# Microbiology sampling of cystic fibrosis patients - when, how and what to expect from the laboratory

Helle Krogh Johansen, Professor, MD, DMSc

Department of Clinical Microbiology, Rigshospitalet, Copenhagen

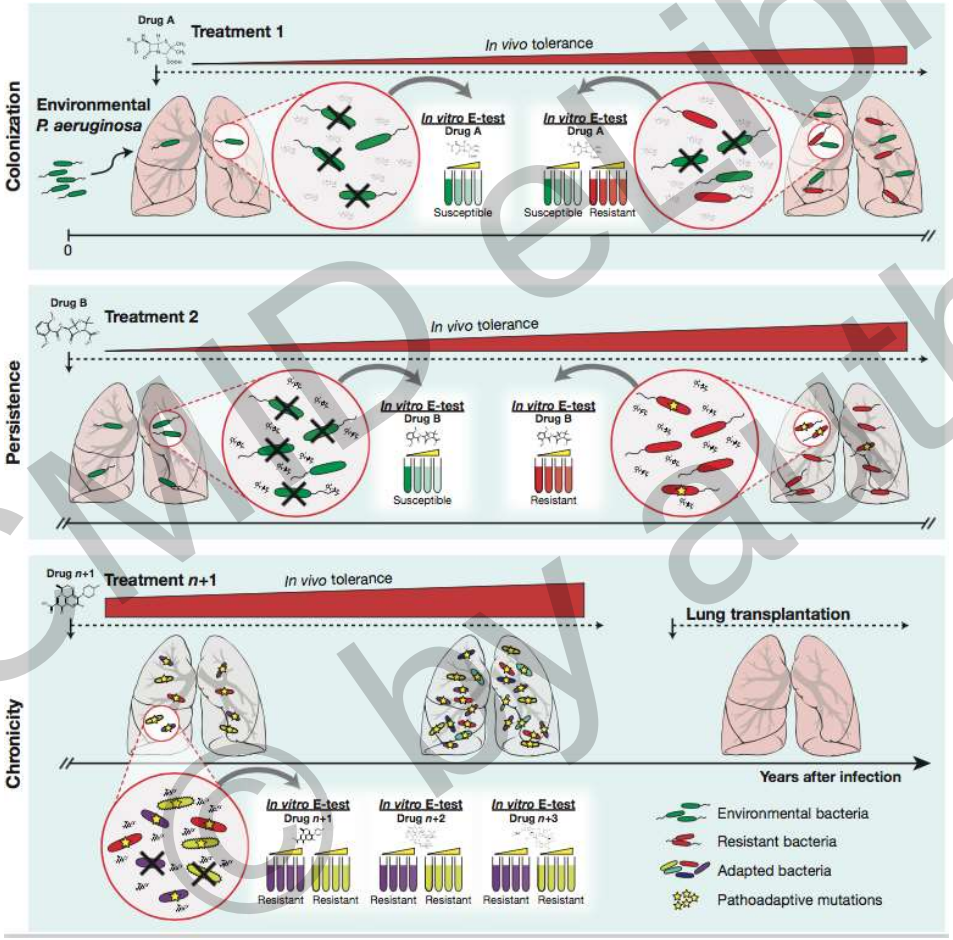
and

Department of Clinical Medicine, Faculty of Health Sciences, University of Copenhagen

and

The Novo Nordisk Foundation, Center for Biosustainability, The Technical University of Denmark, Denmark

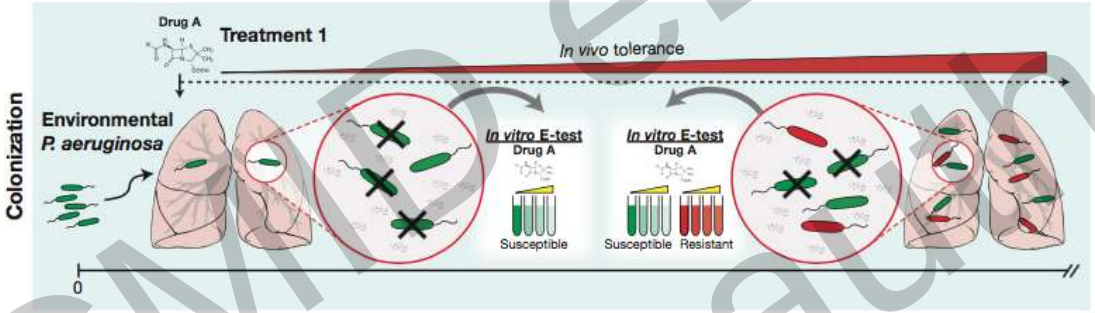
# CF airway infections



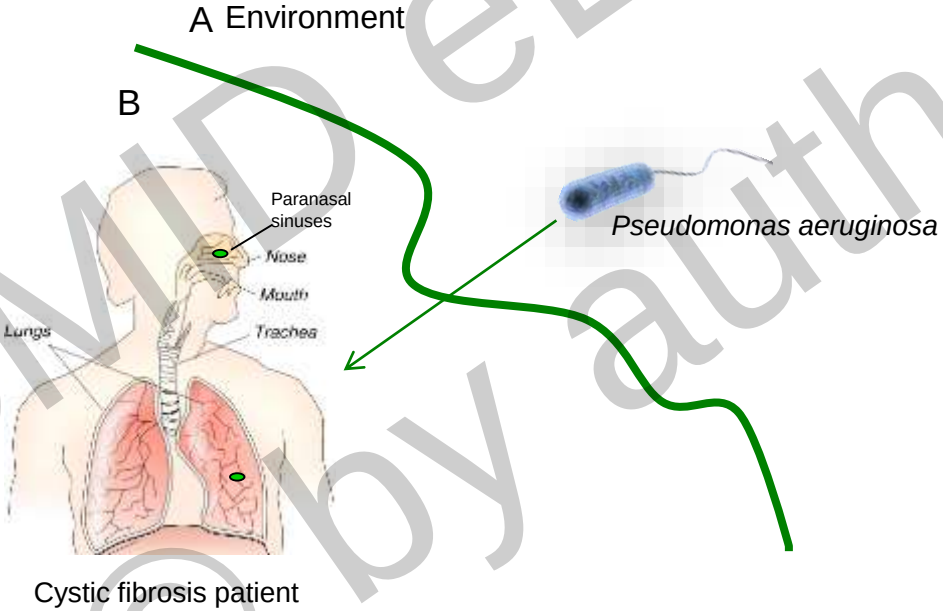



Ruggero la Rosa

# CF airway infections - colonisation



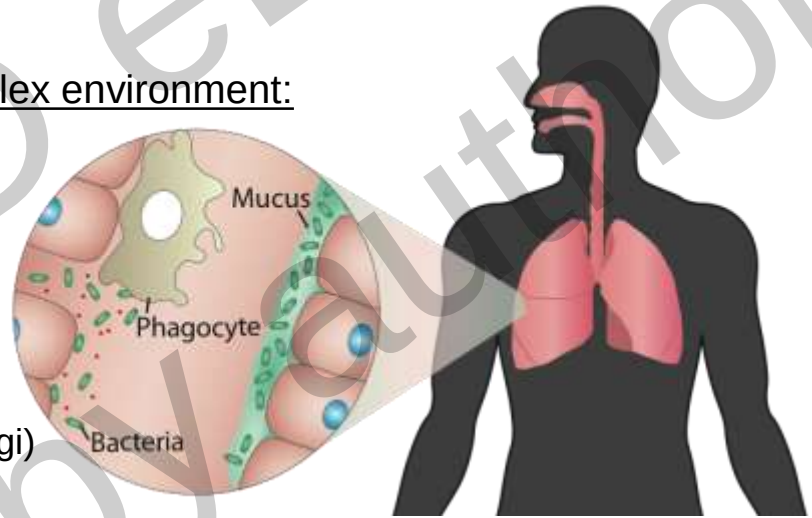
# Initial colonisation



# The niche: the upper and lower airways

<u>The CF airways is a highly complex environment:</u>

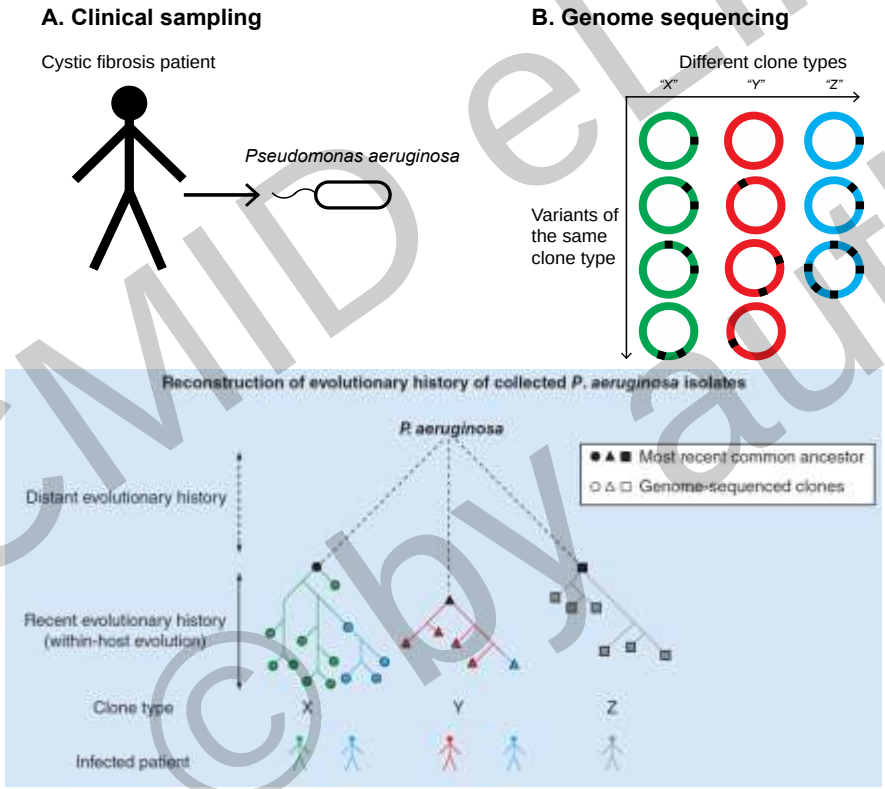
- Spatial heterogeneity
- Heterogeneous distributions of:
  - Oxygen
  - Resources (C & N)
  - Immune response
  - Microorganisms (bacteria, fungi)
  - ….and antibiotics



# Current principle for antibiotic treatment

| | |
|---|---|
| Main objective: | prevention or delay of chronic infection |
| Strategy: | aggressive antibiotic treatment |
| Result: | only 15% chronically infected CF patients 15 years after first *P. aeruginosa* culture |
| Validation: | is the delay of chronic infection supported by evidence? |

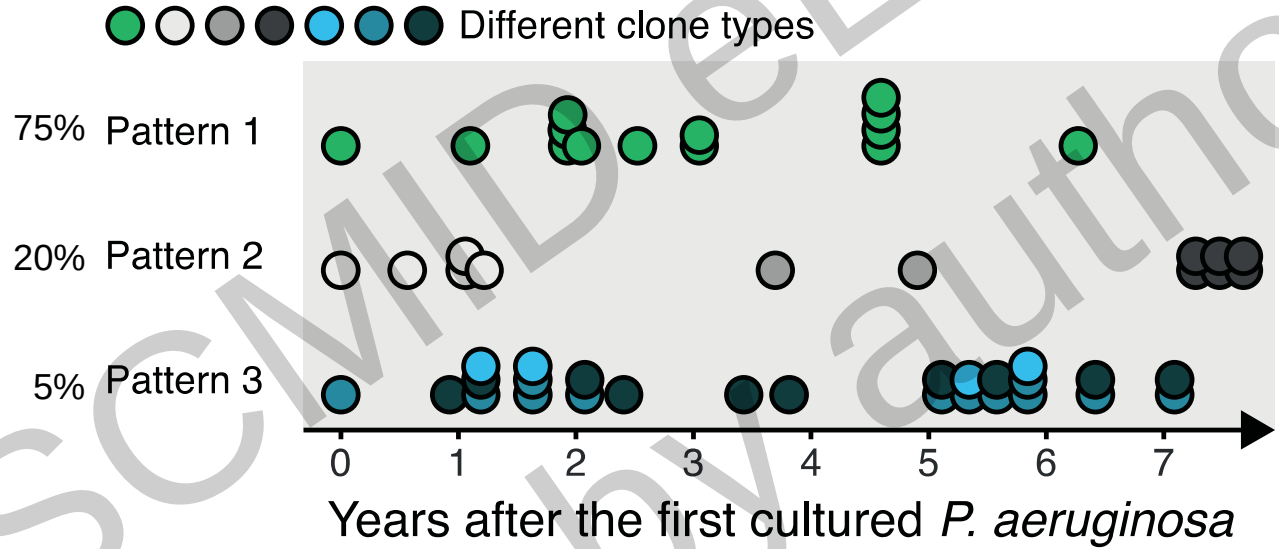
# Library of 500 *P. aeruginosa* isolates collected from 40 patients during the first 10 years of colonisation



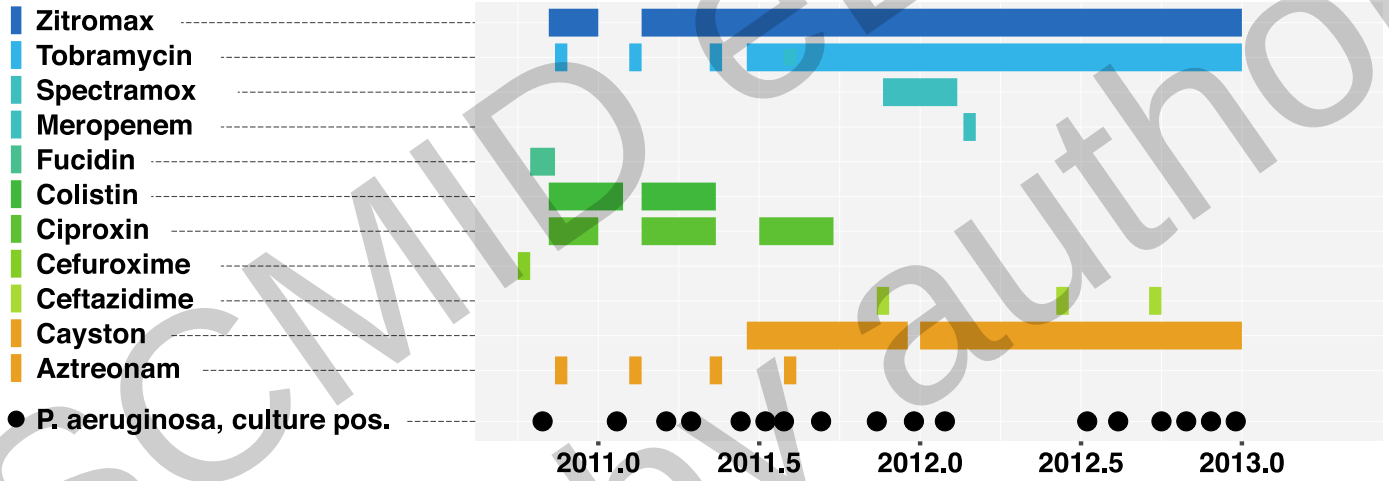



Marvig et al. Nat Genet. 2015 Jan;47(1):57-64

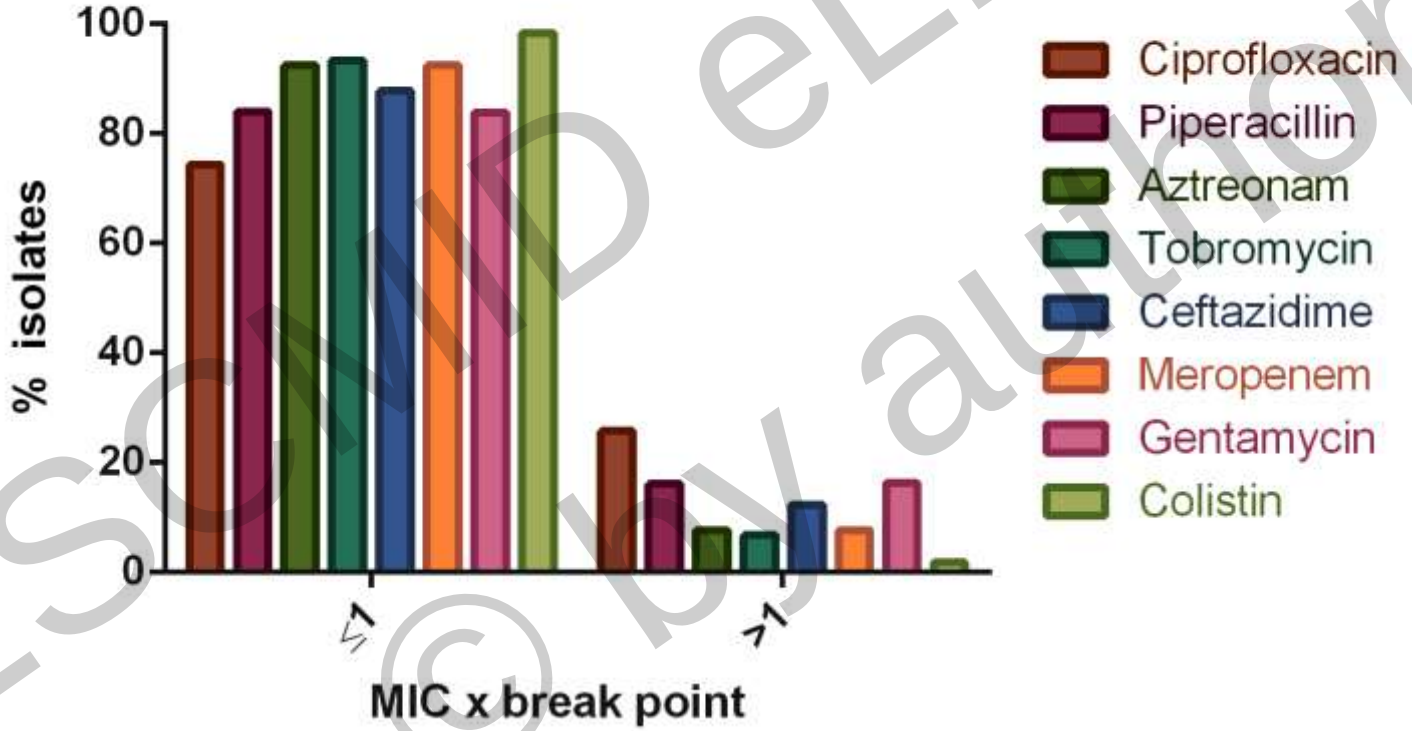
# In most CF children *P. aeruginosa* is not eradicated although the bacteria are susceptible



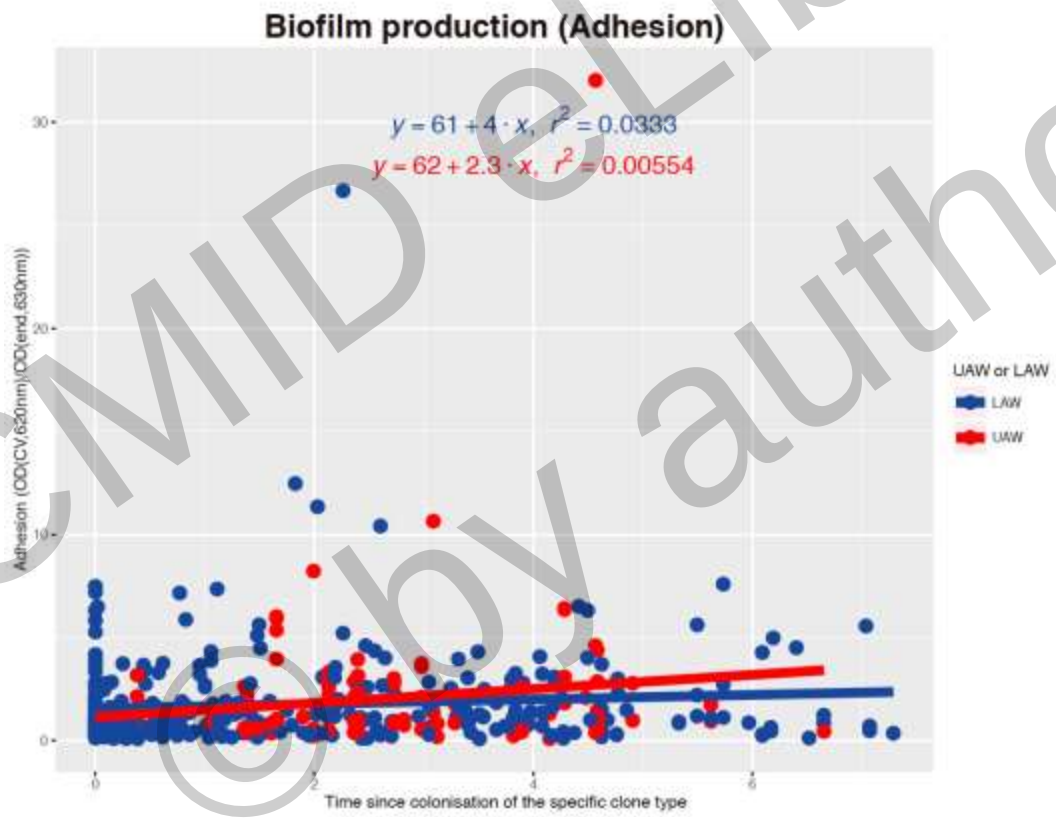
# Antibiotic treatment, an example



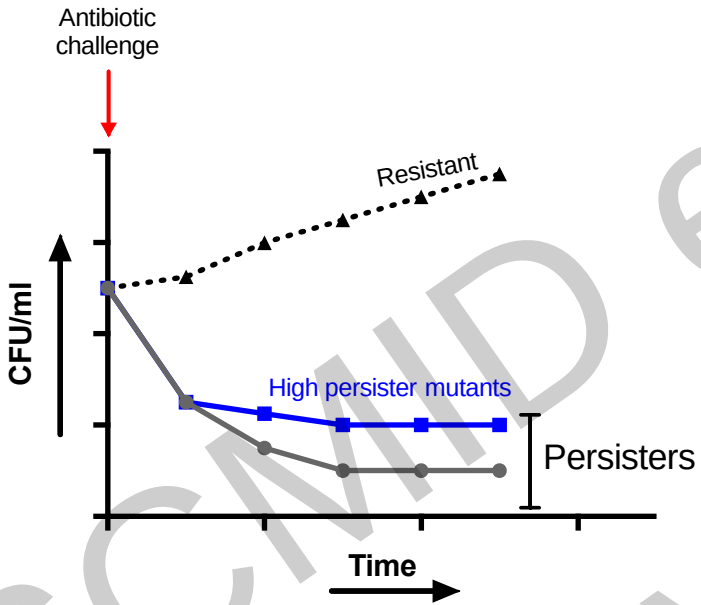
## Global antibiotic sensitivity of all isolates during more than 10 years

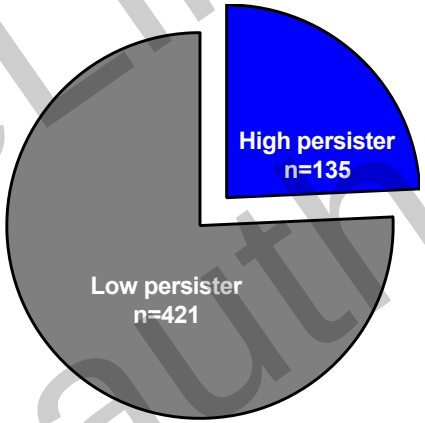


# Biofilm production over time of infection



# Antibiotic tolerant ‘persister cells’




- Persisters are a small subpopulation of bacterial cells that are tolerant to antibiotics
- High persister mutants emerge over the course of chronic infection
- Mechanisms underlying the formation of these cells is unclear

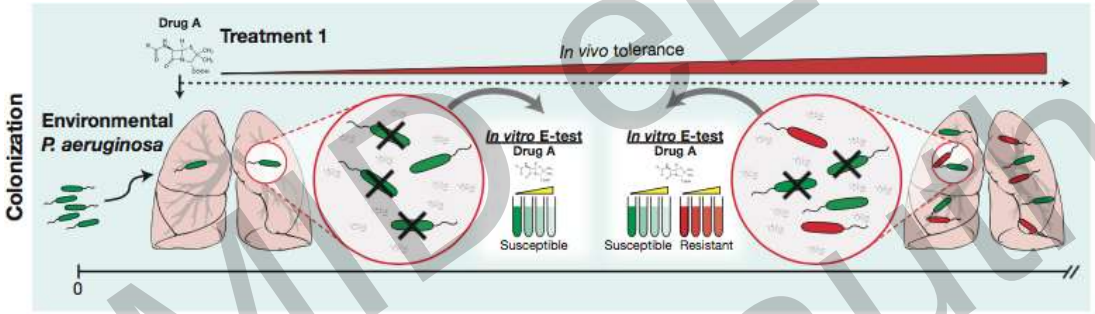
Mojsoska & Cameron

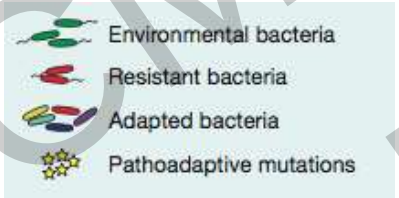
# Conclusions

Persistence after initial colonisation is not entirely explained by

- ✓ Antibiotic resistance development
- ✓ Biofilm formation
- ✓ Persisters

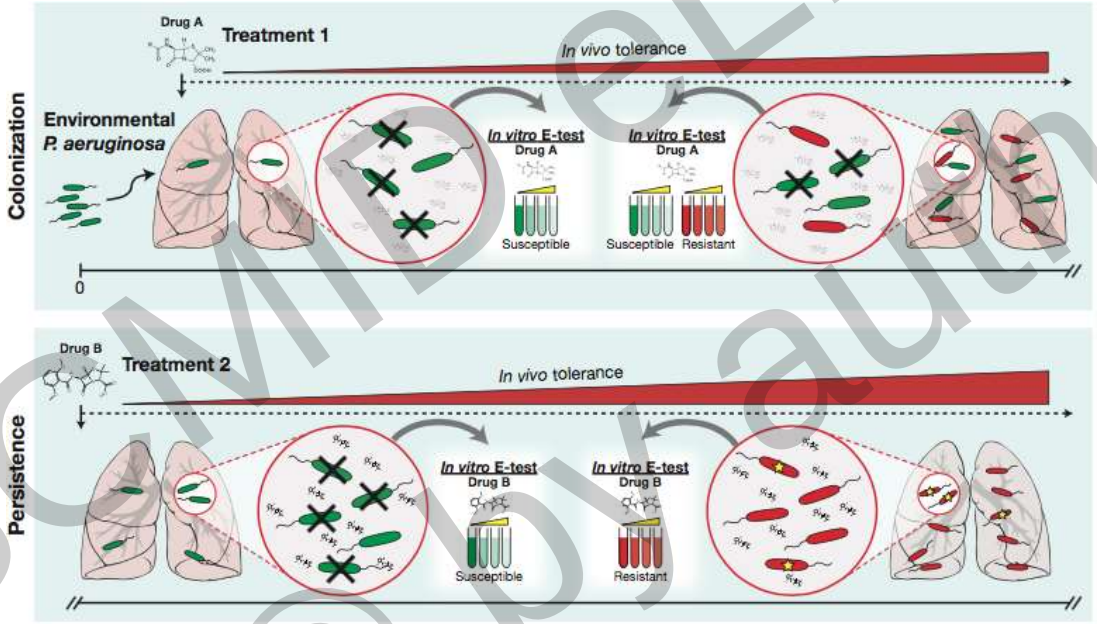
# CF airway infections - colonisation



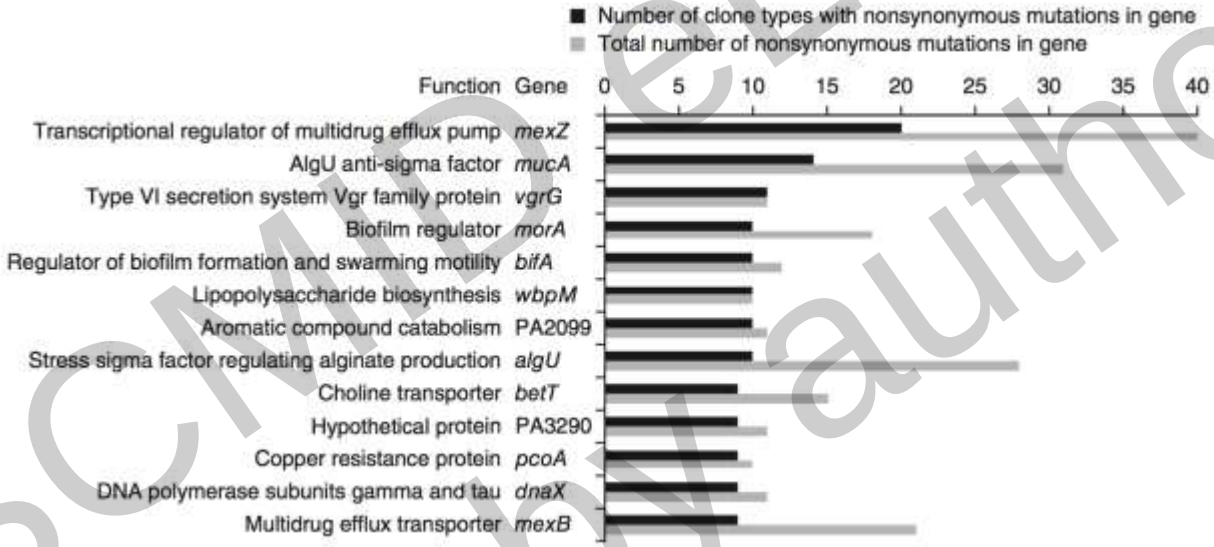




- Colonising susceptible bacteria - few survivors after antibiotic treatment
- Continued treatment results in appearance of few resistant bacteria
- Resistant bacteria do not persist

# CF airway infections - persistence



# Genes mutated more often than would be expected: Markers for persistent infection



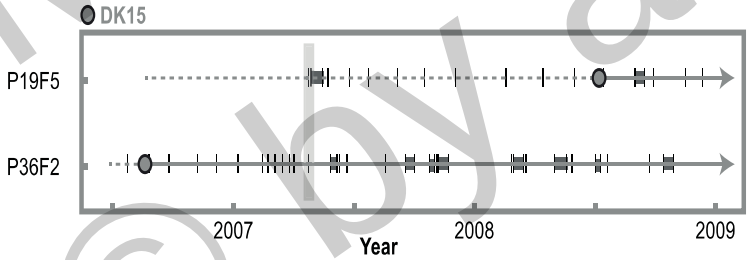

- ✓ Antibiotic resistance
- ✓ Mucoidity
- ✓ Biofilm formation (loss-of-function)

Marvig et al. Nat Genet. 2015 Jan;47(1):57-64

# A case: P19F5 - infected by DK15

P19F5 is a 16 years old girl - homozygous Δ508

In 2007 she most likely meets (directly or indirectly) the adult CF patient P36F2 in our CF Clinic. During this encounter one of P36F2' 3 strains of *P. aeruginosa*, DK15, is transmitted to her, where it establishes a persistent infection



# DK15 in two CF patients

Pathoadaptive genes | Antibiotics against *P. aeruginosa*, MIC values

| | Sample_ID | Genotype | Year | # NS mutations in pathoadaptive genes | Colistin | Piperacillin | Aztreonam | Tobramycin | Ceftazidim | Meropenem | Ciprofloxacin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| P36F2 | 427 | DK15 | 2006 | 1 | 6 | 2,00 | 0,75 | 2,00 | 0,38 | 0,19 | 1,50 |
| | 436 | DK15 | 2007 | 0 | 1,5 | 6,00 | 2,00 | 0,75 | 1,50 | 0,38 | 0,19 |
| | 423 | DK15 | 2011 | 2 | 4 | 3,00 | 1,00 | 2,00 | 0,50 | 0,19 | 1,50 |
| P19F5 | 135 | DK15 | 2008 | 2 | 1 | 6,00 | 3,00 | 0,75 | 2,00 | 0,50 | 0,25 |
| | 136 | DK15 | 2009 | 3 | 1 | 12,00 | 4,00 | 1,50 | 4,00 | 0,75 | 0,19 |
| | 139 | DK15 | 2010 | 9 | 1 | 1,00 | 0,19 | 1,00 | 2,00 | 0,38 | 1,50 |
| | 148 | DK15 | 2011 | 9 | 1 | 0,50 | 0,19 | 0,75 | 1,00 | 0,13 | 0,75 |
| | 140,1 | DK15 | 2011 | 9 | 1 | 1,00 | 0,19 | 0,75 | 1,50 | 0,09 | 0,75 |
| | 138 | DK15 | 2012 | 9 | 1 | 1,50 | 0,25 | 1,50 | 1,50 | 0,13 | 1,50 |
| | 141 | DK15 | 2012 | 8 | 1 | 1,50 | 0,25 | 2,00 | 2,00 | 0,25 | 2,00 |
| | 134 | DK15 | 2012 | 20 | 0,75 | 1,50 | 0,50 | 1,50 | 2,00 | 0,19 | 6,00 |
| | 142 | DK15 | 2012 | 8 | 1,5 | 24,00 | 16,00 | 1,50 | 8,00 | 1,50 | 3,00 |
| | 143 | DK15 | 2012 | 8 | 0,75 | 1,00 | 0,25 | 3,00 | 1,00 | 0,13 | 1,00 |
| | LR17 | DK15 | 2013 | 14 | 1 | 8,00 | 8,00 | 3,00 | 8,00 | 0,25 | 0,75 |

Hypermutator

Susceptible

Intermediate

Resistant

DK15 is a naïve strain of *P. aeruginosa* with only few mutations and no sign of CF pathogen phenotype. It is present as a minor sub-population in P36F2's airways. The genome of the first DK15 isolate from P19F5 is 100% identical to that of the contemporary DK15 isolate from P36F2 - no mutations separate the two.

Resistance is not fixed in the population - despite intensive antibiotic therapy

Shift from a naïve state of colonisation without patho-adaptive mutations to an adapted persistent state

Accumulation of several patho-adaptive mutations

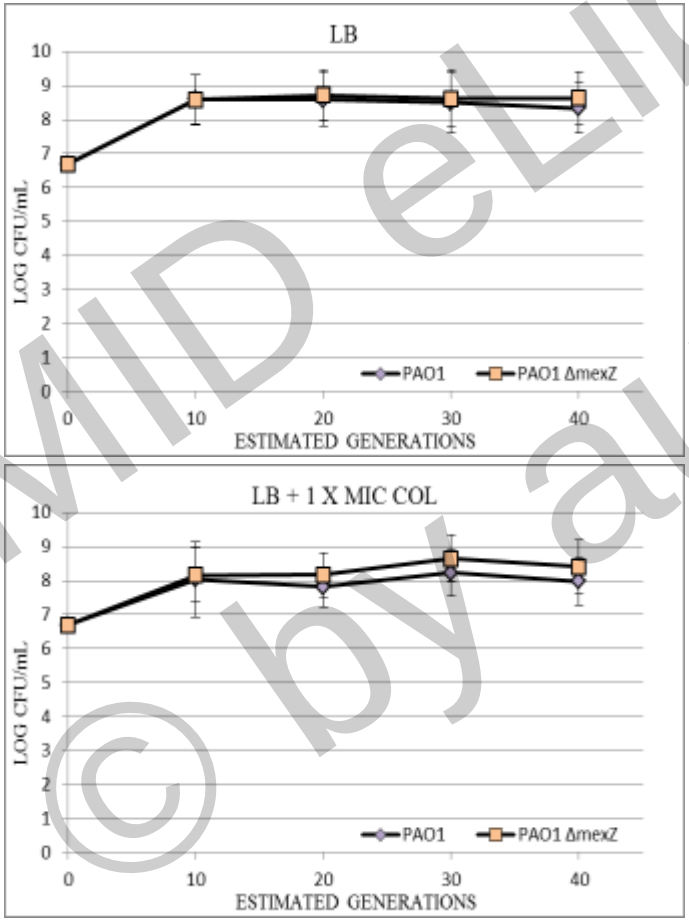
| Sample_ID | Patient | Date | Genotype | dato | muc | alg | mex | opr | gyr | wsp | chp | mut | Misc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 427 | P36F2 | 38958 | DK15 | 2006,66 | | | | | | | | | |
| 436 | P36F2 | 39112 | DK15 | 2007,08 | | algQ | | | | | | | |
| 439,1 | P36F2 | 40424 | DK15 | 2010,68 | | | | | | | | | |
| 423 | P36F2 | 40598 | DK15 | 2011,15 | | algQ | | | | | | | |
| | | | | | | | | | | | | | |
| 135 | P19F5 | 39645 | DK15 | 2008,54 | | | | | | | | | |
| 136 | P19F5 | 39819 | DK15 | 2009,02 | | | | | | | | | pilQ |
| 136,1 | P19F5 | 39819 | DK15 | 2009,02 | | | | | | | | | pilQ |
| BM51 | P19F5 | 40085 | DK15 | 2009,73 | | | | | | | | | pilQ |
| 139 | P19F5 | 40533 | DK15 | 2010,98 | mucA3 | algQ | mexR, mexB2, mexZ | | gyrA | | | | pilQ |
| 148 | P19F5 | 40552 | DK15 | 2011,03 | | algU3 | mexR, mexA1, mexA2, mexA3, mexZ | | gyrA | | | | pilQ |
| 147 | P19F5 | 40552 | DK15 | 2011,03 | mucA1 | algU1 | mexR, mexB2, mexZ | | gyrA | | | | pilQ |
| 140,1 | P19F5 | 40631 | DK15 | 2011,25 | mucA1 | algU4, algQ | mexR, mexB2, mexZ | | gyrA | | | | pilQ |
| 145 | P19F5 | 40631 | DK15 | 2011,25 | mucA5 | | mexR, mexZ | | gyrA | | | | pilQ |
| 140 | P19F5 | 40631 | DK15 | 2011,25 | mucA1, mucR | | mexR, mexB2, mexZ | | gyrA | | | | pilQ |
| 138,1 | P19F5 | 40926 | DK15 | 2012,05 | | algF1, algF2 | mexR, mexA1, mexA2, mexZ | | gyrA | | | | pilQ, pelA1 |
| 137 | P19F5 | 40926 | DK15 | 2012,05 | mucA2 | | mexR, mexA2, mexZ | | gyrA | | | | pilQ |
| 138 | P19F5 | 40926 | DK15 | 2012,05 | | algU2, algQ | mexR, mexA1, mexA2, mexZ | | gyrA | | | | pilQ, pelA1 |
| 141 | P19F5 | 41011 | DK15 | 2012,28 | | | mexR, mexA1, mexA2, mexZ | | gyrA | | | | pilQ, pelA1 |
| [redacted] | P19F5 | 41088 | [redacted] | 2012,49 | mucA4 | algG3, algF3, algQ | mexR, mexB1, mexB2, mexB3, mexB4, mexZ | oprM | gyrA | | chpA2 | mutL | pilQ |
| [redacted] | P19F5 | 41088 | [redacted] | 2012,49 | mucA1, muc | algF3 | mexR, mexB2, mexB4, mexZ | | gyrA | wspA | | mutL | pilQ |
| 142 | P19F5 | 41206 | DK15 | 2012,82 | | algU1 | mexR, mexZ | | gyrA | | | | pilQ |
| [redacted] | P19F5 | 41206 | [redacted] | 2012,82 | mucR | algU5, algF1, algF2 | mexR, mexA1, mexA2, mexZ | | gyrA | | | mutS | pilQ, pelD |
| [redacted] | P19F5 | 41383 | [redacted] | 2013,30 | | algG1, algG2, algF1, algF2, algB | | | gyrA | | chpA1 | mutS, mutM | pilQ, pelD |
| [redacted] | P19F5 | 41383 | [redacted] | 2013,30 | mucP | algI, algQ | mexI | | gyrA | wspR | | mutS | pilQ, pelA2, pelD |

# Resistance phenotype of mexZ mutants

| | Minimal inhibition concentration (MIC) is given in µg/mL | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AMK | TOB | GEN | CEF | MER | AZT | CIP | COL |
| PAO1 | 3 | 0.38 | 1.5 | 1.5 | 0.5 | 3 | 0.064 | 2 |
| PAO1 ΔmexZ | 6 | 0.5 | 2 | 1.5 | 0.5 | 3 | 0.19 | 2 |
| PAO1 ΔmexY | 1 | 0.125 | 0.25 | 1.5 | 0.5 | 3 | 0.047 | 2 |
| PA14 | 2 | 0.25 | 1 | 1.5 | 0.125 | 3 | 0.047 | 0.5 |
| PA14 ΔmexZ | 3 | 0.25 | 1 | 1.5 | 0.25 | 3 | 0.125 | 0.5 |

Values in green are sensitive, yellow are intermediate resistant and red resistant

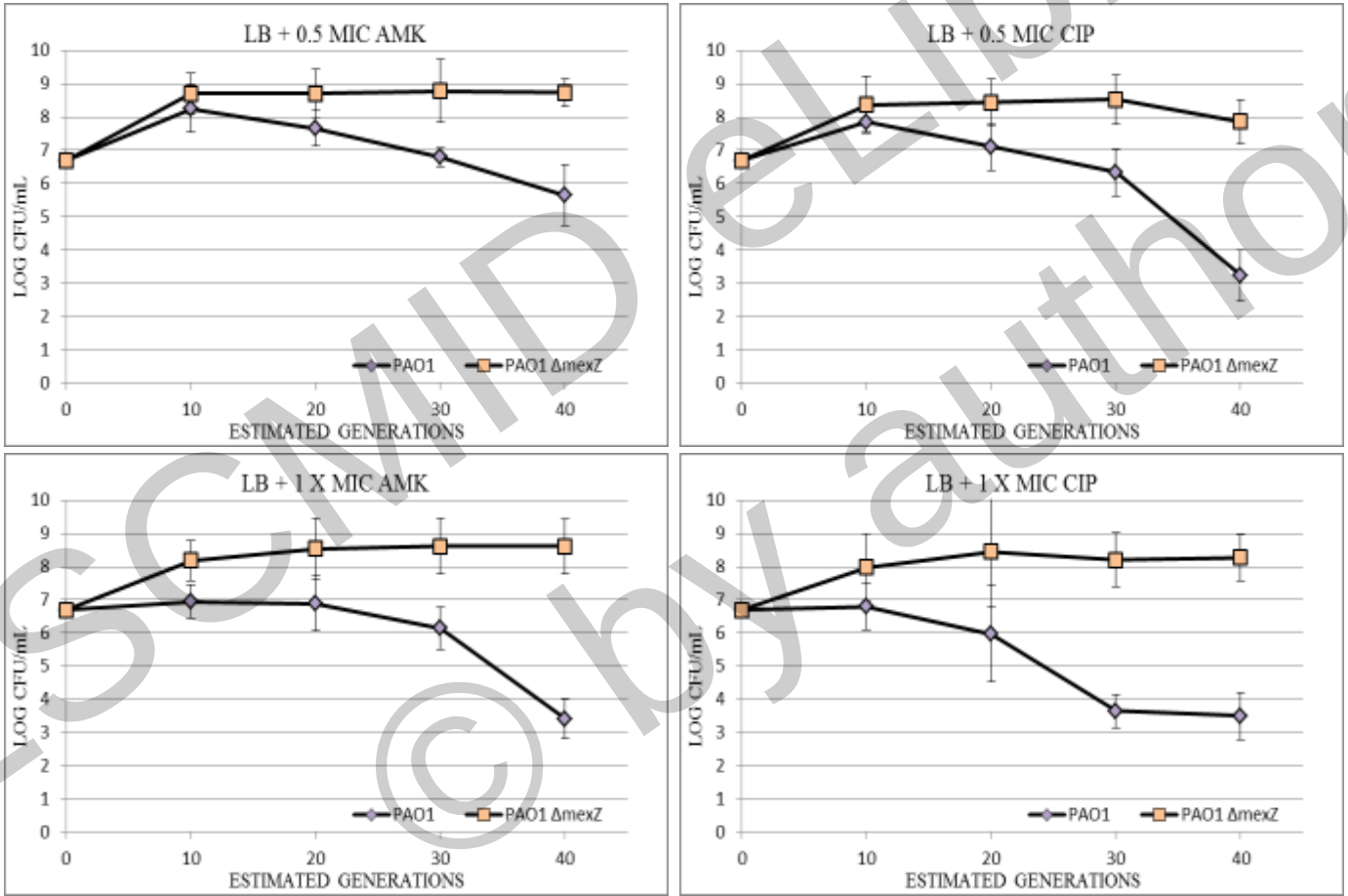
# Competition experiment (control) similar growth






Frimodt-Møller J

# Competition experiment
## introduction of mexZ mutation in 'a clean background' PAO1





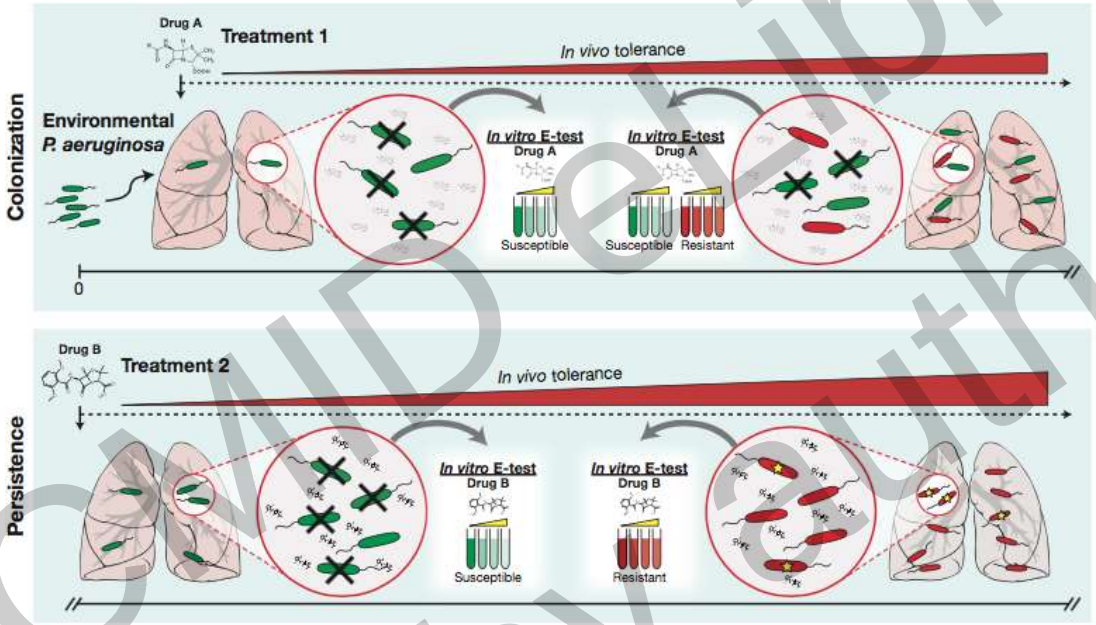

Frimodt-Møller J

# Antibiotic resistance mutations do not always correlate with antibiotic resistance

- Mutations in mexZ lead to induction of the MexYX efflux pump
- No significant change in MIC for aminoglycosides or fluoroquinolones
- Resistant strains show no increased frequency of mexZ mutations
- Clinical problem:
  - ✓ Resistance testing does not detect mexZ variants with increased competitive advantage in presence of antibiotics

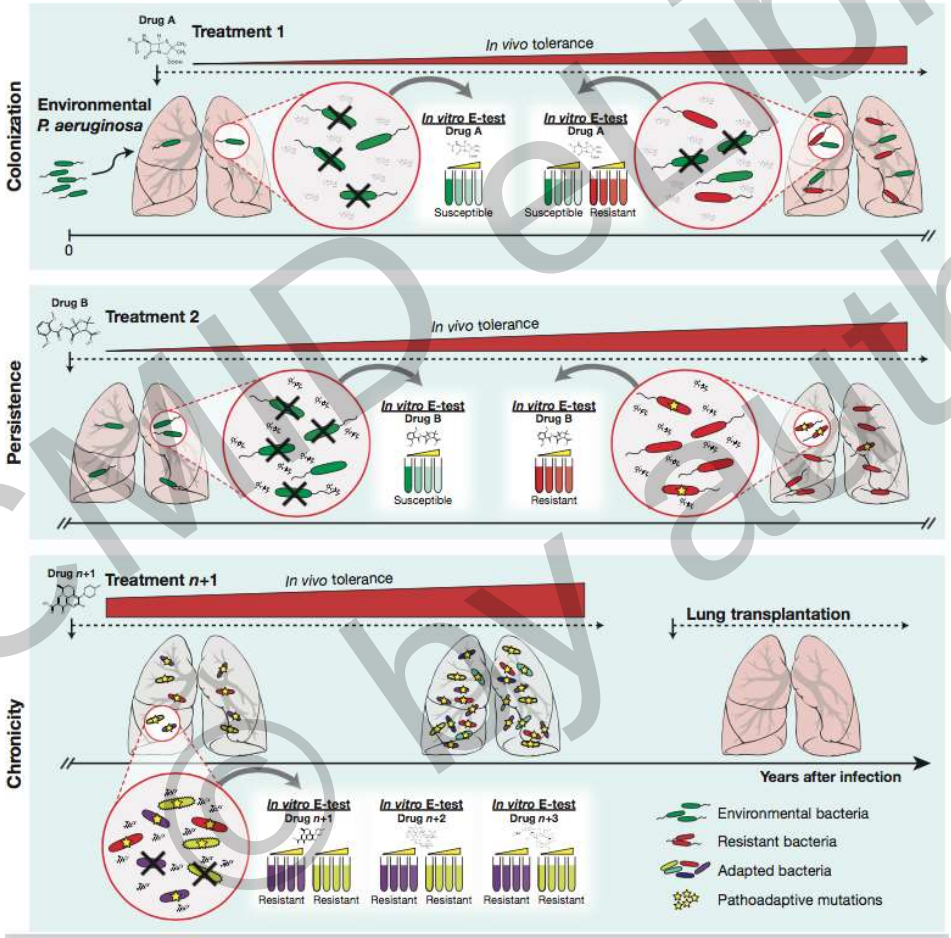
Persistence is associated with pathoadaptive mutations

# CF airway infections - persistence



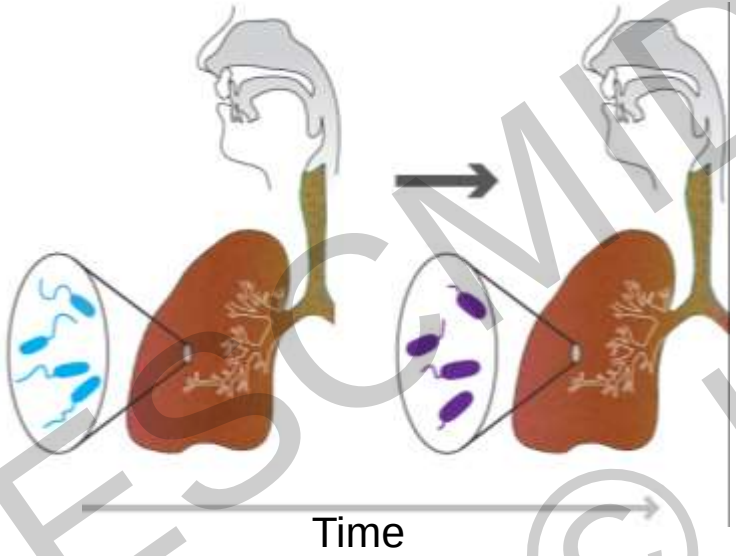

- Disappearance of drug A resistant bacteria - few survivors after drug B treatment
- Accumulation of patho-adaptive mutations
- Continued treatment results in appearance of resistant population of bacteria

# CF airway infections - chronicity



# *P. aeruginosa* DK2 is a successful chronically infecting pathogen in the airways of CF patients

Genetic adaptation to host airways



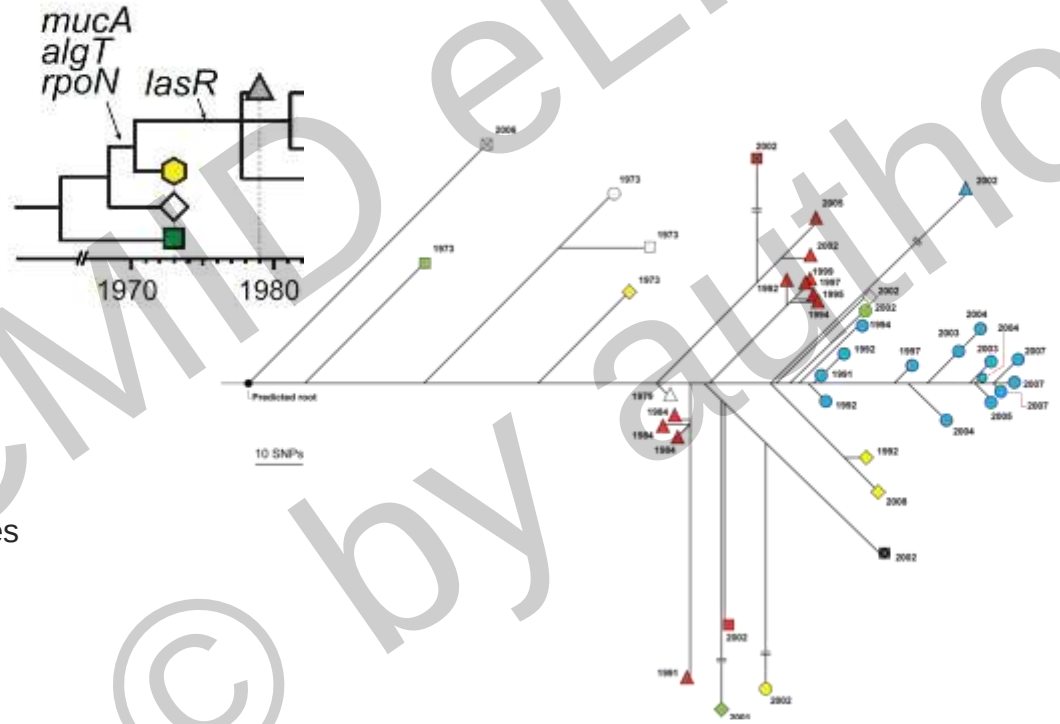

The DK2 clone has disseminated through a cohort of >40 Danish CF patients over a period of >40 years (approx. 200,000 bacterial generations)

Why is the DK2 clone type so successful?

- What is the genetic basis of adaptation?
- What are the evolutionary trajectories?

Yang et al. PNAS, 2011

# Phylogenetic relationships of DK2 clones from chronically infected CF patients - extreme variant

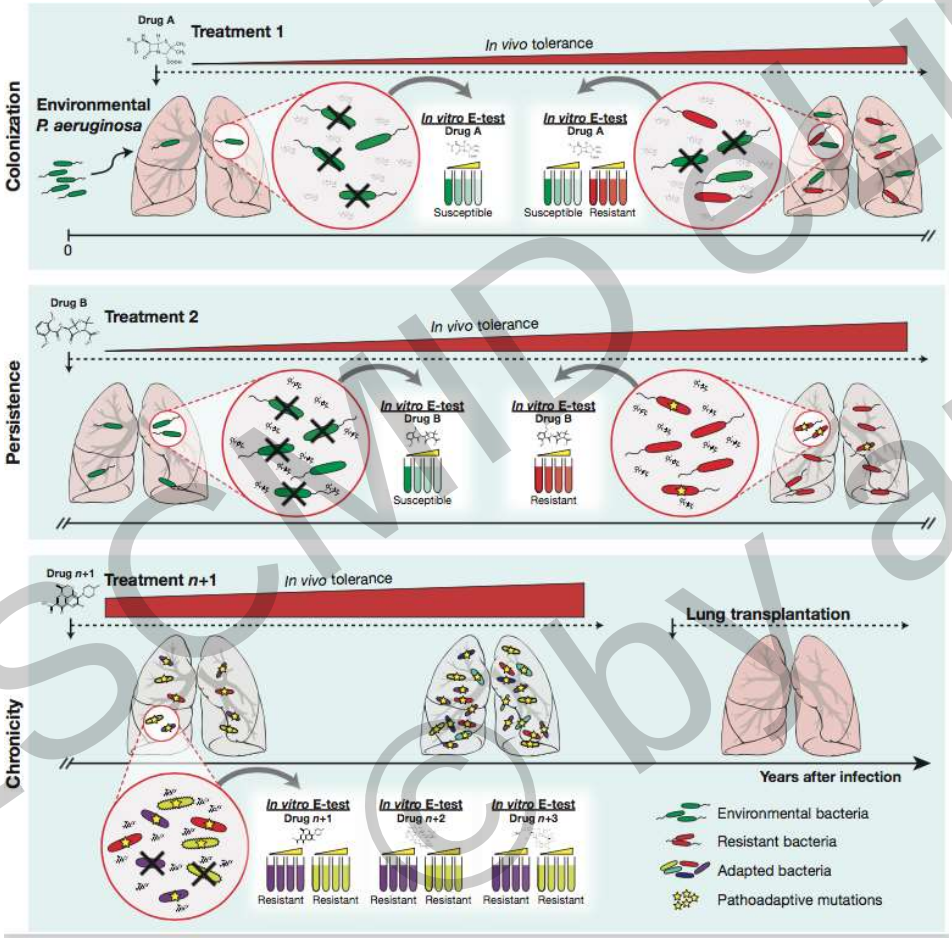



Mutations of large phenotypic impact accumulated early

Mutations in four global regulator genes contributed significantly to the “evolved very fit phenotype”

Marvig et al. PloS Genetics 2013

# CF airway infections - chronicity




Fully adapted clones:

- Multi-resistant
- Many patho-adaptive mutations
- Outcompete less adapted clone types

Ruggero la Rosa

# Conclusions

- Initial colonisation frequently results in persistent infections
- There is no single explanation for this transition
- Among the genetic changes behind persistence a small number of patho-adaptive mutations seem to be especially important
- Most patho-adaptive mutations can not be detected in the clinical microbiology laboratory
- Genetic changes may not always correlate with phenotypic changes
- Particular fitness increasing mutations can drive persistence into chronic infection
- We need to translate the genomic information into useful biomarkers for infection

David Cameron

Lea M. Sommer

Rasmus Marvig



novo nordisk fonden

LUNDBECKFONDEN

DET FRIE FORSKNINGSRÅD
DANISH COUNCIL FOR INDEPENDENT RESEARCH

REGION Rigshospitalet

REGION Region Hovedstaden