# *Pseudomonas aeruginosa* pneumonia: from microbial physiopathology to treatment

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# *Pseudomonas aeruginosa*

## The facts:

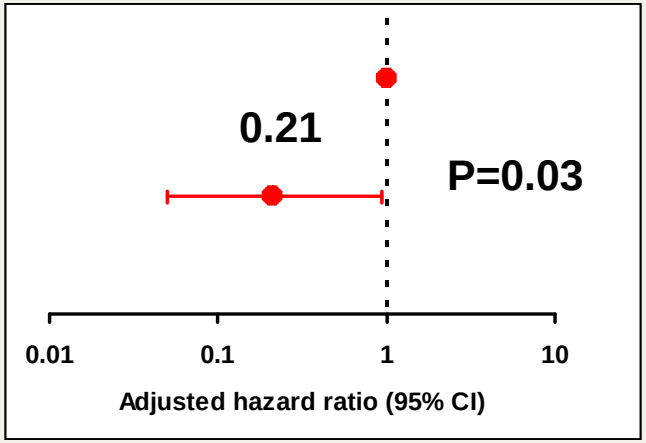
- **opportunist pathogen**
- responsible for ~30% of nosocomial infections
  - ↳ 47% of ventilator associated pneumonia (VAP)
  - ↳ leading cause of bacteremia associated with high mortality (> 40%)
- therapeutic approaches are limited because of:
  - ↳ broad intrinsic antimicrobial resistance
  - ↳ its tendency to rapidly acquire resistance during antimicrobial therapies

# Impact of primary infection site on mortality

| Primary site | Cases | Mortality % | P-value |
|---|---|---|---|
| Unknown | 58 | 13 | - |
| Respiratory tract | 24 | 55 | 0.03 |
| Urinary tract | 22 | 0 | ND |
| Line infections | 5 | 0 | ND |

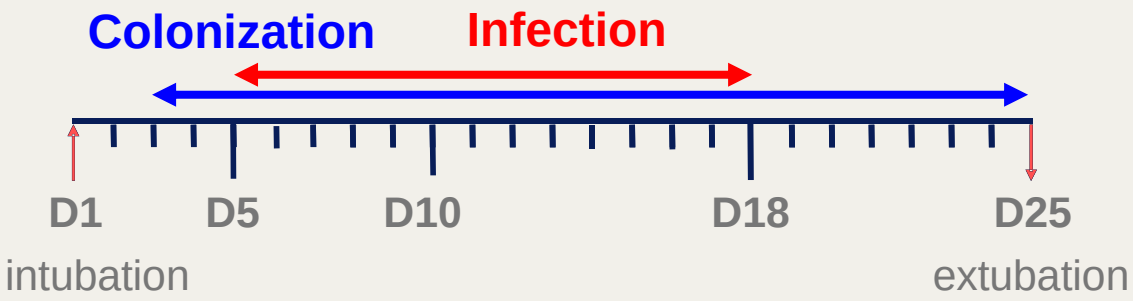
**Respiratory**

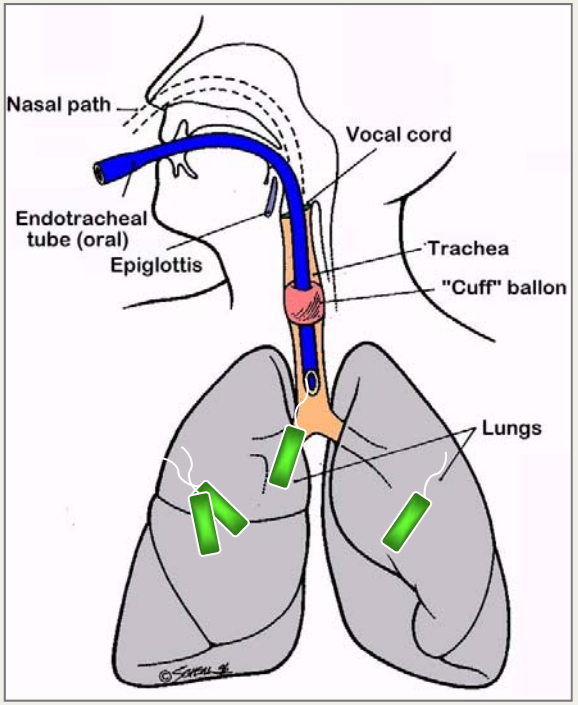
**Urinary/vascular**




*AAC* **2003**;47:2756

# *P. aeruginosa* and intubated patients




**Risk for colonization increases with time of intubation**

↓

**10-20% of colonized patients develop *P. aeruginosa* VAP**

↓

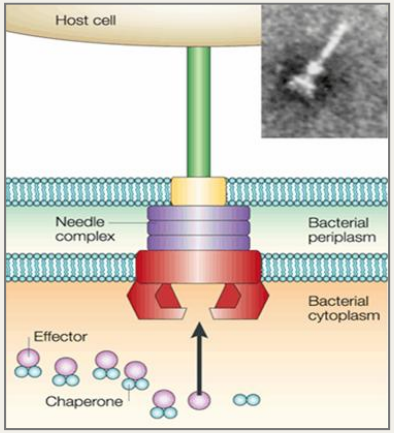
**30 - 40% mortality due to VAP**

# Are there microbiological determinants that influence the outcome of *P. aeruginosa* infections ?

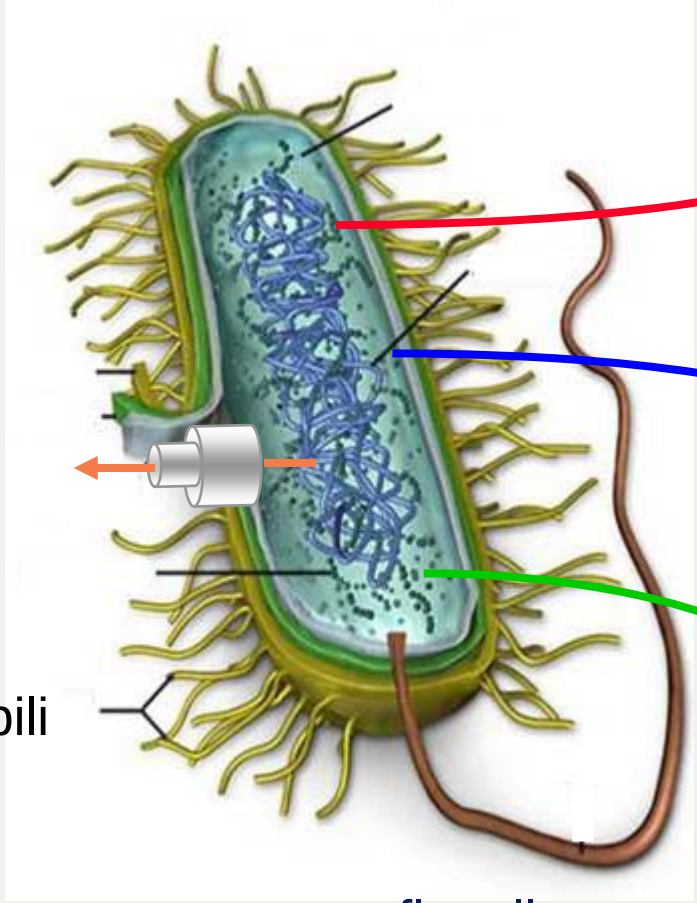
Is the expression of specific virulence determinants

(phenotypes) associated with a worse outcome ?

# Major virulence determinants




Cytotoxicity
TTSS



Type IV pili

flagellum

Siderophores:
pyoverdine
pyochelin

Quorum sensing

**elastase**
phospholipase C
lipase
> 100 genes

**rhamnolipids**
pyocyanin
cyanide
> 100 genes

# Could outcome be linked to specific strains ?

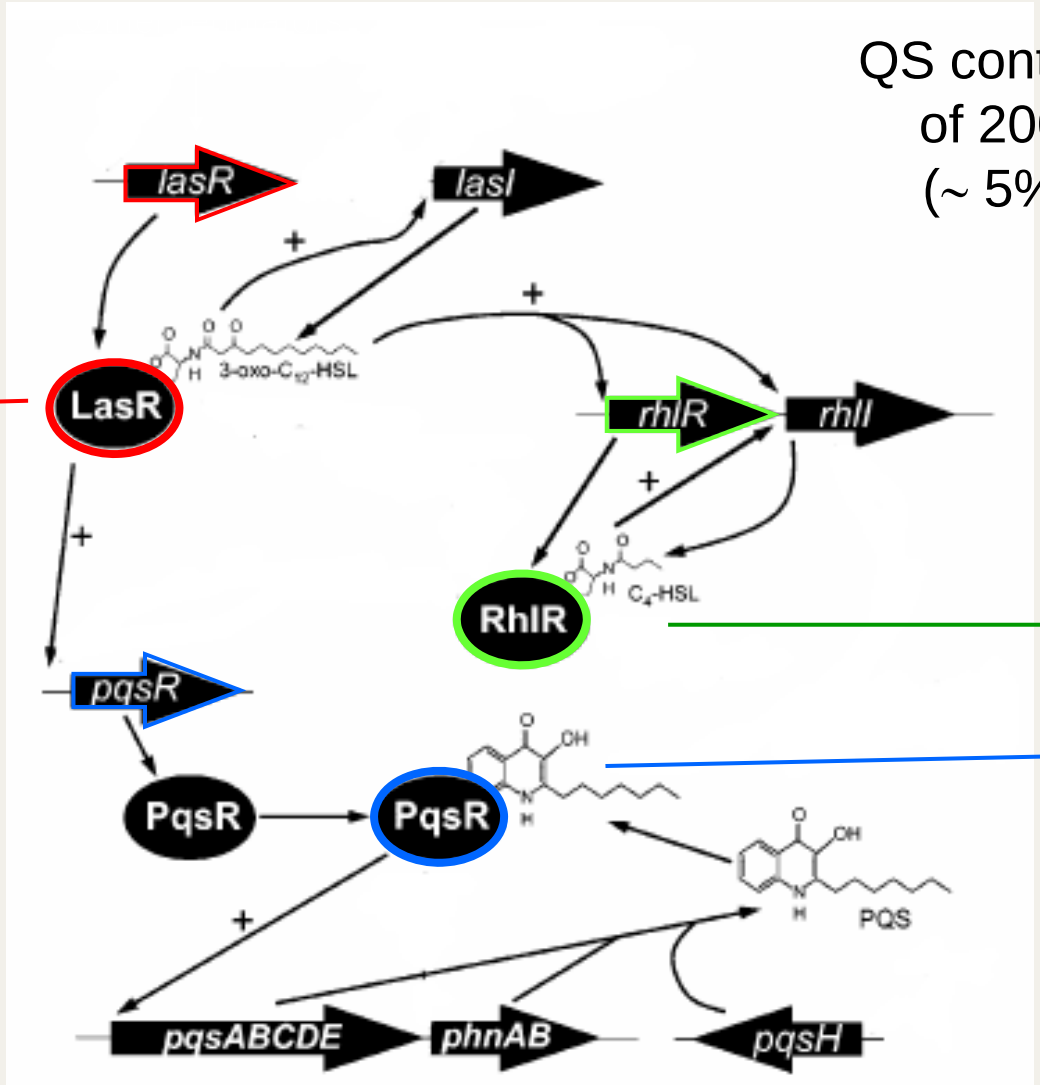
- Type III secretion system
  - 35 VAP isolates
    - 27 (77%) produced type III secreted proteins in vitro
      - 22 (81%): severe disease (death or relapse)
    - 8 strains didn't produce type III secreted proteins
      - 3 (38%): severe disease ($p<0.05$)
    - 10 strains produced ExoU
      - 9 (90%): severe disease
- VAP with isolates producing type III secretion-dependent exoproducts, especially ExoU, *in vitro* are associated with worse clinical outcome. However these studies didn't analyze whether cytotoxicity is associated with infections

*Crit Care Med* **2002**;30:521

# QS regulation in *P. aeruginosa*

QS controls expression
of 200-300 genes
(~ 5% of genome)

elastase
lipase

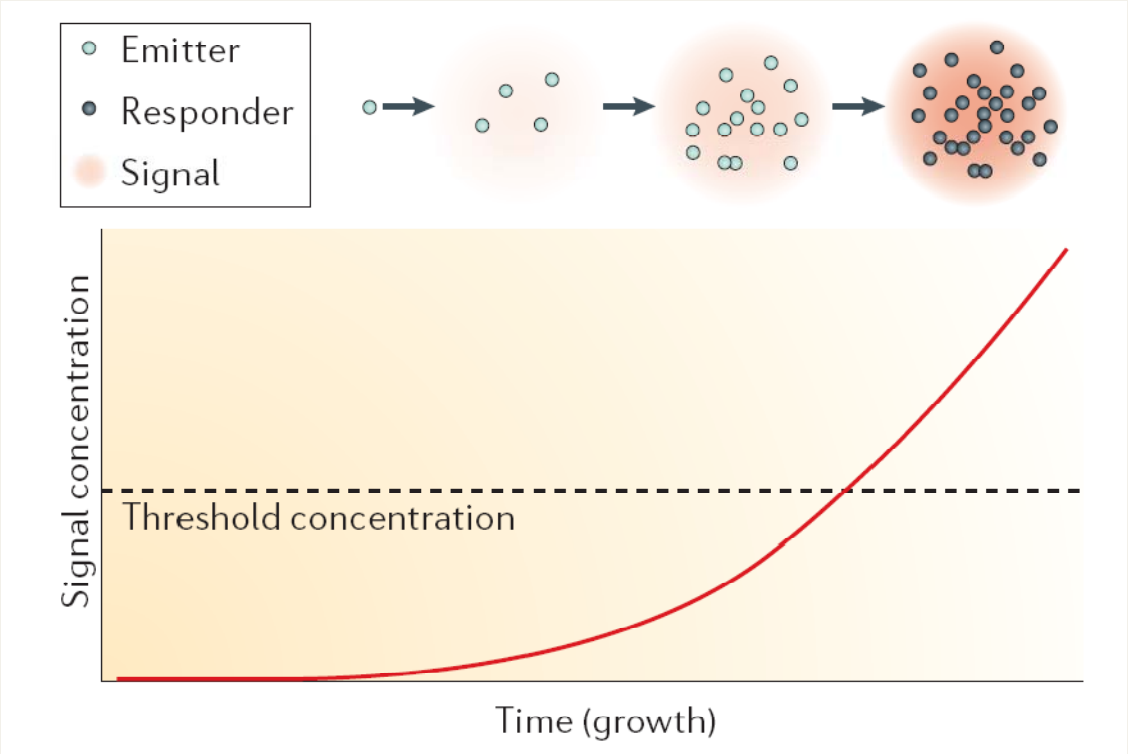



rhamnolipid
pyocyanin
lipase
cyanide

rhamnolipid
pyocyanin
cyanide
mexGHI-opmD

*Adapted from Wade et al. J. Bacteriol. 2005*

# Inter-cellular communication




Quorum

Allows a bacterial population to coordinate

Keller and Surette, Nat Rev Microbiol. 2006

# QS essential for *P. aeruginosa* virulence in...

Plants (*Arabidopsis*)
(Lettuce)

Nematodes (*C. elegans*)

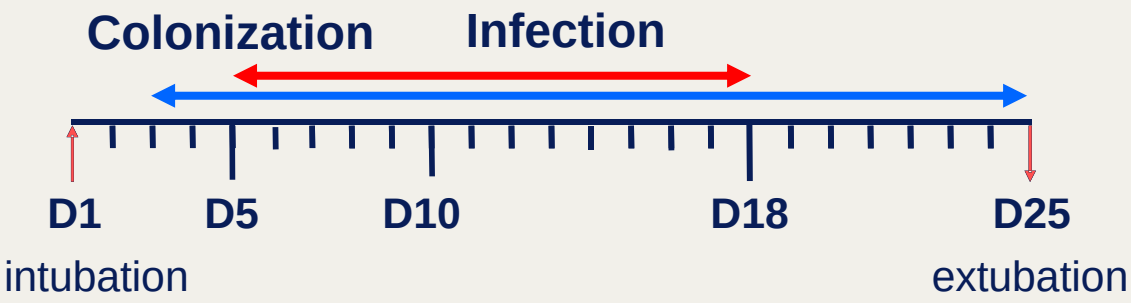
Insects (*Drosophila*)

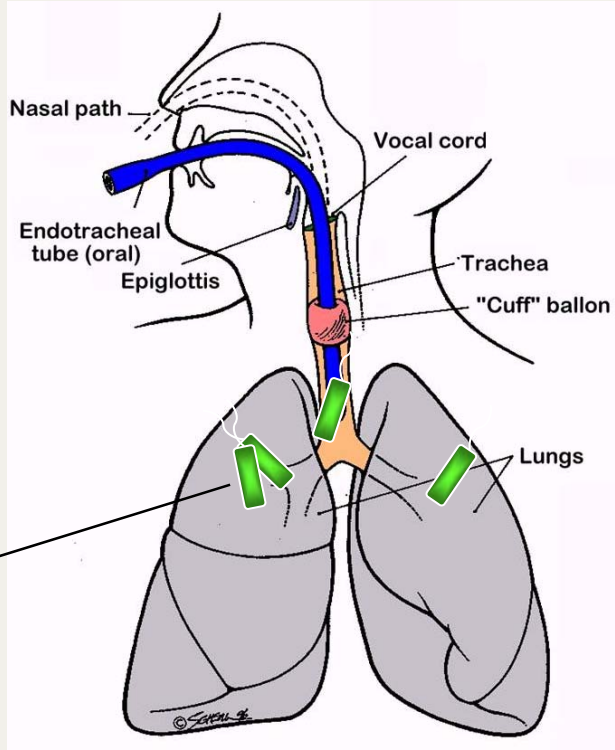
Amoeba (*D. discoideum*)

Mouse

Human infections

# Prospective study on P. aeruginosa colonization in the absence of antibiotic treatment




**13 European ICUs: 31 patients**

**Daily tracheal aspirate**

one *P. aeruginosa* isolate

- total genomic DNA
- total RNA
- autoinducer

# QS-proficiency and rhamnolipid production of initial colonizing isolates is associated with pneumonia in the placebo group

| Patient | % of PAO1 | | sequence | | Exo | PAPI-1 | PAPI-2 | SNP |
|---|---|---|---|---|---|---|---|---|
| | Ela | Rha | *lasR* | *rhlR* | S/U | | | type |
| PAO1 | 100 | 100 | wt | wt | S | 0 | 0 | 000A |
| 05101 | 151 | 116 | wt | wt | S | 0 | 0 | 6C26 |
| 19105 | 153 | 110 | wt | wt | S | 0 | 1 | 7C2E |
| 16101 | 189 | 104 | wt | wt | S | 0 | 0 | 0C2E |
| 13128 | 137 | 103 | wt | wt | S | 0 | 0 | 0C2E |
| 13111 | 109 | 101 | wt | wt | S | 0 | 2 | 85AA |
| 30101 | 345 | 91 | wt | wt | U | 2 | 3 | D421 |
| 24101 | 292 | 102 | wt | wt | S | 0 | 1 | 2C26 |
| 21107 | 24 | 91 | A231V | wt | S | 0 | 2 | 6D92 |
| 13108 | 61 | 88 | wt | wt | S | 0 | 1 | 1BAE |
| 13104 | 190 | 86 | wt | wt | S | 0 | 2 | 0C1A |
| 26102 | 152 | 75 | wt | wt | U | 0 | 2 | F469 |
| 13112 | 123 | 73 | wt | wt | S | 0 | 2 | C40A |
| 13122 | 122 | 90 | wt | wt | U | 0 | 2 | F661 |
| 15102 | 215 | 79 | wt | wt | U | 0 | 1 | E429 |
| 13106 | 127 | 72 | wt | wt | S | 0 | 2 | 4F8A |
| 19101 | 34 | 80 | Δ or IS | wt | U | 0 | 2 | F469 |
| 19102 | 47 | 80 | A231V | wt | S | 0 | 1 | 6D92 |
| 27104 | 24 | 78 | L110Q | wt | S | 0 | 2 | 0812 |
| 13118 | 42 | 41 | wt | wt | S | 0 | 2 | 239A |
| 10103 | 35 | 0 | 199IS | wt | U | 2 | 3 | D421 |
| 15101 | 89 | 0 | wt | wt | S | 0 | 1 | 0C2E |
| 13121 | 16 | 0 | wt | wt | S | 0 | 2 | 239A |
| 26104 | 25 | 0 | wt | T121I | U | 0 | 2 | F469 |
| 13114 | 28 | 0 | L148P | Δ64 bp | U | 0 | 2 | F469 |
| 13117 | 19 | 0 | L148P | Δ64 bp | U | 0 | 2 | F469 |
| 06104 | 0 | 0 | 47 IS | wt | S | 0 | 2 | AF9A |
| 27101 | 1 | 0 | Δ or IS | A111D | S | 0 | 0 | EC4A |
| 22101 | 1 | 0 | P74L | Δ or IS | U | 0 | 2 | F469 |
| 13116 | 1 | 0 | T222I | Δ64 bp | U | 0 | 2 | F469 |
| PA14 | 99 | 95 | wt | wt | U | 2 | 3 | D421 |

| | % of PAO1 | | |
|---|---|---|---|
| Ela,Rha | >90% | 90-10% | <10% |

*Thorax* **2010**;65:703

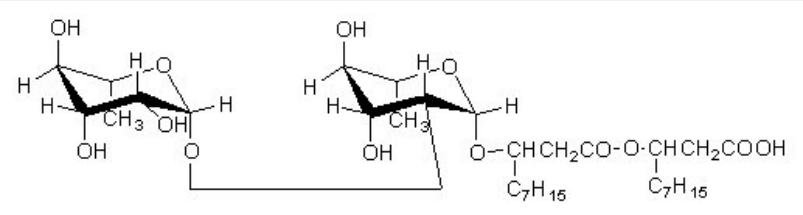
**Table 1 : Virulence determinants in VAP and non-VAP patients**

| **Expression or presence of virulence determinant** (total number of patients with a positive initial isolate) | **VAP (n=6)** | **Non-VAP (n=23)** | **P Value** |
|---|---|---|---|
| Elastase (13)* | 4 | 9 | 0.364 |
| Rhamnolipid (8)* | 5 | 3 | 0.003 |
| *exoS* (18)† | 5 | 13 | 0.362 |
| *exoU* (11)† | 1 | 10 | 0.362 |
| PAPI-1 (2)† | 0 | 2 | 1.000 |
| PAPI-2 (25)† | 4 | 21 | 0.180 |

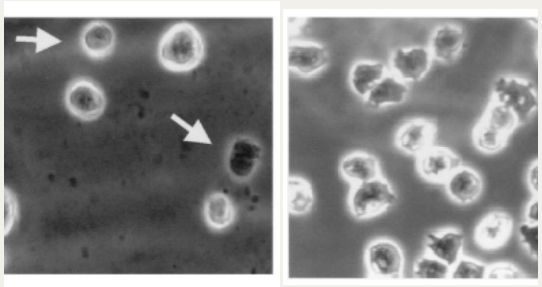
- 57% of patients initially colonized by QS-proficient isolates versus 9% colonized by QS-deficient isolates developed VAP (P= 0.018)

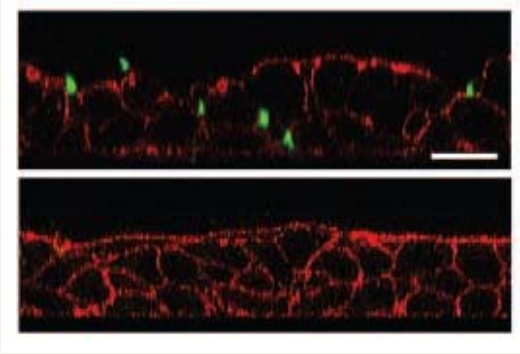
- Production of the QS-dependent virulence factor rhamnolipids is associated with VAP (P= 0.003)

# Role of rhamnolipids



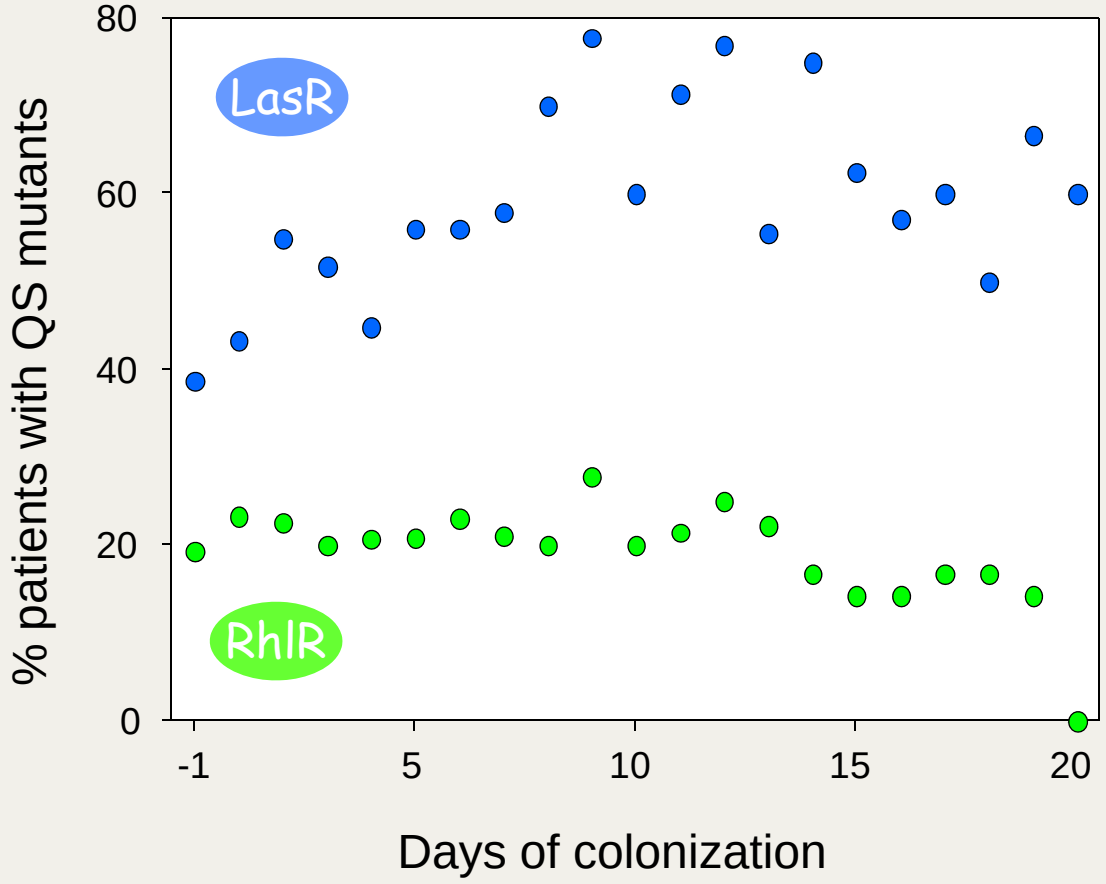

- Uptake of hydrophobic molecules (1992)
- Surfactant for swarming motility (2000)
- Lysis of amoeba (*D. discoideum*) (2002)
- Maintain biofilm structure (2003)
- Disrupt tight junctions in human airway epithelia (2006)
- Lyse PMNs *in vitro* (2007)





# QS-deficient isolates (LasR mutants) increase during colonization

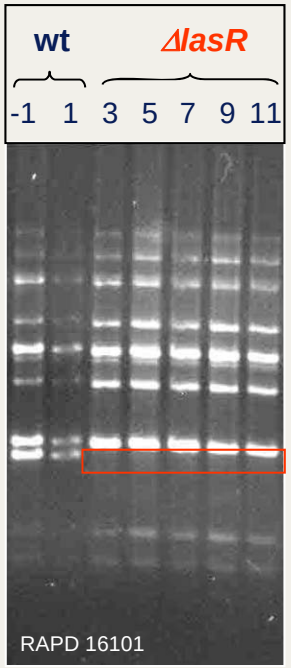
31 placebo patients

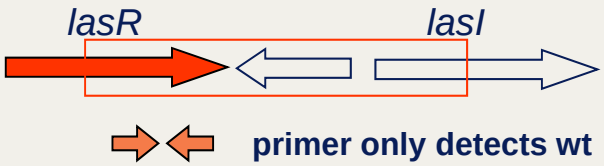



*PNAS* **2009**;106:6339

# *In patient* population dynamics: **one** genotype

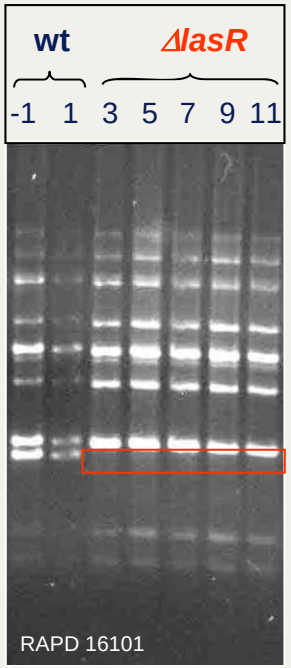
**Isolate (*in vitro*)**



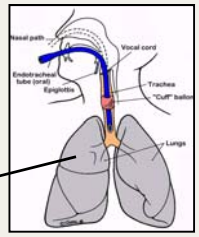
# *In patient* population dynamics: **one** genotype

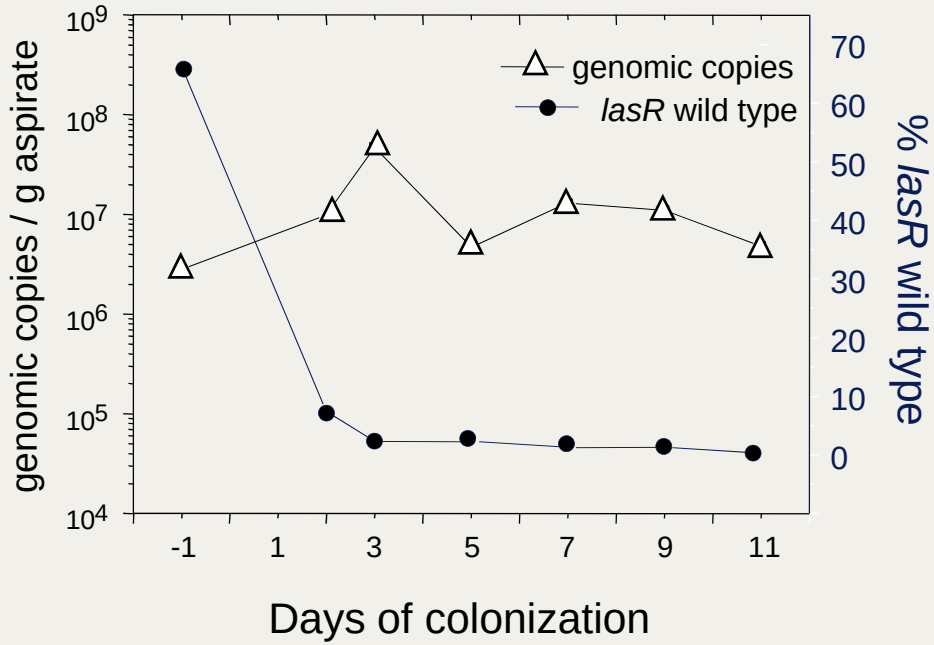
**Isolate**
***in vitro***

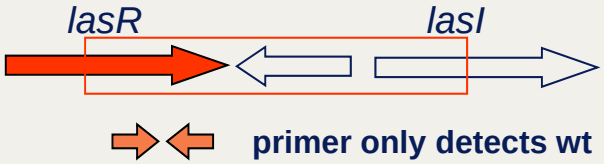



**Population**
***in patient***

**Genomic DNA**





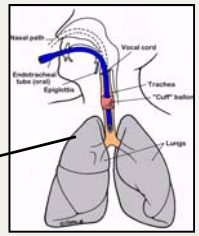




… *lasR* mutants dominant in the population !!!

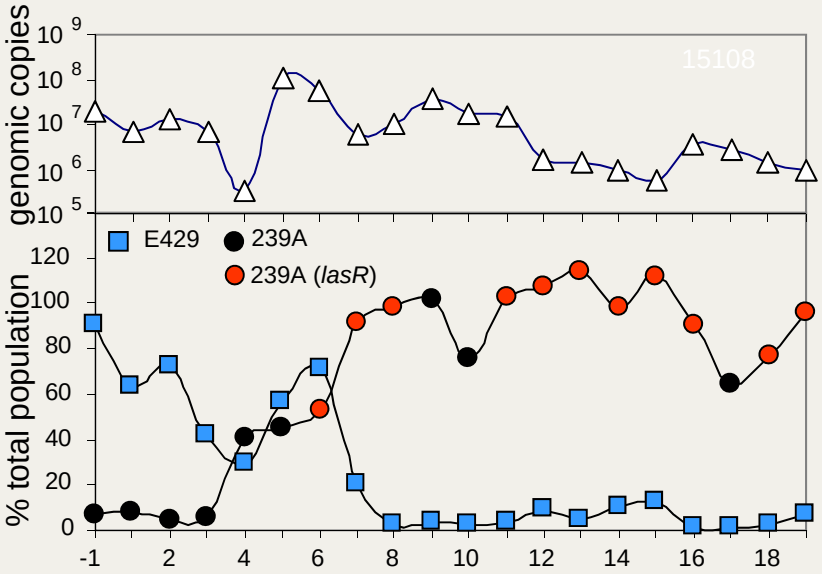
# *In patient* population dynamics: **two** genotypes

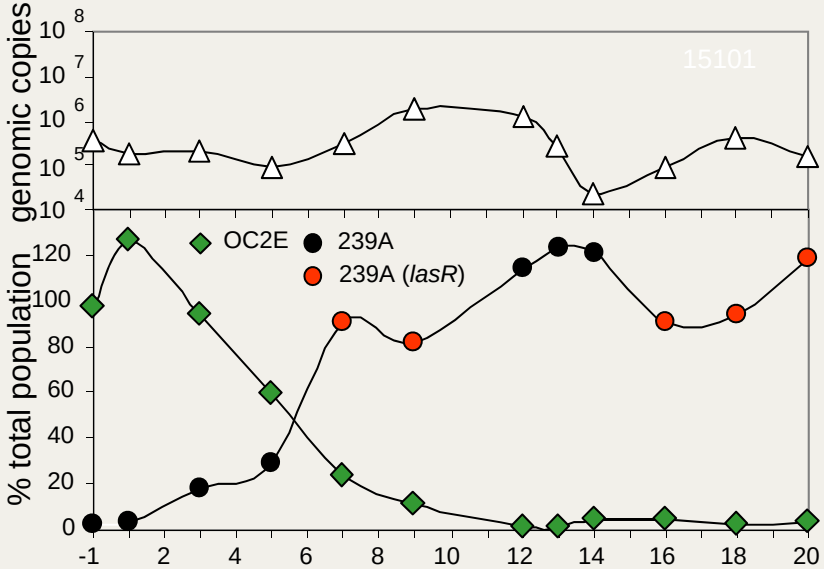
**Population**
***in patient***

**Genomic DNA**



● *lasR* wt ● *lasR* mutant

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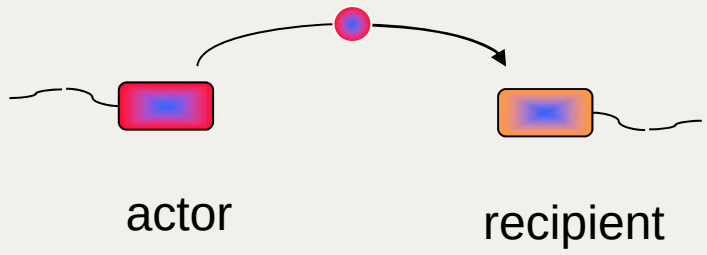
# Bacterial social behaviours




| | | Effect on recipient | |
|---|---|---|---|
| | | pos | neg |
| Effect on actor | pos | mutual benefit | selfishness |
| | neg | altruism | spite |

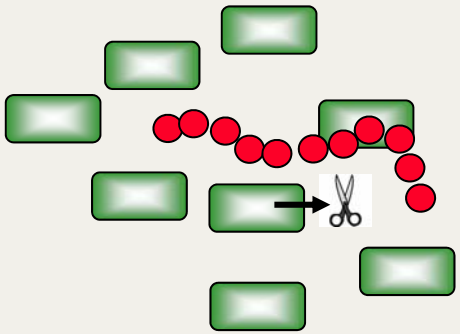
Signal : elicits response in recipient, induced response is beneficial for the actor

Public good : resource that is costly to produce and provides benefit to all individuals in the population

Cooperation : behavior that benefits another individual (recipient) and that is maintained because of its beneficial effect on the recipient

Cheater : individual who does not cooperate, but gain benefit from others cooperating

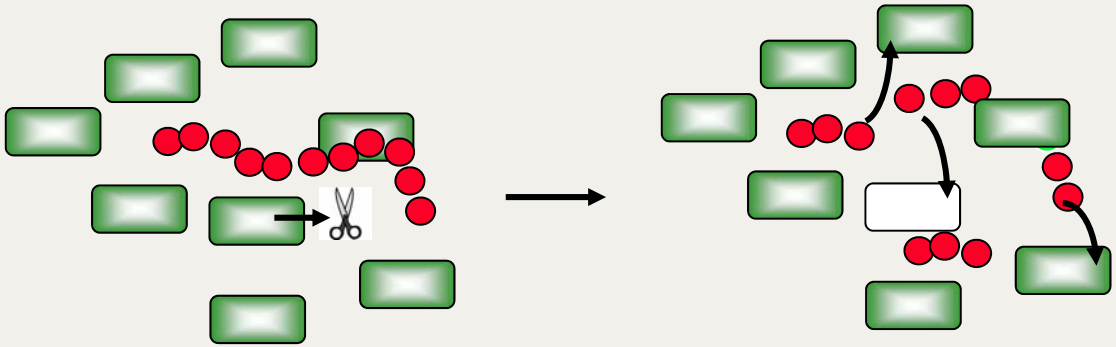
# Why do *lasR* mutants outcompete wt ?




**Cooperator**
(ex: QS wild type isolate)

**Public goods**
(ex: polypeptides, produced by elastase)

# Quorum sensing as a social behavior

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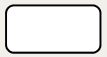

**Non-cooperator** or **cheater**
(ex: a *lasR* mutant)

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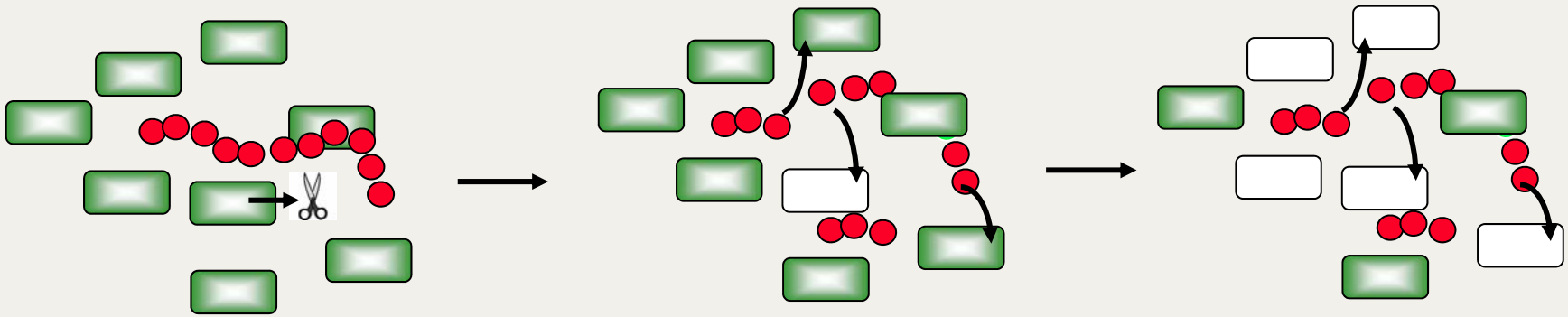


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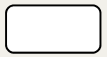
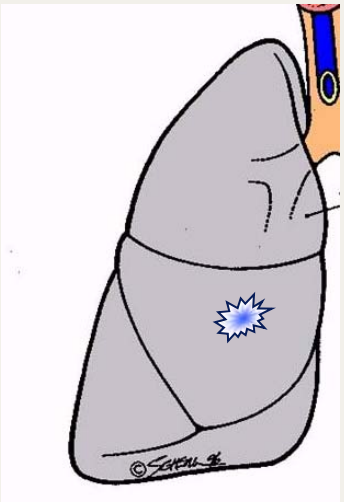


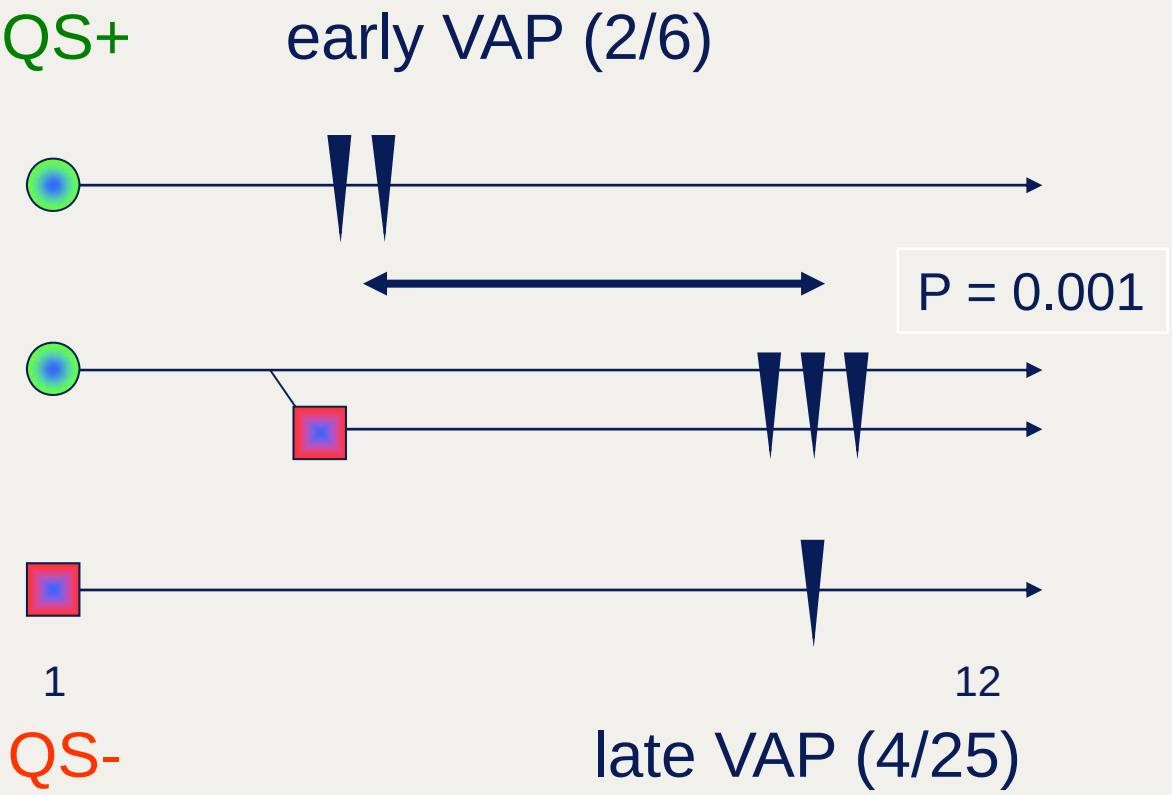
**Non-cooperator** or **cheater**
(ex: a *lasR* mutant)

**QS cheaters (*lasR* mutants) have fitness advantage BUT only in the presence of QS cooperators !!**

*PNAS* **2009**;106:6339

# QS is important for development of VAP





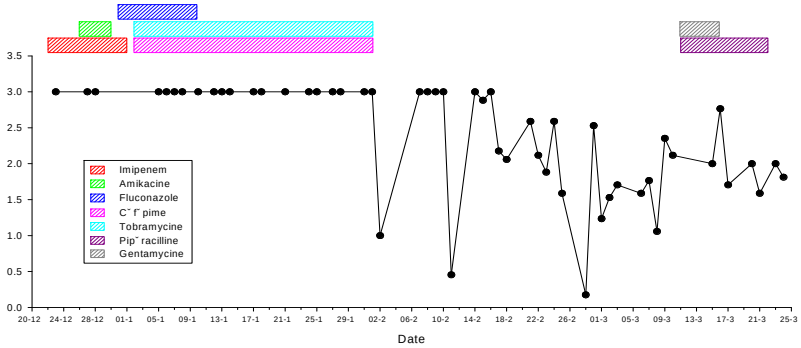

- VAP occurs earlier in patients colonized by QS-proficient isolates

- Progressive accumulation of QS-deficient isolates might protect from VAP

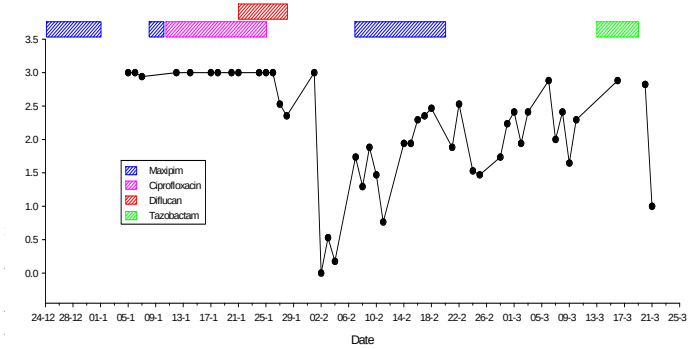
*PNAS* **2009**;106:6339

# Antibiotic therapy and virulence factor production

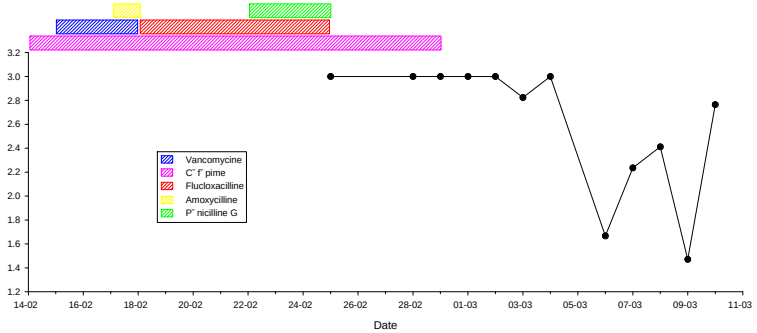
## Patient A




## Patient B




## Patient C

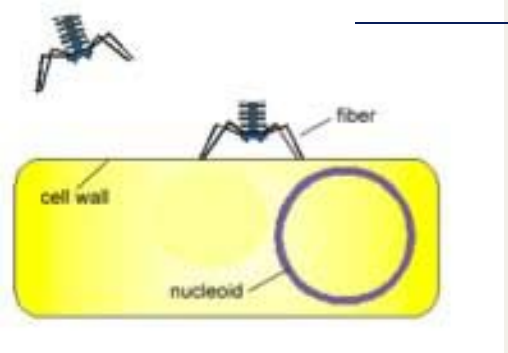



*Van Delden et al*, unpublished results

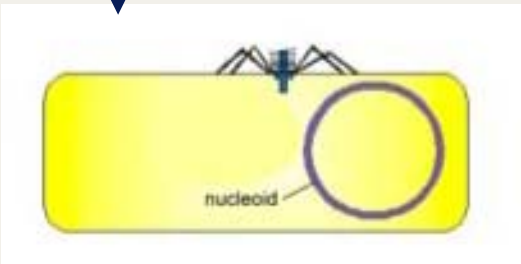
*Conclusion*:

1. fluctuations of quorum-sensing dependent virulence factor production appear after discontinuation of antimicrobial therapies
2. antimicrobial therapies might select quorum-sensing proficient isolates

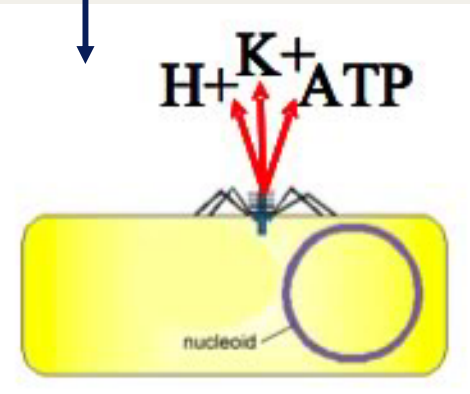
# Bacterial warfare: R-pyocin mediated killing



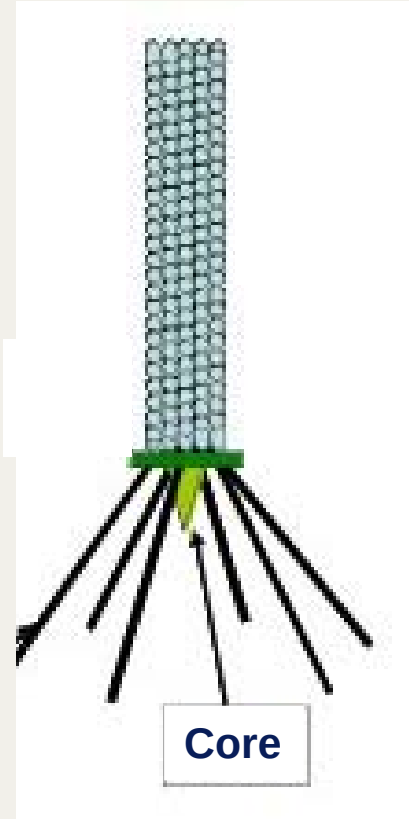

Landing



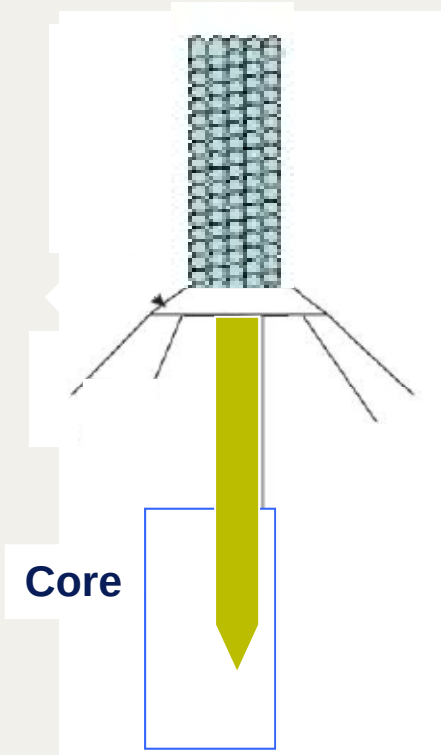

Drilling




Killing

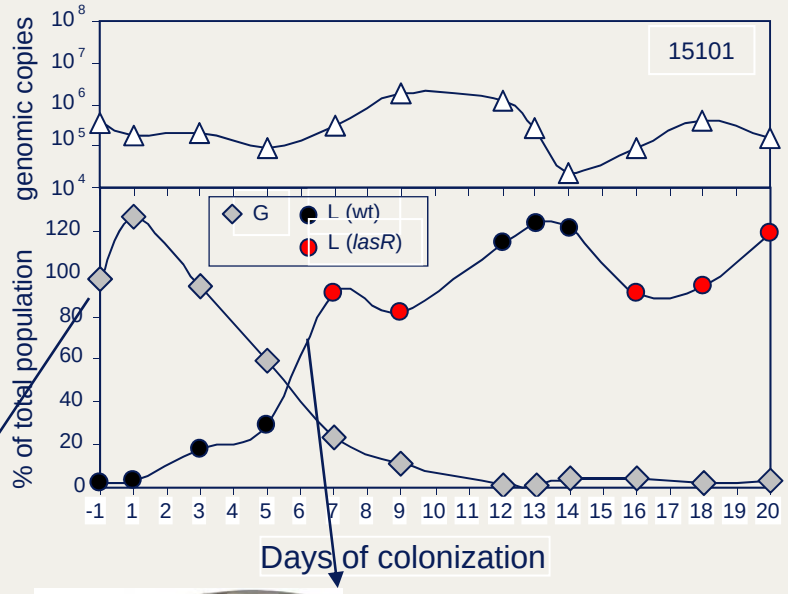


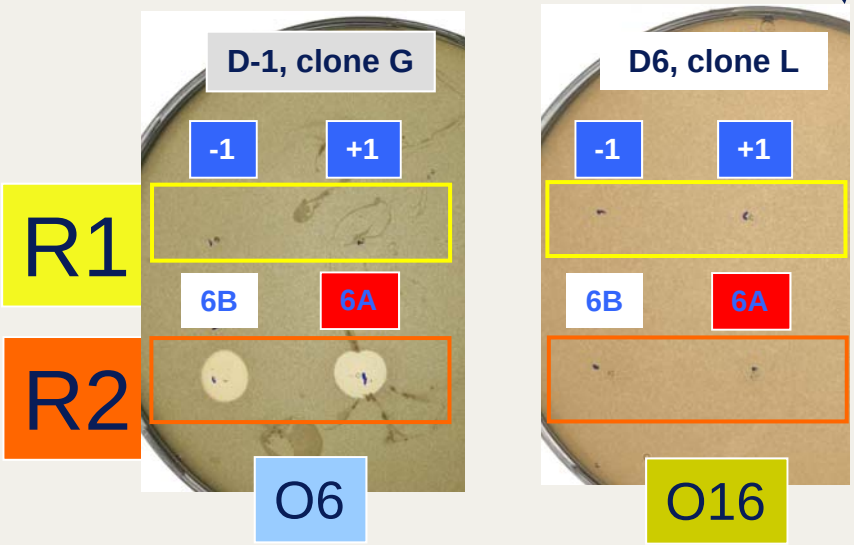

relaxed




contracted

# R-pyocin warfare *in vivo* ?

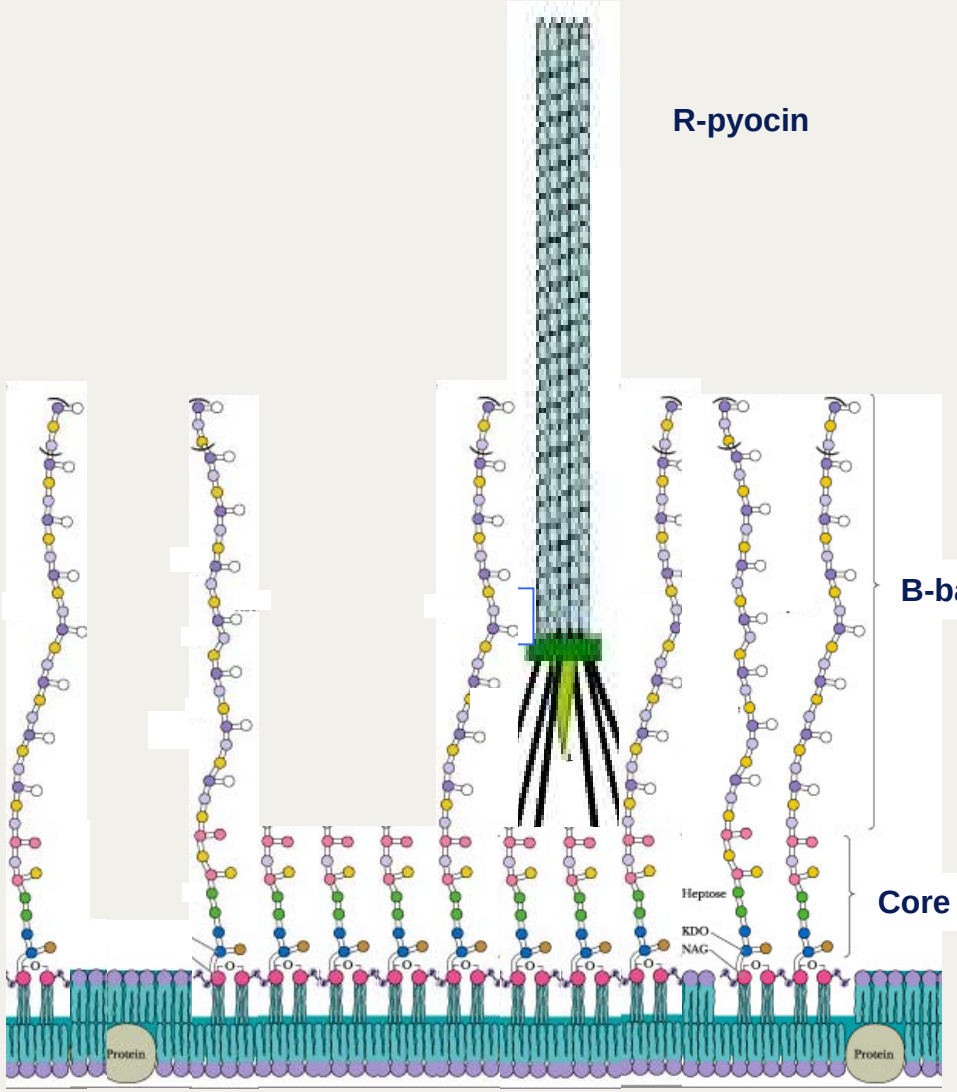


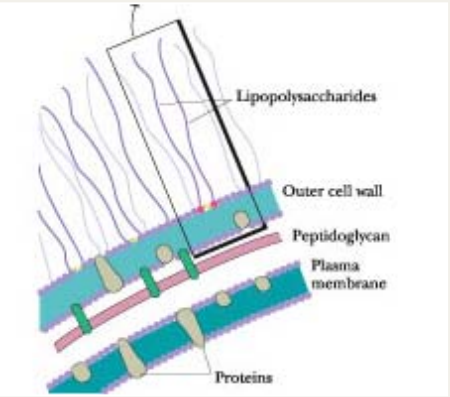




**Initial clone G is killed by clone L by R2 pyocin**

*J Bact* **2010**;192:1921

# Working model for R-pyocin – LPS interaction




**Shield**

**Receptor**

**Other serotypes: receptors may be the same, but shielding differs according to B-band charge and packaging**

# Summary

- Phenotype and NOT genotype associated with *P. aeruginosa* VAP
- Rhamnolipid production (*rhlR* QS system) high risk factor for VAP
- *P. aeruginosa* adapts to lung environment by mutation of *lasR*

  → Many patients co-colonized by wt and *lasR* mutants

  → *lasR* mutants: social « cheaters » or part of cooperative strategy ?

  → one genotype: *lasR* mutant out-competes wild-type population

  → multiple genotypes: other factors such as bacterial warfare determine population dynamics

# How should we treat Pseudomonas infections ?


# Resistance of *P. aeruginosa* can be predicted

| Antipseudomonal agent, included in previous therapy | Resistance of the bacteremic strain to this agent: Yes (cases) | No (controls) | OR (95% CI) | *P* |
|---|---|---|---|---|
| Ceftazidime | | | | |
| Yes | 3 | 5 | — | |
| No | 13 | 246 | 11.4 (1.6–64.7) | .008 |
| Piperacillin[a] | | | | |
| Yes | 3 | 6 | — | |
| No | 26 | 231 | 4.4 (0.67–22.1) | .06 |
| Imipenem[a] | | | | |
| Yes | 11 | 25 | — | |
| No | 30 | 186 | 2.7 (1.1–6.5) | .02 |
| Ciprofloxacin | | | | |
| Yes | 0 | 9 | — | |
| No | 15 | 243 | 0.0 (0.0–9.1) | 1.0 |
| Aminoglycoside | | | | |
| Yes | 6 | 26 | — | |
| No | 37 | 198 | 1.2 (0.39–3.4) | .61 |

**Table 3. Multivariate association, averaged across antipseudomonal agents, of previous exposure to an agent, and resistance to that same agent in 267 bacteremic strains of *Pseudomonas aeruginosa*.**

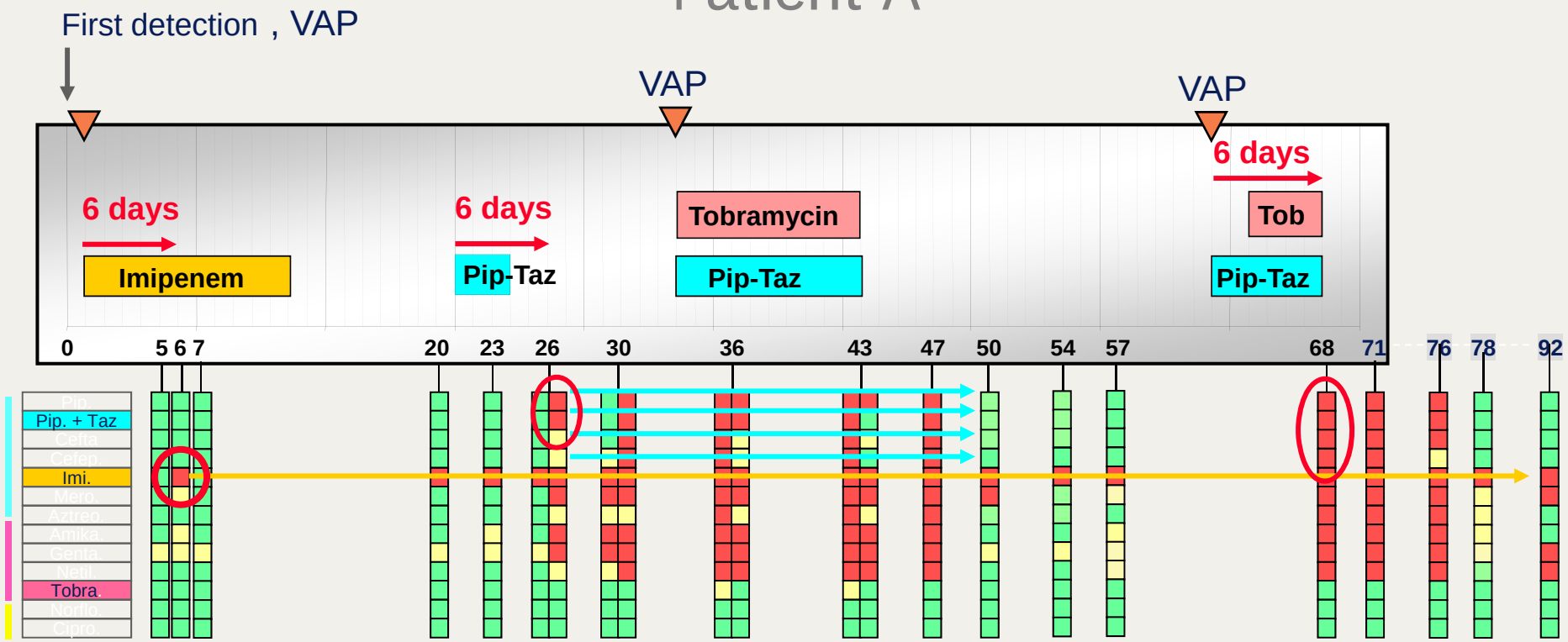
| Characteristic | Adjusted OR (95% CI) | *P* |
|---|---|---|
| Previous monotherapy with the agent | 2.5 (1.3–4.8) | .006 |
| Previous combination therapy including the agent | 1.8 (0.55–5.6) | .34 |
| Severe sepsis or septic shock | 1.6 (0.94–2.6) | .08 |

*CID* **2001**;33:1859

*Conclusion*: preceding ceftazidime and imipenem exposure, especially as monotherapy, was associated with resistant *P. aeruginosa* bacteremic isolates

# Evolution of antibiotic resistance

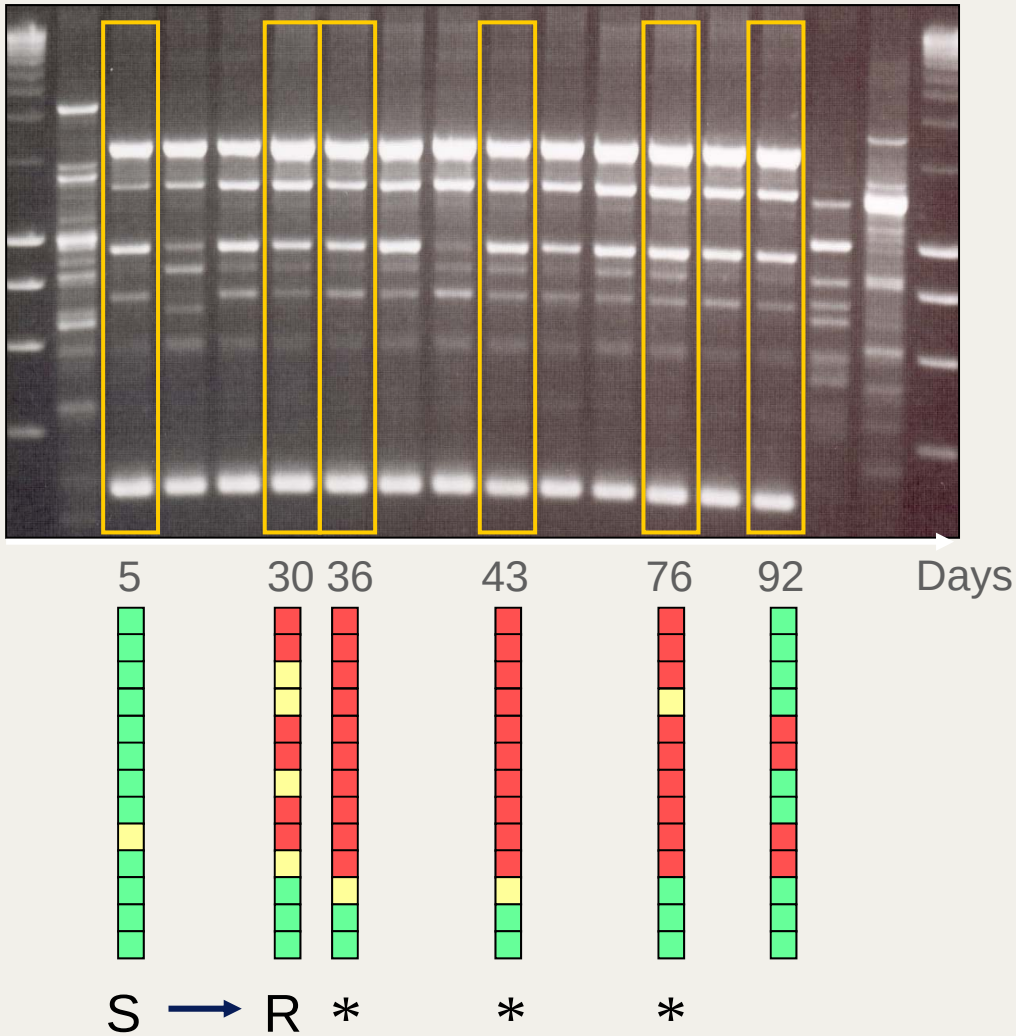
## Patient A



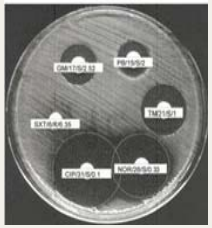

- Susceptible
- Intermediate
- Resistant

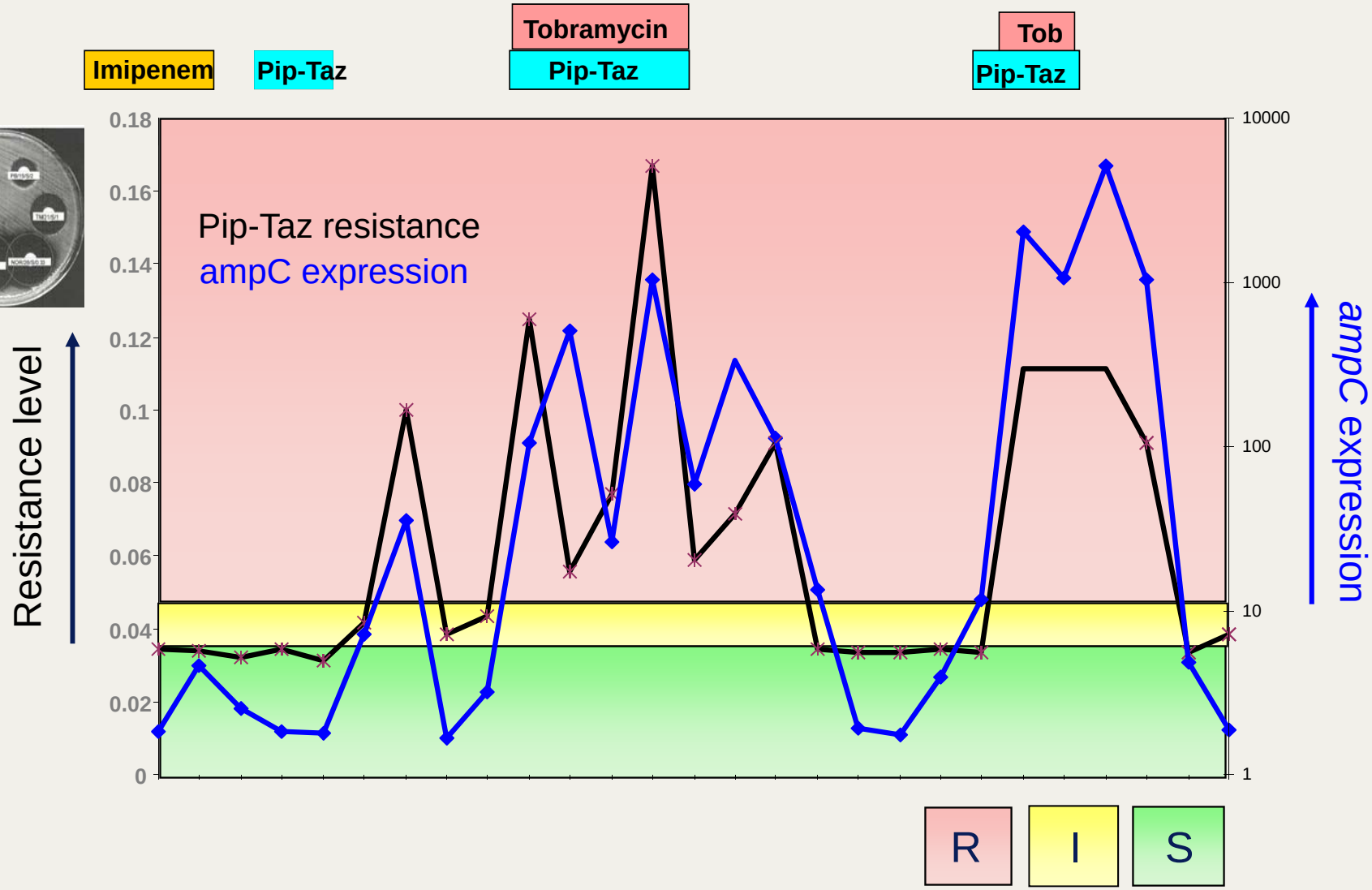
Reinhardt et al., AAC 2007

# Emergence and NOT acquisition of resistance



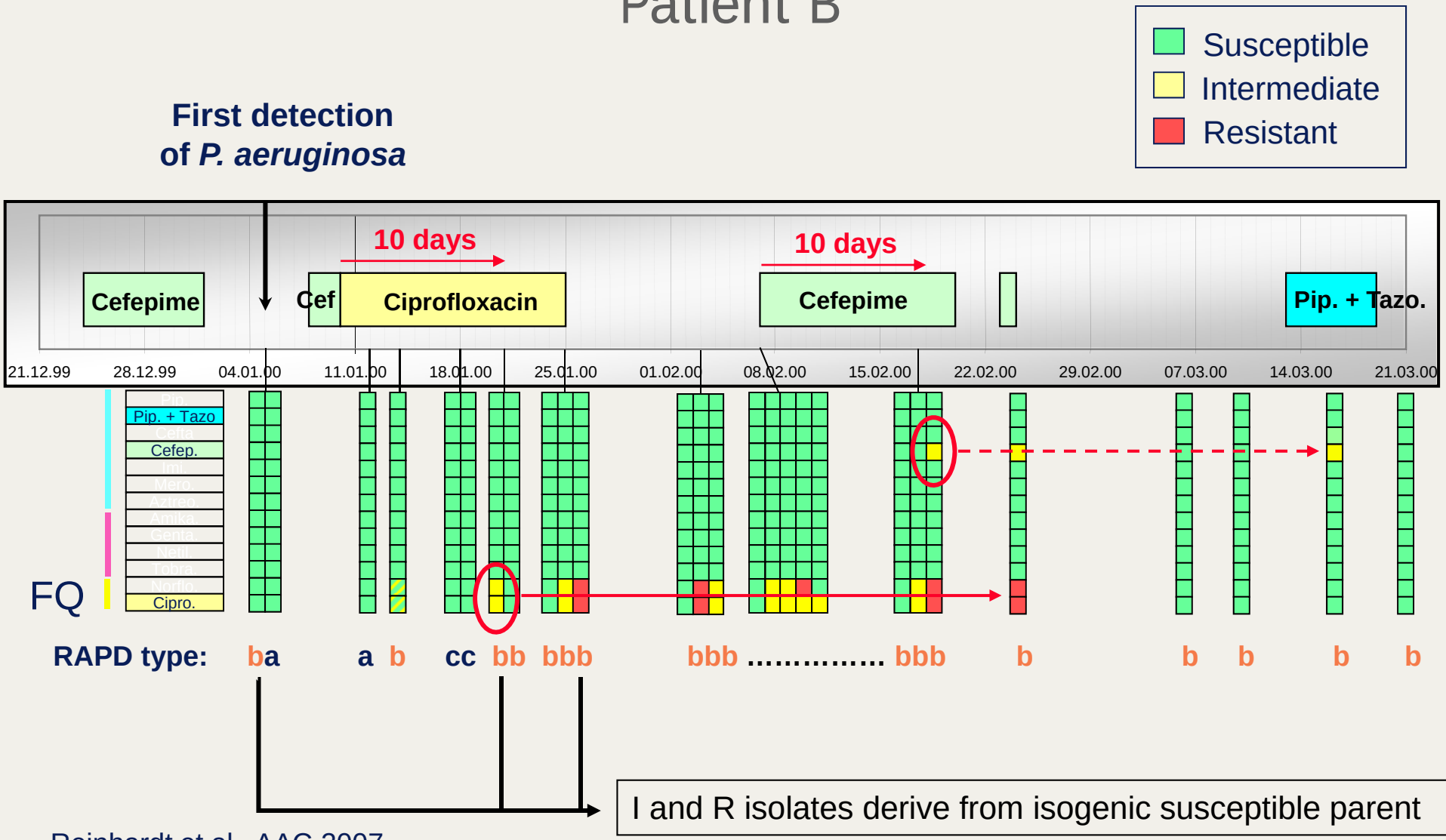
# Pip-Taz resistance correlates with *ampC* expression





# Evolution of antibiotic resistance

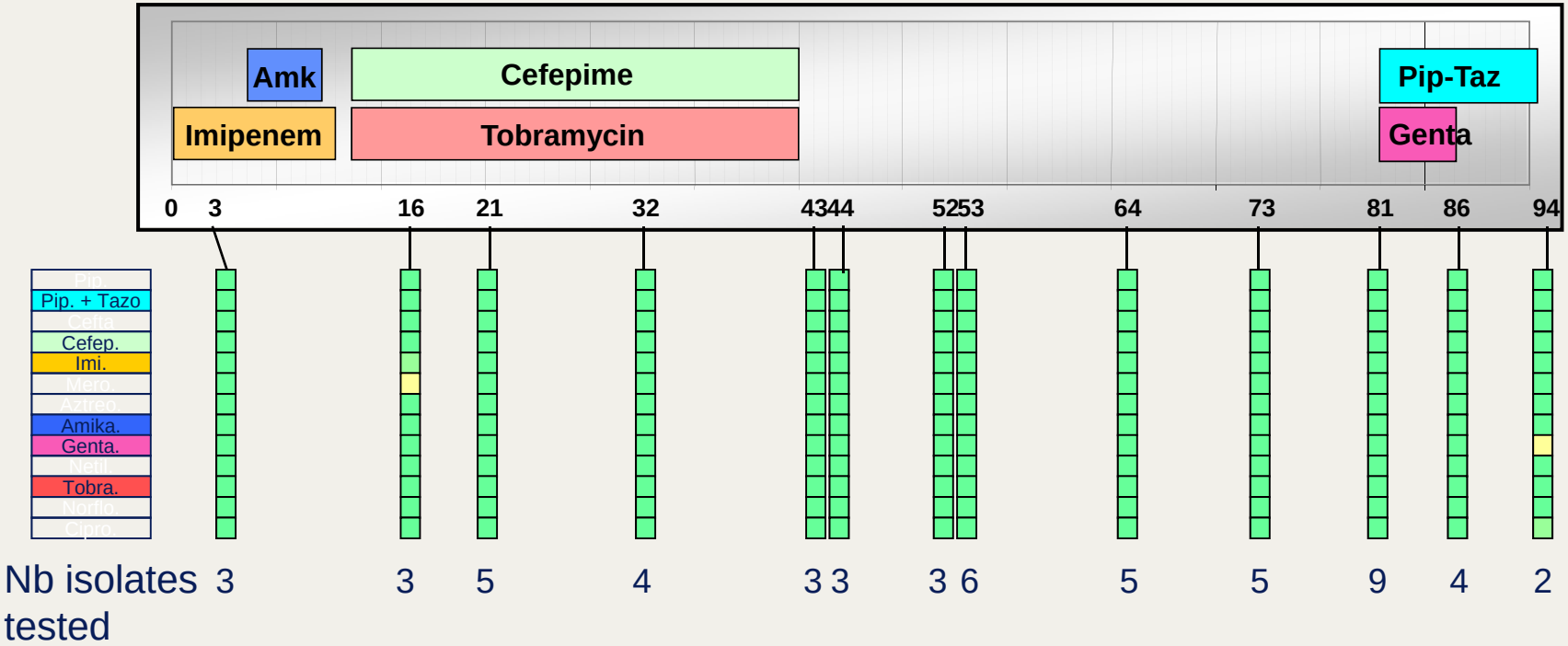
## Patient B




Reinhardt et al., AAC 2007

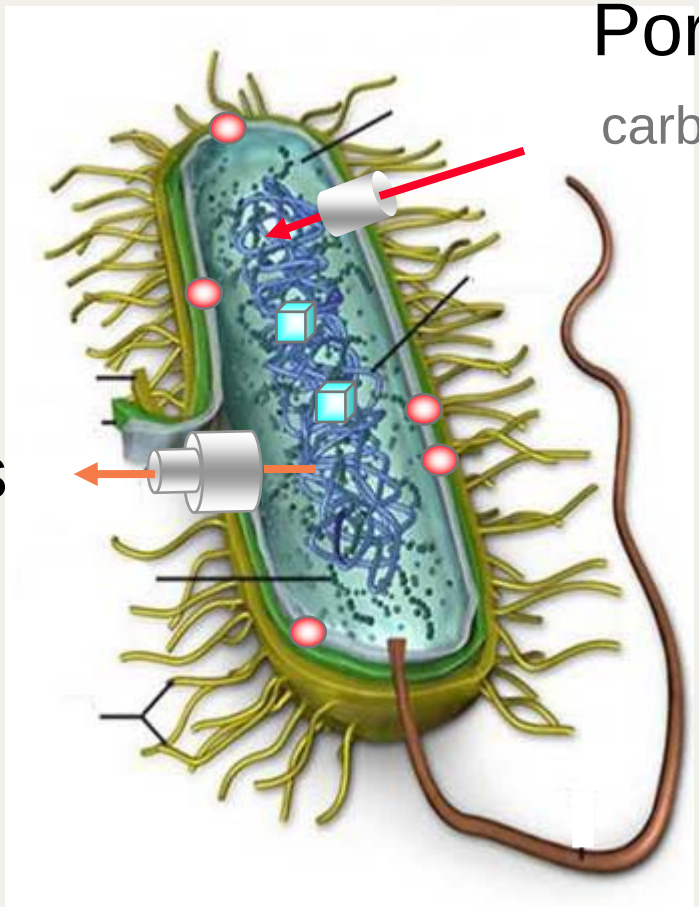
# Evolution of antibiotic resistance

## Patient C




All isolates remain susceptible !

# Major antibiotic resistance mechanisms



Porin OprD

carbapenem

Efflux pumps

all classes of antibiotics

AmpC β-lactamase

penicillins
cephalosporins

Topoisomerases

quinolones

# Dynamics of antibiotic resistance

| Pat. | treatment | mechanism | emergence | stability[1] |
|---|---|---|---|---|
| A | imipenem | ~~OprD~~ ↓ | 6 days | > 80 days |
| | pip/taz | AmpC ↑ | 6 days | < 7 days |
| B | ciprofloxacin | MexCD ↑ | 10 days | < 40 days |
| | cefepime | MexXY ↑ | 10 days | < 15 days |
| C | amik+imi | none | NA | NA |
| | cefep+tobra | none | NA | NA |

[1] after treatment stop
NA, not applicable

**Combination therapy prevented resistance emergence ?**

# Is combination therapy better than monotherapy ?

Combination therapy

- The pros
  - Decreases the risk of an inappropriate empirical therapy
  - Might reduce the risk of selection of resistant isolates
  - The interaction might be synergistic and increase the killing

- The contras
  - Higher costs
  - More side effects
  - Possibly higher risk of superinfection with fungi due to wider spectrum

# Do meta-analyses help us ?

β lactam monotherapy versus β lactam-aminoglycoside combination therapy for sepsis in immunocompetent patients: systematic review and meta-analysis of randomised trials

Mical Paul, Ishay Benuri-Silbiger, Karla Soares-Weiser, Leonard Leibovici

*BMJ* **2004**

No advantage for *P. aeruginosa* bacteremia

**Does combination antimicrobial therapy reduce mortality in Gram-negative bacteraemia? A meta-analysis**

*Lancet Inf Dis* **2004**;4:519

**Nasia Safdar, Jo Handelsman, and Dennis G Maki**

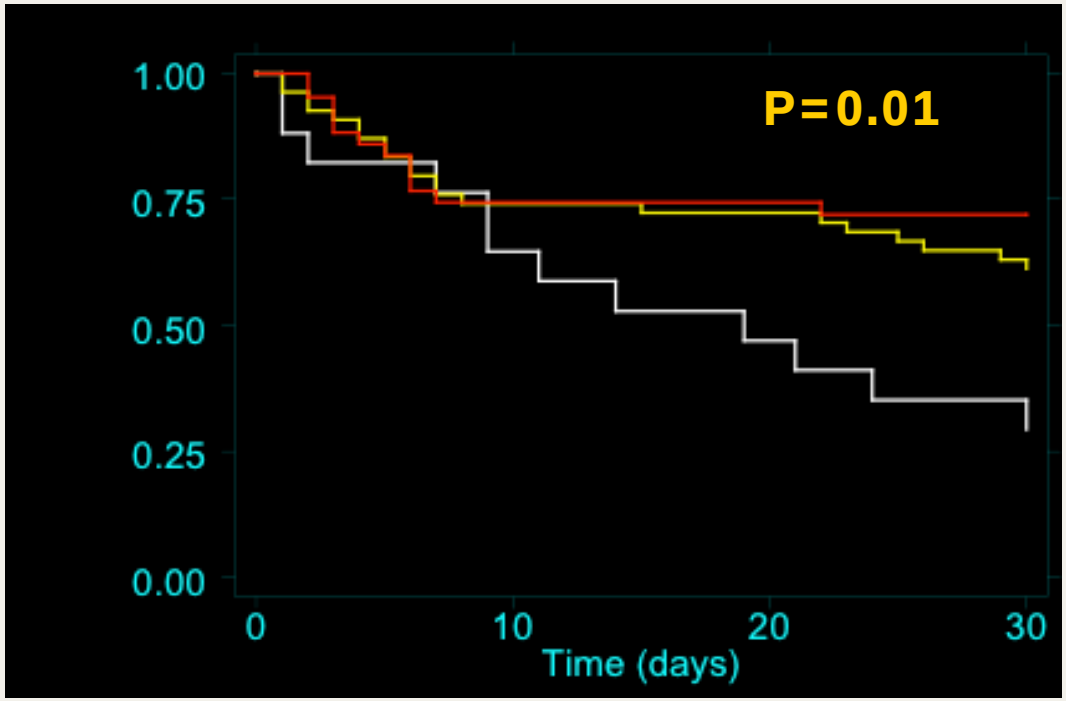
Significant survival benefit for *P. aeruginosa* bacteremia

# Potential biases from meta-analyses

No compensation possible for:

- No evaluation of adequacy of the empirical and definitive therapies
- Inclusion of patients receiving aminoglycoside monotherapy
- Low number of documented *P. aeruginosa* bacteremia in each trial

# Time to death during *P. aeruginosa* bacteremia




n = 115

**Combination tt**

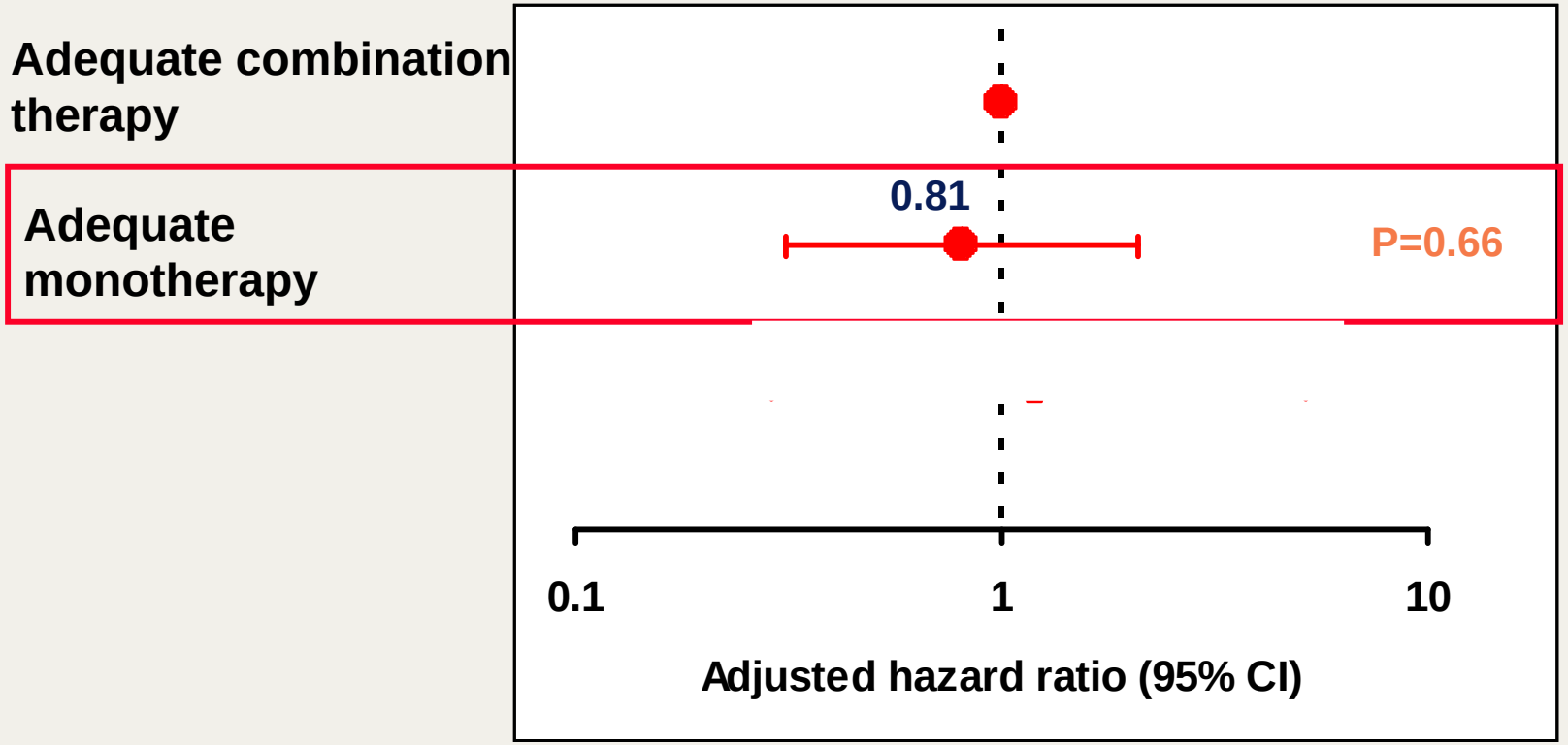
**Monotherapy**

**Inadequate tt**

*Conclusion*: 16 of 45 (35%) patients who died did die within the first 5 days

*AAC* **2003**;47:2756

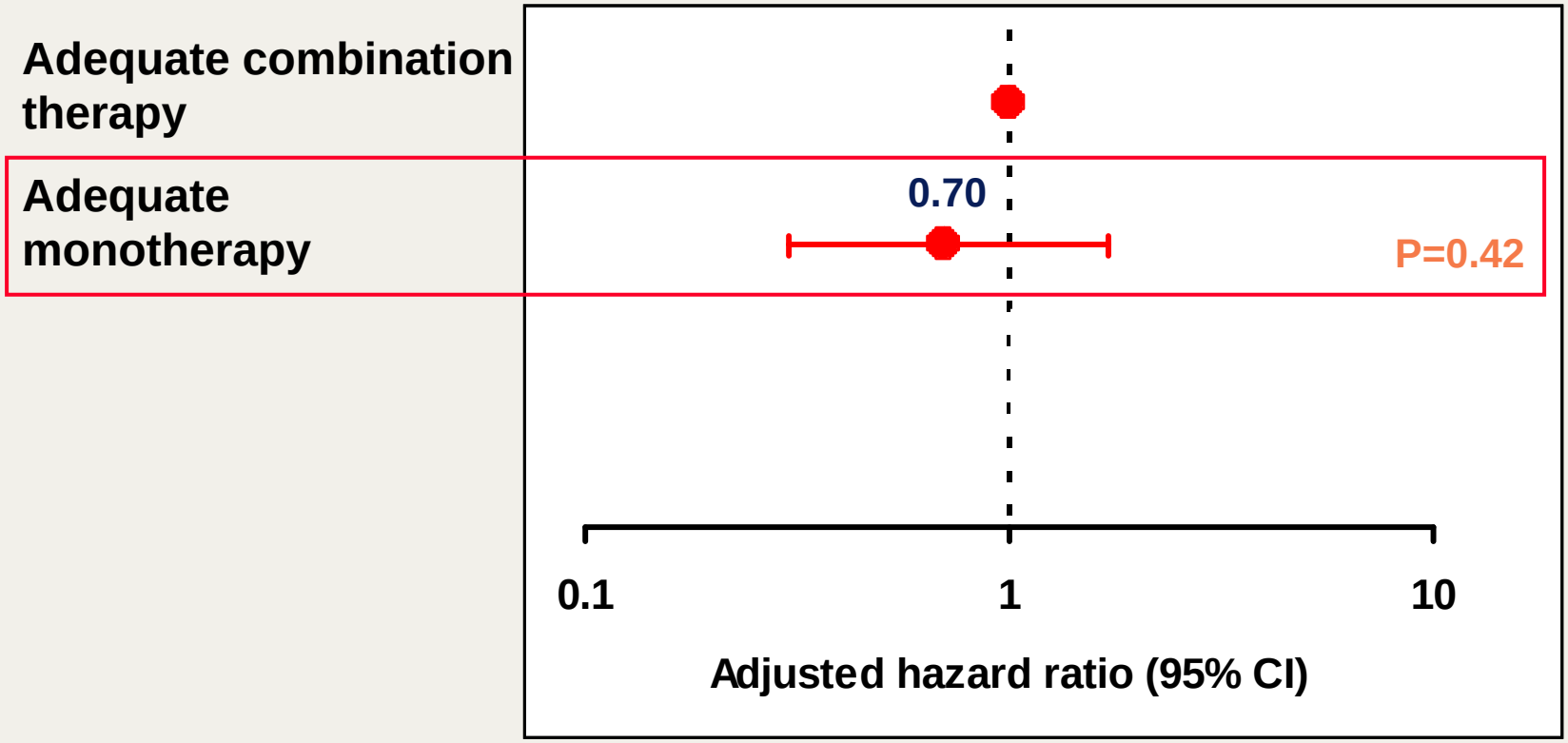
# Impact of mono versus combination empirical therapy on early deaths




*Conclusion*: empirical combination therapies do not improve the outcome of those patients that are so sick that they will die within the first days

*AAC* **2003**;47:2756

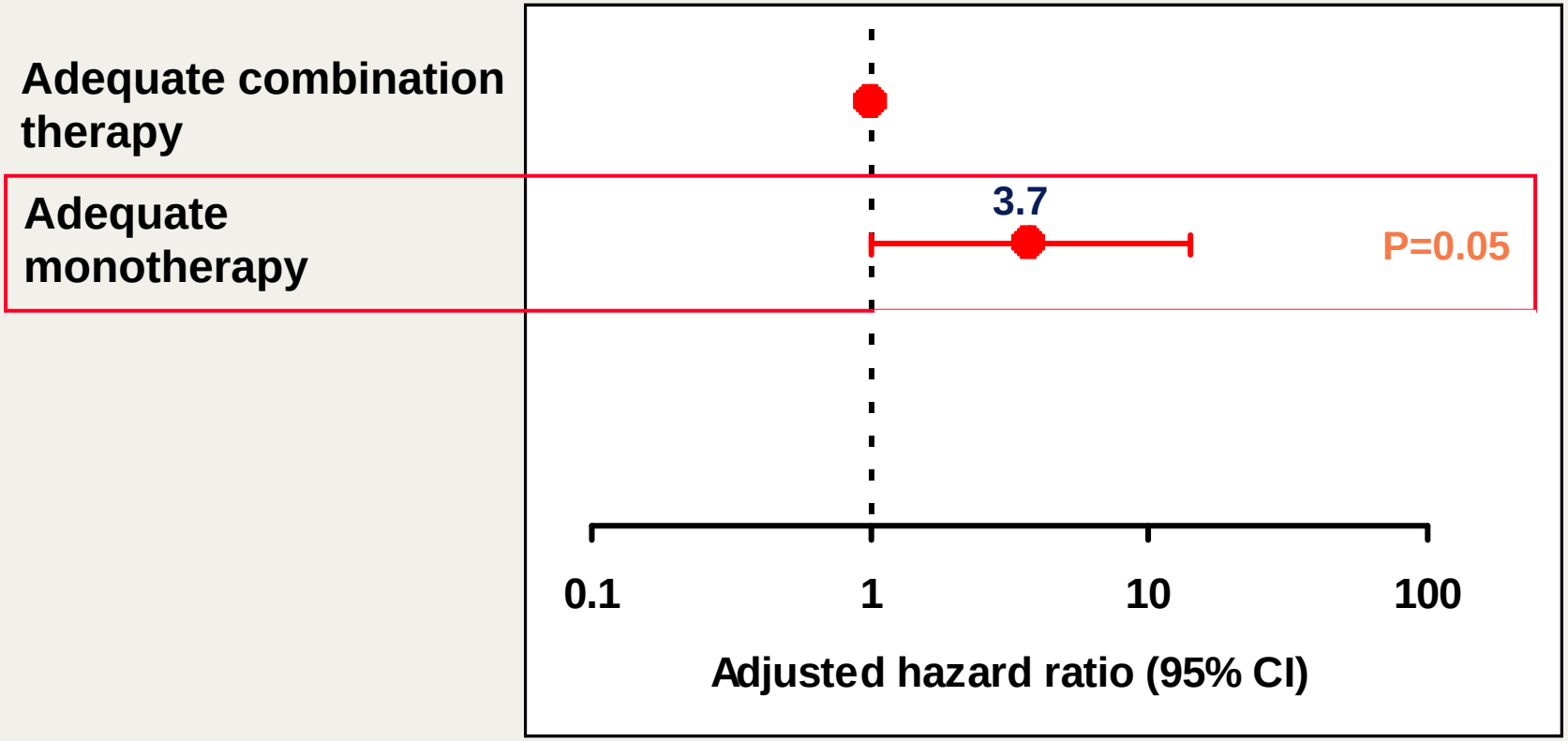
# Impact of mono versus combination definite therapy on late deaths




*Conclusion*: a definitive combination therapy does not improve the outcome

*AAC* **2003**;47:2756

# Impact of mono versus combination empirical therapy on late deaths




*Conclusion*: empirical combination therapy improves the outcome at 30 days after censuring for patients that die within the first 5 days

*AAC* **2003**;47:2756

# Anti-virulence strategies

## Classical antibiotics :

Antibiotic → Essential target:
- DNA replication
- Protein synthesis
- Cell wall synthesis

→ Selection for Antibiotic resistance

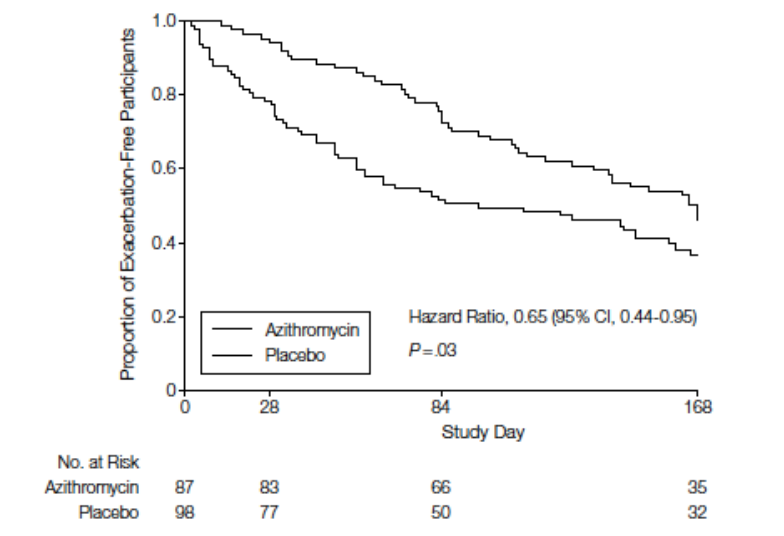
## Anti-virulence strategies:

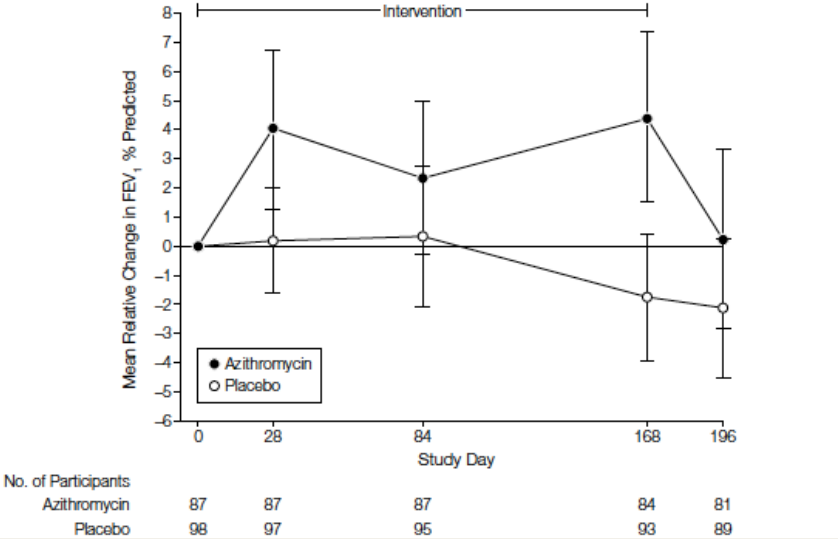
Anti-virulence molecule → Non-essential target:
- flagella (vaccine)
- virulence factor synthesis (QS)

→ Theoretically no selection pressure for resistance

# Azithromycin is beneficial in CF patients colonized by *Pseudomonas*

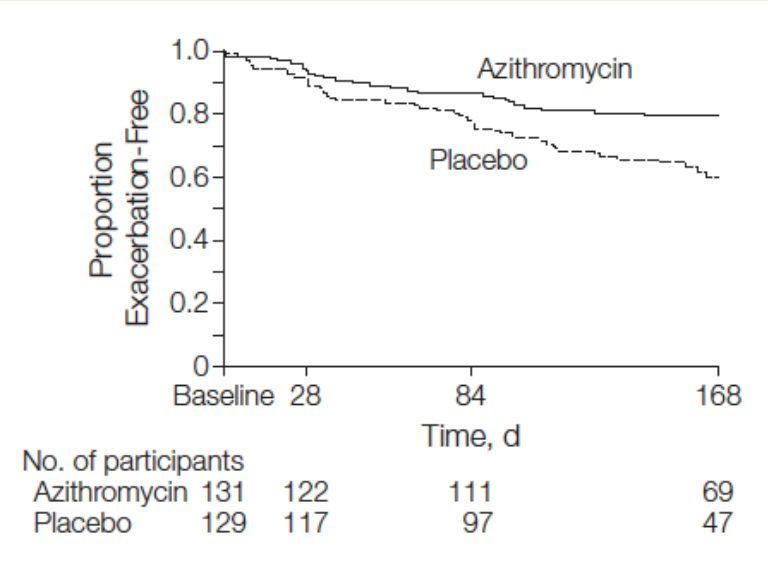
- Azithromycin improves FEV1, reduces acute exacerbations and increases weight gain in CF patients colonized by *Pseudomonas*

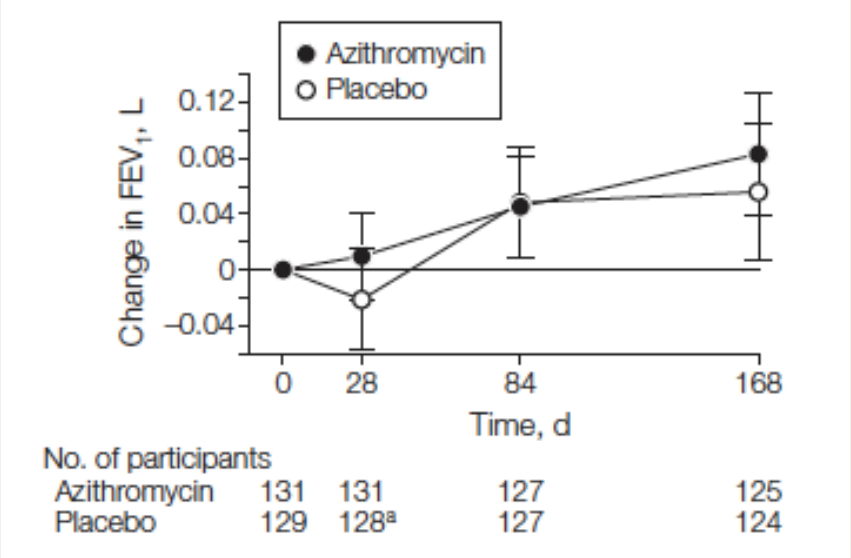



*JAMA* **2003**;290:1749

# Azithromycin does not improve pulmonary function in the absence of *Pseudomonas*




The beneficial effect of azithromycin in CF patients is restricted to patients colonized by *Pseudomonas*

*JAMA* **2010**;303:1707

# Azithromycin and bronchiolitis obliterans

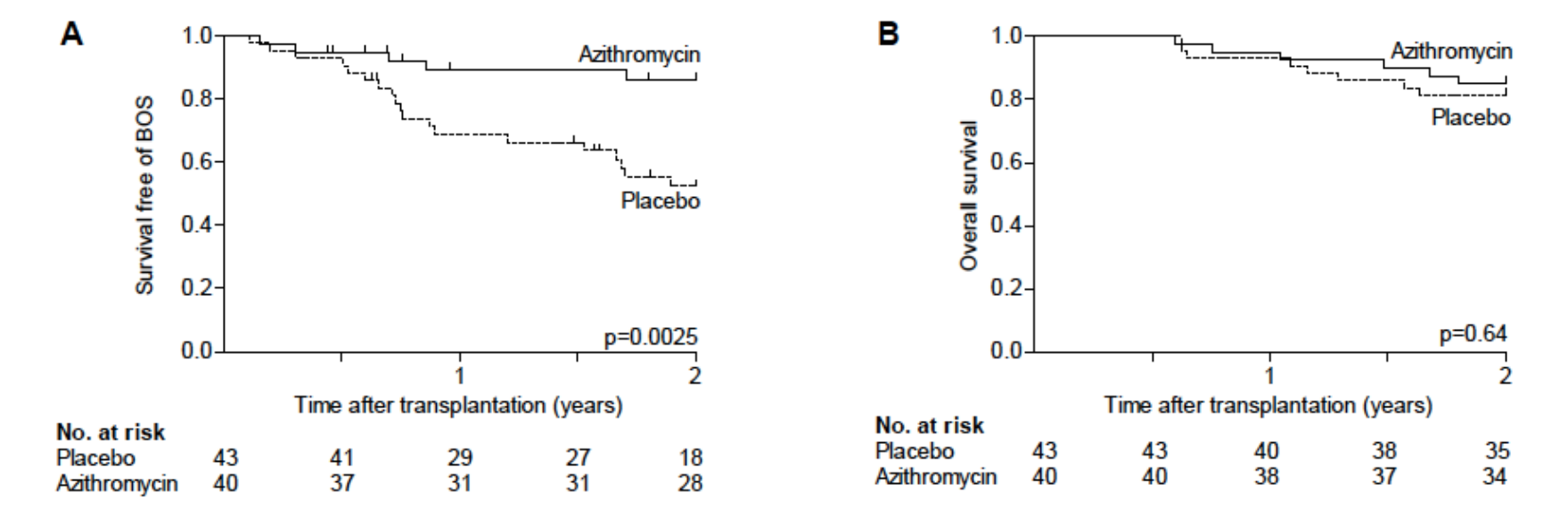
**Table 3** Multivariate Cox Regression for Progression of BOS Stage 1 to Death[a]

| | Death | |
|---|---|---|
| Variable | Hazard ratio (95% CI) | *p* |
| Azithromycin treatment[b] | 0.30 (0.10–0.88) | 0.03 |
| $FEV_1$ at BOS Stage 1 | 0.53 (0.35–0.81) | 0.003 |
| *Pseudomonas* culture positive | 1.96 (1.06–3.60) | 0.03 |

- Azithromycin initiated before BOS Stage 2 is associated with reduced mortality in multivariate analysis
  - Retrospective, 78 treated compared to 95 non treated

(*J Heart Lung Transpl* **2010**;29:531)

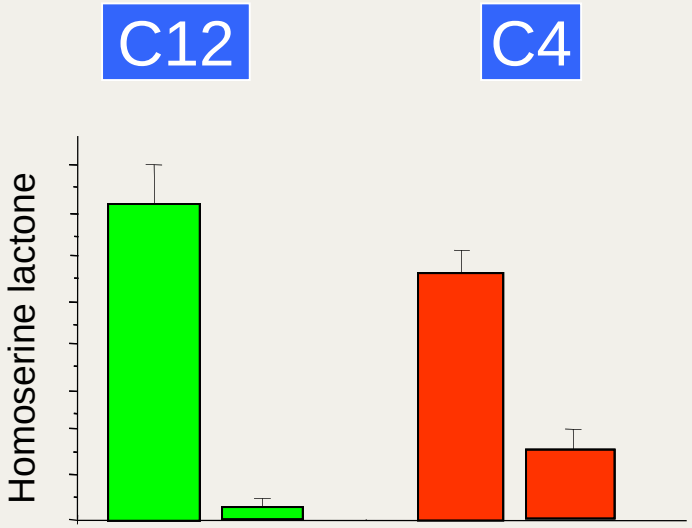
# Azithromycin increases BOS free survival But not overall survival



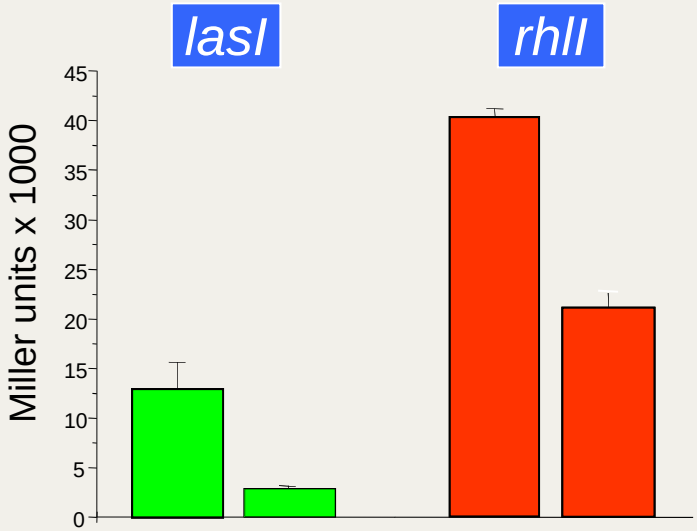

*ERJ* **2010**, in press

# AZM decreases QS gene expression *in vitro*

Autoinducer




Expression of
Autoinducer synthase gene




2 mg/l AZM - + - + - + - +

Minimal inhibitory concentration for *P. aeruginosa*: 128 mg/l AZM

Tateda et *al*. AAC, 2001

# Prophylactic azithromycin prevents VAP

**Placebo group**

VAP

Days of colonization

| Patient | SCORE | -1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 05101 * | **1.20** | 2 | 0 | 2 | 0 | 2 | | | | | | | | | | | | | | | | | |
| 06104 | **0.00** | 0 | 0 | 0 | 0 | | | | | | | | | 0 | 0 | 0 | 0 | | | | | | |
| 10103 | **0.25** | 0 | 0 | 0 | 0 | | | | | | | | | | | | | | | | | | |
| 13104 * | **1.17** | 1 | 1 | NI | 1 | | | | | | | | | | | | | | | | | | |
| 13106 * | **1.00** | 1 | 1 | 1 | 1 | | | | | | | | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | |
| 13108 | **1.00** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | | |
| **13111 *** | **2.00** | 2 | 2 | 2 | 2 | 2 | | | | | | | | | | | | | | | | | |
| 13112 * | **1.00** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | | | | | | | | | |
| 13114 | **0.00** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | |
| **13116** | **0.00** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | | |
| 13117 | **0.00** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | |
| 13118 | **0.78** | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | | | | | | | | | | | | | |
| 13121 | **0.29** | 0 | 1 | 0 | 0 | 0 | 1 | 0 | | | | | | | | | | | | | | | |
| 13122 * | **1.10** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 2 | 1 | 2 | 0 | 2 | 2 | 0 | 2 | 2 | 1 | |
| **13128 *** | **2.00** | 2 | 2 | 2 | 2 | 2 | | | | | | | | | | | | | | | | | |
| 15101 | **0.05** | 0 | 1 | 0 | NI | 0 | NI | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 15102 * | **1.00** | ND | 1 | 1 | 1 | 1 | 1 | 1 | ND | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | |
| **16101** | **1.42** | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | | | | | | | | | | |
| 19101 | **1.00** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | |
| 19102 * | **1.08** | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 0 | 1 | 2 | 1 | 0 | 1 | | | | | | | | | |
| 19105 * | **1.13** | 2 | 2 | 2 | 2 | NI | 2 | 1 | 0 | 0 | 1 | NI | 2 | 0 | NI | 2 | 1 | 0 | NI | 0 | NI | 1 | |
| **21107 *** | **1.55** | 2 | 1 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 1 | | | | | | | | | | | |
| 22101 | **0.00** | 0 | 0 | 0 | 0 | 0 | ND | 0 | | | | | | | | | | | | | | | |
| **24101** | **2.00** | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | | | | | | | | | | |
| 26102 | **0.33** | 1 | 0 | 0 | NI | NI | | | | | | | | | | | | | | | | | |
| 26104 | **0.27** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | | | | | | | | | | | |
| 27101 | **0.00** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NI | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 27104 | **1.00** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | | | | | | | | | |
| 30101 | **0.33** | 2 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | | | | | | |
| **n=29** | **0.79** | -1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |

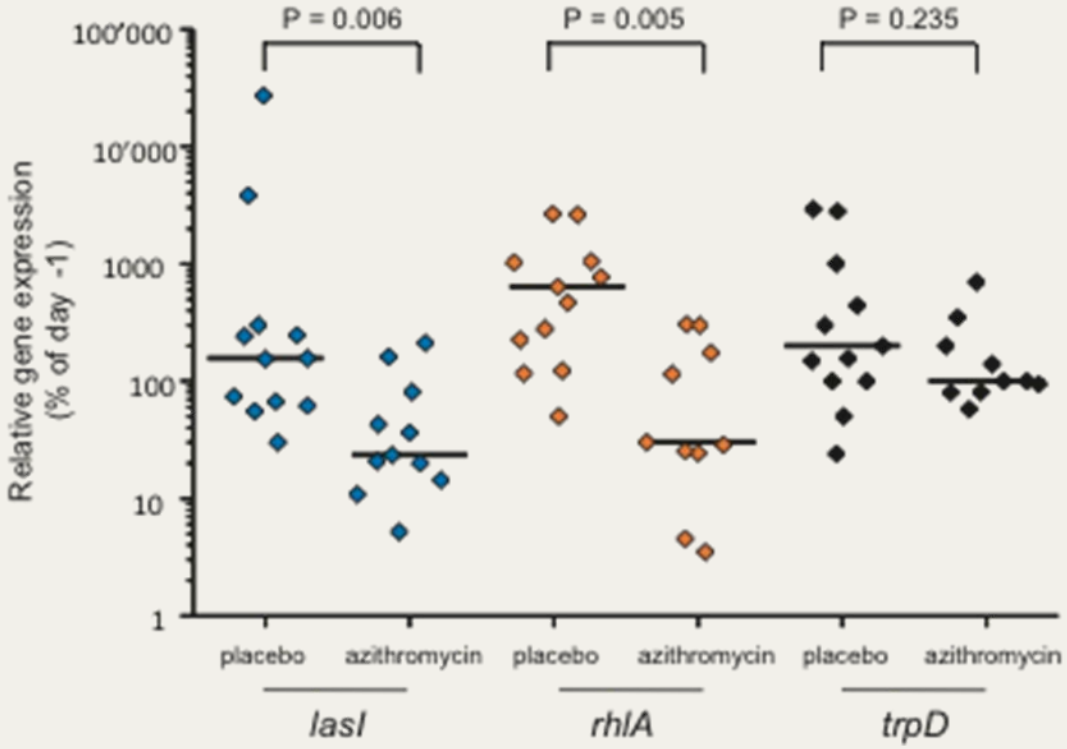
| RHAM | | |
|---|---|---|
| PAO1 | >90% | 2 |
| PAO1 | 90-10 | 1 |
| PAO1 | <10% | 0 |

**Azithro group**

| Patient | SCORE | -1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 01101 | **0.00** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | | |
| 03101 * | **1.80** | 2 | NI | 1 | 2 | 2 | 2 | | | | | | | | | | | | | | | | |
| 03103 | **1.00** | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | |
| 06101 * | **2.00** | 2 | 2 | 2 | 2 | 2 | 2 | | | | | | | | | | | | | | | | |
| 06103 | **1.00** | NI | 2 | NI | 2 | 0 | 1 | 1 | 1 | 0 | 1 | | | | | | | | | | | | |
| 08101 | **0.00** | 0 | ND | NI | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | | | |
| 08102 | **0.00** | 0 | 0 | 0 | 0 | 0 | 0 | ND | 0 | ND | 0 | ND | 0 | ND | 0 | ND | 0 | ND | 0 | ND | 0 | ND | 0 |
| 10102 | **1.06** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | | | 1 | | |
| 10105 | **0.40** | 0 | 0 | 1 | 1 | 0 | | | | | | | | | | | | | | | | | |
| 10107 * | **2.00** | 2 | 2 | 2 | 2 | 2 | | | | | | | | | | | | | | | | | |
| 13105 * | **1.00** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | | | | | | | | | | |
| 13109 | **0.00** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | |
| 13110 | **1.00** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | | | | | | | | | | |
| **13113** | **2.00** | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | | | | | | | | |
| **13115** | **0.00** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | | | | |
| 13119 | **0.57** | 1 | 1 | 1 | 0 | 0 | 0 | 1 | | | | | | | | | | | | | | | |
| 13120 | **1.20** | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 0 | 2 | 1 | 1 | | | | | | | |
| 13123 | **0.17** | 0 | 0 | 0 | 0 | 1 | 0 | 0 | | | | | | | | | | | | | | | |
| 13124 | **0.00** | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | | | | | | | |
| 15104 * | **1.07** | 2 | 0 | 1 | 1 | 1 | 2 | NI | 2 | 1 | 0 | 1 | 1 | ND | 1 | ND | 1 | ND | 1 | ND | 1 | | |
| 15107 * | **1.00** | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | | | | | | | | | | | |
| 15109 * | **1.00** | 1 | 1 | NI | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | |
| 19103 | **1.25** | 1 | 1 | 2 | 2 | 1 | 2 | NI | NI | 1 | 1 | 1 | NI | NI | NI | NI | 1 | NI | 1 | NI | 1 | | |
| 21102 * | **0.83** | 1 | 1 | 1 | 0 | NI | 1 | NI | 1 | | | | | | | | | | | | | | |
| 21106 | **0.83** | 1 | 0 | 1 | 1 | 1 | 1 | | | | | | | | | | | | | | | | |
| 26101 * | **0.69** | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | | | | | | |
| 26103 * | **0.50** | 2 | 0 | 0 | 1 | 0 | 0 | | | | | | | | | | | | | | | | |
| 27102 * | **1.53** | 1 | 0 | 1 | 0 | 1 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | | | |
| 27103 | **1.00** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | | | | | | | | | | |
| 28101 | **0.56** | 1 | 0 | 1 | 0 | 0 | NI | 1 | 0 | 1 | 1 | | | | | | | | | | | | |
| **n=30** | **0.85** | -1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |

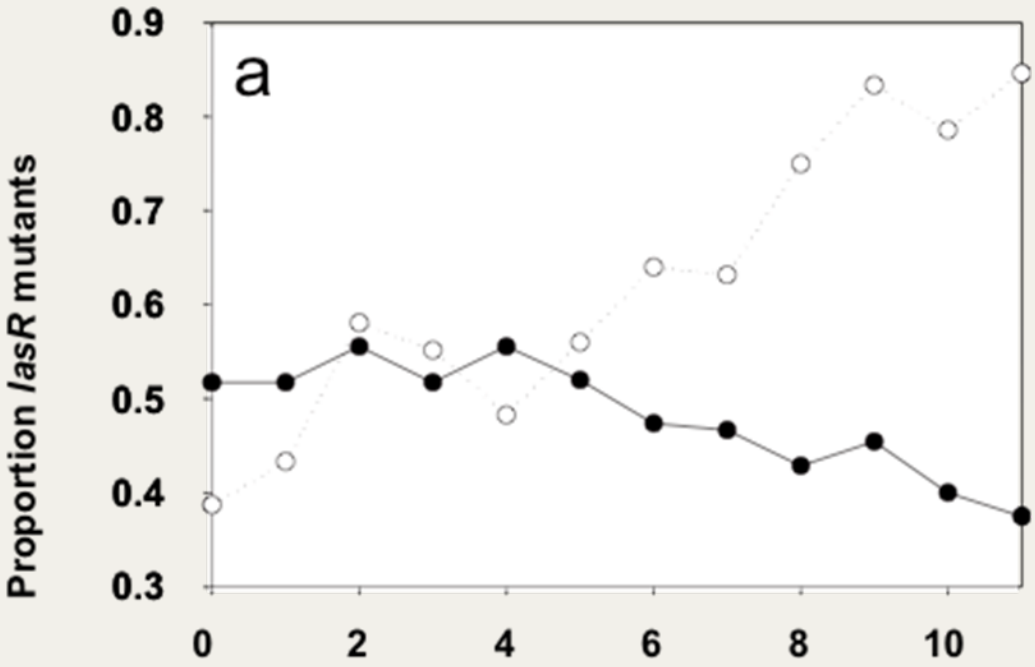
Azithromycin treatment has prevented 5 putative cases of VAP

# Azithromycin inhibits "*in patient*" the production of QS genes




*Plos Pathogens* **2010**; 6: e1000883

# Inhibition of QS by azithromycin selects for persistent colonization with QS-proficient isolates




placebo

P < 0.001

azithromycin

- Whereas placebo treated patients are progressively colonized by QS-deficient isolates, azithromycin treated patients remain colonized by QS-proficient isolates

*Plos Pathogens* **2010**; 6: e1000883

# Mechanisms of action of azithromycin on Pseudomonas infections

- Chronic colonization by *Pseudomonas* expressing virulence genes increases local inflammation potentially responsible for decrease in lung function seen in both CF and BOS
  - Azithromycin might have a direct anti-inflammatory effect
  - Part of the clinical benefit observed with azithromycin in CF and BOS might be due to an indirect anti-inflammatory effect due to inhibition of quorum-sensing dependent virulence of *Pseudomonas*

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