Homeland Defense & Security
Information Analysis Center

Predicting Antibiotic Resistance

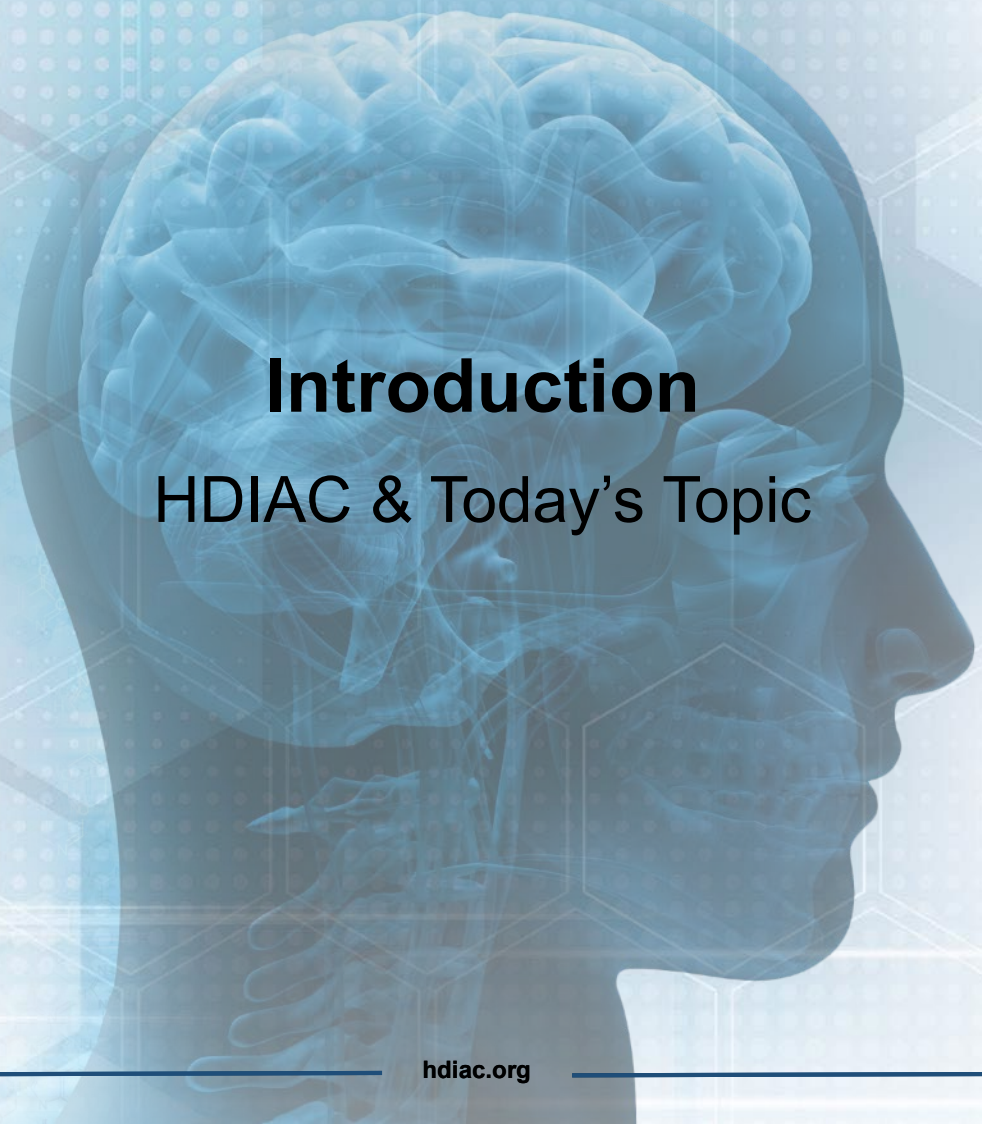
Moving from Reaction to Preemption of Emerging Pathogen Threats

Yousif Shamoo, Ph.D.

Rice University

June 12, 2019

The views presented are those of the speaker and do not necessarily represent the views of DoD or its components.



Introduction

HDIAC & Today's Topic

HDIAC Overview

What is the Homeland Defense & Security Information Analysis Center (HDIAC)?

One of three Department of Defense Information Analysis Centers

Responsible for acquiring, analyzing, and disseminating relevant scientific and technical information, in each of its eight focus areas, in support of the DoD and U.S. government R&D activities

HDIAC's Mission

Our mission is to be the go-to R&D/S&T and RDT&E leader within the homeland defense and security (HDS) community, by providing timely and relevant information, superior technical solutions, and quality products to the DoD and HDS Communities of Interest/Communities of Practice.

HDIAC Overview

HDIAC Subject Matter Expert (SME) Network

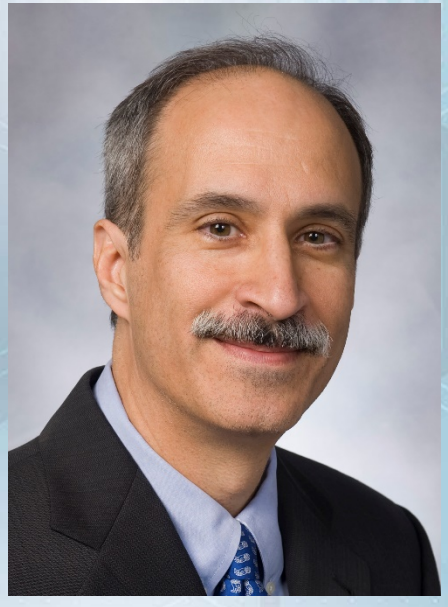
HDIAC SMEs are experts in their field(s), and, typically, have been published in technical journals and publications.

SMEs are involved in a variety of HDIAC activities

- Authoring HDIAC Journal articles
- Answering HDIAC Technical Inquiries
- Engaging in active discussions in the HDIAC community
- Assisting with Core Analysis Tasks
- Presenting webinars

If you are interested in applying to become a SME, please visit HDIAC.org or email info@hdiac.org.

Presenter



Yousif Shamoo, Ph.D.
Vice Provost for Research, and Professor, Biosciences
Rice University

Yousif Shamoo, Ph.D., serves as the Vice Provost for Research at Rice University, overseeing strategic planning for the university research enterprise including engagement with industry through research and technology transfer, since 2014. He was appointed Professor of BioSciences in 2012, and first joined the Rice University faculty in 1998. He served as Rice's Director of the Institute of BioSciences and Bioengineering from 2008-2014, leading the coordination of interdisciplinary research among Rice faculty. He received his Ph.D. degree in Molecular Biophysics and Biochemistry from Yale University in 1988.Dr. Shamoo's research lab studies the dangerous rise of multi-drug resistant bacteria.

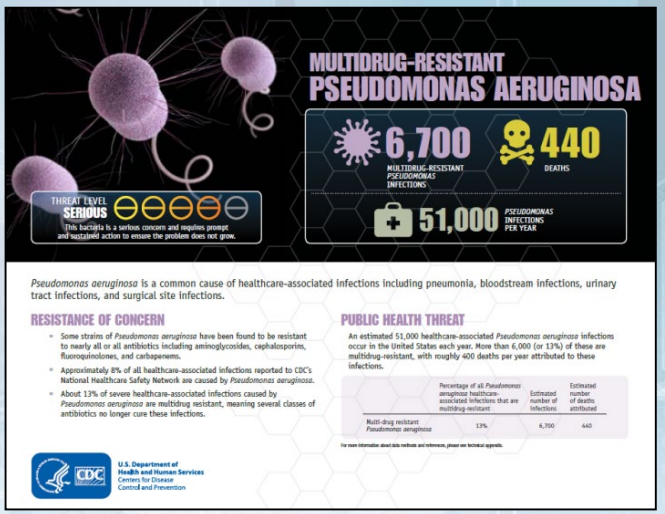
Dr. Shamoo currently receives research support from the National Institutes of Health and the Department of Defense. He has served as a reviewer for study sections for the National Institutes of Health, National Science Foundation, and Department of Defense. He is the recipient of the American Society for Microbiology Distinguished Lecturer award from 2011-2013 and Rice's top teaching award, the George R. Brown Award for Excellence in Teaching in 2015. He also received the George R. Brown Award for Superior Teaching three times since 2009.

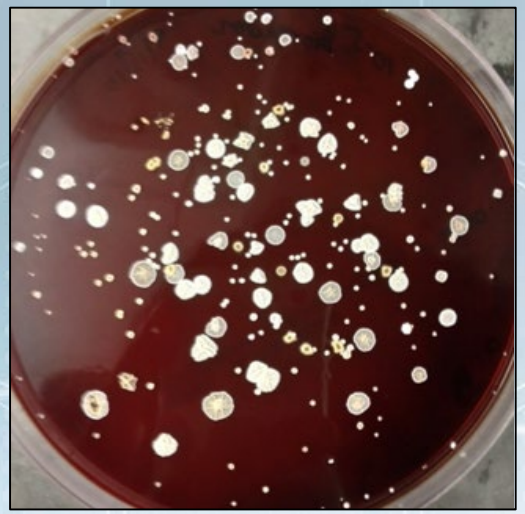
Overview

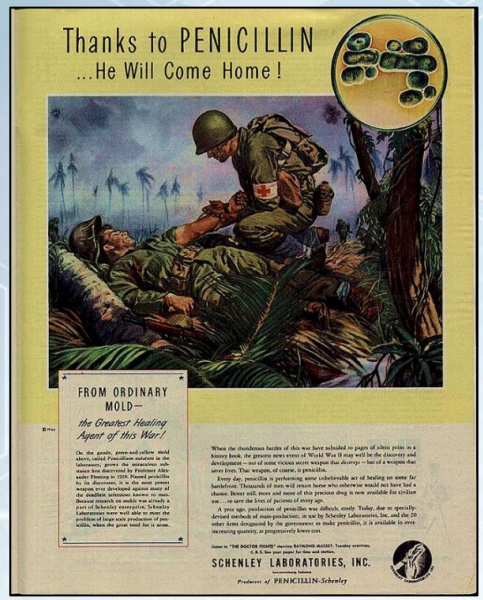
Three stories of bugs and drugs:

Experimental evolution to identify the genetic and biochemical basis of resistance

- ***Nocardia nova* & *N. cyriacigeorgica* to *Trimethoprim*-Sulfamethoxazole (TMP-SMX) (an emerging organism)**
- ***P. aeruginosa* to colistin (hypermutation as driver)**
- ***Francisella tularensis adaptation* to DOX/CIPRO (using *F. holoarctica* Live Vaccine Strain) (combinatorial therapy)**







How do we re-capitulate evolution?

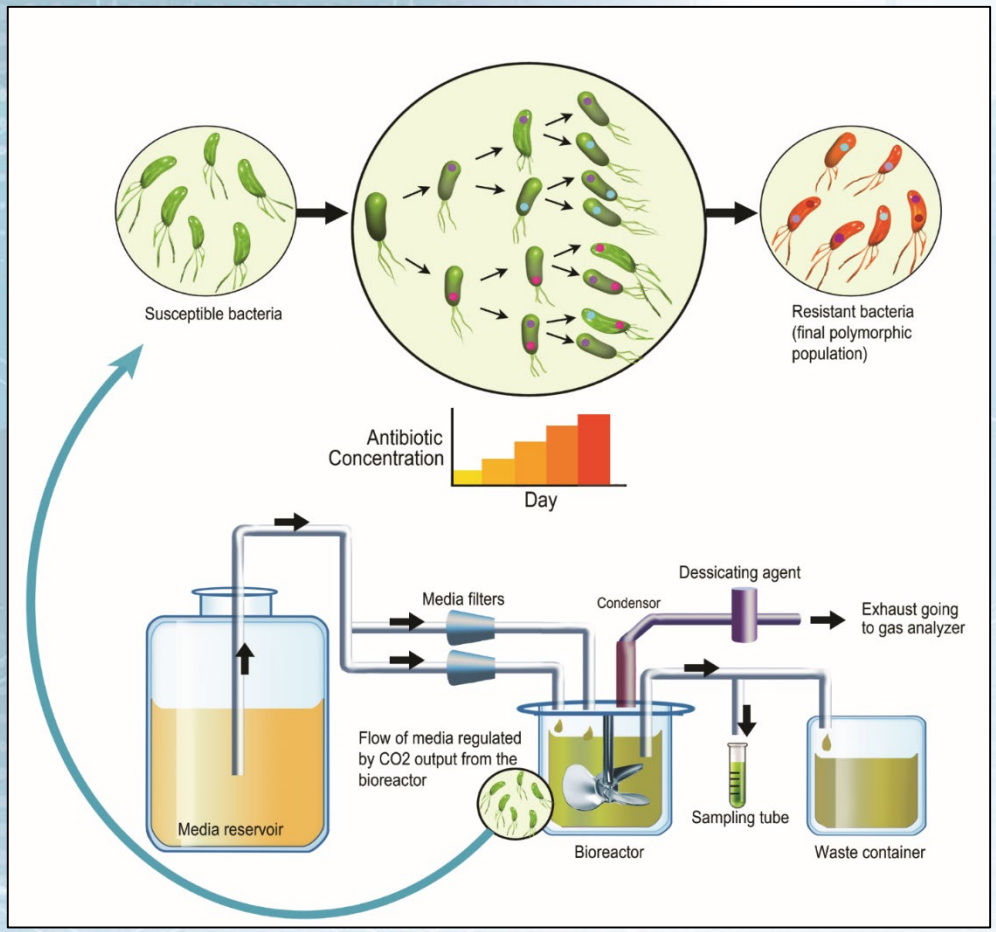
1. Antibiotic concentration is slowly increased (sub-MIC).
2. Large polymorphic population
3. Biofilms favored
4. Intermediates and end-points of adaptation are characterized.

Are they similar to the patient?

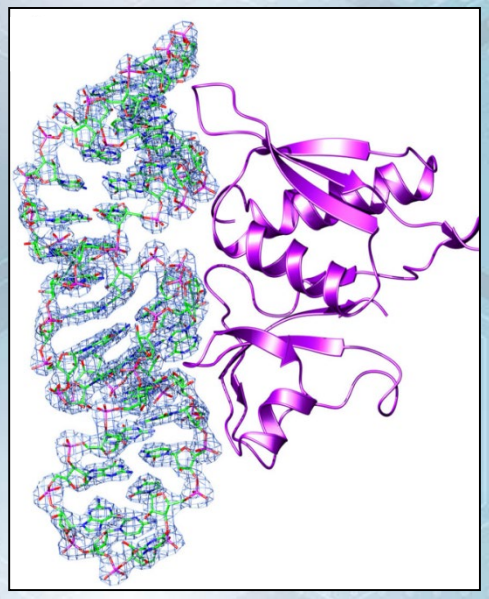
Miller *et al.,* AAC (2013) Beabout *et al.,* MBE (2015), *ACS Infect Dis* (2016) Mehta et al., AAC (2018) *J. Antibiotics* (2017)

Are results reproducible?

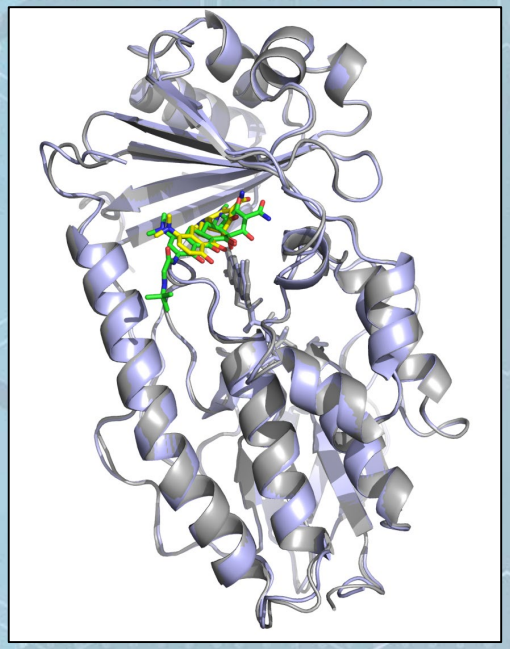
Couñago *et al.*, Mol. Cell (2006) Peña *et al.*, Mol. Sys Biol. (2010)



Pipeline approach to the prediction of resistance: evolution, genomes and atoms



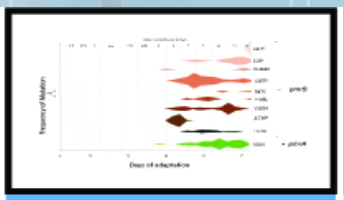
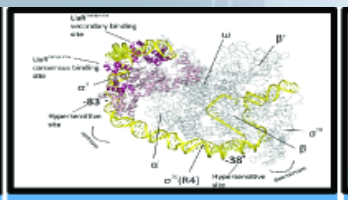
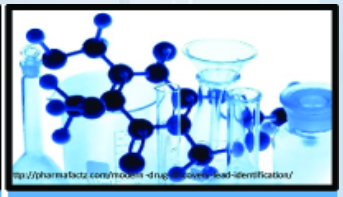
Bacillus anthracis
S8: RNA aptamer



tetX2:tigecycline
oxidoreductase

E. faecalis LiaR DBD Domain
with DNA

Pipeline approach to the prediction of resistance: evolution, genomes and atoms

				
Evolution to antibiotic resistance	Identify frequency and order of mutations	Identify linkages between mutations	Validation of identified targets	Rank targets for drug development
- Maintain diversity - Sub-inhibitory drug concentration - Maximum possible growth rate - Allow biofilm formation	- Deep sequence daily population data - Construct timeline of resistance	- Phenotypically characterize end-point isolates - Whole genome sequence diverse isolates	- Biochemical and biophysical characterization of resistance conferring pathways or proteins	- Determine which genes can act as appropriate targets for the development of “anti-evolution” co-drugs

Story 1: Nocardia
Emerging or just poorly recognized?

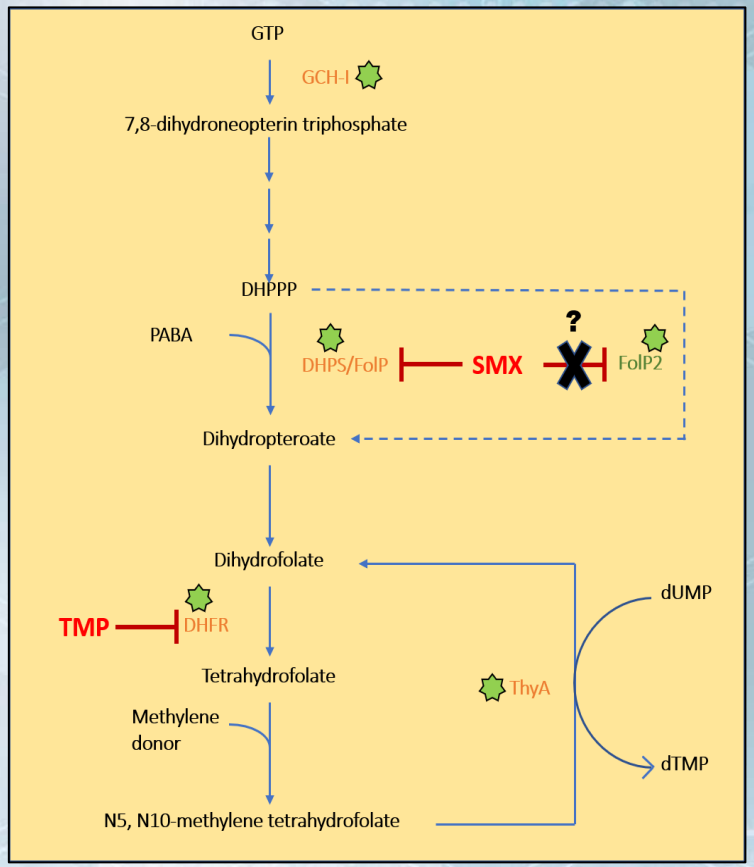
- **Gram positive, aerobic actinomycetes that are ubiquitously found in soil and water**
  - Very slow growing
- **Opportunistic pathogen affecting immunocompromised individuals (cancer & transplant)**
  - but one-third of nocardiosis patients are immunocompetent
- **Treatment usually involves long term antimicrobial therapy that can last up to a year.**
  - combination therapy using folate biosynthesis pathway inhibitors, trimethoprim and sulfamethoxazole (TMP-SMX)

Mehta et al., AAC 2018
Mehta et al., J. Antibiotics, 2018

So little known we had to start with the basics…

- **Assemble ref genomes:**
  - PacBio and Short reads *N. cyriacigeorgica*
- **Do evolution on wide range of genomes**
  - 8 *N. nova* and 2 *N. cyriacigeorgica* (7 MDACC (Dr. Han) 3 ATCC)
- **Took two experimental evolution approaches**
  - Serial transfer (8 pops in duplicate) to get at reproducibility
  - Bioreactor with deep sequencing of whole populations each day and end point isolates
    - Establishes order and frequency of each allele as a longitudinal time study

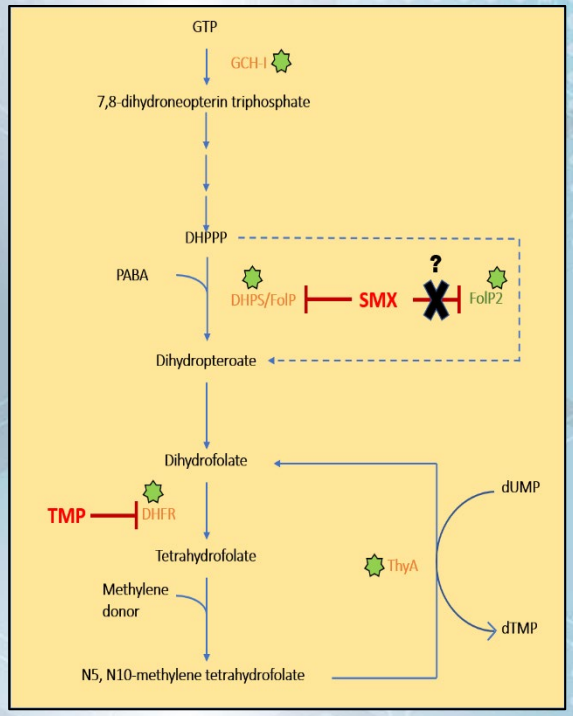
Adaptive mutations cluster within folate biosynthetic pathway



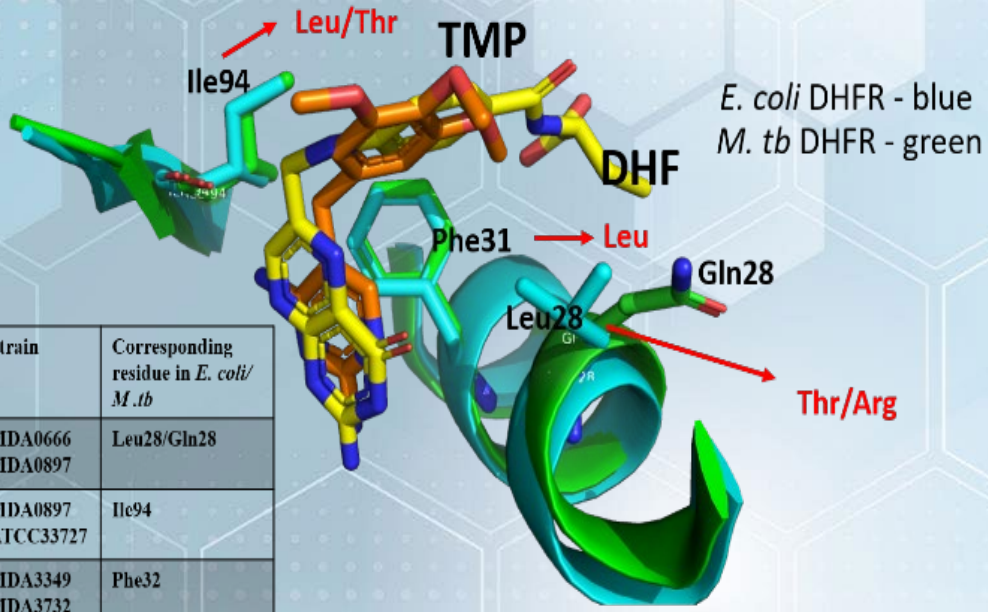
- **TMP binds dihydrofolate reductase (DHFR)**
- **SMX binds Dihydropteroate synthase (DHPS or FolP)**
- **Adaptive Mutations in DHFR/DHPS seen in Mycobacteria, *E. coli* and others**

Genes with mutations

DHFR mutations fall mostly at DHF/TMP binding pocket

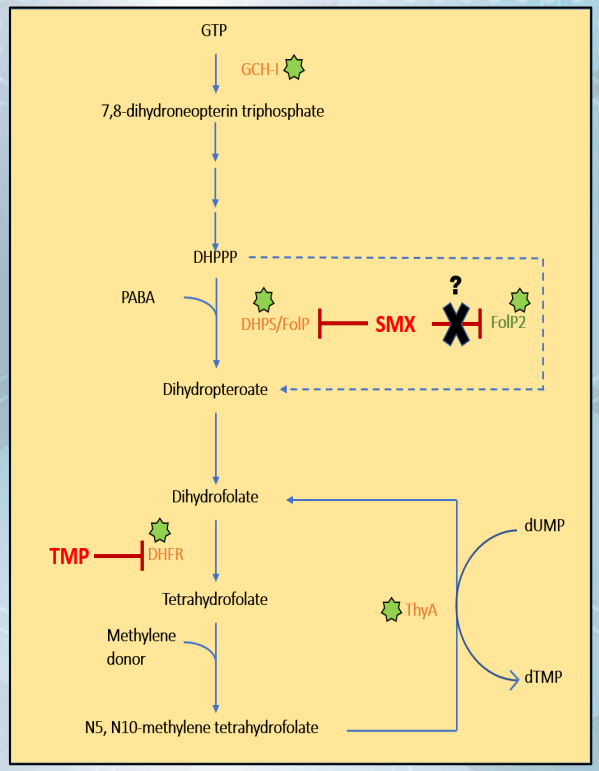


DHF = dihydrofolate

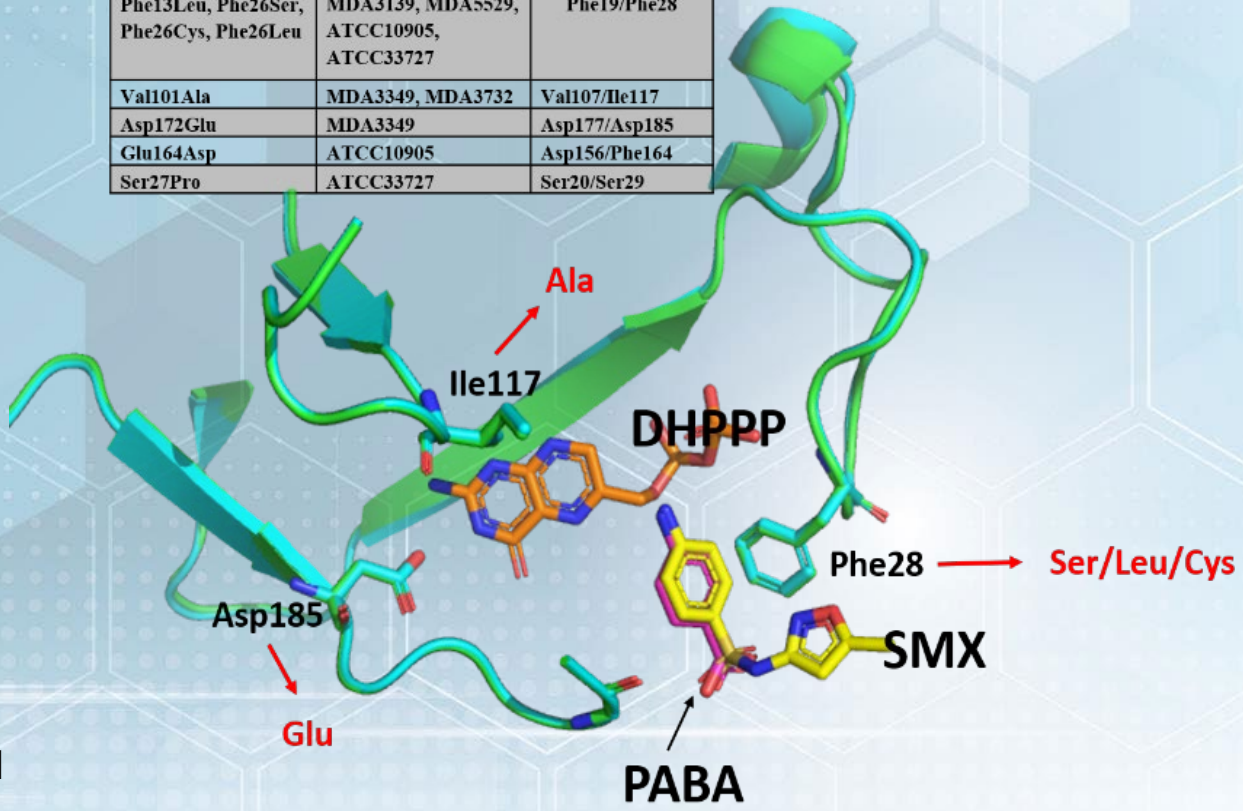


Mutation in *Nocardia*	Strain	Corresponding residue in *E. coli*/ *M .tb*
Ile30Thr Met30Arg	MDA0666 MDA0897	Leu28/Gln28
Ile99Leu Ile99Thr	MDA0897 ATCC33727	Ile94
Phe36Leu	MDA3349 MDA3732	Phe32
Ile155Thr	MDA3139 ATCC10905	Phe153/Leu153

DHPS mutations fall mostly at ligand/SMX binding pocket



Mutation in Nocardia	Strain	Corresponding residue in M .tb/Y. pestis
Phe13Ser, Phe13Cys, Phe13Leu, Phe26Ser, Phe26Cys, Phe26Leu ★	MDA3349, MDA0666, MDA0897, BAA2227, MDA3139, MDA5529, ATCC10905, ATCC33727	Phe19/Phe28
Val101Ala	MDA3349, MDA3732	Val107/Ile117
Asp172Glu	MDA3349	Asp177/Asp185
Glu164Asp	ATCC10905	Asp156/Phe164
Ser27Pro	ATCC33727	Ser20/Ser29

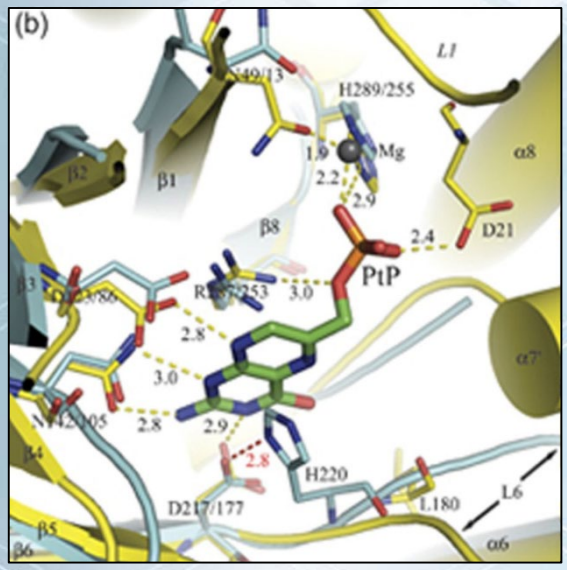


DHPPP= 6-hydroxymethyl 7,8-dihydropterin pyrophosphate

PABA = para-amino benzoic acid

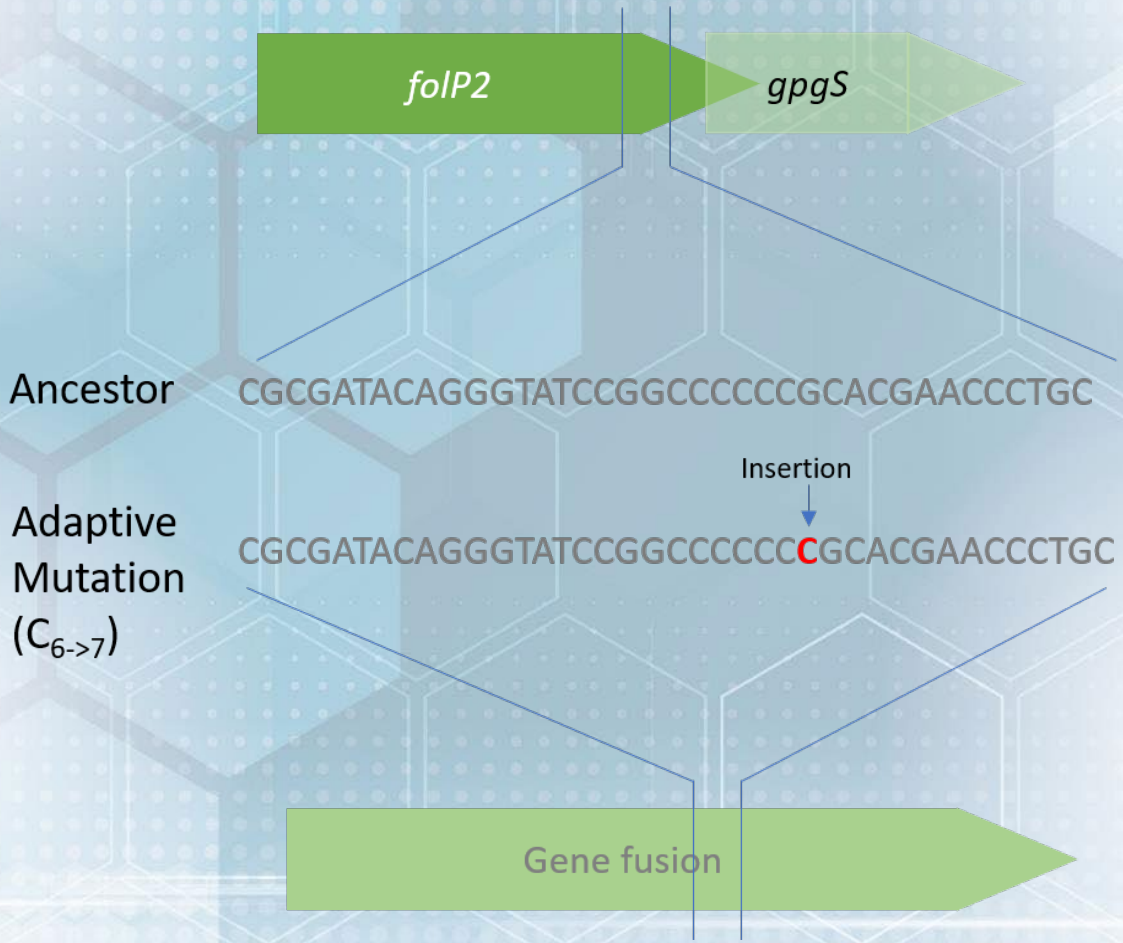
Mutations also seen in a "non-functional" homolog of DHPS?

- **36% sequence identity with the first copy of DHPS (MDA3349)**
- **Other actinomycetes like *Mycobacterium, Corynebacterium* and *Rhodococcus* also have a second copy of DHPS Original copy of DHPS in MDA3349**
- **In *M. leprae* the second copy cannot complement DHPS**
- **In *M. tuberculosis* showed the purified homolog could not carry out reaction**
- **In *M. smegmatis* knockout of second copy**
  - Causes 8-fold decrease in SMX MIC



Gengenbacher M. et al., 2008

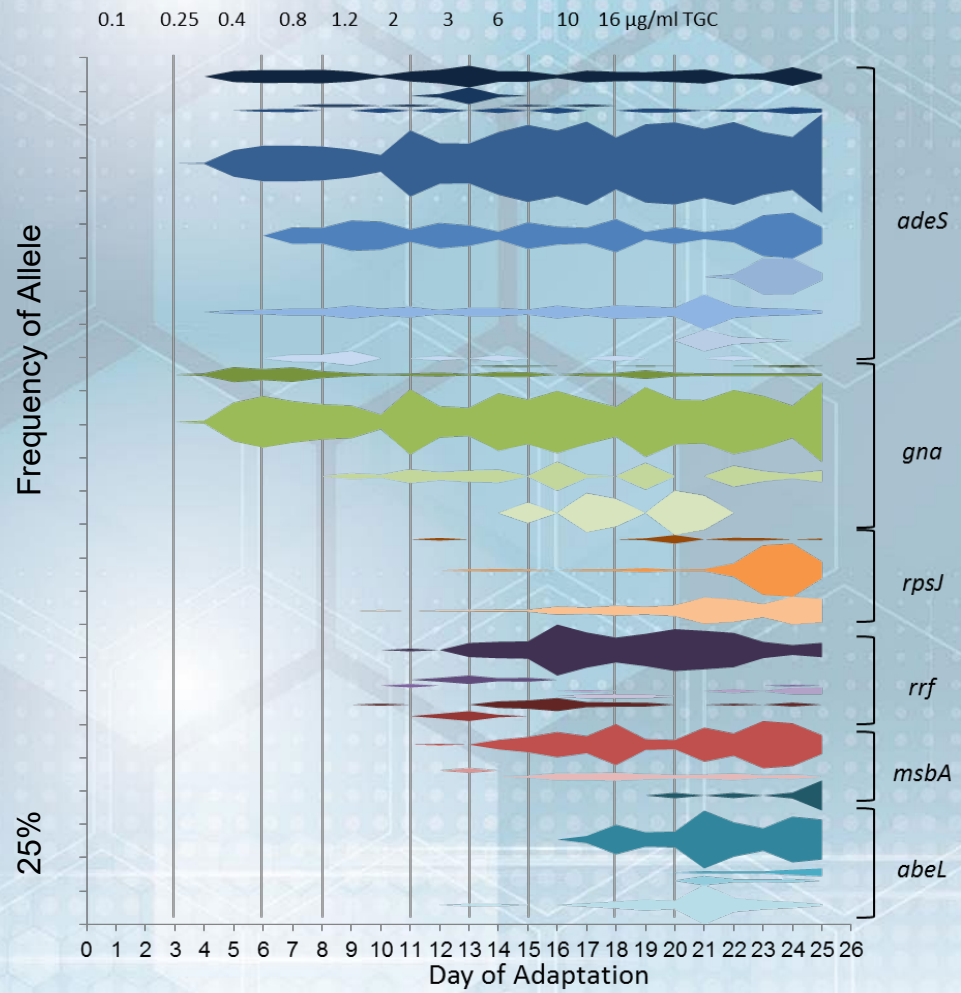
A new bifunctional enzyme? Why?



A new bifunctional enzyme? Why?

- A frameshift mutation in *folP2* leads to a gene fusion. Non-functional DHPS *(folp2)* and *gpgS overlap and* are brought in frame by the insertion of a single cytosine ($C_{6->7}$) (multiple times)
- GpgS= glucosyl-3-phosphoglycerate synthase: in *M. tuberculosis,* makes an intermediate in the synthesis of the cytoplasmic methylated polysaccharide, methylglucose lipopolysaccharide (MGLP). MGLP stabilizes medium and long chain fatty acids and stimulates activity of fatty acid synthase I (snps in *gpgS* also seen)

Strange results suggest bioreactor was different from flask transfers



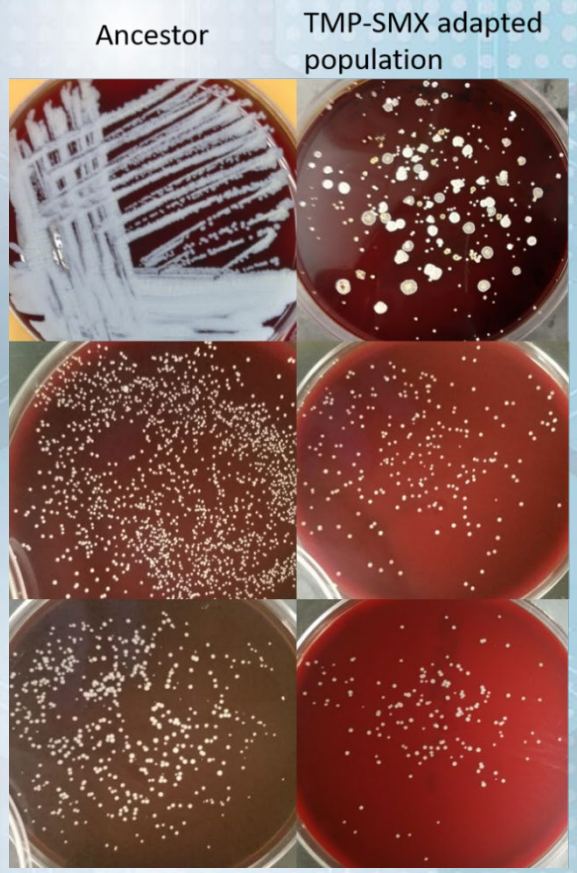
Expected

1. Adaptive mutation arise in population
2. Increase in frequencies
3. Other mutations occur
4. Repeat 1-3

Observed

1. No sweeps or rise of any particular genotype
2. About 1/3 are sensitive to TMP-SMX even though drug is 4x MIC for over a week

Weak selection in a strong selection environment?



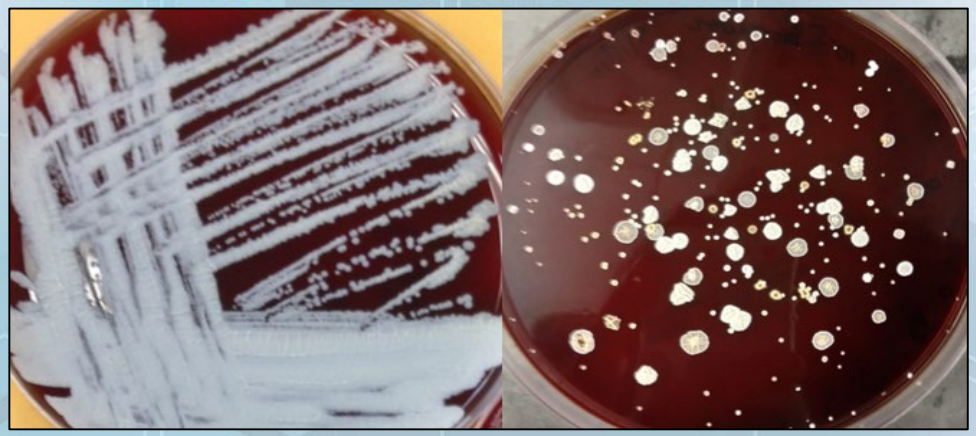
N. cyriacigeorgica MDA3349 ← Bioreactor

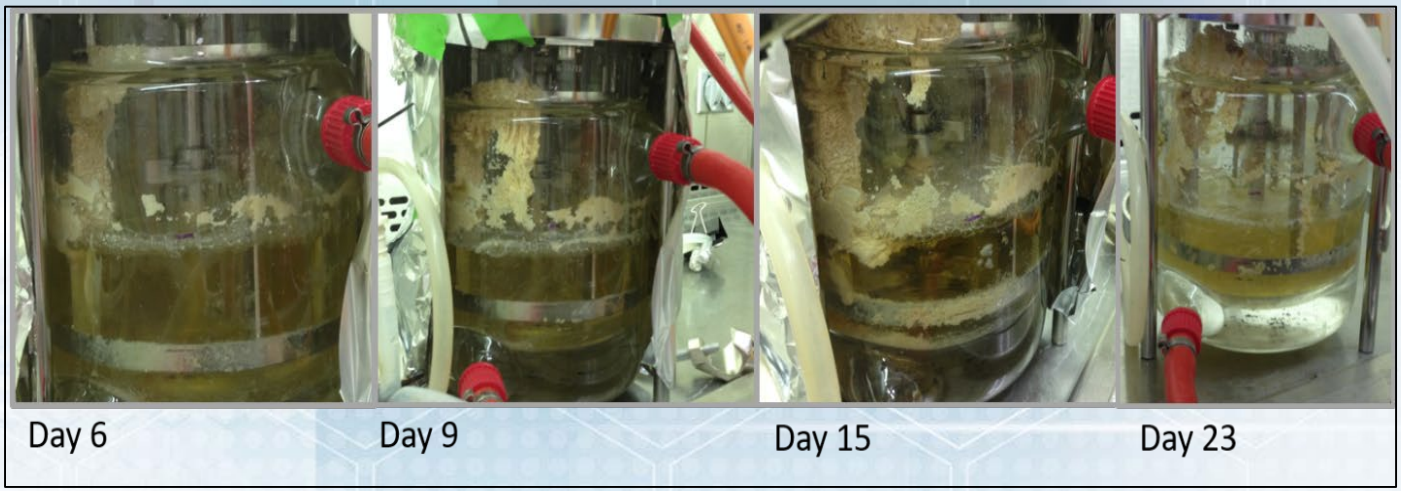
N. nova MDA0897 ← Serial Flask Transfer

N. cyriacigeorgica MDA3732 ← Serial Flask Transfer

- **Biofilms provide much stronger than usual protection against TMP-SMX**

Nocardiosis is very recalcitrant to treatment



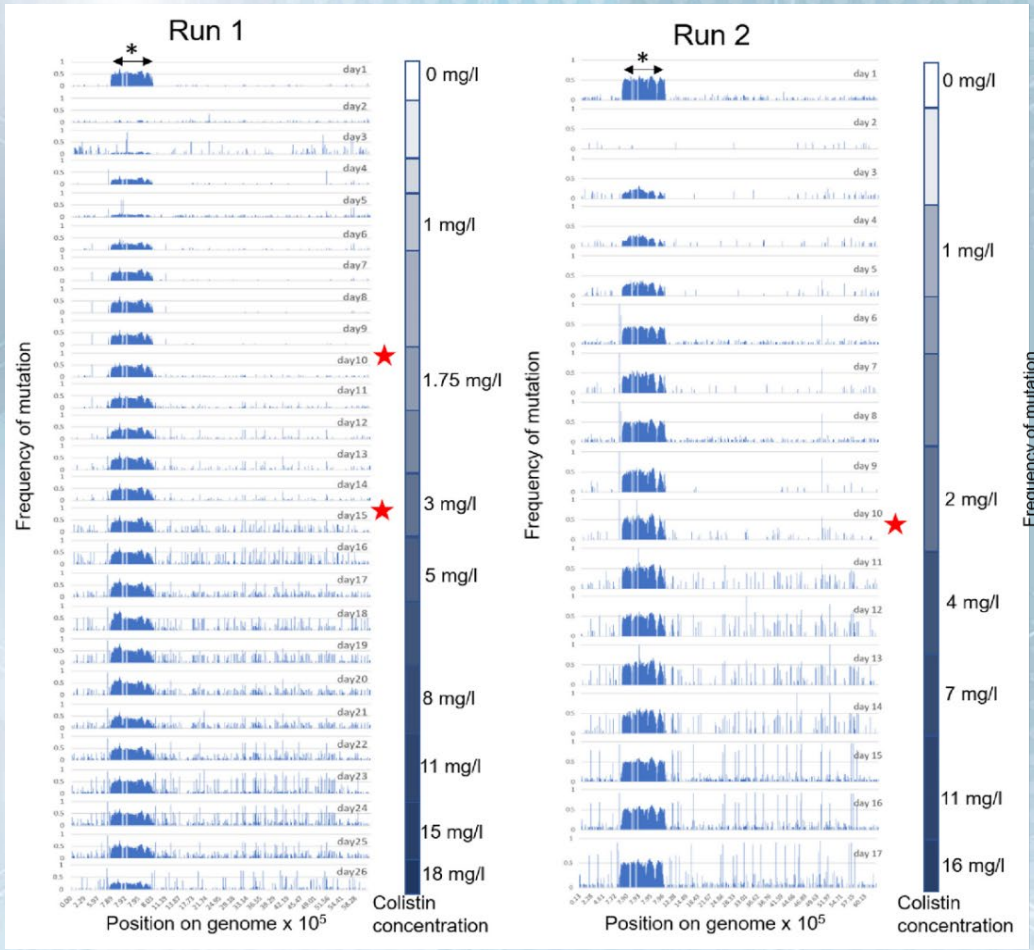


Pseudomonas aeruginosa adaptation: Hypermutation as a driver for rapid adaptation

- ***Pseudomonas aeruginosa* is common in long term infections of cystic fibrosis patients**
- **Adaptation to antibiotics is recurrent and highly problematic**
- **Hypermutation in patients is paradoxical.**
  - e.g., hypermutation leads to the accumulation of many non-adaptive mutations
  - Mueller's Ratchet suggests that hypermutator while advantageous in the short term have increasingly decreased overall fitness
  - Clinical isolate data suggest either new invasions of PA or in some cases:
    - a subpopulation waiting in the wings to re-infect

Mehta et al., AAC *in press* (2019)

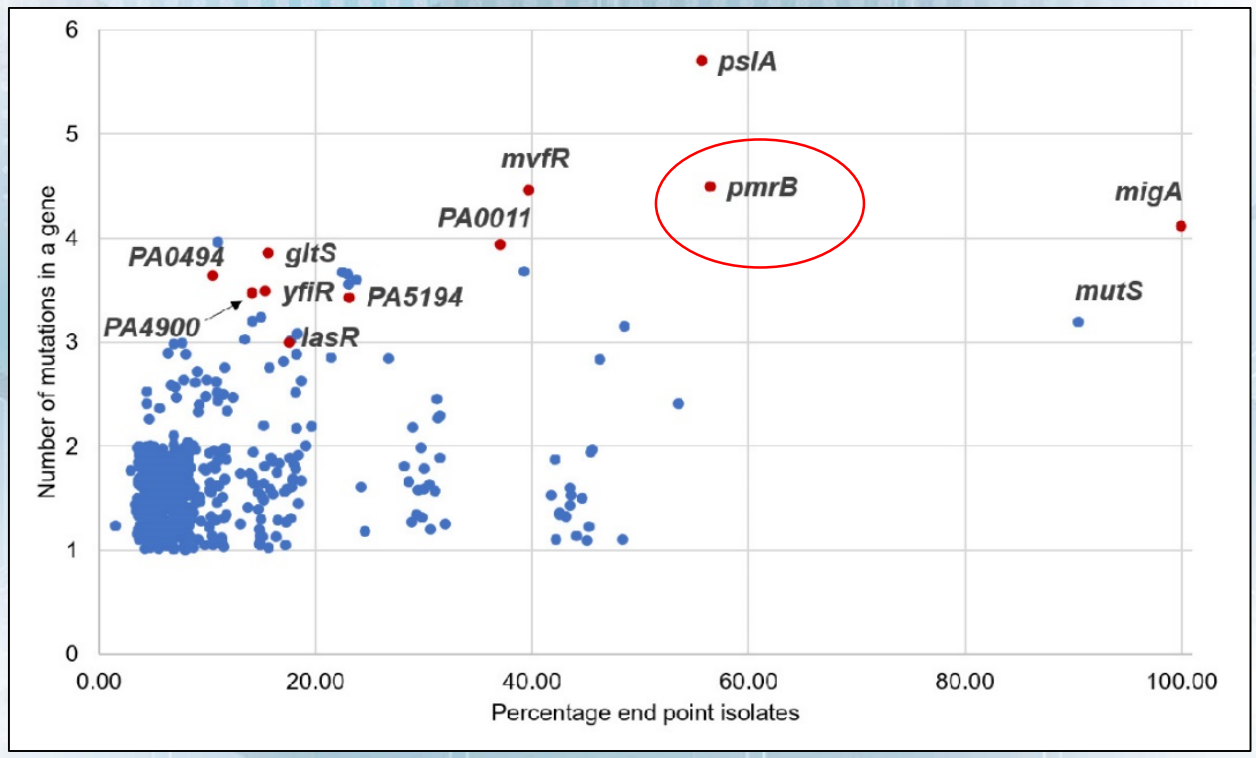
Experimental evolution shows repeated evolution of hypermutation



★ Day at which MutS mutates to generate higher mutation frequency

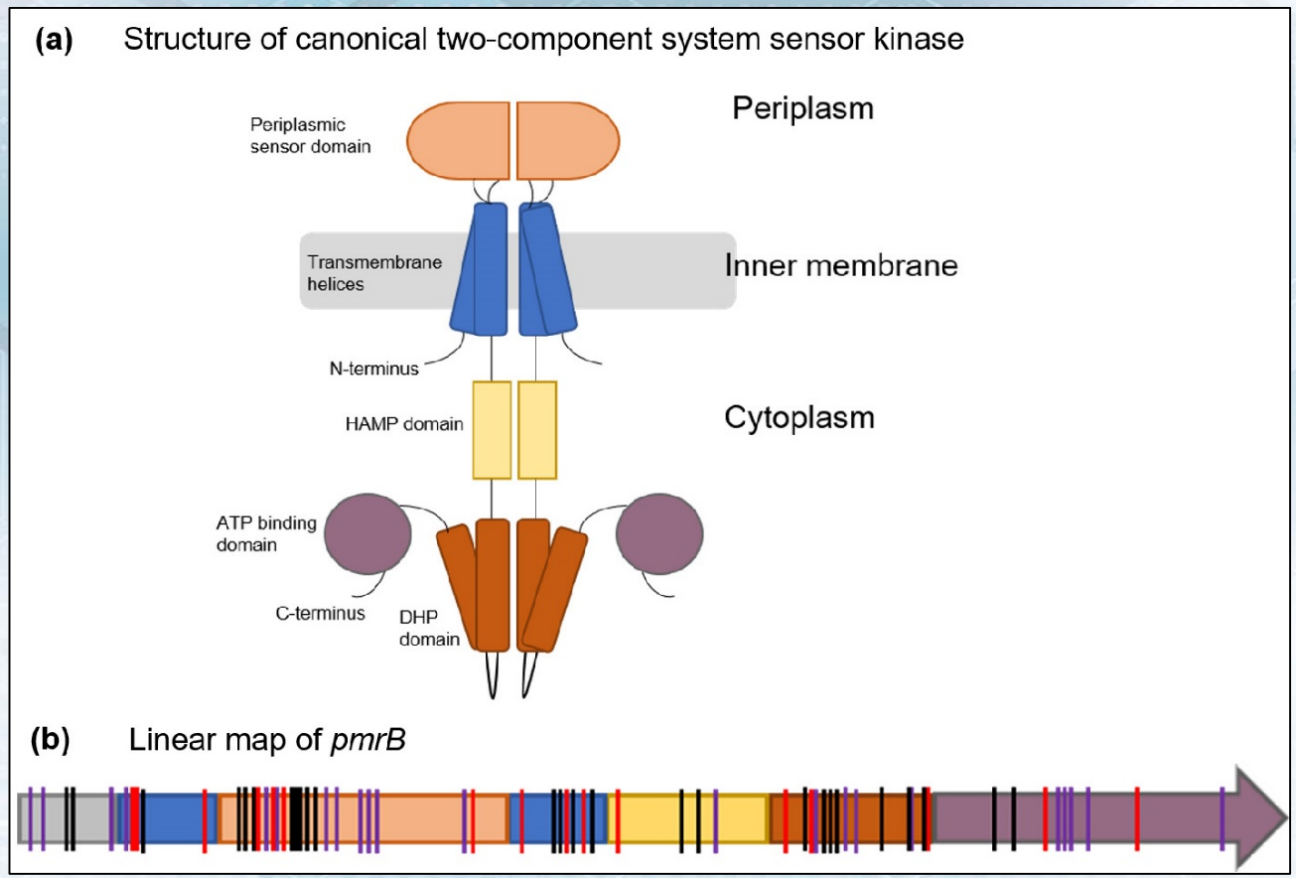
Linearized genome, not to scale

Sorting wheat from chaff: Identifying adaptive mutations

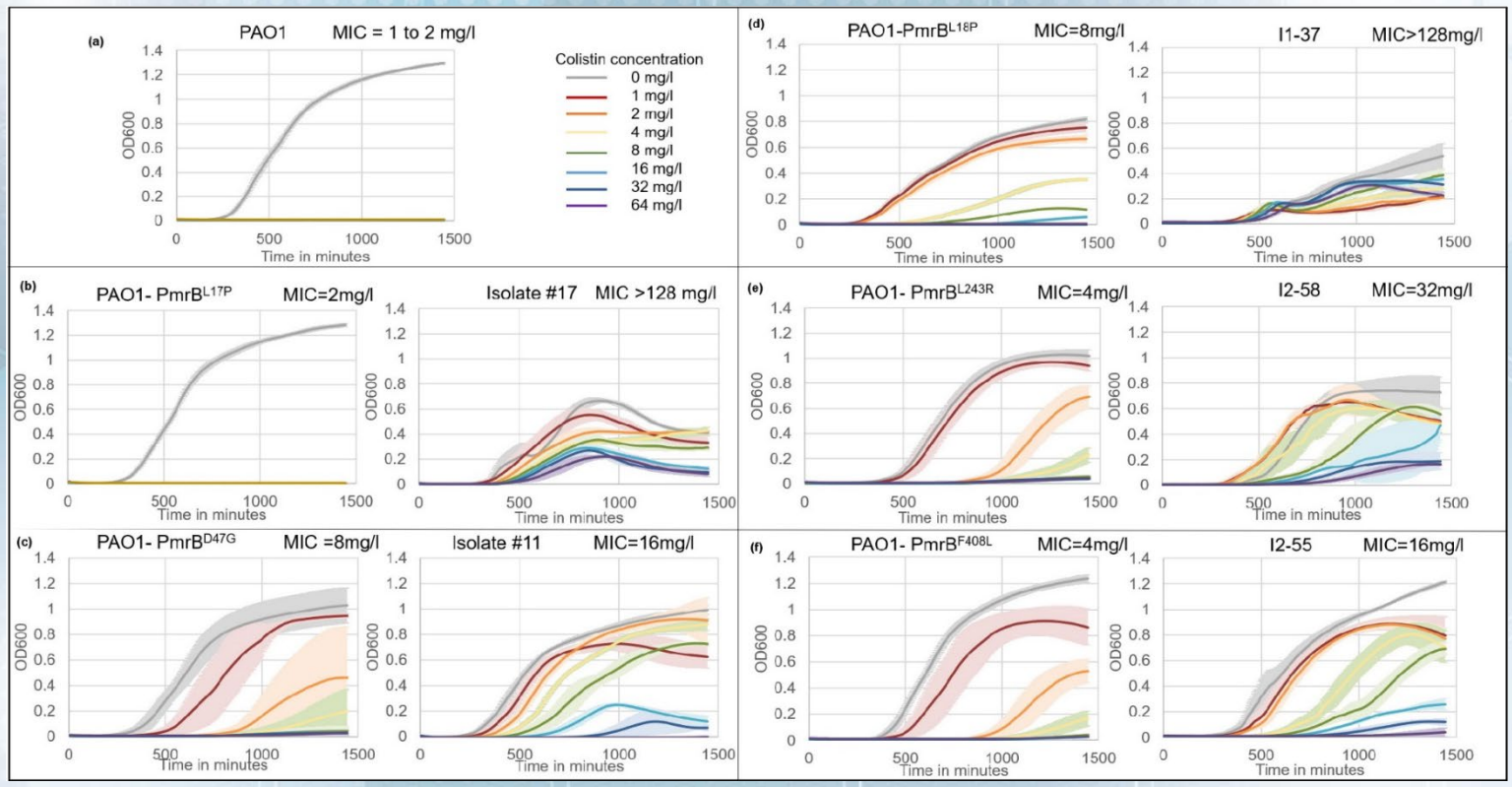


- **The 29 sequenced end point isolates had cumulatively acquired 761 total mutations affecting 563 genes.**
- **Among the daily populations evolving to colistin from both runs, 1,197 genes were mutated and a total of 2657 mutations were identified at ≥ 5% frequency.**

Hypermutation of pmrB shows remarkable plasticity suggesting modest changes in function produce resistance



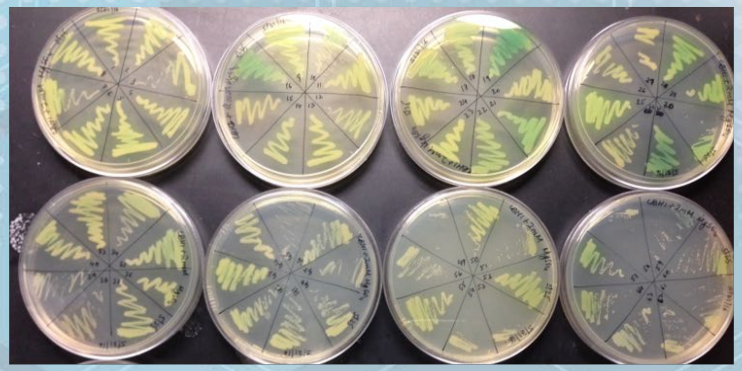
Hypermutation reduces fitness overall



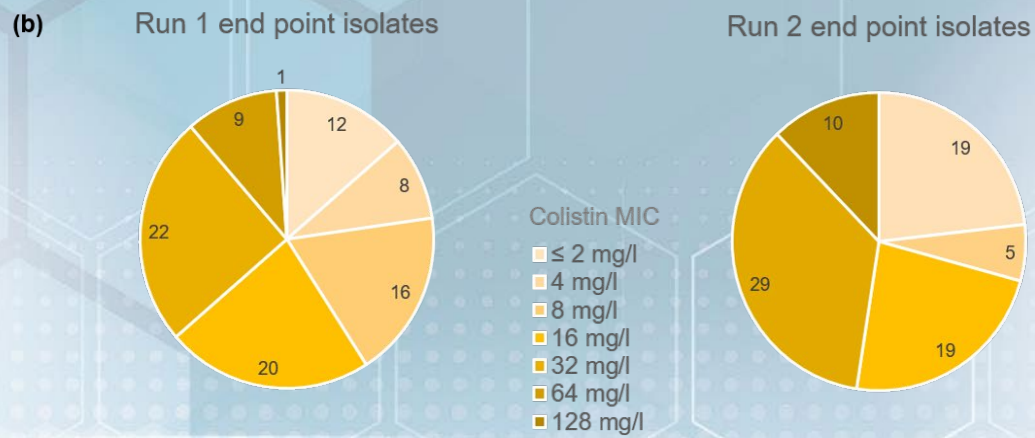
- **Also shows that other mutations after pmrB contribute to colistin resistance**

Polymorphic populations: Subpopulations matter

(a)



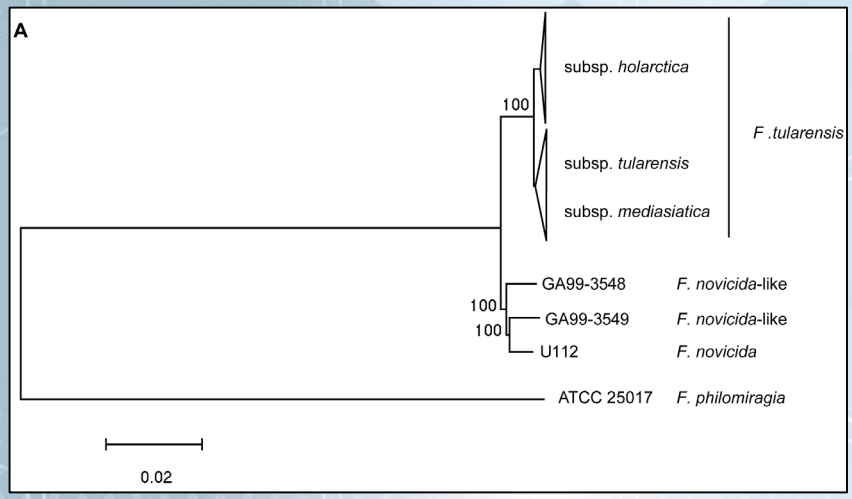
(b)



- **Subpopulations of wild type are the seeds of future resistance**

Francisella tularensis : How effective will a combinatorial therapy be?

- **Gram negative, intercellular pathogen – can survive in macrophages**
- **Category A select agent – highly virulent (infective dose can be <10 bacteria)**
- **Potential bioterrorism agent**
- **4 subspecies; subsp. tularensis being the most virulent (BSL-3)**
- **Subsp. holarctica has lower virulence but is more closely related to subsp. tularensis**
- **LVS (live vaccine strain):**
  - Attenuated holarctica strain: Our strain of choice

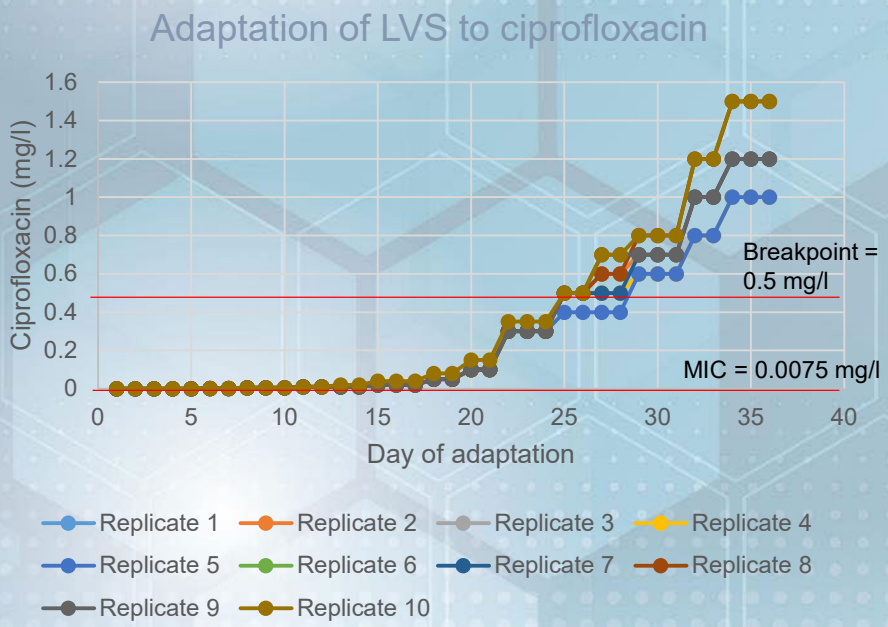


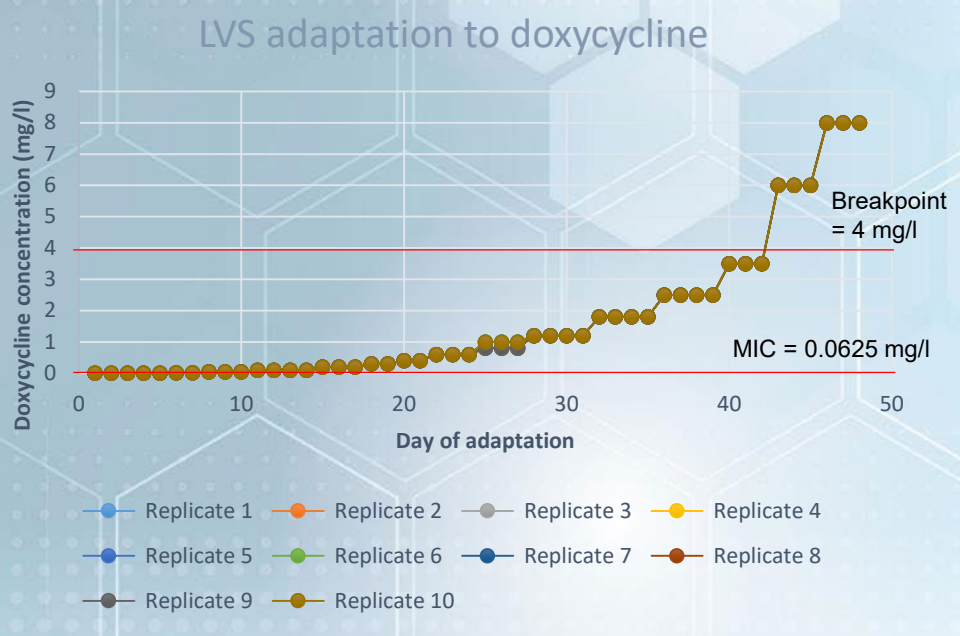
Larsson et al. 2009 PLoS Pathogens

Current treatment strategies

- Doxycycline and streptomycin are approved by the FDA for treating tularemia
- CDC maintains stockpiles of doxycycline and ciprofloxacin for mass casualty settings and postexposure prophylaxis
- Bacteriostatic Doxycycline has a higher failure rate and requires longer duration of therapy
- The rising threat of antimicrobial resistance could make doxycycline ineffective

Adaptation timelines during single drug exposure

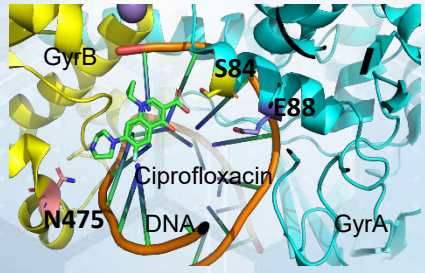




Relevant mutations identified in single drug evolved populations
Note: This is a partial list, as this is in progress

Ciprofloxacin evolved populations

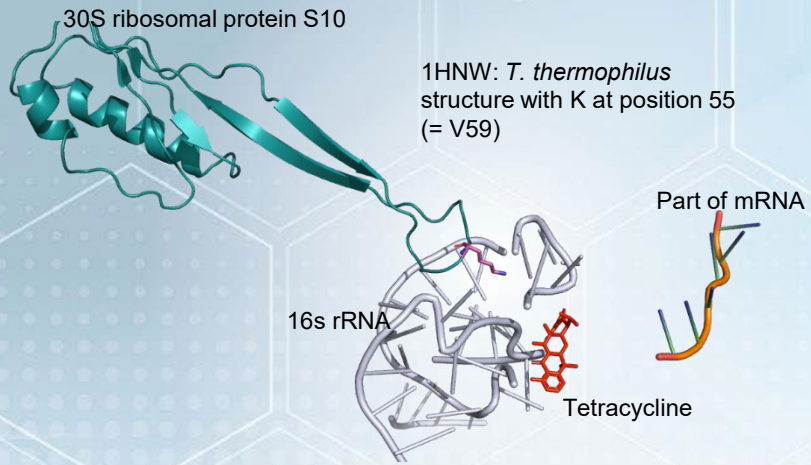
Population	Topo IV subunit A		Gyrase subunit A			Topo IV subunit B				Gyrase subunit B	
	H78N	E125D	T83K	T83I	D87Y	G96S	S458Y	S493Y	R585I	P746T	S465Y
Cipro 2				94.40%	5.20%						
Cipro 3		34.10%		100%				63.40%			
Cipro 7			100%		100%				100%		
Cipro 8	16.30%			72.80%	29.60%	23%				15.60%	30.70%
Cipro 9				100%			6.90%				



2XCT: *S. aureus* gyrase complex with cipro and DNA
S84=T83; E88=D87; N475=S465

Doxycycline evolved populations

Population	30S ribosomal protein S10	Conserved protein FTL1107 (TolC homolog)	Outer membrane protein TolC precursor FTL1865			Efflux protein RND family MFP subunit
	V59L	L405R	I436T	I430R	G429A	L182F
Doxy 1	61.10%		94.10%			
Doxy 3	100%	100%				
Doxy 5	100%			100%	100%	
Doxy 7	100%	100%				
Doxy 10	100%					100%

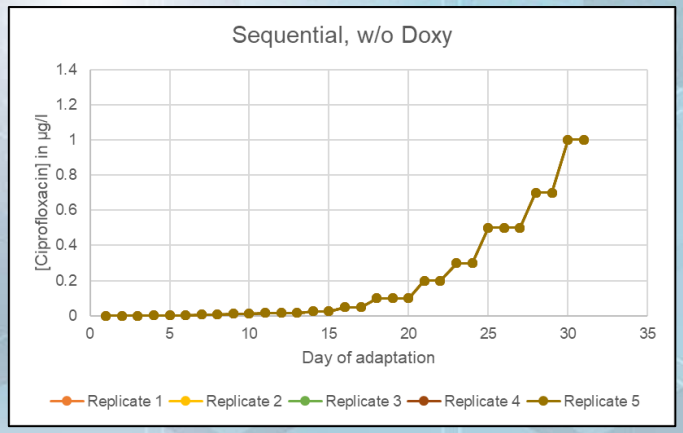


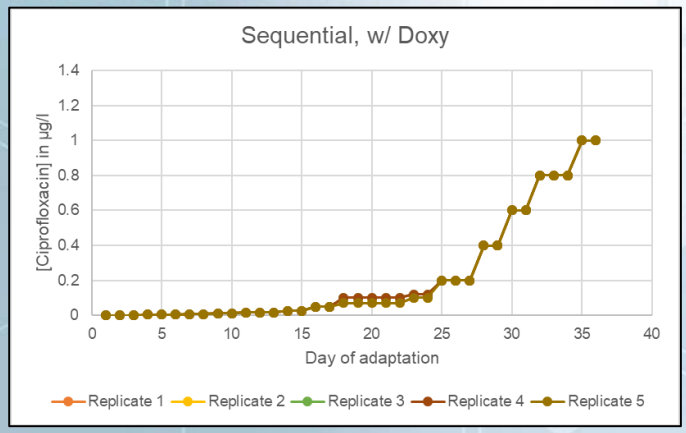
1HNW: *T. thermophilus* structure with K at position 55 (= V59)

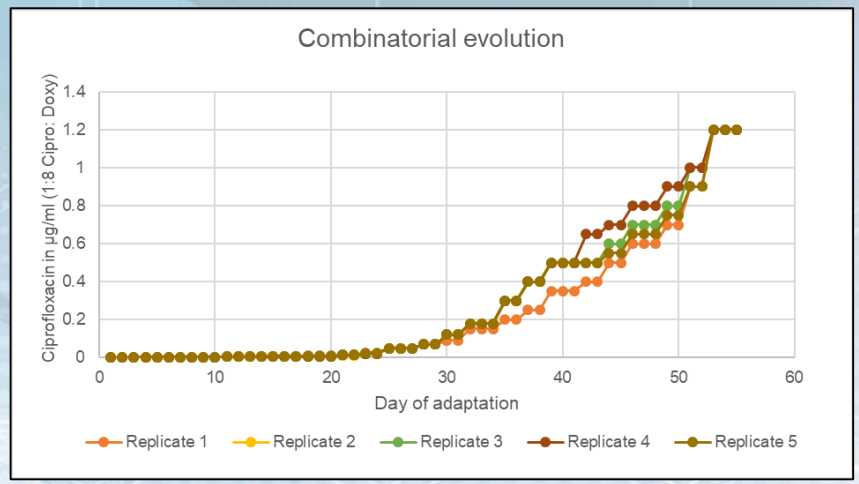
Deletion of TolC can cause increased susceptibility to a variety of antibiotics

We identified in AB/EF/SA/EC To TIG (Beabout AAC 2016)

Francisella sequential and combinatorial evolution suggest monotherapy should remain effective



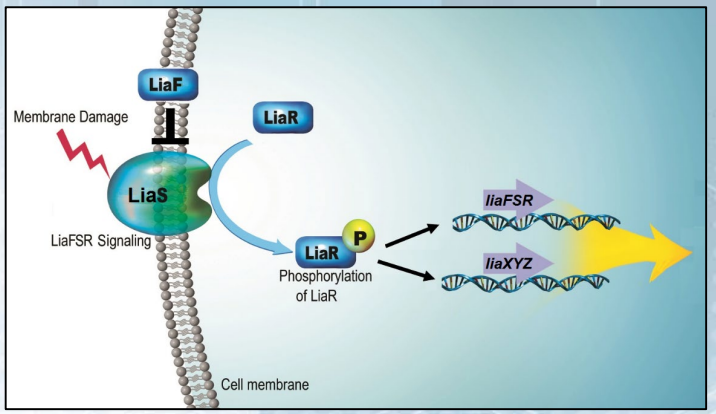




- Resistance onset delayed when DOXY and CIPRO are used together (30 vs 50 days) but not more than if each drug were used in series.

Conclusions

- **We can predict the emergence of resistance reliably.**
- **Strategies towards resistance are highly varied for each pathogen and drug**
- **We can use predictions to target <u>HOW</u> an organism is going to adapt to identify new small molecules that act as co-drug to limit resistance and in some cases induce hypersusceptibility to current drugs.**



Example: Knockout this pathway in enterococci and you induce hypersusceptibility.

Rincon S. et al. (2019) J. Infect. Dis.
Tran, T.T. et al., (2016) Curr. Opin. Micro.

Thank you.

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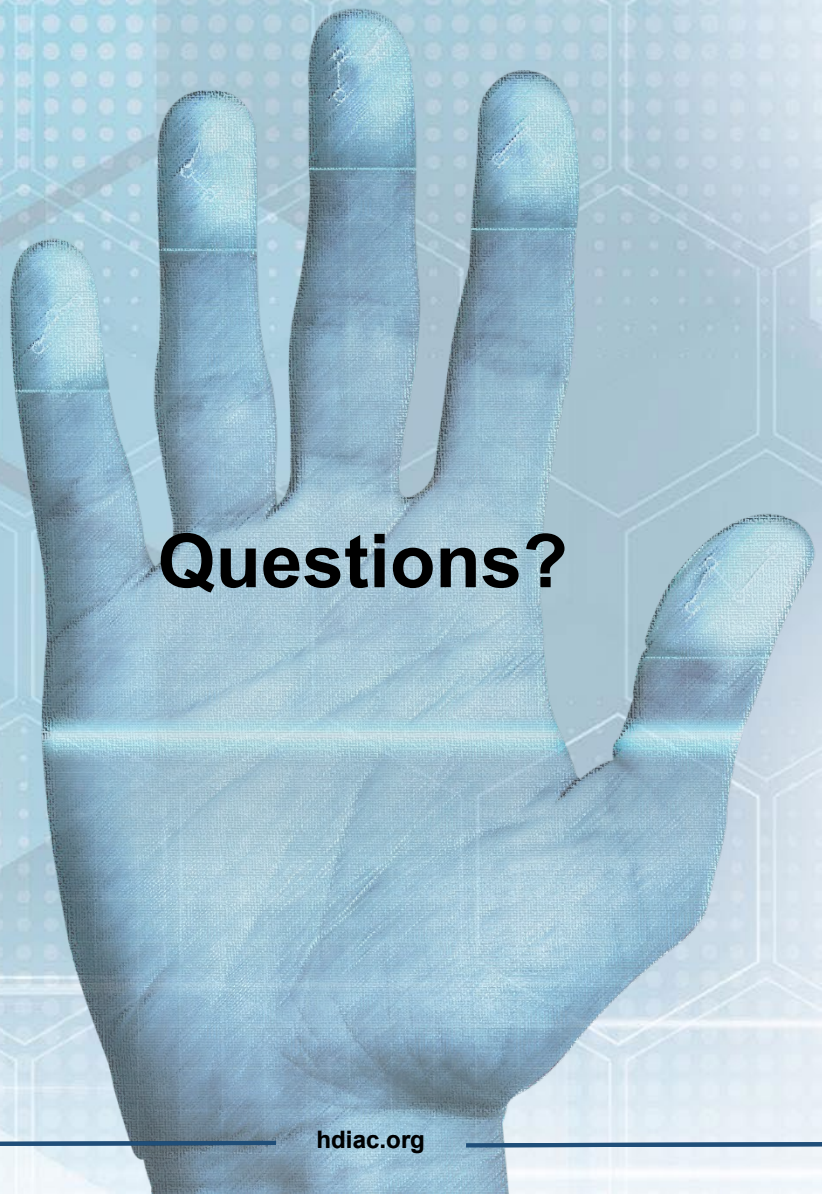

Questions?