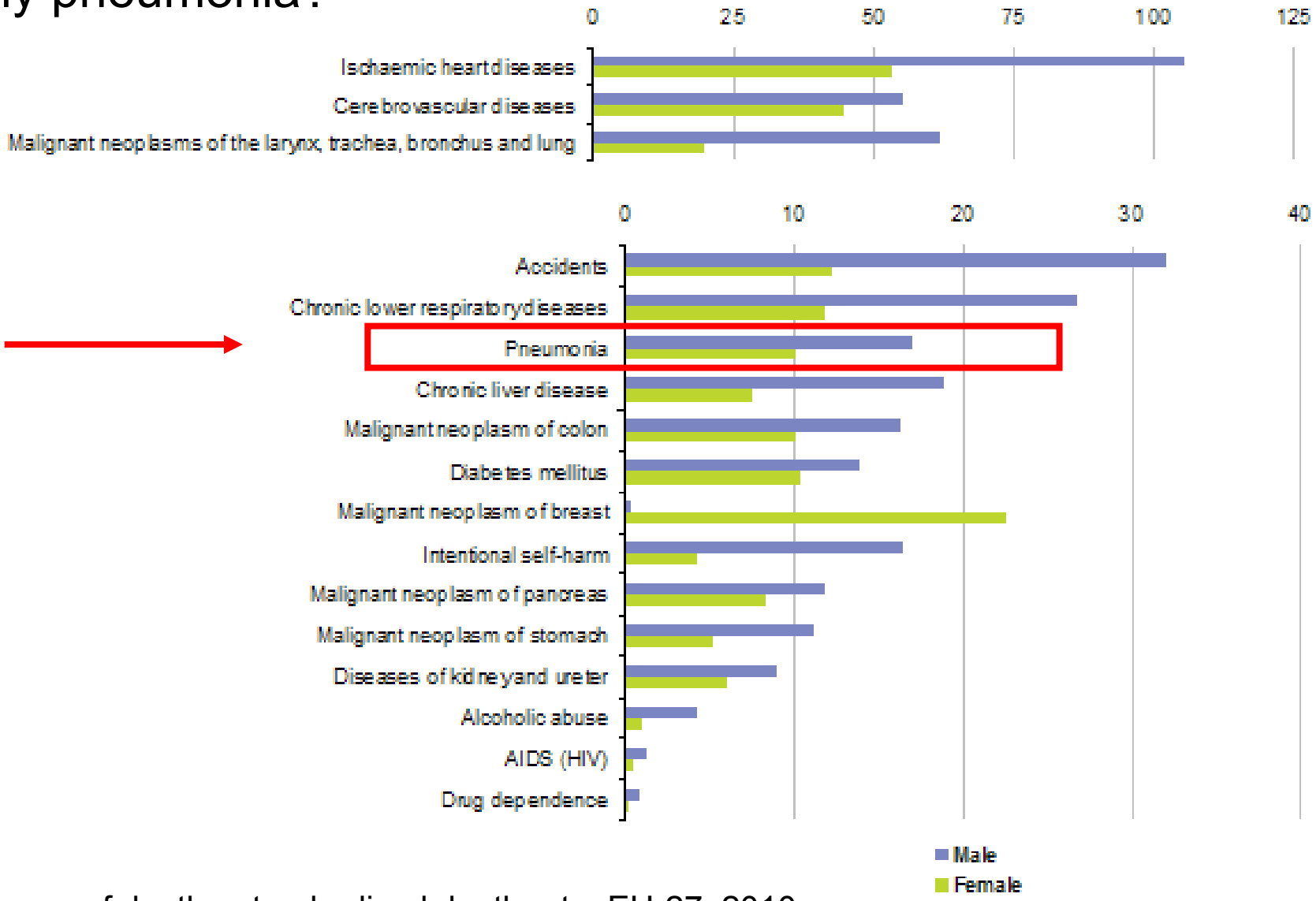
Antimicrobial resistance in *Streptococcus pneumoniae* isolates from Belgian community acquired pneumonia, with special reference to efflux mechanism.

Ann Lismond

Pharmacologie cellulaire et moléculaire
Promoteurs: Pr. F. Van Bambeke, Pr. P. Tulkens

Why pneumonia?



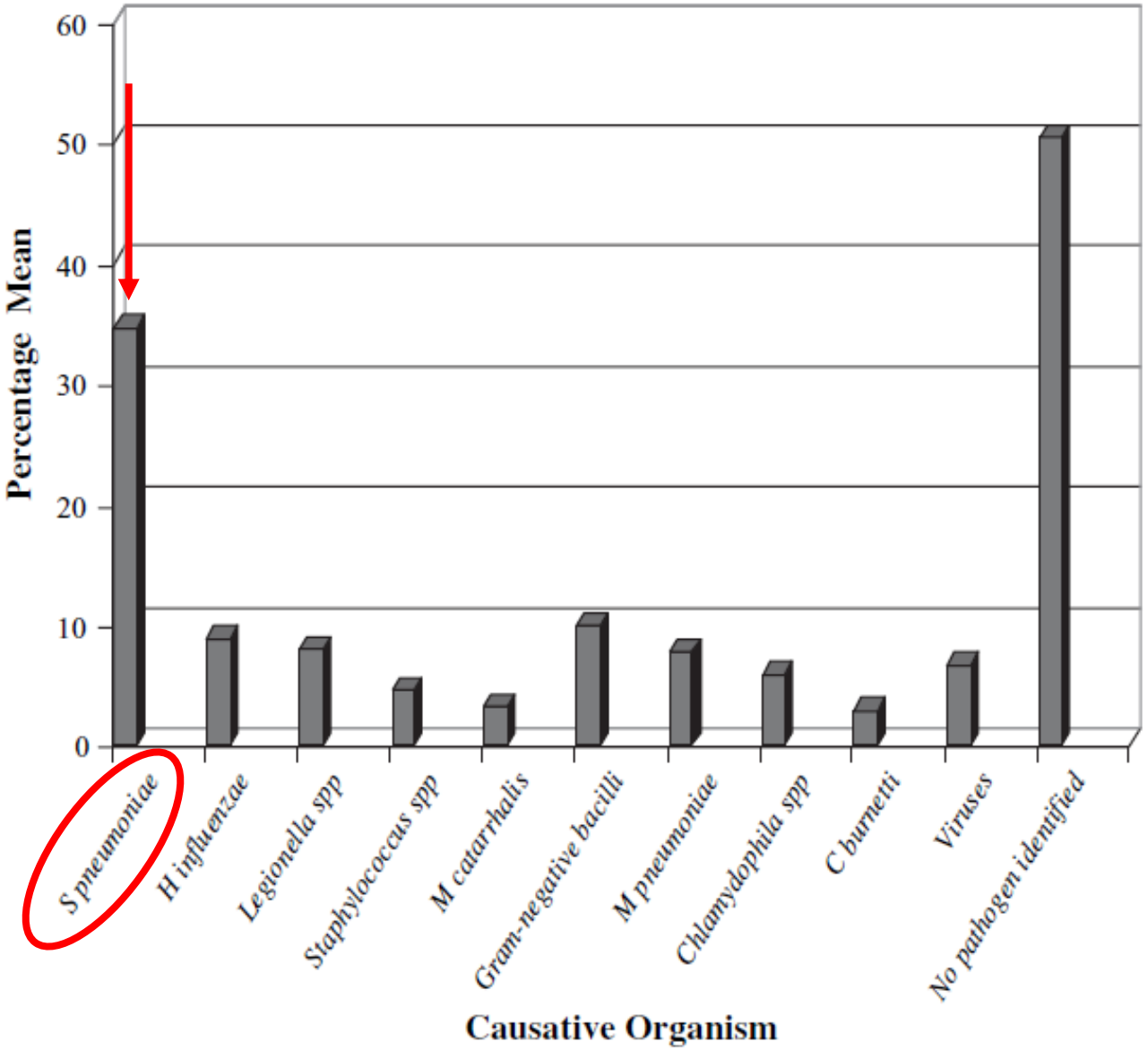
Causes of death - standardised death rate, EU-27, 2010
(per 100 000 inhabitants)

http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Causes_of_death_statistics

Community-Acquired Pneumonia

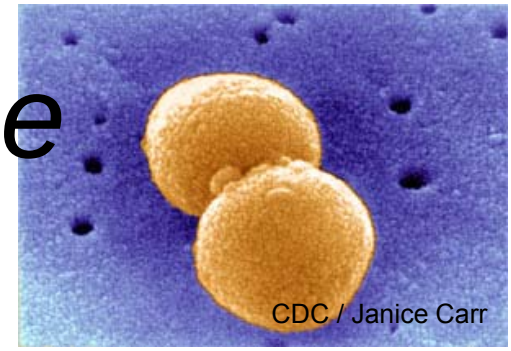
- Leading cause of morbidity & mortality worldwide
- Mortality: <1% to ~48% associated to severity & risk factors
- Incidence varies depending on period of the year & age
- Long-term prognosis worst for pneumococcal CAP

Causative organisms:



Welte T *et al.* Thorax (2010). PM:20729232.

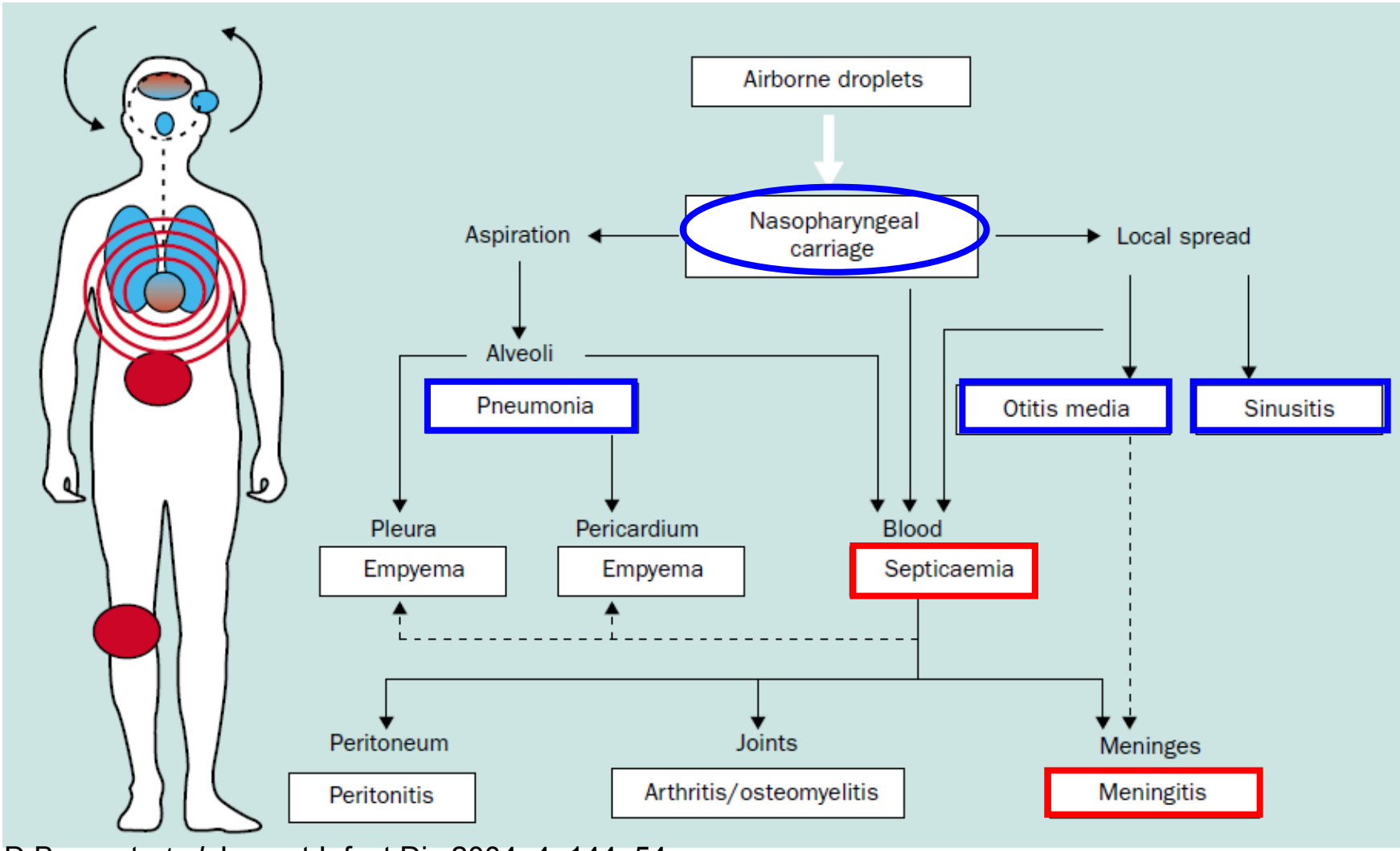
Streptococcus pneumoniae



- Gram + cocci, non motile
- polysaccharidic capsule (>90 serotypes)

  → target of current vaccine
- Extremely adaptive: naturally competent (transformation) & recombination
- Upper respiratory tract commensal (20-70% adults)

- Cause of
  - Mucosal infections: AOM, CAP, sinusitis, AECB…
  - Invasive infections: bacteraemia, sepsis, meningitis,…

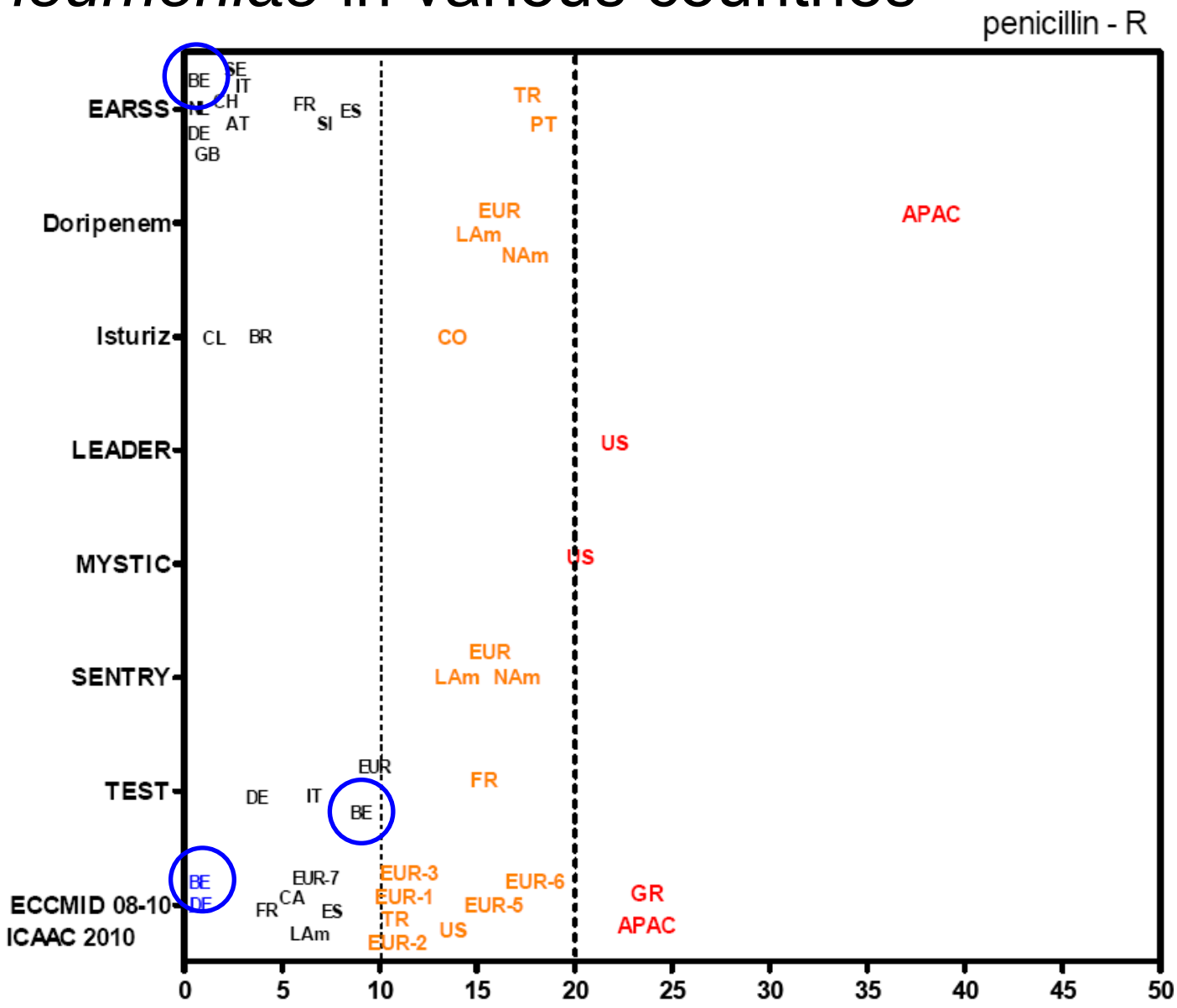


D Bogaert *et al.* Lancet Infect Dis 2004; 4: 144–54

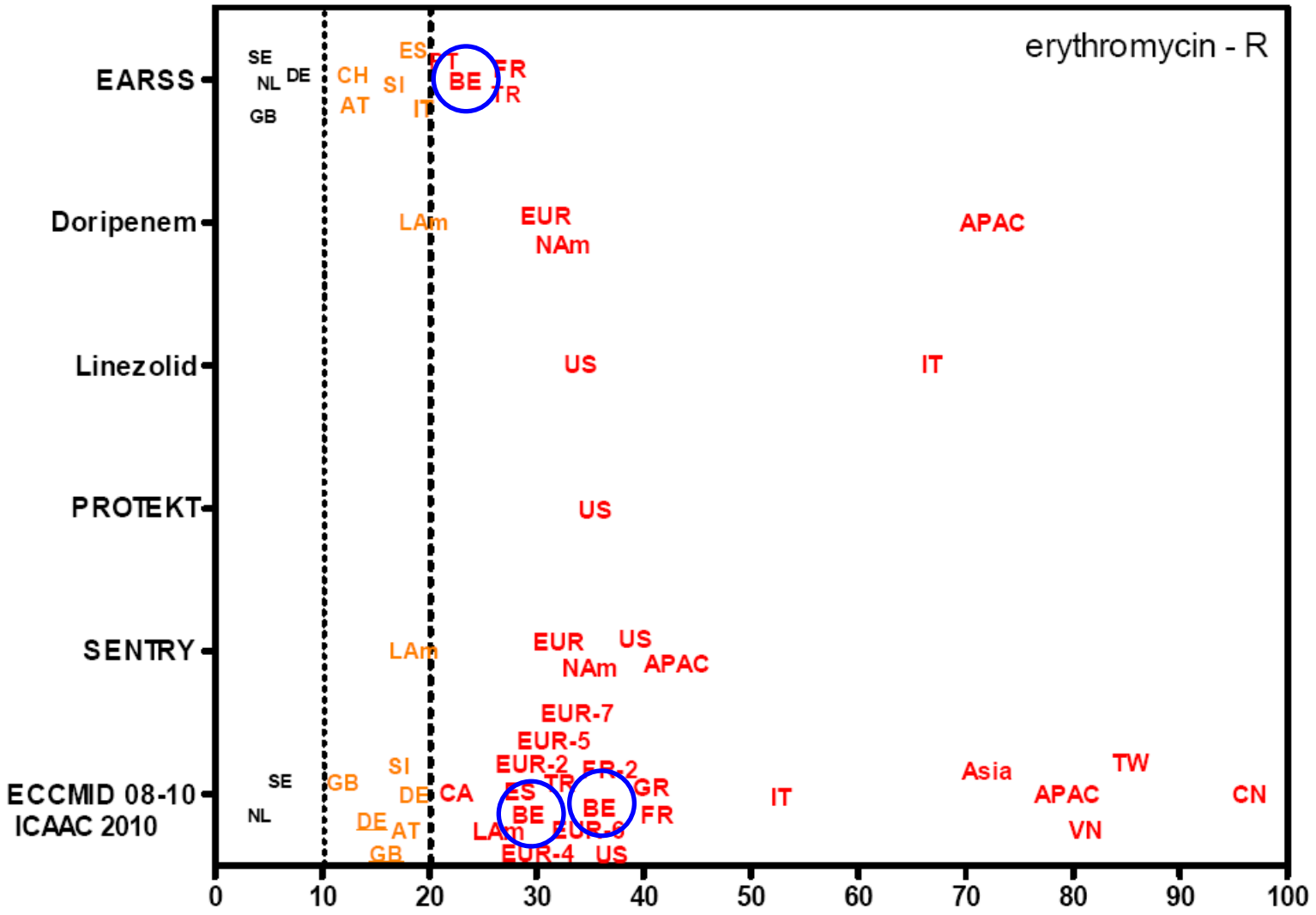
Main antibiotic classes used against *S. pneumoniae*

	S. pneumoniae resistance mechanisms:
• β-Lactams	•Decreased affinity of PBP (mosaic genes)
• Macrolides	•Ribosomal alteration: - Ribosomal methylation (23S) - Ribosomal mutation (23S domain V) - Mutation in riboproteins L4 or L22 •Efflux: pumps MefA/E/I
• Fluoroquinolones	•Mutations in Topoisomerase IV / DNA Gyrase •Efflux: pumps PmrA, PatA, PatB

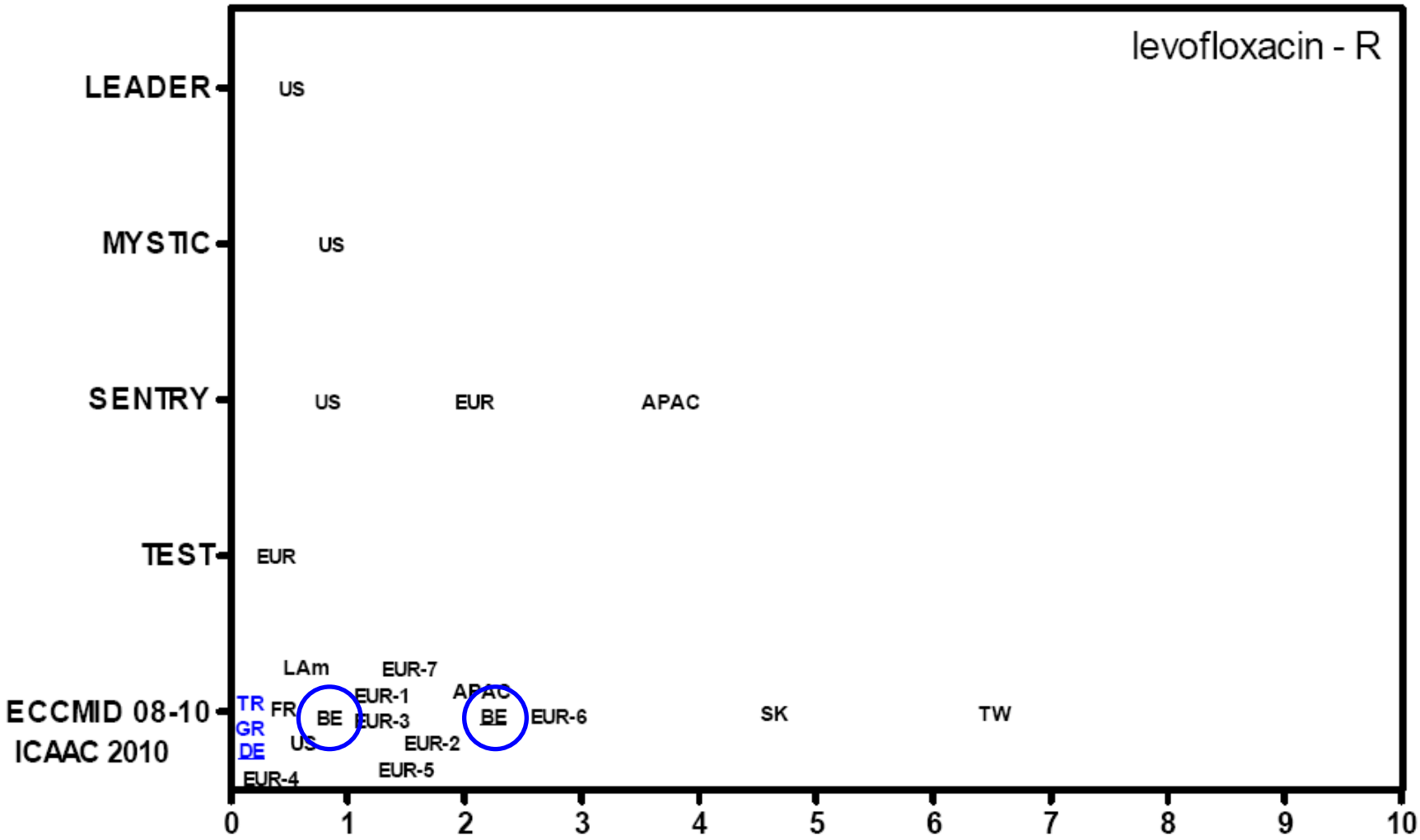
Comparison of antibiotic resistance rates of *S. pneumoniae* in various countries



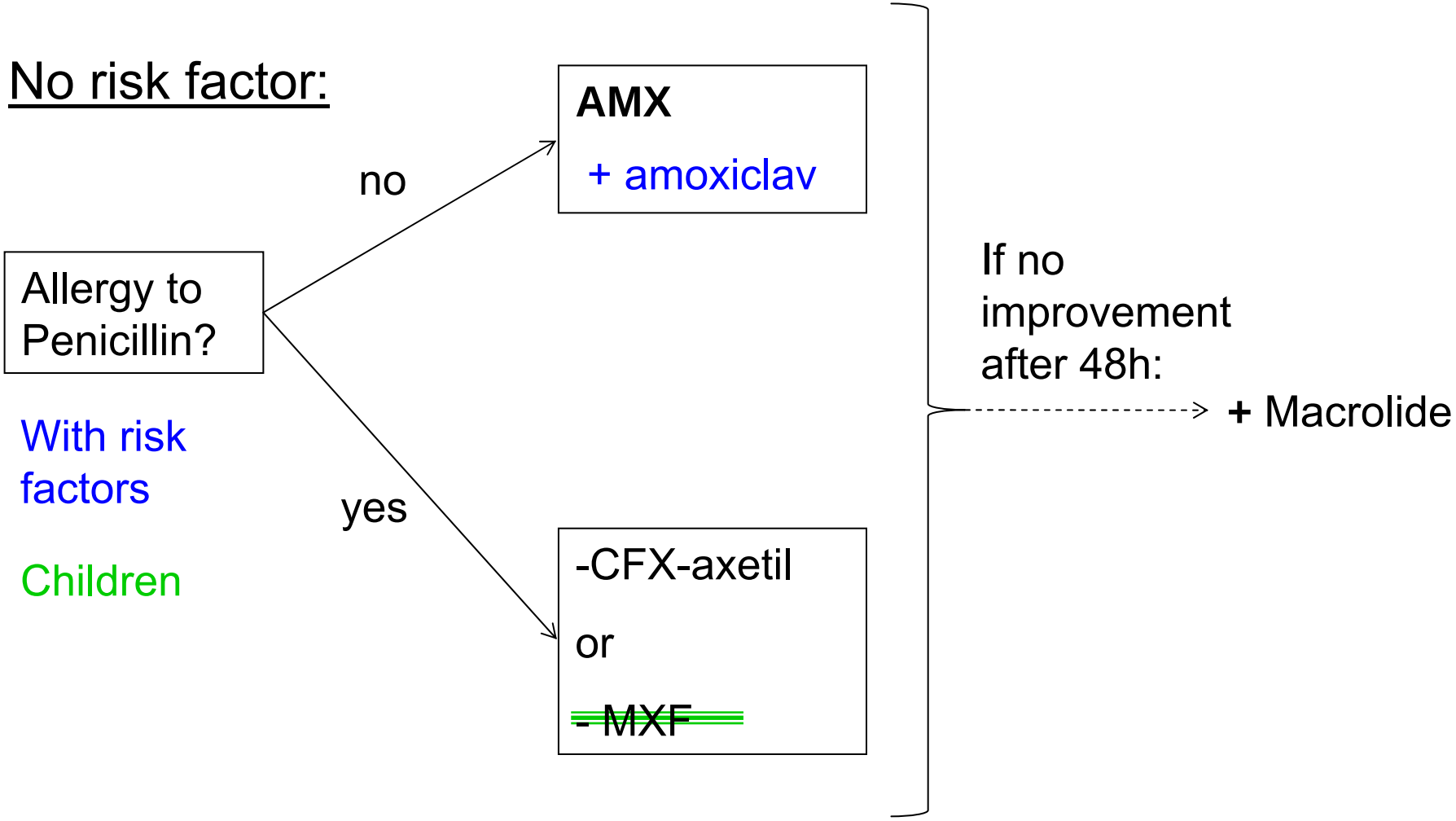
Comparison of antibiotic resistance rates of *S. pneumoniae* in various countries



Comparison of antibiotic resistance rates of *S. pneumoniae* in various countries



Belgium guidelines for initial oral empiric antibiotic therapy for outpatients with CAP (2008)



BAPCOC: Belgian Antibiotic Policy Coordination Committee (Belgium)

OBJECTIVES

- Pneumonia treatment optimal?
  - Antibiotics recommended ~ resistance ?
  - Vaccine coverage ~ prevalent serotypes ?
  - Interest of new molecules in development ?

- Fluoroquinolones active efflux:
  - Prevalence & clinical relevance?
  - Identification of transporter & Substrate specificity?
  - Expression & Inducibility?

Belgian collection of *S. pneumoniae*

Antimicrobial susceptibility of *Streptococcus pneumoniae* isolates from vaccinated and non-vaccinated patients with a clinically confirmed diagnosis of community-acquired pneumonia in Belgium

Ann Lismond[a], Sylviane Carbonnelle[a,1], Jan Verhaegen[b], Patricia Schatt[c], Annelies De Bel[d], Paul Jordens[e], Frédérique Jacobs[f], Anne Dediste[g], Frank Verschuren[h], Te-Din Huang[i,2], Paul M. Tulkens[a,*], Youri Glupczynski[j], Françoise Van Bambeke[a]

[a] *Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain,*
[b] *Laboratorium microbiologie, Universitair Ziekenhuis Gasthuisberg, Leuven, Belgium*
[c] *Laboratoire de microbiologie, Cliniques Notre-Dame de Grâce, Gosselies, Belgium*
[d] *Microbiologie en ziekenhuishygiëne, Universitair Ziekenhuis Brussel, Brussels, Belgium*
[e] *Afdeling pneumologie, O.L.V. Ziekenhuis, Aalst, Belgium*
[f] *Clinique des maladies infectieuses, Hôpital Erasme, Brussels, Belgium*
[g] *Laboratoire de microbiologie, CHU Saint-Pierre, Brussels, Belgium*
[h] *Service des urgences, Cliniques universitaires Saint-Luc, Brussels, Belgium*
[i] *Laboratoire de microbiologie, Cliniques universitaires Saint-Luc, Brussels, Belgium*
[j] *Laboratoire de microbiologie, CHU Mont-Godinne, Yvoir, Belgium*

General protocol

Patient with suspicion of pneumonia

Sampling for microbiology → Isolation of SP

Clinical examination, X-ray → CAP diagnostic

CAP diagnostic → *signal* → **Clinic :** clinical file (Dr. Carbonnelle)

Microbiology (A. Lismond) ⇢ confirmation ⇢ **Clinic :** clinical file (Dr. Carbonnelle)

Isolation of SP → Analysis of the SP → MIC, efflux

Clinic : clinical file (Dr. Carbonnelle)

Analysis of the case
- Symptoms, severity
- X-ray
- AB: previous, current
- Contact of GP
- Reason(s) of referral to hospital

Analysis of the SP → MIC, efflux

Analysis of the case → Microbiological & clinical data are assembled
(anonymous)

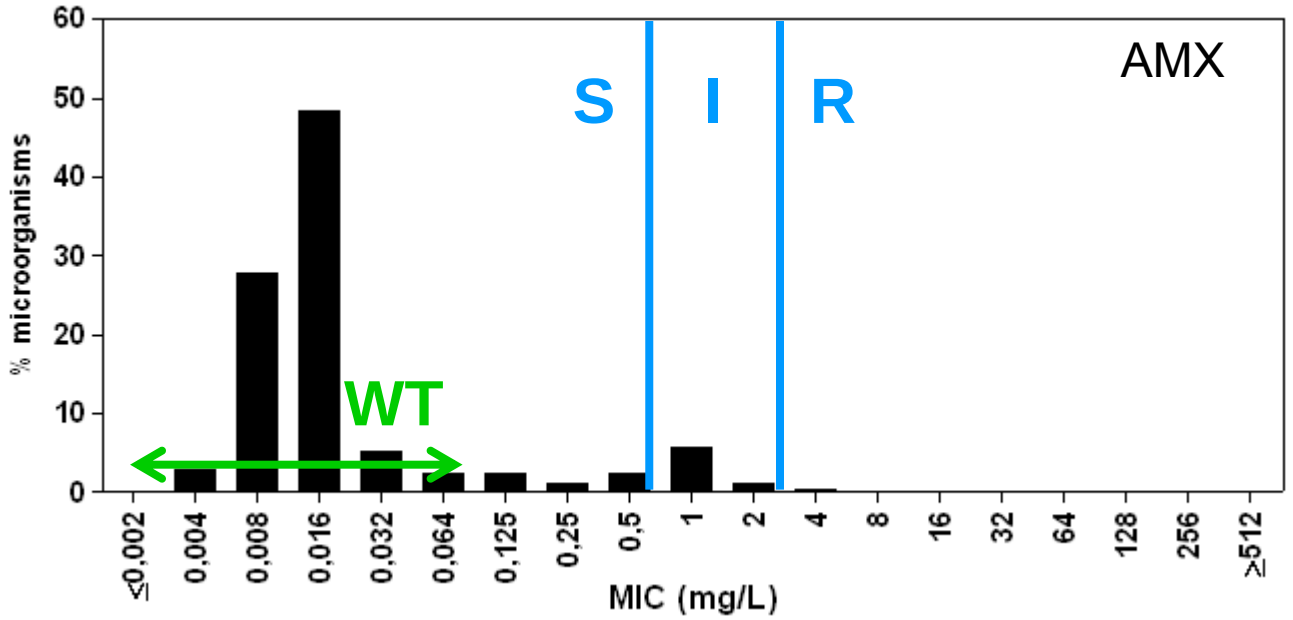
for 249 isolates, collected between 04/2007 and 03/2009

European Committee on Antimicrobial Susceptibility Testing (EUCAST)

MIC = Minimal Inhibitory Concentration

Wild type (WT) : organism characterized by the **absence of acquired and mutational resistance mechanisms** to the drug.

Wild type micro-organisms may or may not respond clinically to antimicrobial treatment.



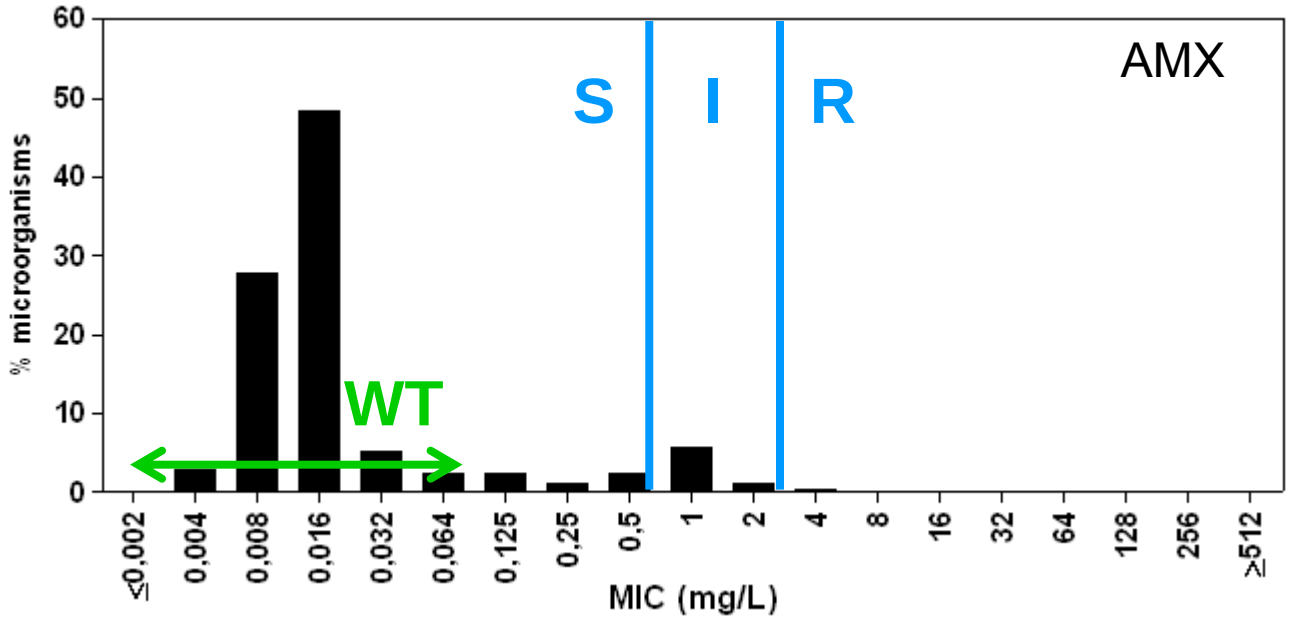
Clinical resistance (EUCAST)

A micro-organism is defined as

- **Susceptible (S)** by a level of antimicrobial activity associated with a **high likelihood of therapeutic success**

- **Intermediate (I)** by a level of antimicrobial agent activity associated with **uncertain therapeutic effect**

- **Resistant (R)** by a level of antimicrobial activity associated with a **high likelihood of therapeutic failure**

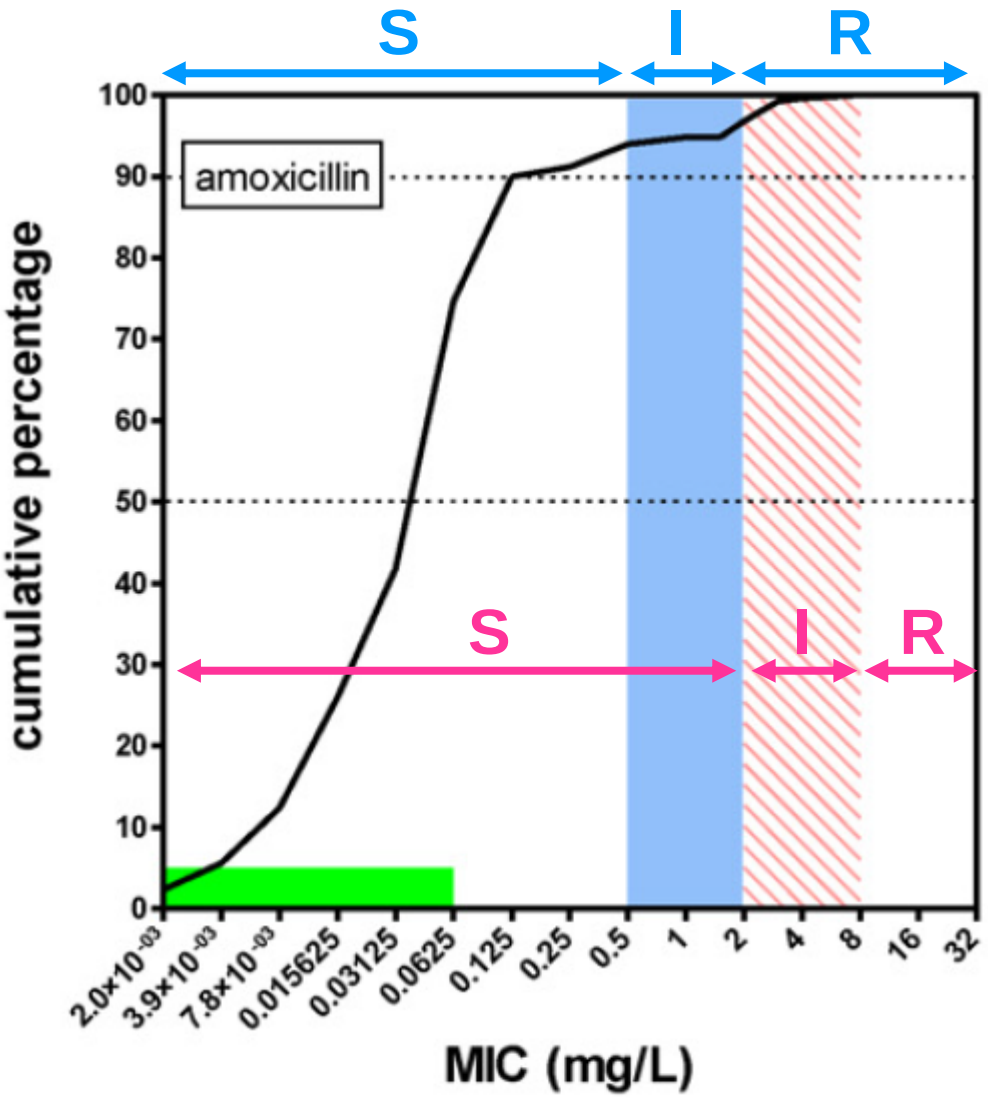


OBJECTIVES

- Pneumonia treatment optimal?
  - Antibiotics recommended ~ resistance ?
  - Vaccine coverage ~ prevalent serotypes ?
  - Interest of new molecules in development ?

- Fluoroquinolones active efflux:
  - Prevalence & clinical relevance?
  - Identification of transporter & Substrate specificity?
  - Expression & Inducibility?

Antibiotics susceptibility



clinical breakpoint:

EUCAST

S ≤ 0.5	94%
I	2.8%
R > 2	3.2%

CLSI

S ≤ 2	96.8%
I	2.8%
R ≥ 8	0.4%

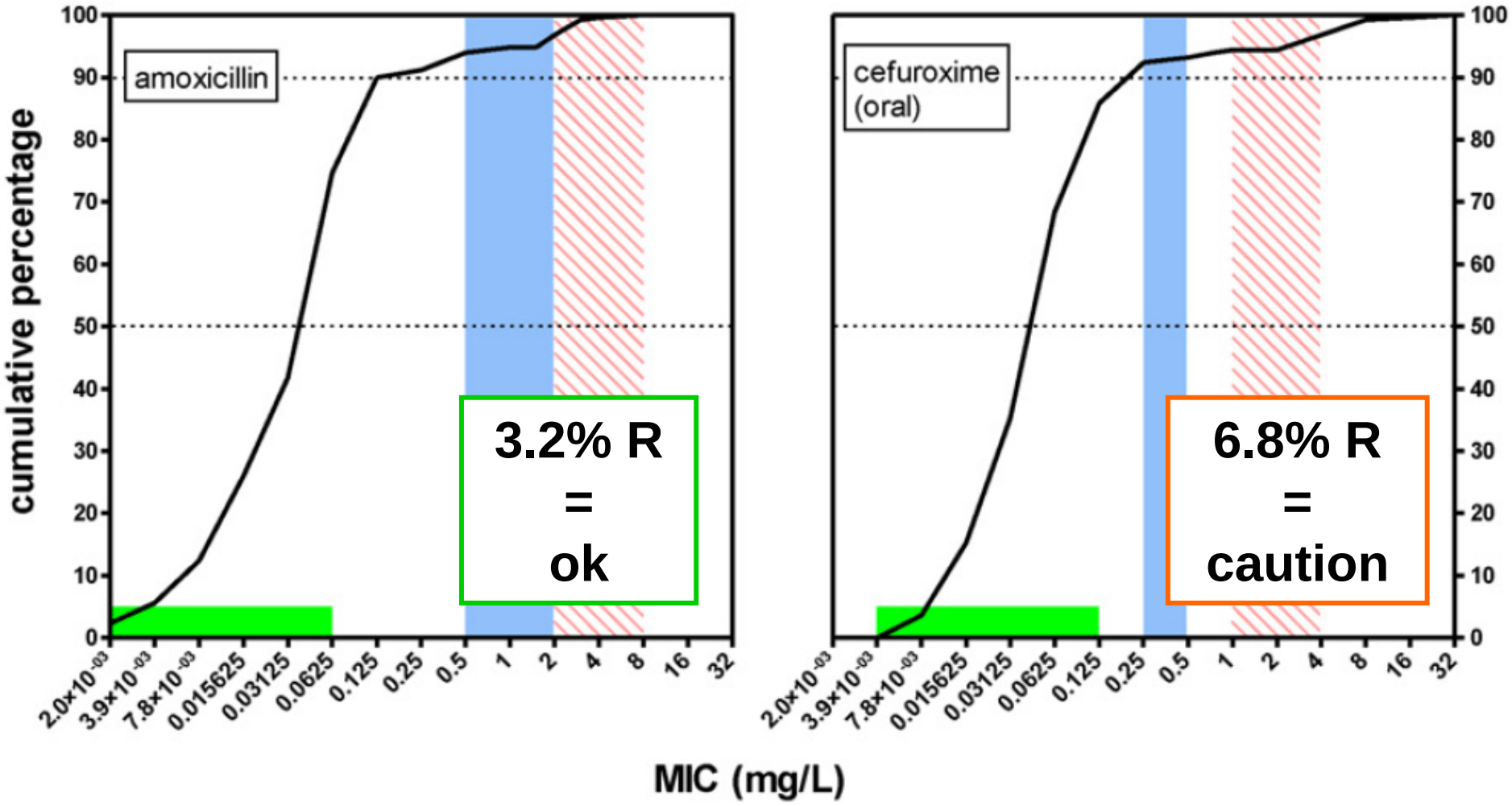
wild-type population

EUCAST

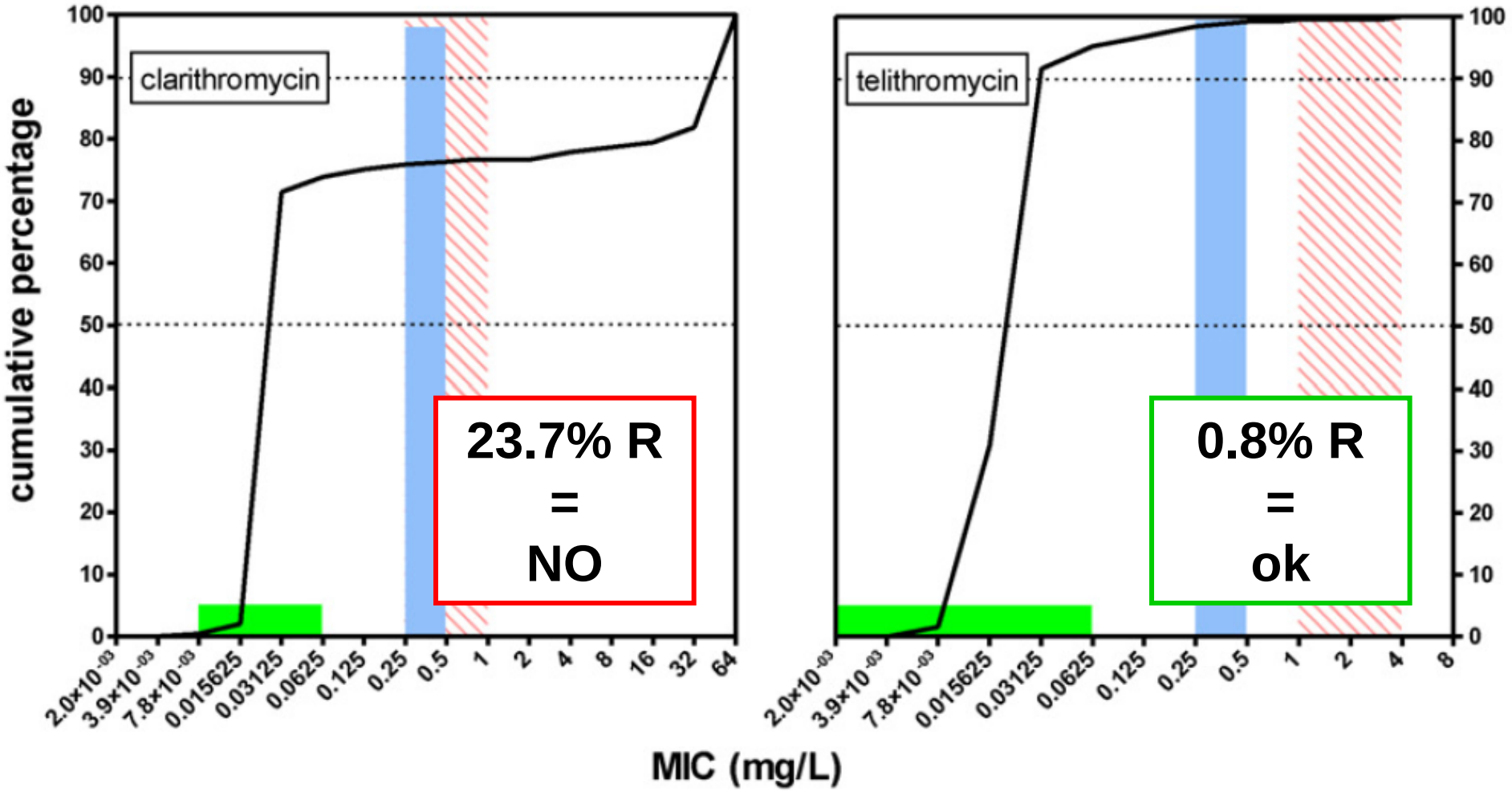
WT pop → 0.06 µg/ml

CLSI = Clinical and Laboratory Standards Institute

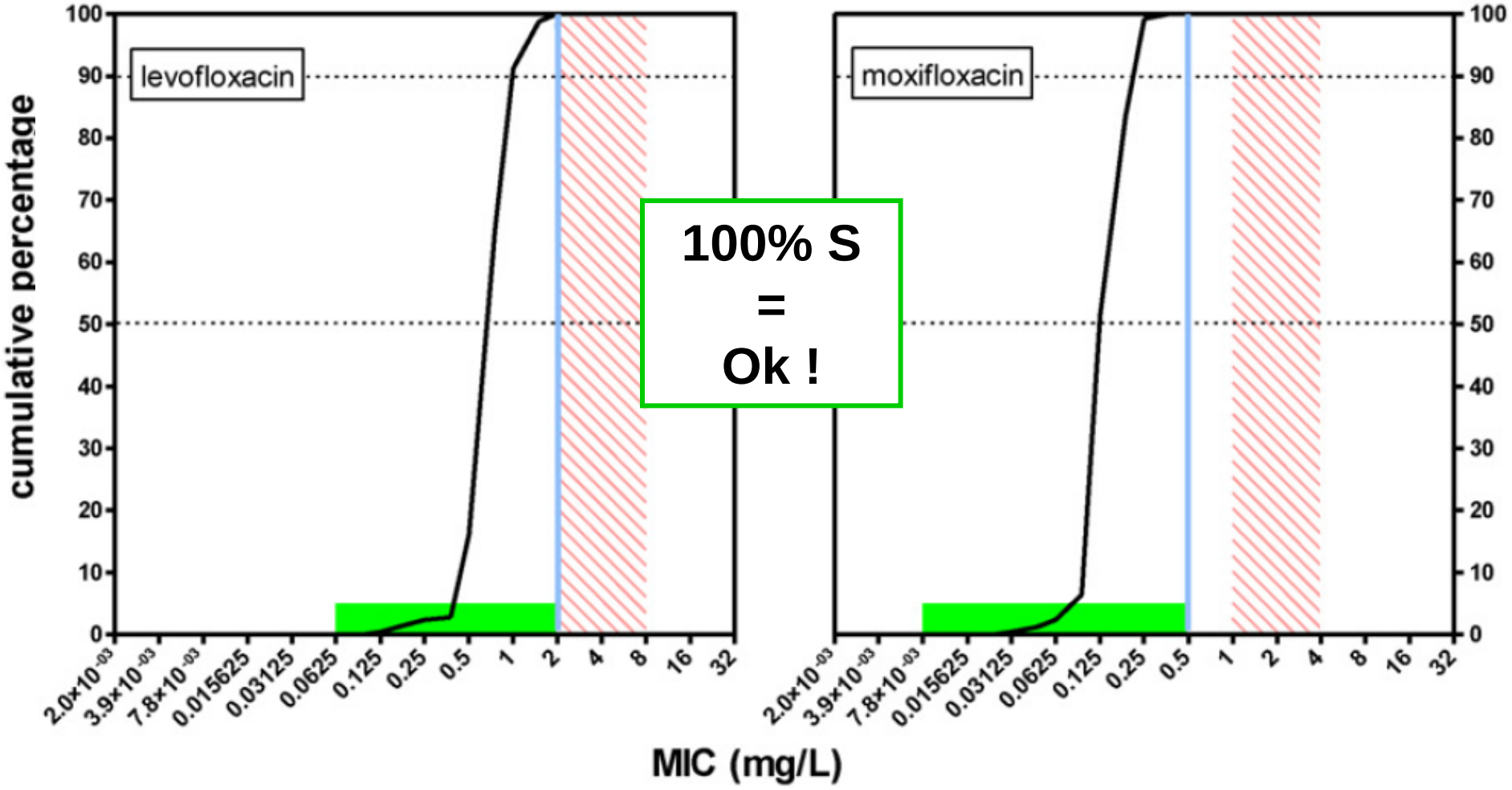
Susceptibility to β-Lactams:



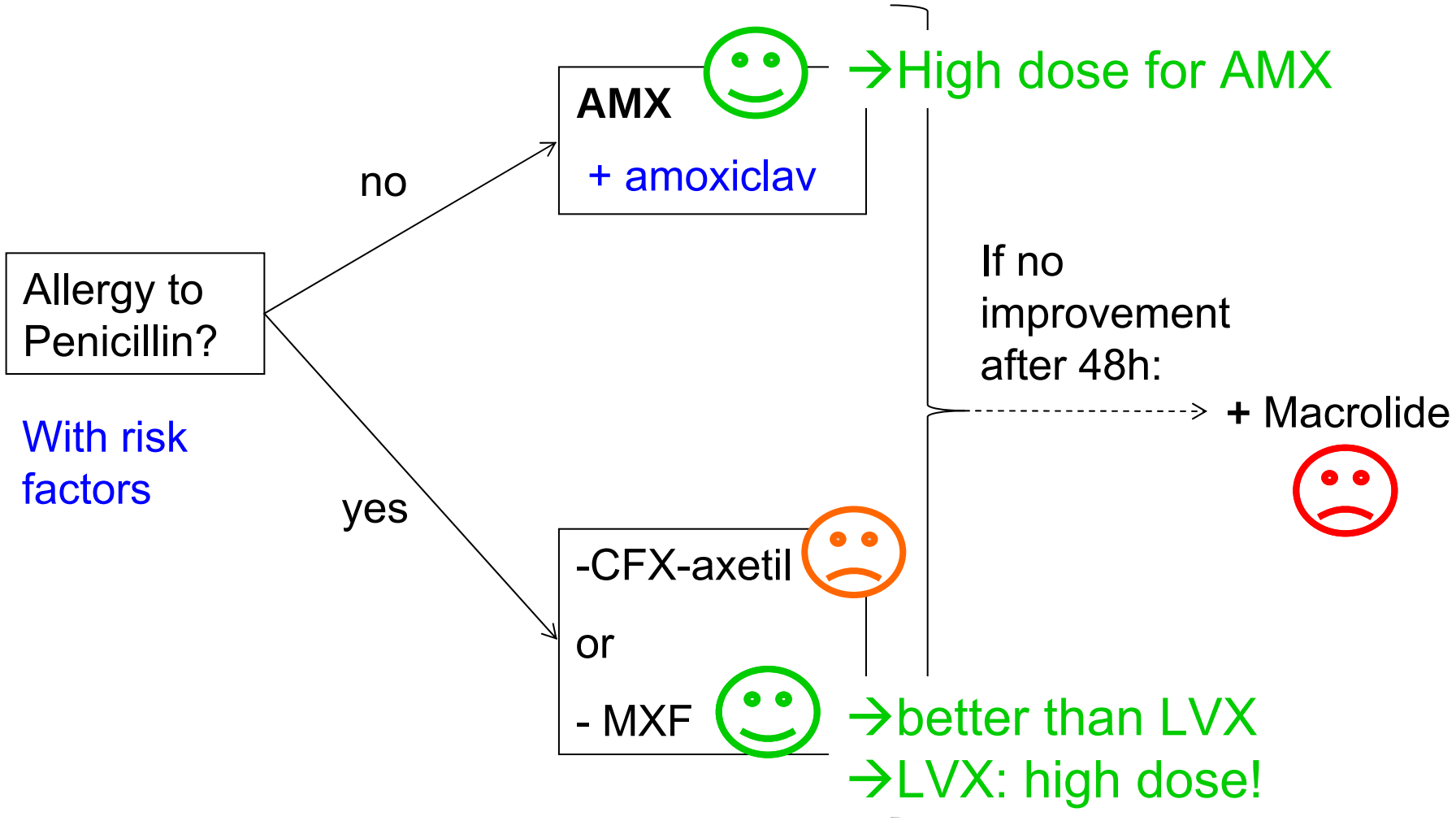
Susceptibility to Macrolides:



Susceptibility to Fluoroquinolones:



Belgium guidelines for initial oral empiric antibiotic therapy for outpatients with CAP (2008)



BAPCOC: Belgian Antibiotic Policy Coordination Committee (Belgium)

OBJECTIVES

- Pneumonia treatment optimal?
  - Antibiotics recommended ~ resistance ?
  - Vaccine coverage ~ prevalent serotypes ?
  - Interest of new molecules in development ?

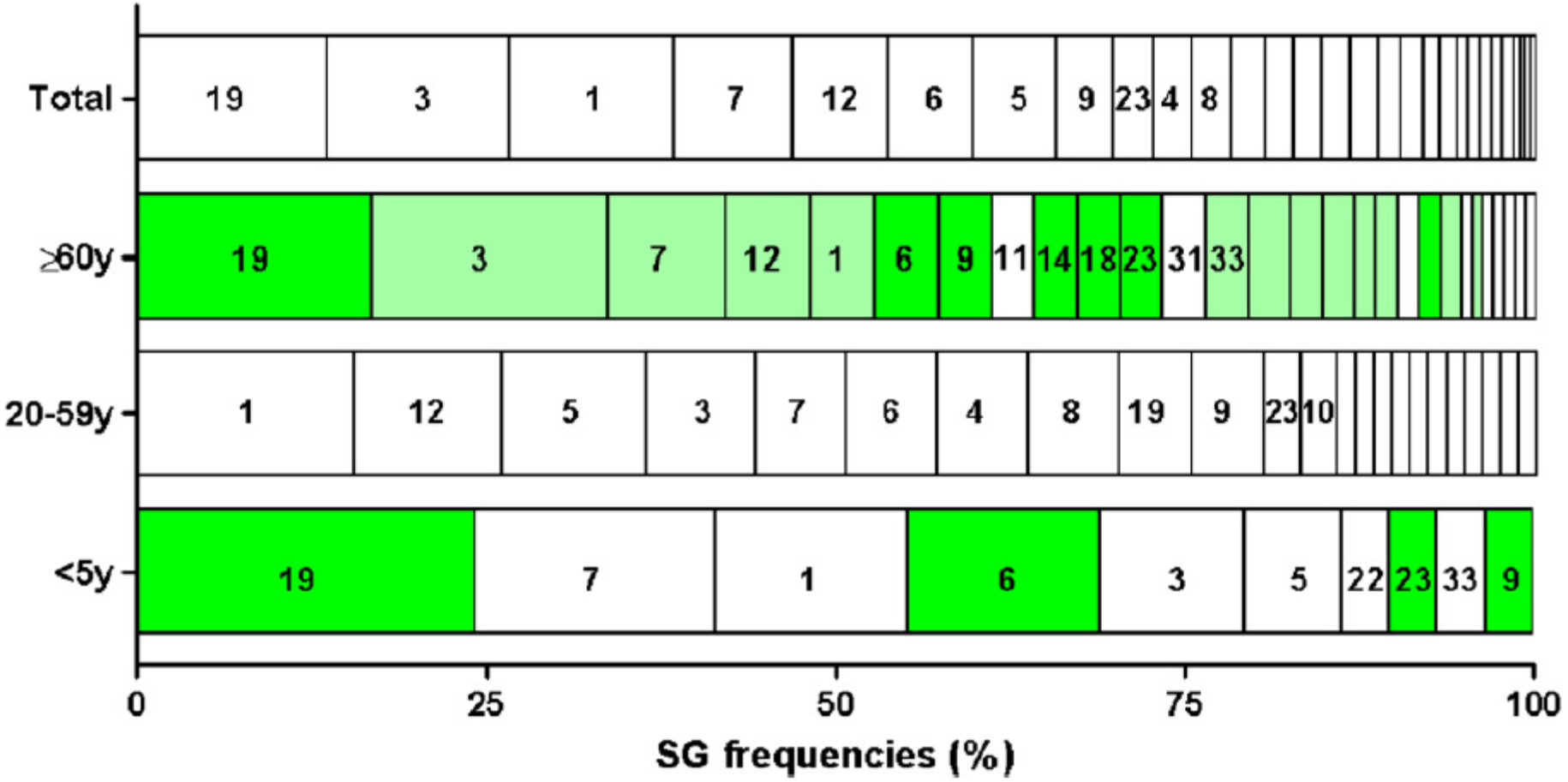
- Fluoroquinolones active efflux:
  - Prevalence & clinical relevance?
  - Identification of transporter & Substrate specificity?
  - Expression & Inducibility?

Pneumococcal vaccines

Serotypes	PPV23
1	x
2	x
3	x
4	x
5	x
6A	
6B	x
7F	x
8	x
9N	x
9V	x
10A	x
11A	x
12F	x
14	x
15B	x
17F	x
18C	x
19A	x
19F	x
20	x
22F	x
23F	x
33F	x

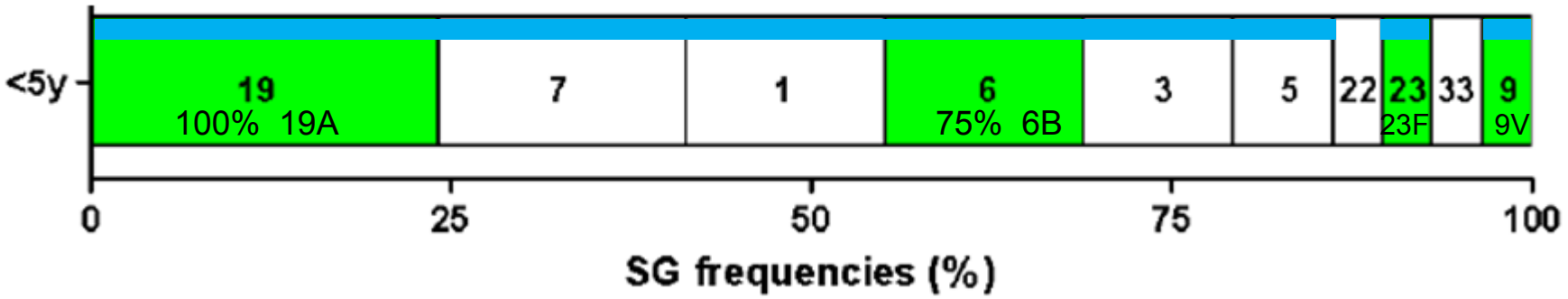
- >90 ST → 24 covered by vaccines

Serogroups prevalence in collection



Vaccines for children

N=29



Serotypes	PCV7
1	
3	
4	x
5	
6A	
6B	x
7F	
9V	x
14	x
18C	x
19A	
19F	x
23F	x

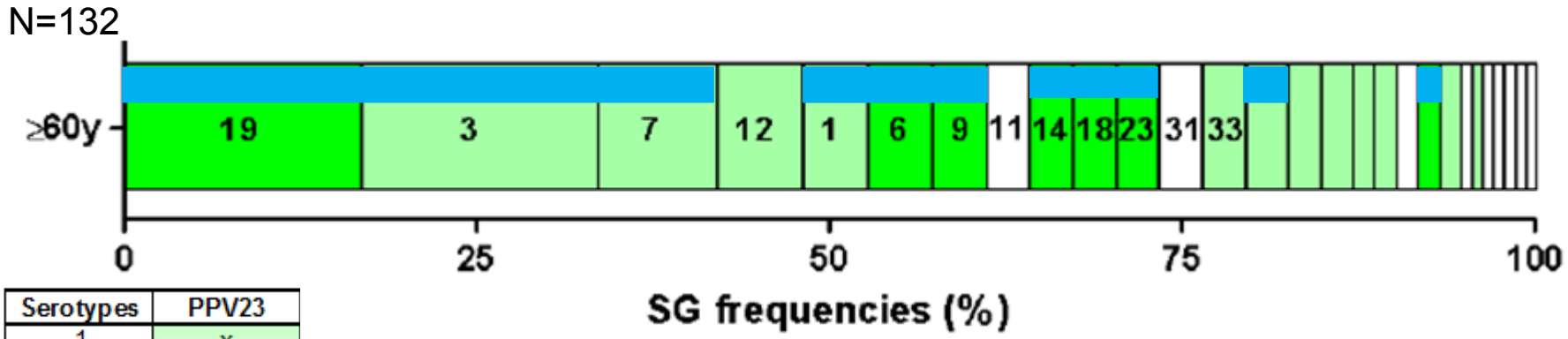
Apparent coverage of SG: ~45%

PCV-7 theoretical coverage in <5y → **~17%** of isolates

Vaccination status: ~60% yes, ~38% no

PCV-13 theoretical coverage in <5y → **~93%** of isolates

Vaccines for adults



Serotypes	PPV23
1	x
2	x
3	x
4	x
5	x
6A	
6B	x
7F	x
8	x
9N	x
9V	x
10A	x
11A	x
12F	x
14	x
15B	x
17F	x
18C	x
19A	x
19F	x
20	x
22F	x
23F	x
33F	x

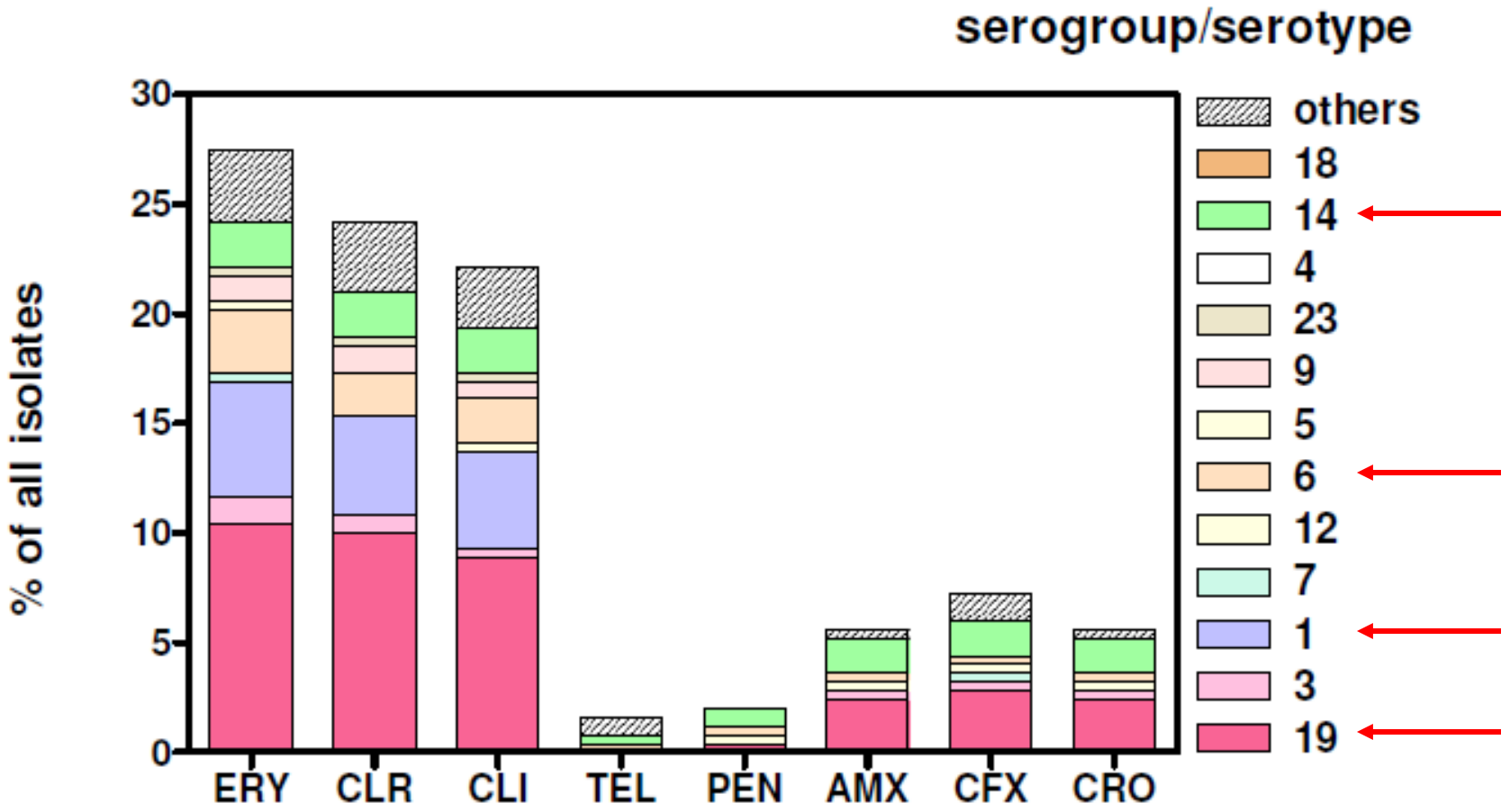
PPV-23 theorical coverage in ≥60y → 58-87%

Vaccination status: ~20% yes , ~60% no, ~20% unknown

PCV-13 theorical coverage in ≥60y → 55-67%

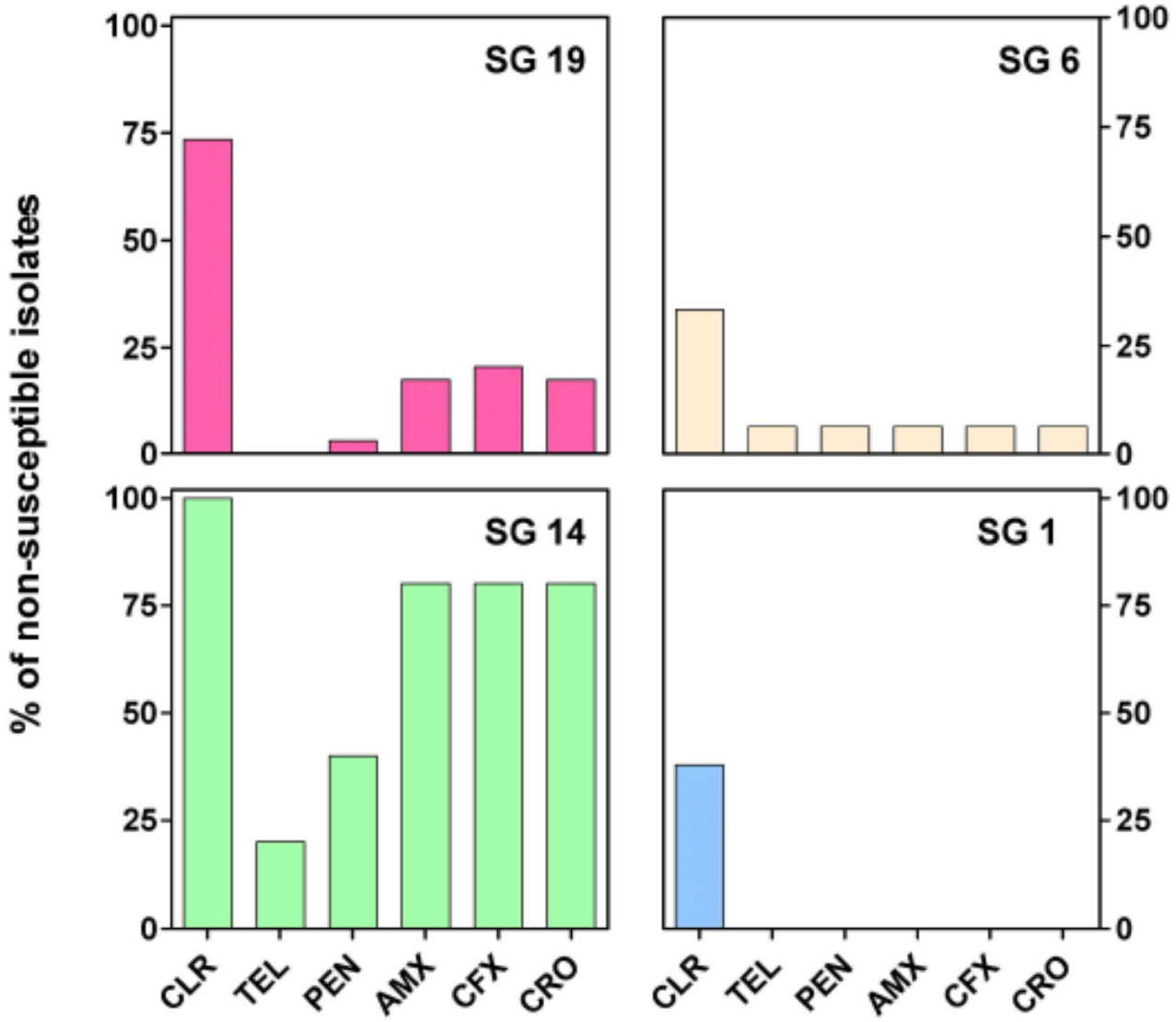
→ clinical trials to combine PCV13 & PPV23

Relationship non-susceptibility and serogroups



Anibiotic non-susceptibility is mainly in SG-19, -1, -6, -14!

Percentage of non-susceptible strains in specific serogroups:



→ included in PPV23 and PCV13!

↓

Protection from antibiotic resistant strains!

→ Strong association between serogroup & antibiotic non-susceptibility!

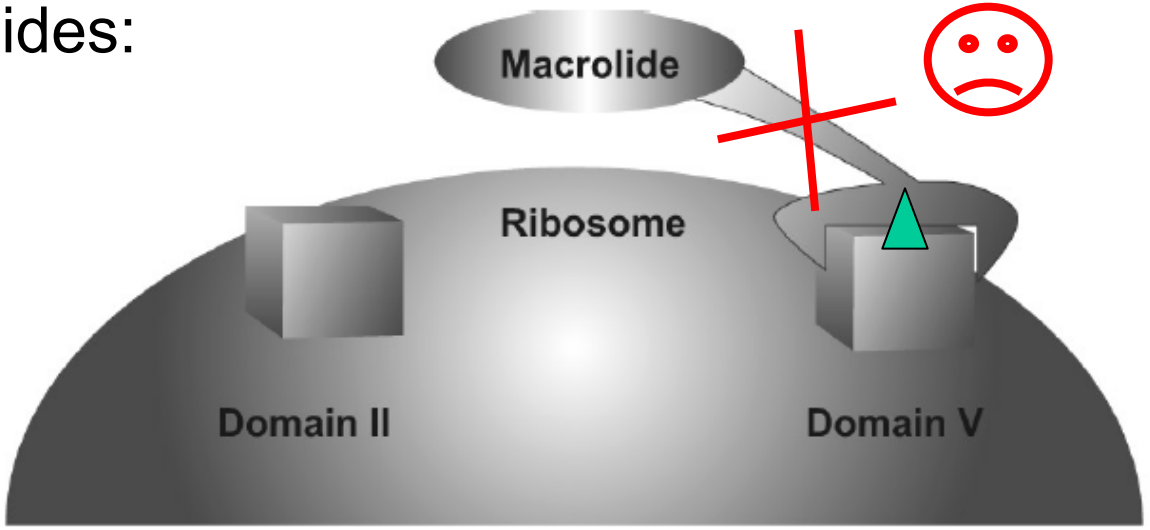
OBJECTIVES

- Pneumonia treatment optimal?
  - Antibiotics recommended ~ resistance ?
  - Vaccine coverage ~ prevalent serotypes ?
  - Interest of new molecules in development ?

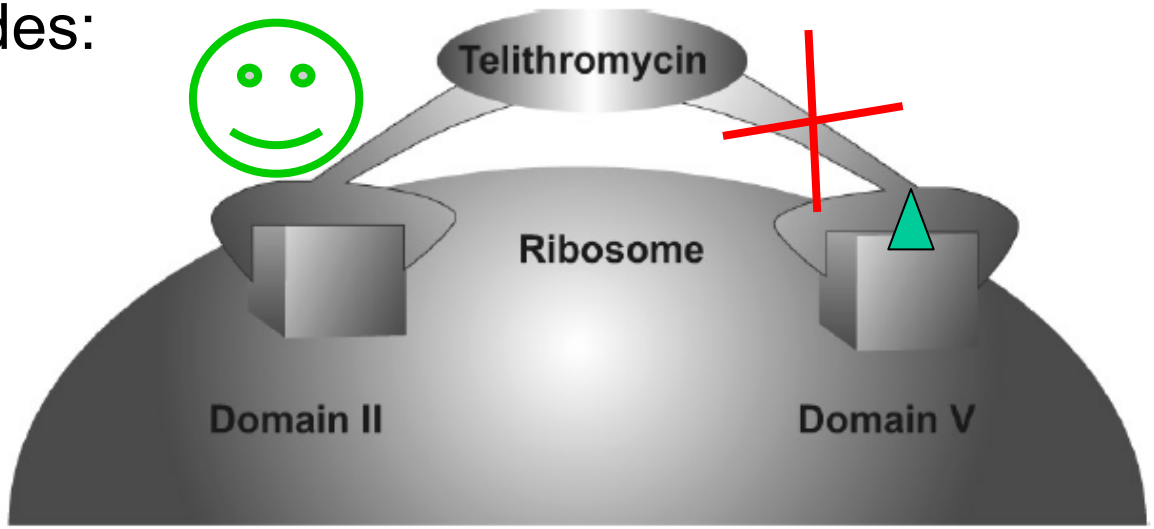
- Fluoroquinolones active efflux:
  - Prevalence & clinical relevance?
  - Identification of transporter & Substrate specificity?
  - Expression & Inducibility?

Interest of new molecules for CAP?

Macrolides:



Ketolides:

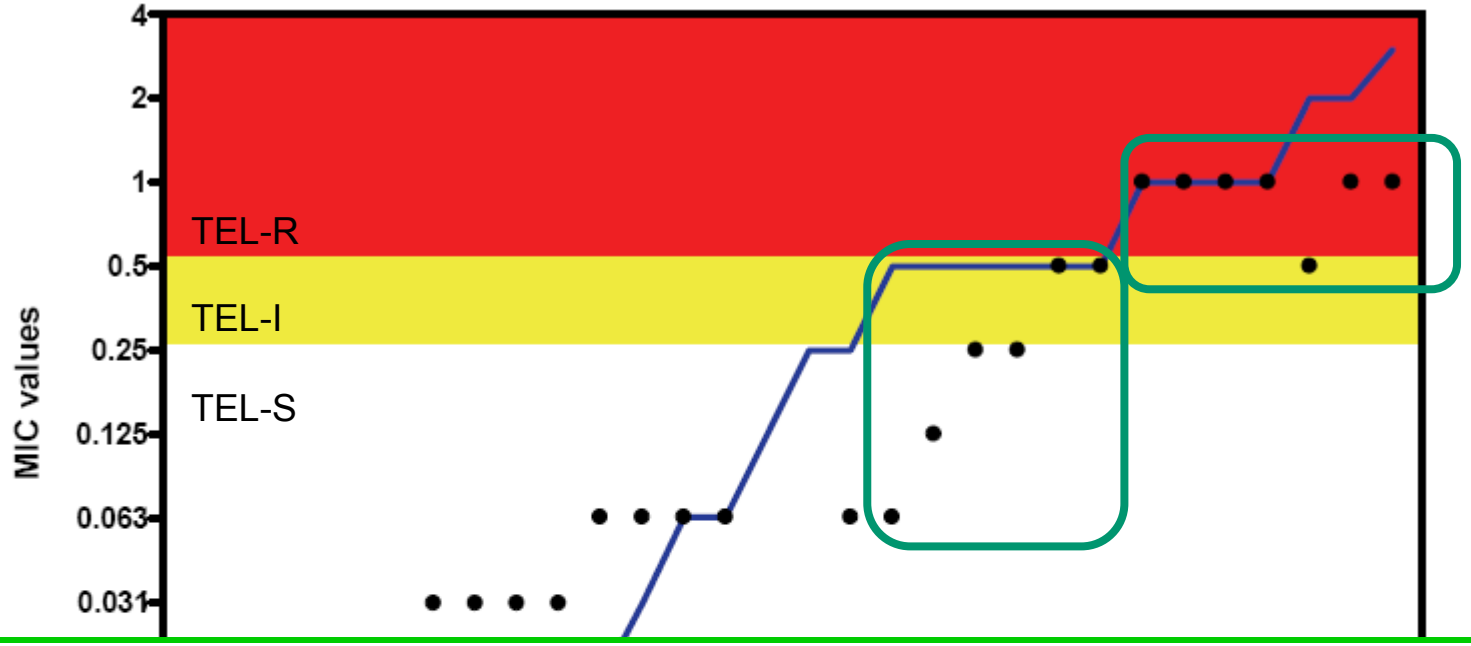


Telithromycin

Solithromycin

K.P. Klugman, International Journal of Antimicrobial Agents 29 Suppl. 1 (2007) S6–S10

Interest of new molecules for CAP?



Recruiting — Efficacy and Safety Study of Intravenous to Oral Solithromycin (CEM-101) Compared to Intravenous to Oral Moxifloxacin in Treatment of Patients With Community-Acquired Bacterial Pneumonia

Condition: Community-acquired Bacterial Pneumonia

Interventions: Drug: Solithromycin; Drug: Moxifloxacin

Recruiting — Efficacy and Safety Study of Oral Solithromycin (CEM-101) Compared to Oral Moxifloxacin in Treatment of Patients With Community-Acquired Bacterial Pneumonia

Condition: Community-acquired Bacterial Pneumonia

Interventions: Drug: Solithromycin; Drug: Moxifloxacin

OBJECTIVES

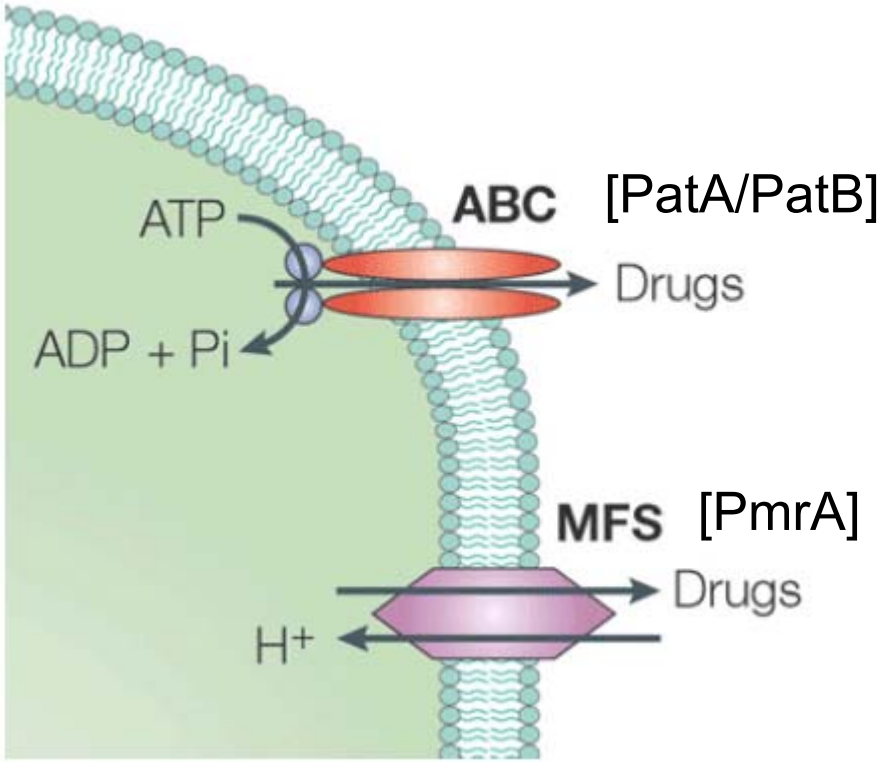
- Pneumonia treatment optimal?
  - Antibiotics recommended ~ resistance ?
  - Vaccine coverage ~ prevalent serotypes ?
  - Interest of new molecules in development ?

- Fluoroquinolones active efflux:
  - Prevalence & clinical relevance?
  - Identification of transporter & Substrate specificity?
  - Expression & Inducibility?

Efflux pumps

- Transmembrane transporter proteins
- Ubiquitous mechanism
- Purpose: expulse toxic substrates out of the cell

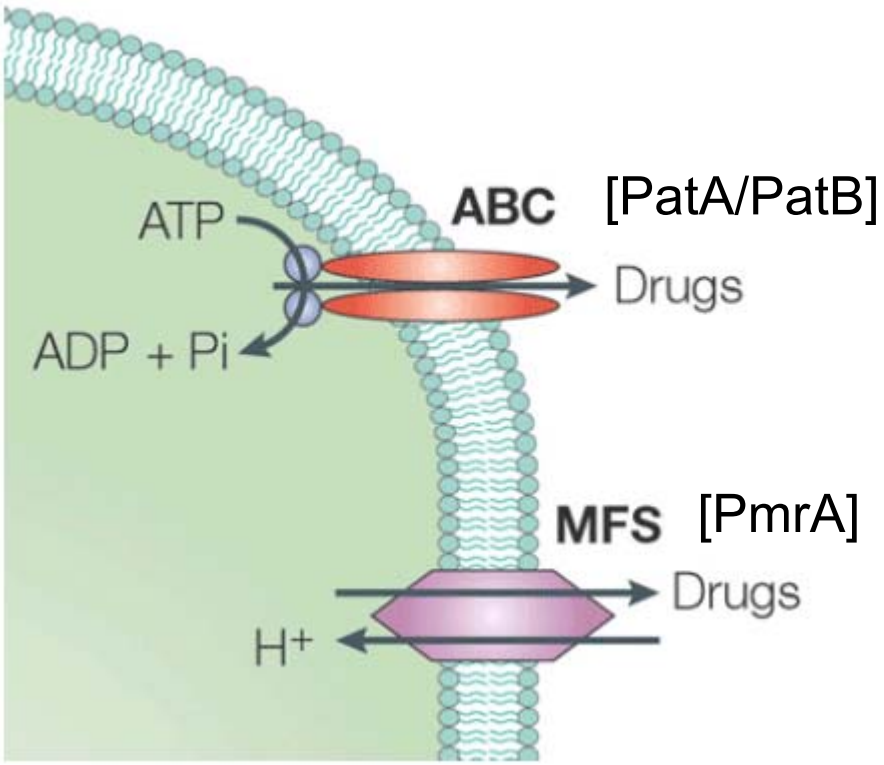
  → AB can be recognized as substrate
- Effect: decrease AB concentration within cell



→ less AB reach target

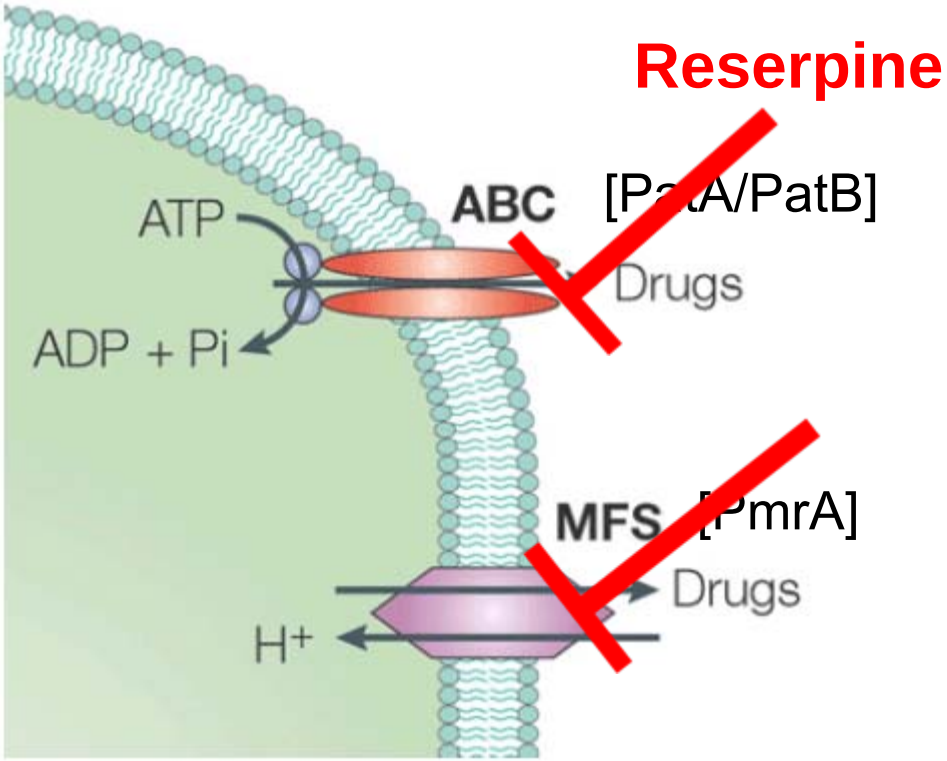
→ Low level resistance

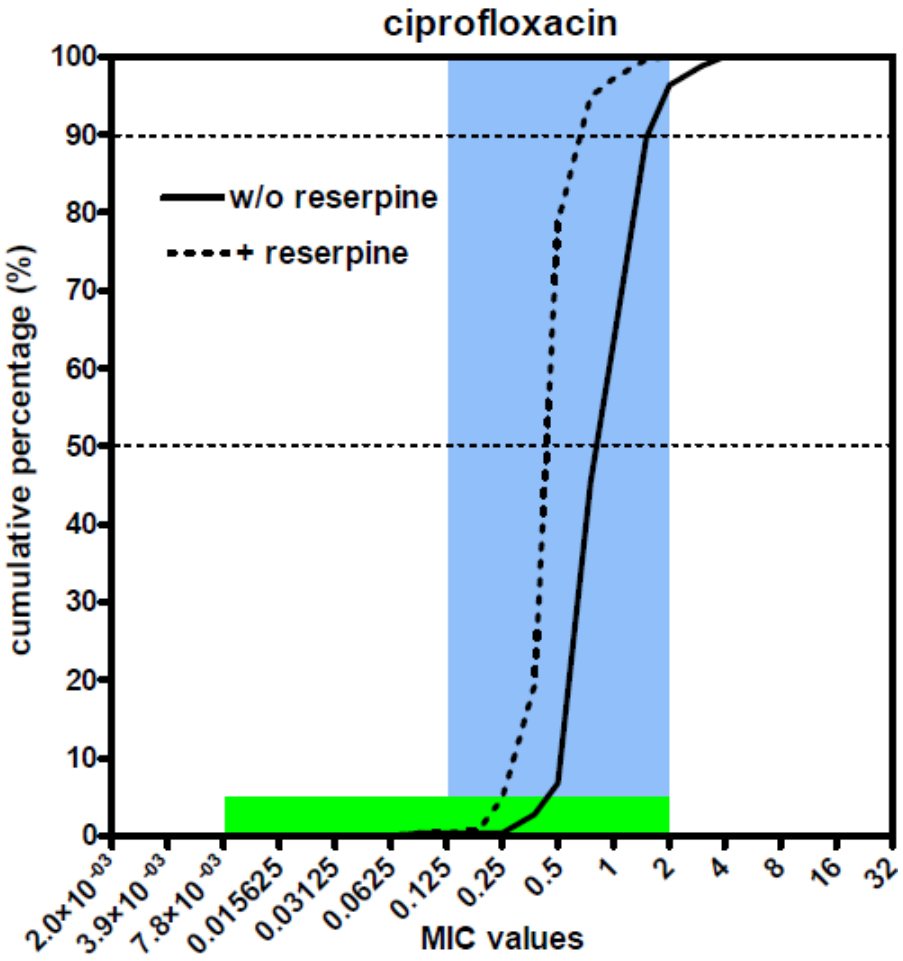
Efflux pumps



- Every bacterial genome has various pumps
- Same AB can be substrate of different pumps
- Narrow spectrum (Gram +) : 1 pump can recognize 1 AB class
- Substrate specificity varies within 1 AB class

Fluoroquinolones active efflux in *S. pneumoniae*



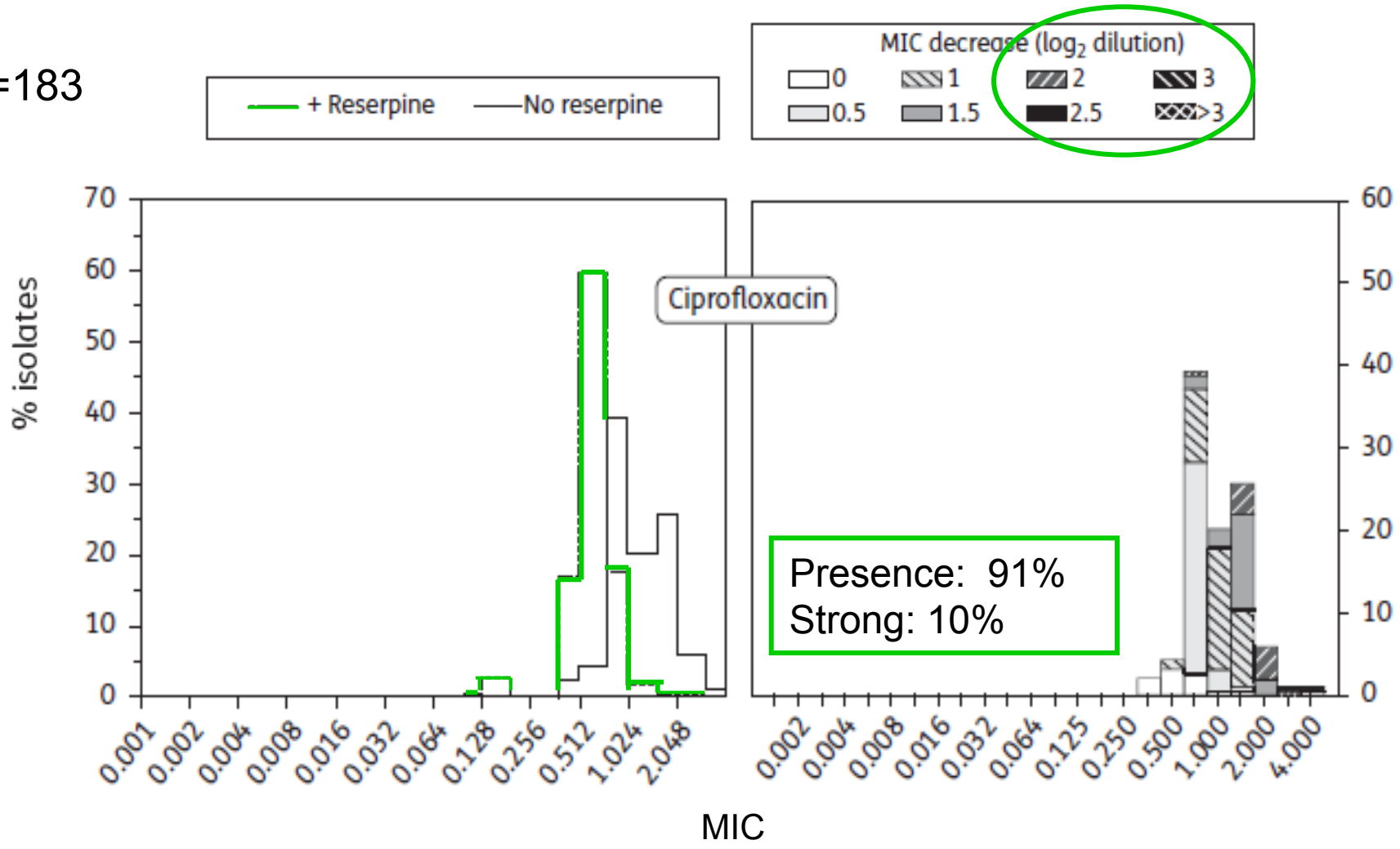


3 FQ transporters described:

- PmrA (Gill *et al.* 1999)
- PatA] (Marrer *et al.* 2006)
- PatB]

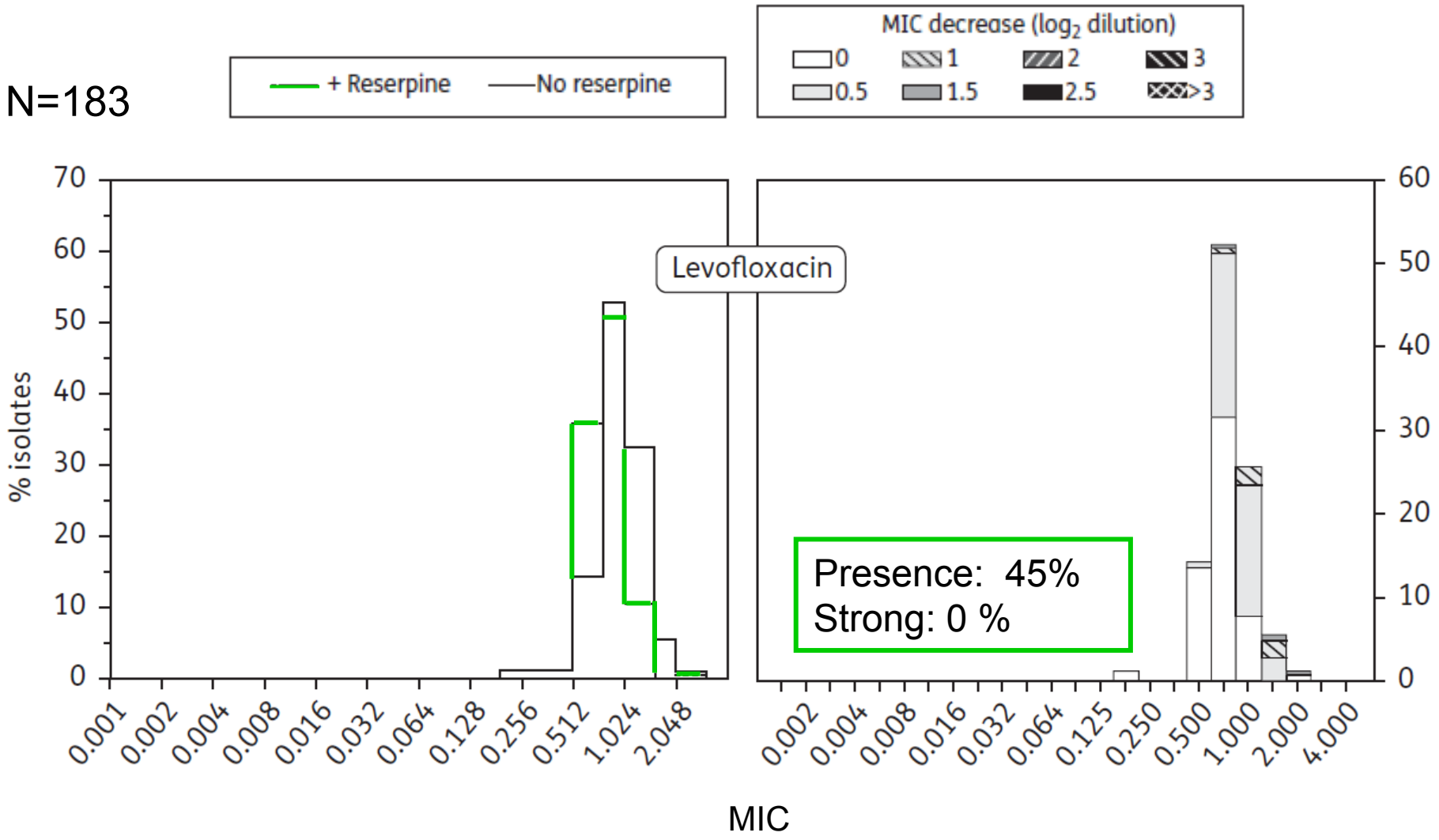
Prevalence & clinical relevance?

N=183



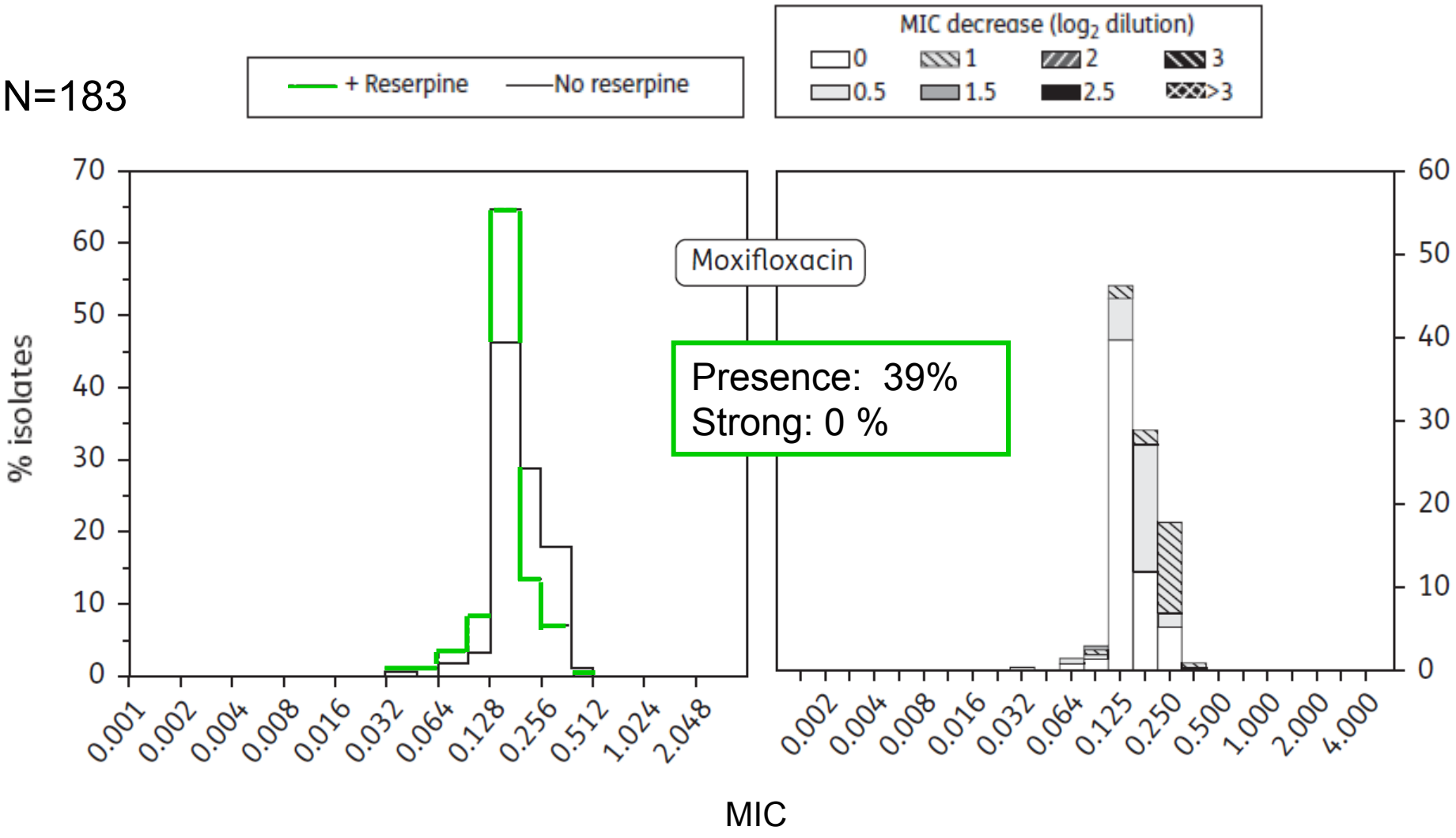
Prevalence & clinical relevance?

N=183

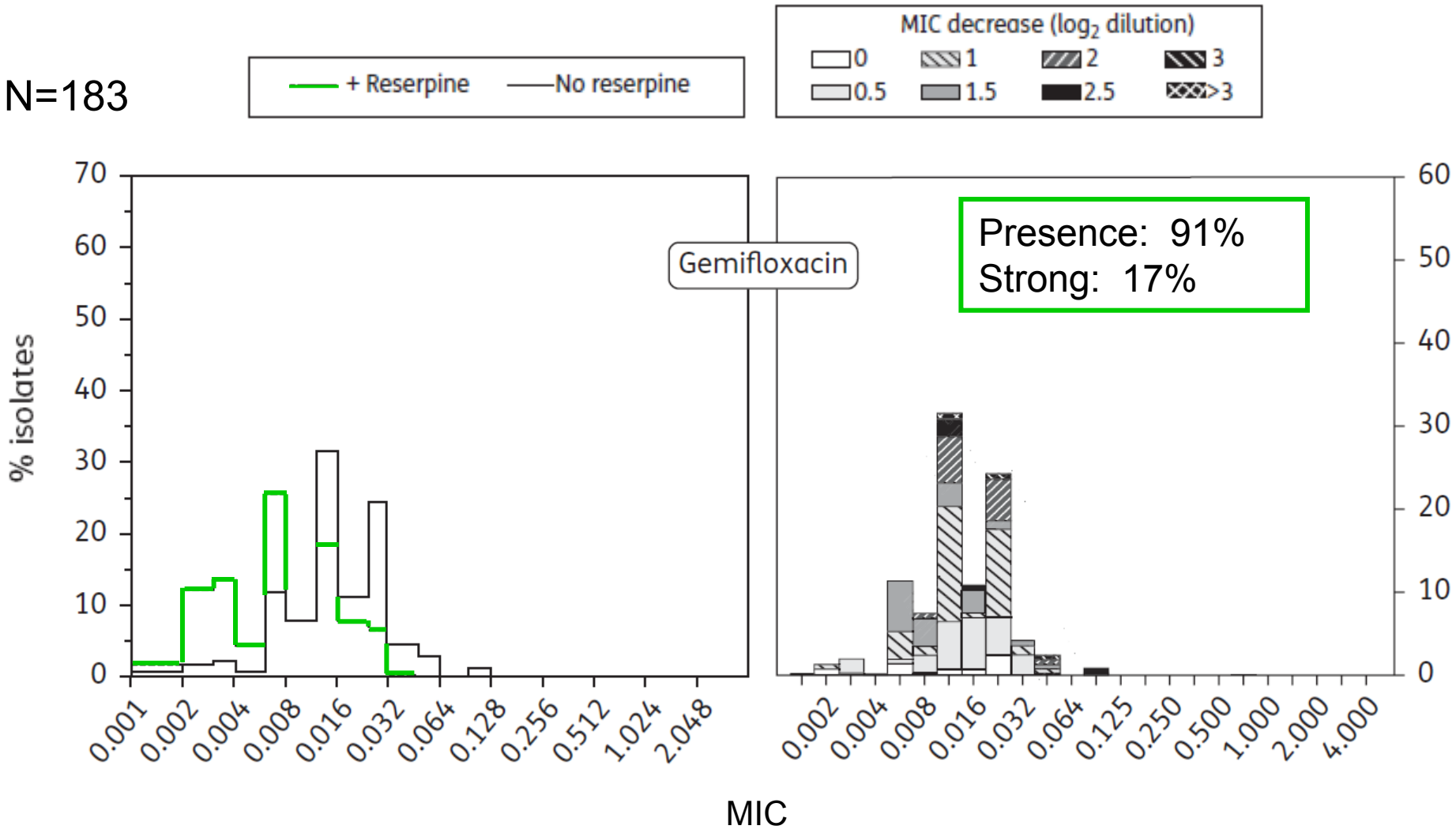


Prevalence & clinical relevance?

N=183



Prevalence & clinical relevance?



Prevalence & clinical relevance?

		CIP	LVX	MXF	GAR	GEM
	Efflux (%)					
	present	91	45	39	61	91
	≥2 dil°	10	-	-	-	17
EUCAST	Breakpoints					
S≤ /R>	S≤ /R>	0.12 / 2	2 / 2	0.5 / 0.5	wt ≤ 0.12	*0.12 / 0.5*
CLSI	I / R (%)	98 / 2	-	-	0.5 > wt	-
S≤ /R≥	S (%)	0	100	100	99.5 wt	100

Efflux presence? → YES!

Prevalence? → 39 to 91% depending on fluoroquinolone

Effect? → Modest >< Strong: CIP (10%) & GEM (17%)

Clinical significance? → None for anti-pneumococcal fluoroquinolones (MXF, LVX & GEM) → MIC < bkpt

→ Risk of selection of resistant strains

OBJECTIVES

- Pneumonia treatment optimal?
  - Antibiotics recommended ~ resistance ?
  - Vaccine coverage ~ prevalent serotypes ?
  - Interest of new molecules in development ?

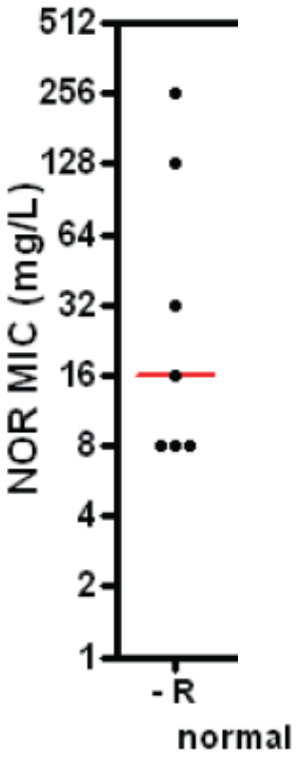
- Fluoroquinolones active efflux:
  - Prevalence & clinical relevance?
  - Identification of transporter & Substrate specificity?
  - Expression & Inducibility?

Identification of transporter & substrate specificity

Pump? → PatA/PatB > > PmrA

heterodimer

Specificity? NOR / CIP >> GEM > LVX > MXF



OBJECTIVES

- Pneumonia treatment optimal?
  - Antibiotics recommended ~ resistance ?
  - Vaccine coverage ~ prevalent serotypes ?
  - Interest of new molecules in development ?

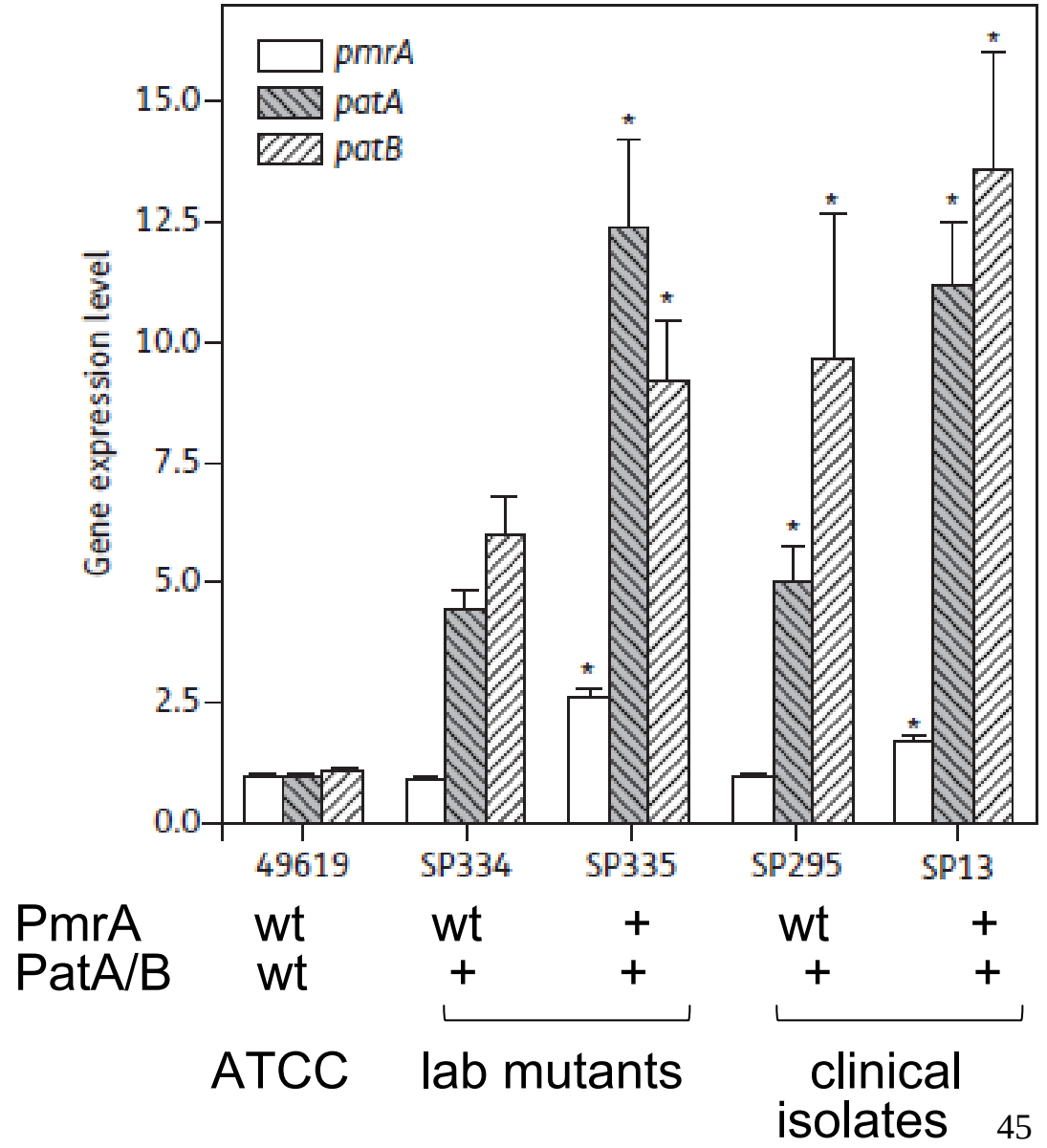
- Fluoroquinolones active efflux:
  - Prevalence & clinical relevance?
  - Identification of transporter & Substrate specificity?
  - Expression & Inducibility?

Expression & Inducibility?

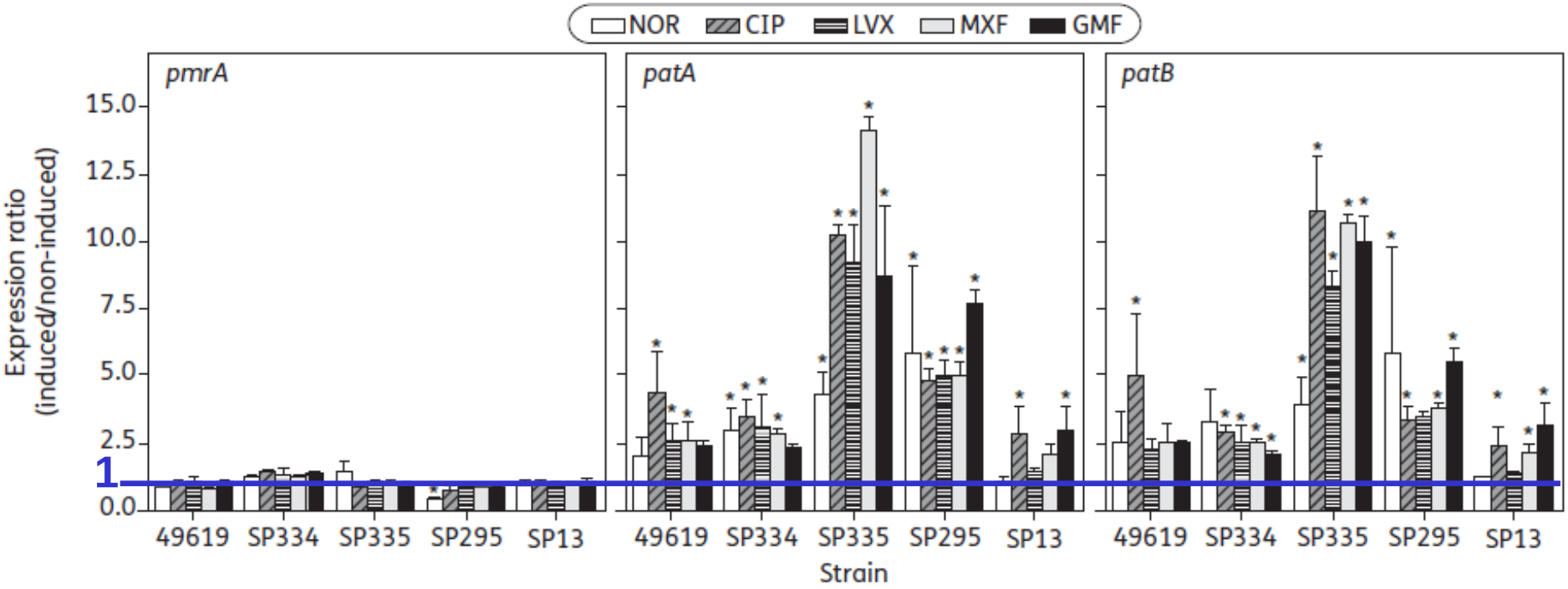
	CIP
ATCC-49619	0.5
SP-334	4
SP-335	32
SP-295	2
SP-13	16

Over-expression?

→Impact on MICs
→Also in clinical isolates



Expression & Inducibility?



Induction? →Only patA & patB
→By all FQ tested, even if not substrates
→In lab strains & in clinical isolates

→Reversible
→Time & dose dependant

CONCLUSIONS & PERSPECTIVES

- Pneumonia treatment
  - Continuing surveillance of **antibiotics** susceptibilities and **serotypes** distribution!

    → **local & up-to-date epidemiology**
  - Guidelines to review regularly:
    - Antibiotic resistance rate (CFX)
    - Availability of new molecules (SOL)
  - Vaccines:
    - Formulation to update regularly
    - Excellent coverage for children (PCV13)
    - PCV13 is ‘accepted’ for adults while should be ‘recommended’
    - PCV13 given earlier: from 50y
    - New vaccine independent from capsular polysaccharides?

CONCLUSIONS & PERSPECTIVES

- Fluoroquinolones active efflux:
  - Present but not clinically relevant (MXF, LVX)
  - Transporter = heterodimeric PatA/PatB (> PmrA)
  - Over-expression impacts MIC
  - Even non-substrates can induce over-expression

    → important for design of new molecules:

    non-substrates + non-inducers!

  - Can non-antibiotics induce over-expression?
  - In the clinics: can efflux be triggered by previous antibiotic treatment?

    → new collection from AECB

Thank you !