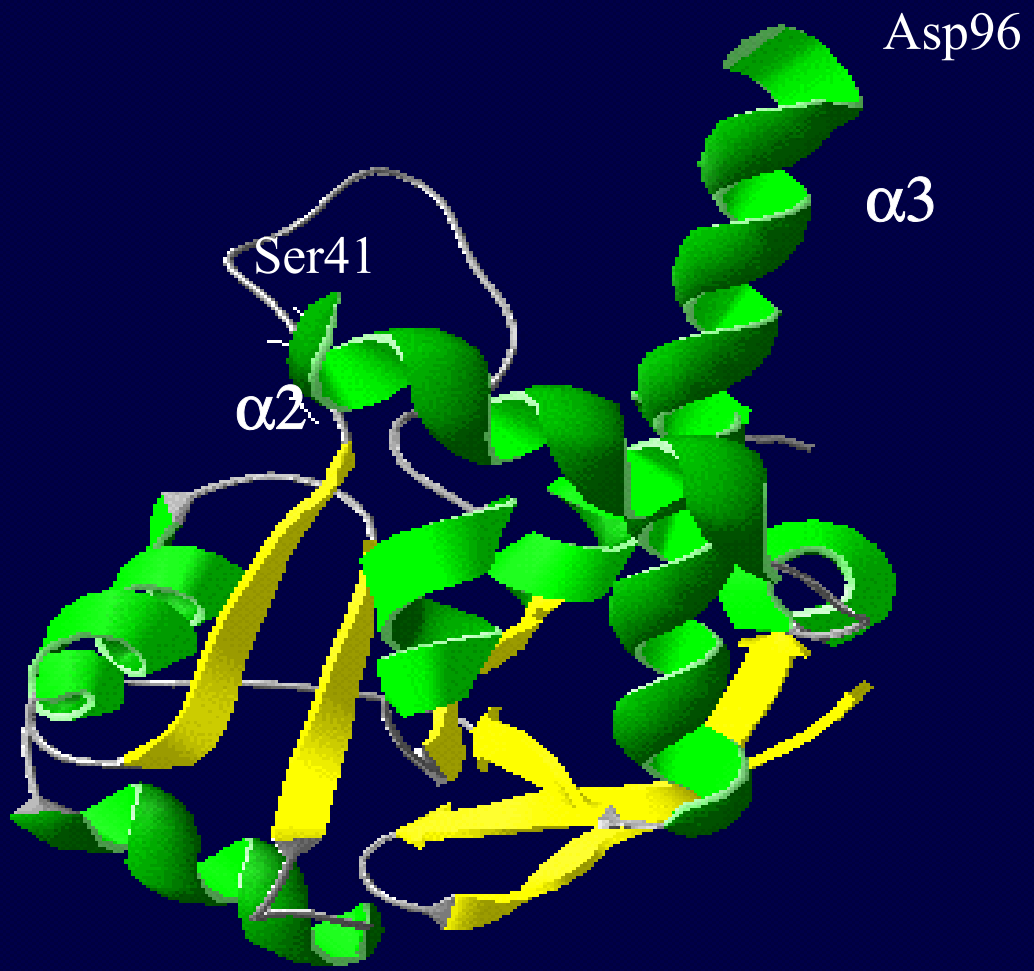
[a] Tait-Kamradt et al. Antimicrob Agents Chemother, 2000

[b] Tait-Kamradt et al. Antimicrob Agents Chemother, 2000

[c] Malbruny et al., ICAAC 2001

[d] Canu et al., ICAAC 2001
Pihlajamaki, M., et al. Antimicrob. Agents Chemother 2002

Ribbon diagram of *Thermotoga maritima* L4 protein



Worbs et al. EMBO J, 2000, 19, 807-16.

High incidence of macrolide resistance in *S. aureus* from cystic fibrosis (CF) patients

- Increase in the incidence of macrolide resistance in *S. aureus* strains isolated from CF patients in our hospital (15 to 31 children/adults each year)
  - 26.7% in 1997
  - 54.8 % in 1998
  - 72% in 1999

Azithromycin 10 vs 20 mg/kg/d for 3 days in acute GAS pharyngitis (1)

- Inclusion
  - Children between 2-12 years of age
  - Acute tonsillopharyngitis
  - Positive card test for streptococcal antigen confirmed by positive throat culture for GAS
- Exclusion
  - Antibiotics within 8 days
  - Hypersensitivity to beta-lactams or macrolides, severe underlying disease, previous inclusion, symptoms suggestive of viral infection
- Treatment
  - Azithromycin 10 mg/kg/d vs 20 mg/kg/d; 3 days (double-blinded)
  - Penicillin V 45 mg/kg/d; 10 days (open)
  - Clinical and bacteriological follow-up: days 14 and 30

Cohen R et al. Pediatr Infect Dis J. 2002;21:297-303

Outcome on day 14 (ITT-assessable patients)

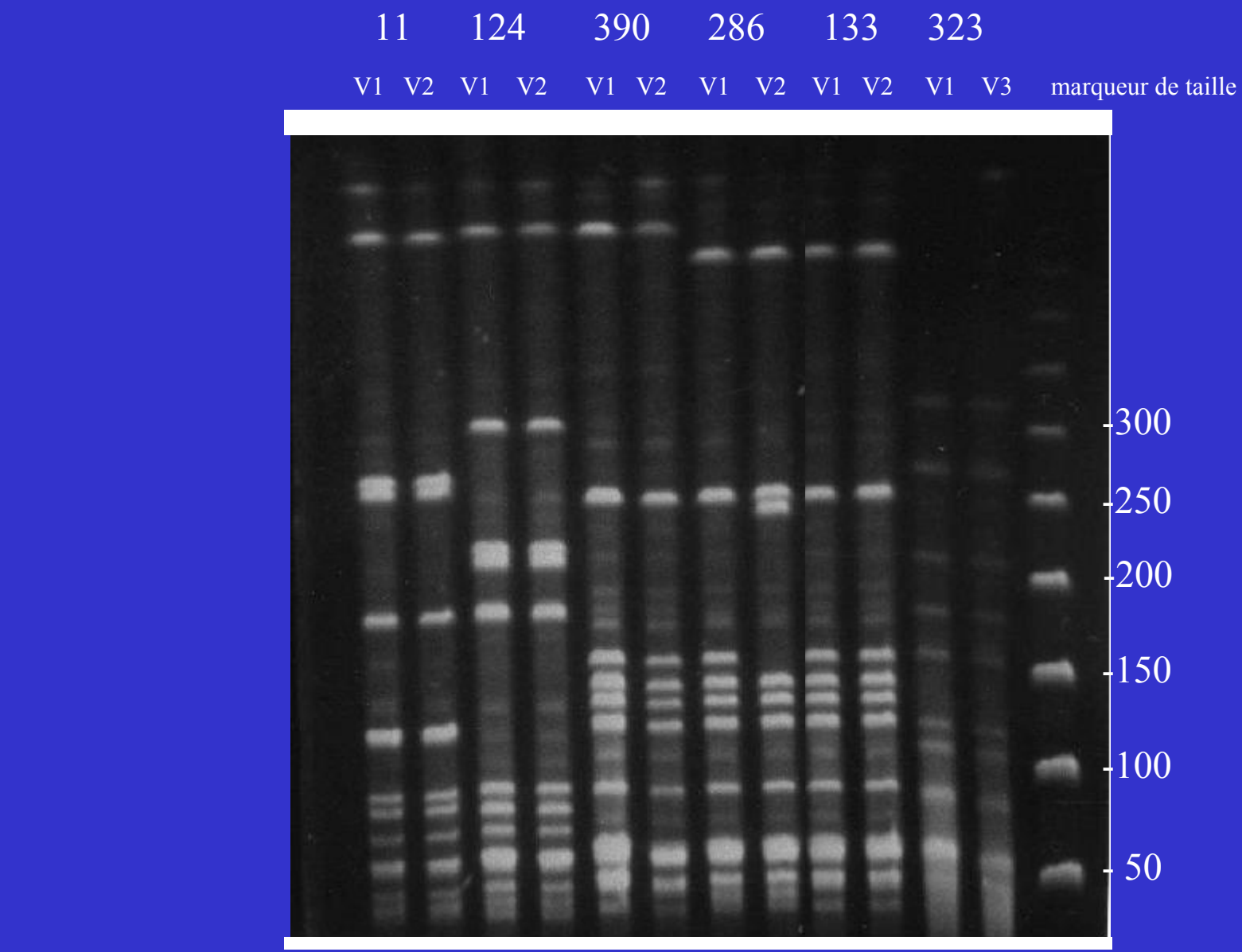
	AZM 10 mg/kg/d	AZM 20 mg/kg/d	Penicillin V
Microbiological outcome			
Total no of patients	158	157	154
No of failures (%)	79 (50)	22 (14)	27 (17.5)
Clinical outcome			
Total no of patients	169	165	167
No of failures (%)	28 (16.6)	14 (8.5)	12 (7.2)

Pre- and post-treatment GAS isolates

Isolate	MIC of (mg/L)			Mechanism of R
	Ery	Azi	Jos	
Pre-treatment strains	**0.064**	**0.12 -0.25**	**0.5**	**None**
Post-treatment Strains				
11 V2	**0.5**	**2**	**1**	**L4 mutation**
124 V2	**0.5**	**1**	**1**	**L4 mutation**
390 V2	**1**	**2**	**2**	**L4 mutation**
286 V2	**>128**	**>128**	**>128**	***erm*(B)**
133 V2	**8**	**8**	**2**	**unknown**
323 V2	**32**	**>128**	**128**	**unknown**
508	**8**	**8**	**0.5**	***mef*(A)**

All pre- and post-treatment strains were related (PFGE and RAPD).
Strains 286 and 508 acquired a DNA fragment with *erm*(B) and *mef*(A), respectively

Bingen et al. Antimicrob Agents Chemother 2002 ; 46:1199


11
124
390
286
133
323
V1 V2 V1 V2 V1 V2 V1 V2 V1 V2 V1 V3
marqueur de taille
300
250
200
150
100
50
Mécanisme de résistance
L4
L4
L4
erm
?
?

Macrolides in cystic fibrosis

- Macrolides are used in the treatment of pulmonary infections due to *Staphylococcus aureus*

- Non antibiotic use of macrolides
  - Indirect effect at subinhibitory concentrations against *Pseudomonas aeruginosa* (laboratory-based evidence)
  - Anti-inflammatory effects
  - Prophylactic use of azithromycin in our institution since 1997

Macrolide use and emergence of resistance to macrolides

- Among 90 patients consulting at the paediatric department between 1998 and 2001, 25 were colonized with *S. aureus* over a 4-year period. All but three were treated with macrolides (7 received azithromycin prophylaxis, 250 mg bid, 5 days a week, 3 months- >1 year)

Prevalence of *erm* and *msr*(A) genes in *Staphylococcus aureus*

- 143 *S. aureus* clinical isolates resistant to erythromycin
  - 102 *erm*(A)
  - 37 *erm*(C)
  - 1 *erm*(B)
  - 3 *msr*(A)

→ Presence of *erm* or *msr*(A) genes accounts for erythromycin resistance in all strains

Lina et al., Antimicrob Agents Chemother 1999, 43, 1062-6

Staphylococcal strains

- 9 CF patients with long-term (>18 months) colonization with *S. aureus*
  - 7 received azithromycin prophylaxis --> emergence of resistance in 3 of 4 patients for whom an initial S strain was available.

- Strains
  - 24 *S. aureus* strains
  - 20 macrolide-resistant strains and 4 macrolide- susceptible strains.

- Pulsed field gel electrophoresis
    - 4 patients were colonized over 18 months by persistent strains.

erm, *msr*(A) genes and ribosomal mutations in 20 erythromycin-resistant strains

Patient (strain)	Resistance genes	Type or number of mutations			
		Domain V	Domain II	L4	L22
1 (A)	-	A2058T	-	-	-
1 (B)	-	A2059G	-	-	-
1 (C), 1(D)	-	A2058G	-	-	-
2 (B)	*erm*	-	-	-	-
2 (C)	-	A2058G	-	-	-
3 (A)	*erm*	4	8	6	-
3 (C)	*erm*	16	-	-	-
3 (D)	*erm*	-	-	-	-
4 (A), 4 (B)	*msr* (A)	T2089C, C2207T	-	-	-
5 (B)	*erm*	C2163T	7	14	1
5 (C), 5 (D), 5 (E)	-	A2058G C2207T	-	-	-
6 (B)	-	A2058G +3 others	-	-	-
6(C)	-	A2058G	-	-	1
7	-	-	-	2	-
8	-	A2059G	-	-	-
9	-	A2058G	-	-	-

Number of *rrl* gene mutated copies

- ***S. aureus*** **may have 6 (*S. aureus* Mu50) or 5 copies (*S. aureus* N315) of the *rrl* gene**

- **Amplification and sequence of each copy of the *rrl* genes in four strains**
  - **1C and 7H** **4 mutated copies /5 (A2058G)**
  - **1D** **4 mutated copies /5 (A2058U)**
  - **8** **3 mutated copies/5 (A2059G)**

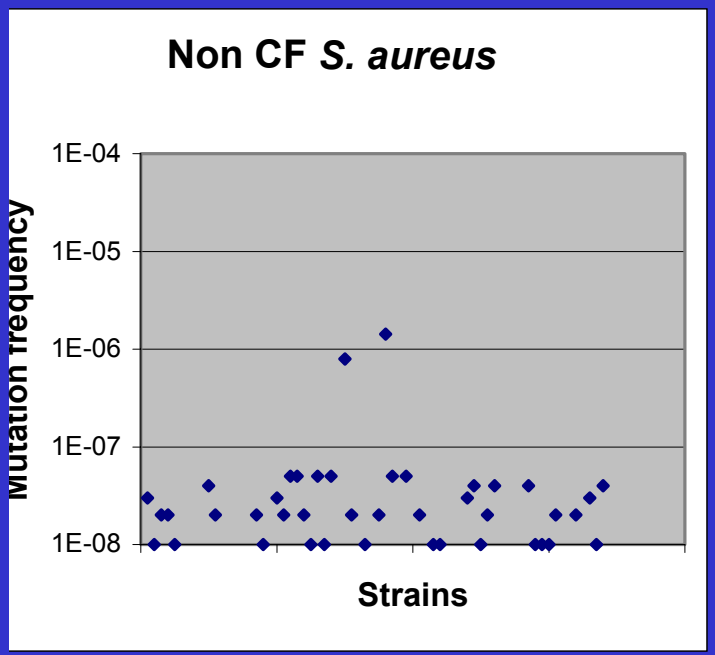
Accumulation of mutations in *S. aureus* from CF patients

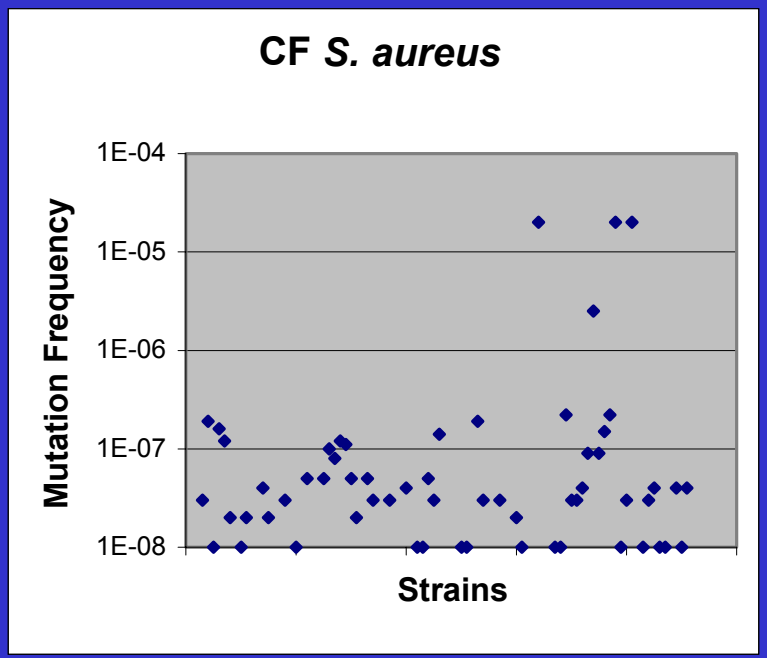
- Unusual mechanism of resistance to macrolides
- Several silent mutations which did not confer any advantage in terms of resistance
- Hypermutable *S. aureus* in CF patients as shown for *P. aeruginosa* (Oliver et al., Science, 2000, 288:1251-3) ?

Hypermutable phenotype

- Screening for hypermutable phenotype
  - 91 strains in 25 CF patients (various phenotypes)
  - 71 strains in non CF patients [isolates from broncho-pulmonary secretions (n=51) and blood (n=20)]
  - Determination of mutation frequencies for rifampin (100 mg/L)
  - Inoculum: 10^8; in triplicate
  - Control with streptomycin for hypermutable strains

Frequencies of rifampin resistance mutations





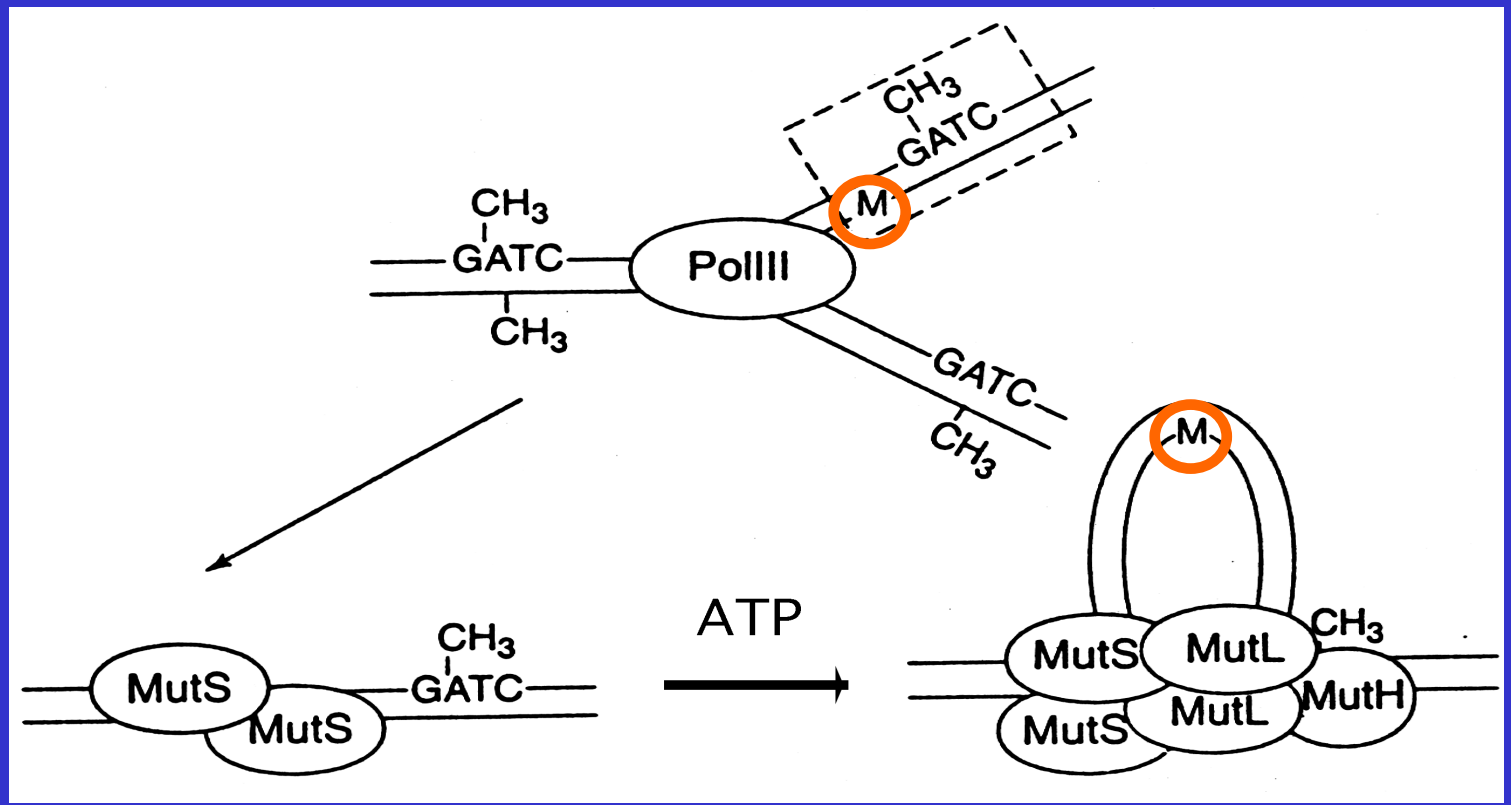
2/71 displayed a mutation frequency > 10^{-7}.

15/91 strains displayed a mutation frequency > 10^{-7}.

$p<0.025$

Results confirmed for hypermutable strains with streptomycin (11 vs 1, $p<0.01$)

Properties of MutS, MutL and MutH proteins: *dam*-directed mismatch repair



From Horst et al, Trends in Microbiology, 1999, 7:29-36

mutS in *S. aureus*

- **Identification of the *mutS* gene**
  - **Identified on the basis of homology of *mutS* from *E. coli* with sequences from *S. aureus* COL, *S. aureus* Mu50 and *S. aureus* N315**
  - **MutS highly conserved**

- **Analysis of *mutS* in 1 hypermutable strain and 7 strains with numerous mutations (5 patients).**
  - **One strain: large deletion (41 aa)**
  - **Five strains: several mutations (unknown relevance)**
  - **By PCR, 2 strains lacked *mutS*.**

Conclusion

- **Macrolide resistance in *S. aureus* isolated from CF patients is mostly due to ribosomal mutations**

- **Emergence of resistance related to the presence of hypermutable strains + azithromycin prophylaxis (and curative treatment)**

- **Hypermutable strains may have an advantage over other strains: easy adaptation to the pulmonary environment in CF patients (*P. aeruginosa*, Oliver et al., Science, 2000)**

Diversity of macrolide resistance types

- In recent years, both the incidence of macrolide resistance in Gram positive organisms and the variety of resistance mechanism have increased sharply.

- It is conceivable that the use of new long-acting macrolides with different pharmacokinetics may have contributed to modulation of the selective pressure exerted against pneumococci and to the selection of new resistance genotypes