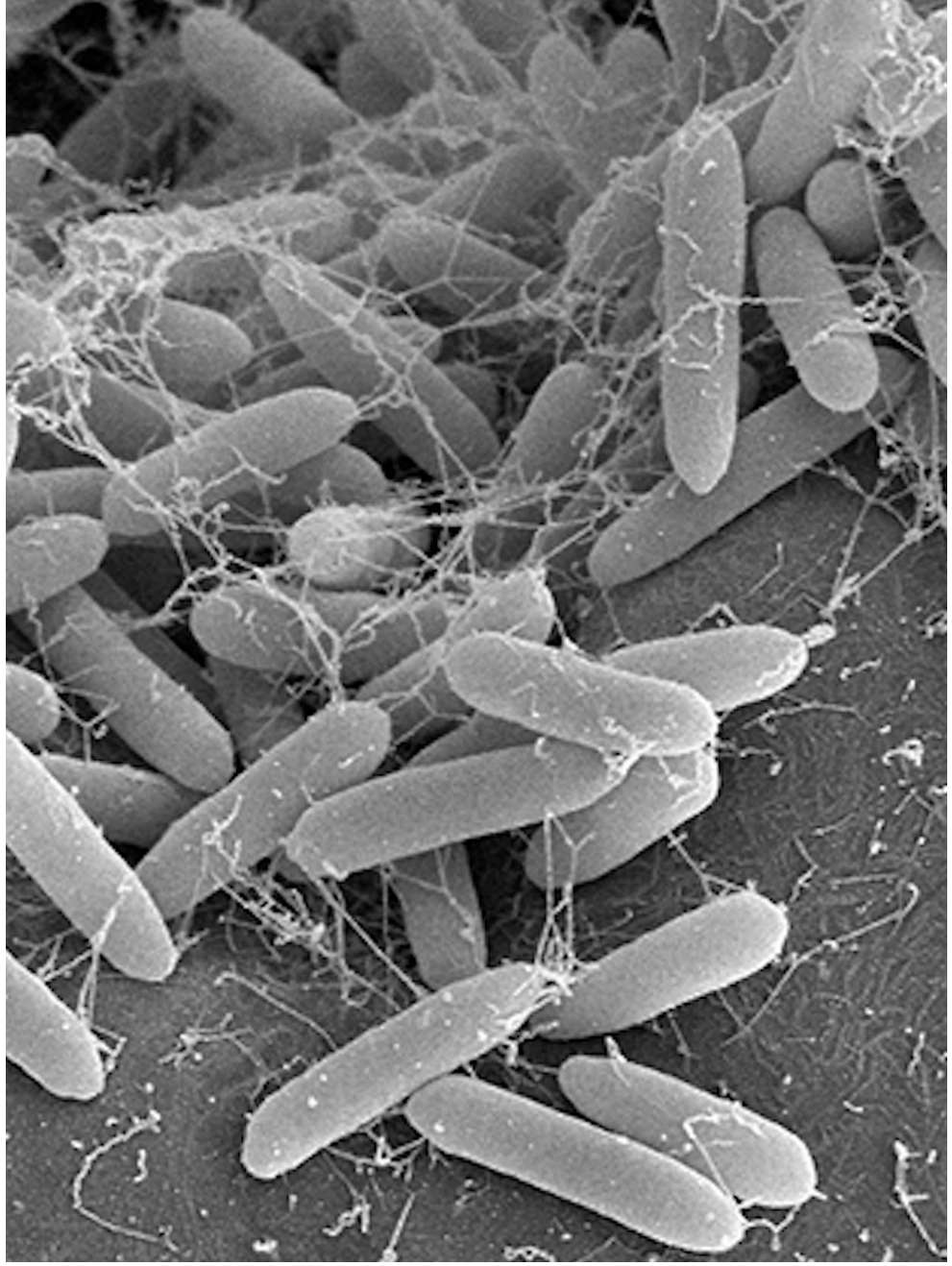
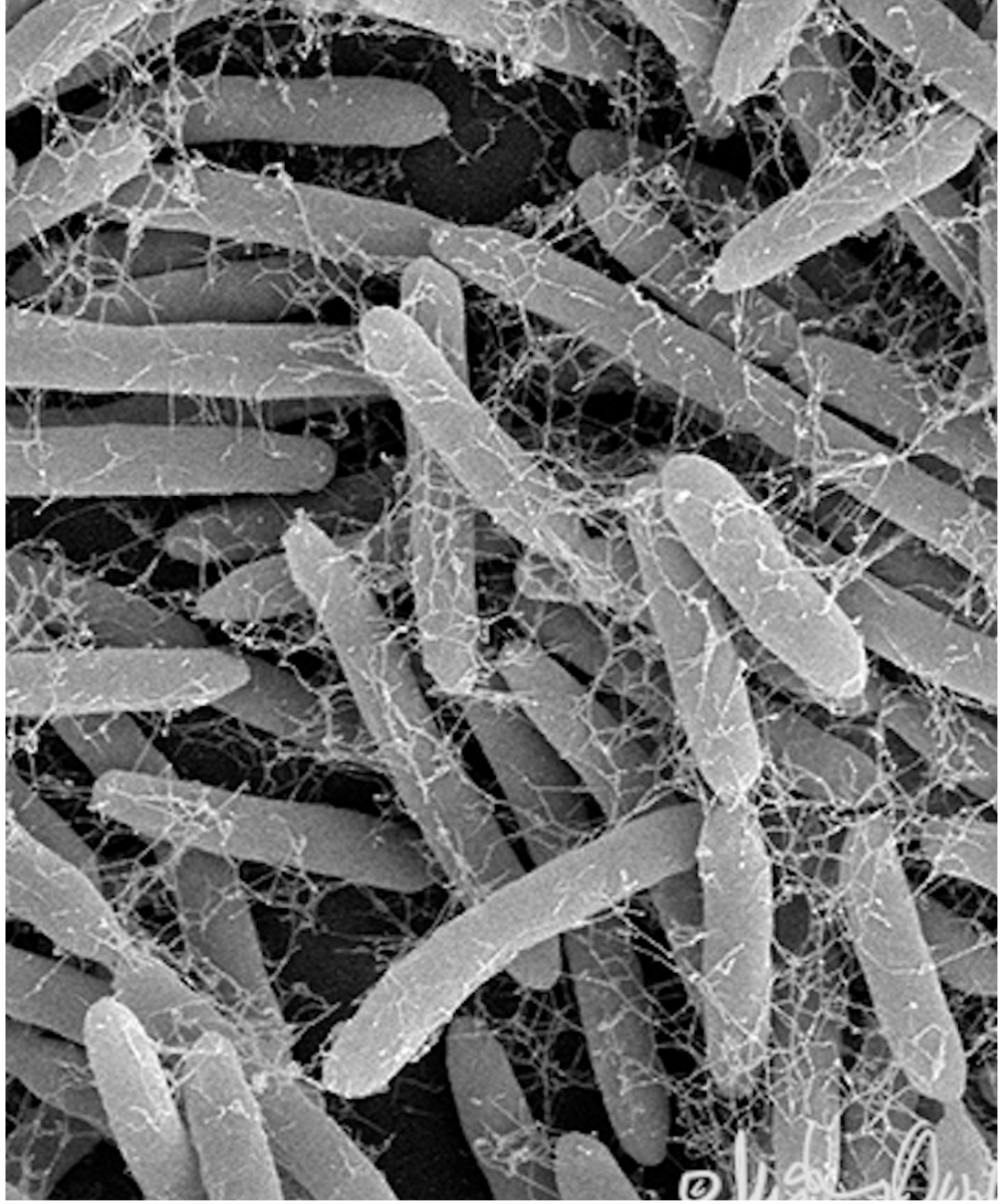


Pseudomonas aeruginosa w/o Piperacillin Tazobactam



Pseudomonas aeruginosa with Piperacillin Tazobactam

Detecting Antimicrobial Resistance: The Silent Pandemic

-

AMR in other bacteria

Prof. Adrian Egli, MD PhD
Institute for Medical Microbiology, University of Zurich

25.10.2022

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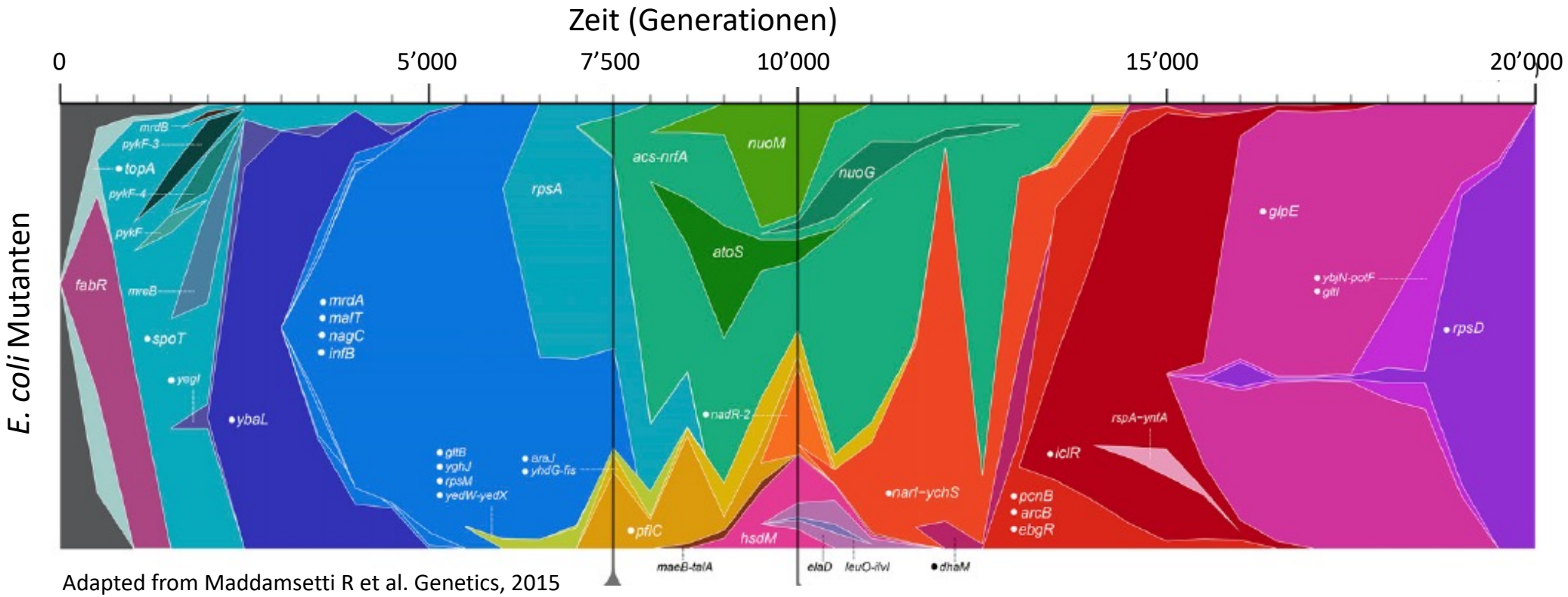
@AppliedMicrobi2

Universität Zürich[UZH]

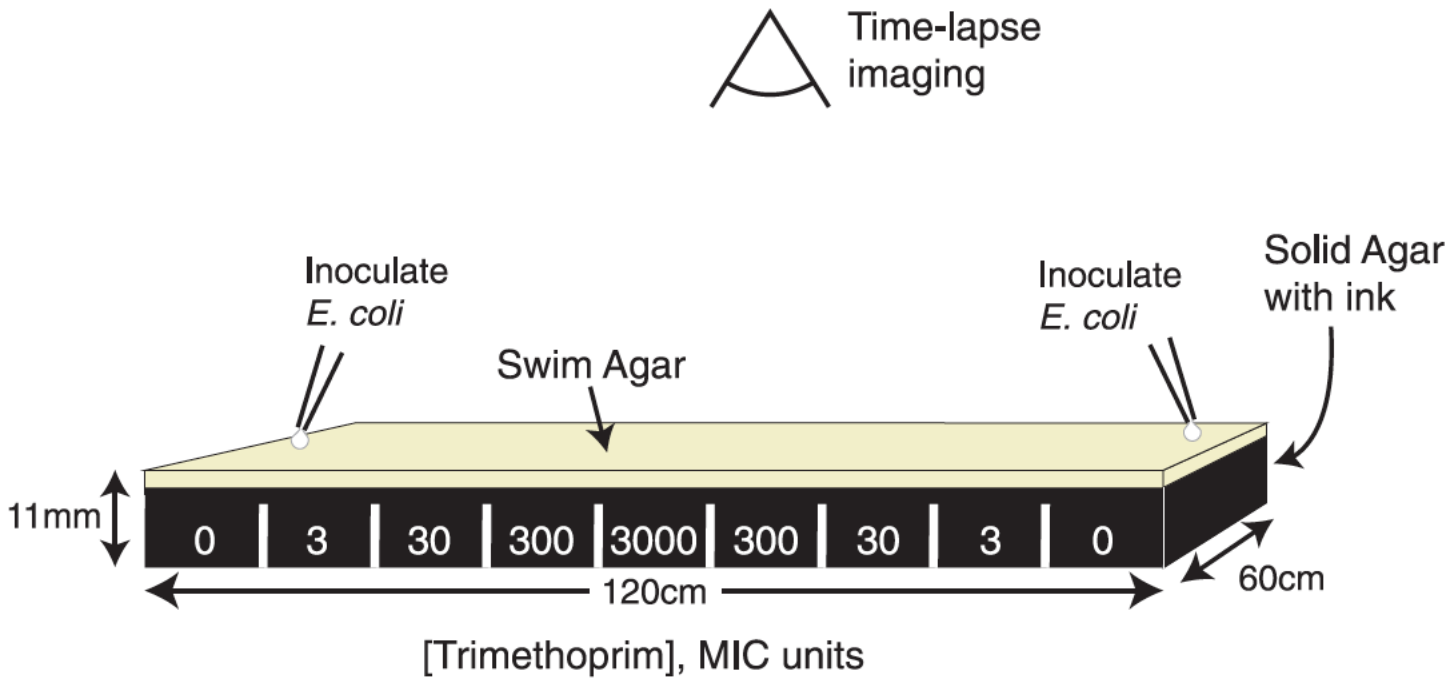
The Lenski experiment: a world of variants

- > 25 years of *E. coli* in culture with more than 60’000 passages
- Stochastical element of evolution



Adapted from Maddamsetti R et al. Genetics, 2015

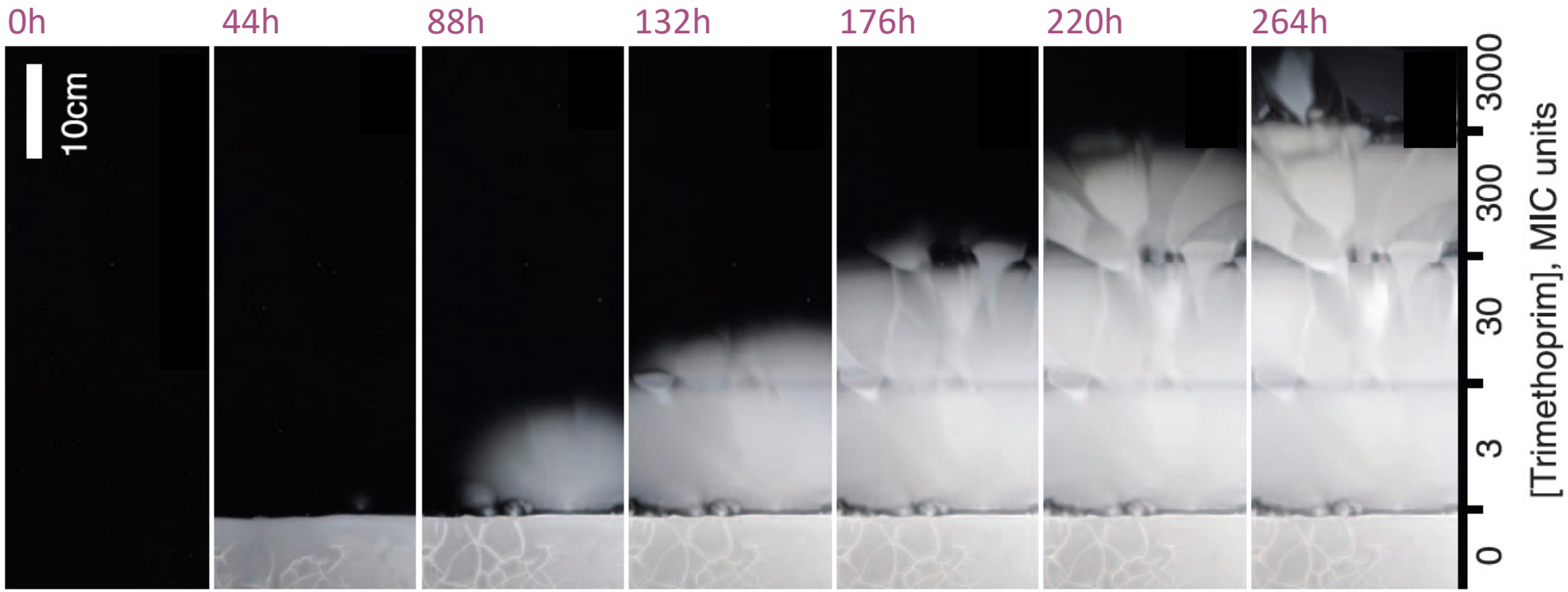
The mega plate: visualize real-time evolution



Agarplate with increasing concentration of an antibiotic (Trimethoprim)

Baym M et al. Science 2016

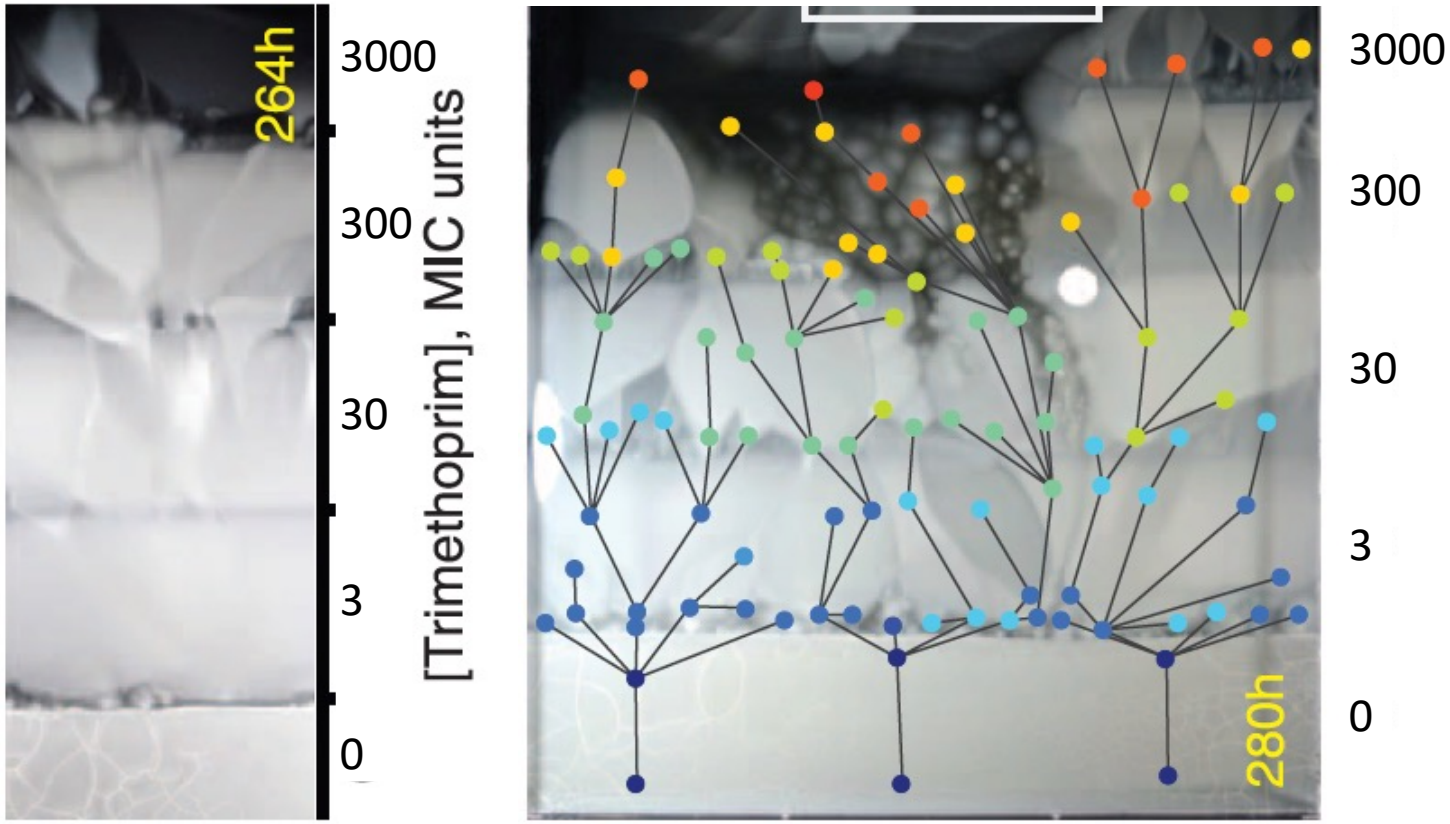
Rapid evolution against antibiotics



Resistance against very high antibiotic conctrations within 2 weeks

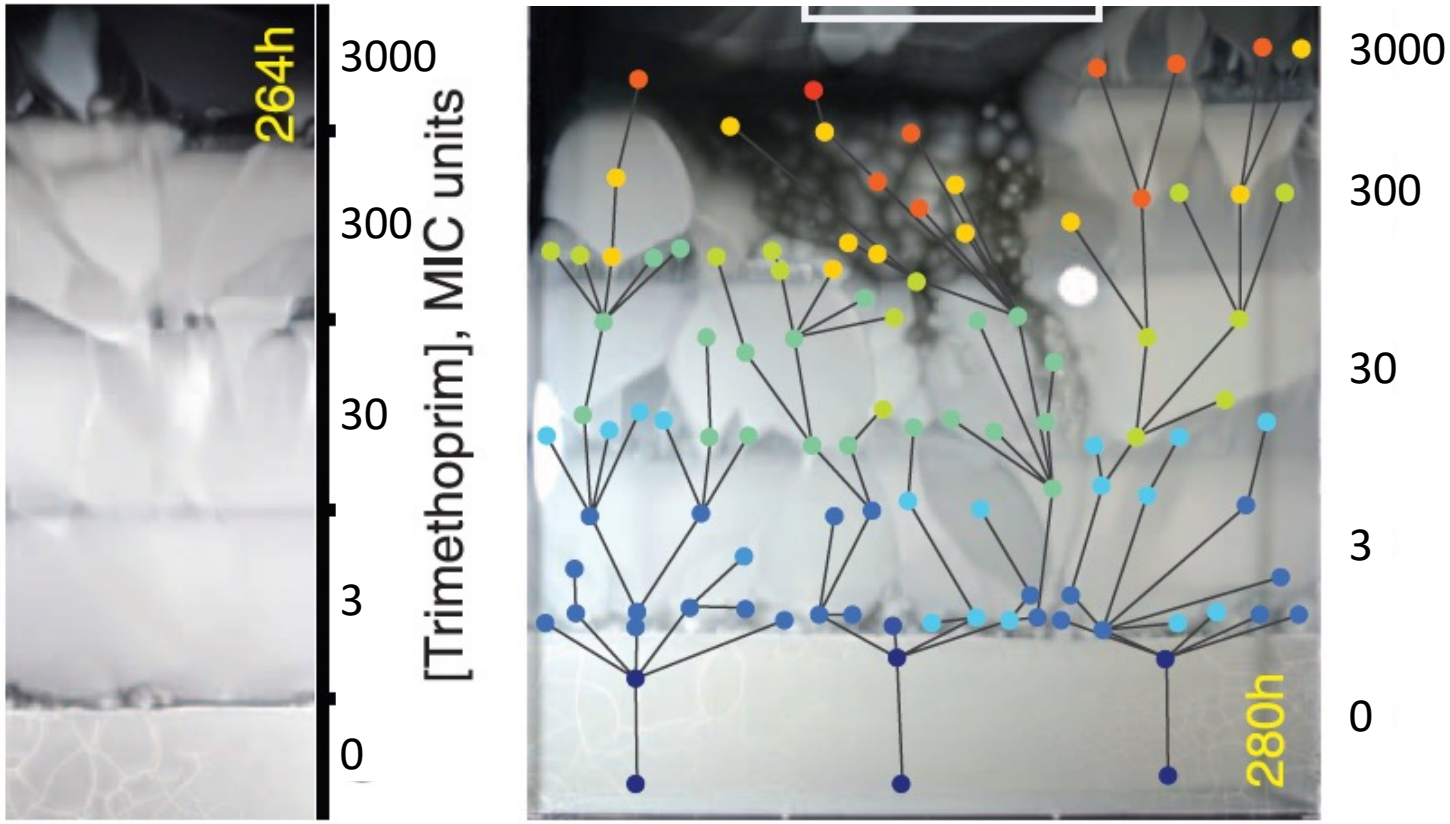
Adapted from Baym M et al. Science 2016

Evolution through point mutations



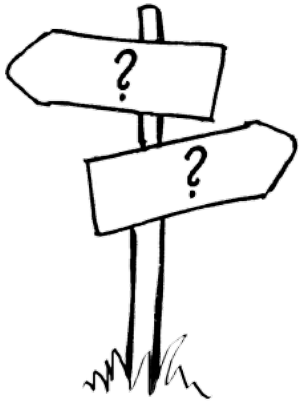
Baym M et al. Science 2016

Evolution through point mutations



1. Training required: **Step wise increase!**

2. Many ways lead to Rome: **many ways to resistance!**

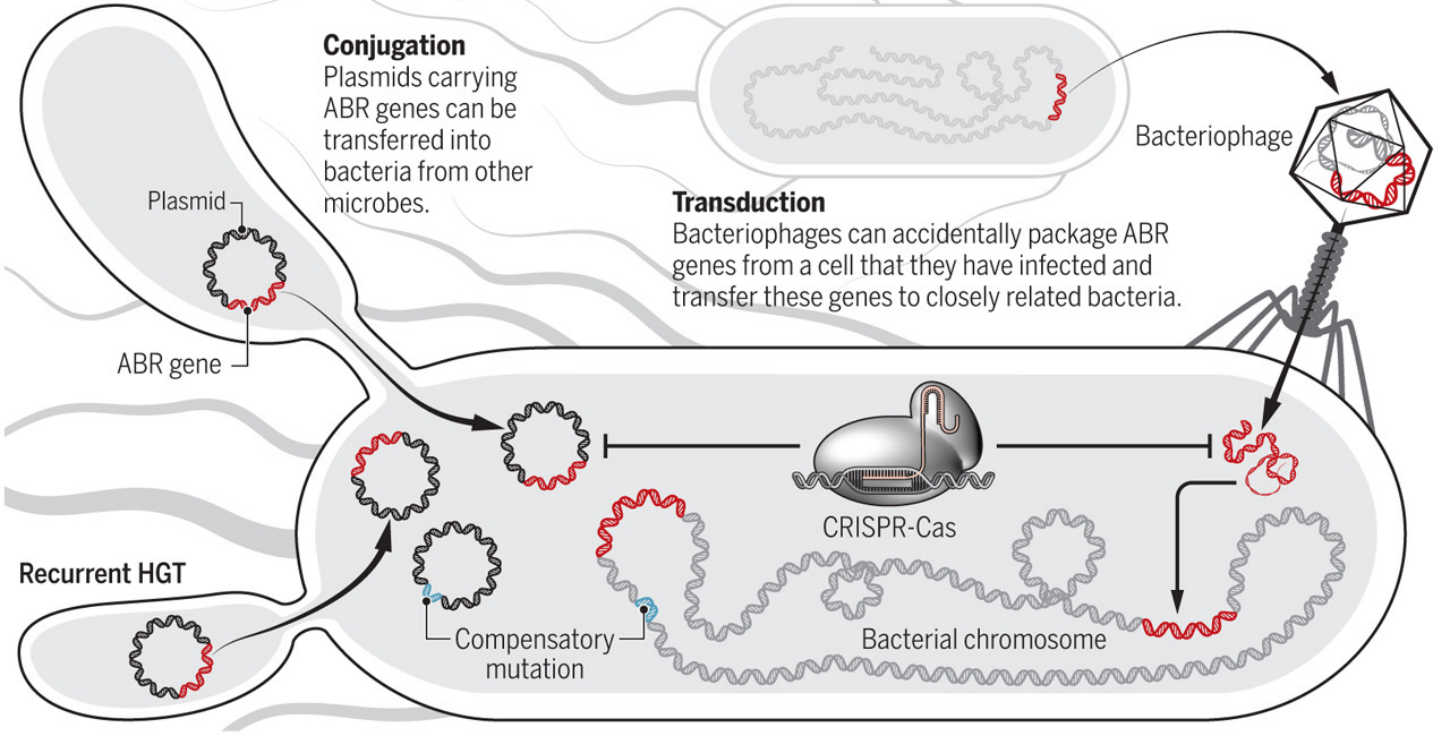


Baym M et al. Science 2016

Acquisition of antibiotic resistance

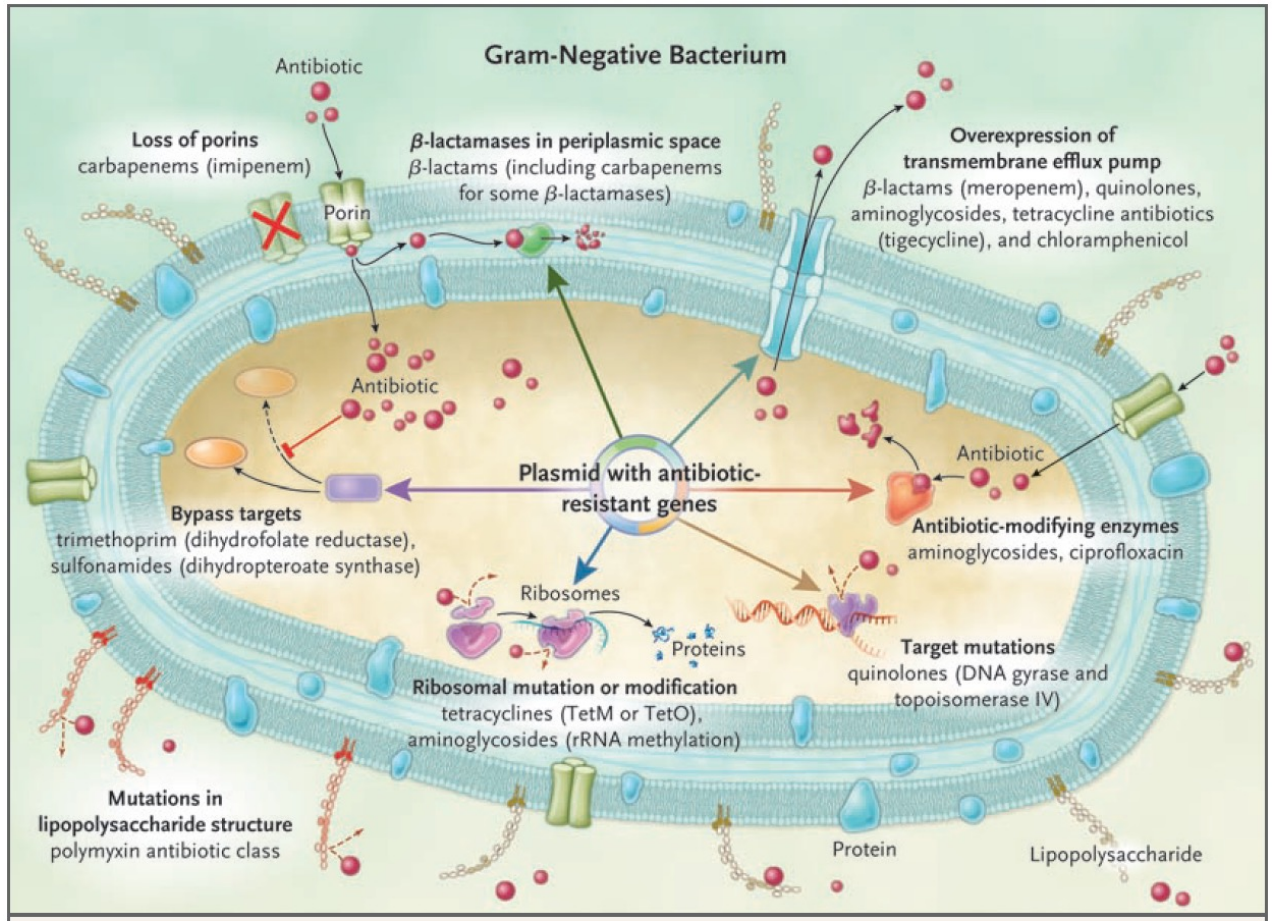
Mechanisms of mobile antibiotic resistance

Pathogenic bacteria can acquire antibiotic resistance (ABR) genes through two main mechanisms of horizontal gene transfer (HGT): conjugation and transduction. Compensatory mutations alleviate the fitness costs imposed by ABR genes, contributing to their stabilization. CRISPR-Cas–based systems may selectively destroy DNA carrying mobile ABR genes.



MacLean RC et San Millan A, Science 2019

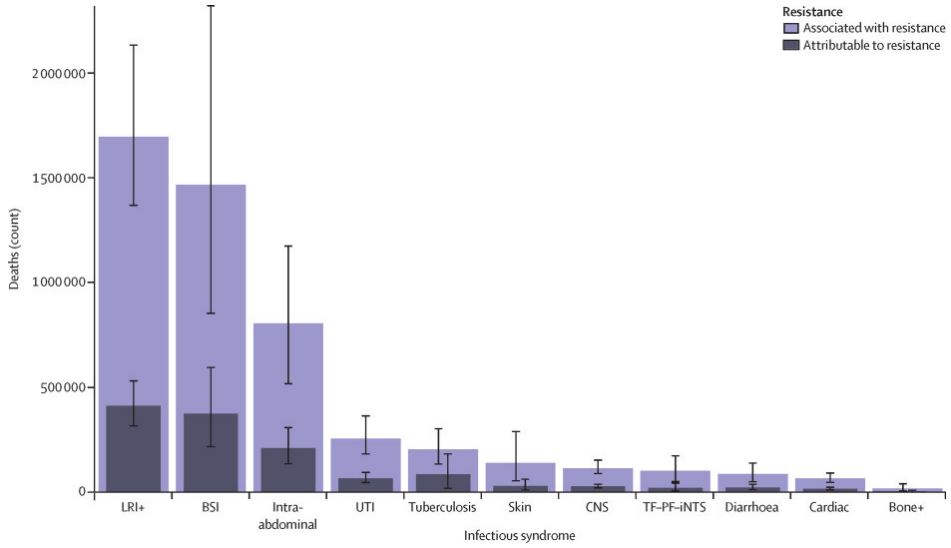
Resistance mechanisms basics



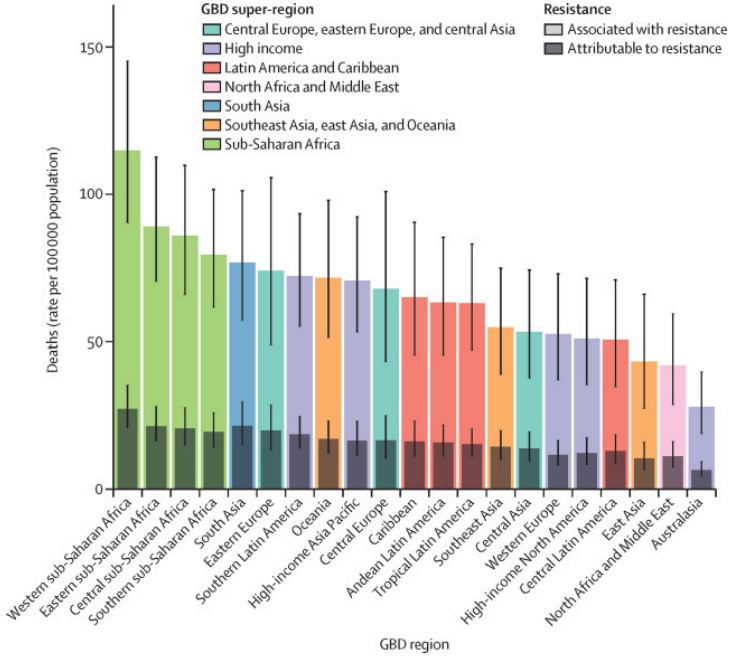
- 1. **efflux pump**
- 2. **porin loss**
- 3. **target modification**
- 4. **enzymatic cleavage**

Peleg AY et Hooper DC; New Engl J Med 2010

Antibioticaresistance: a massive problem



Antimicrobial resistance collaborators, Lancet 2022, 399, p629-55



→ Approx. 4,95 millionen (3,62–6,57) death with bacterial AMR in 2019, incl. 1,27 million (95% UI 0,911–1,71) death due to bacterial AMR

AMR in different age groups

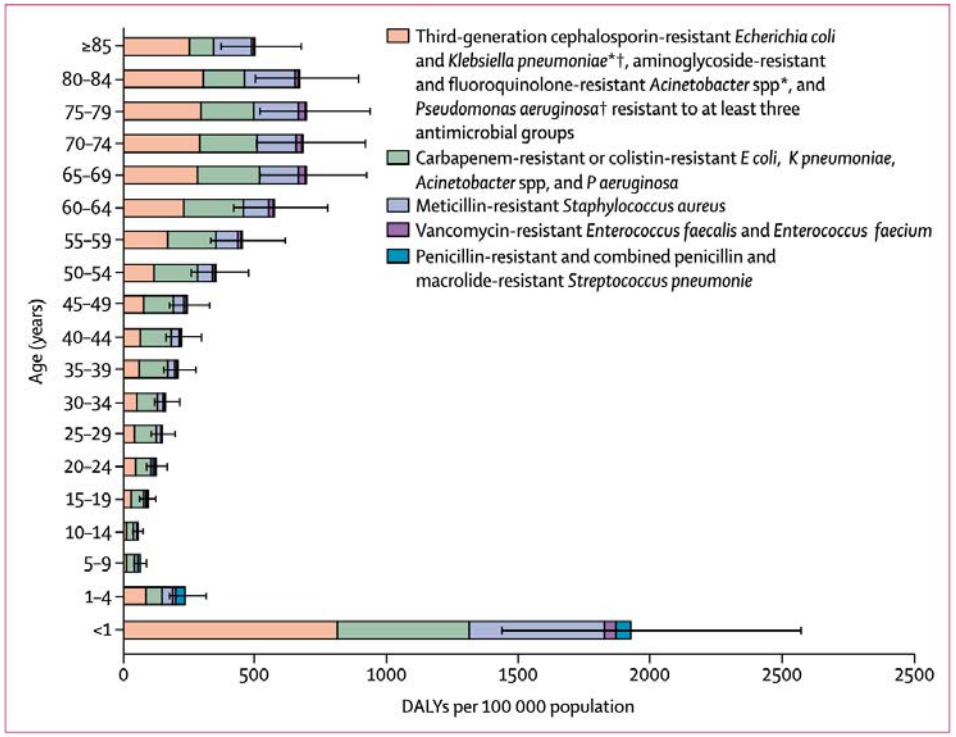


Figure 2: **Model estimates of the burden of infections with antibiotic-resistant bacteria of public health importance in DALYs, by age group, EU and European Economic Area, 2015**
Error bars are 95% uncertainty intervals. DALYs=disability-adjusted life-years. *Excludes those resistant to carbapenem or colistin. †In 2015, most of the third-generation cephalosporin-resistant *E coli* (88·6%) and *K pneumoniae* (85·3%) isolates reported to the European Antimicrobial Resistance Surveillance Network produced an extended-spectrum β-lactamase.[9]

→ the most weak in society are affected most:
<1 year
> 65 years

Cassini A, Diaz Högberg L, et al. Lancet Infect Dis 2019, 19:56-66

Most prevent resistance mechanisms

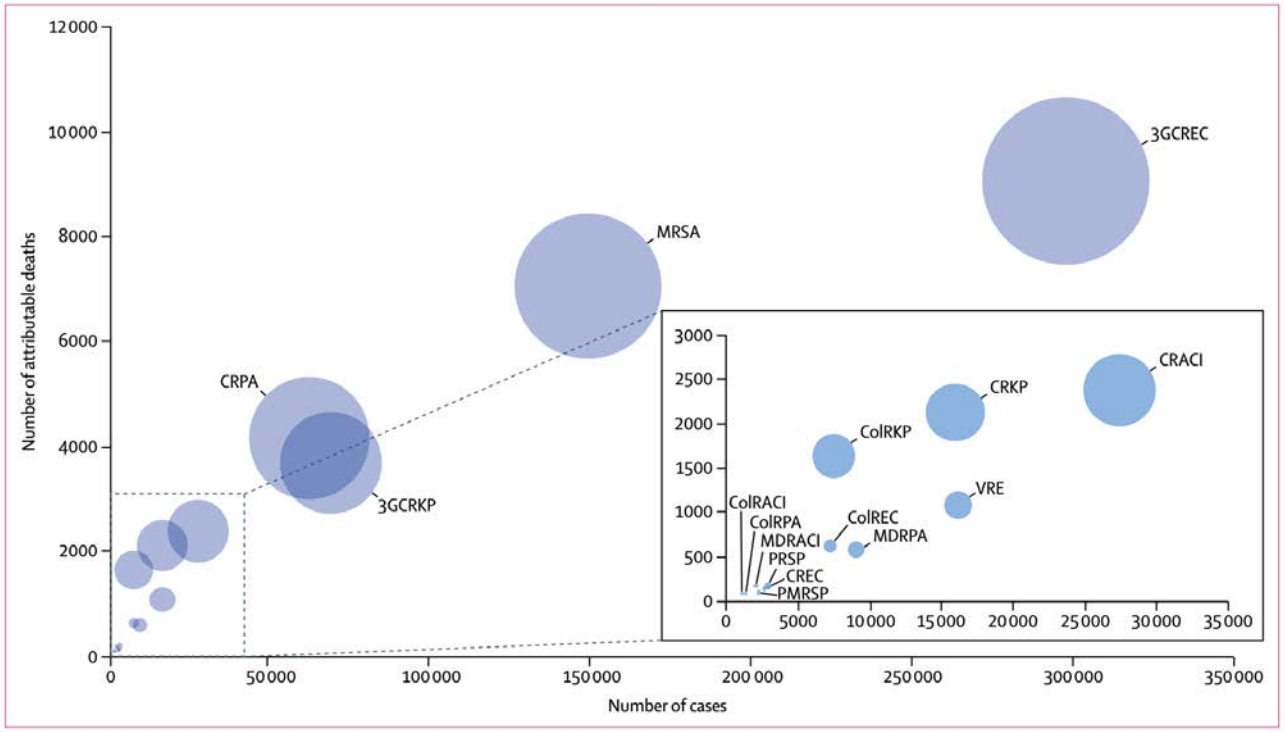


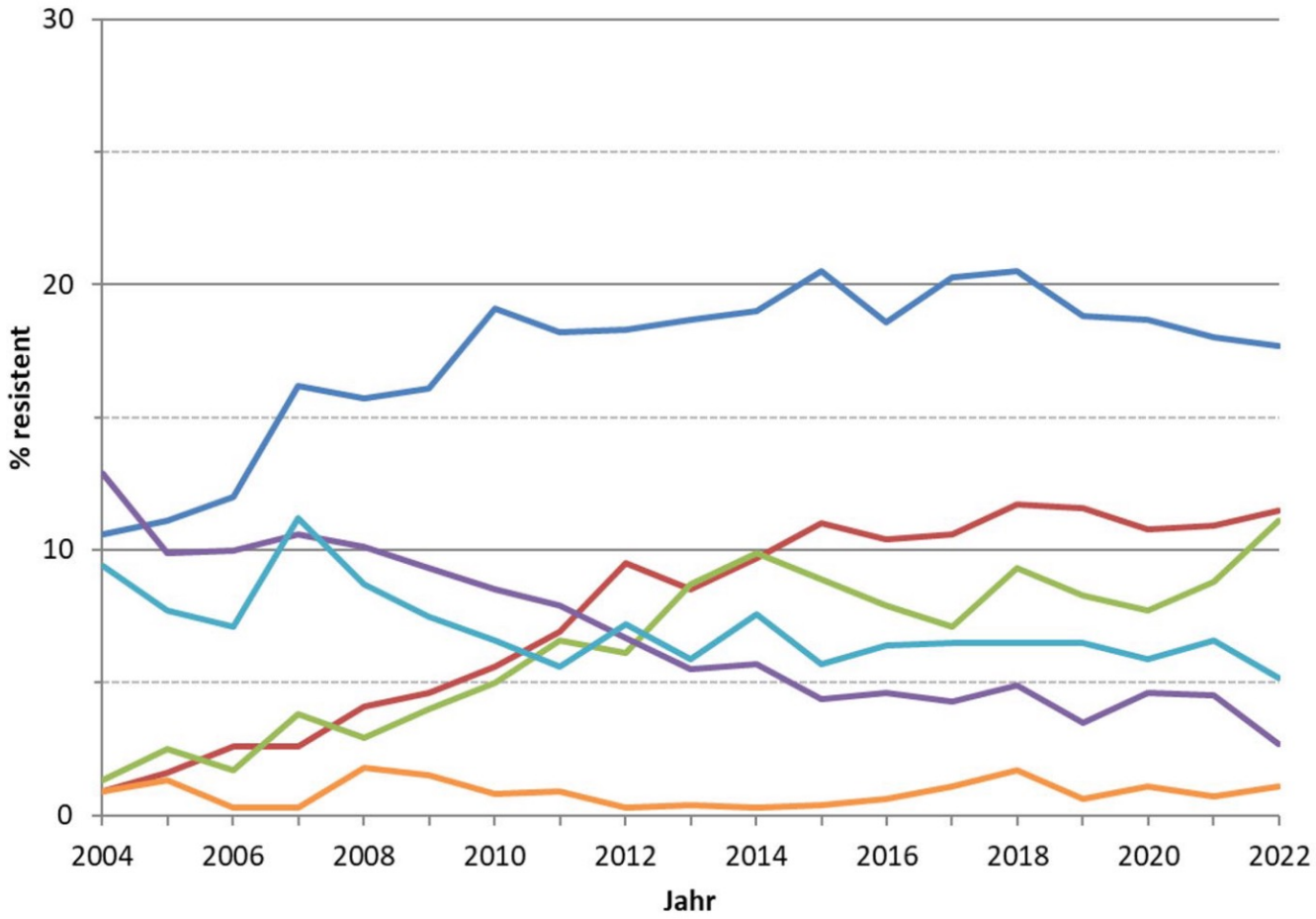
Figure 1: **Infections with antibiotic-resistant bacteria, EU and European Economic Area, 2015**
Diameter of bubbles represents the number of disability-adjusted life-years. ColRACI=colistin-resistant *Acinetobacter* spp. CRACI=carbapenem-resistant *Acinetobacter* spp. MDRACI=multidrug-resistant *Acinetobacter* spp. VRE=vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*. ColREC=colistin-resistant *Escherichia coli*. CREC=carbapenem-resistant *E coli*. 3GCREC=third-generation cephalosporin-resistant *E coli*. ColRKP=colistin-resistant *Klebsiella pneumoniae*. CRKP=carbapenem-resistant *K pneumoniae*. 3GCRKP=third-generation cephalosporin-resistant *K pneumoniae*. ColRPA=colistin-resistant *Pseudomonas aeruginosa*. CRPA=carbapenem-resistant *P aeruginosa*. MDRPA=multidrug-resistant *P aeruginosa*. MRSA=meticillin-resistant *Staphylococcus aureus*. PRSP=penicillin-resistant *Streptococcus pneumoniae*. PMRSP=penicillin-resistant and macrolide-resistant *S pneumoniae*.

- ESBL in *E. coli* (=3GCREC)
- ESBL in *K. pneumoniae* (=3GCRKP)
- Methicillin resistant *S. aureus*
- Carbapenem resistant *P. aeruginosa*

- Do not forget:
- Sexually transmitted diseases
- *Salmonella thyphimurium*

Cassini A, Diaz Högberg L, et al. Lancet Infect Dis 2019, 19:56-66

Antimicrobial resistance in Switzerland



ESBL increases.
MRSA decreases.
VRE shows local outbreaks.

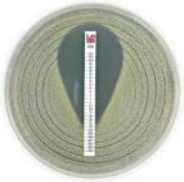
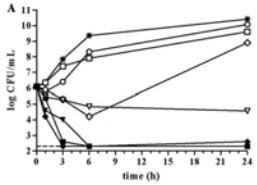
FQR-E. coli	(Fluorochinolon resistente *E. coli*)
ESCR-E. coli	(Ceftriaxon resistente *E. coli*)
ESCR-KP	(Ceftriaxon resistente *Klebsiella pneumoniae*)
MRSA	(Methicillin resistente *Staphylococcus aureus*)
PNSP	(Penicillin resistente *Streptococcus pneumoniae*)
VRE	(Vancomycin resistente *Enterococcus faecium*)

Data from Anresis (www.anresis.ch)
BAG Bulltin July 2022

Determination of AMR in the lab

- **Phenotypic profile** e.g. microdilution, disks, e-tests, (kill-curves)



  - Principle: bacterial growth in presence of an antibiotic drug[1]
  - Complex situation: living organisms are exposed to stress, usually very rich media

- **Genotypic profile** e.g. PCR, sequencing

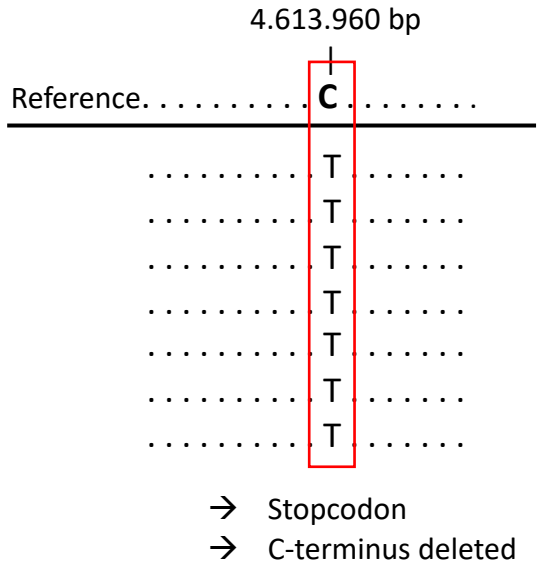


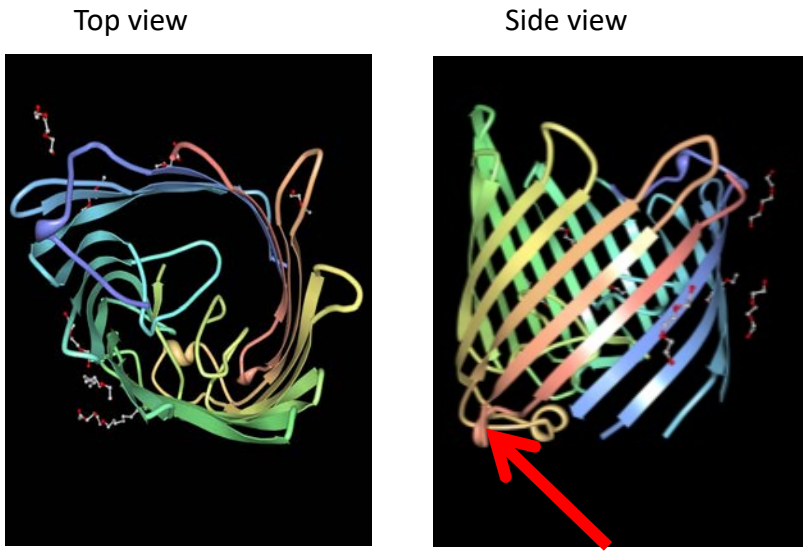
  - Principle: Prescence or Abscence of a Gene linked to resistance e.g. CTX-M1 oder Oxa-48
  - Usually no quantitative expression of the gene, no MICs

Example: Porin loss of oprD in *Pseudomonas aeruginosa*

- Influx mechanisms (porins)

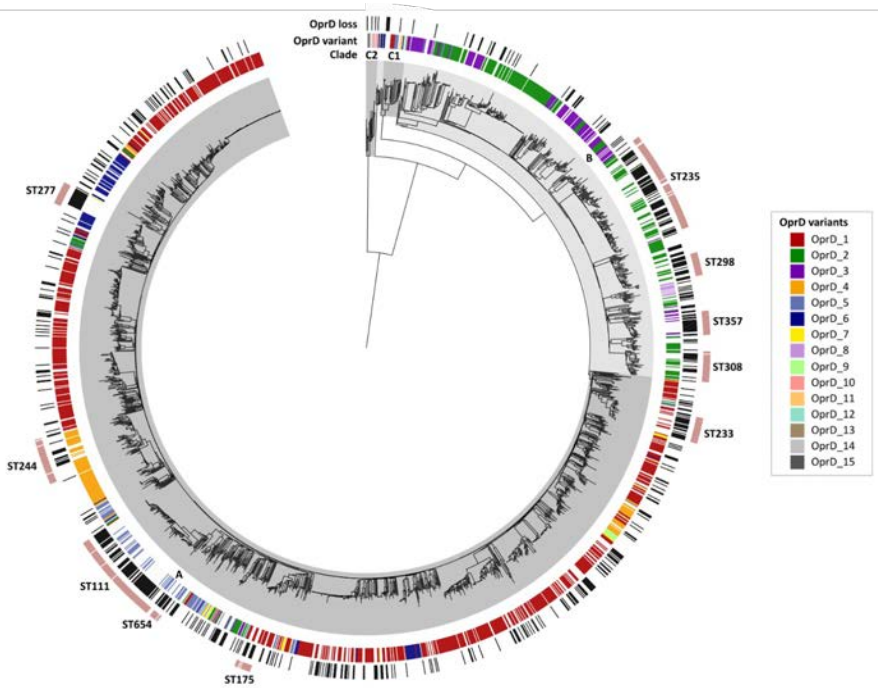
  e.g. Carbapenem resistence due to porin loss (oprD) with *Pseudomonas aeruginosa*





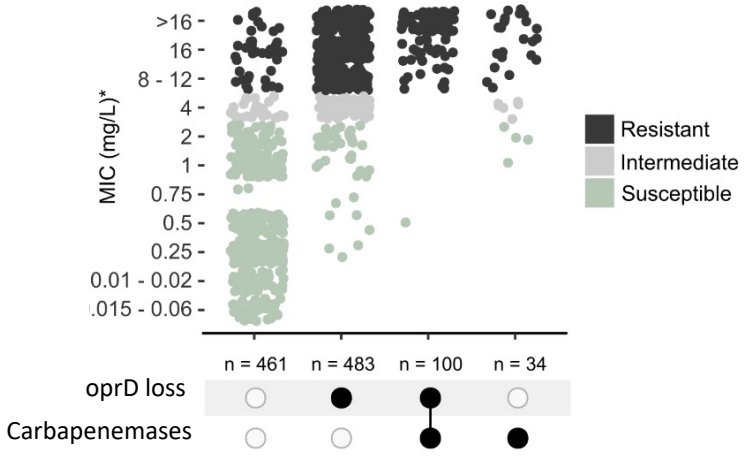
Deletion of the red β sheet

Prediction of Phenotype from Genotype? Example of oprD in *P. aeruginosa*



Biggel M, … et Seth-Smith HMB, 2022 submitted

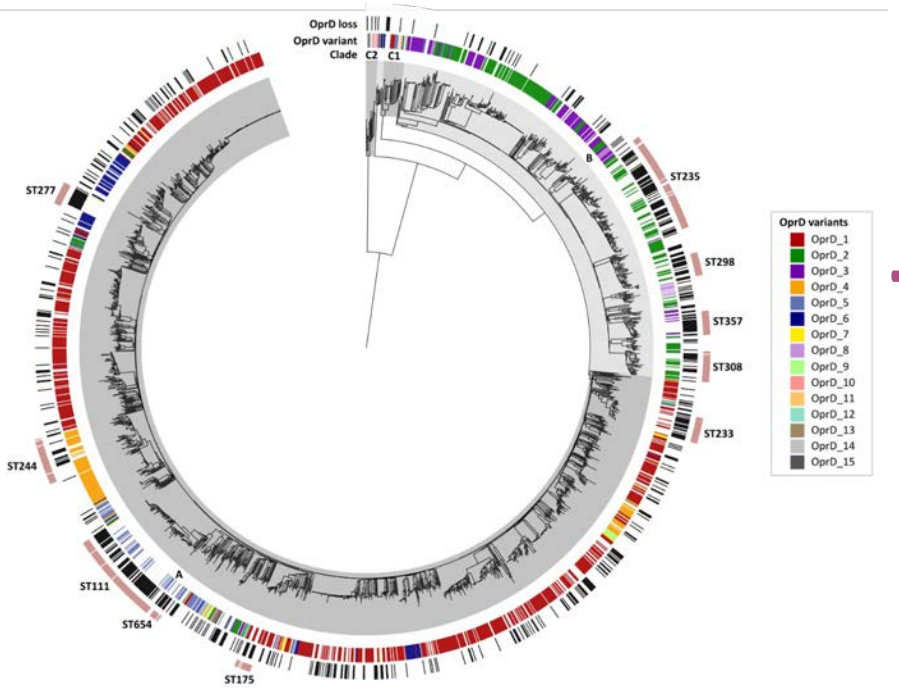
?



Holy grail!

- 2000+ *Pseudomonas aeruginosa* genomes
- In CH: Porin loss is about 10x higher than Carbapenemases

Research: Combination of genotype and function to predict the MIC



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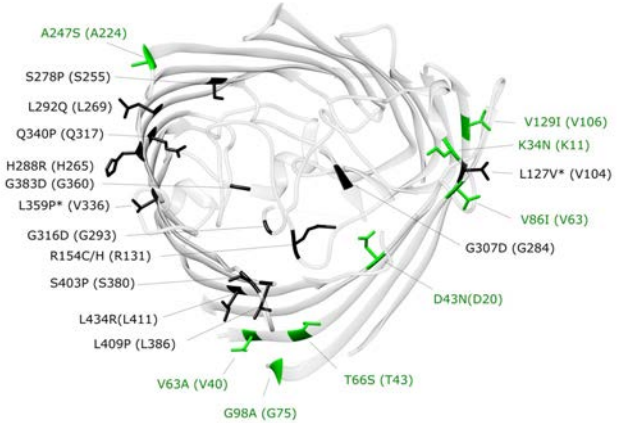

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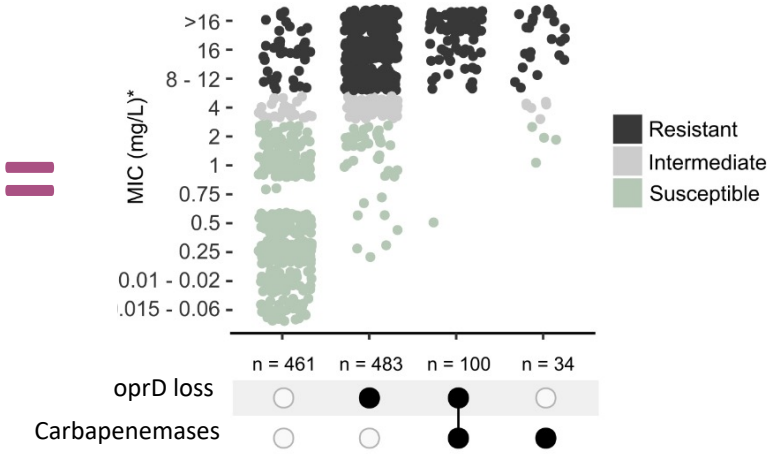
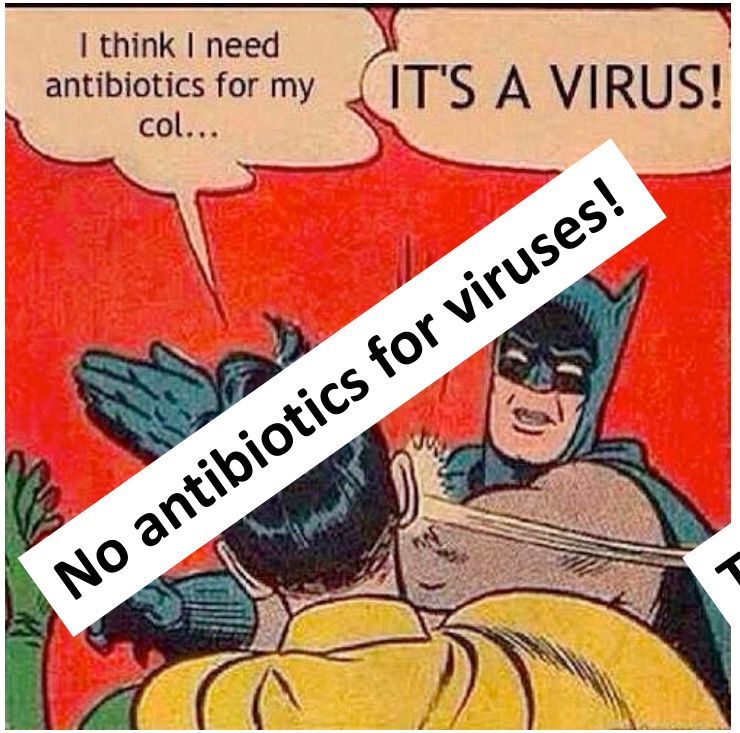
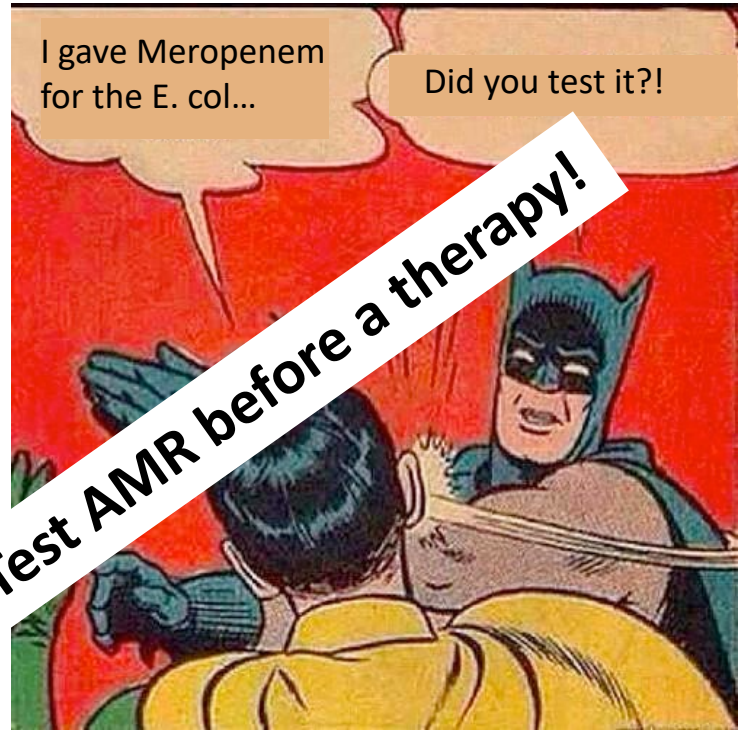


Biggel M, … et Seth-Smith HMB, 2022 submitted

- 2000+ *Pseudomonas aeruginosa* genomes
- **Next idea**: AI-based analysis to lern mutations and functional implications

How can we reduce the spread of antibiotic resistance?





Key aspects:

- **ANTIBIOTIKA STEWARDSHIP!**
- **DIAGNOSTIC STEWARDSHIP!**
- **One Health!**

- **Data quality is key**
- **FAIR** (findable, accessible, interoperable, re-usable) **data**

Take home message

- **Antibiotic resistance**
  - De novo -> by mutation and selection pressure
  - With plasmid/gene transfer and selection pressure.
- **Four basic mechanisms of resistance:**
  - Efflux pump, porin loss, modification of the target, cleavage by enzymes
- **Antibiotic Stewardship**
  - Essential in combating the development of resistance
  - Concept: One Health -> humans and animals form one unit.
  - Innovative diagnostics is key!

THANK YOU for your attention! Questions?

- Contact:

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@AppliedMicrobi2

Website: www.imm.uzh.ch

Universität Zürich[UZH]

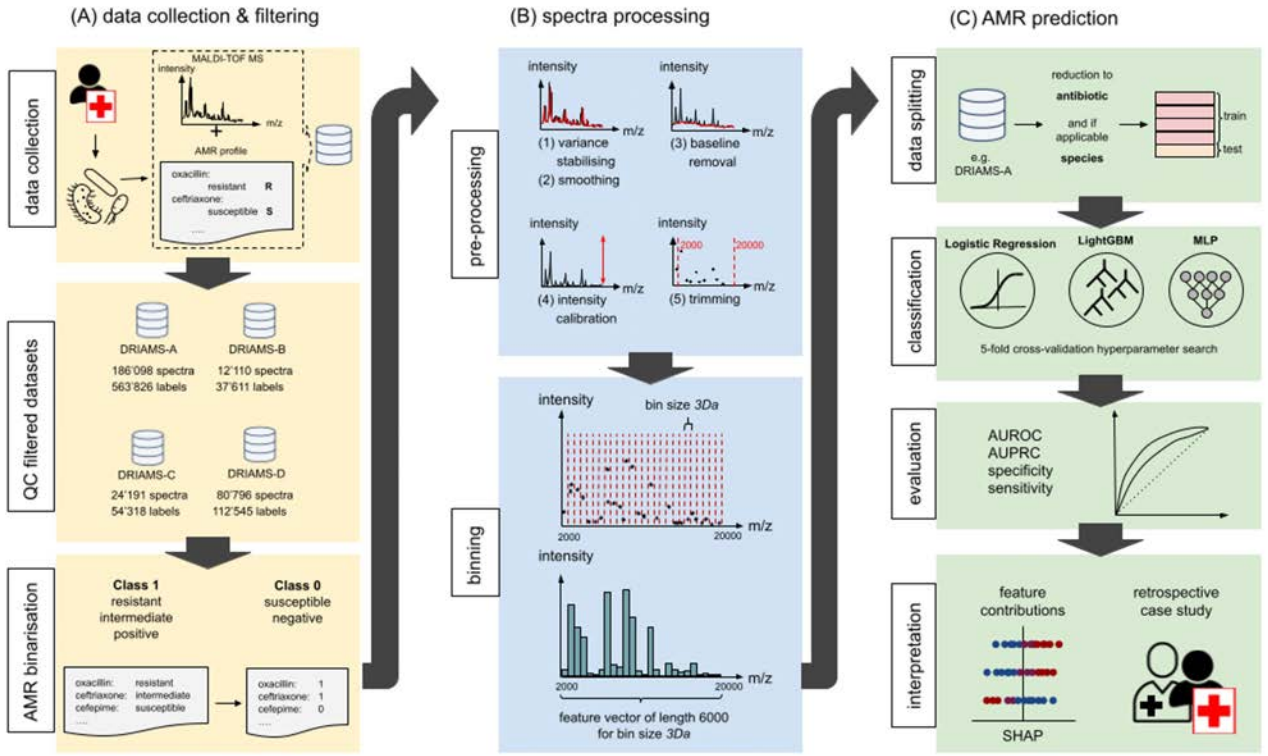
Research: MALDI-TOF MS analysis to predict antibiotic resistance

- MALDI-TOF MS usually used for ID
- Collaboration:

University Hospital Basel
University of Basel
ETH Zürich
Kantonsspital Aarau
VIOLLIER
Kantonsspital Baselland

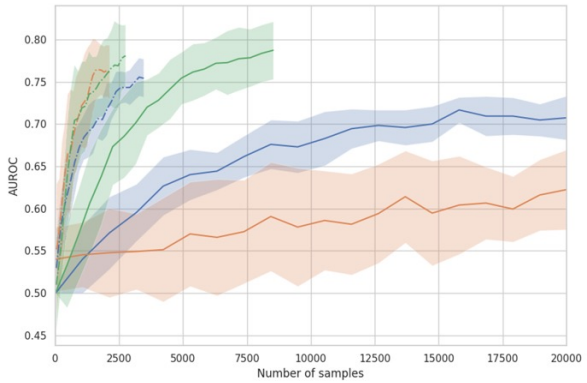
- Re-analysis of spectrum profiles with machine learning based algorithms

- 303‘195 (!) spectra
- 78 commonly used antimicrobials



Weis C, Cuénod A, … et Egli A; Nature Medicine, 2021

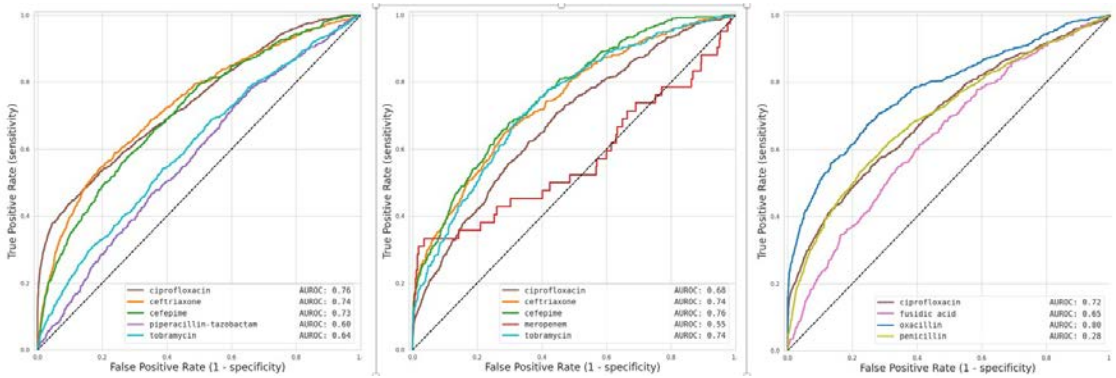
Prediction of AMR category is good, but needs more work for routine application



→**Deliverable**:
Prediction of AMR directly from the MALDI-TOF spectrum

- **Prediction of susceptibility:**
- *S. aureus*: MRSA (0.8 AUC)
- *E. coli*: Ceftriaxon R (0.74 AUC)
- *K. pneumoniae*: Ceftriaxon R (0.74 AUC)

E. coli (lightGMB) *K. pneumoniae* (MLP) *S. aureus* (lightGMB)



→ **Next step:**
Improve algorithm
Prospective validation of the algorithm
Expansion to prediction of invasiveness

Weis C, Cuénod A, … et Egli A; Nature Medicine, 2021