Washington University in St. Louis
School of Medicine

Center for Genome Sciences & Systems Biology

Understanding and Combatting Resistome Exchange Across Commensal, Environmental, and Pathogenic Microbes

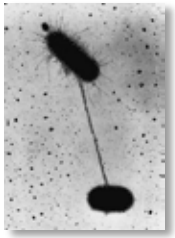
Gautam Dantas, PhD

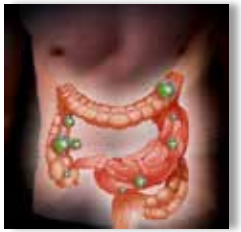
Associate Professor

Department of Pathology & Immunology

Department of Biomedical Engineering

Department of Molecular Microbiology



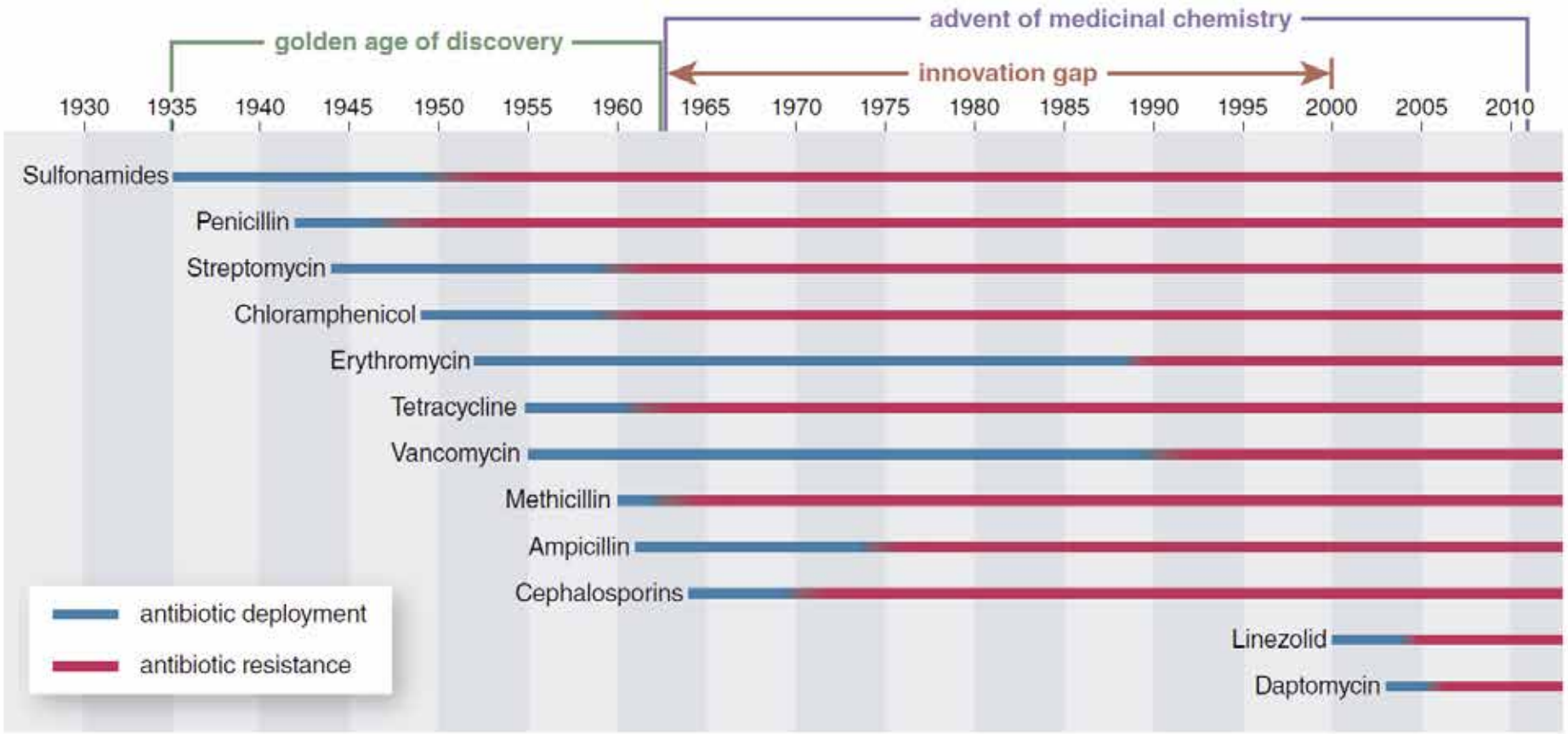

DANTAS LAB

Washington University in St. Louis

www.dantaslab.org

Alumni

Clinical resistance rapidly follows deployment for ALL antibiotics



Dantas and Sommer, ***American Scientist*** *(2014)*

Treatment of antibiotic resistant infections is an increasing challenge

Antibiotic Resistant Infections Are A Leading Cause of Death

700,000 deaths in 2014

10 Million *estimated* deaths in 2050

(UK Prime Minister's AMR Report, 2014)

Treatment of Antibiotic Resistant Infections Is Expensive

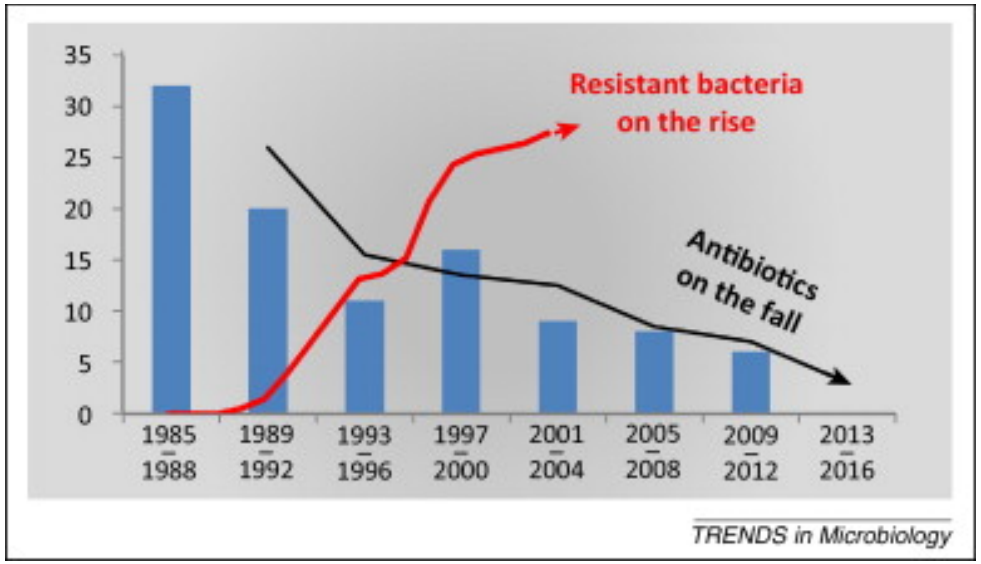
$55 Billion cost to the US economy in 2013

(US CDC, 2013)

$100 Trillion *estimated* cost to global economy by 2050

(UK Prime Minister's AMR Report, 2014)

Resistant Infections Are Increasing BUT New Antibiotic Discovery Is Decreasing



(Tschäberle & Hack, 2014)

Methods for studying antibiotic resistance in microbial communities

KNOWN

CULTURE & PHENOTYPE

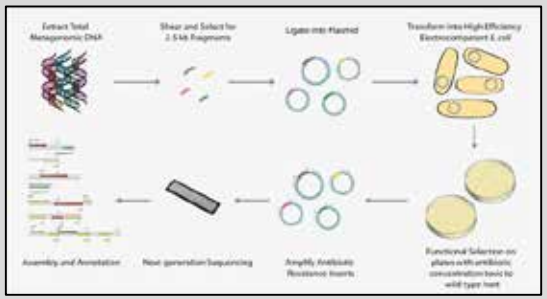
- Clinical resistance levels
- Direct clone to (multidrug) resistance connection
- Culture bias

SHOTGUN METAGENOME SEQUENCING

- No culture bias
- Large sampling depth
- **Only previously identified genes**
- Relative abundance

Known, Readily Cultured
Known, Not Readily Cultured
Unknown

Antibiotic Resistance Reservoir (**RESISTOME**)

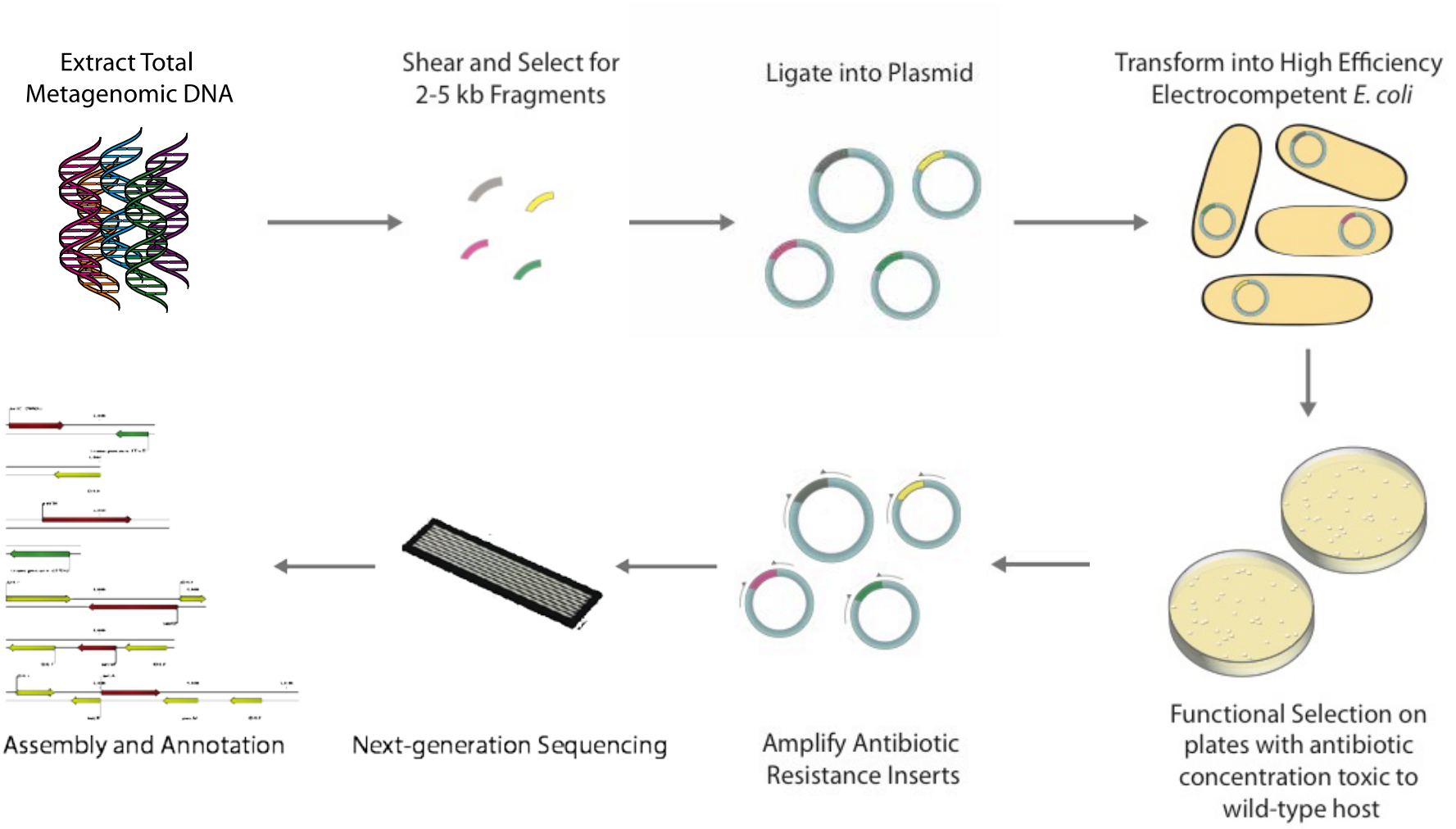


FUNCTIONAL METAGENOMIC SELECTIONS

- No culture bias
- Large sampling depth
- **Function confirmed**
- **Can identify novel genes**

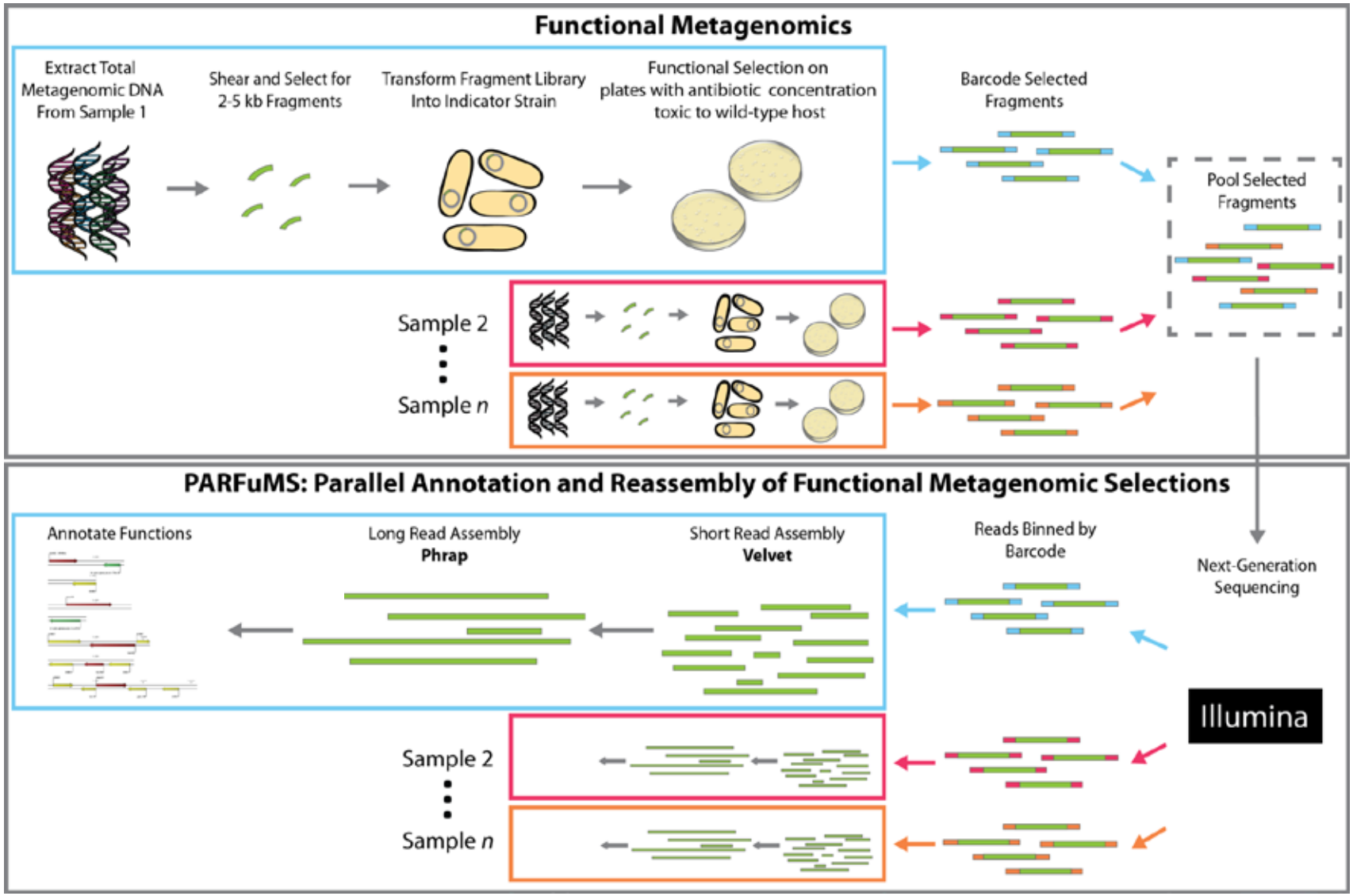
UNKNOWN

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)*
Reviewed in *Handelsman et al.* ***Chem Bio*** *(1998); Allen et al.* **Nat Rev Micro** *(2010); Dantas et al.* **Annu Rev Micro** *(2013)*

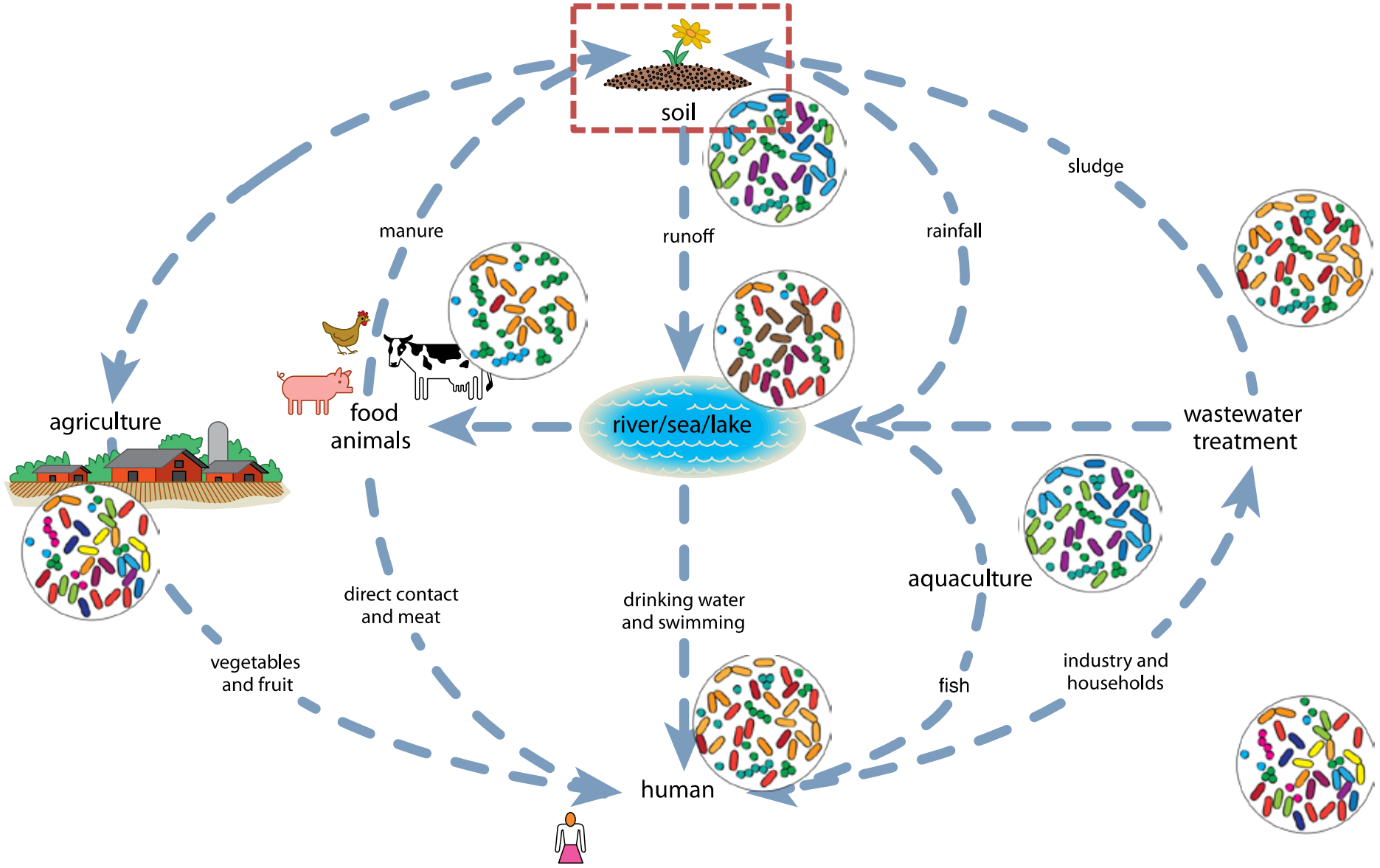
Increasing functional metagenomic throughput via next-gen sequencing



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)*

Reviewed in *Dantas et al.* **Annu Rev Micro** *(2013); Dantas et al.* **American Scientist** *(2014); Crofts et al.* **Nature Reviews Micro** *(2017)*

Transmission networks of microbiomes and resistomes across habitats



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

MDR soil Proteobacteria exchange resistance genes with pathogens
BUT majority of extensive soil resistome has low potential for exchange

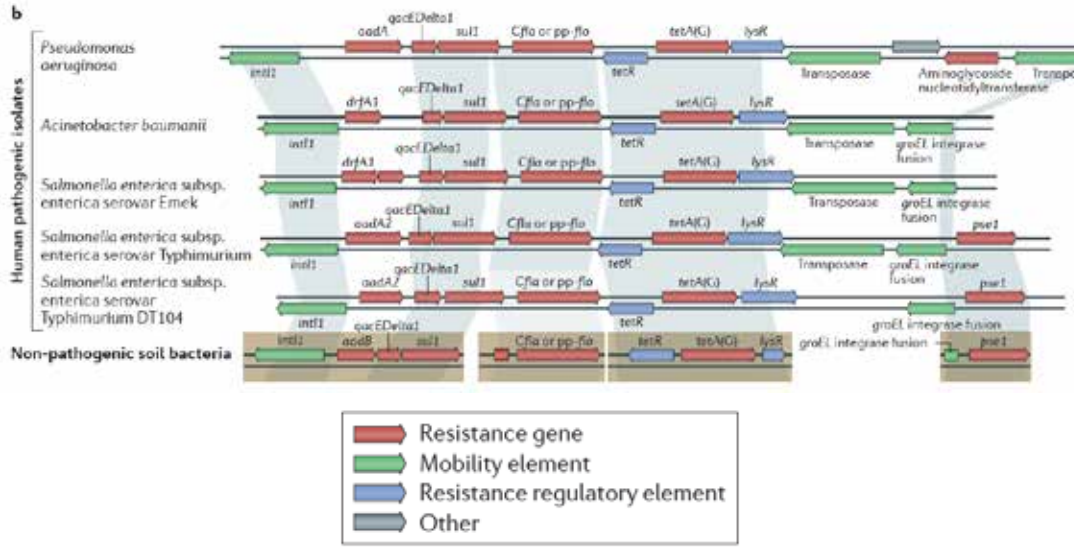
The Shared Antibiotic Resistome of Soil Bacteria and Human Pathogens

Kevin J. Forsberg,[1*] Alejandro Reyes,[1*] Bin Wang,[1,2] Elizabeth M. Selleck,[3] Morten O. A. Sommer,[4,5]† Gautam Dantas[1,2]†

www.sciencemag.org **SCIENCE** VOL 337 31 AUGUST 2012

Kevin Forsberg

Alejandro Reyes



Soil Proteobacteria share MULTIDRUG resistance gene clusters with human pathogens

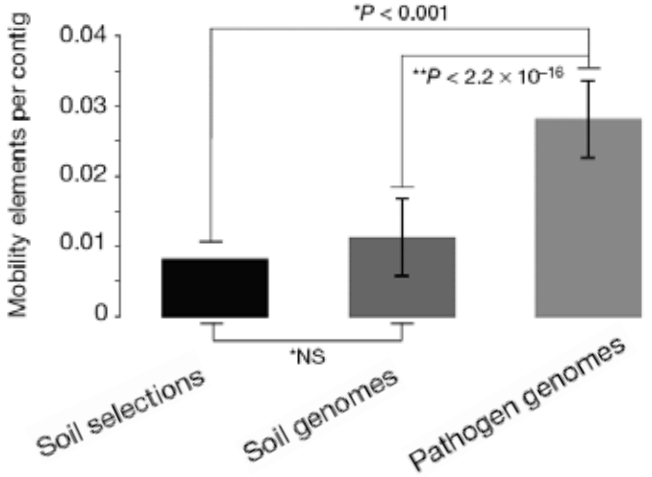
Bacterial phylogeny structures soil resistomes across habitats

Kevin J. Forsberg[1*], Sanket Patel[1,2*], Molly K. Gibson[1], Christian L. Lauber[3], Rob Knight[4,5], Noah Fierer[3,6] & Gautam Dantas[1,2,7]

612 | NATURE | VOL 509 | 29 MAY 2014

Kevin Forsberg

Sanket Patel



But MOST soil resistance genes are novel and co-localized with fewer mobilization genes than pathogens

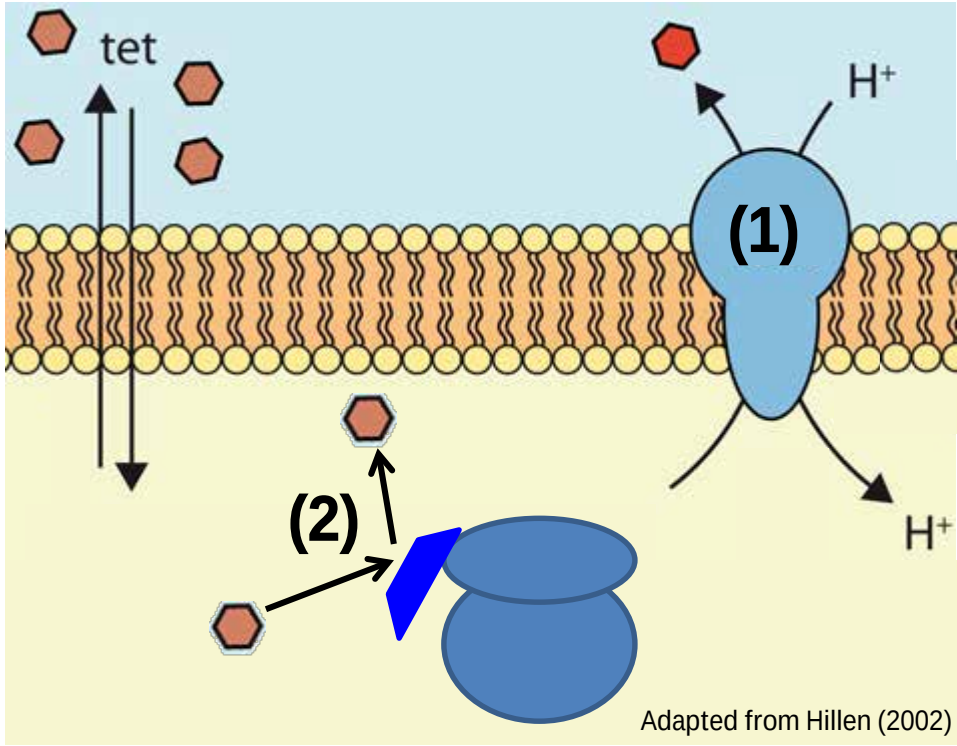
BUT cryptic soil resistance genes are still clinically-relevant
e.g. tetracycline resistance

- **Two major mechanisms** of tetracycline resistance:
  - Active Efflux **(1)**
  - Ribosomal Protection **(2)**
  - **Both prevalent in pathogens**

- **3rd mechanism**: tetracycline **inactivation**
  - 3 genes from human commensals
  - Tet(X) only characterized enzyme
  - **Not seen in pathogens until 2013**
  - Oxidizes drug via FAD cofactor

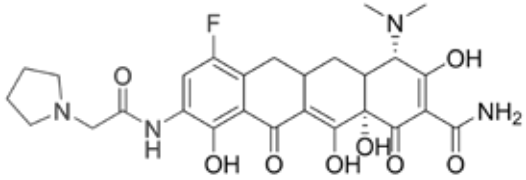
- **Drug inactivation** is large clinical threat
  - *e.g.* β-lactamases, acetyltransferases
  - Allows survival of "cheaters"
  - Eliminates drug, energetically favorable

Common Tetracycline Resistance Mechanisms



Adapted from Hillen (2002)

Playing with fire: Touting drugs “unaffected” by “common” resistance mechanisms



Eravacycline

AAC
Journals.ASM.org

Target- and Resistance-Based Mechanistic Studies with TP-434, a Novel Fluorocycline Antibiotic

Trudy H. Grossman,[c] Agata L. Starosta,[a] Corey Fyfe,[c] William O'Brien,[c] David M. Rothstein,[c]* Aleksandra Mikolajka,[a] Daniel N. Wilson,[a,b] and Joyce A. Sutcliffe[c]

Gene Center, Department of Biochemistry,[a] and Center for Integrated Protein Science Munich (CiPSM),[b] University of Munich, Germany, and Tetraphase Pharmaceuticals, Inc., Watertown, Massachusetts, USA[c]

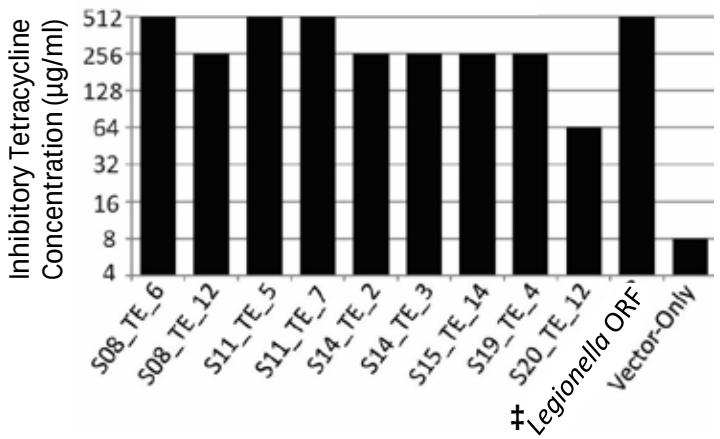
TP-434 is a novel, broad-spectrum fluorocycline antibiotic with activity against bacteria expressing major antibiotic resistance mechanisms, including tetracycline-specific efflux and ribosomal protection. The mechanism of action of TP-434 was assessed using both cell-based and *in vitro* assays. In *Escherichia coli* cells expressing recombinant tetracycline resistance genes, the MIC of TP-434 (0.063 μg/ml) was unaffected by *tet*(M), *tet*(K), and *tet*(B) and increased to 0.25 and 4 μg/ml in the presence of *tet*(A) and *tet*(X), respectively. Tetracycline, in contrast, was significantly less potent (MIC ≥ 128 μg/ml) against *E. coli* cells when any of these resistance mechanisms were present. TP-434 showed potent inhibition in *E. coli in vitro* transcription/translation (50% inhibitory concentration [IC_{50}] = 0.29 ± 0.09 μg/ml) and [^{3}H]tetracycline ribosome-binding competition (IC_{50} = 0.22 ± 0.07 μM) assays. The antibacterial potencies of TP-434 and all other tetracycline class antibiotics tested were reduced by 4- to 16-fold, compared to that of the wild-type control strain, against *Propionibacterium acnes* strains carrying a 16S rRNA mutation, G1058C, a modification that changes the conformation of the primary binding site of tetracycline in the ribosome. Taken together, the findings support the idea that TP-434, like other tetracyclines, binds the ribosome and inhibits protein synthesis and that this activity is largely unaffected by the common tetracycline resistance mechanisms.

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

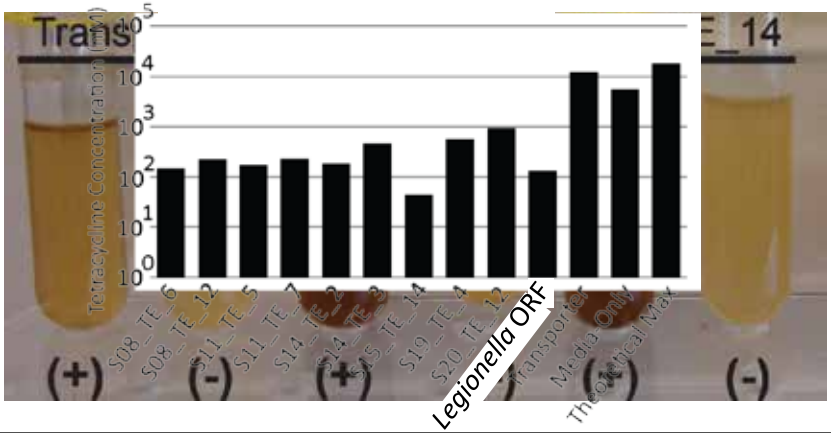
Predicted Function: "*FAD-Dependent Oxidoreductase*"

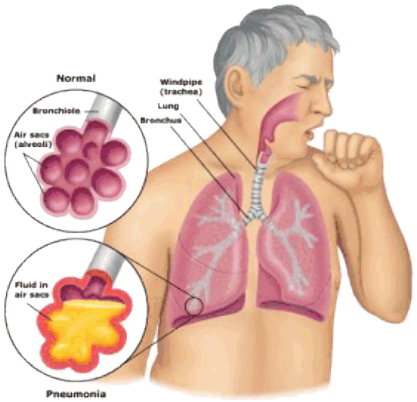
Kevin Forsberg

Sequence unlike any tetracycline resistance gene



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



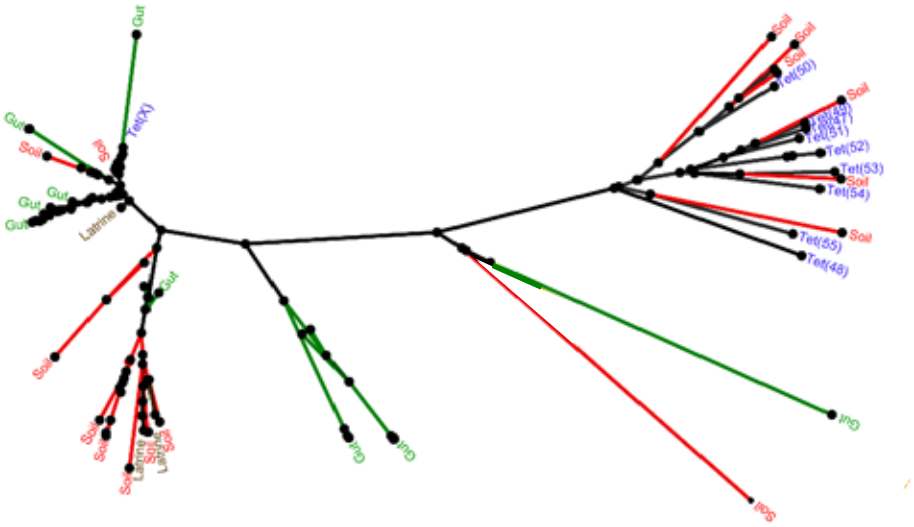


Only homolog from pathogenic *Legionella*‡

Resistance conferred by tetracycline inactivation

Forsberg, Patel, Wencewicz, Dantas. ***Chemistry & Biology****, (2015)*

Tetracycline destructases are widespread in diverse metagenomes and pathogens



Tet(X) identified in MDR pathogens:

E*. faecium*

S*. aureus*

K*. pneumoniae* ***ü*** (Leski *et al.* 2013)

A*. baumanii* ***ü*** (Deng *et al.* 2014)

P*. aeuruginosa* ***ü*** (Leski *et al.* 2013)

E*nterobacter spp.* ***ü*** (Leski *et al.* 2013)

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

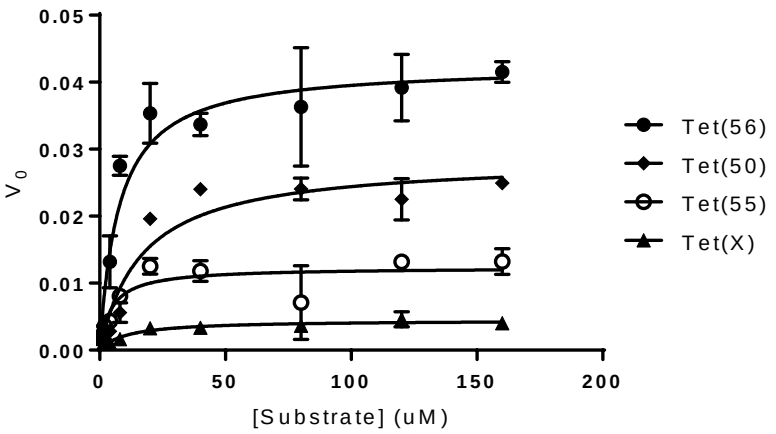
- ***Soil***
- ***Gut***
- ***Latrine***
- ***Previously described***

Drew Gasparrini

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

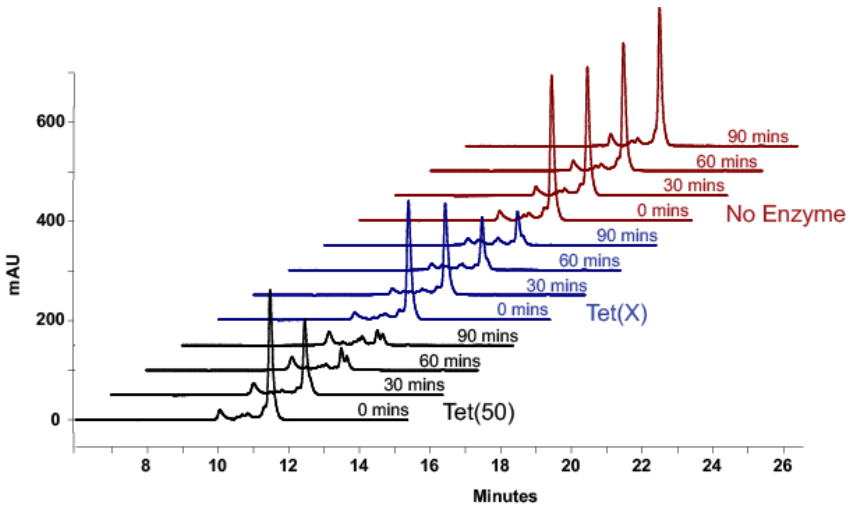
Gasparrini et al., ***unpublished***

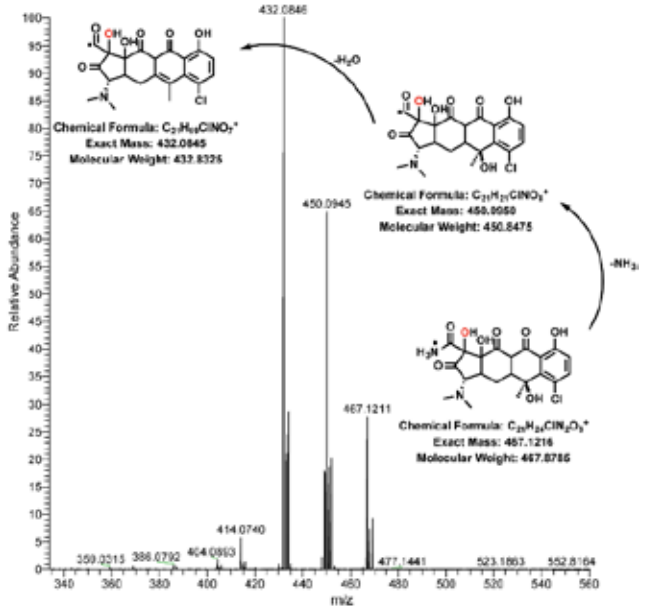
Biochemical and structural elucidation of novel mechanism of resistance *(in collaboration with Tim Wencewicz and Niraj Tolia)*



	K_m (μM)	V_{max} (s^{-1})	k_{cat}/K_m ($\mu M^{-1}s^{-1}$)
Tet(56)	7.665 ± 1.630	0.0425 ± 0.002051	0.013862
Tet(50)	16.84 ± 3.555	0.02852 ± 0.001555	0.004234
Tet(55)	4.562 ± 1.684	0.01233 ± 0.0009667	0.006757
Tet(X)	10.77 ± 2.613	0.00448 ± 0.0002618	0.00104

Catalytic efficacy of tetracycline destructases is **4-15 fold greater than only previously described** tetracycline inactivating enzyme

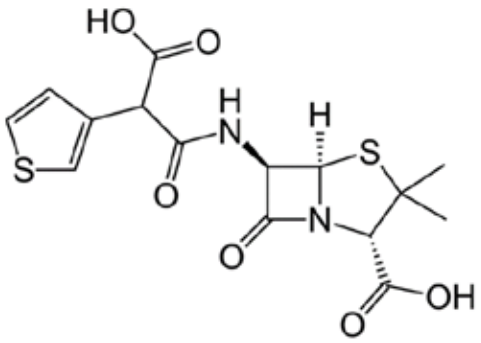




Tetracycline destructases produce **novel decay products** of tetracycline antibiotics, characterized by HPLC, HR-MS/MS

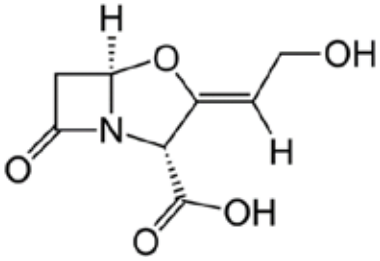
Anhydrotetracycline inhibits tetracycline destructases

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



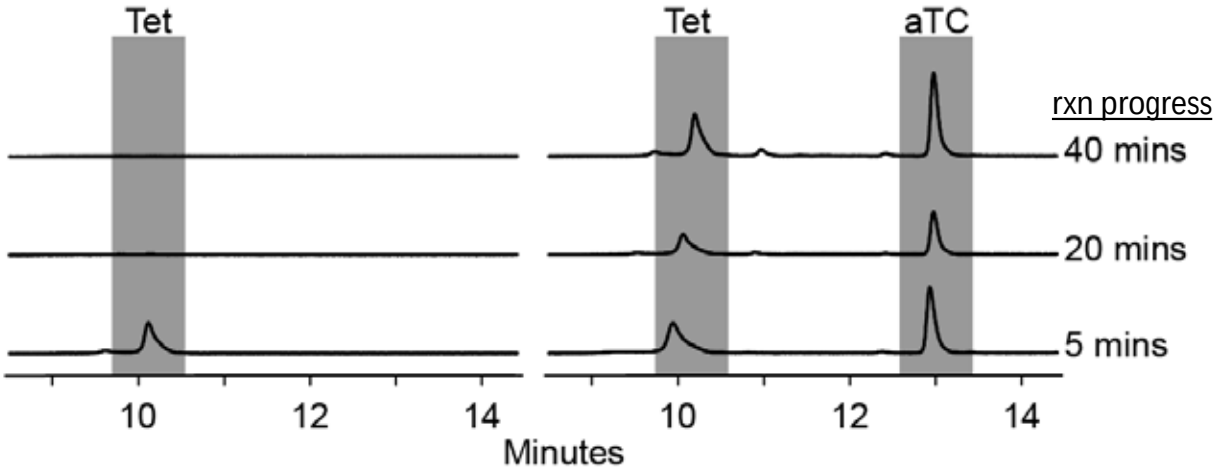
Ticarcillin
(β-lactam antibiotic)

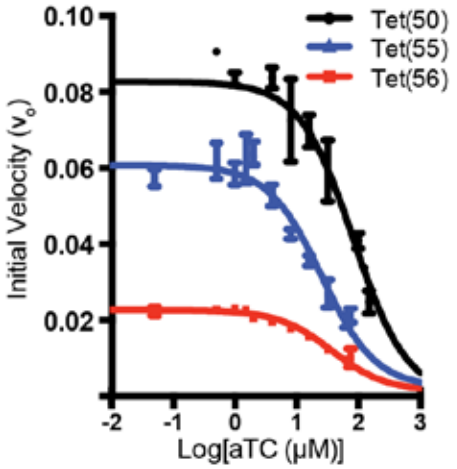
+



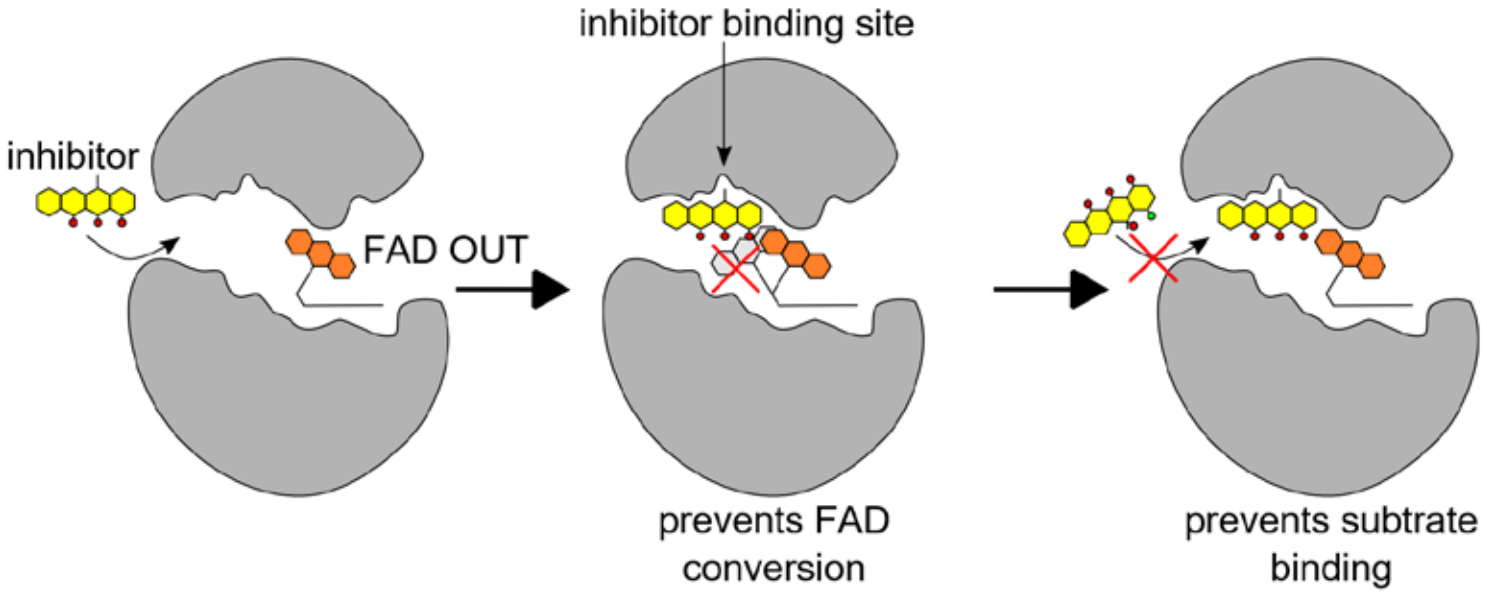
Clavulanate
(β-lactamase inhibitor)

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





A structural basis for anhydrotetracycline inhibition

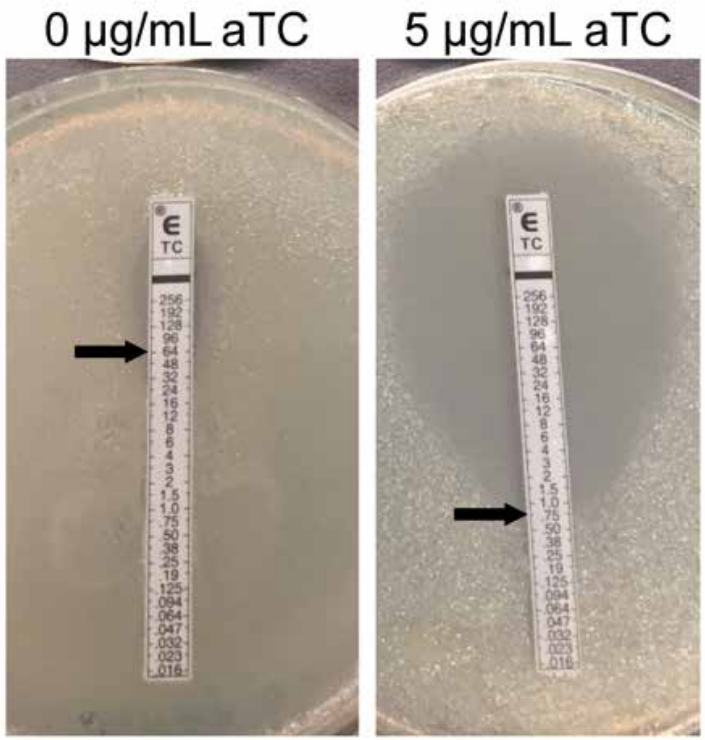


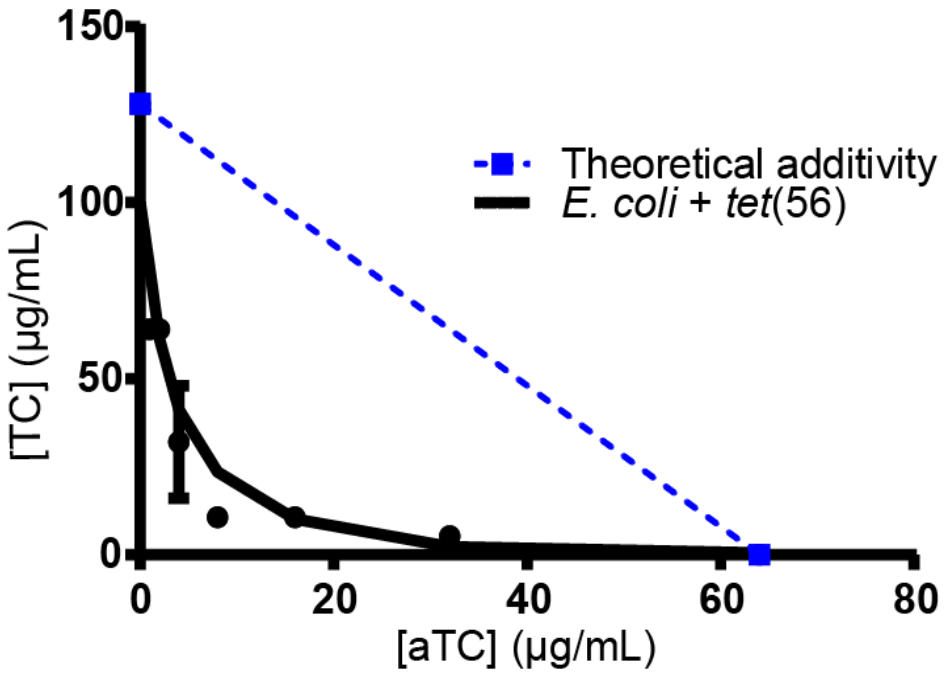
- aTC binds at distinct “inhibitor binding site” to **(a)** lock FAD cofactor in the unproductive OUT conformation and **(b)** block substrate binding

Park, Gasparrini*, Reck, Symister, Elliot, Vogel, Wencewicz‡, Dantas‡, Tolia‡.* ***Nature Chemical Biology*** *(2017)*

Inhibiting tetracycline destructase activity rescues tetracycline efficacy

Anhydrotetracycline **synergistically rescues tetracycline antibiotic activity** against *E. coli* expressing *tet(56)*

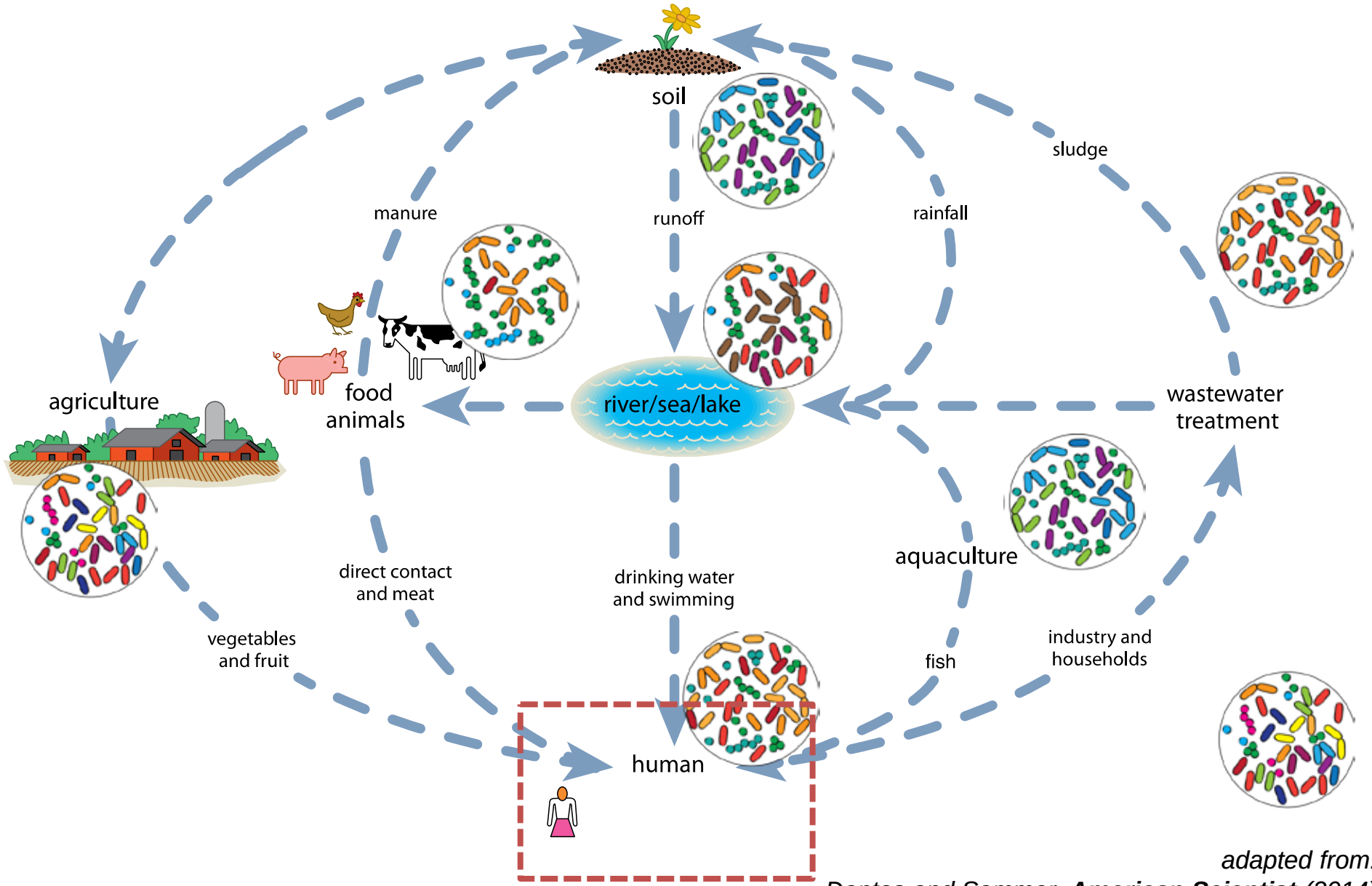




Tet(56) FICI = 0.1875

Park, Gasparrini*, Reck, Symister, Elliot, Vogel, Wencewicz‡, Dantas‡, Tolia‡.* ***Nature Chemical Biology*** *(2017)*

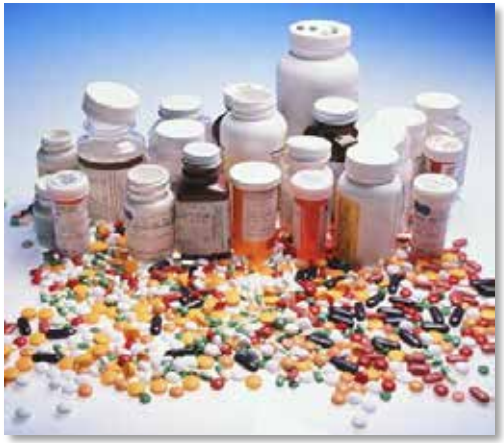
Transmission networks of microbiomes and resistomes across habitats

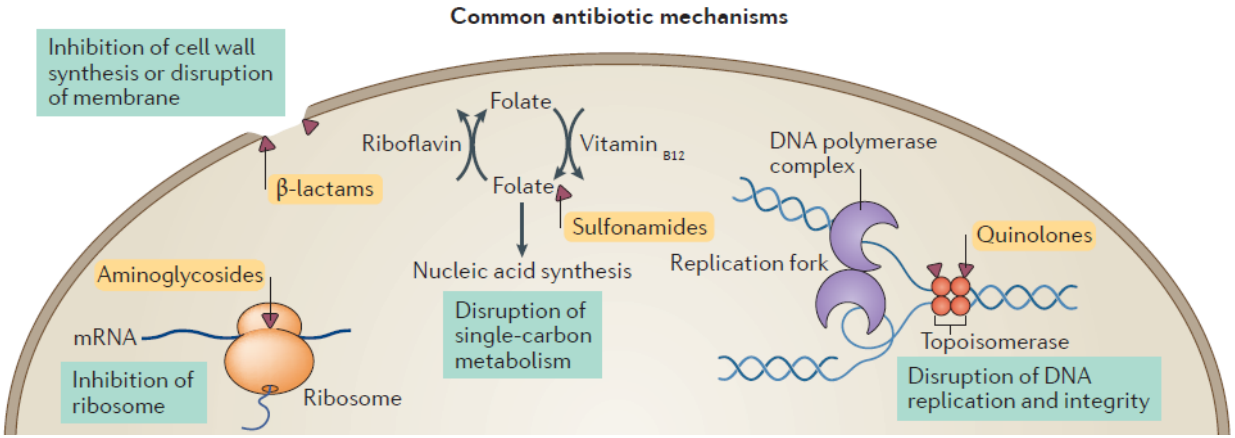


adapted from:
Dantas and Sommer, ***American Scientist*** *(2014)*

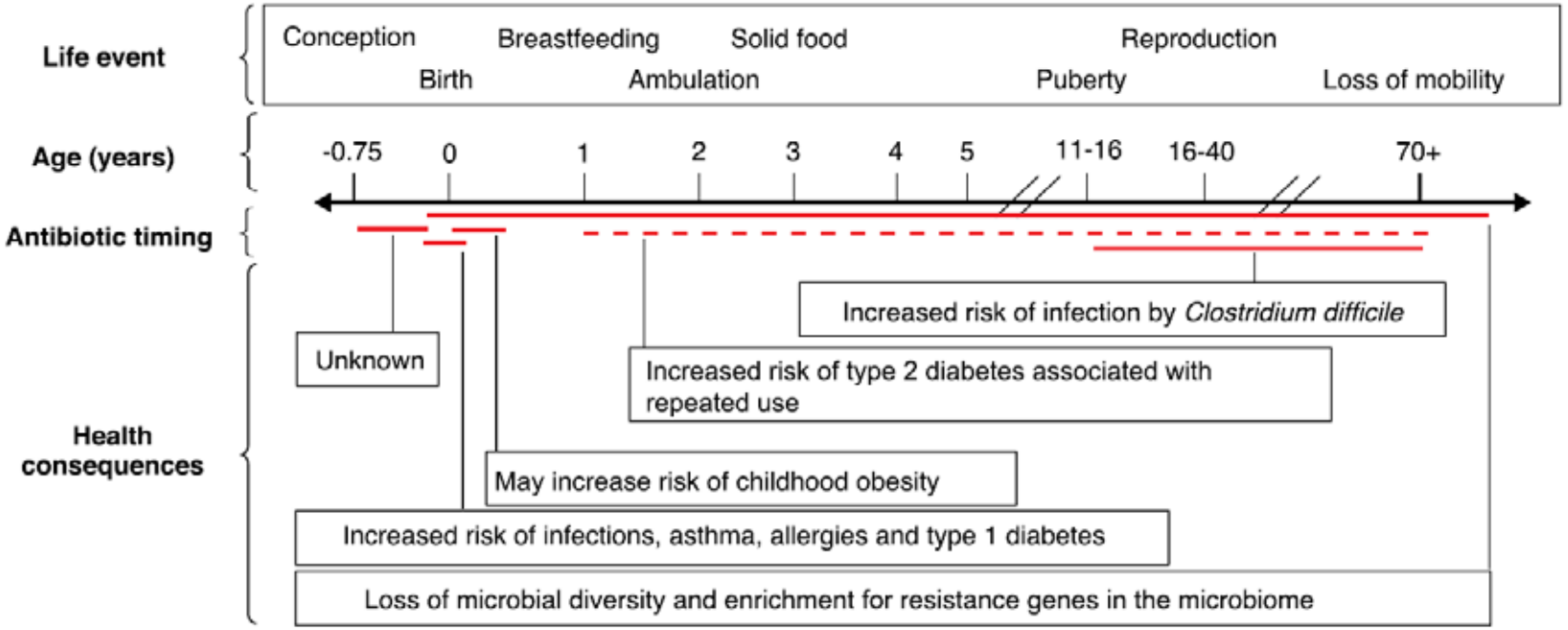
Resistance spreads across habitats

Antibiotic perturbation of the human microbiome can be dysbiotic



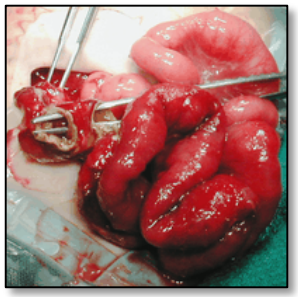


Crofts, Gasparrini, Dantas. **Nature Reviews Micro** *(2017)*



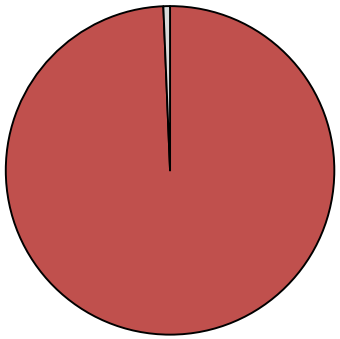
Langdon, Crook, Dantas. **Genome Medicine** *(2016)*

Antibiotics are the most prescribed medication for preterm infants



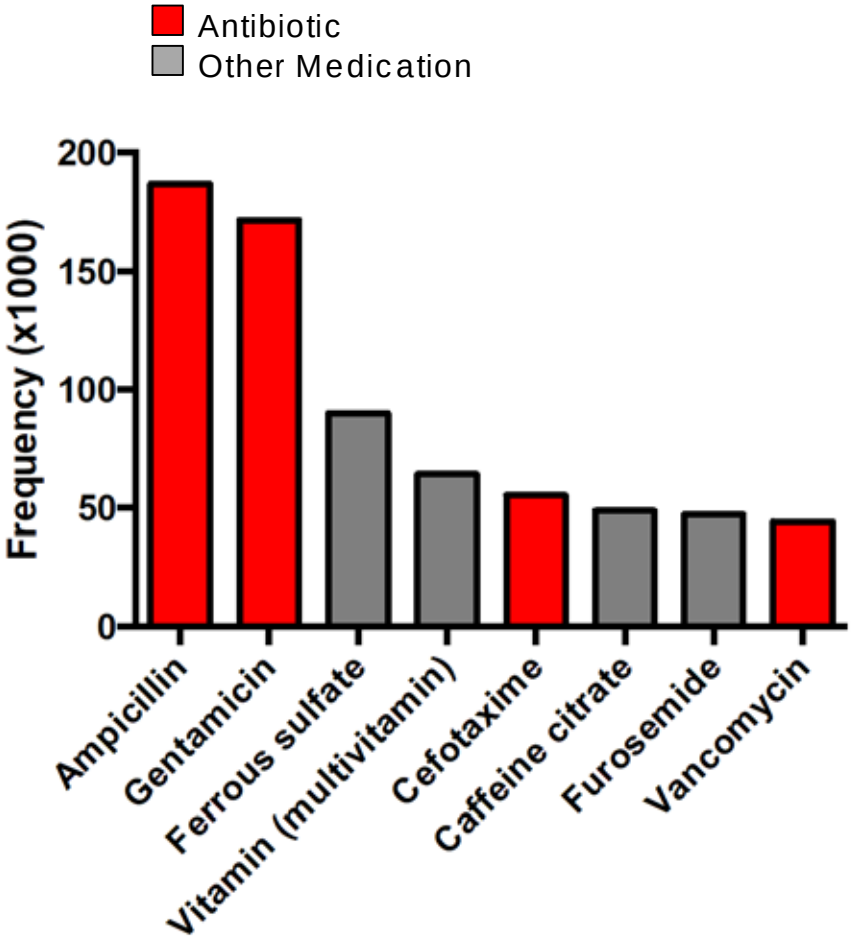
Preterm birth is **leading cause of infant death**

Preterm infants are highly susceptible to infections



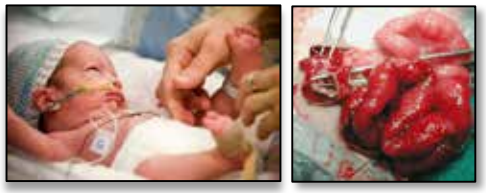
99%

of VLBW infants receive **antibiotics** in the **1st two days of life**

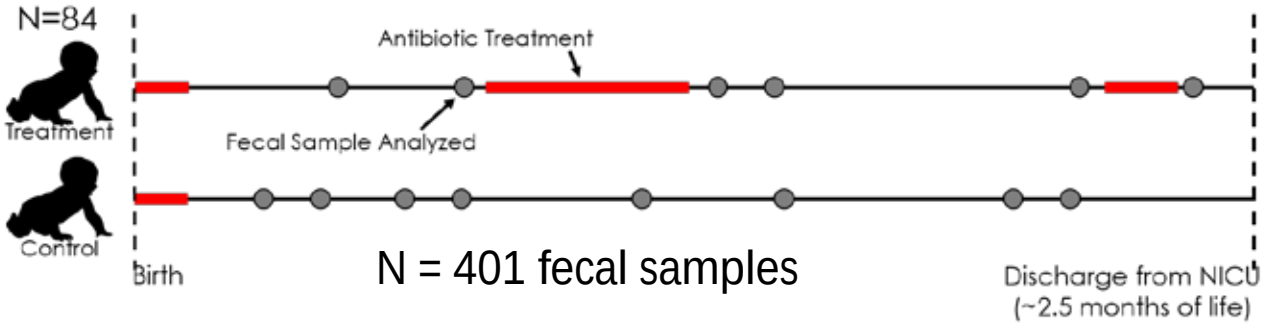


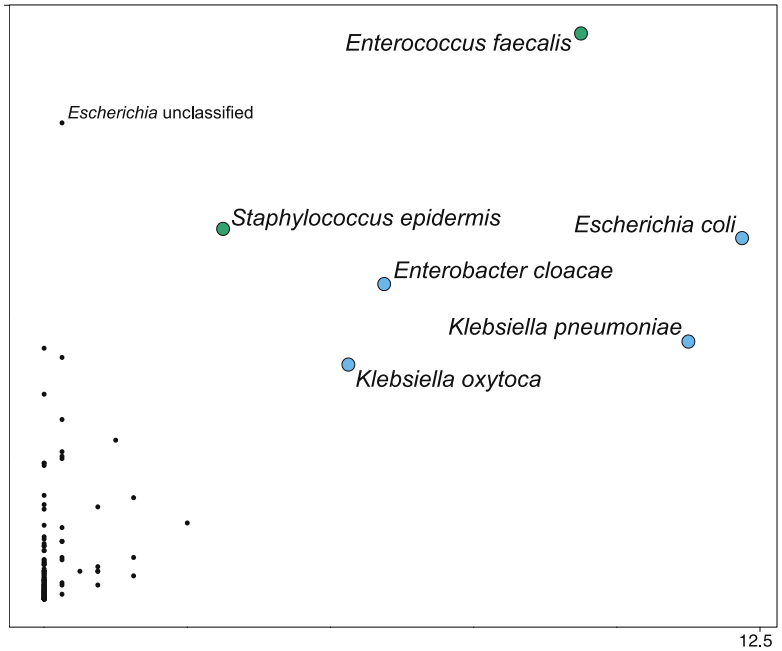
Gasparrini, Crofts*, Gibson, Tarr, Warner, Dantas.* ***Gut Microbes****, (2016)*

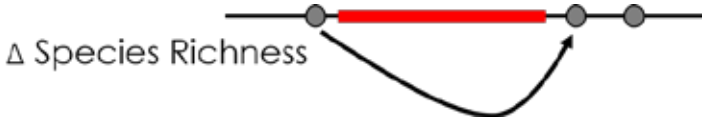
Gut microbiomes of preterm infants are dominated by MDROs
We can predict microbiome and resistome responses to antibiotics

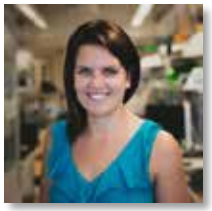


Preterm birth is **leading cause of infant death**







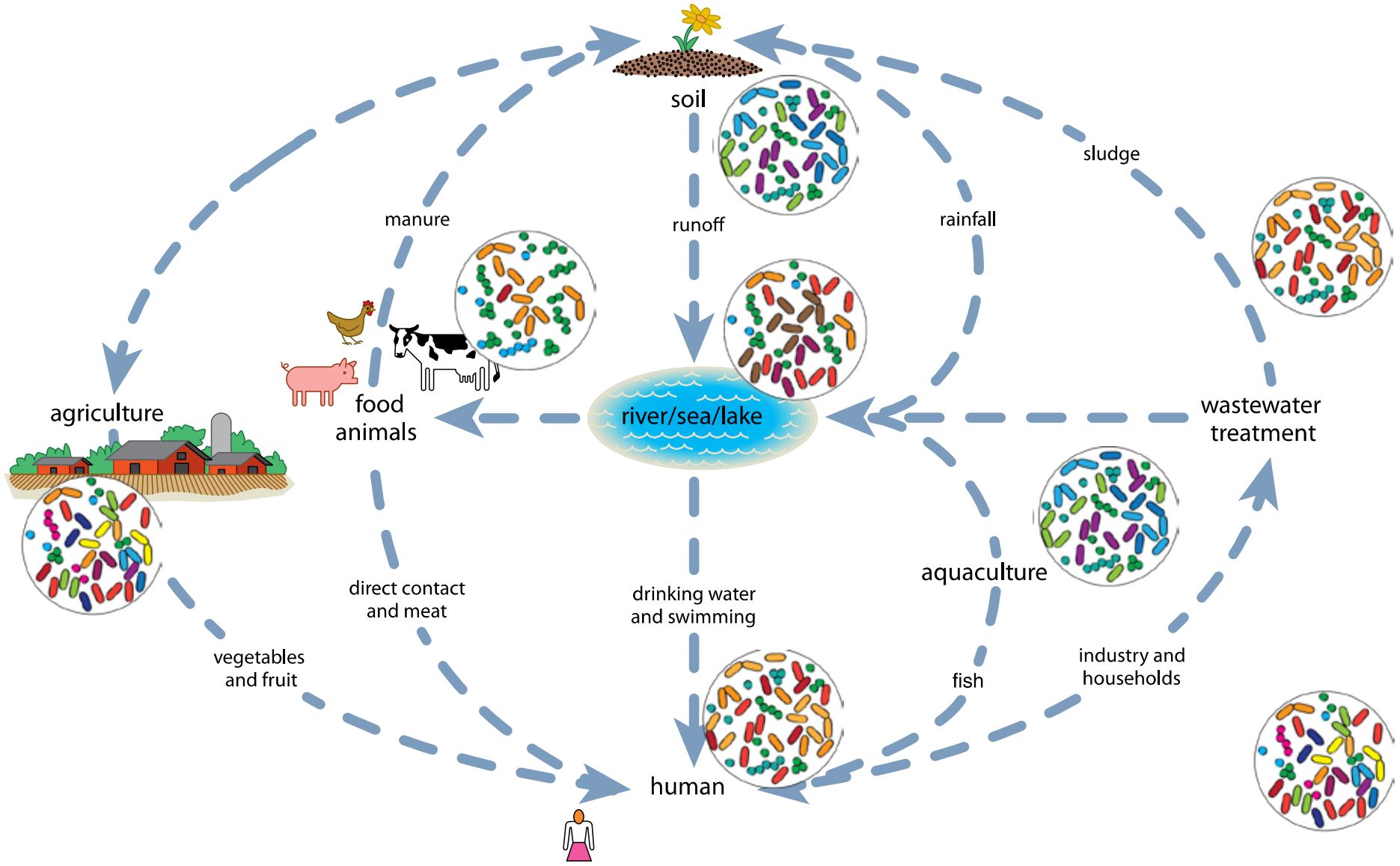


Molly Gibson

85% prediction accuracy based on 4 variables

Gibson, Wang, Ahmadi, Burnham, Tarr, Warner, Dantas. ***Nature Microbiology****, (2016)*

Transmission networks of microbiomes and resistomes across habitats

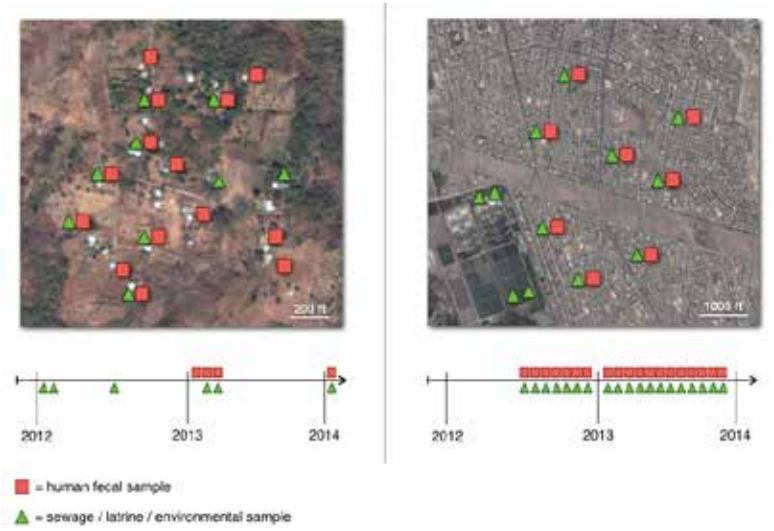


adapted from:
Dantas and Sommer, ***American Scientist*** *(2014)*

Gut microbiomes across the globe are structured by lifestyle
Resistomes are structured by phylogeny and habitat

Village in **R**ural **E**l **S**alvador (RES)

Peri-urban **S**hanty-**T**own (PST) in Peru

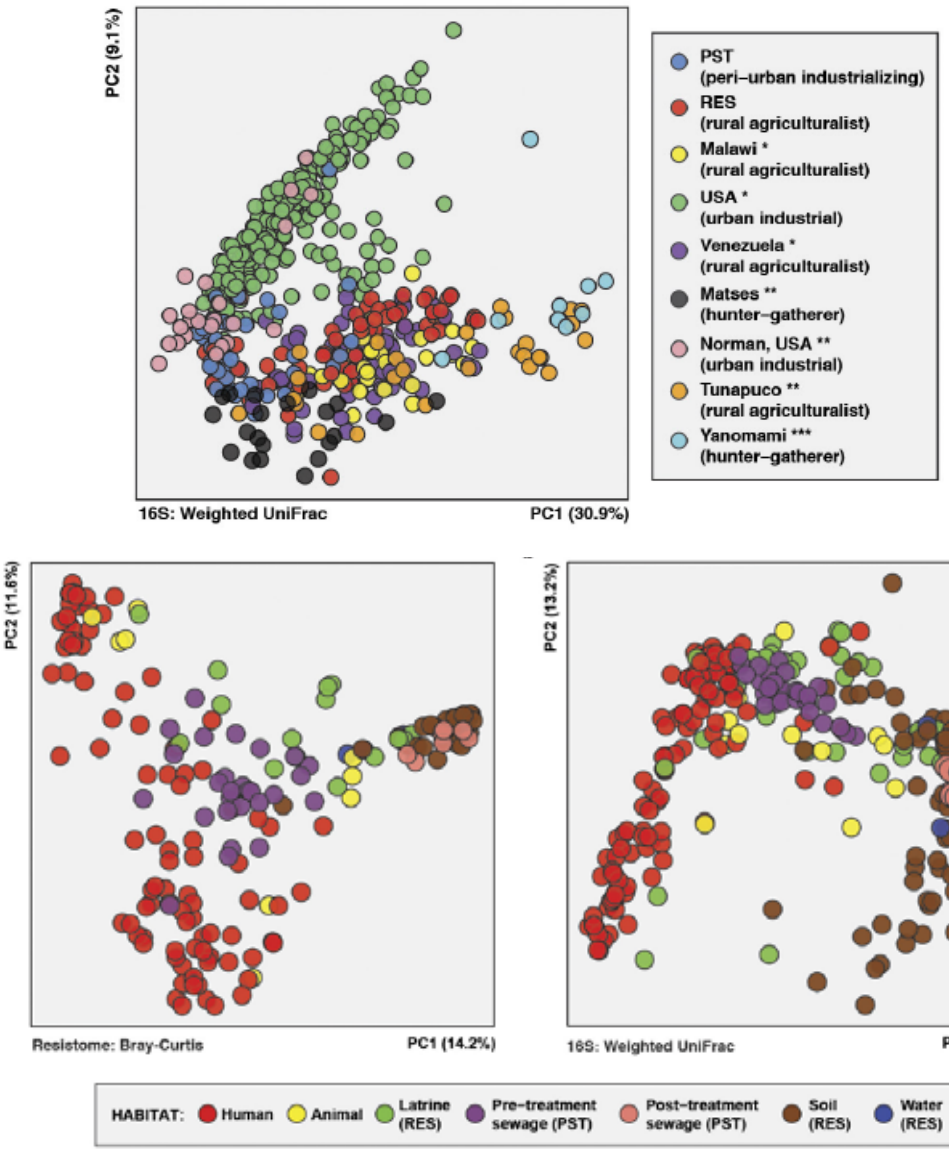


263 fecal samples from 115 individuals from 27 houses

209 environmental samples from animals, soils, sewage

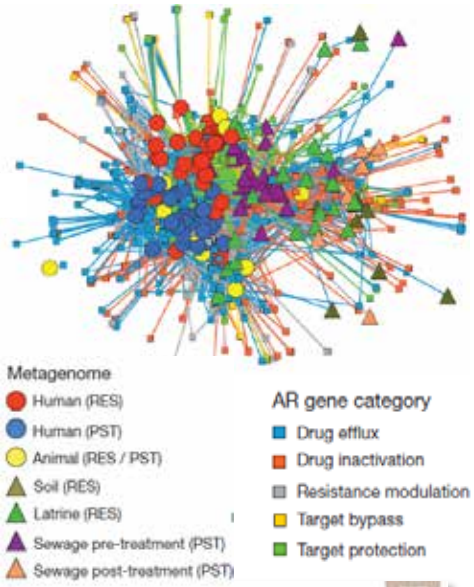
Erica Pehrsson

Pablo Tsukayama



Pehrsson, Tsukayama*, Patel, Mejia, Sosa, Navarette, Calderon, Cabrerra, Hoyos, Bertoli, Berg, Gilman, Dantas.* ***Nature*** *(2016)*

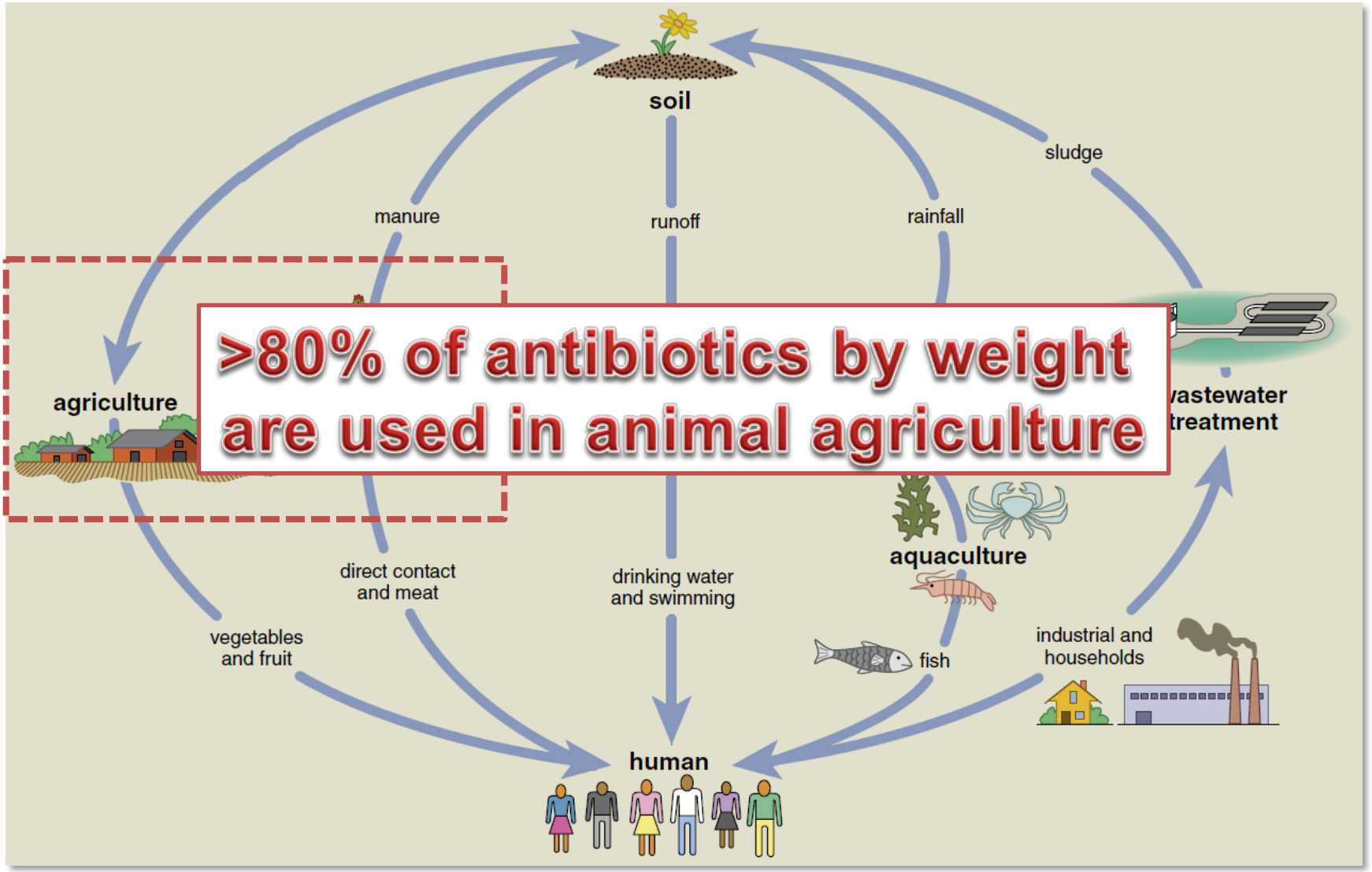
Identification of resistome dissemination hotspots may help with surveillance



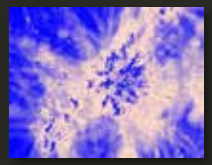
Chicken coops (El Salvador) and **Sewage treatment plant** (Peru) were **hotspots for resistome exchange** between humans and the environment

Pehrsson, Tsukayama*, et al.* ***Nature*** *(2016)*

Antibiotic resistance is an ECOLOGICAL problem



Dantas & Sommer, ***American Scientist*** *(2014)*



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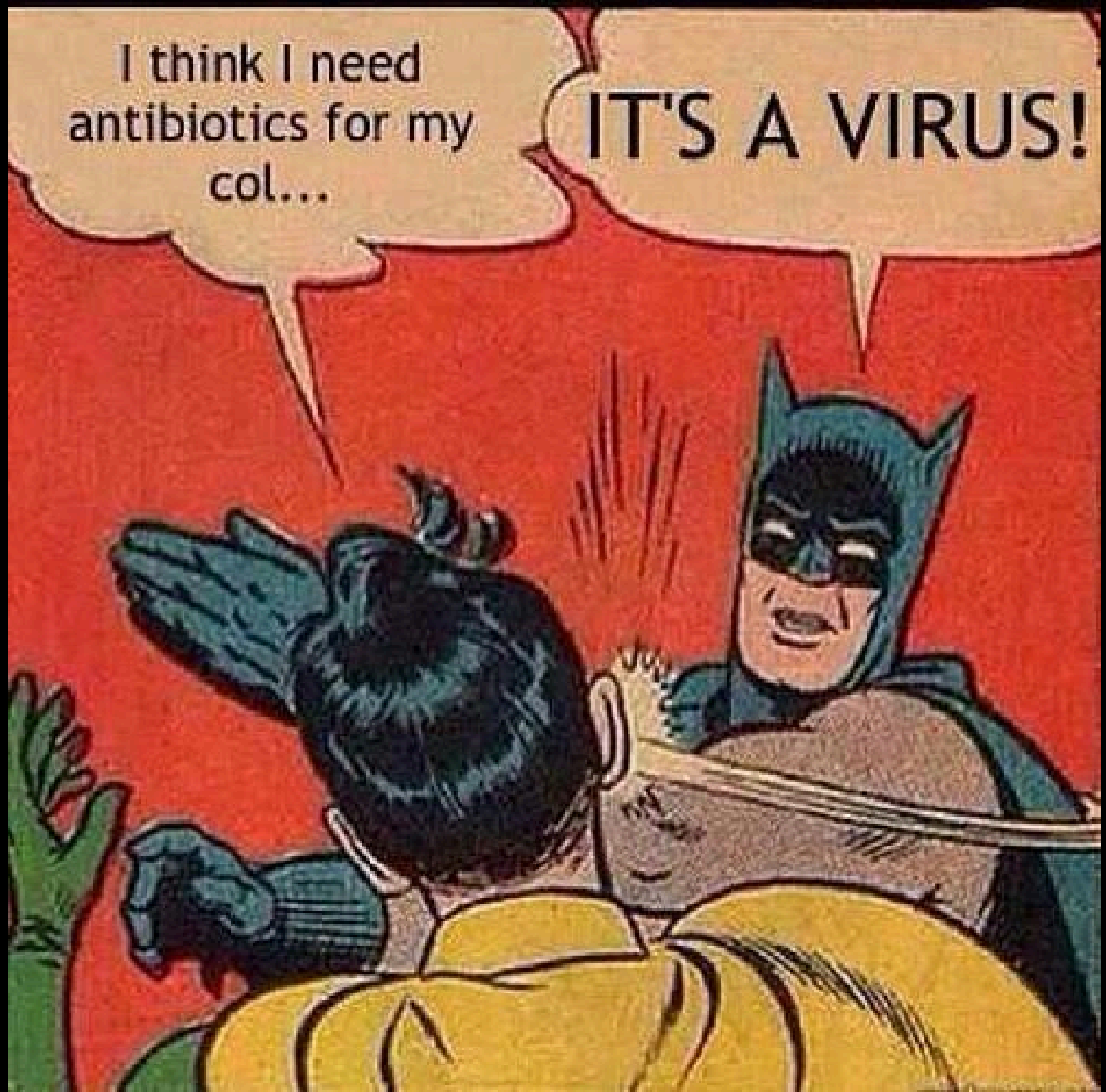
I think I need antibiotics for my col...
IT'S A VIRUS!