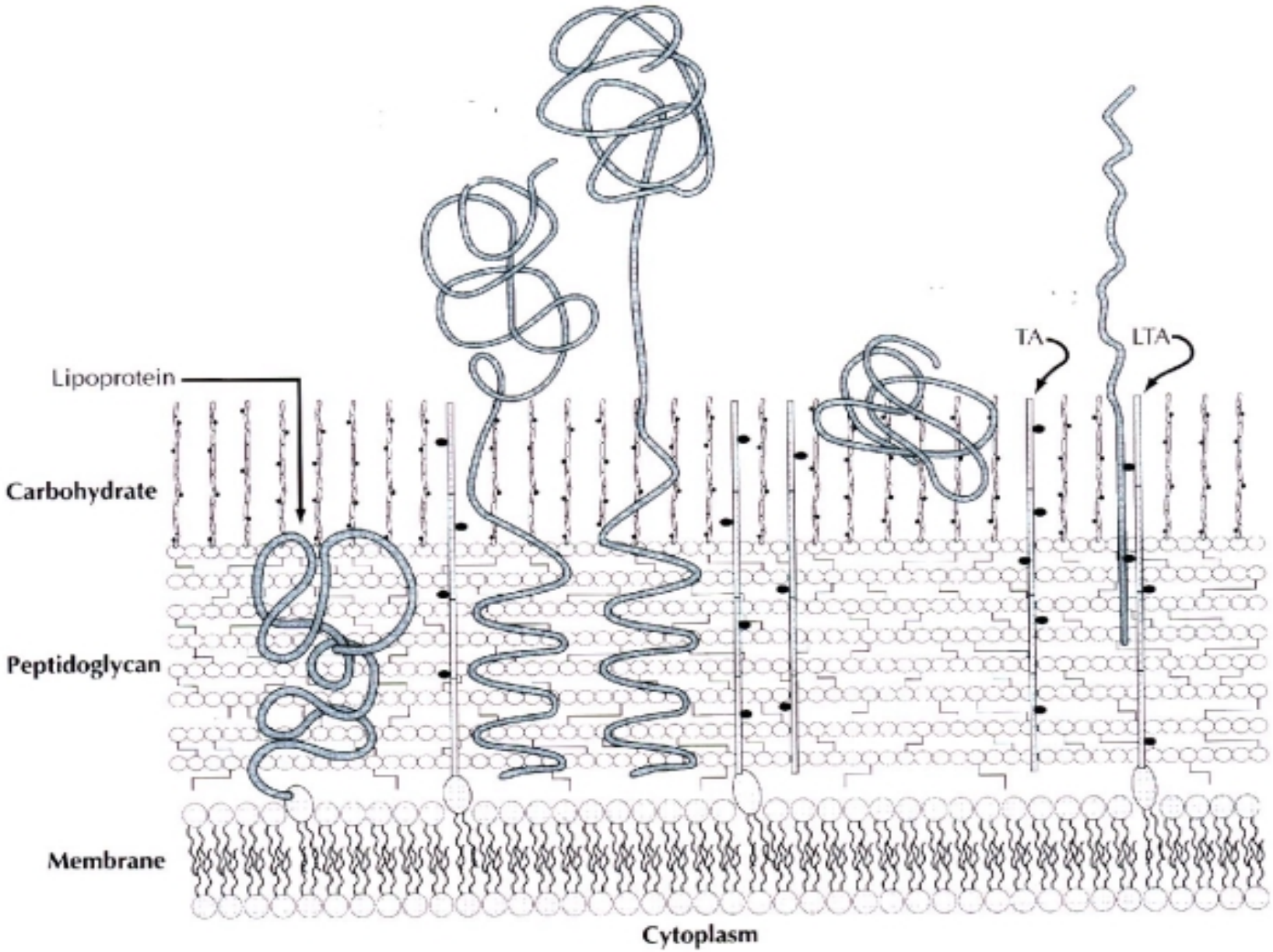
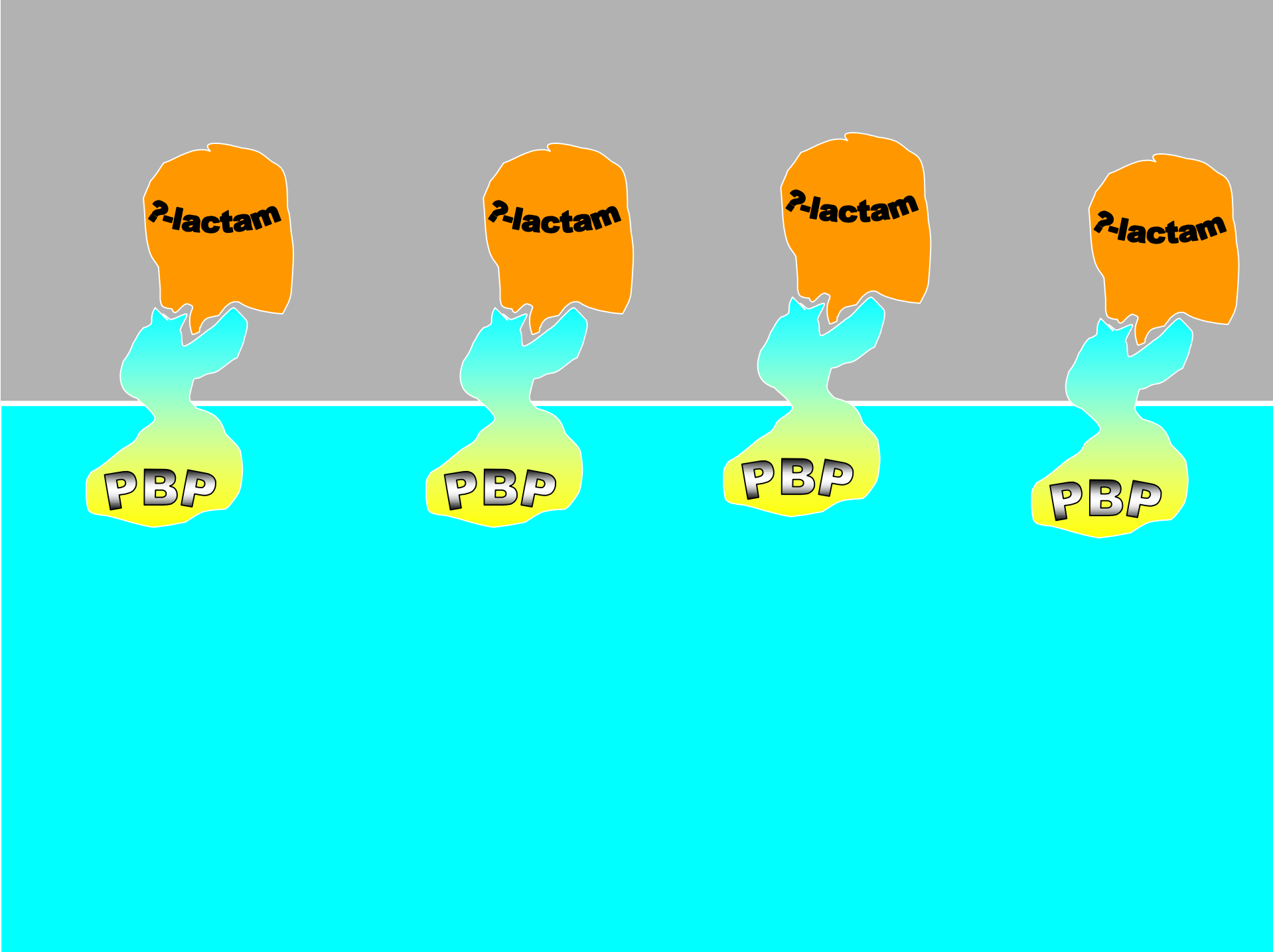
Emerging Resistance in *Streptococcus pneumoniae*

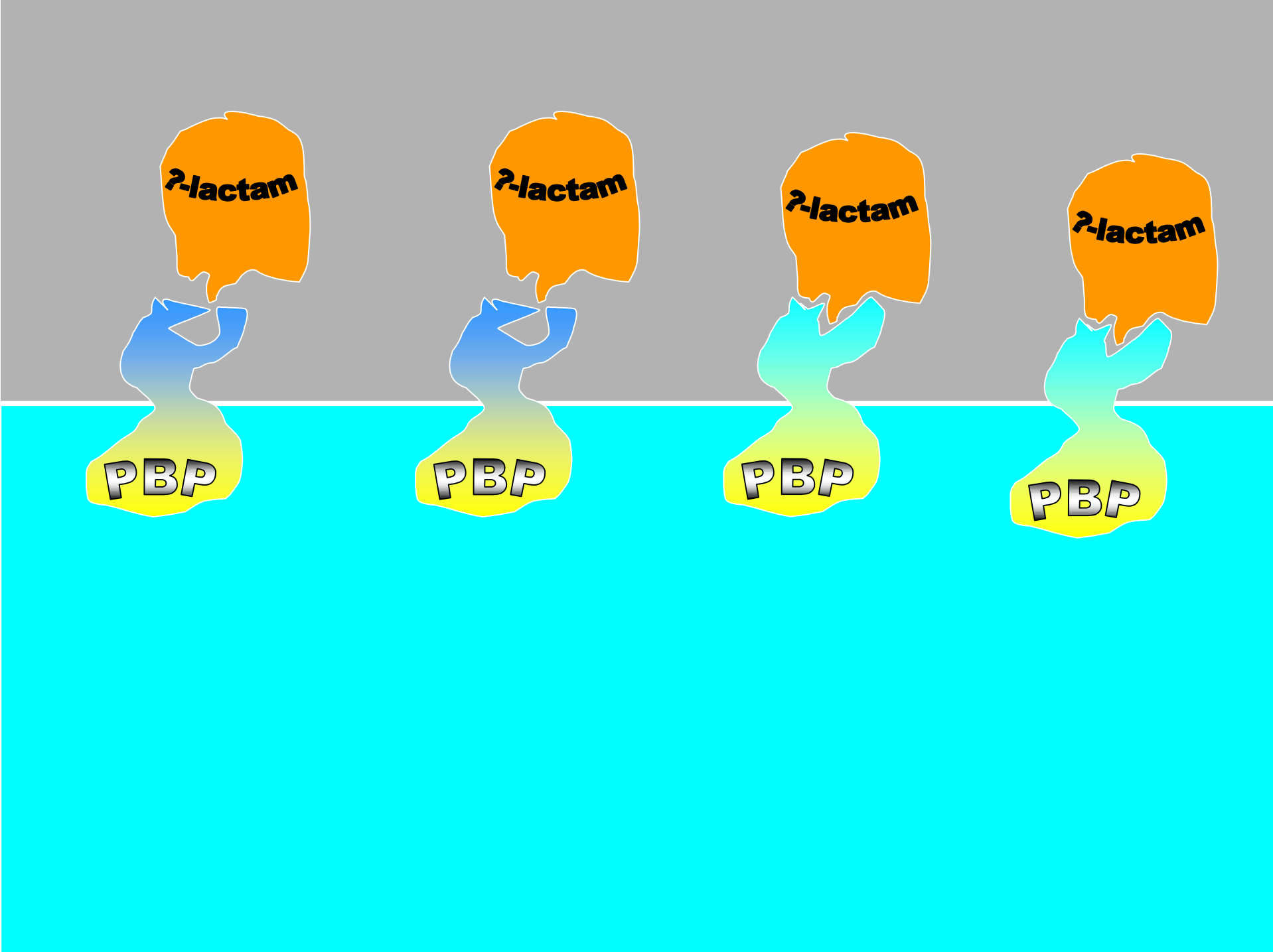
Antimicrobial Resistance Mechanisms

- β-Lactams
- Macrolides
- Quinolones

β-Lactams


Lipoprotein
Carbohydrate
Peptidoglycan
Membrane
TA
LTA
Cytoplasm


β-lactam
β-lactam
β-lactam
β-lactam
PBP
PBP
PBP
PBP


β-lactam
β-lactam
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PBP
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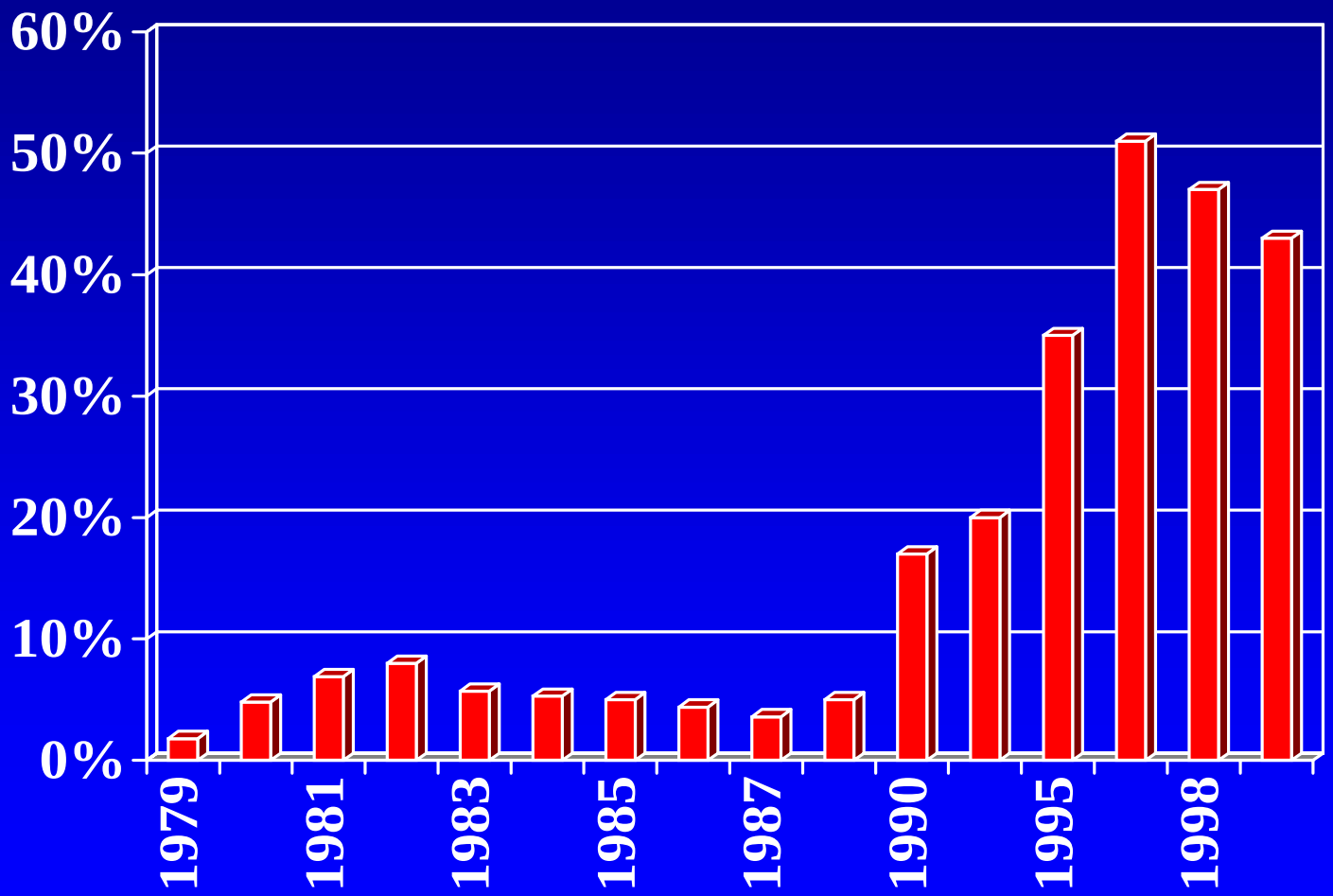
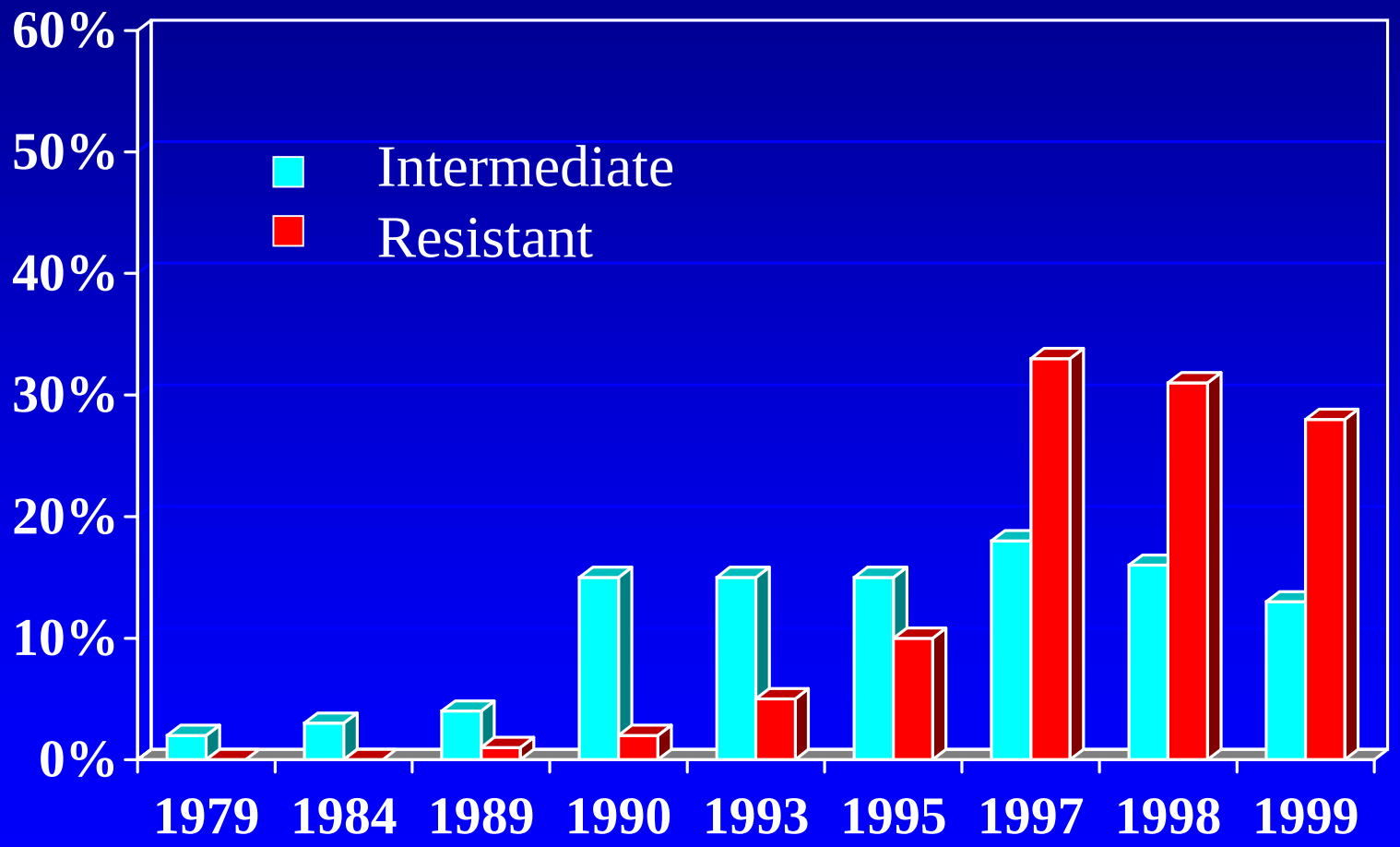
The Emergence of Penicillin Non-Susceptible Pneumococci in the US



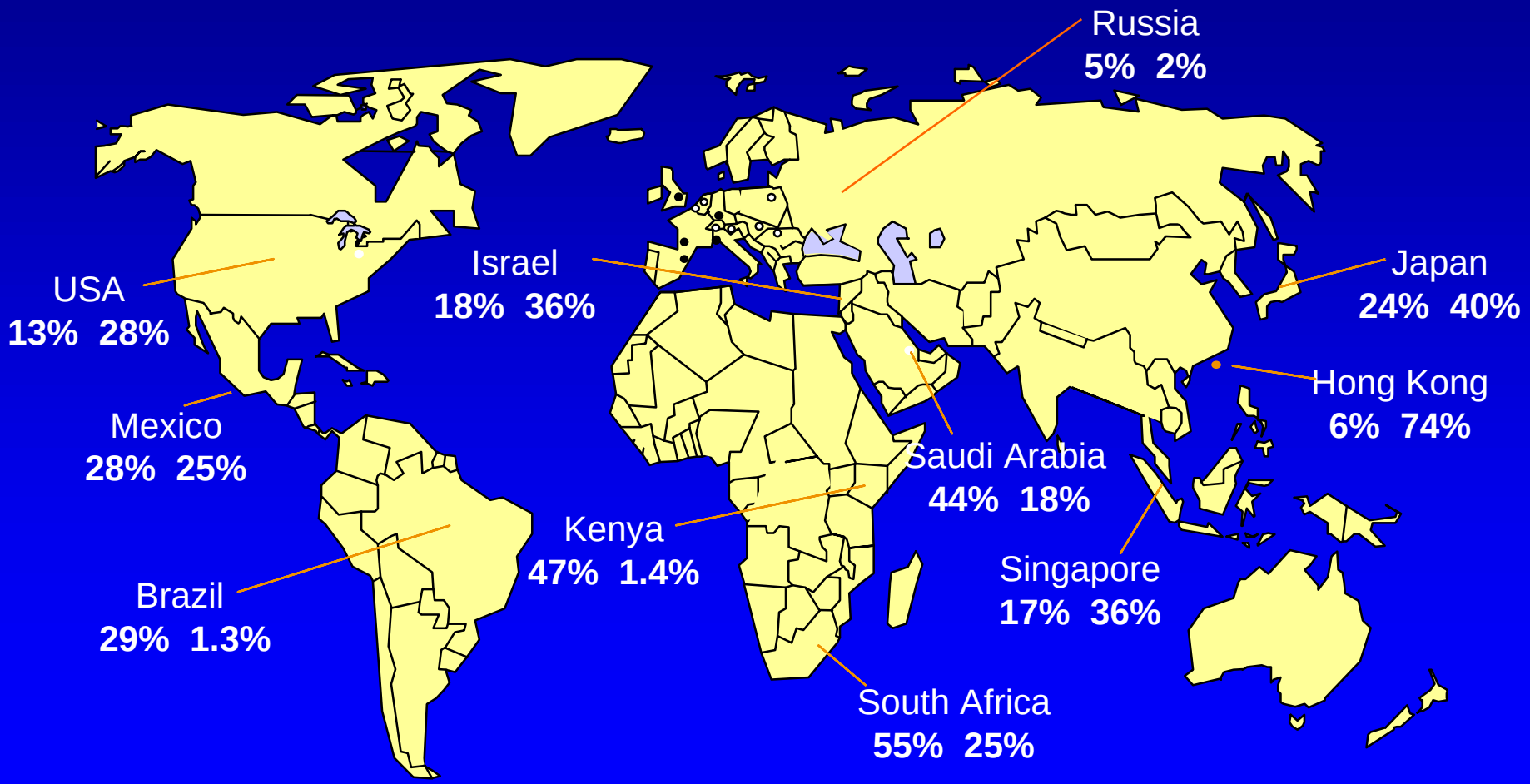
Assessing resistance and mortality

- For β-lactams, resistance is gradual - isolates that are “non-susceptible” likely still respond to therapy, but highly resistant less likely
- supported by PK/PD data that would argue for a penicillin resistant breakpoint of ≥4 μg/ml rather then ≥2 μg/ml

The Emergence of Penicillin Non-Susceptible Pneumococci in the US



The Alexander Project 1999: *S. pneumoniae*, Pen-I and Pen-R



Logistic regression analysis of penicillin resistance and mortality, invasive pneumococcal pneumonia

Pen MIC	Risk of death	Risk of death after 4th hospital day
≥4	2.3	7.1*
2.0	1.3	0.65
0.12-1.0	1.4	1.0
<0.12	Ref	Ref

Feikin et al., Am J Public Health 2000

Impact of penicillin susceptibility on medical outcomes for adult patients with bacteremic pneumococcal pneumonia

Metlay et al., Clin Infect Dis 2000

- retrospective population based study in Atlanta
- 192 patients infected with pneumococci, 44 (23%) infected with PNSP
- Compared to PSP

In-hospital medical outcomes for patients with pneumococcal pneumonia, adjusted for severity of illness

Metlay et al., Clin Infect Dis 2000

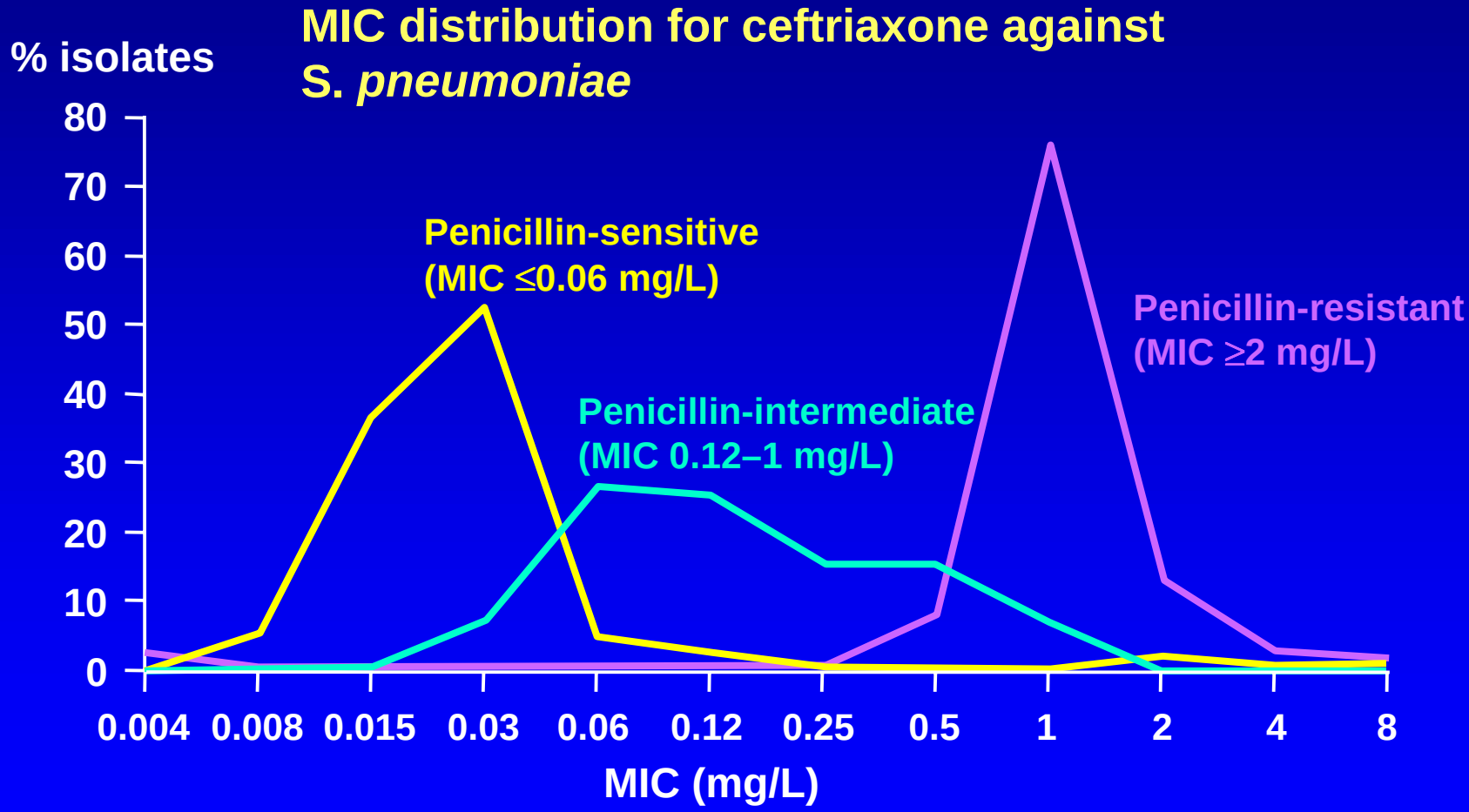
Outcome	Relative risk (Pen NS vs. Pen S)
Death	1.7 (0.8,3.4)
Respiratory failure	1.5 (0.7, 3,1)
Admission to ICU	1 (0.3, 2.9)
Suppurative complications	4.8 (1.2, 18.8)

Proposed NCCLS ceftriaxone/cefotaxime/cefepime susceptibility breakpoint changes

Proposed NCCLS ceftriaxone/cefotaxime/cefepime susceptibility breakpoint changes

Susceptible	≤ 1 μg/ml
Intermediate	2 μg/ml
Resistant	≥ 4 μg/ml

Penicillin-resistant *S. pneumoniae* tend to be resistant to other β-lactams



Goldstein et al. J Antimicrob Chemother 1996;38(Suppl. A):71–84

Distribution of 1707 Pneumococcal MICs in Canada: 2000

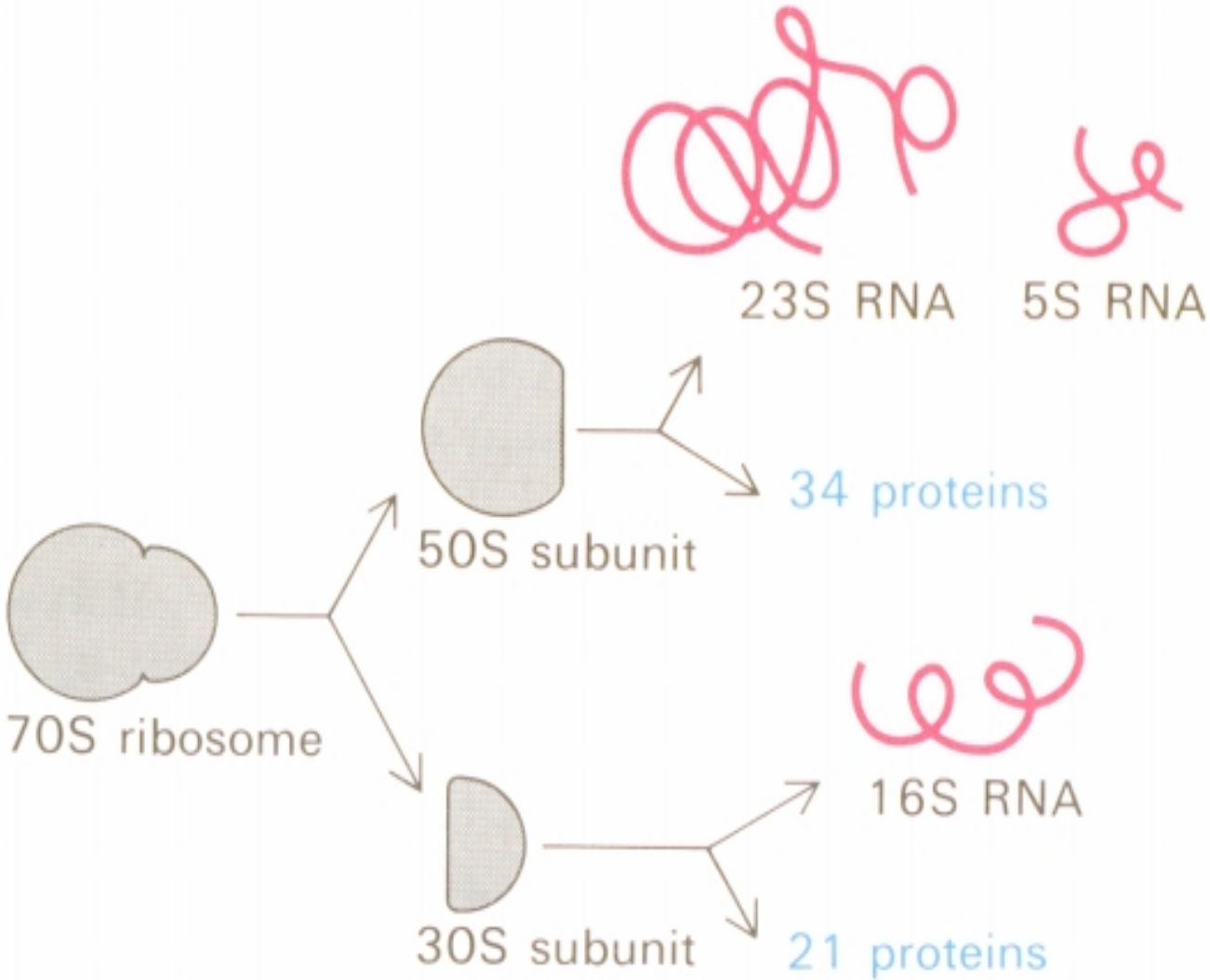
	MIC Range								
	.03	.06	.12	.25	.5	1	2	4	8
Penicillin	1438	45	66	24	14	32	77	17	2
Ceftriaxone	1429	65	46	30	34	80	17	6	0

Intermediate range: Current ..... Proposed ——

Macrolide Resistance

Ribosome

- Ribosomal RNA
- Ribosomal protein


23S RNA
5S RNA
50S subunit
34 proteins
70S ribosome
16S RNA
30S subunit
21 proteins

Mechanism of Action of MLS Antibiotics

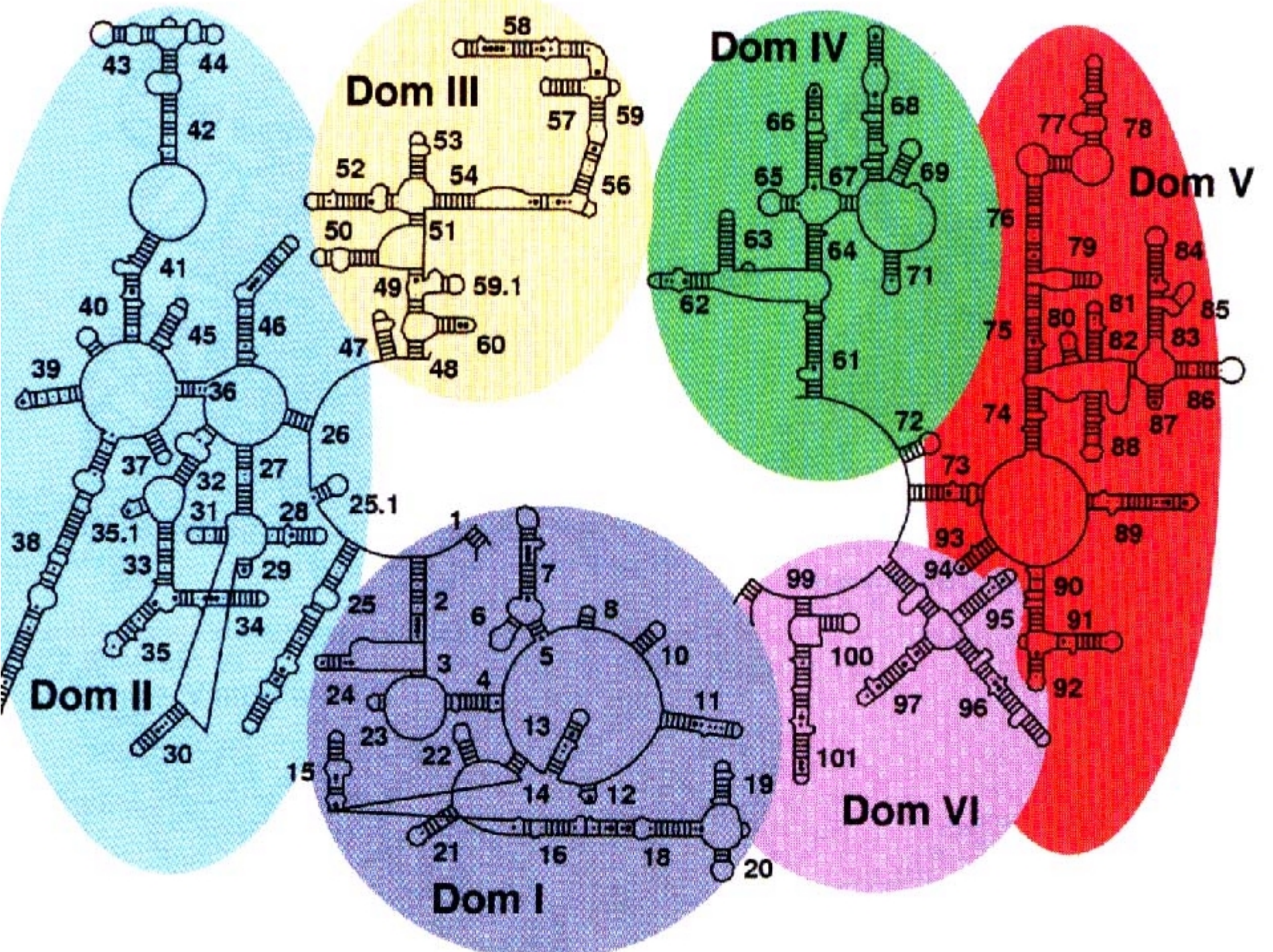
- inhibit protein synthesis by their action on the 23 rRNA of the 50S ribosomal subunit

Mechanisms of Resistance

- Target modification
- Efflux

Target modification: methylation

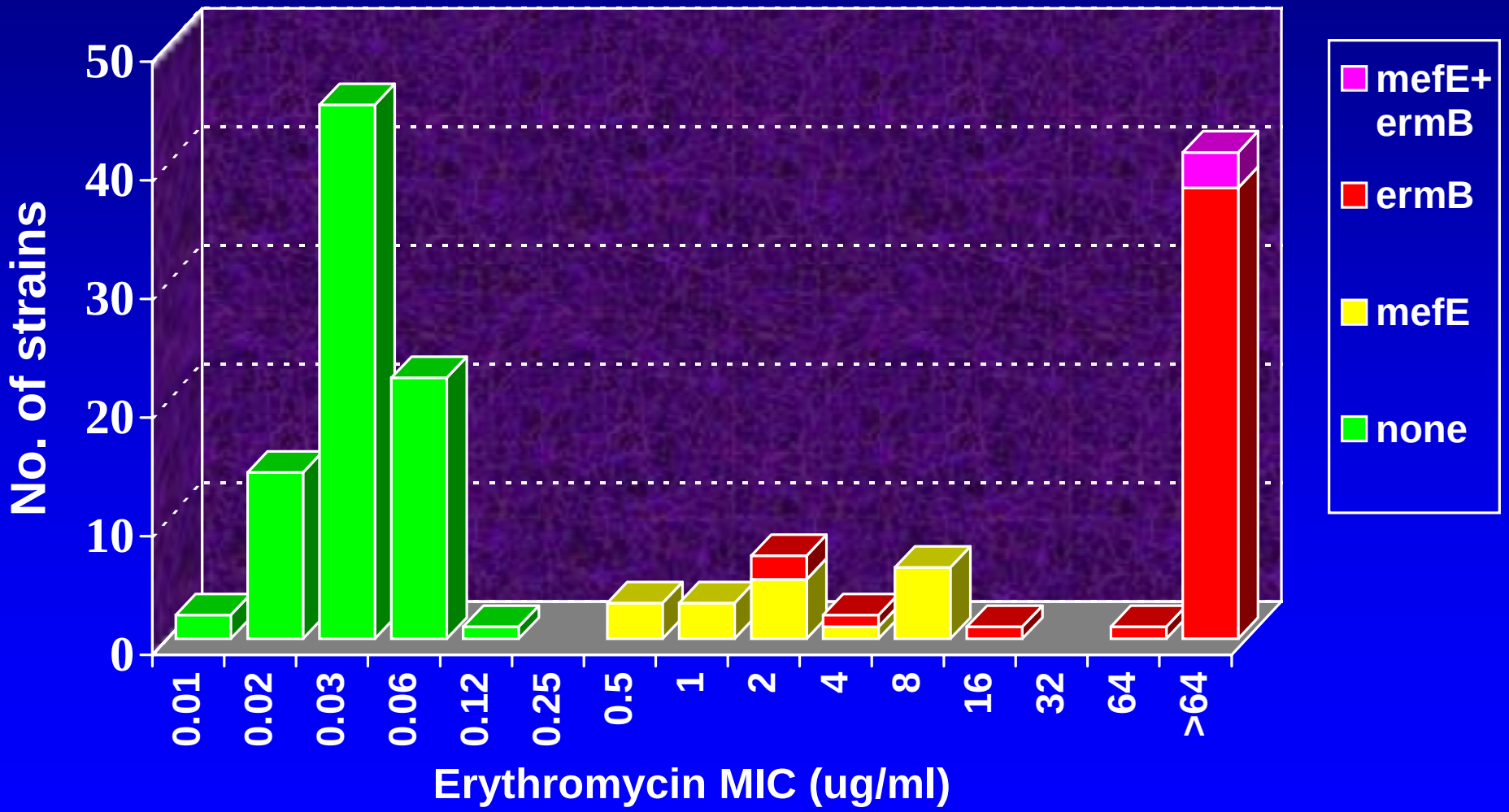
- methylation of A2058 of ribosome
- *ermB*
- produces the MLS_B phenotype
  - macrolide and clindamycin resistance


Dom I
Dom II
Dom III
Dom IV
Dom V
Dom VI

Efflux in *S. pneumoniae*

- *mefA*
- results in the active efflux of 14- and 15-membered macrolides
- produces the M phenotype
  - erythromycin, clarithromycin, and azithromycin resistance
  - clindamycin susceptibility

Correlation between erythromycin MICs and resistance mechanisms

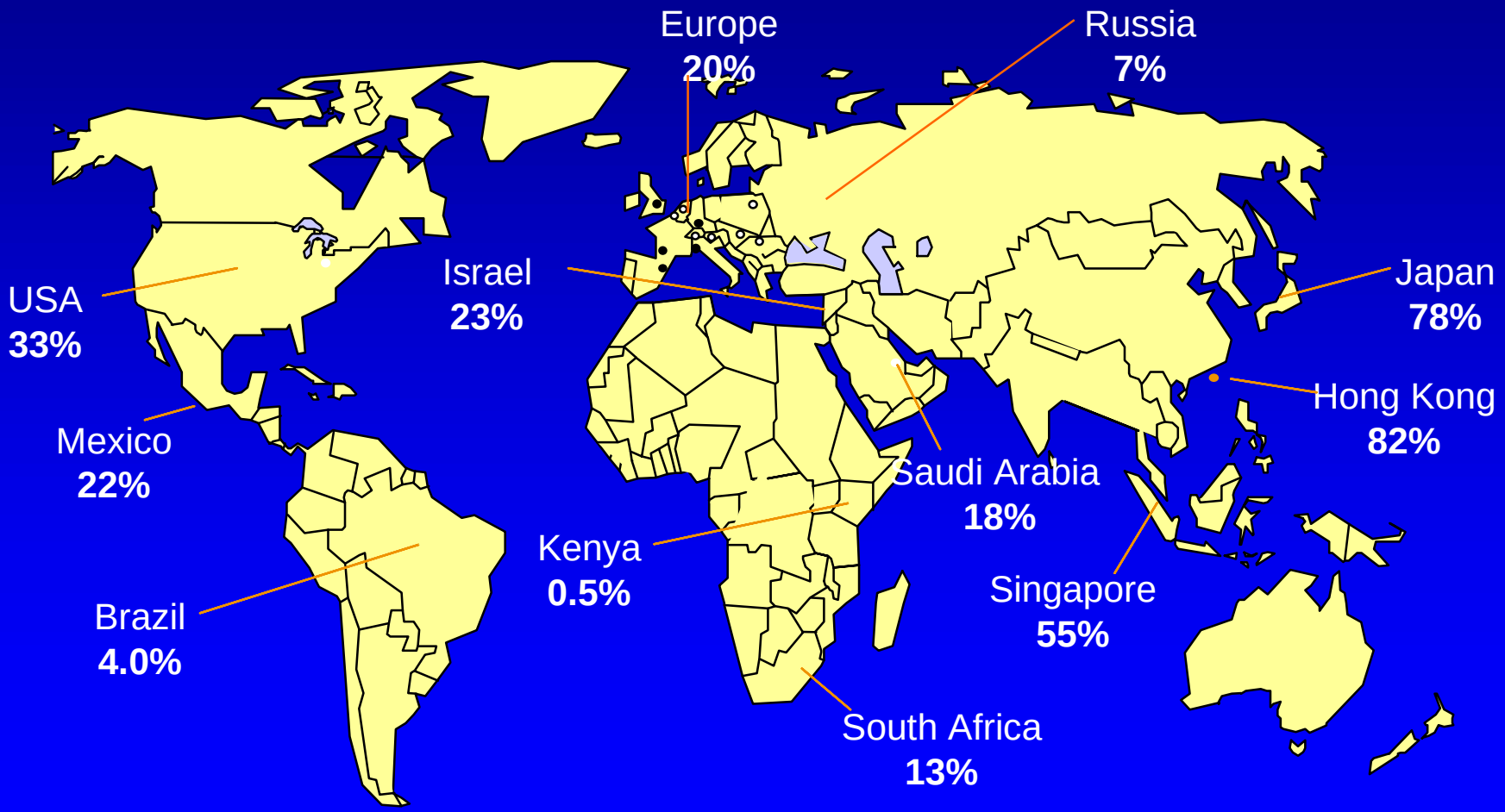


Nagai ICAAC 2000, abstr # 892

Prevalence and mechanisms of macrolide resistance in *S. pneumoniae*

Country	Prevalence of macrolide resistance	Mechanism of resistance	
		Efflux	Target site
US	19%,	70%	30%
Italy	33%,	6%	94%
Far East	80%	50%	50%

The Alexander Project 1999: *S. pneumoniae*, Macrolide Resistance



Resistance defined as erythromycin MIC ≥1mg/L

Why haven't we seen treatment failures in macrolide resistant pneumococci treated with macrolides?

- usually mortality is used as outcome measure
- patients treated with macrolide alone as outpatient, mortality <1%
- sicker patients admitted to hospital treated with combination of cephalosporin and macrolide

Macrolide treatment failures with macrolide resistant *S. pneumoniae*

- 12 patients (7 adults, 5 children) on oral macrolides were hospitalized with bacteremic pneumococcal infections (9 in Spain, 3 in US)
- 11 patients had pneumonia, 1 patient had bacteremia only
- Macrolides being used were: erythromycin (3), azithromycin (4), clarithromycin (3), and josamycin (2)
- 11 of the isolates had *ermB* and 1 *mefE* gene

Garau et al., ICMASK 2000, abstract 7.09

Fluoroquinolones

Topoisomerases: critical enzymes in DNA replication

- topoisomerase IV (***parC***, *parE*)
- DNA gyrase (***gyrA***, *gyrB*)

How do fluoroquinolones work

- DNA first binds to topoisomerases
- Fluoroquinolone traps the topoisomerases/DNA complex
- Cell dies

Cabral *et al.,* Nature, 1997

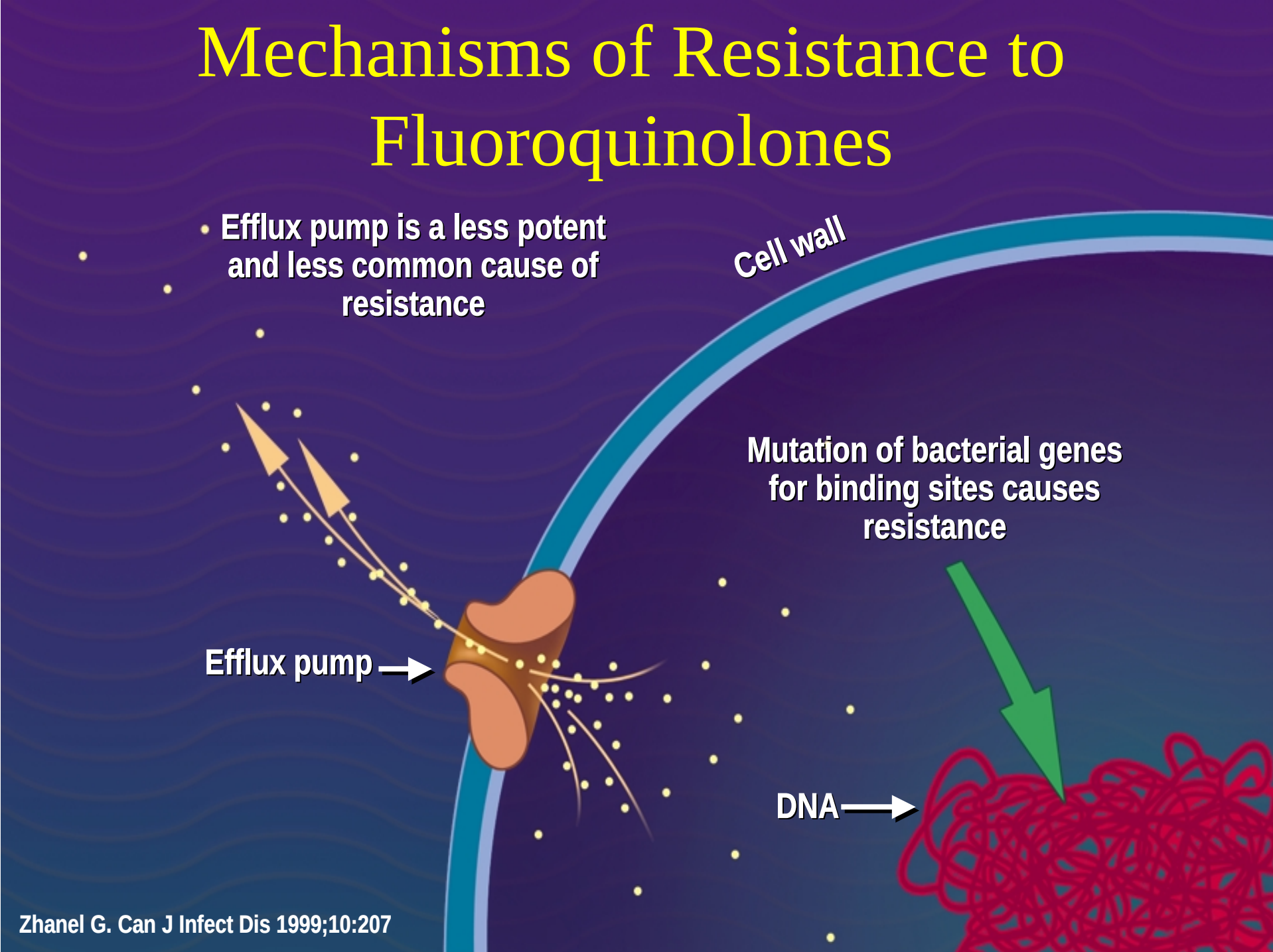
Targets for the fluoroquinolones

- Two targets: GyrA and ParC
- Fluoroquinolones preferentially binds to one of the targets over the other:

Development of Resistance

- De novo
  - up regulation of intrinsic PmrA efflux pump

Mechanisms of Resistance to Fluoroquinolones

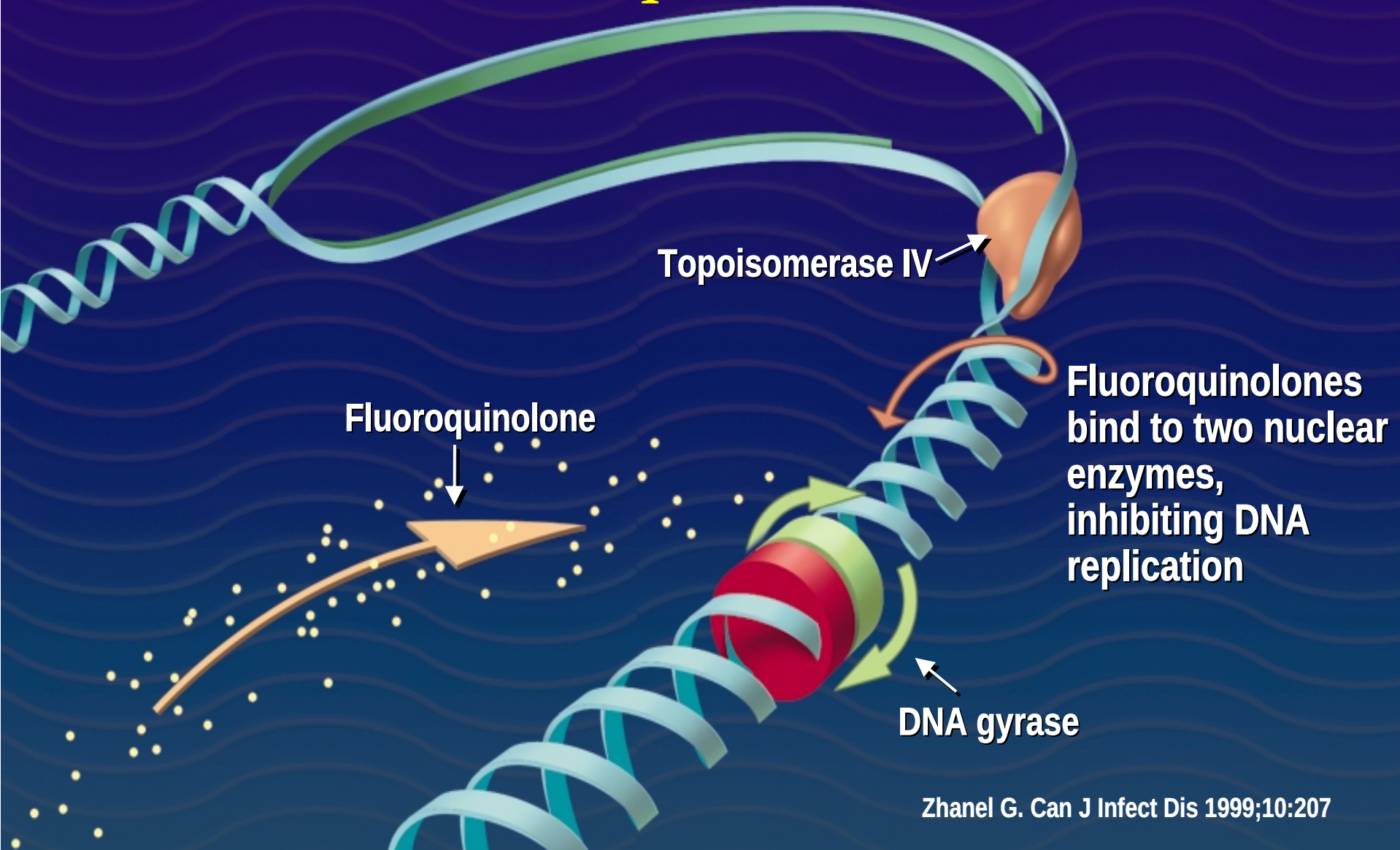


Zhanel G. Can J Infect Dis 1999;10:207

Development of Resistance

- De novo
  - up regulation of intrinsic PmrA efflux pump
  - spontaneous mutations in primary target which lowers the affinity of the fluoroquinolone and increases the MIC: *parC* or *gyrA*

Mechanism of Action of Fluoroquinolones



Zhanel G. Can J Infect Dis 1999;10:207

Spontaneous mutation

- The frequency of a spontaneous mutation to fluoroquinolone resistance in *S. pneumoniae* is $1/10^7$ to 10^8

Burden of pneumococci during infection

Acute exacerbation of chronic bronchitis

- 10^5 CFU during remission
- 10^8 during exacerbation

(Hill)

Pneumonia

- 10^{12} to 10^{14} CFU

(Frisch AW, J Exp Med 1942)

Issues

- Fluoroquinolones are becoming de facto first line antibiotics for treatment of CAP
  - 1 billion $US/year
- CAP guidelines
  - IDSA
  - Canadian
  - ATS

Issues

- Sub-optimal therapy may increase prevalence of resistance and/or lead to clinical failures
  - Marginally effective compounds (PK/PD)
  - Fluoroquinolone active but either not being absorbed or patient non-compliant

Clinical Fluoroquinolone and Oral Cephalosporin Failures

Weiss et al, Clin Infec Dis, In Press

- Within a 2-month period in 1995, 9 patients were infected/colonized with a PRSP on same pulmonary ward
  - **MIC to cipro of 4 μg/ml**
  - all strains were 23F and same PFGE
  - all mutations in same *parC* site

Clinical Fluoroquinolone and Oral Cephalosporin Failures

- Subsequently, there was an additional 7 isolates during 1996 and 1997 on same ward
  - **MIC to cipro 16 μg/ml**
  - each isolate 23F and identical by PFGE
  - all with same mutations in *parC* and now mutation in *gyrA*

Clinical Fluoroquinolone and Oral Cephalosporin Failures

- Of the 16 patients,
  - 13 met criteria for AECB
  - 3 met criteria for pneumonia
- AECB
  - Cefuroxime given to 6, 5 of which failed
  - Ciprofloxacin given to 5, all failed
- HAP
  - 3/3 died

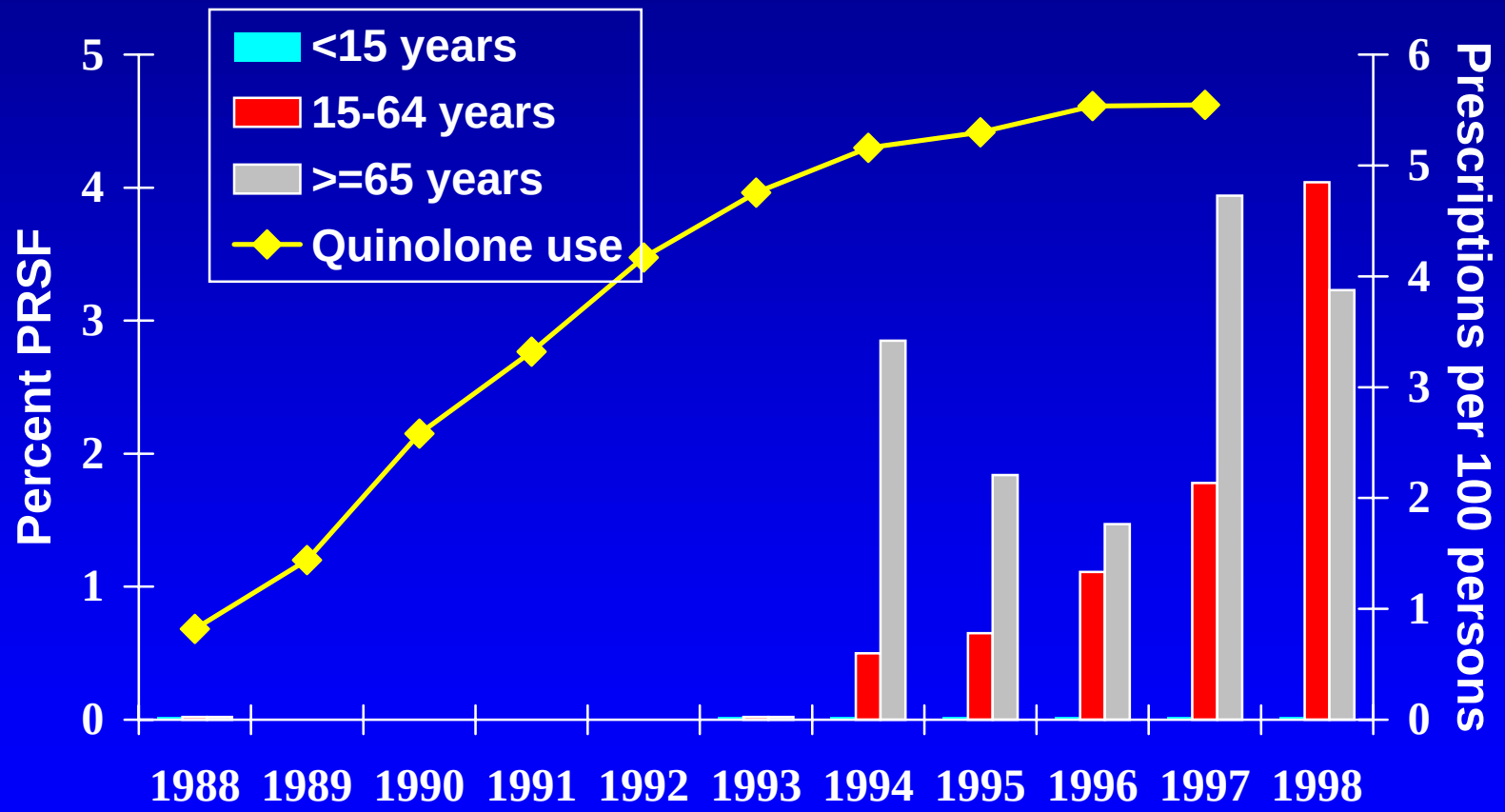
Fluoroquinolone resistance rates in pneumococci

- Spain (Linares et al. NEJM Nov 99)
  - 3% in 1997
- Hong Kong (Ho et al. AAC March 99)
  - 12% in 1998
- N. Ireland (Goldsmith et al. JAC March 98)
  - 15% in isolates isolated between 1994 and 1998

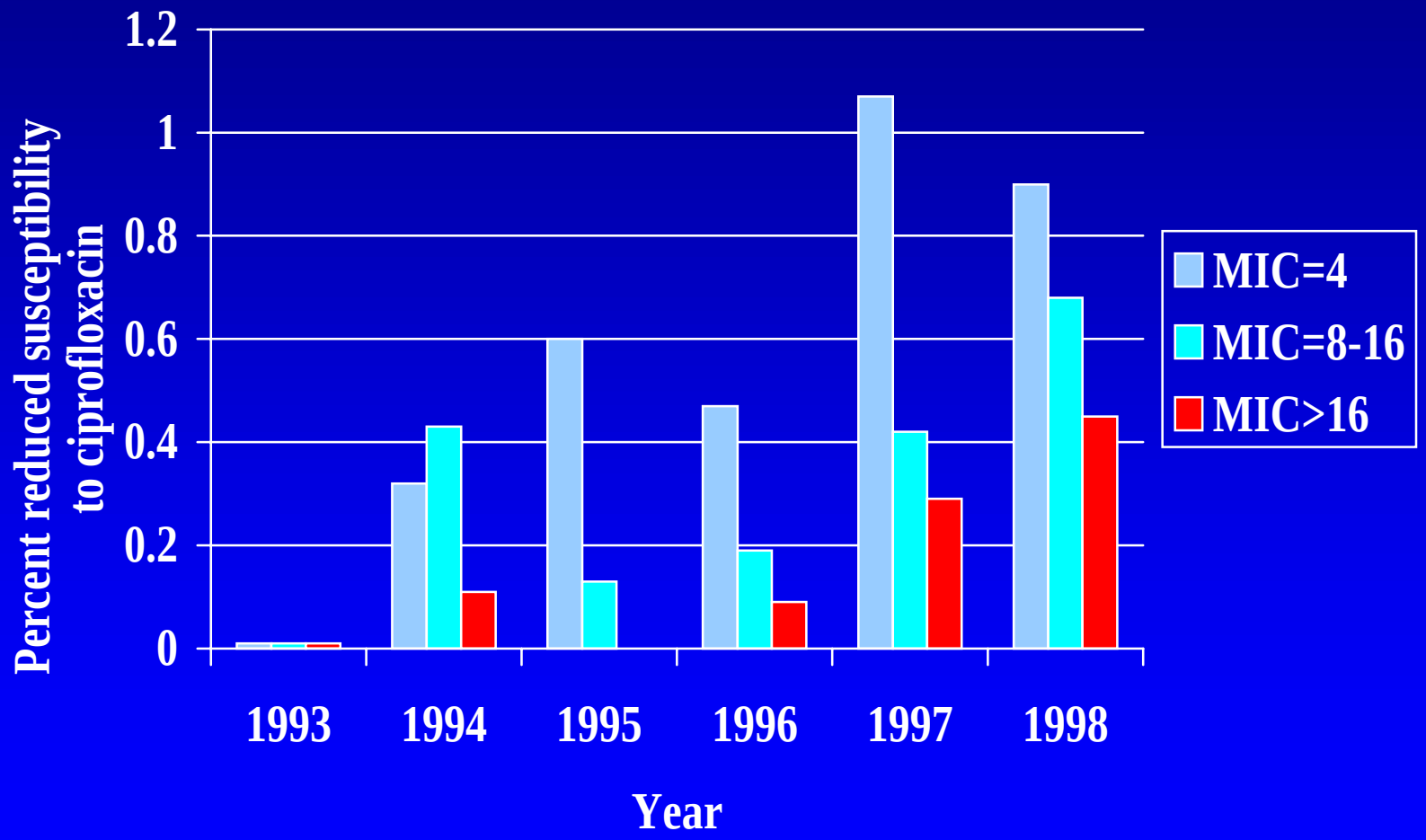
Decreased susceptibility of *S. pneumoniae* to fluoroquinolones in Canada

Chen et al., 1999 NEJM

Fluoroquinolone use and PRSF Canada, 1988-1998



CBSN *S. pneumoniae* MICs for ciprofloxacin, 1993-8



Analysis of ciprofloxacin activity against *S. pneumoniae* after 10 years use in US

Sahm et al., AAC, 2000

- 5,640 isolates collected from 377 geographically distributed US hospitals
- collected over 1997-98 respiratory season
- 0.3% of isolates had cipro MICs of ≥4 μg/ml
- resistance strains significantly associated with:
  - >64 years of age
  - respiratory source
  - penicillin resistance

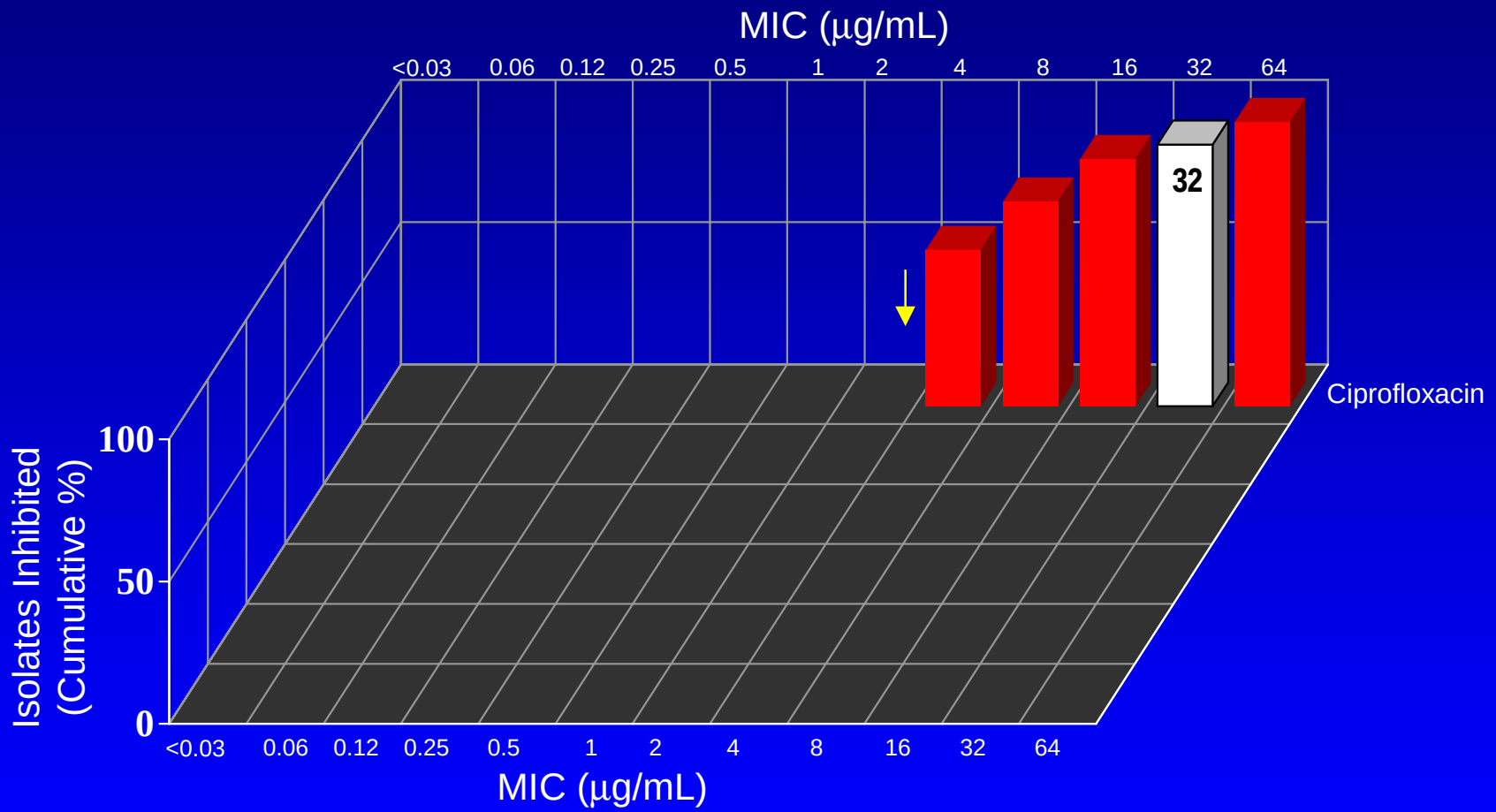
Levofloxacin resistance in pneumococci

	No. of isolates	Percent resistant
United States	172	2.9%
Canada	350	1.43%

PROTEKT 2000

Implications

Quinolone Activity Against Ciprofloxacin-Resistant *S. pneumoniae**



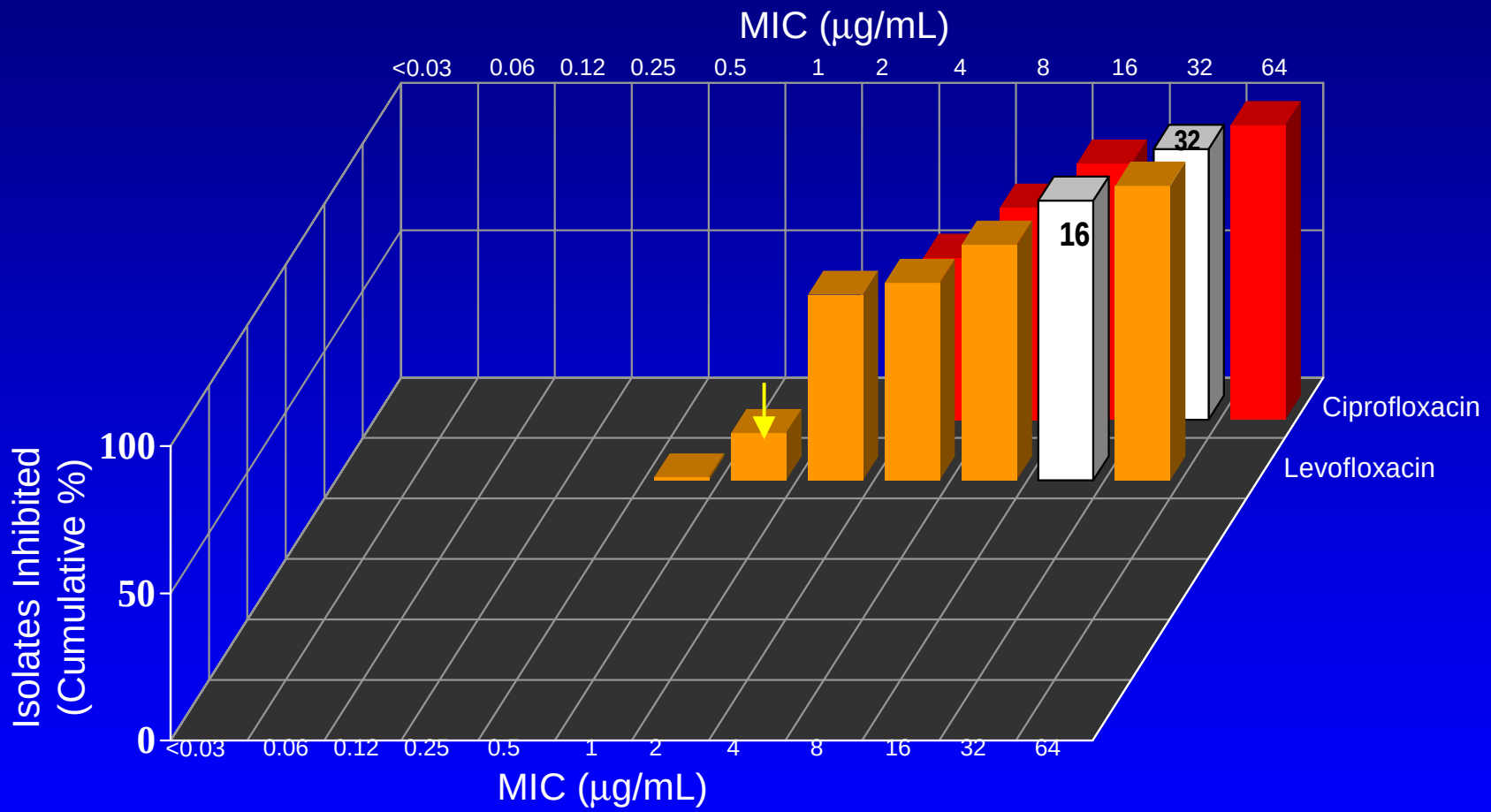
*Resistance to ciprofloxacin defined as ≥4 µg/mL.

Adapted from Chen et al. *N Engl J Med*. 1999.

Yellow arrow = MIC_{90} in wild type strains

White Bar = MIC_{90} in cipro resistant strains

Quinolone Activity Against Ciprofloxacin-Resistant S. pneumoniae*



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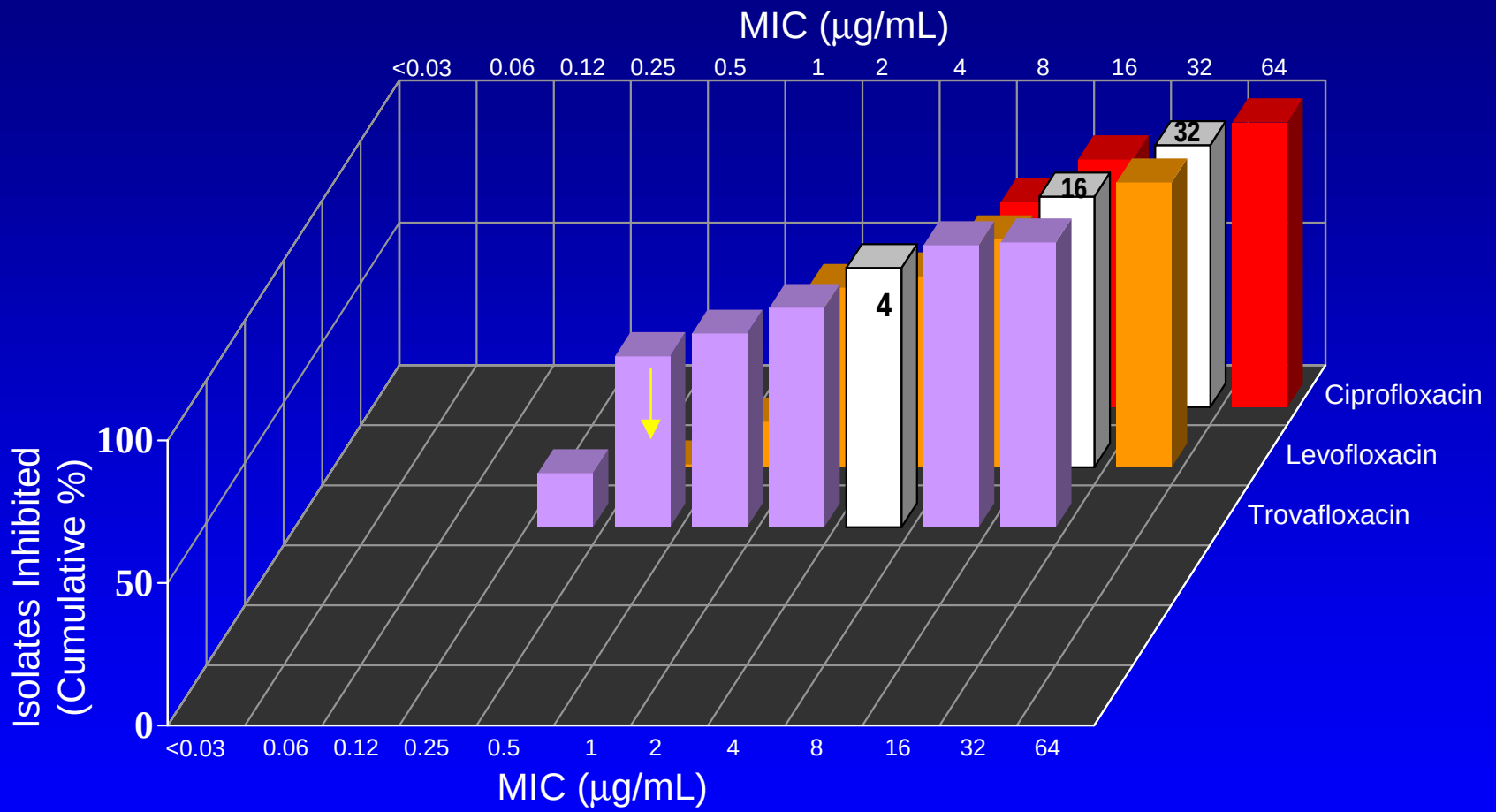

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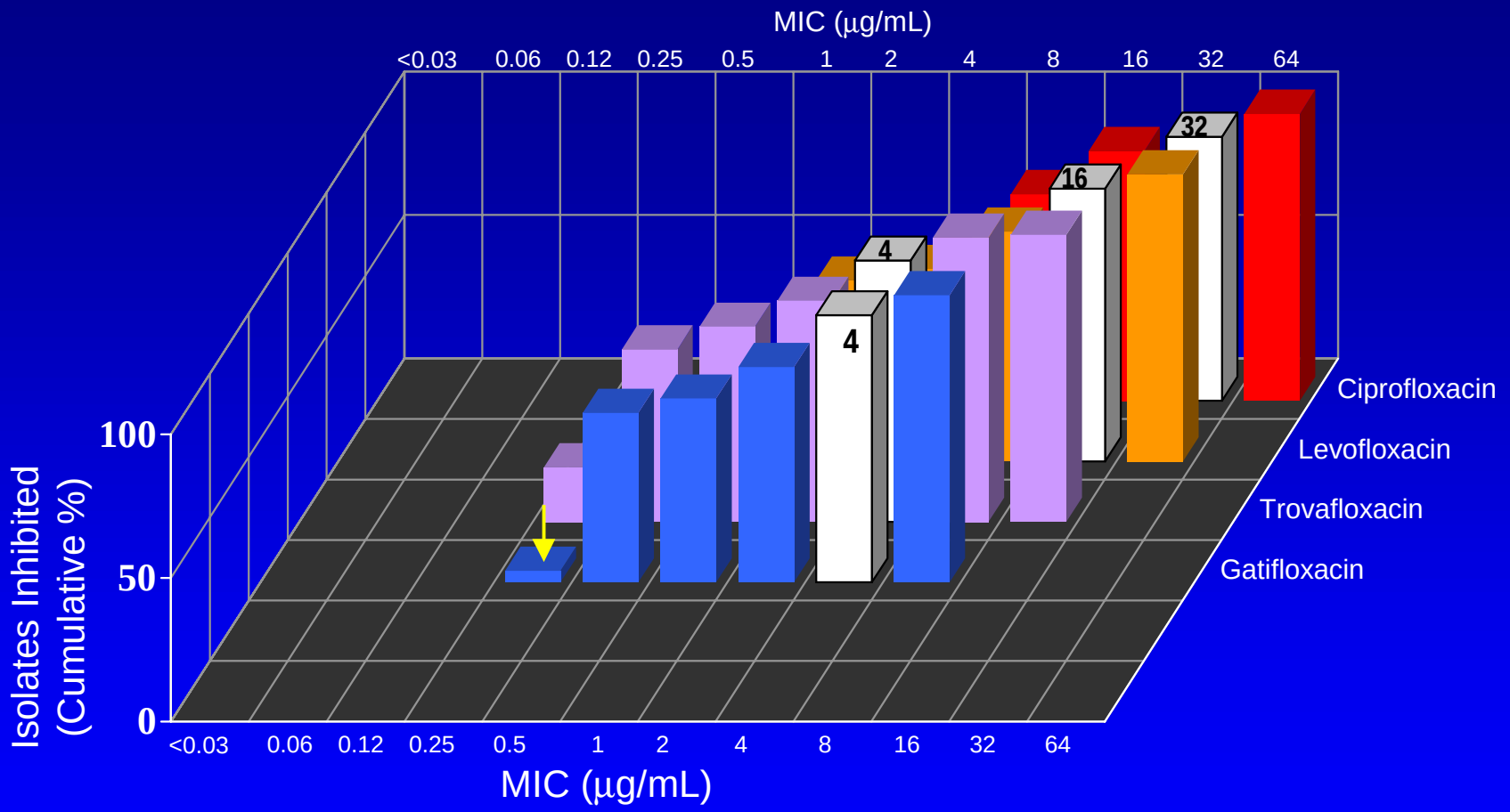

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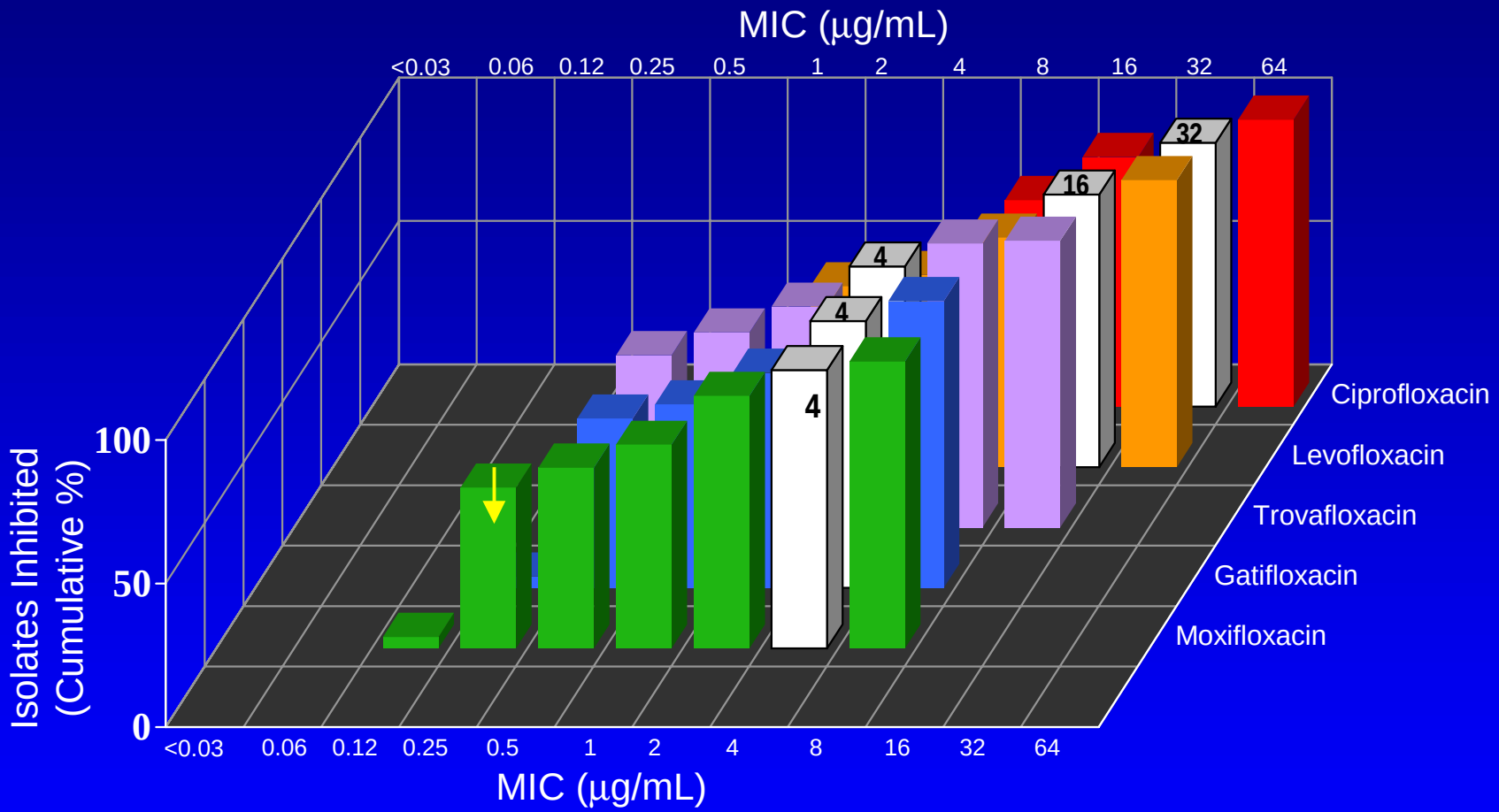

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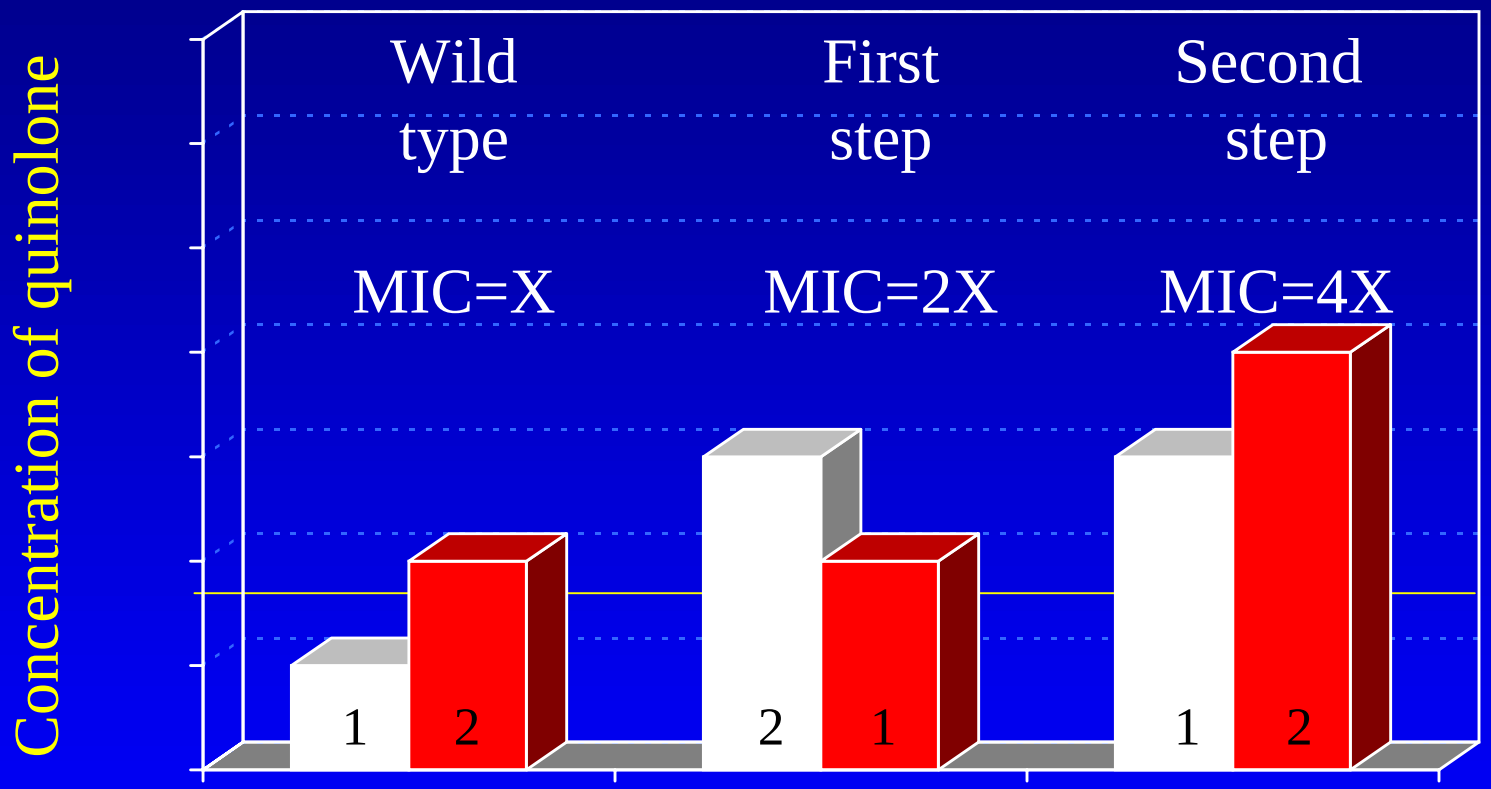
Issues

- Increasing prevalence of first-step mutants

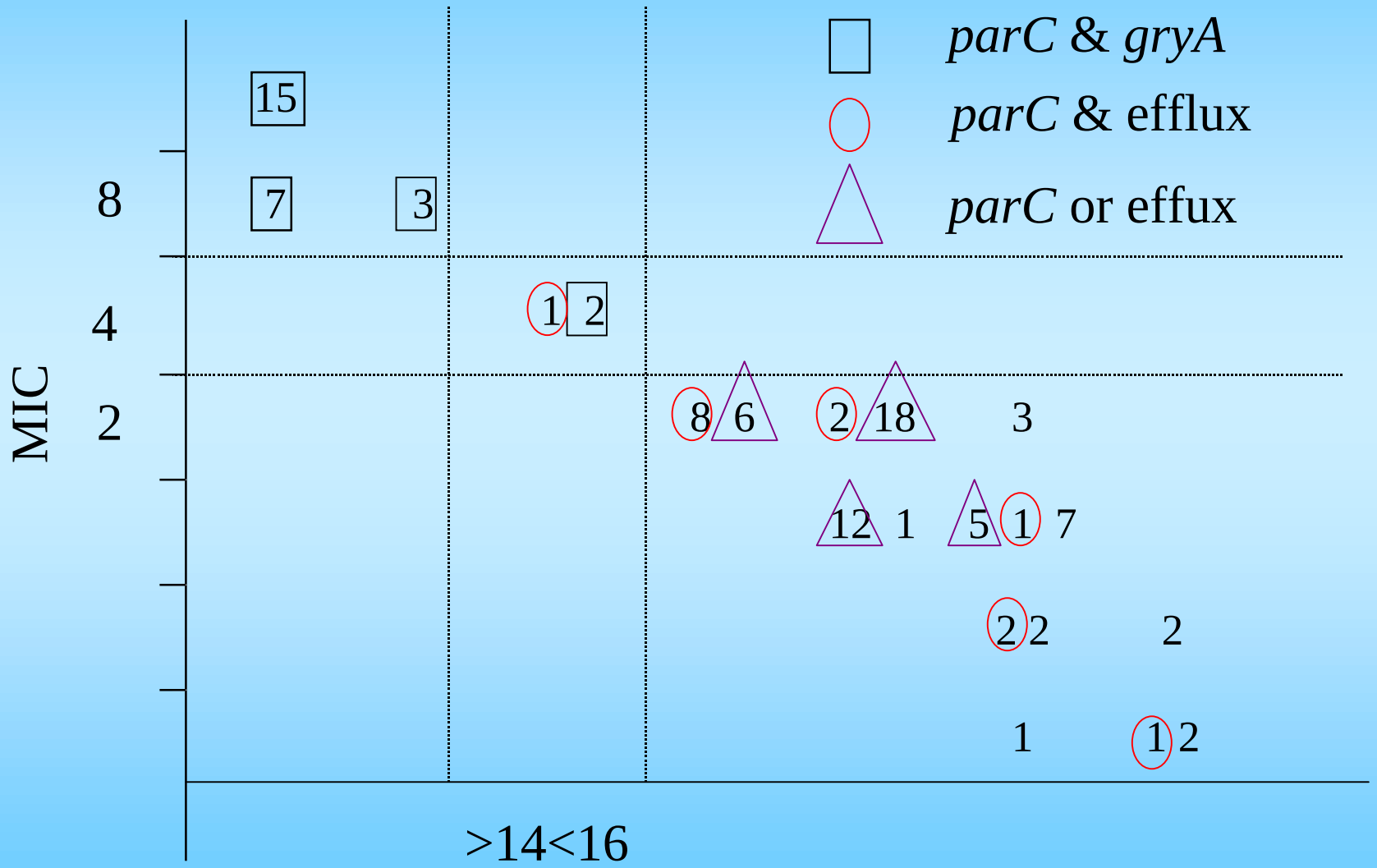
The amount of quinolone needed (MIC) to saturate primary target



Issues

- Increasing prevalence of first-step mutants
- Laboratory testing
  - Routine testing of all pneumococcal isolates
  - Detecting first step mutants

Correlations among levofloxacin MICs, zone diameters, and resistance mechanisms (101 isolates tested)



Richardson, JCM, 2001

What does it mean clinically?

Failure of treatment of pneumococcal pneumonia with levofloxacin

Case 1

- 64 yo M
  - presented with history and clinical findings of CAP
  - no prior hx of fluoroquinolone use
  - treated with levofloxacin 500 mg po 10 days
  - sputum grew *S. pneumoniae*
- One week after completing therapy
  - diagnosed with recurrent pneumonia
  - sputum grew *S. pneumoniae*

Case 1

Sputum isolate	PFGE pattern	MICs (μg/ml)			Mutations	
		Levo	Pen	Eryth	*parC*	*gyrA*
Pre-Tx	A	1	<0.06	<0.25	-	-
Post-Tx	A	8	<0.06	<0.25	S79F	S81F

Case 2

- 37 yo F with x-ray proven CAP
  - no prior fluoroquinolone therapy
  - treated with levofloxacin 500 mg po 10 days
  - Sp isolate susceptible to levofloxacin by DD
- 3 days into her therapy
  - admitted to hospital because clinically no improvement
  - responded after switched to ceftriaxone and erythromycin

Case 2

Sputum isolate	PFGE pattern	Disk diffusion testing	MICs (μg/ml)			Mutations	
			Levo	Pen	Eryth	*parC*	*gyrA*
Pre-Tx	B	S	4	<0.06	<0.25	S79F	-
During-Tx	B	R	16	<0.06	<0.25	S79F	S81F

Case 3

- 66 year old female
- COPD
- Penicillin allergy (rash)

Case History

- June 7: ciprofloxacin 500 mg po BID for flu
- June 13: fever, chills, R pleuritic chest pain
- June 15: seen in ER (on cipro)
  - CXR: RLL infiltrate +/- effusion
- Admitted to hospital
- Started empirically on levofloxacin 500 mg po od

Course in Hospital

- Day 1
  - blood cultures: *S. pneumoniae*
- Day 4
  - CT: consolidation of RML, RLL and LLL and loculated effusion.
- Day 5
  - SpO2 saturation 85% on 5L, HR 122, BP 100/60
  - increasing R sided chest pain
  - pleural fluid culture: *S. pneumoniae*

Course in Hospital

- Day 6
  - SpO2 deteriorating on FiO2 100%
  - admitted to ICU and intubated
  - started on cefotaxime 2g q 12H
- Day 7
  - refractory shock
  - expired

Case 3

Source of isolate	MICs (μg/ml)			Mutations		
				parC		*gyrA*
	Levo	Pen	Eryth	1st	2nd	
Blood (6/15)	16	<0.06	<0.25	S79F	-	S81Y
Pleural fluid (6/20)	16	<0.06	<0.25	S79F	D83Y	S81Y

Case 4

- Patient with AECB treated with ciprofloxacin
- Developed pneumonia and switched to levofloxacin
- Failed therapy-levo-resistant isolate of pneumococci