

Predicting and Combating Antimicrobial Resistance Across Diverse Microbiomes



Gautam Dantas, PhD

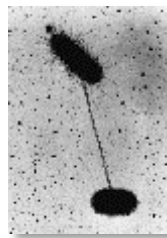
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Department of Pathology & Immunology

Department of Biomedical Engineering

Department of Molecular Microbiology

Edison Family Center for Genome Sciences &
Systems Biology



DANTAS LAB

Washington University in St. Louis

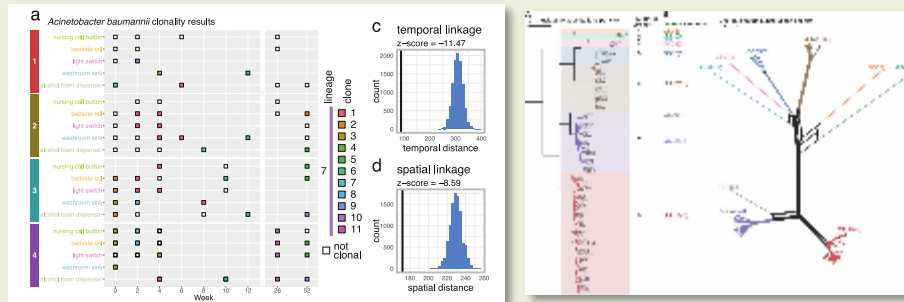
www.dantaslab.org



Dantas Lab - Major Research Themes

TRANSLATIONAL MICROBIOLOGY

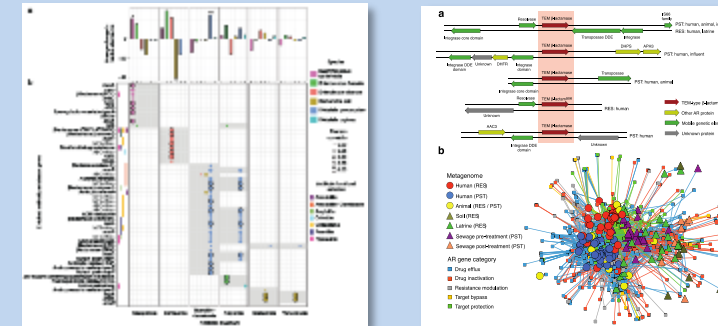
Goal: Understand and diagnose infections



Crofts *et al.*, **Nature Chemical Biology**, 2018
D'Souza, Potter *et al.*, **Nature Communications**, 2019
Park, Gasparini *et al.*, **Nature Chemical Biology**, 2017
Gonzales *et al.*, **Nature Chemical Biology**, 2015
Potter *et al.*, **Clinical Chemistry**, 2019

MICROBIAL ECOLOGY

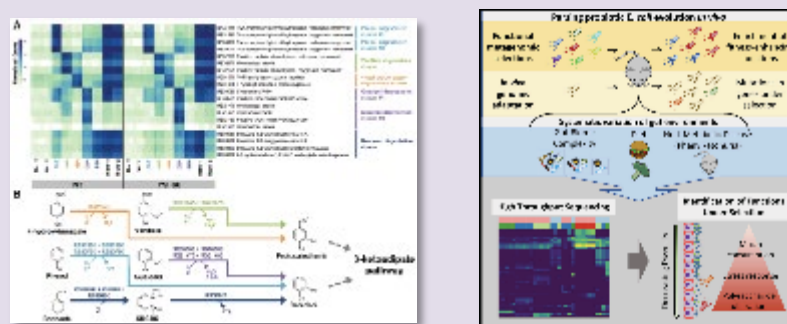
Goal: Understand and predict microbiome function



Forsberg, Patel, *et al.*, **Nature**, 2014
Gibson *et al.*, **Nature Microbiology**, 2016
Baumann-Dudenhoeffer *et al.*, **Nature Medicine**, 2018
Gasparini *et al.*, **Nature Microbiology**, 2019
Perhsson, Tsukayama, *et al.*, **Nature**, 2016

MICROBIAL ENGINEERING

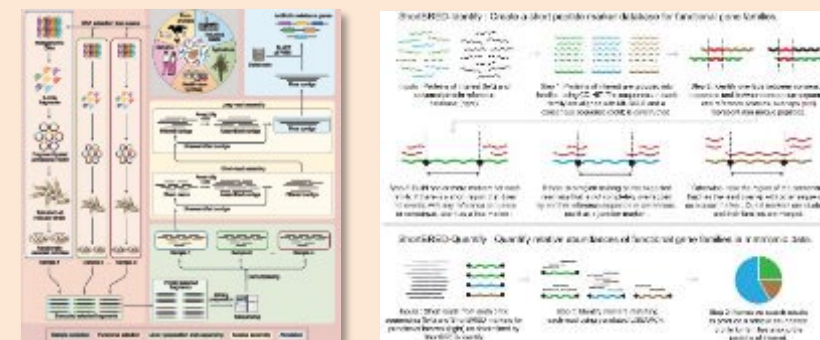
Goal: Design novel therapies and value chemicals



Yoneda, Henson, *et al.*, **Nucleic Acids Research**, 2016
Henson, Campbell, DeLorenzo *et al.*, **Metabolic Engineering**, 2018
Gibson, Pesesky *et al.*, **Journal of Molecular Biology**, 2014
Crook, Ferreiro *et al.*, **Cell Host & Microbe**, 2019.

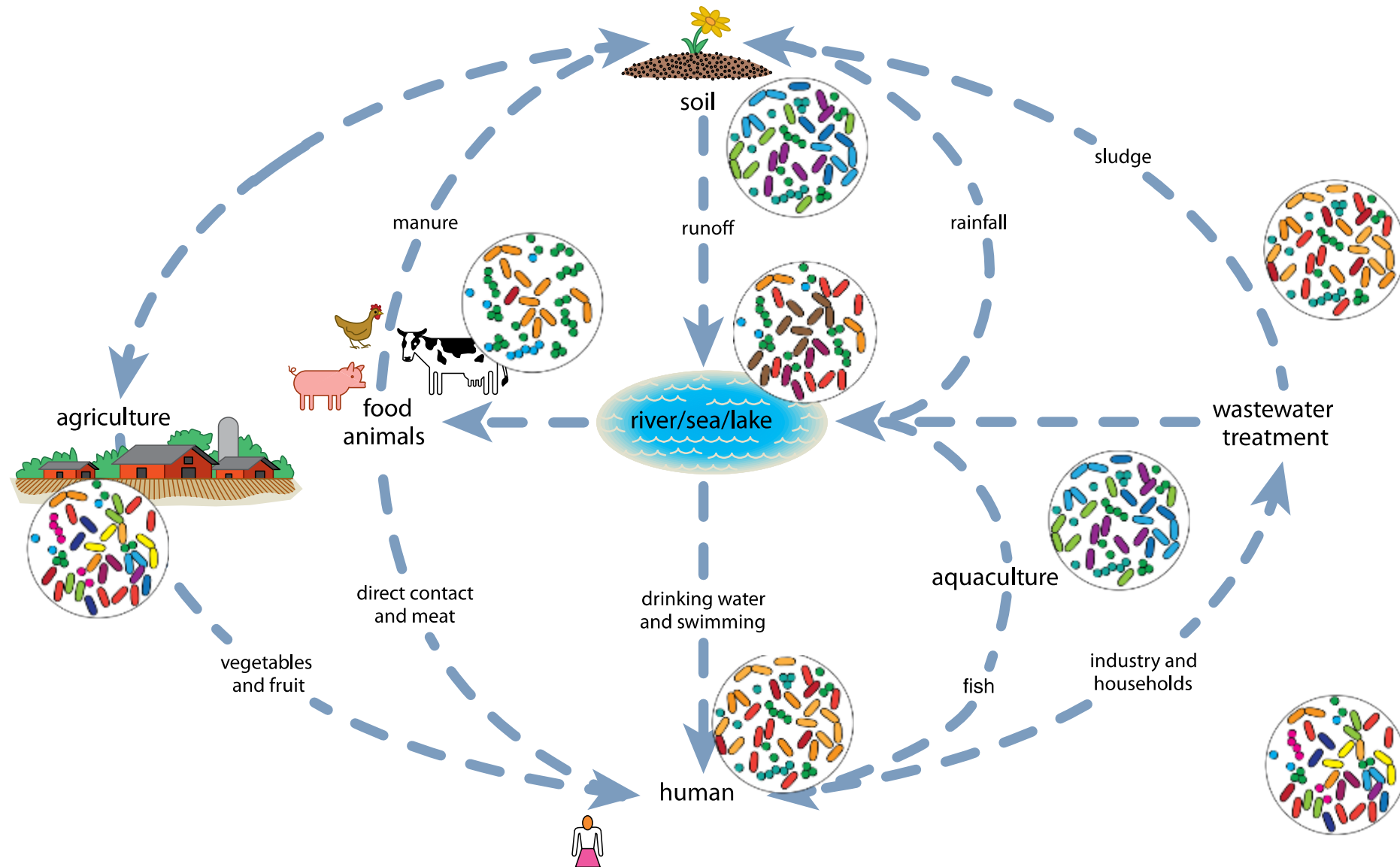
TECHNOLOGY DEVELOPMENT

Goal: Develop new tools to enable better science



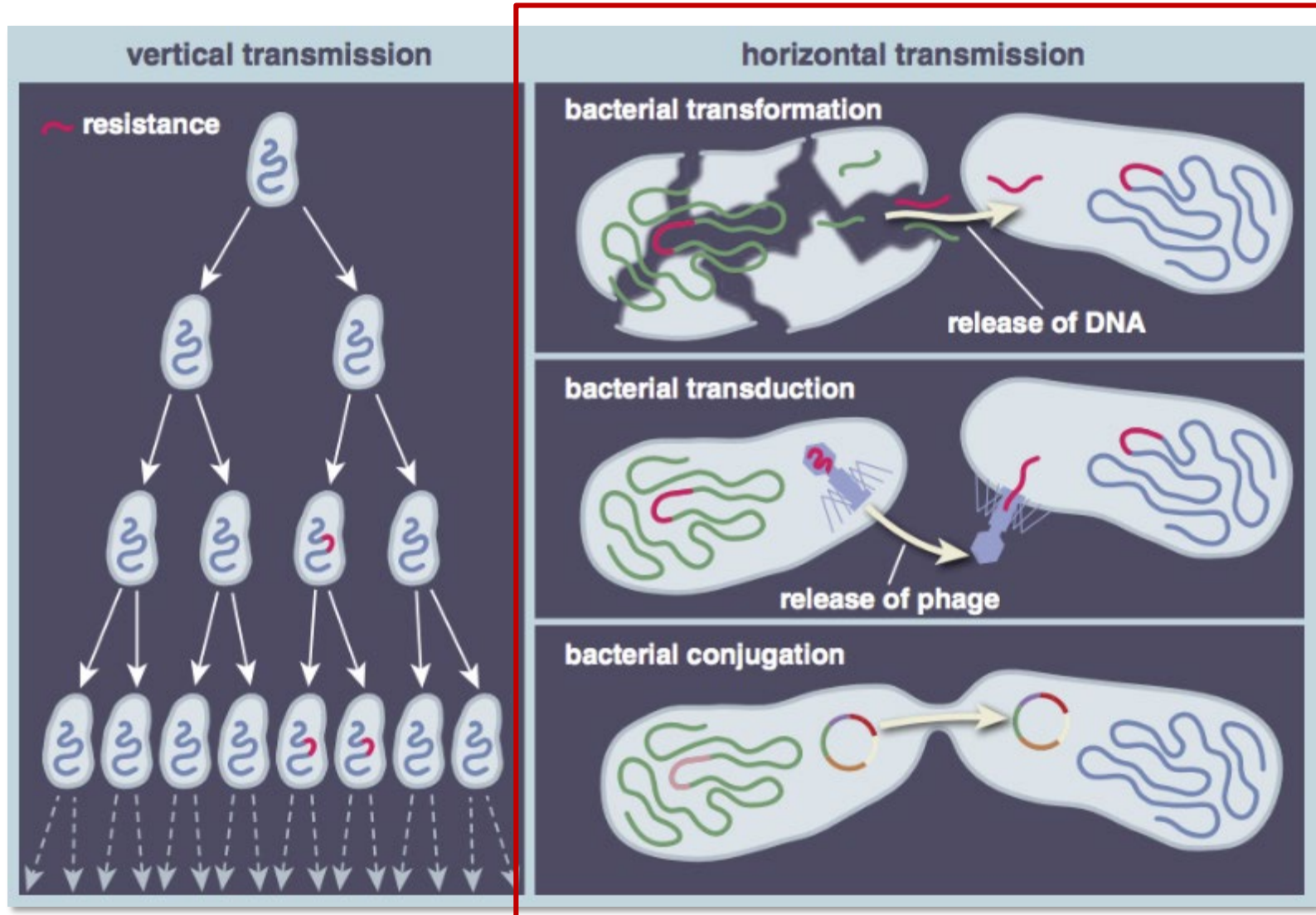
Forsberg, Reyes, *et al.*, **Science**, 2012
Gibson *et al.*, **ISME Journal**, 2015
Kaminski, Gibson *et al.*, **PLoS Computational Biology**, 2015
Boolchandani, D'Souza *et al.*, **Nature Reviews Genetics**, 2019

Transmission networks of microbiomes and resistomes across habitats



adapted from:
Dantas and Sommer, American Scientist (2014)

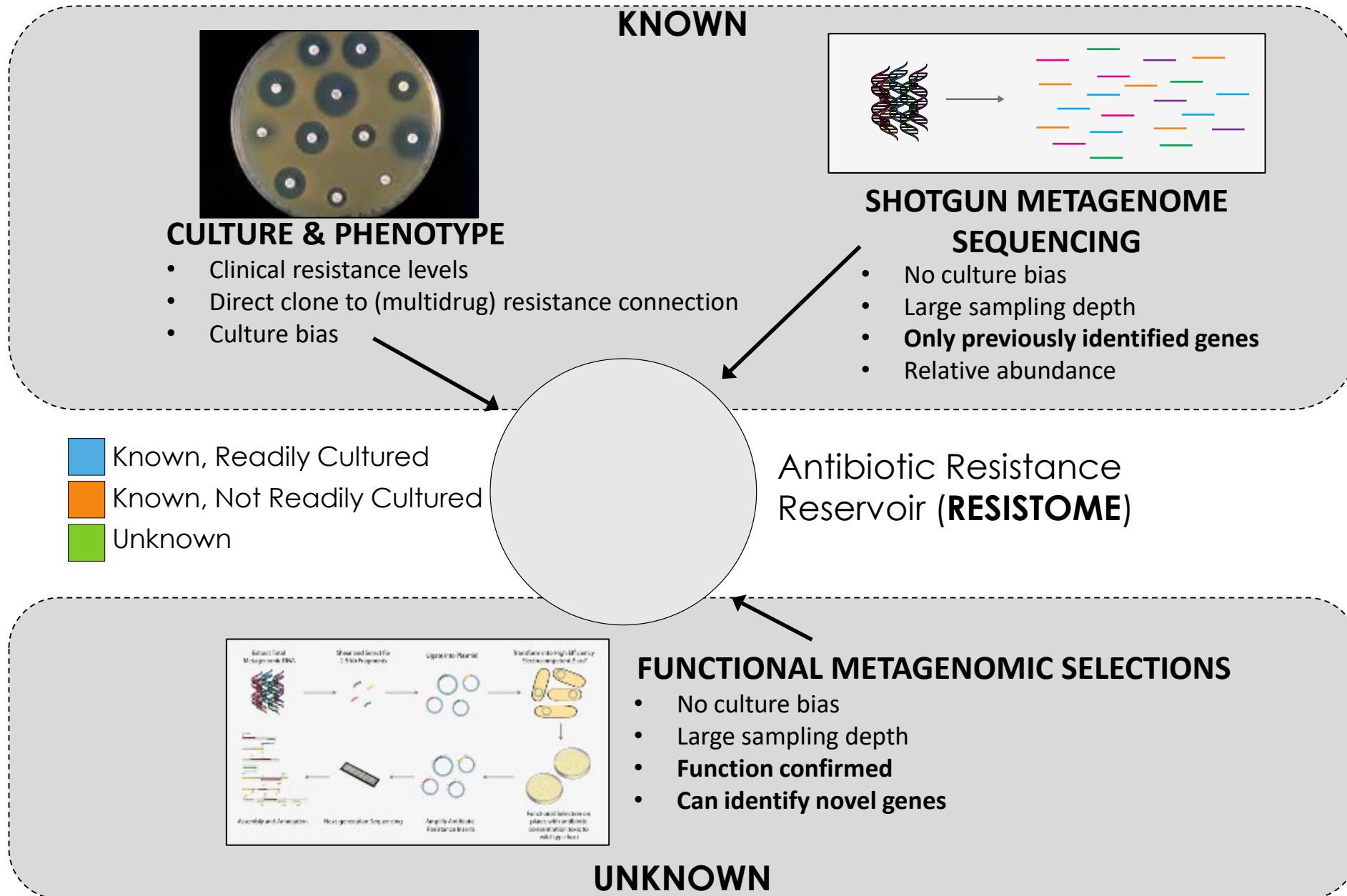
Horizontal gene transfer enables rapid TRANSFER of resistance



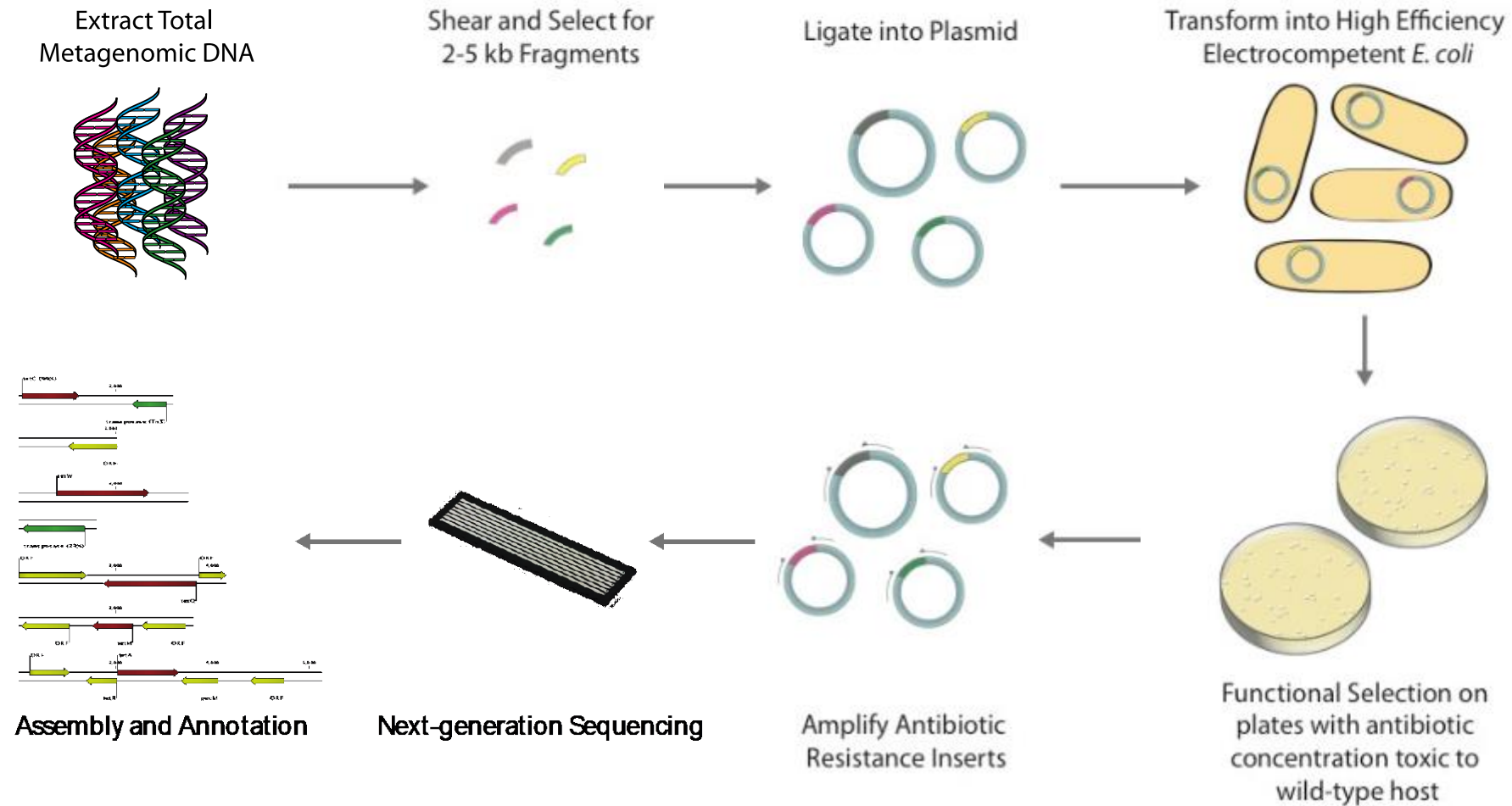
Dantas & Sommer,
American Scientist (2014)

Enriched for antibiotic resistance genes
Smillie et al., *Nature* (2011)

Methods for studying antibiotic resistance in microbial communities

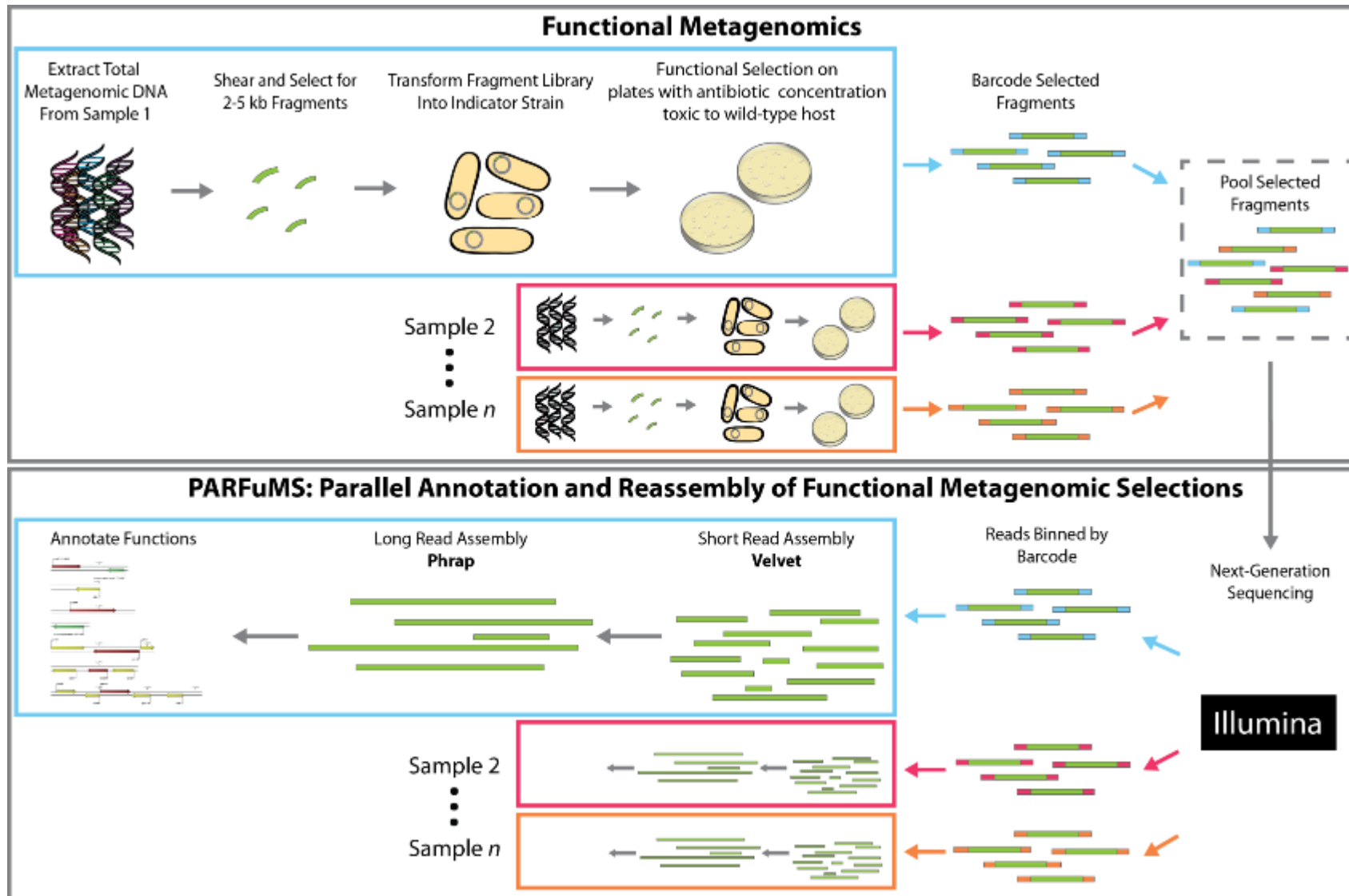


Functional metagenomic selections identify novel antibiotic resistance genes in microbial communities



Applied in Rondon et al. **ISME** (2000); Sommer et al. **Science** (2009); Forsberg et al. **Science** (2012); Forsberg et al. **Nature** (2014); Clemente et al. **Science Advances** (2015); Moore et al. **Microbiome** (2015); Gibson et al. **Nature Microbiology** (2016); Pehrsson et al., **Nature** (2016); Park et al. **Nature Chemical Biology** (2017); Gasparini et al. **Nature Microbiology** (2019)
Reviewed in Handelsman et al. **Chem Bio** (1998); Allen et al. **Nat Rev Micro** (2010); Dantas et al. **Annu Rev Micro** (2013); Crofts et al. **Nature Reviews Micro** (2017); Ferreiro et al. **Cell** (2018); Boolchandani et al., **Nature Reviews Genetics** (2019)

Increasing functional metagenomic throughput via next-gen sequencing



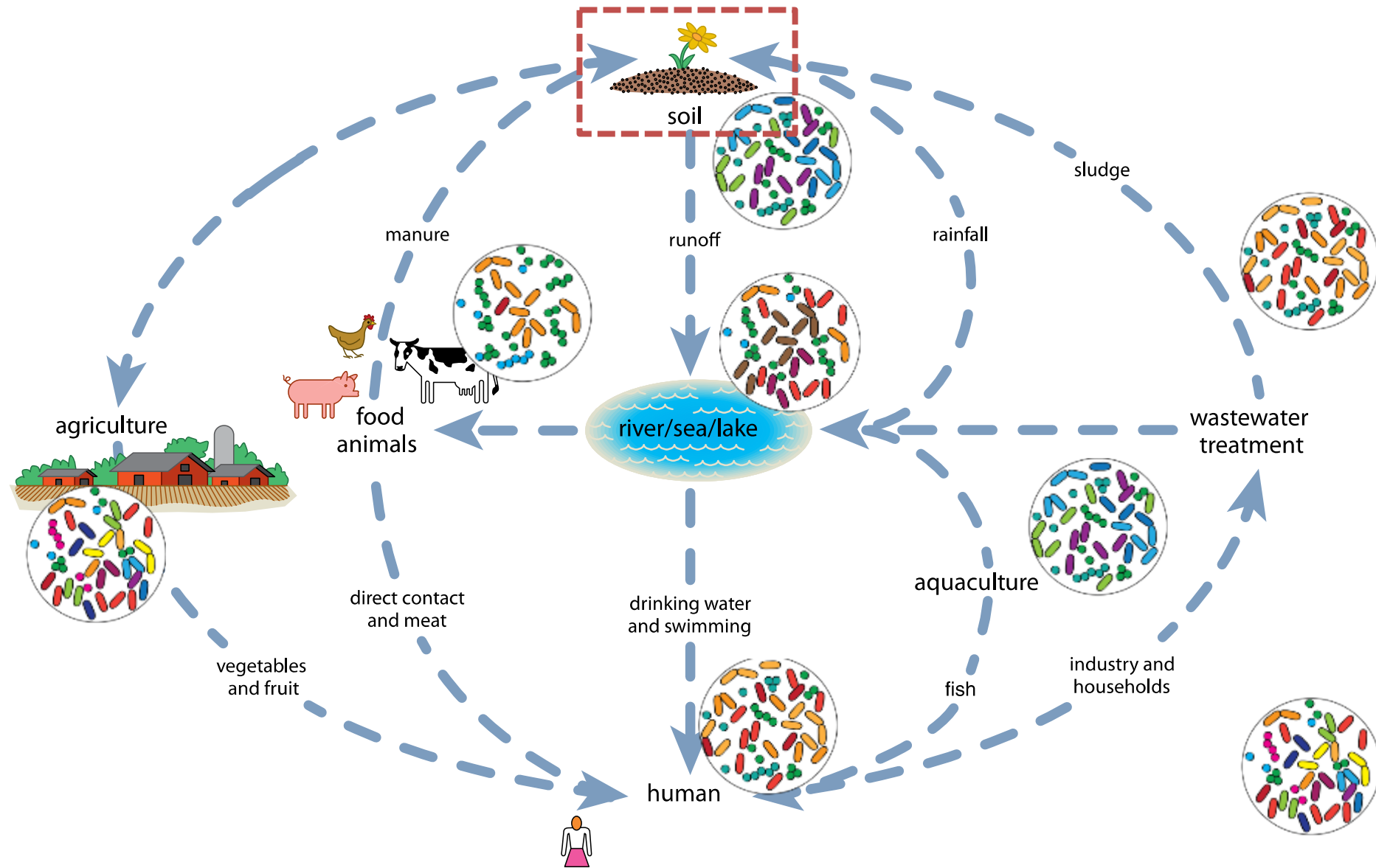
Applied in Sommer et al. **Science** (2009); Forsberg et al. **Science** (2012); Forsberg et al. **Nature** (2014); Clemente et al. **Science Advances** (2015); Moore et al., **Microbiome** (2015); Gibson et al. **Nature Microbiology** (2016); Pehrsson et al., **Nature** (2016); Park et al. **Nature Chemical Biology** (2017); Gasparini et al. **Nature Microbiology** (2019)

Reviewed in Dantas et al. **Annu Rev Micro** (2013); Dantas et al. **American Scientist** (2014); Crofts et al. **Nature Reviews Micro** (2017); Ferreiro et al. **Cell** (2018); Boolchandani et al., **Nature Reviews Genetics** (2019)

Resistance spreads across habitats



Transmission networks of microbiomes and resistomes across habitats



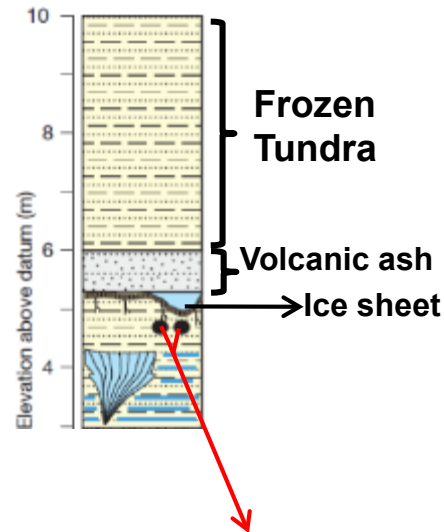
adapted from:
Dantas and Sommer, American Scientist (2014)

The soil resistome is ancient, diverse, and growing:

Is it in RECENT exchange with pathogens?

Antibiotic resistance is ancient

D'Costa...Wright, *Nature* (2011)

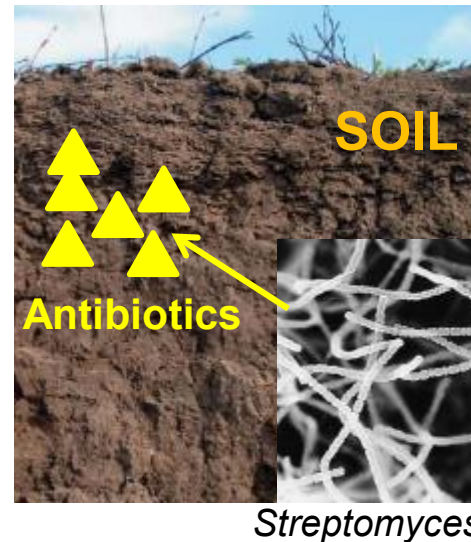


30,000 year-old preserved DNA Resistance to 3 antibiotic classes:

- β -lactams
 - e.g. Penicillin, Amoxicillin
- Tetracyclines
 - e.g. Tetracycline, Minocycline
- Glycopeptides
 - e.g. Vancomycin

Producer Hypothesis

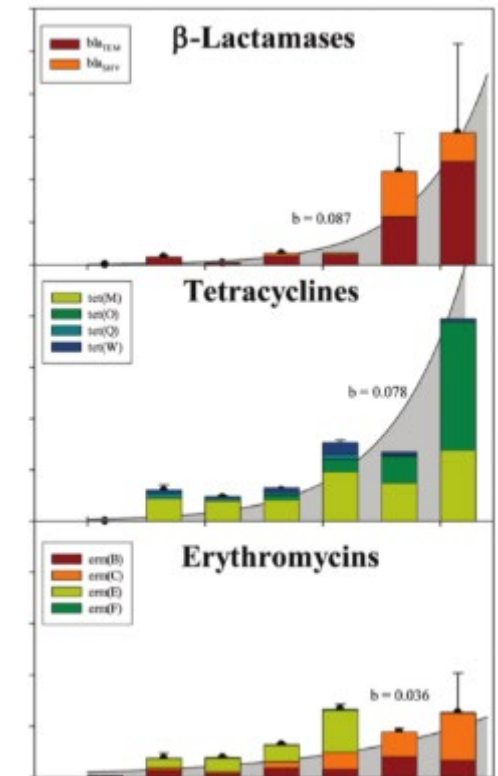
Benveniste, Davies, *PNAS* (1973)



- **Most clinical antibiotics are produced by soil bacteria**
- **Producer resistome billions of years old**

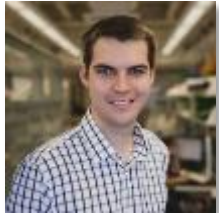
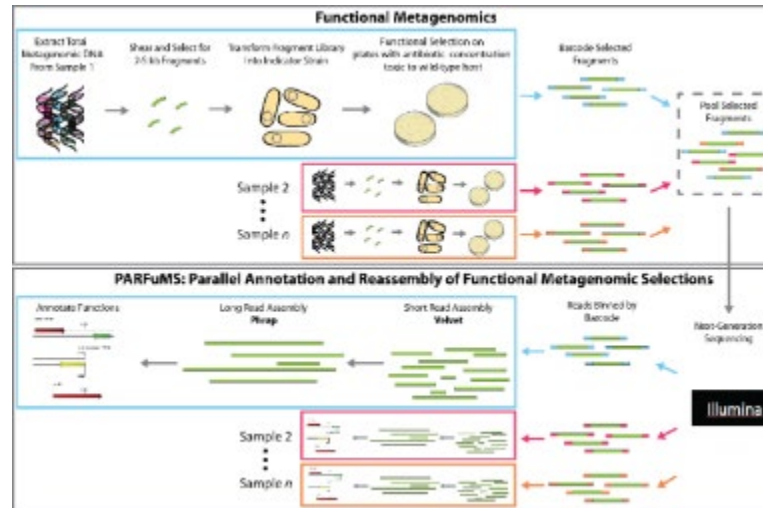
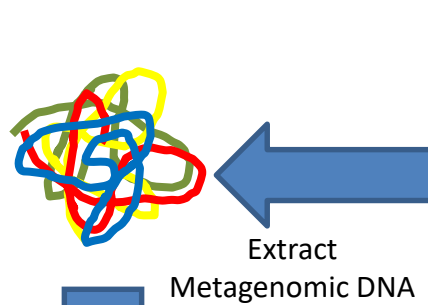
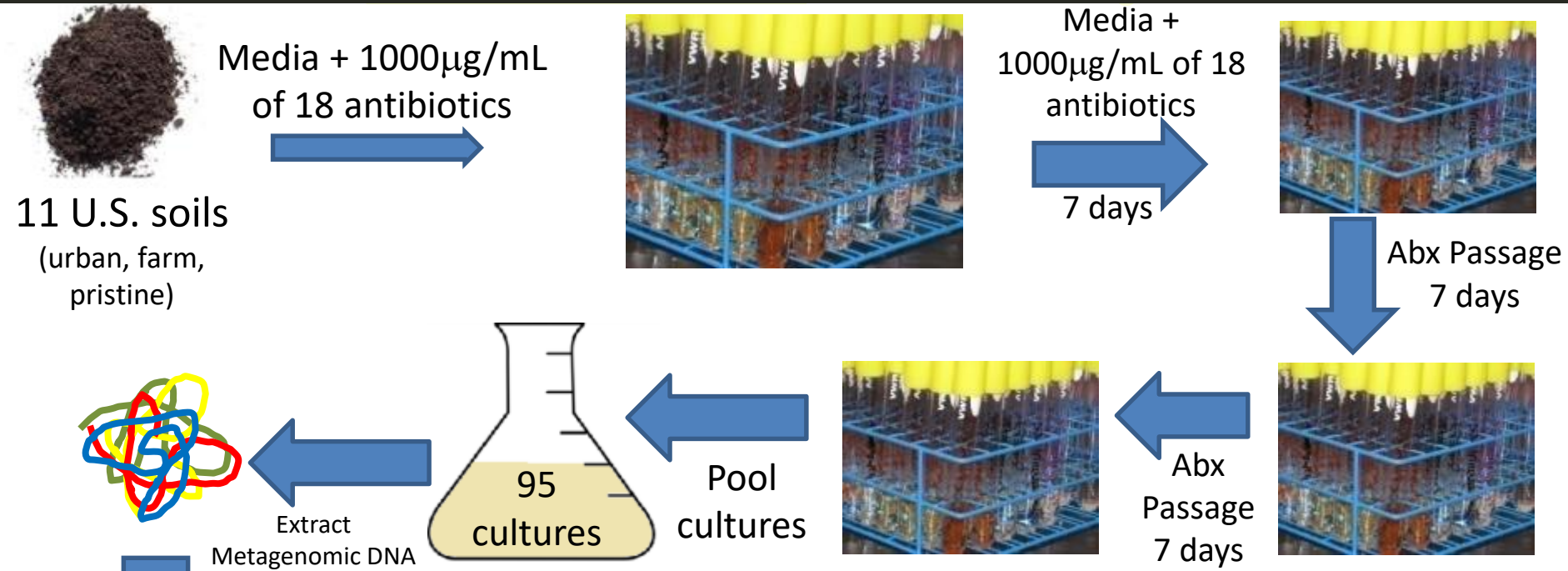
Increased Gene Abundance

Knapp...Graham, *PNAS* (2010)



- **Antibiotic resistance in soil enriched over last 80+ years**

Resistome of highly multi-drug resistant soil bacteria



Kevin Forsberg



Alejandro Reyes

Soil resistance genes with 100% nucleotide ID to globally-distributed pathogens

Gene Name	# of Selections	Antibiotic Class	Resistance Mechanism	Identical Pathogenic Hits
AB95_PI_68.1	4	β -lactam	enzymatic cleavage	<i>A. baumannii</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>S. typhimurium</i> , <i>P. mirabilis</i>
AB95_CH_13.1	1	Amphenicol	efflux	<i>A. baumannii</i> , <i>P. aeruginosa</i>
AB95_TE_2.2	3	Tetracycline	efflux	<i>A. baumannii</i> , <i>S. typhimurium</i>
AB95_TE_1.1	3	Tetracycline	efflux	<i>A. baumannii</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>S. typhimurium</i>
AB95_GE_3.3	2	Aminoglycoside	covalent modification	<i>E. cloacae</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>S. typhimurium</i>
AB95_GE_3.1	2	Sulfonamide	target modification	<i>C. diphtheriae</i> , <i>E. cloacae</i> , <i>K. pneumoniae</i> , , <i>P. aeruginosa</i> , <i>S. typhimurium</i> , <i>Yersinia pestis</i>
AB95_CH_21.1	1	Aminoglycoside	covalent modification	<i>A. baumannii</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>S. typhi</i>

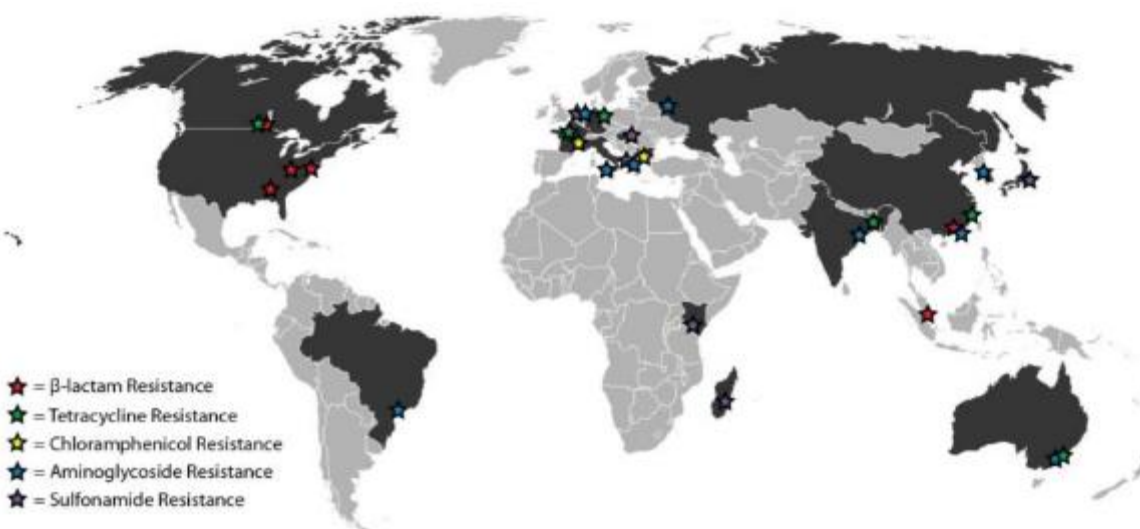
- 5 antibiotic classes

- **ALL major resistance mechanisms**

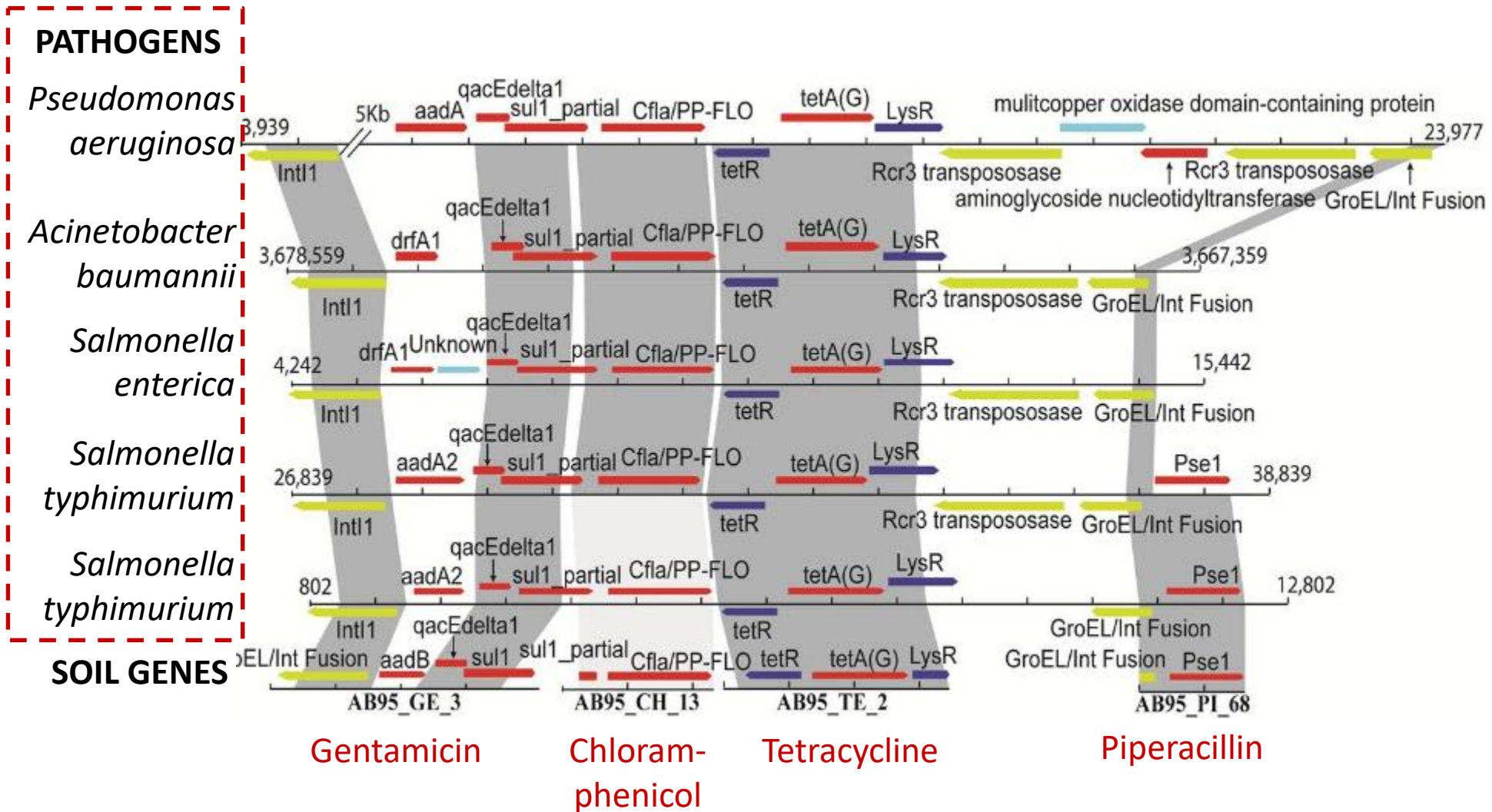
- Pathogens are **Gram +/-** clinical isolates from **across the world**

- Pathogen genes on plasmids and chromosomes

Forsberg*, Reyes*, Wang, Selleck, Sommer, Dantas. **Science**, (2012)



Soil bacteria and human pathogens share MULTIDRUG resistance clusters



OBSERVATION:

Multidrug-Resistant Proteobacteria from the Soil
are Exchanging Antibiotic Resistance Genes
with Pathogens in the Clinic

QUESTION:

Is this the RULE or the EXCEPTION?



Kevin Forsberg



Sanket Patel

Soil resistomes are structured by bacterial phylogeny and have lower HGT potential than human pathogens

18 Soils From 2 Long-Term Experimental Plots

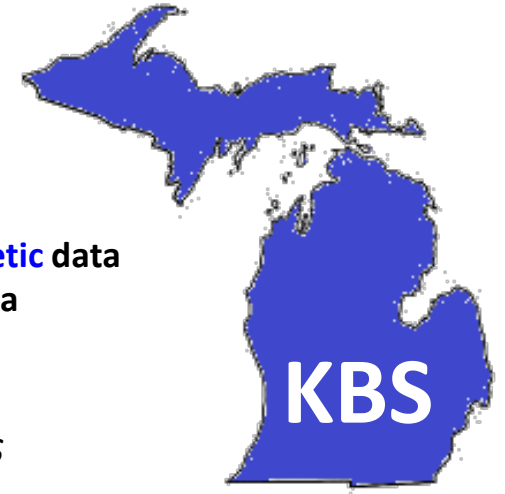


Successional Grassland (CC) (9 soils)

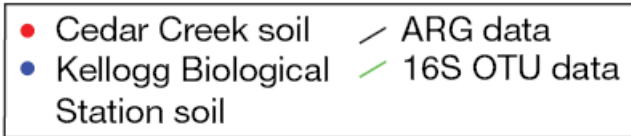
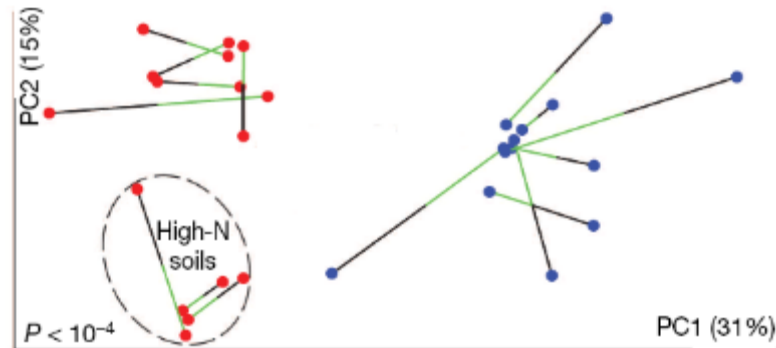
- 8 Soils with 16S phylogenetic data
- 9 Soils with resistome data

Agricultural Field (KBS) (9 soils)

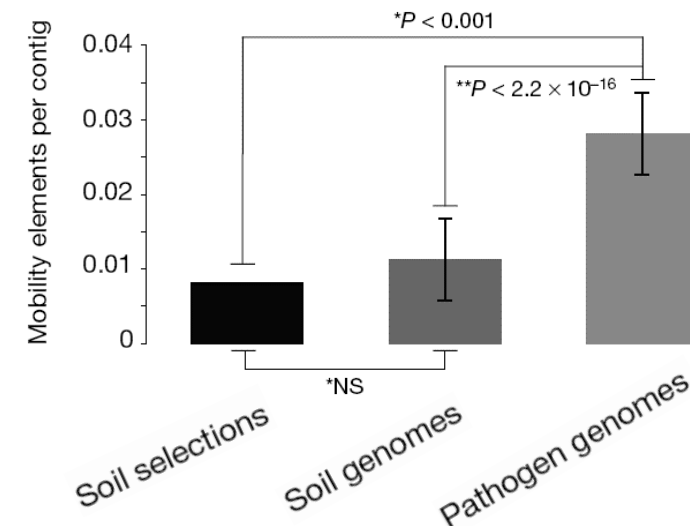
- 9 soils with 16S phylogenetic data
- 7 soils with resistome data



219 of 324 selections yielded > 3000 antibiotic resistance genes



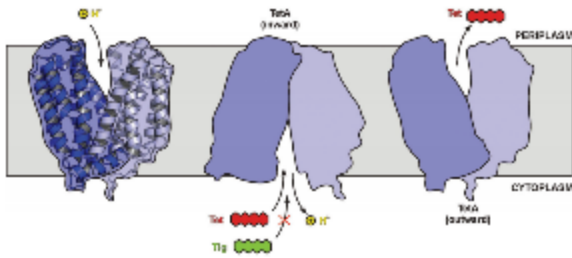
Soil resistomes are linked to 16S phylotypes



Soil resistance genes are co-localized with fewer mobilization genes than pathogens

BUT cryptic environmental resistance genes are still clinically-relevant *e.g. tetracycline resistance*

1. Efflux

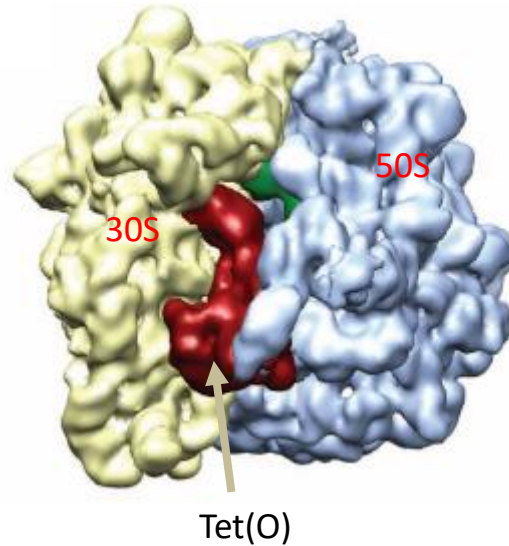


Known tetracycline efflux pumps:

<i>tet(A)</i>	<i>tetA(P)</i>	<i>tet(40)</i>
<i>tet(B)</i>	<i>tet(V)</i>	<i>tet(41)</i>
<i>tet(C)</i>	<i>tet(Y)</i>	<i>tet(42)</i>
<i>tet(D)</i>	<i>tet(Z)</i>	<i>tet(45)</i>
<i>tet(E)</i>	<i>tet(30)</i>	<i>tetAB(46)</i>
<i>tet(G)</i>	<i>tet(31)</i>	<i>tcr(3)</i>
<i>tet(H)</i>	<i>tet(33)</i>	<i>otr(C)</i>
<i>tet(J)</i>	<i>tet(35)</i>	<i>otr(B)</i>
<i>tet(K)</i>	<i>tet(38)</i>	
<i>tet(L)</i>	<i>tet(39)</i>	

Nguyen et al., Biol. Chem., 2014

2. Ribosomal Protection

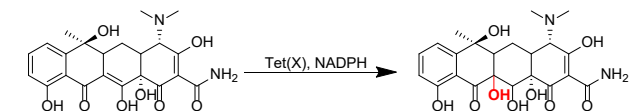


Known ribosomal protectors:

<i>tet(M)</i>	<i>tet(T)</i>	<i>tet(36)</i>
<i>tet(O)</i>	<i>tet(W)</i>	<i>tet(44)</i>
<i>tet(Q)</i>	<i>tetB(P)</i>	<i>otr(A)</i>
<i>tet(S)</i>	<i>tet(32)</i>	<i>tet</i>

Li et al., Nature Comm., 2013

3. Enzymatic inactivation



Known tetracycline inactivators:

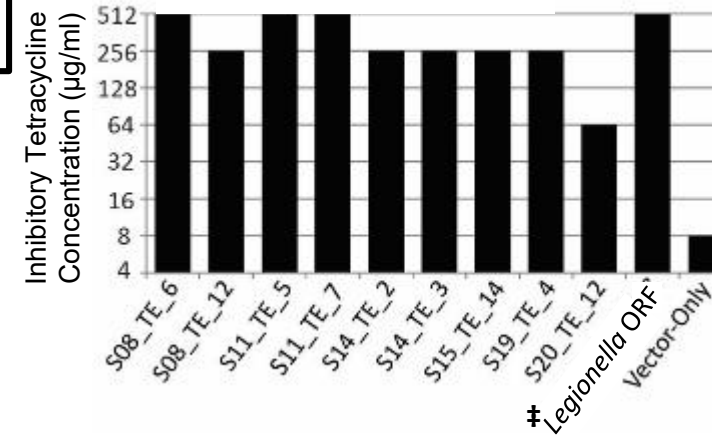
tet(X)
[*tet(37)*]
[*tet(34)*]

Volkers et al., FEBS, 2011

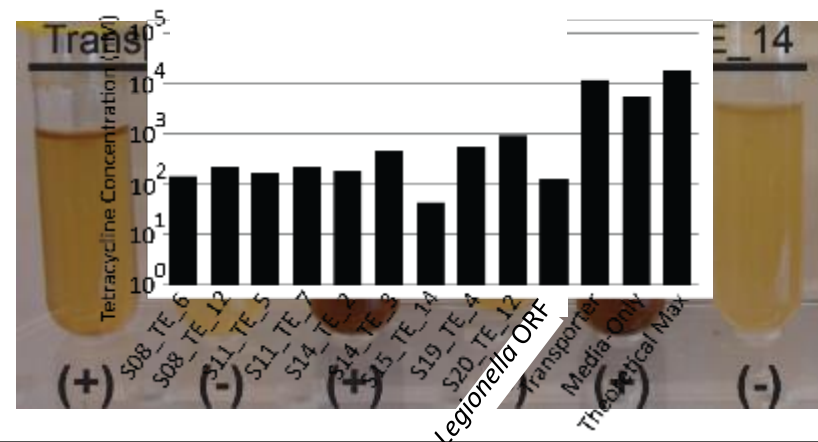
NINE new tetracycline inactivating enzymes (*Tet-Destructases*) from SIX soils

Predicted Function: "*FAD-Dependent Oxidoreductase*"

Sequence unlike any tetracycline resistance gene

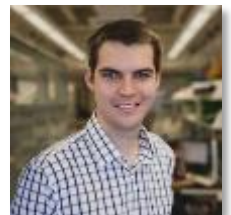
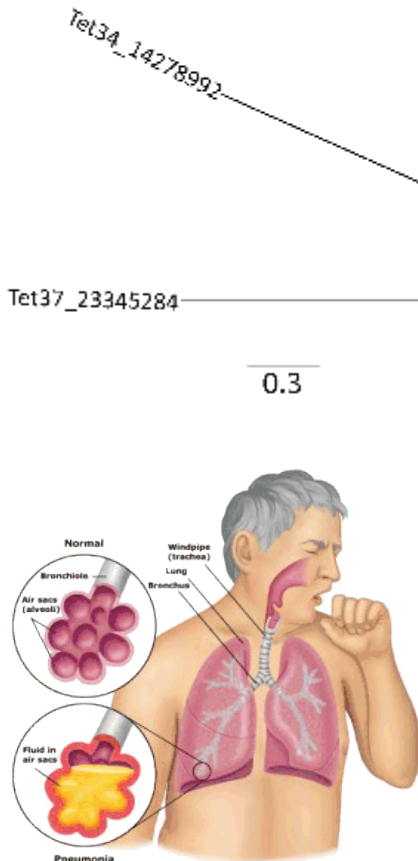


Resistance up to 64-fold higher than vector-only control



Only homolog from pathogenic *Legionella*‡

Resistance conferred by tetracycline inactivation

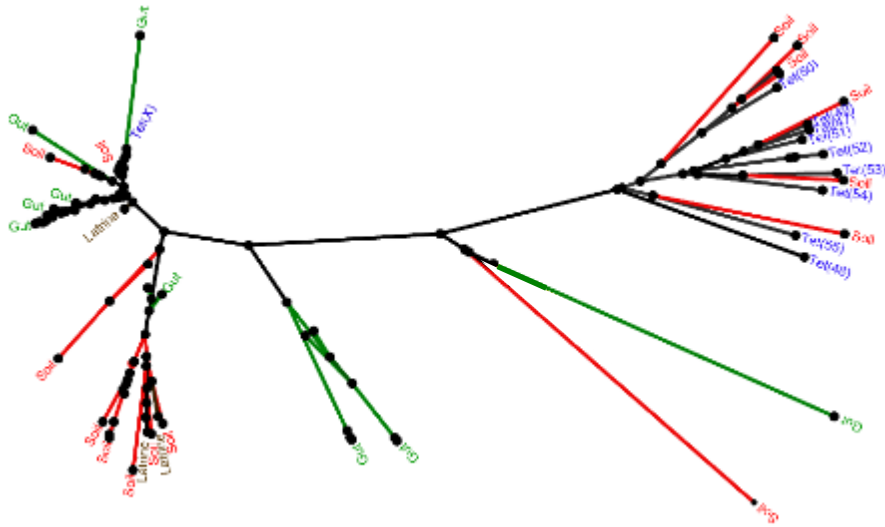


Kevin Forsberg



Tim Wencewicz

Tetracycline destructases are widespread in diverse metagenomes and pathogens



Tet(X) identified in MDR pathogens:

E. faecium

S. aureus

K. pneumoniae ✓ (Leski *et al.* 2013)

A. baumannii ✓ (Deng *et al.* 2014)

P. aeruginosa ✓ (Leski *et al.* 2013)

Enterobacter spp. ✓ (Leski *et al.* 2013)

**69 additional potential
tetracycline destructases were
computationally predicted from
diverse metagenomes:**

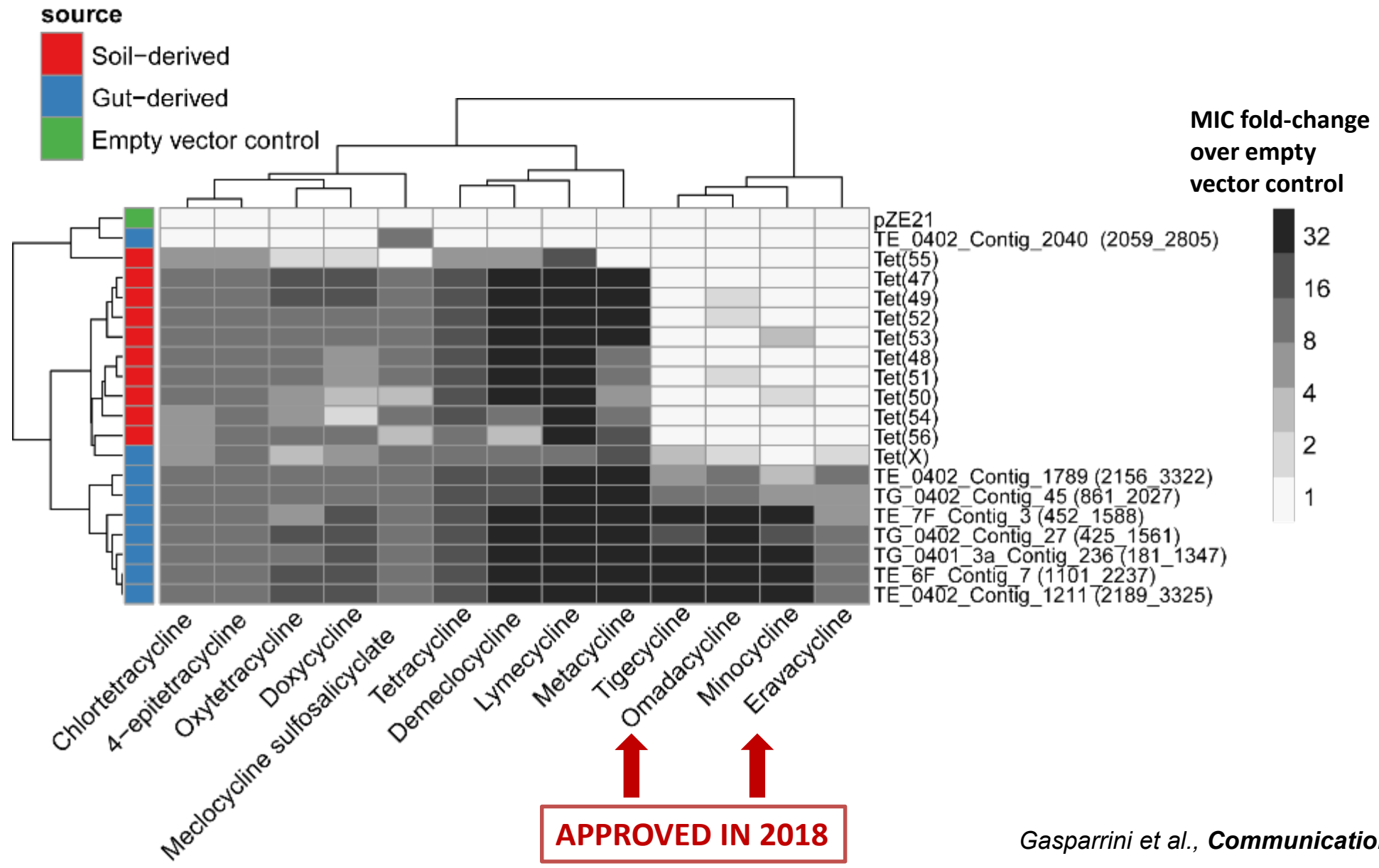
- **Soil**
- **Human Gut**
- **Latrines**
- **Previously described**

**Tetracycline inactivation is an
emerging mechanism of clinical
resistance to a crucial class of drugs!**



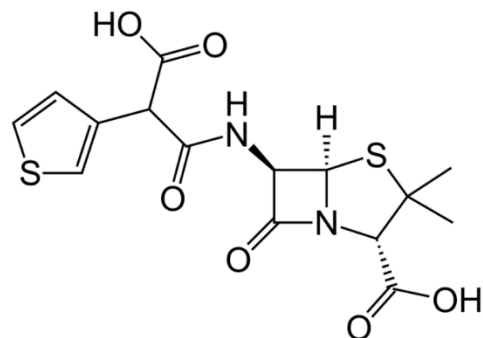
Drew Gasparriini

Tetracycline destructase resistance phenotypes cluster by habitat



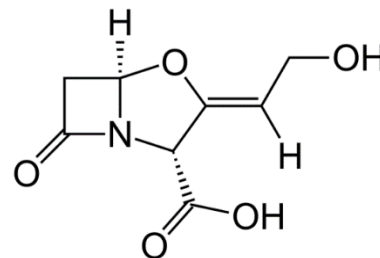
Anhydrotetracycline inhibits tetracycline destructases

Inhibition of antibiotic inactivating enzymes is a powerful tool for combating resistance



Ticarcillin
(β -lactam antibiotic)

+



Clavulanate
(β -lactamase inhibitor)

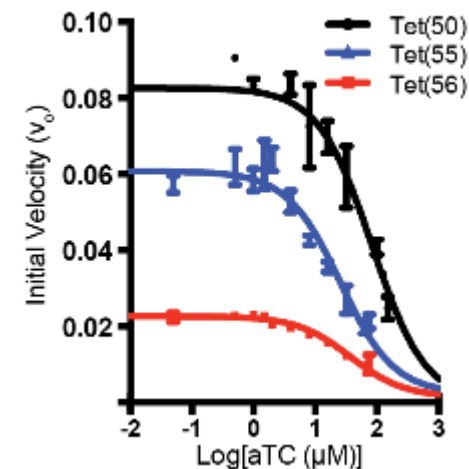
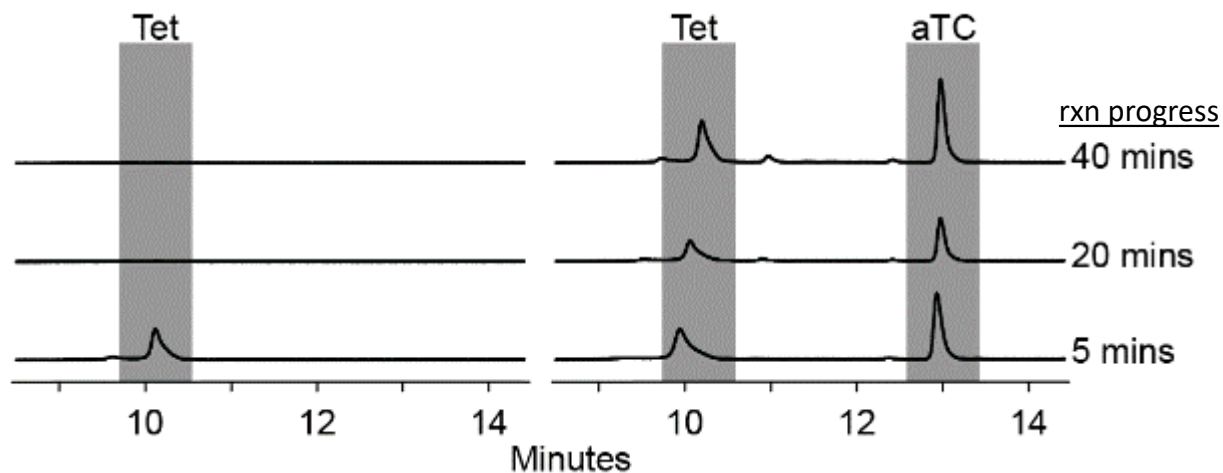


Drew Gasparini



Jooyoung Park

Anhydrotetracycline prevents **enzymatic tetracycline degradation** by Tet(56) and other tetracycline inactivating enzymes *in vitro*



Tim Wenciewicz



Niraj Tolia

Crystal structures illuminate common vs unique mechanisms of tetracycline inactivation by gut vs soil tetracycline destructases

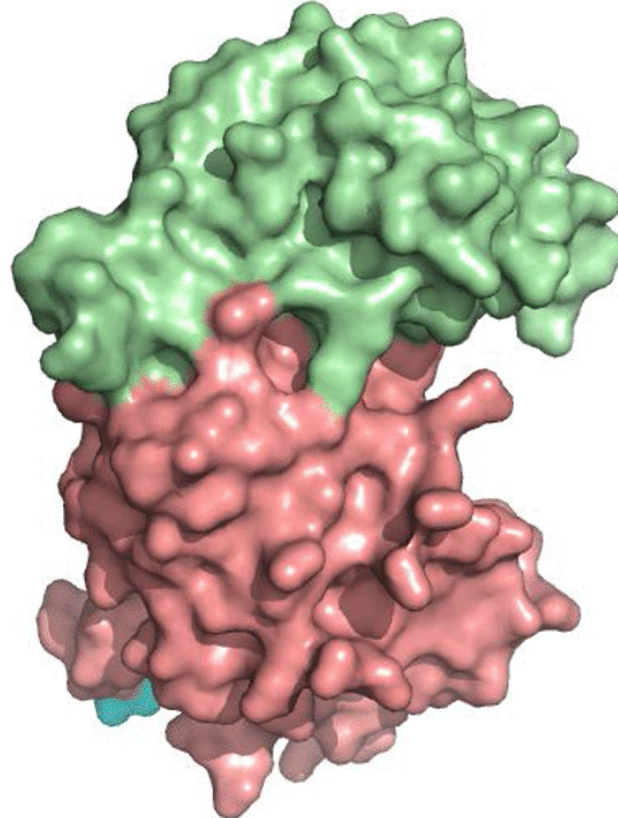
Gut-derived: Tet(X)

Soil-derived: Tet(50)

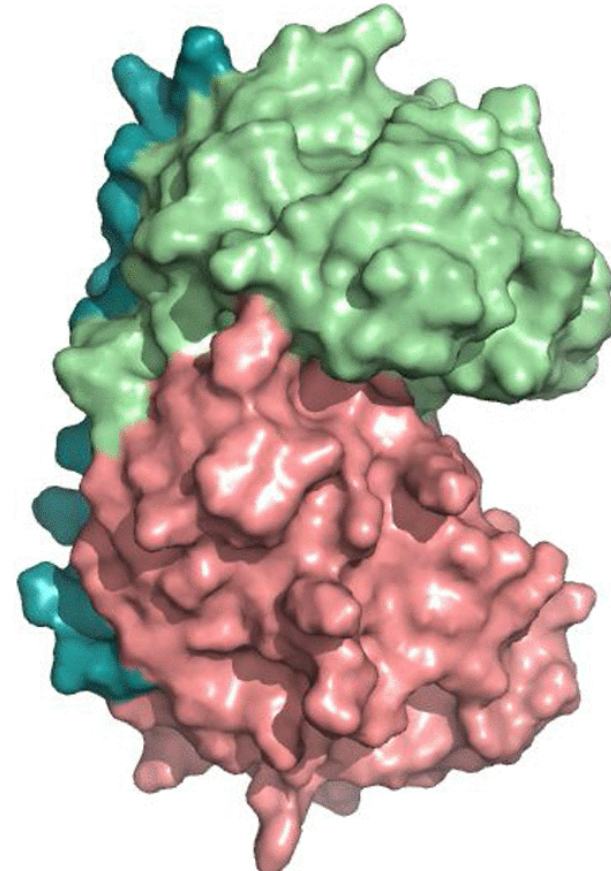
TET-binding
domain

C-terminal
 α -helix(es)

FAD-binding
domain



Volkers et al. (2011) *FEBS Lett.*

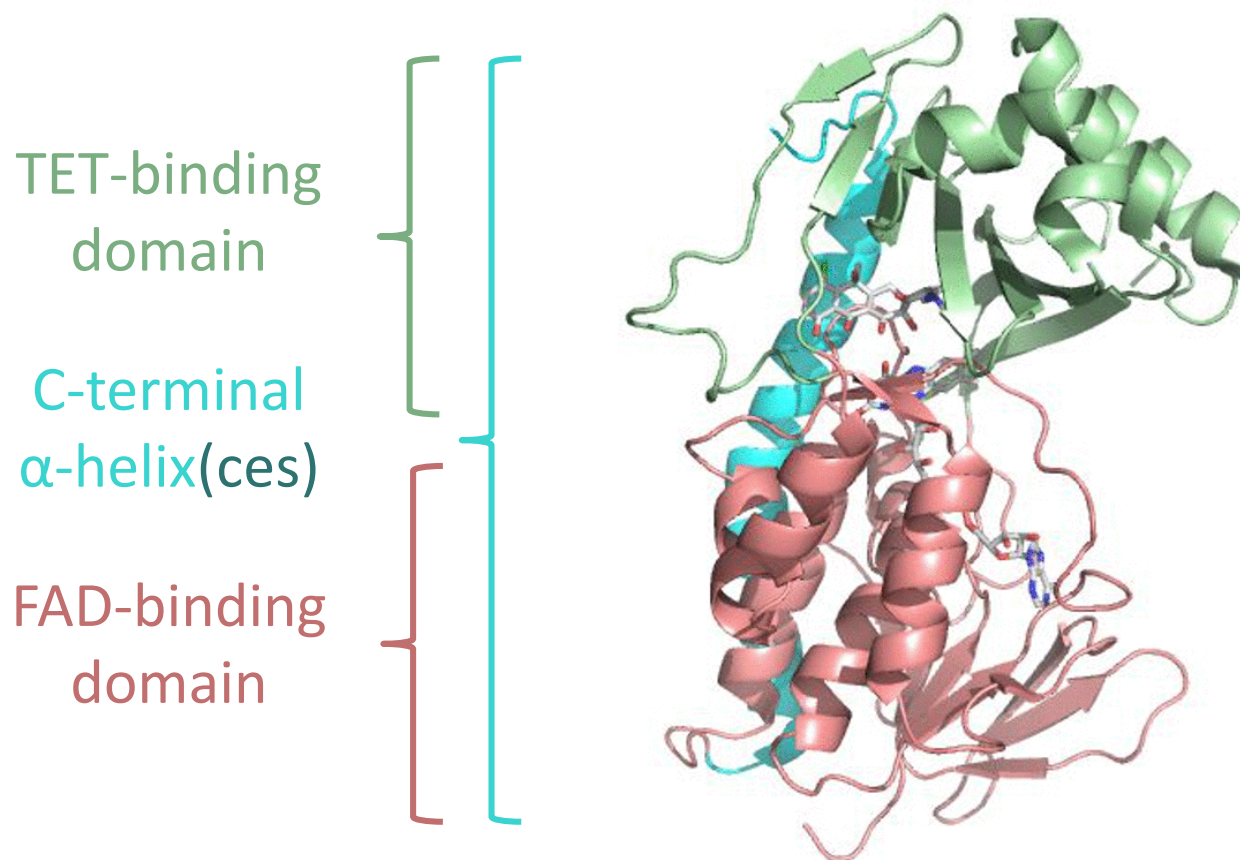


Park*, Gasparrini*, et al. (2017) *Nature Chem. Biol.*

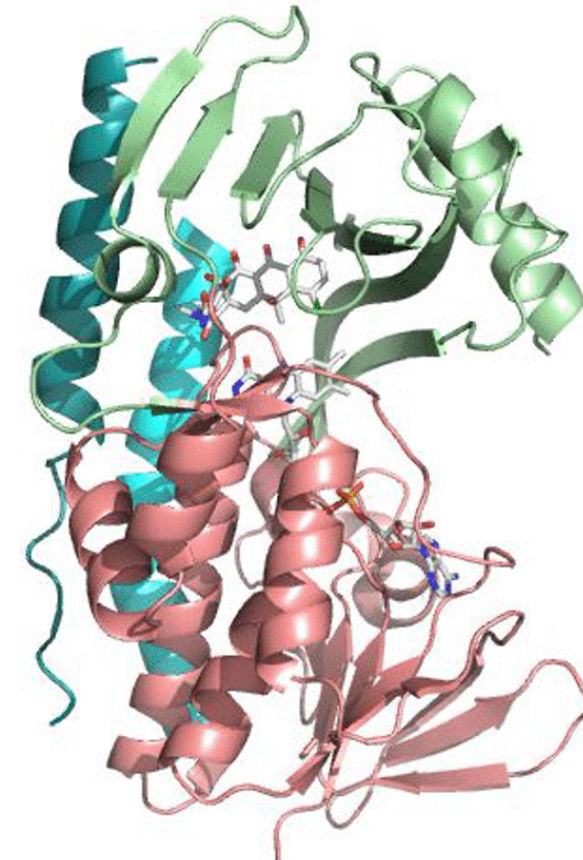
Crystal structures illuminate common vs unique mechanisms of tetracycline inactivation by gut vs soil tetracycline destructases

Gut-derived: Tet(X)

Soil-derived: Tet(50)



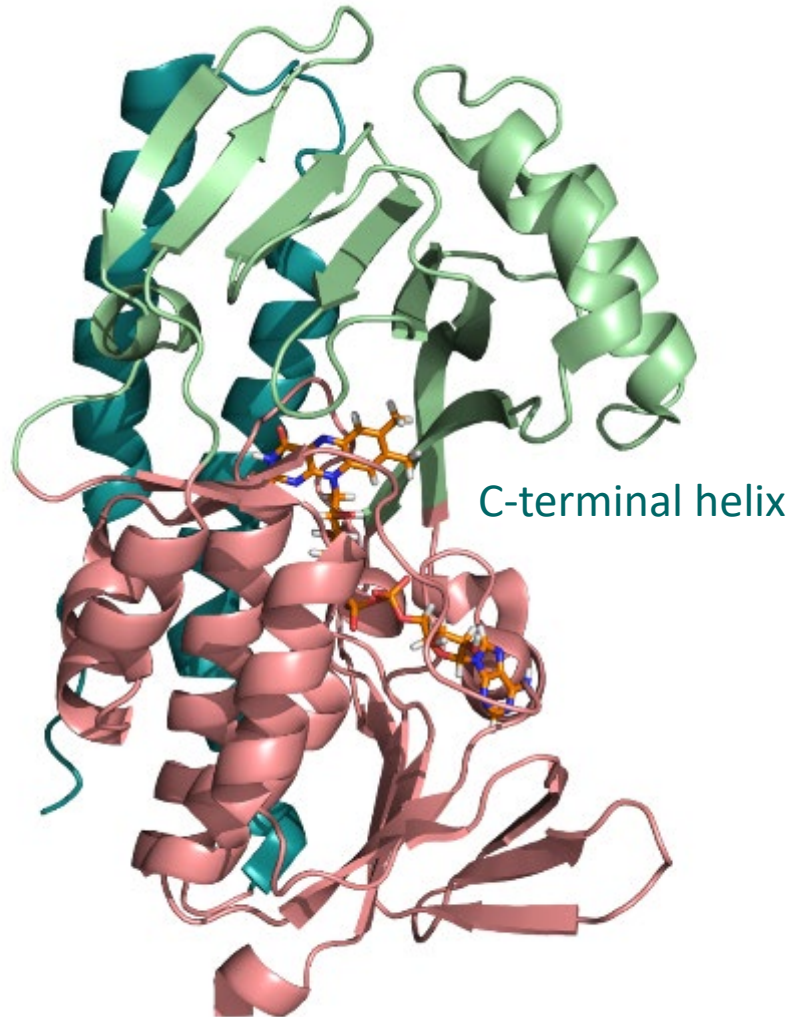
Volkers et al. (2011) *FEBS Lett.*



Park*, Gasparrini*, et al. (2017) *Nature Chem. Biol.*

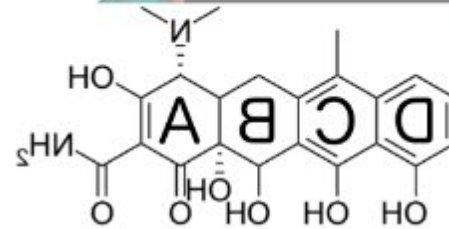
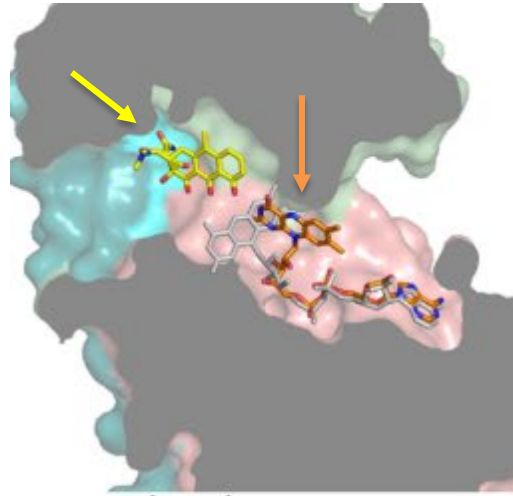
A structural basis for anhydrotetracycline inhibition

Substrate binding domain

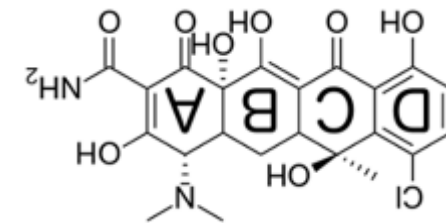
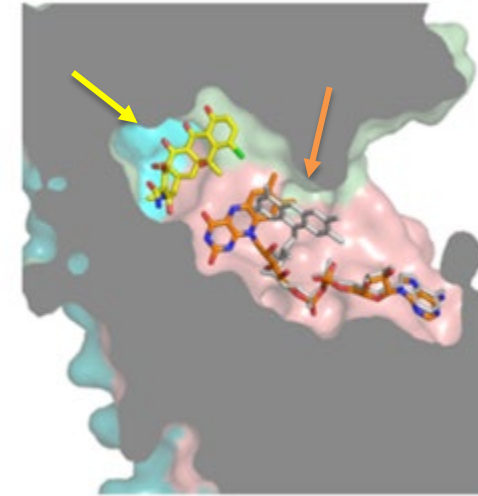


FAD-binding domain

Tet(50) + inhibitor



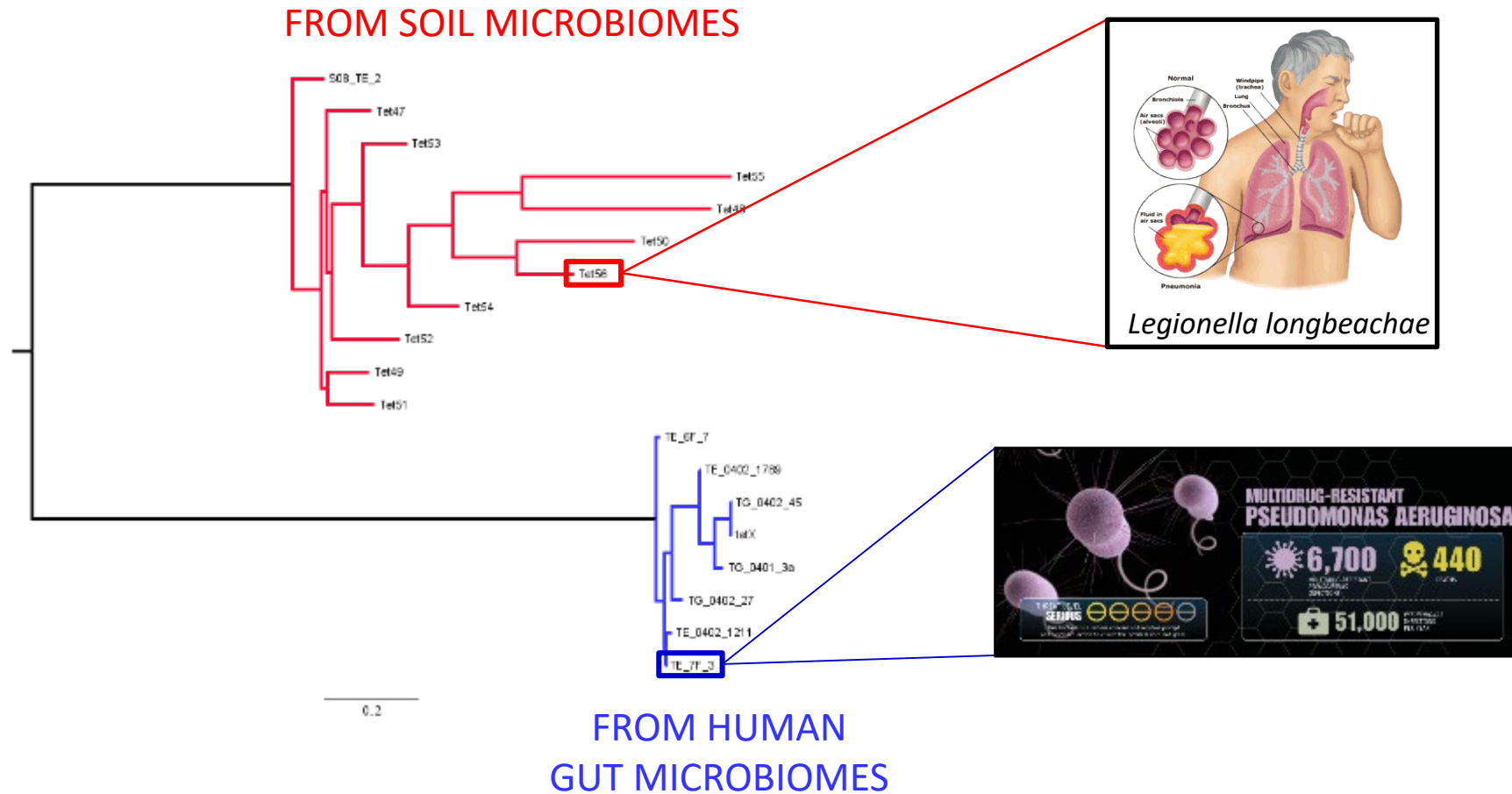
Tet(50) + substrate



- Inhibitor binds in flipped orientation
- Inhibitor binds at distinct binding site
- Inhibitor locks FAD in OUT conformation

Tetracycline destructases from soil and human microbiome identical to pathogens:

Legionella longbeachae and *Pseudomonas aeruginosa*



Park*, Gasparriini*, et al., *Nature Chemical Biology*. (2017)

Gasparriini et al., *Communications Biology*, (2020)

Mobile tetracycline destructases [*tet*(X3) and *tet*(X4)] on conjugative plasmids in Enterobacteriaceae and Acinetobacter isolated from humans, meat for consumption, and animals in China

nature
microbiology

Letter | Published: 27 May 2019

Emergence of plasmid-mediated high-level tigecycline resistance genes in animals and humans

Tao He, Ran Wang, Dejun Liu, Timothy R. Walsh, Rong Zhang, Yuan Lv, Yuebin Ke, Qianjiang Ji, Ruicheng Wei, Zhihai Liu, Yingbo Shen, Gang Wang, Lichang Sun, Lei Lei, Ziquan Lv, Yun Li, Maoda Pang, Liyuan Wang, Qiaoling Sun, Yulin Fu, Huangwei Song, Yuxin Hao, Zhangqi Shen, Shaolin Wang, Gongxiang Chen, Congming Wu, Jianzhong Shen  & Yang Wang  - Show fewer authors

Nature Microbiology **4**, 1450–1456 (2019) | [Download Citation](#) 

nature
microbiology

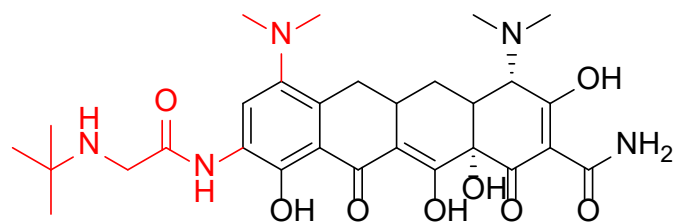
Letter | Published: 24 June 2019

Plasmid-encoded *tet*(X) genes that confer high-level tigecycline resistance in *Escherichia coli*

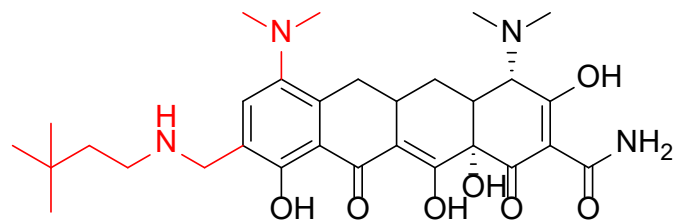
Jian Sun, Chong Chen, Chao-Yue Cui, Yan Zhang, Xiao Liu, Ze-Hua Cui, Xiao-Yu Ma, Youjun Feng, Liang-Xing Fang, Xin-Lei Lian, Rong-Min Zhang, You-Zhi Tang, Kou-Xing Zhang, Han-Mian Liu, Zhi-Hui Zhuang, Shi-Dan Zhou, Jing-Nan Lv, Hong Du, Bin Huang, Fang-You Yu, Barun Mathema, Barry N. Kreiswirth, Xiao-Ping Liao , Liang Chen  & Ya-Hong Liu 

Nature Microbiology **4**, 1457–1464 (2019) | [Download Citation](#) 

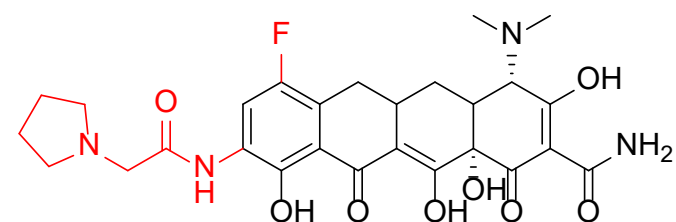
Tet-destructases from pathogenic *Pseudomonas aeruginosa* and human microbiome destroy NEWEST tetracyclines, BUT we have promising inhibitors



Tigecycline (approved 2005)

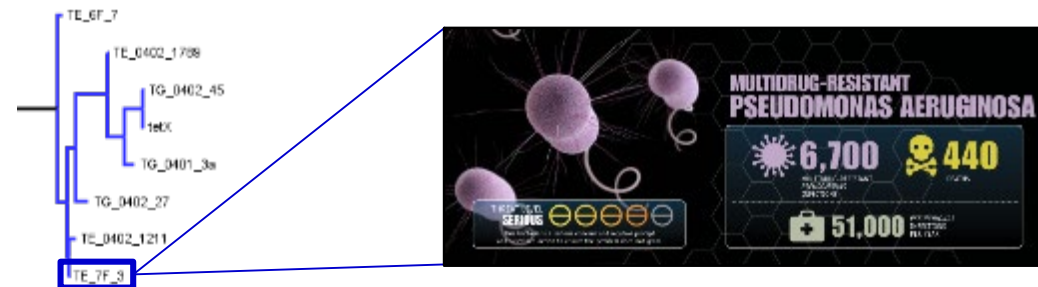


Omadacycline (approved October 2018)



Eravacycline (approved August 2018)

FROM HUMAN GUT MICROBIOMES

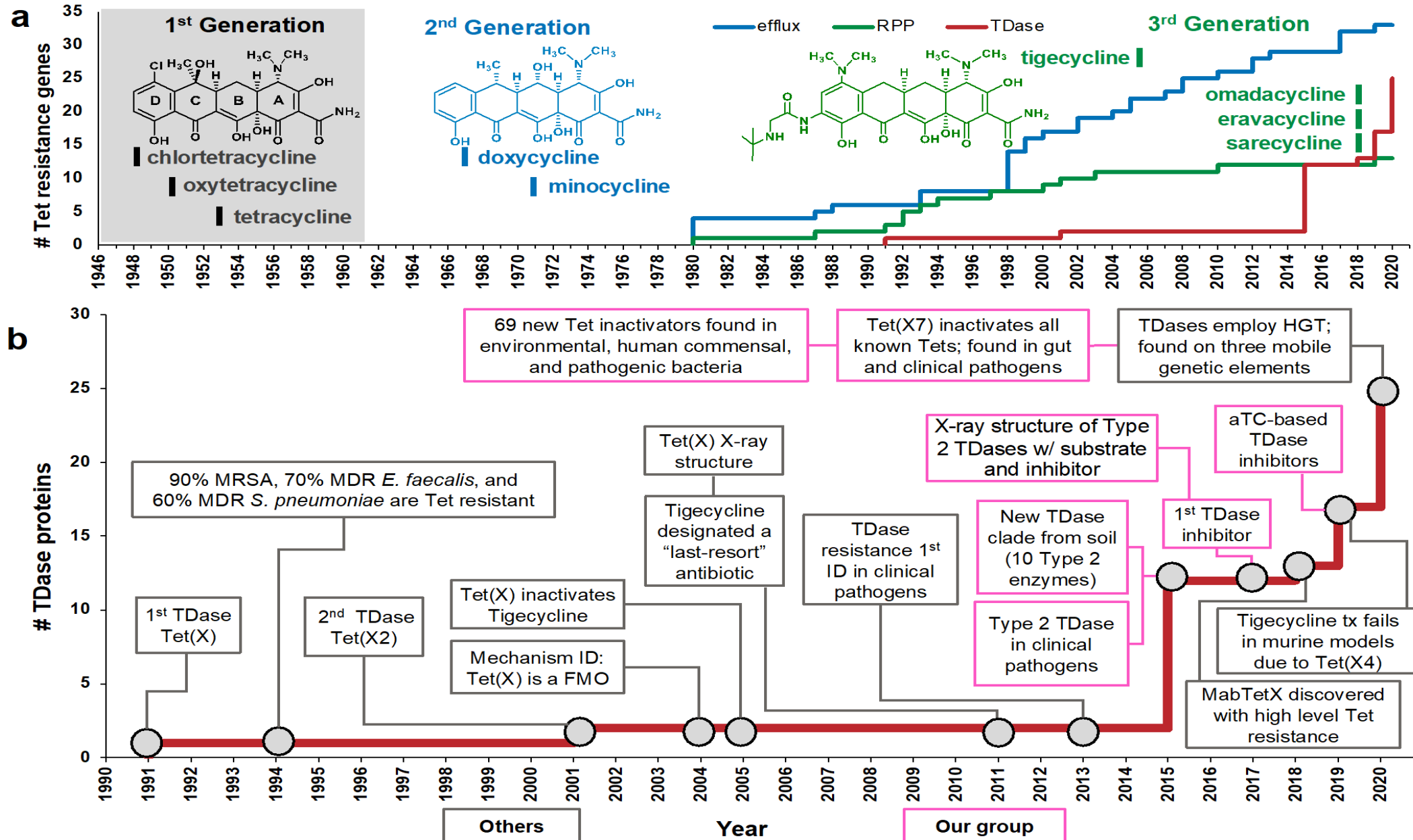


Antibiotic	<i>Pseudomonas aeruginosa</i> Pa-3		<i>Escherichia coli</i> DH10B + TE_7F_3	
	MIC	MIC+ 8 µg/mL aDem	MIC	MIC+ 8 µg/mL aDem
Tetracycline	128 µg/mL	32 µg/mL	256 µg/mL	128 µg/mL
Tigecycline	64 µg/mL	64 µg/mL	32 µg/mL	2 µg/mL
Eravacycline	64 µg/mL	32 µg/mL	8 µg/mL	8 µg/mL
Omadacycline	256 µg/mL	256 µg/mL	64 µg/mL	8 µg/mL

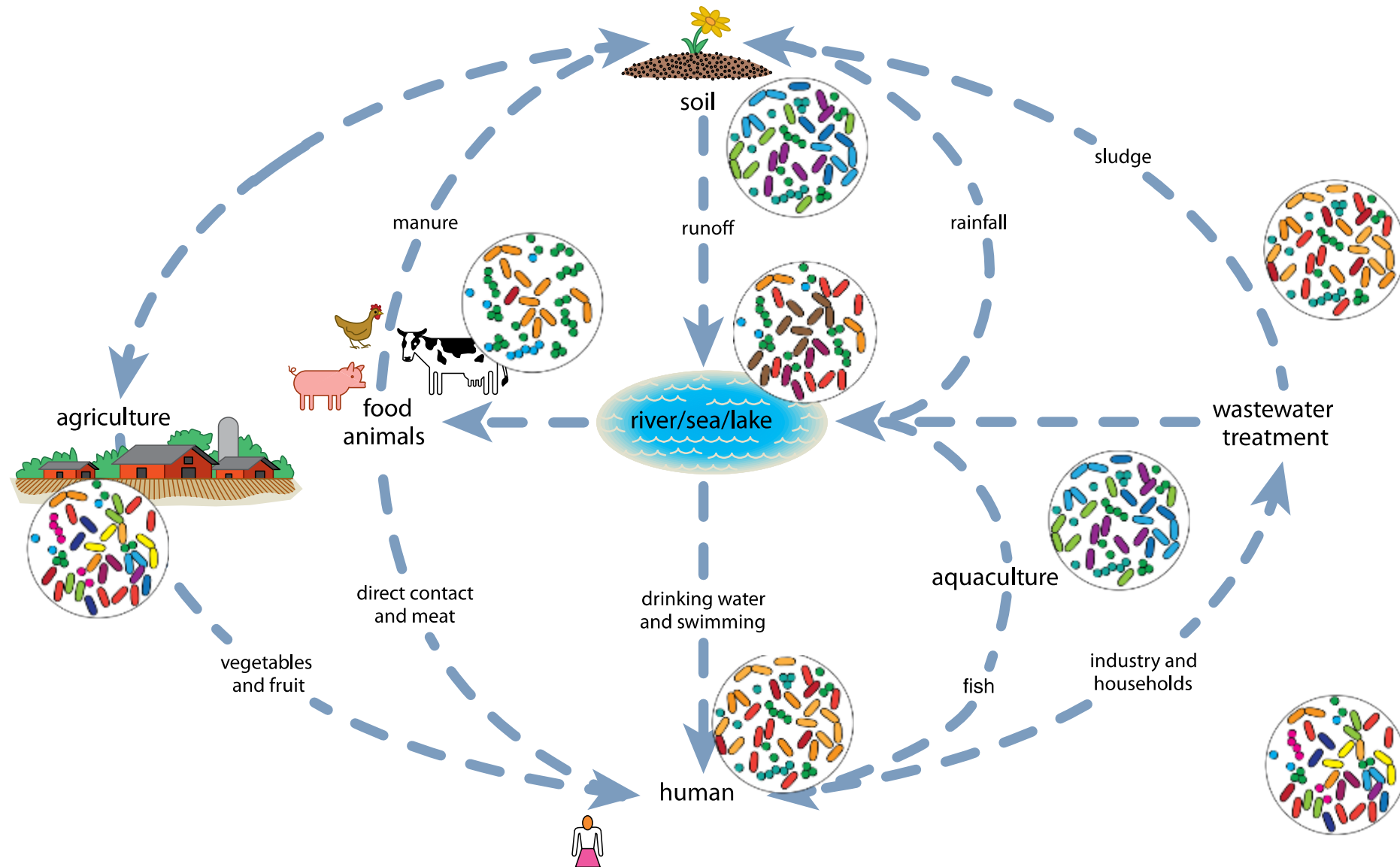
Markley et al., *ACS Infectious Diseases*. (2019)

Gasparrini et al., *Communications Biology*, (2020)

The Tetracycline Destructases Story: A Paradigm for Proactive vs Reactive Prediction and Combatting of Antimicrobial Resistance

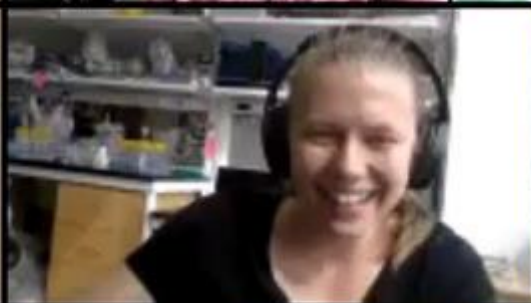
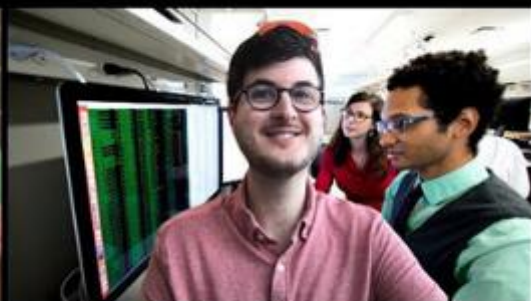


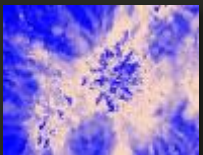
Transmission networks of microbiomes and resistomes across habitats



adapted from:
Dantas and Sommer, American Scientist (2014)







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I think I need
antibiotics for my
col...

IT'S A VIRUS!

