Combinatorials

Novel drugs and drug combinations against bacterial growth, survival and persistence:

From high-throughput screening to mechanism of action

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submission platform jpiamr Joint Programming Initiative on Antimicrobial Resistance

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Combinatorials

Disclosure belangen spreker

(potentiële) belangenverstrengeling	Geen / Zie hieronder
Voor bijeenkomst mogelijk relevante relaties met bedrijven	Bedrijfsnamen
• Sponsoring of onderzoeksgeld • Honorarium of andere (financiële) vergoeding • Aandeelhouder • Andere relatie, namelijk …	• • • •

Aims of the project

To **repurpose** or **improve** FDA-approved drugs and neglected/disused antibiotics, and identify combinations to re-sensitize resistant/tolerant bacteria

1. Perform thousands of pairwise drug combinations on bacterial growth, viability and bacterial persistence in
   - uropathogenic *Escherichia coli*
   - *Staphylococcus aureus*
   - *Streptococcus pneumoniae*

2. To optimize antimicrobial treatment using state-of-the-art and further advance translational pharmacokinetic/pharmacodynamic (PK/PD) modeling

3. Validate top lead combinations:
   - in resistant clinical strains
   - toxicity in cell-based assays and several animal models
   - *in vivo* PK/PD modeling

4. Underlying modes of action of our top 40 synergistic drug combinations

Partners and participants involved in the realisation of the project

Prof. dr. Uhlin
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Umea

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Rijksuniversiteit Groningen
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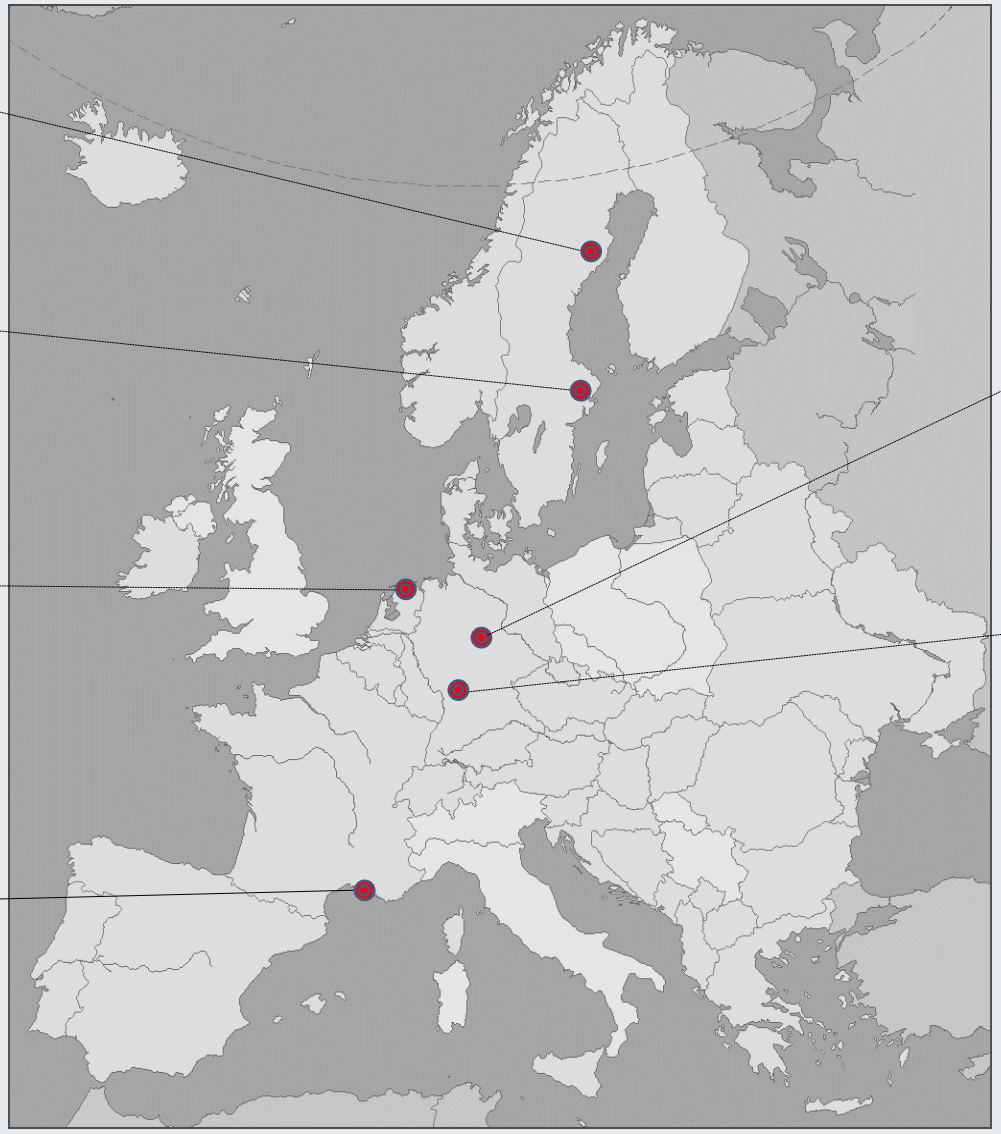
Recherche Scientifique
Marseille



Prof. dr. Kloft
Freie Universitaet
Berlin

Dr. Typas
EMBL
Heidelberg

Targeted bacteria

- ***Streptococcus pneumoniae***
- ***Salmonella typhimurium***
- ***Escherichia coli, uropathogenic***
- ***Bacillus subtilis***

"ESKAPE" pathogens

Enterobacter spp

***Pseudomonas aeruginosa*, MDR**

Acinetobacter baumannii

***Klebsiella pneumoniae*, carbapenem-R**

***Staphylococcus aureus*, MRSA**

***Enterococcus faecium, vancomycin*-R**

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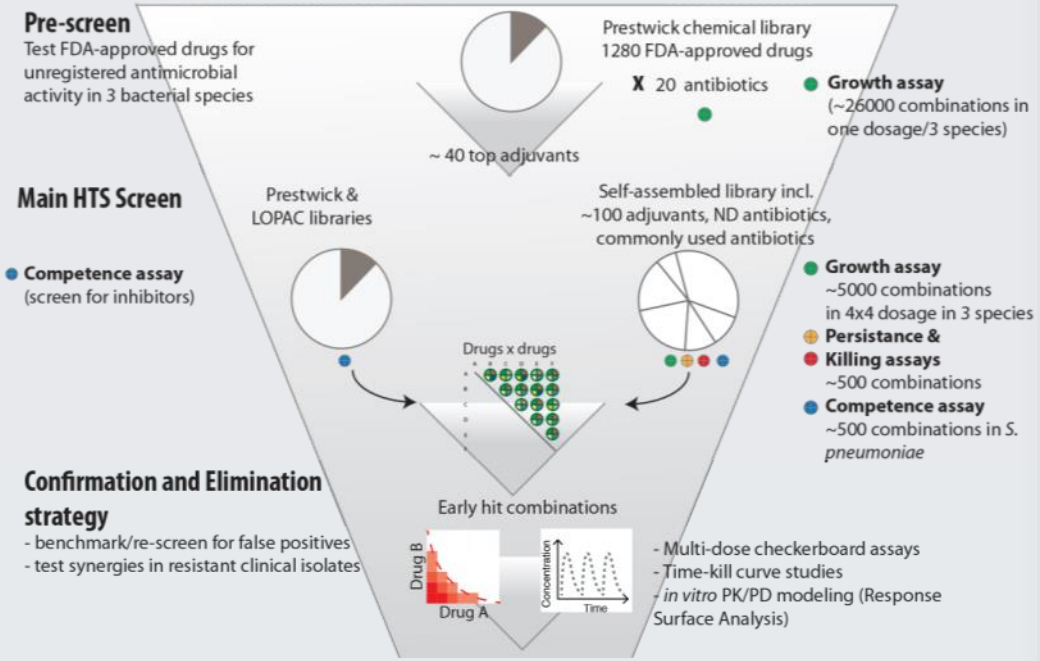
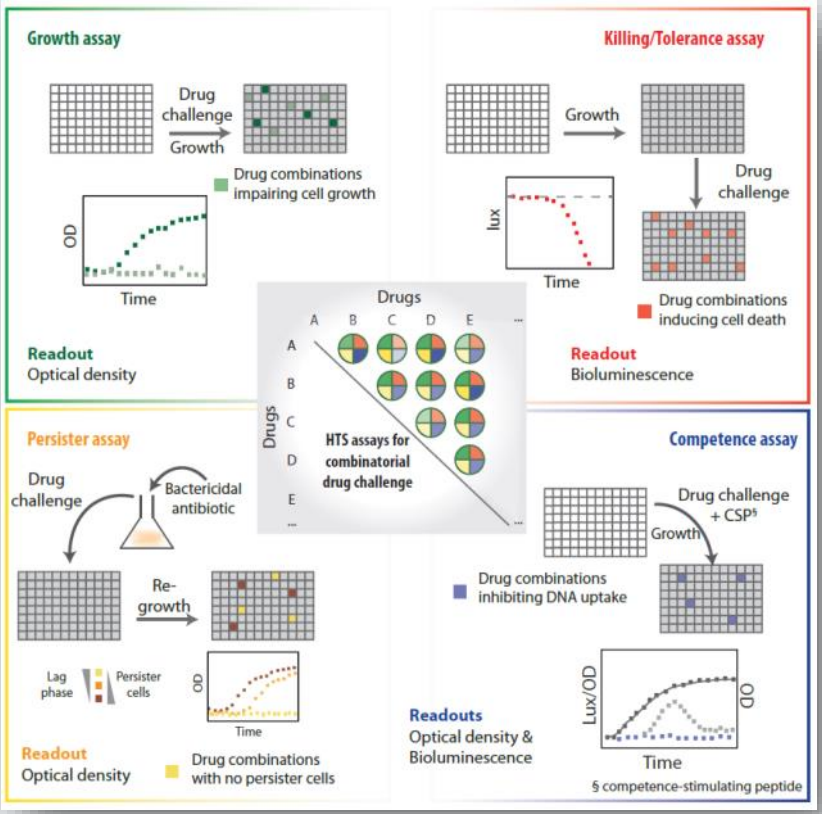
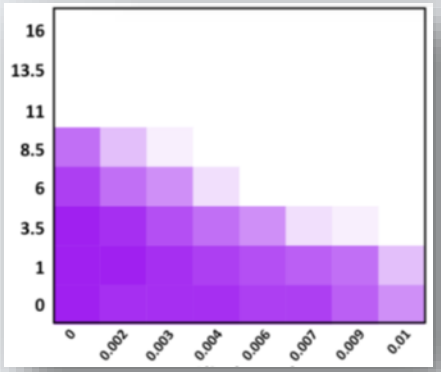
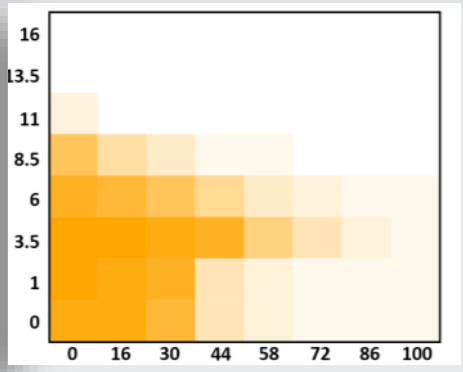
Project aims (I)

1. Identification of antimicrobial adjuvants









S. aureus*, *S. pneumoniae* and *E. coli

Pre-data in S. typhimurium* and *P. aeruginosa

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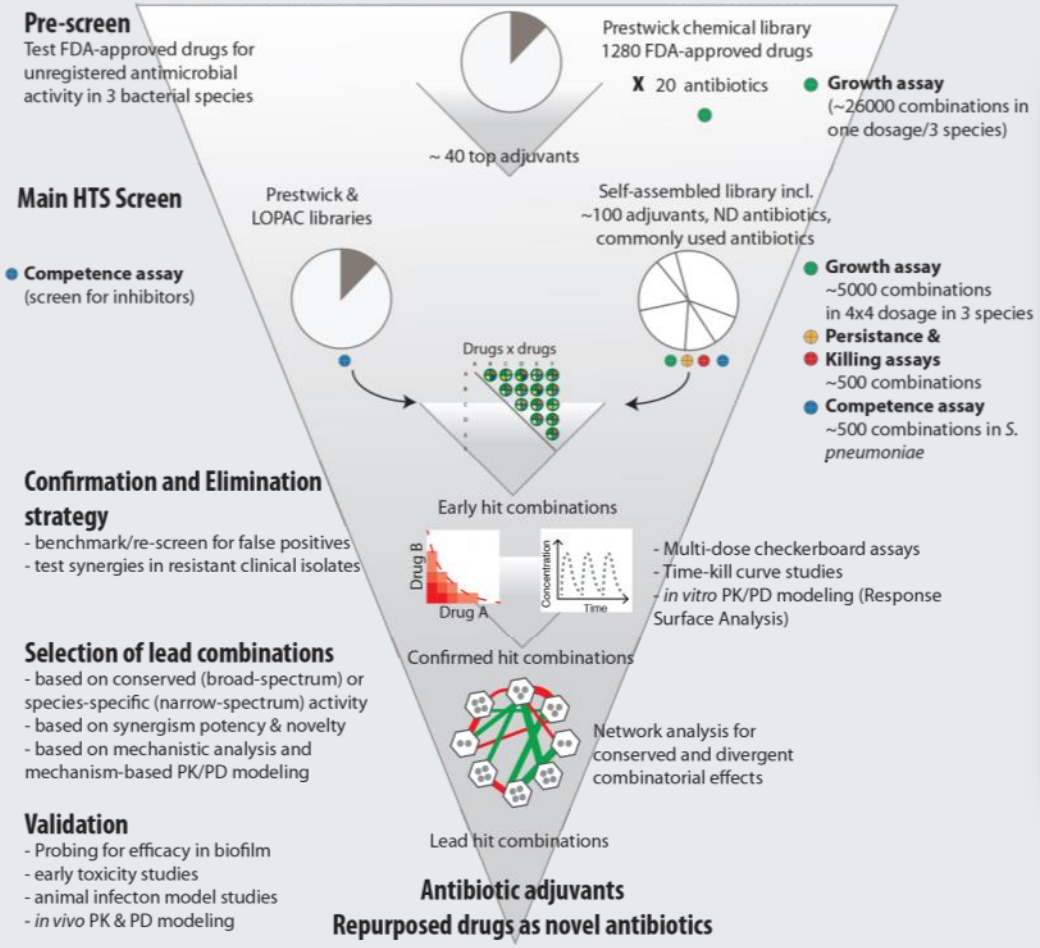
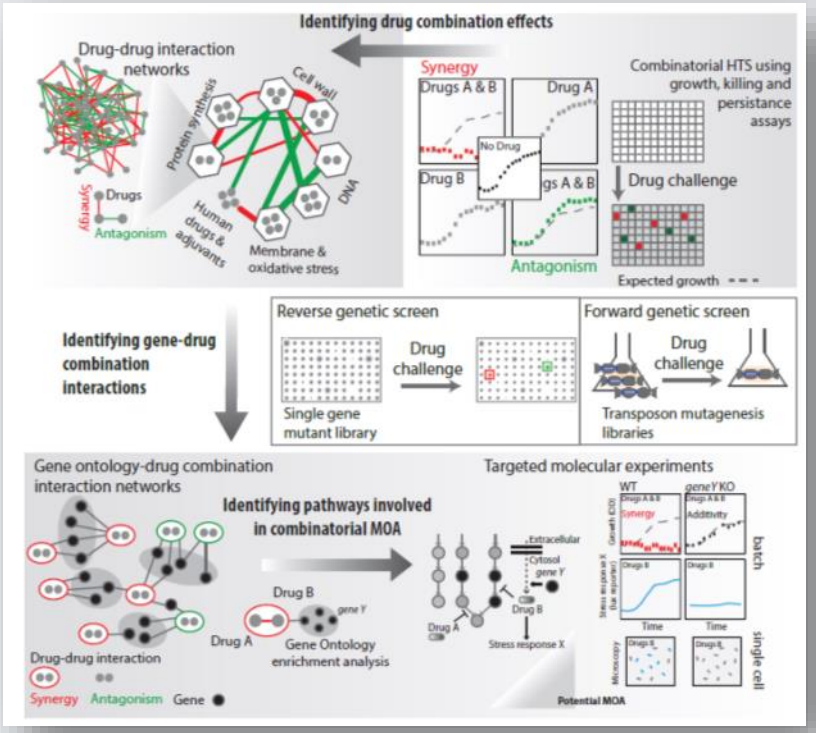
Project aims (II)

2. Mechanistic dissection of drug-drug synergies





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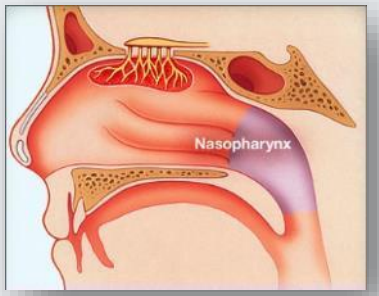
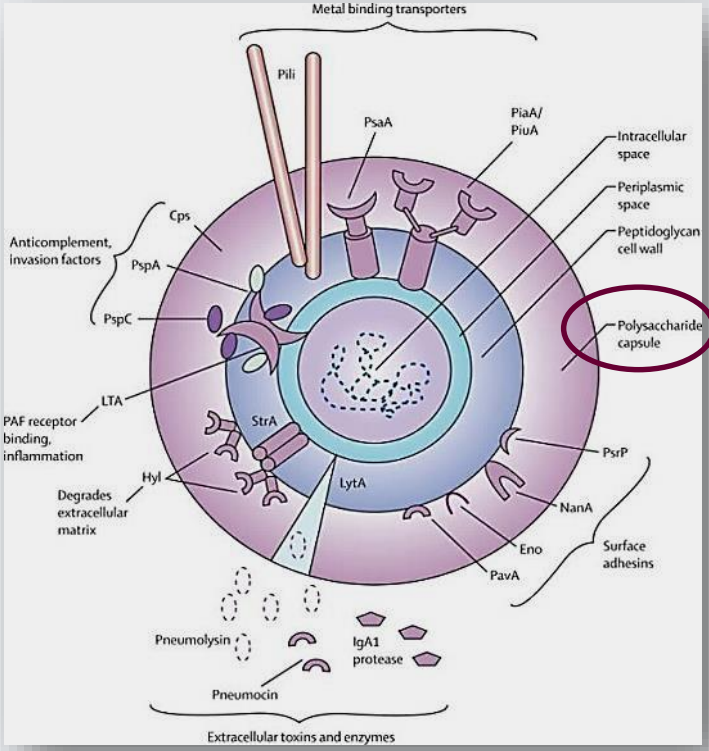
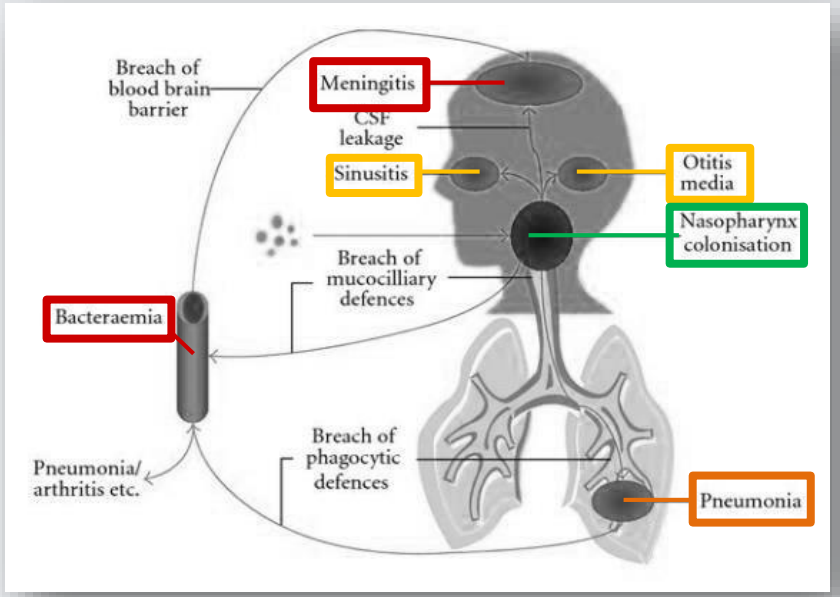
Why to study *S. pneumoniae* (pneumococcus)?

- *S. pneumoniae* **harmlessly** resides in the nasopharynx of children
- > **150** genes involved in **pathogenesis**
- A **major cause** of **morbidity** and **mortality** worldwide





van der Poll T, Lancet 09



1.6 million deaths every **year**

(WHO, 2013)

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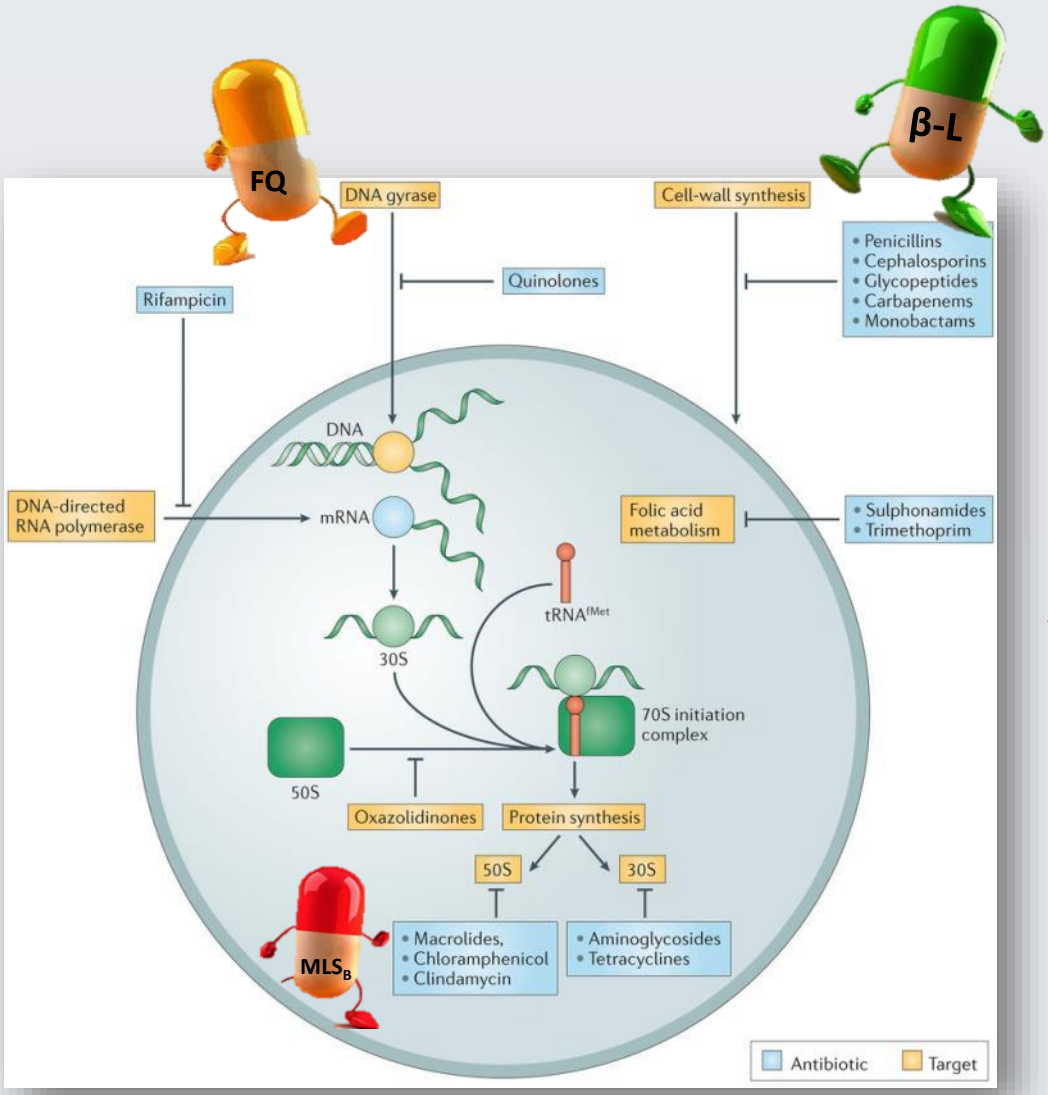
Pneumococcal antibiotic resistance:



(Lewis K, Nature reviews drug discovery 2013)

Emergence of resistance

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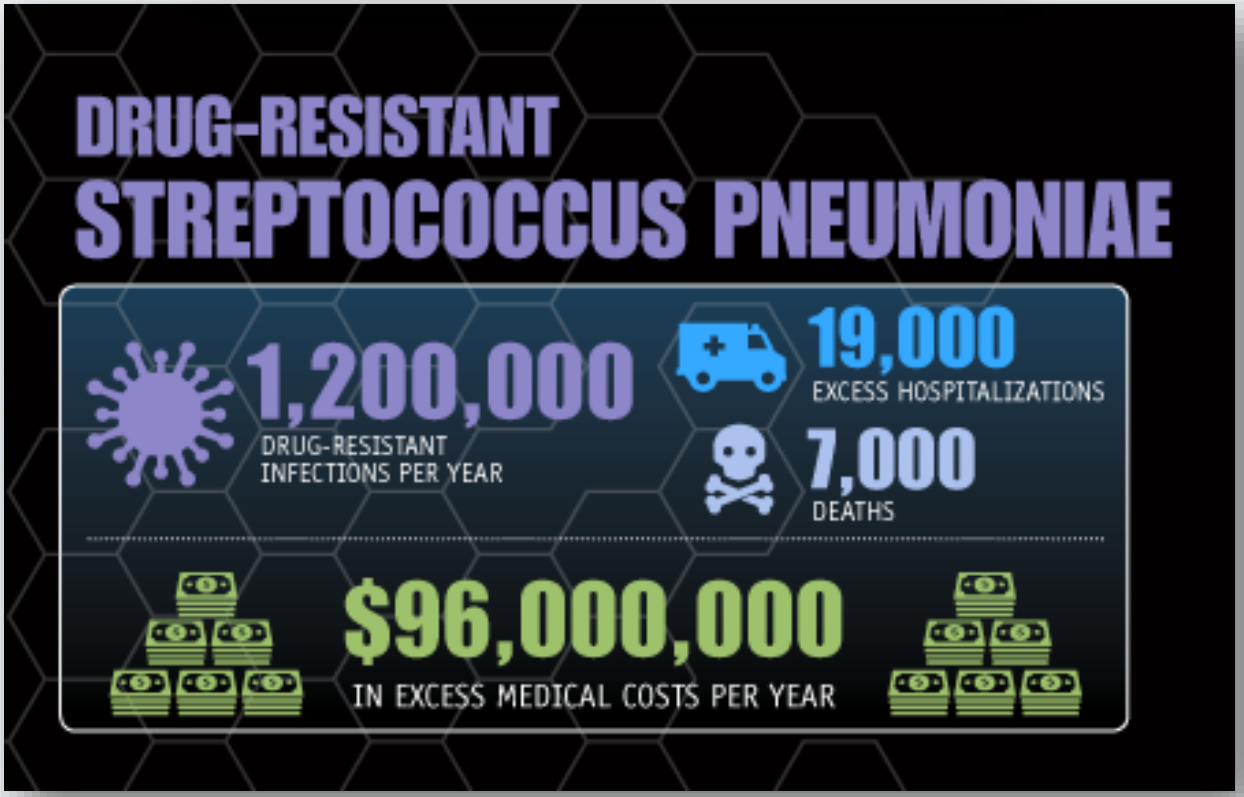
Pneumococcal antibiotic resistance:



www.cdc.gov

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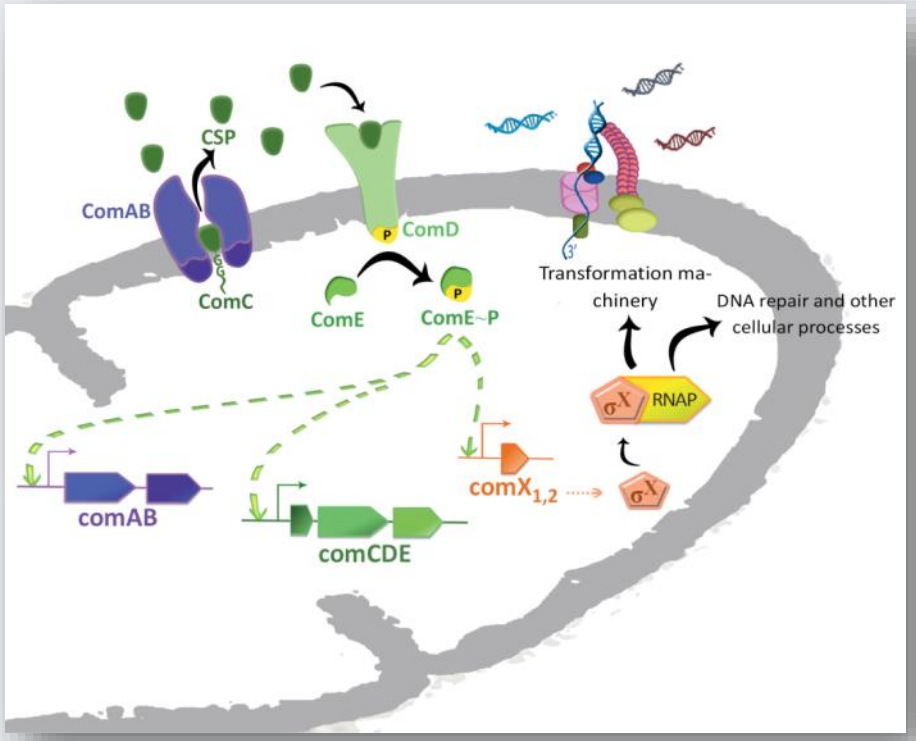
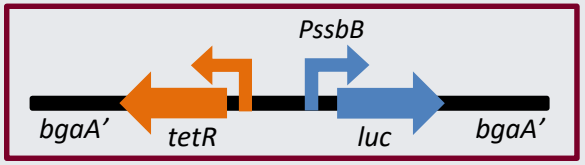
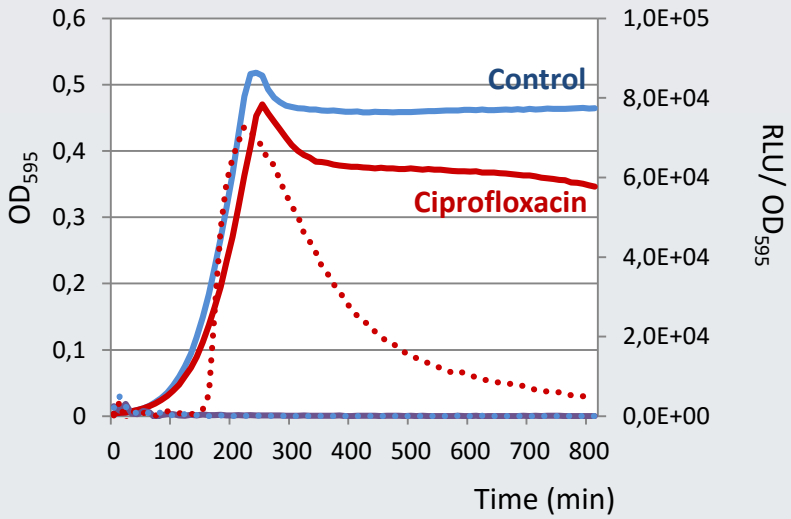
Mechanisms of antimicrobial resistance acquisition:

Competence pathway:



DLA3 strain:





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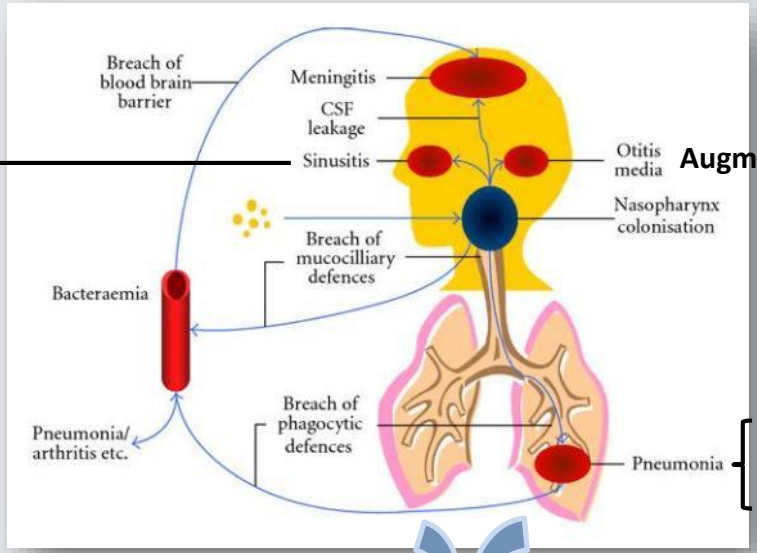
Several antibiotics induces competence in *S. pneumoniae*



Augmentin
fluoroquinolones

Augmentin

augmentin
fluoroquinolones

Uncomplicated COPD
No risk factors:
Age <65 years
FEV_1 >50% predicted
<3 exacerbations per year
No cardiac disease

- Advanced macrolide (azithromycin, clarithromycin) ✗
- Cephalosporin (cefuroxime, cefpodoxime, cefdinir) ✗
- Doxycycline
- Trimethoprim–sulfamethoxazole
- If recent antibiotic exposure (<3 months), use alternative class

Complicated COPD
1 or More risk factors:
Age ≥65 years
FEV_1 ≤50% predicted
≥3 exacerbations per year
Cardiac disease

- Fluoroquinolone (moxifloxacin, gemifloxacin, levofloxacin)
- Amoxicillin–clavulanate
- If at risk for *Pseudomonas* infection, consider ciprofloxacin and obtain sputum culture
- If recent antibiotic exposure (<3 months), use alternative class

Sethi et al NEJM 2008

Selection of the most researched inhaled antibiotics

Substance	Results
Tobramycin	Eradication in 13–35%, reduction of pathogenic load, improved lung function
Colistin	Rise in FEV1, eradication in 3 of 18 cases, fewer exacerbations, reduction of pathogenic load
Aztreonam	CF: improved lung function, longer interval to exacerbation
Liposomal ciprofloxacin Ciprofloxacin	Reduction of pathogenic load Reduction of pathogenic load
Gentamycin	Fewer exacerbations, improved quality of life Eradication of P.a. in 30.8 % improved quality of life

Rademacher et al., Medicine 2011

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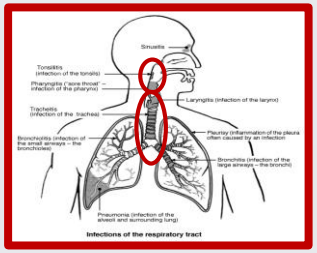
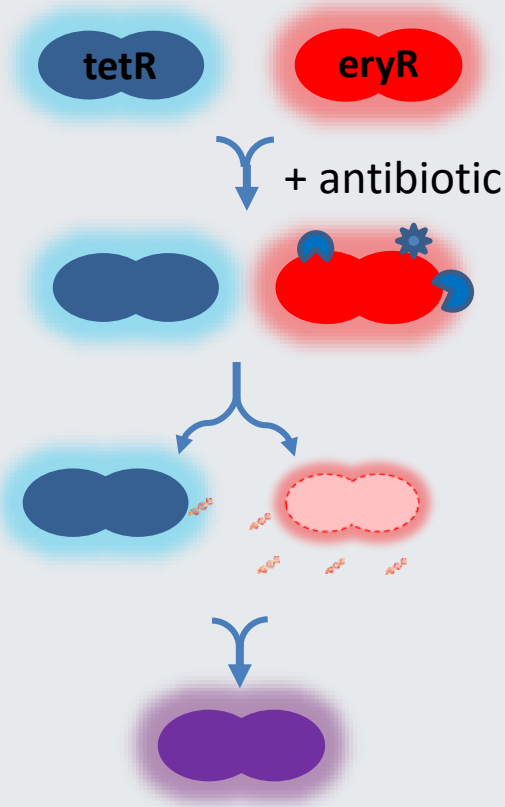
Why is it important?



2 different pneumococci



Viridans group streptococci: a reservoir of resistant bacteria in oral cavities

A. Bryskier

AVENTIS Pharma, Romainville, France

Evolution of penicillin resistance in *Streptococcus pneumoniae*; the role of *Streptococcus mitis* in the formation of a low affinity PBP2B in *S. pneumoniae*

Christopher G. Dowson,[1,*] Tracey J. Coffey,[1] Christopher Kell[1] and Robert A. Whiley[2]

vating the antibiotic via β-lactamases, reducing outer membrane permeability in the case of Gram-negative

Journal of Antimicrobial Chemotherapy (1999) **44**, 19–25 JAC

A variety of Gram-positive bacteria carry mobile *mef* genes

Vicki A. Luna[a], Patricia Coates[b], E. Anne Eady[b], Jonathan H. Cove[b], Thanh T. H. Nguyen[a] and Marilyn C. Roberts[a*]

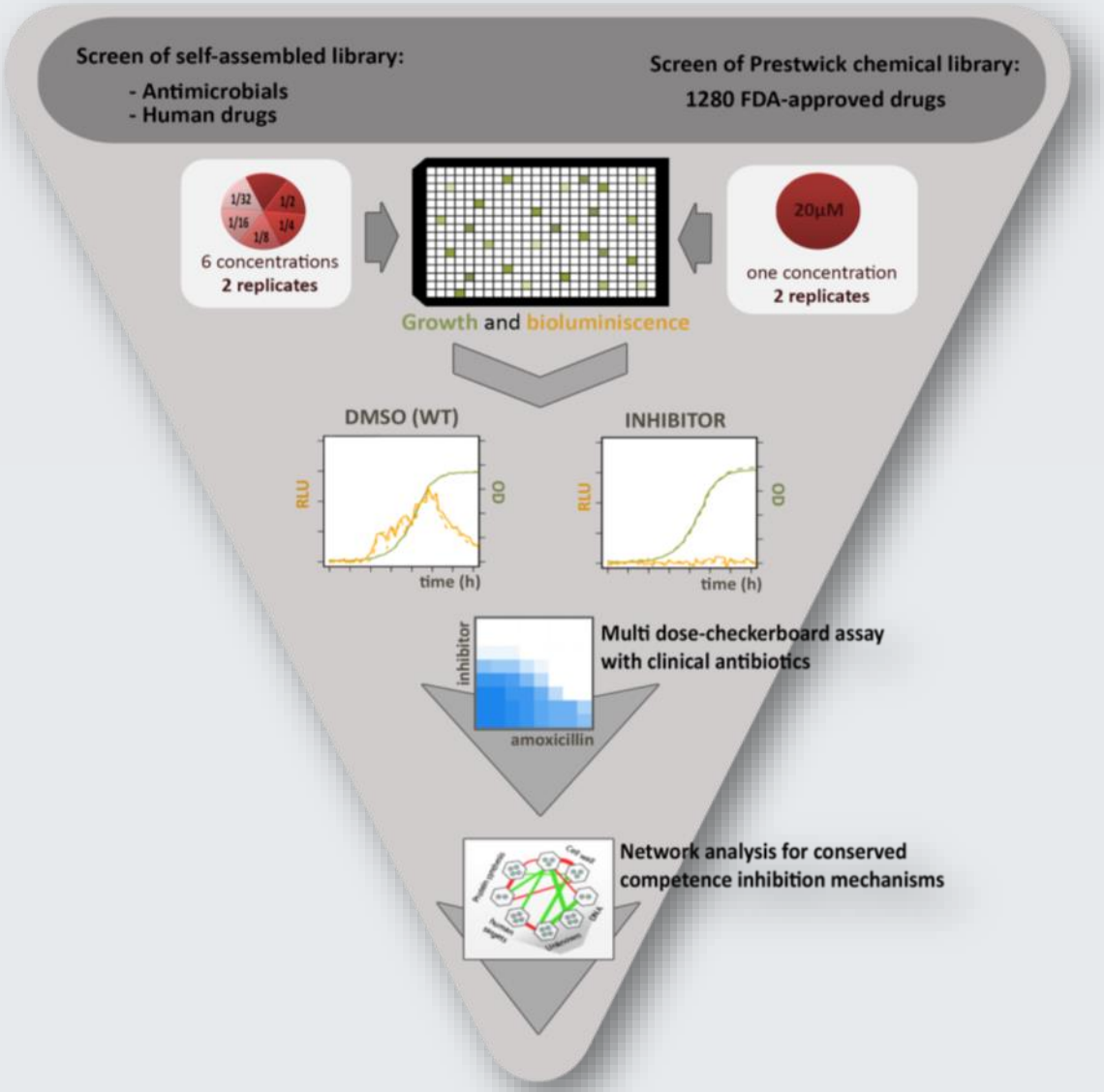
In Vitro Exchange of Fluoroquinolone Resistance Determinants between *Streptococcus pneumoniae* and Viridans Streptococci and Genomic Organization of the *parE-parC* Region in *S. mitis*

Claire Janoir,[1] Isabelle Podglajen,[1] Marie-Dominique Kitzis,[2] Claire Poyart,[3] and Laurent Gutmann[1]

[1]*Laboratoire de Recherche Moléculaire sur les Antibiotiques, Facultés de Médecine Broussais-Hôtel-Dieu et Pitié-Salpêtrière, Université Paris VI, and* [2]*Service de Bactériologie, Hôpital Saint-Joseph, and* [3]*Laboratoire Mixte Pasteur-Necker de Recherche sur les Streptocoques et les Streptococcies, Faculté de Médecine Necker-Enfants Malades, Paris, France*

Project aims (III)

3. Indentification of novel cellular mechanisms: bacterial competence



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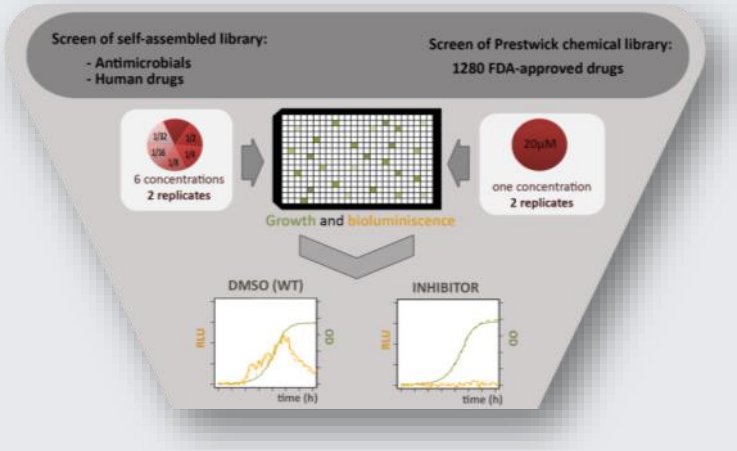
Preliminary results (HTS for competence inhibition)

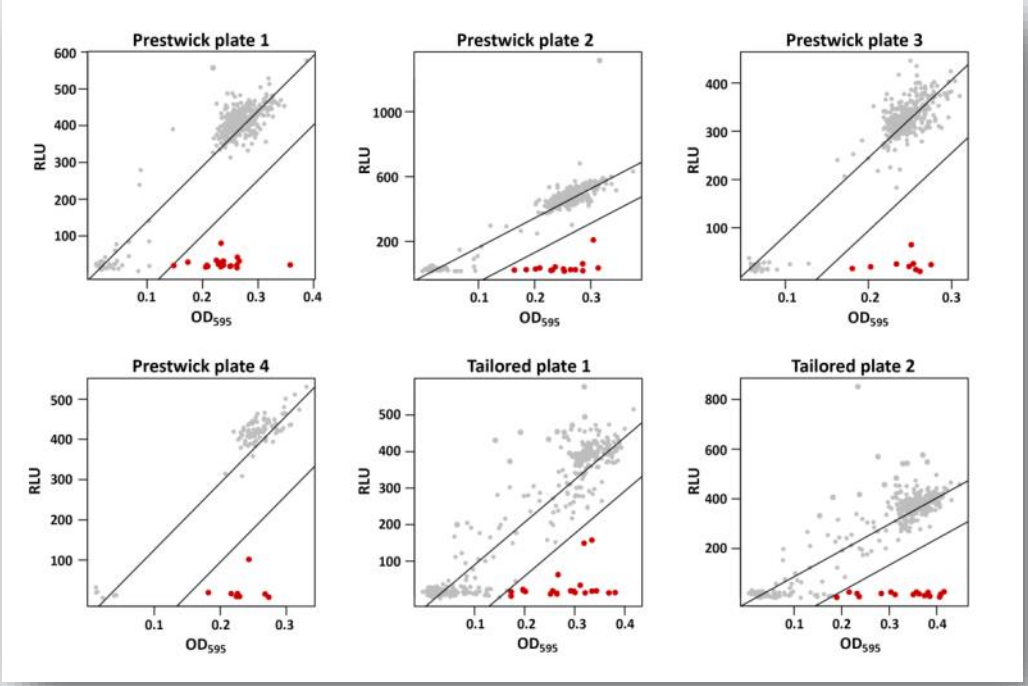


Prestwick: **1280** FDA-approved drugs
Self assembled library: **86** compounds

48 hits found

Selection of best hits based on:

1. Low concentration required
2. Low toxicity
3. Good absortion/excretion



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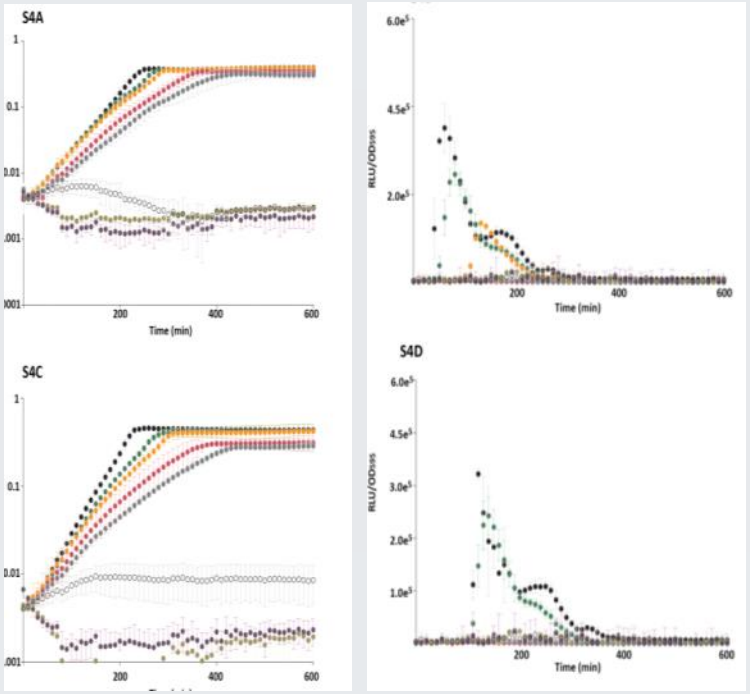
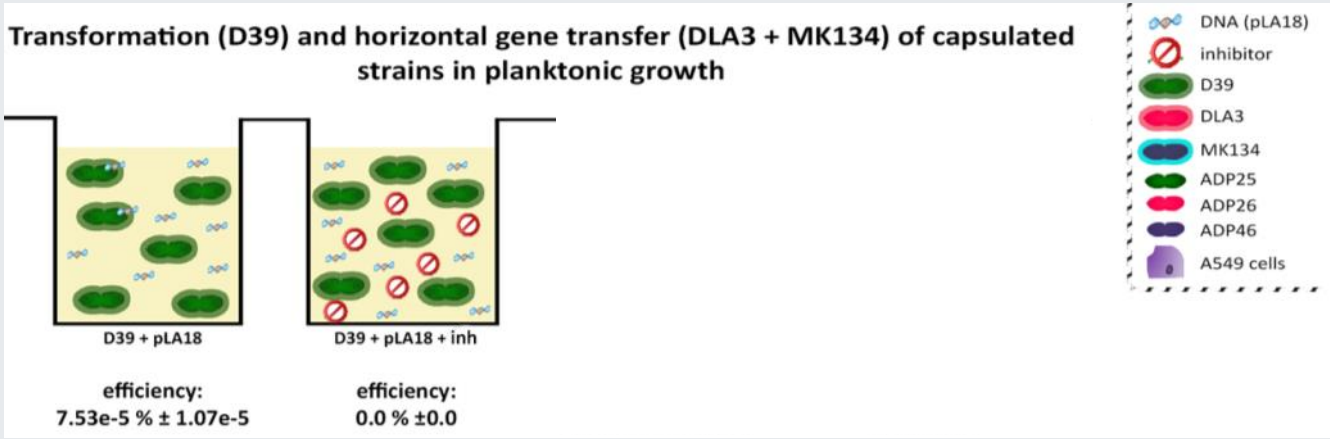
Preliminary results (HTS for competence inhibition)

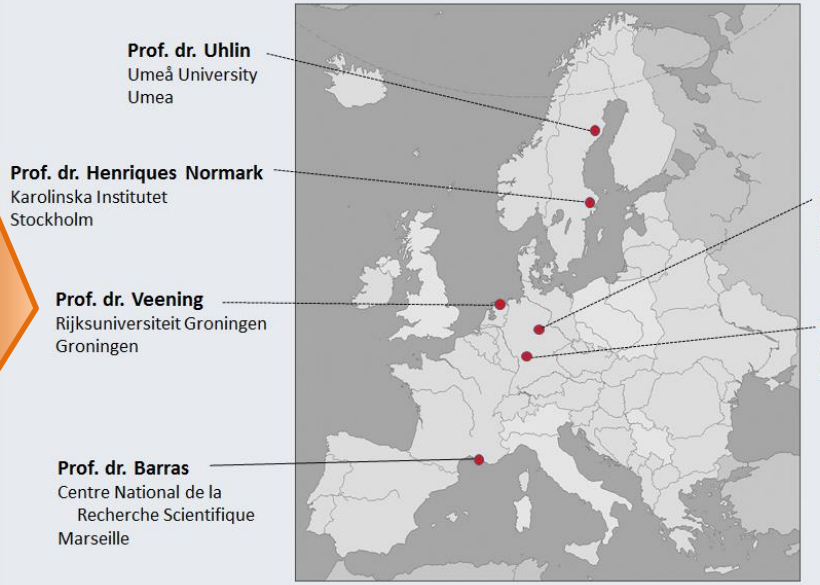
Inhibition also in MDR strains





Acknowledgements

submission platform
jpiamr
Joint Programming Initiative on Antimicrobial Resistance



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