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Evolution of resistance to old (and new) antibiotics:
which are the relevant factors to measure for risk prediction?

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Mutation rates are often used to predict the risk of resistance development (by pharmaceutical industry, researchers etc.)

Is this relevant or just something that is relatively easy to measure?

Main conjecture:

Mutation rate to resistance is a poor predictor
of the risk of emergence of resistance in a patient

Are we basing dosing strategies to reduce emergence of resistance on measurements of the wrong parameters?

Is the pharmaceutical industry discontinuing drug development, because of resistance issues, for the wrong reasons?

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Many factors influence the emergence and transmission of resistant bacteria

1. Selective pressures (antibiotics, heavy metals, biocides)

1. Emergence rates (mutation- and horizontal gene transfer rates, population sizes)

3. Fitness costs of resistance

4. Transmission dynamics (host population structure and density, immunity, migration, hygienic measures etc.)

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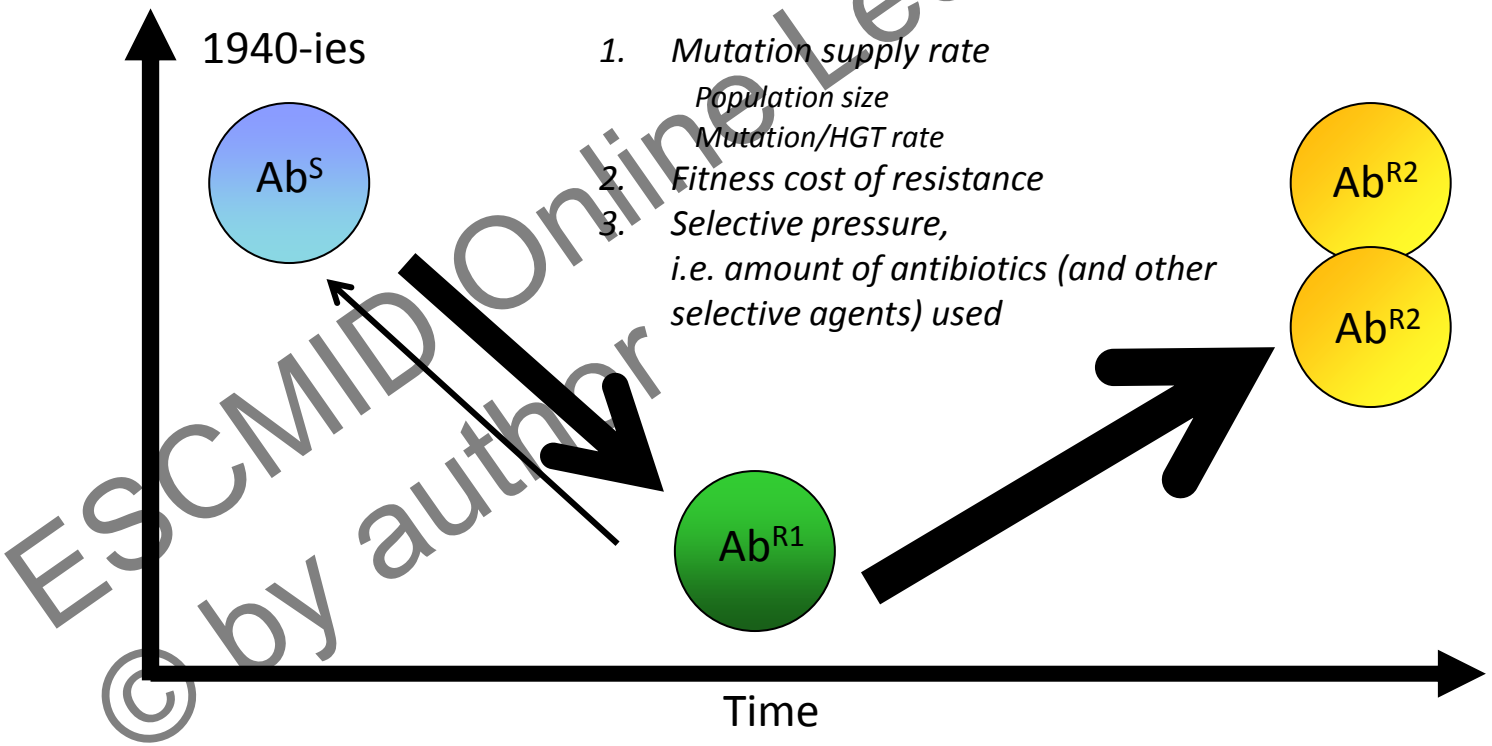
Evolution of antibiotic resistance: a two-step process

RESISTANCE SELECTION **FITNESS SELECTION**

Individual
Population



1. *Mutation supply rate*
   Population size
   Mutation/HGT rate
2. *Fitness cost of resistance*
3. *Selective pressure, i.e. amount of antibiotics (and other selective agents) used*

Björkman et al PNAS 1998, Björkman et al Science 2000, Nilsson et al PNAS 2005, Andersson and Hughes Nature Rev Microbiol 2010, Andersson and Hughes Nature Rev Microbiol 2014

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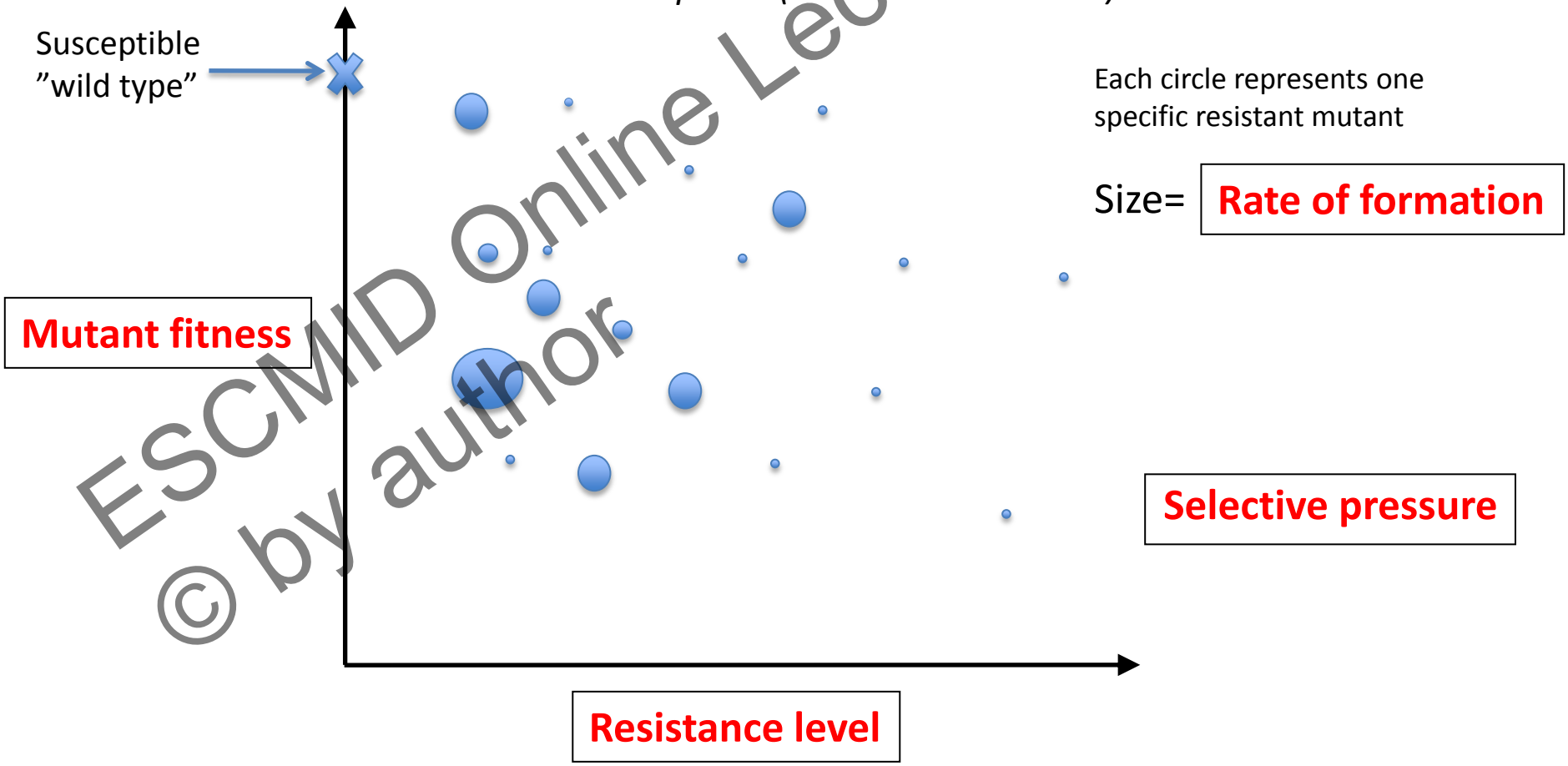
Predicting resistance evolution

Biological factors of importance

"Mutational space" (mutations and HGT)



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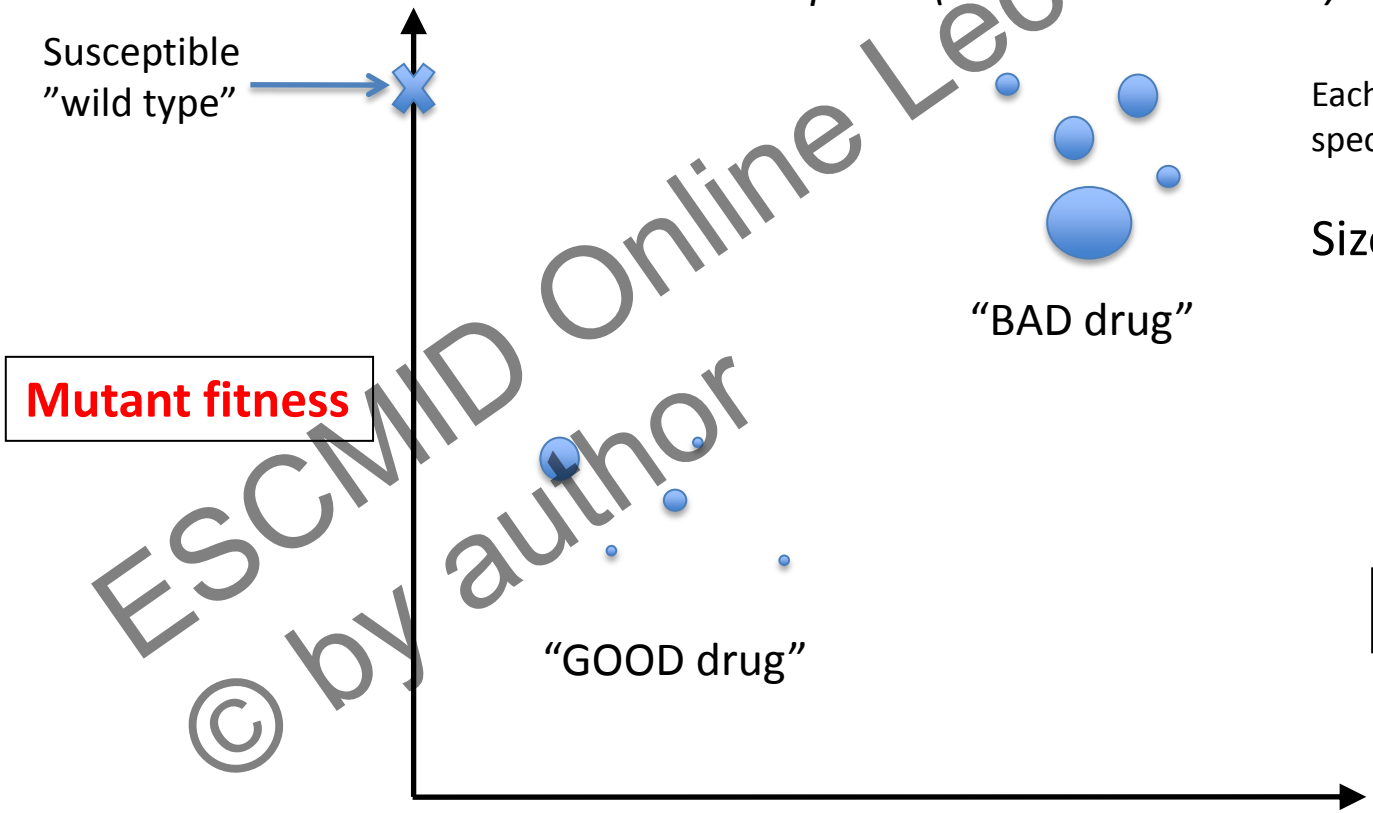
Predicting resistance evolution

Biological factors of importance

"Mutational space" (mutations and HGT)



Each circle represents one specific resistant mutant

Size= **Rate of formation**

Selective pressure

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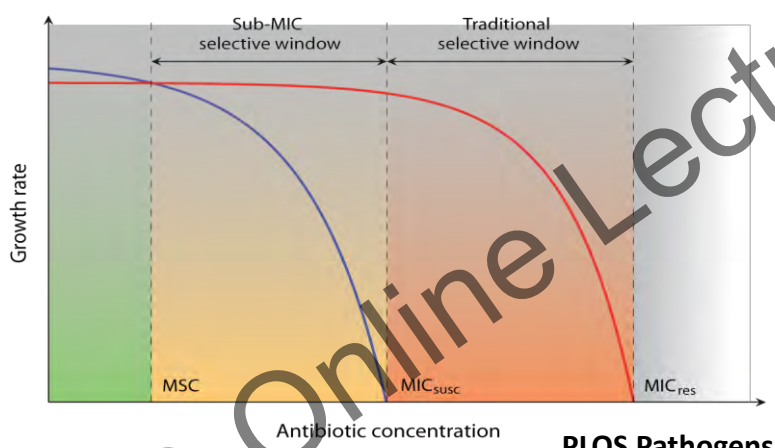
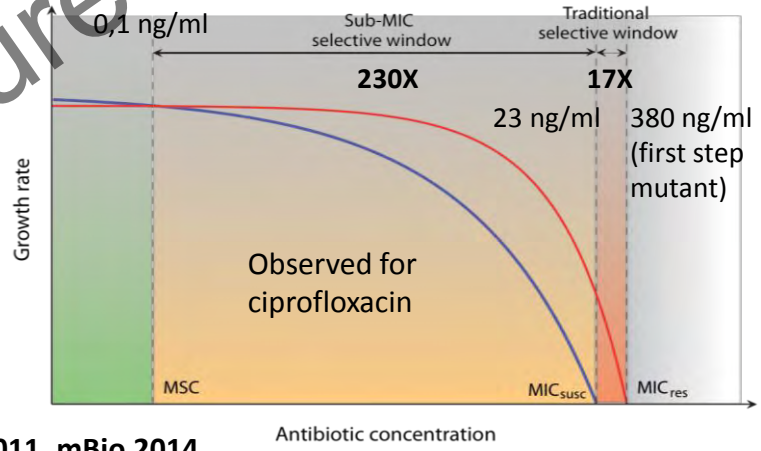
How to measure the relevant parameters?

1. Selective pressures

a. Competition experiments R+S at different antibiotic concentrations
(Classical selective window not sufficient)





PLOS Pathogens 2011, mBio 2014

2. Emergence (mutation) rates

a. Serial passage at increasing AB concentration
(only gives a qualitative answer—no rates)
b. Static time-kills with different population sizes
(only gives a semi-qualitative answer)
c. Luria Delbruck fluctuation tests
(the proper way—gives rates)

3. Fitness costs of resistance

In vitro, in animals, in humans, without and with antibiotics

a. Growth in single cultures
b. Competition experiments (R+S)

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Slower resistance development when:

Rates of emergence of resistance are expected to be slow when:

For mutational resistance

1. Multiple targets, e.g. beta-lactams acting on several PBPs
2. Multiple genes encoding same target, e.g. drugs binding to rRNA and inhibiting translation (aminoglycosides, macrolides, tetracyclines etc.)

For horizontally transferred resistance

1. Low ecological connectivity
2. Genetic barriers (low HGT rates, restriction/CRISPR systems, recombination barriers etc.)

Rule of thumb in industry: Mutation rate less than 10^{-8} with selection at 4xMIC of susceptible wt
Presently used AB that would not pass that bar if tested against, for example, E. coli, Salmonella

Aminoglycosides (gidB knockouts)	10^{-7}/cell/generation, 10xMIC
Colistin (pmrAB point mutations)	10^{-6}/cell/generation, 2-35xMIC
Rifampicin (rpoB point mutations)	10^{-8}/cell/generation, >5xMIC
Fosfomycin (5 genes)	10^{-7}/cell/generation, 6-1000xMIC
Mecillinam (>40 genes)	10^{-6}/cell/generation, 10-200xMIC
Nitrofurantoin (nsfAB knockouts)	10^{-7}/cell/generation, 2xMIC
Fluoroquinolones (marR knockouts)	10^{-7}/cell/generation, 2xMIC

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Slower resistance development when:

Fitness cost of resistance is high (and difficult to compensate)

Examples of antibiotics with very high mutation rates but also typically high fitness cost mutations

	Mutation rate, resistance increase
Fosfomycin (about 5 genes)	10^{-7}/cell/generation, 6-1000xMIC
Mecillinam (>40 genes)	10^{-6}/cell/generation, 10-200xMIC
Nitrofurantoin (nsfAB)	10^{-7}/cell/generation, 2xMIC

	Relative fitness (susceptible wt =1)
Fosfomycin (about 5 genes)	0.75-0.85
Mecillinam (>40 genes)	0.29-0.76
Nitrofurantoin (nsfAB)	0.90-0.95

Nilsson et al AAC 2003
Sandegren et al JAC 2008
Thulin et al 2014

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Why mutation rate is not a good predictor of the risk of clinical resistance development

1. Mutation rates to resistance typically in the range of 10^{-10} (e.g. lineozolid) to 10^{-6} (e.g. mecillinam)

2. 1/population size > mutation rate for most types of infections
   → pre-existing resistant mutants in most populations

3. I.e. for many infections the appearance of resistant mutants is not (strongly) limited by the mutation supply rate

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Experimental support: Fosfomycin, mecillinam and nitrofurantoin resistance in *E. coli*

-Fosfomycin, mecillinam and nitrofurantoin are typically used for treatment of lower UTIs

-Fosfomycin inhibits early step in cell wall biosynthesis (MurA)

-Mecillinam inhibits late step in cell wall biosynthesis (MrdA-PBP2)

-Nitrofurantoin is converted by bacterial nitroreductases to a highly reactive electrophilic compound that damages proteins and DNA

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A paradox, sort of…

Mutation rate to resistance high, 10^{-7} to 10^{-6}/cell/gen, because of common loss-of-function mutations

Population size: ca. 10^5-10^8 bacteria/ml

Urine volume: 1-300 ml

Total bacterial population size in bladder: typically >>10^6

Based on these population sizes and mutation rates, modeling shows that probabilities for fixation of fosfomycin, mecillinam and nitrofurantoin resistant mutants during treatment are very high (20-50%)

However:
-Resistance development during treatment low

So how can we account for this?

1. **Population dynamics**
2. **Fitness**

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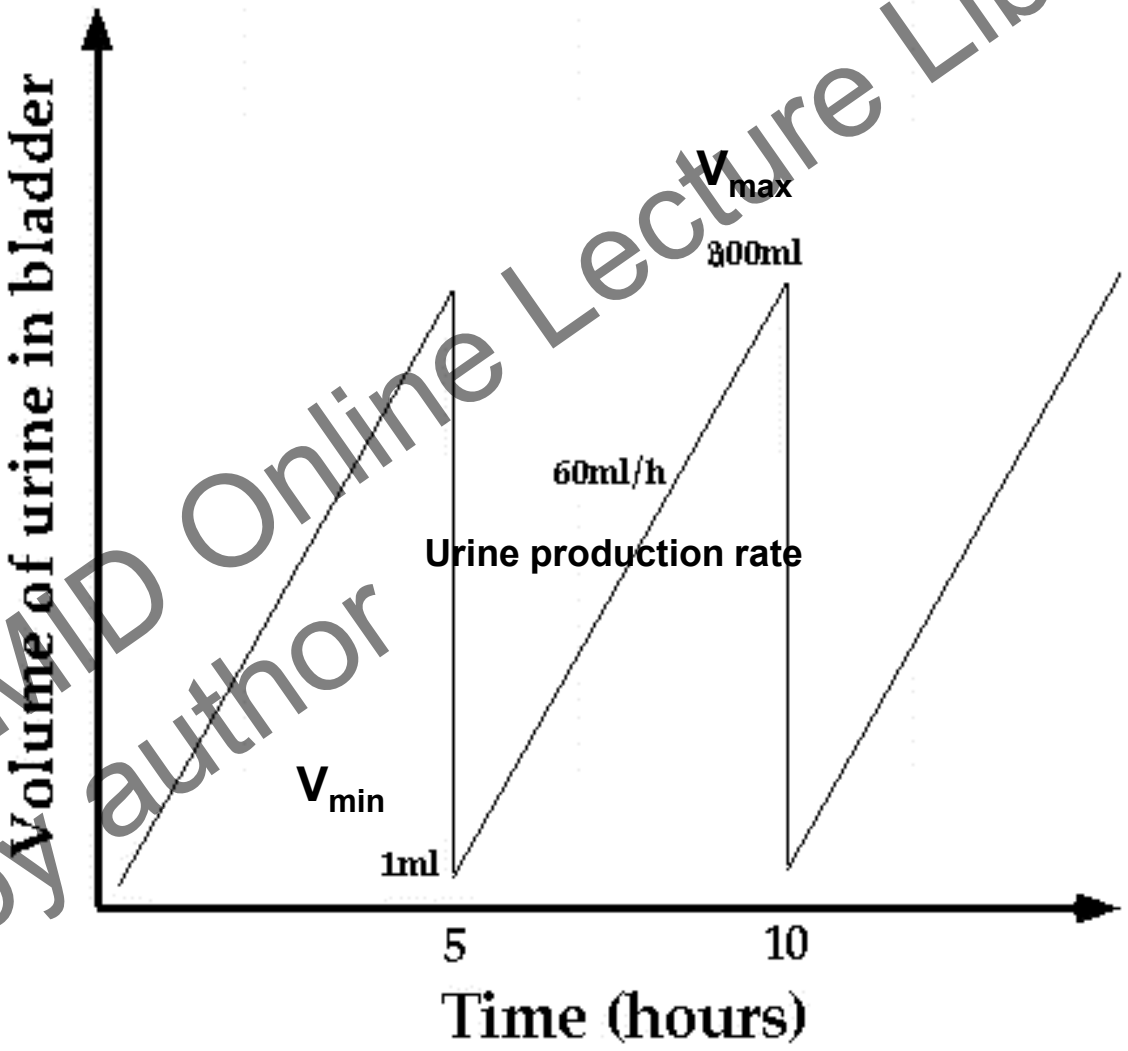
Dynamics of urine flow in a healthy bladder



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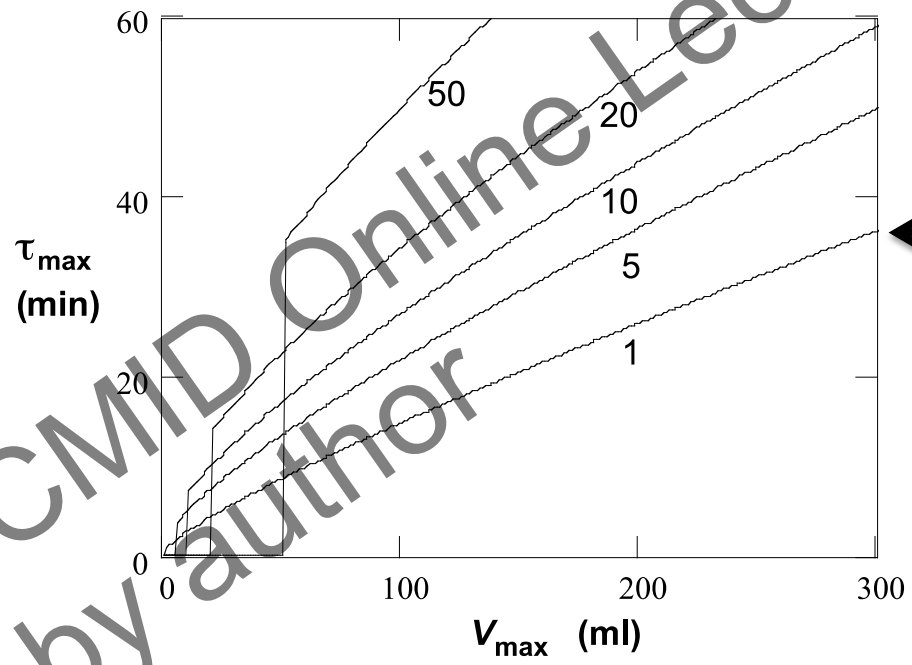
Minimal generation time required to allow establishment in bladder at different V_{max} and V_{min}



In a "normal" bladder t_{gen} has to be 36 min or shorter for bacteria to be maintained

I.e. bacteria have to grow fast enough to balance the effect of dilution by urine production and flush out by micturition

Nilsson et al AAC 2003

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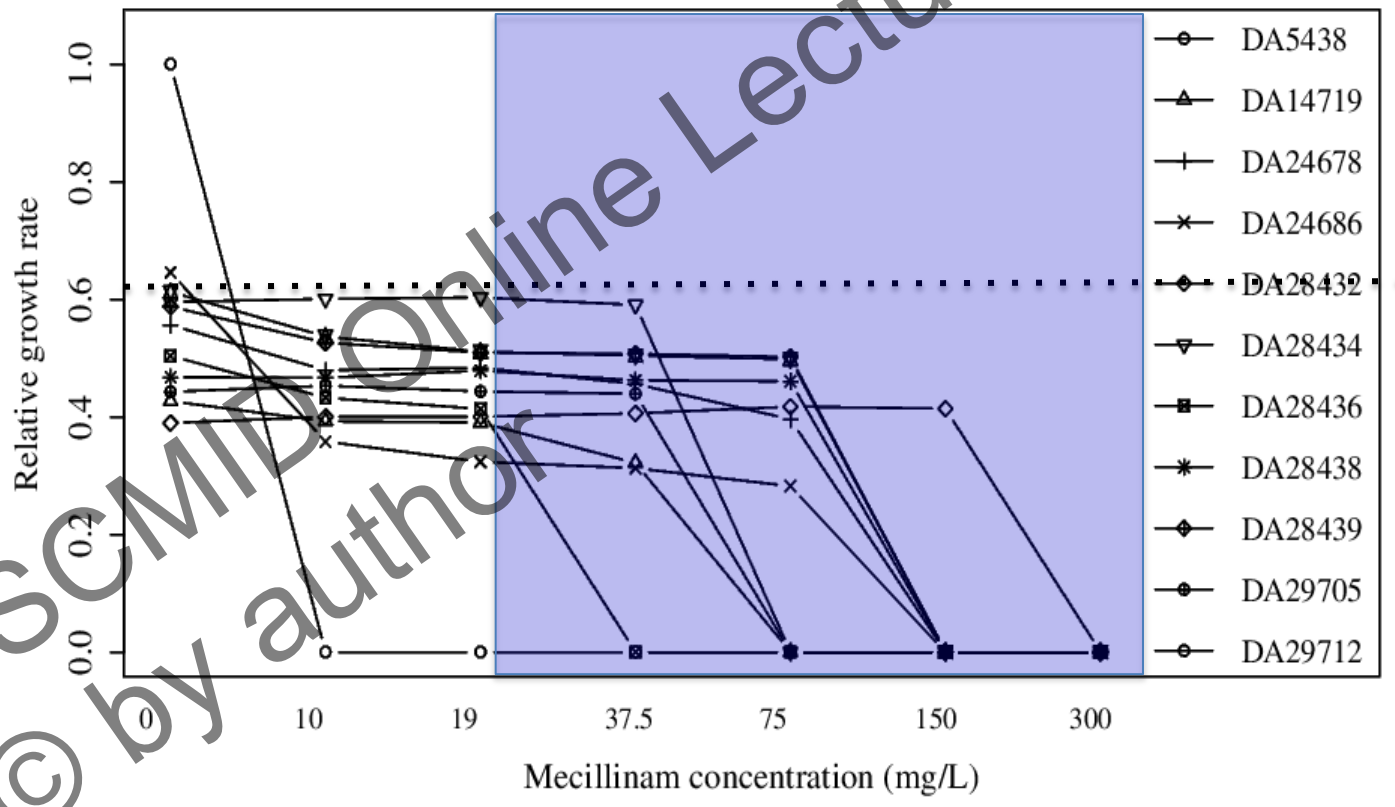
Mecillinam resistant mutants show a reduced fitness in absence, and presence, of drug

MecS

MecR



Thulin et al 2014

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Three factors prevent establishment of frequently occurring resistant mutants:

1. Fitness cost associated with resistance --> grows to slowly to fix

2. Resistance is not complete, i.e. with increasing antibiotic concentration the growth rate is reduced below a threshold level --> grows to slowly to fix

3. Turnover (e.g. bladder flow, immune system) of bacterial population→ even though mutants appear they do not fix

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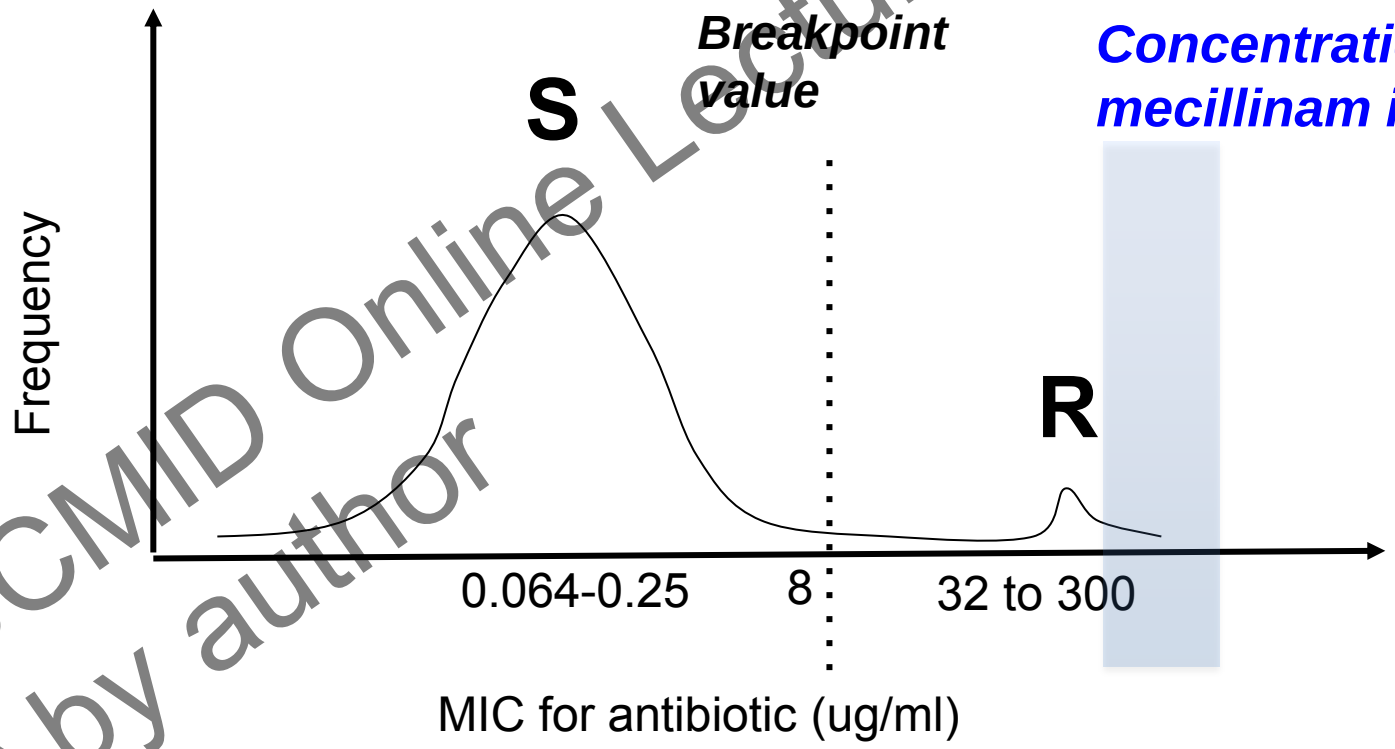
A potential problem with clinical breakpoint values

Mecillinam breakpoint values



Essentially the same conclusion for fosfomycin and nitrofurantoin: Concentration of antibiotic in urine is well above the clinical breakpoint value and the level of resistance of most resistant mutants

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Summary:

1. Mutation rate (and by inference static time-kill) is a poor predictor of the risk of resistance development clinically

2. To perform better predictions we need to:
   a. Determine fitness of resistant mutants in absence and presence of drug, i.e. growth rate = f (antibiotic concentration)

   b. Include in vivo population sizes of the relevant pathogens and infections
   (why has 10^6-10^7 become standard for static time-kill?)

   c. Include bacterial dynamics during infection; e.g. turnover rates, by immune system or other factors (e.g. micturition)
   (will resistance development towards mecillinam, fosfomycin, nitrofurantoin be faster if used for other infections than UTIs?)

3. Too strict implementation of breakpoints might preclude us from using still effective antibiotics