Washington University in St. Louis
School of Medicine

Center for Genome Sciences & Systems Biology

Predicting and Combating Pathogenic and Abiotic Disruptions to Diverse Microbiomes

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Department of Biomedical Engineering

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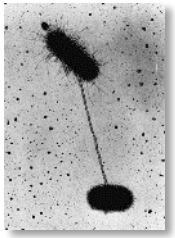
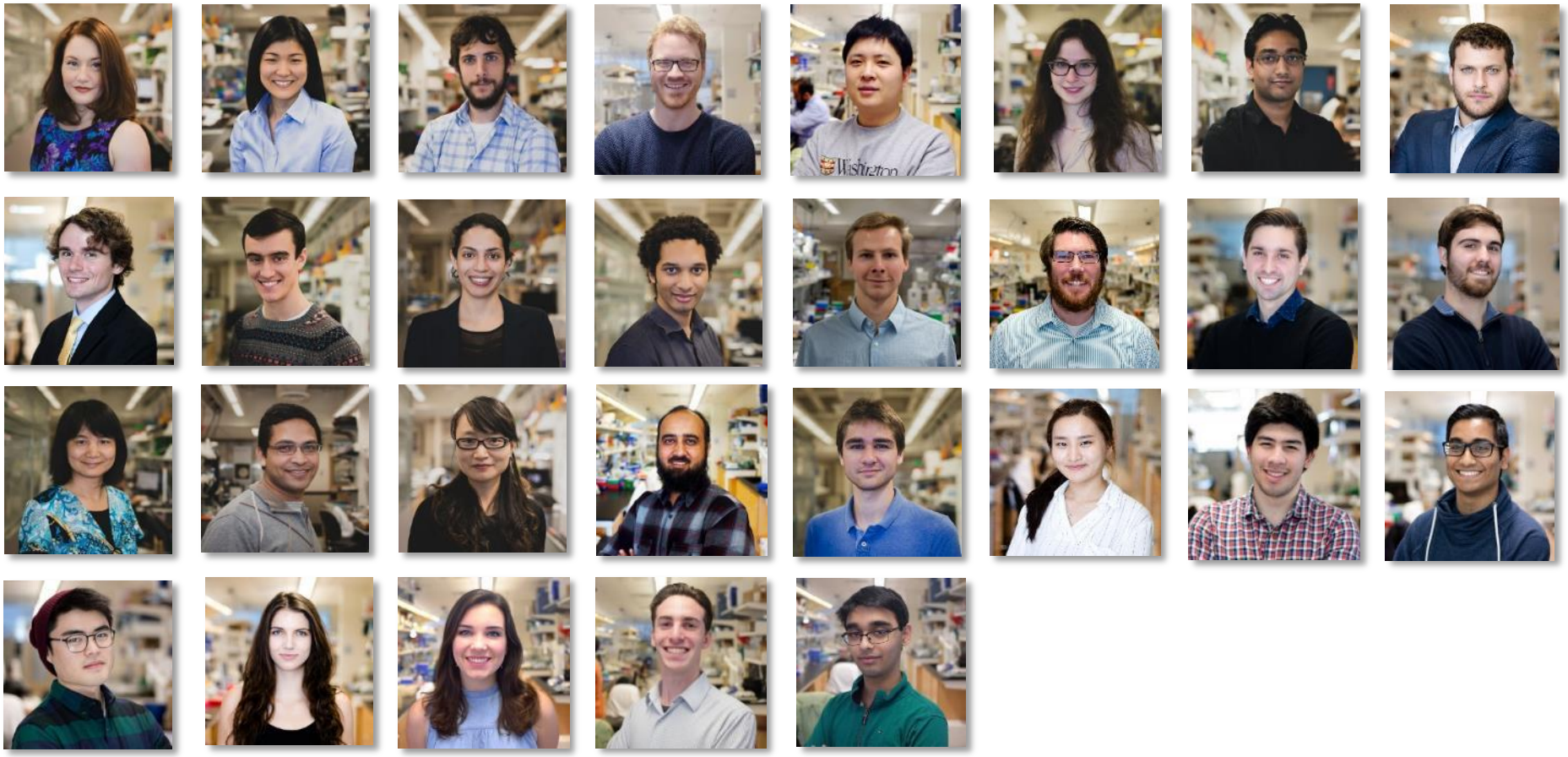





DANTAS LAB

Washington University in St.Louis

www.dantaslab.org



Methods for studying antibiotic resistance in microbial communities

KNOWN

CULTURE & PHENOTYPE

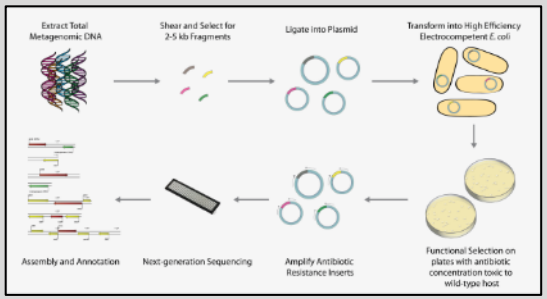
- Clinical resistance levels
- Direct clone to (multidrug) resistance connection
- Culture bias

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**)

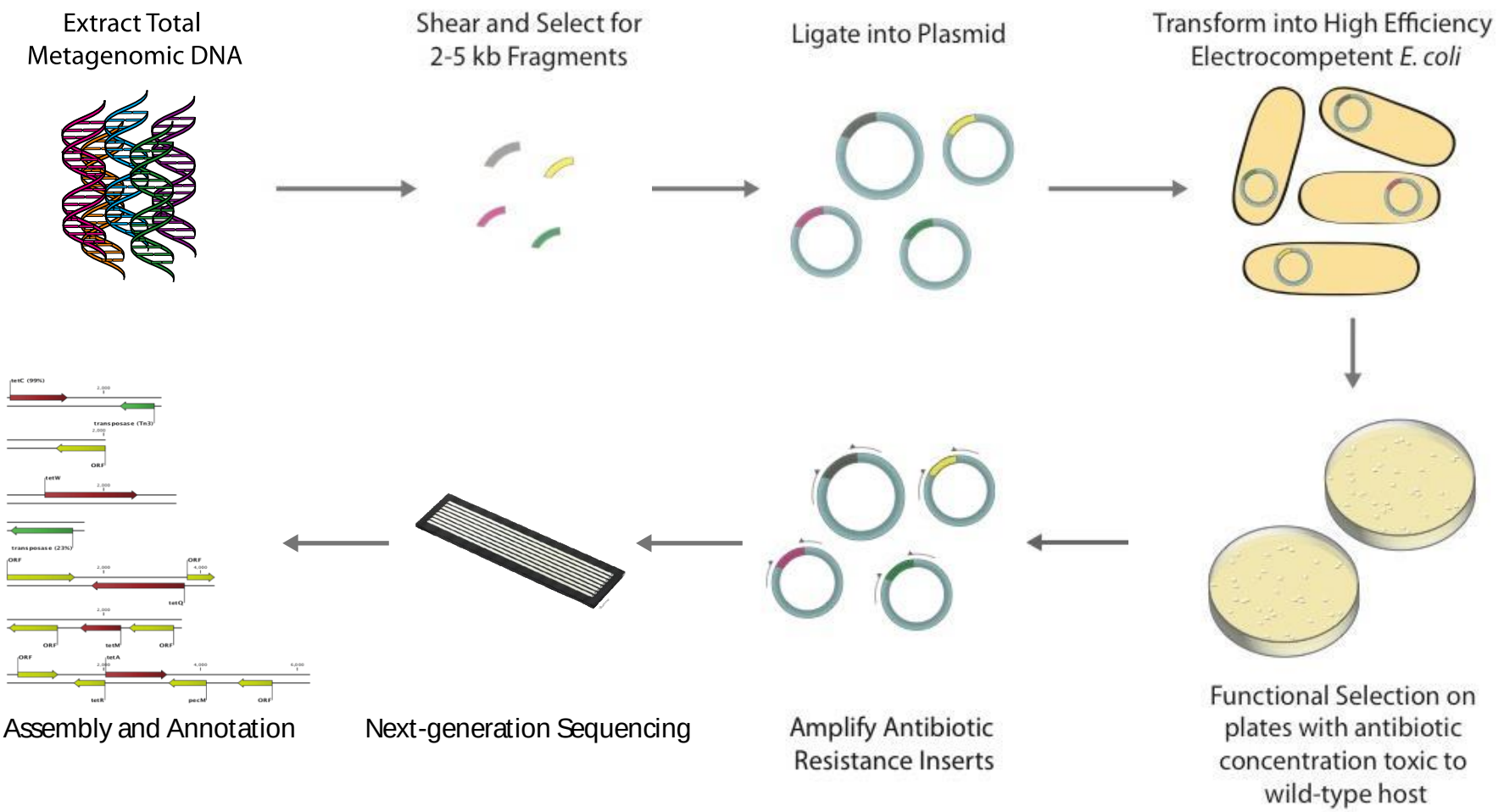


FUNCTIONAL METAGENOMIC SELECTIONS

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

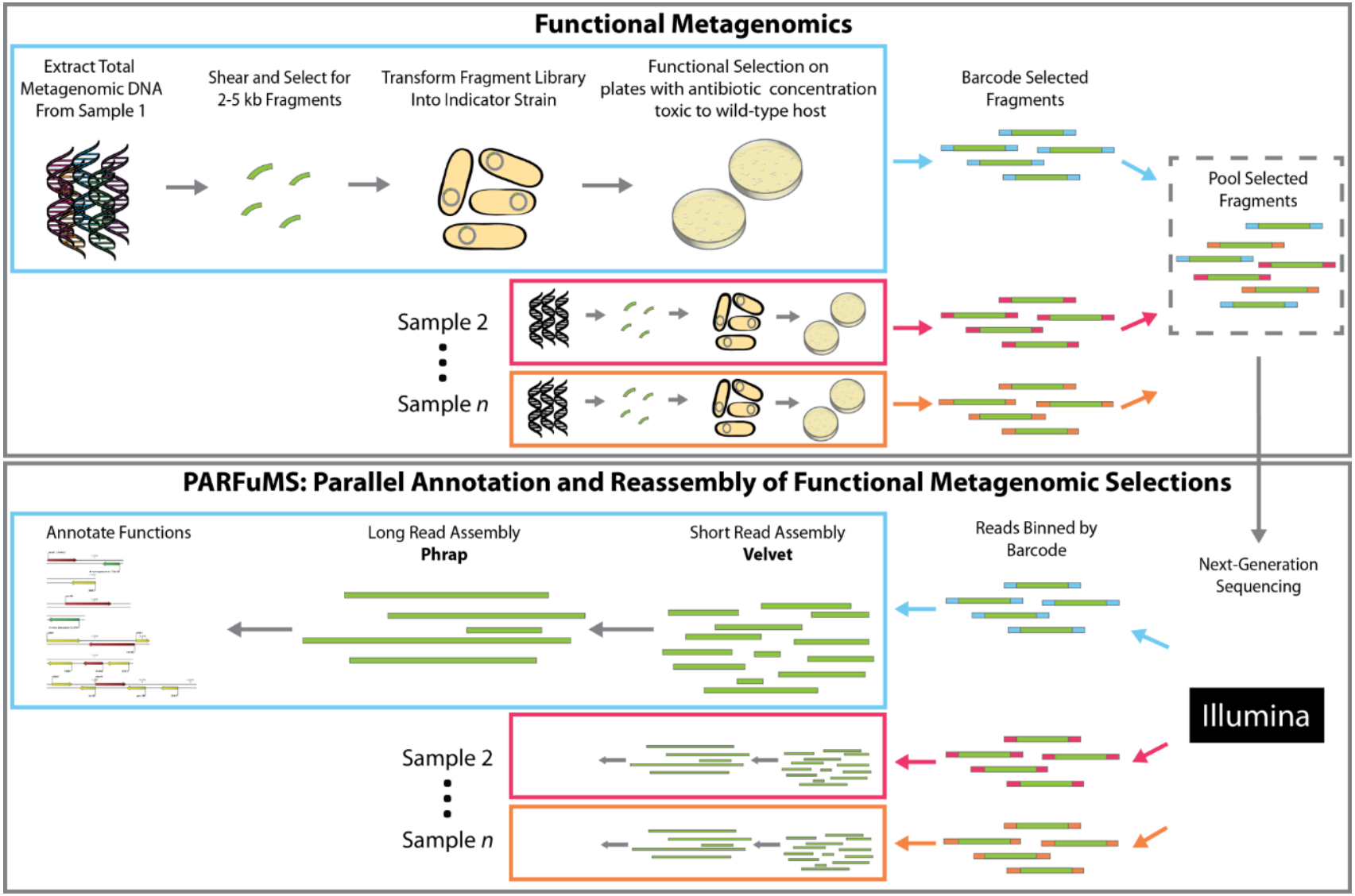
UNKNOWN

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

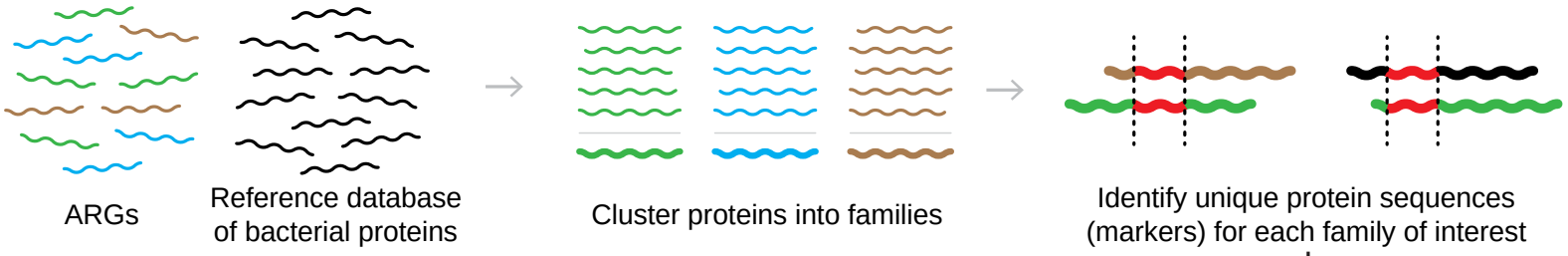


Applied in *Sommer et al.* **Science** *(2009); Forsberg et al.* **Science** *(2012); Forsberg et al.* **Nature** *(2014); Clemente et al.* **Science Advances** *(2015); Moore et al.*, **Microbiome** *(2015); Gibson et al.* **Nature Microbiology** *(2016); Pehrsson et al.*, **Nature** *(2016)*

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

ShortBRED: improved annotation accuracy from short reads

1. Create a short protein marker database for functionally selected antibiotic resistance genes (ARGs)

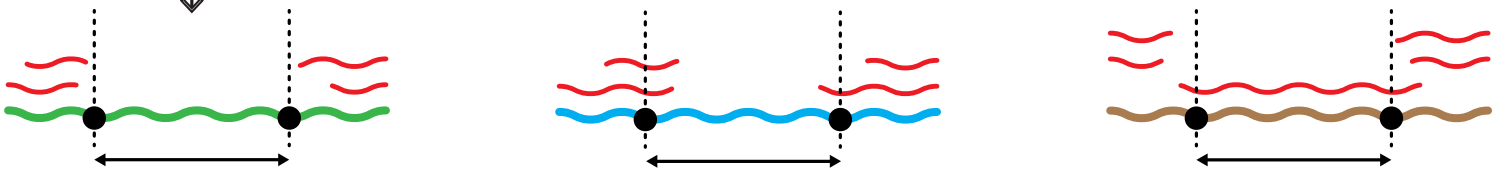


ARGs

Reference database of bacterial proteins

Cluster proteins into families

Identify unique protein sequences (markers) for each family of interest

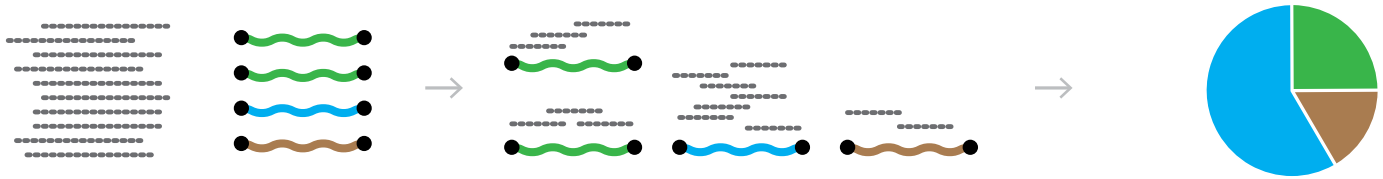


True Markers: no overlap with any reference sequence or consensus

Junction Markers: minor overlap with any reference sequence or consensus

Quasi Markers: least overlap possible; overlapping families are merged

2. Quantify relative abundance of antibiotic resistance genes (ARGs) in whole metagenome shotgun data

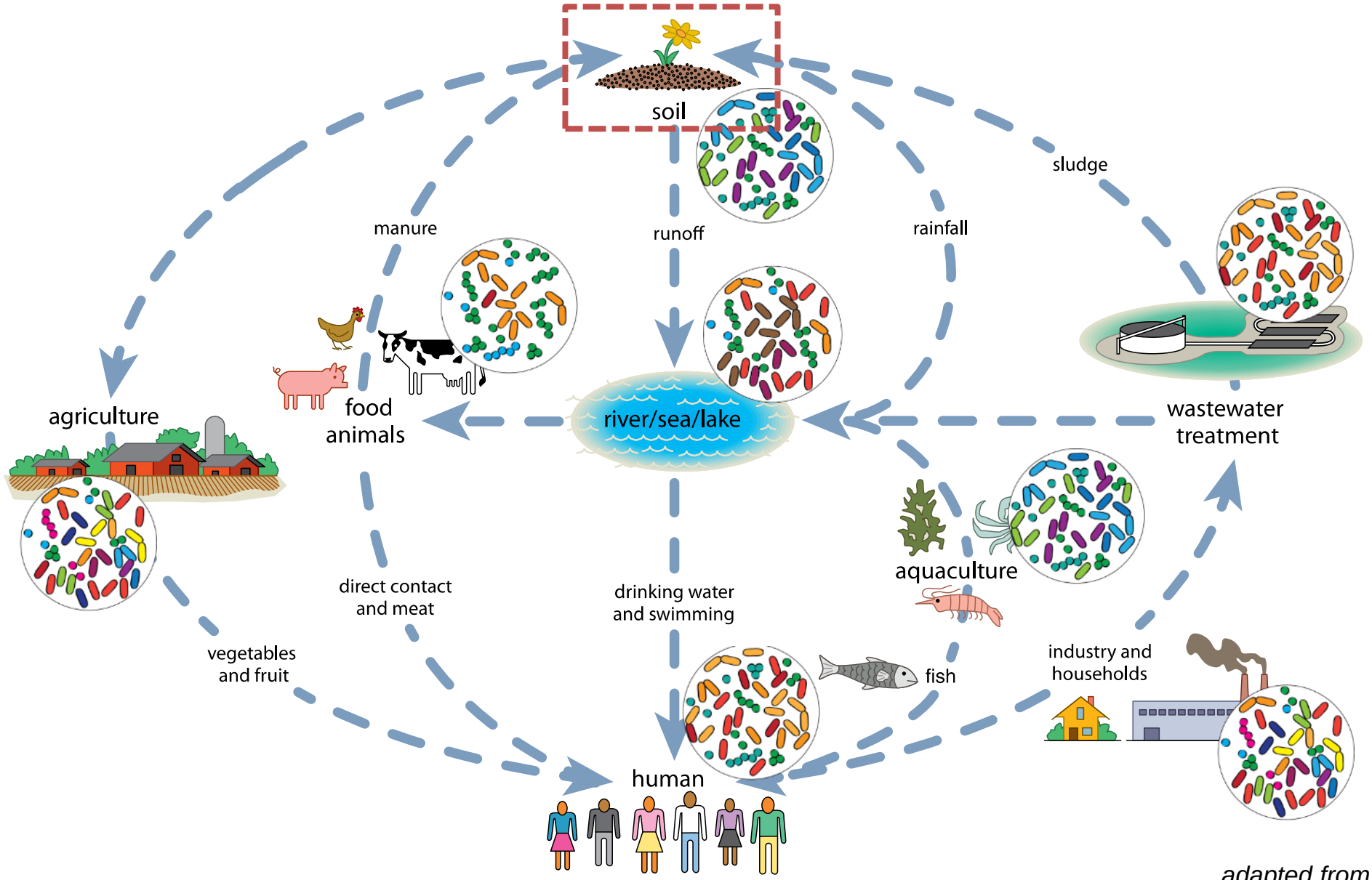


Map short reads to unique markers using USEARCH

Normalize results to produce a relative abundance profile for ARGs across samples

Kaminski, Gibson*, Franzosa, Segata, Dantas*, Huttenhower*.* ***PLoS Comp. Bio.*** *(2015)*

Transmission networks of microbiomes and resistomes across habitats

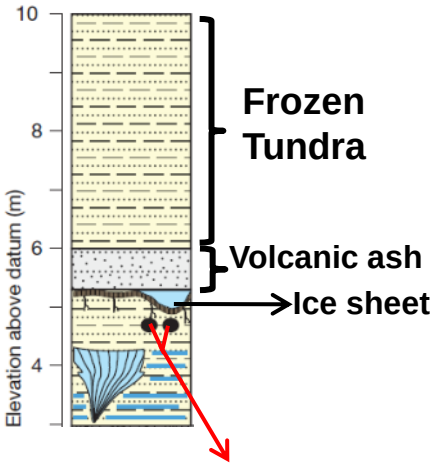


adapted from:
Dantas and Sommer, ***American Scientist*** *(2014)*

The soil resistome is ancient, diverse, and growing:

Is it in RECENT exchange with pathogens?

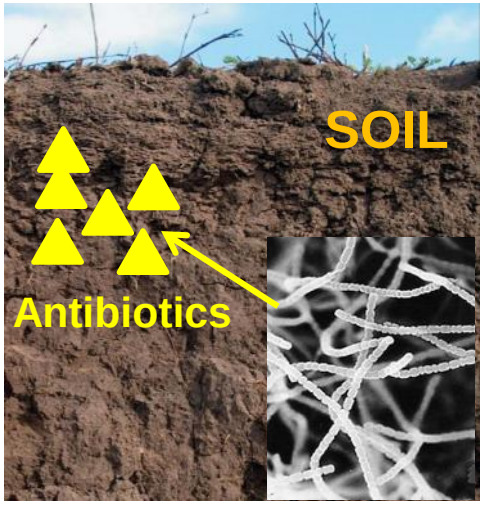
Antibiotic resistance is ancient
*D'Costa…Wright, **Nature** (2011)*



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

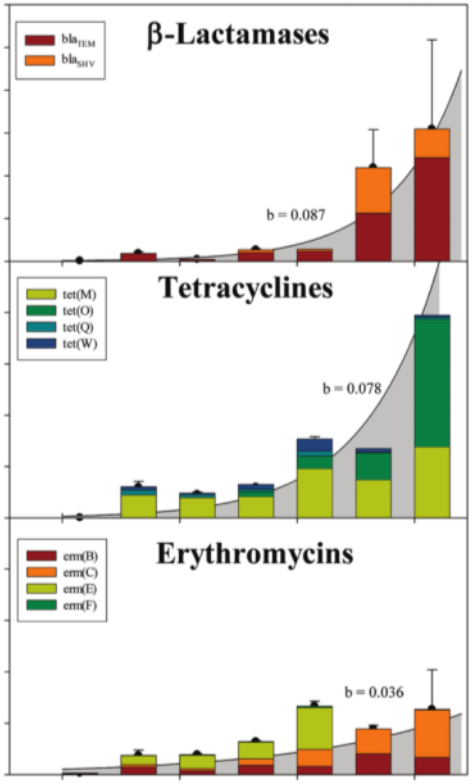
Producer Hypothesis
*Benveniste, Davies, **PNAS** (1973)*



Streptomyces

- **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 70+ years**

Antibiotic catabolizers are even more multidrug resistant than producers

11 U.S. soils
(urban, farm, pristine)

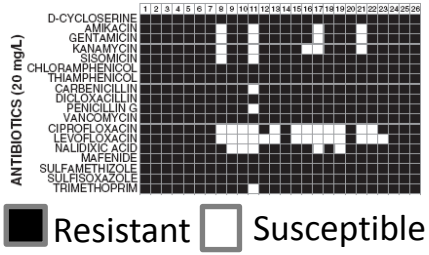
Media + 1000µg/mL of 18 antibiotics

Abx passage 7 days

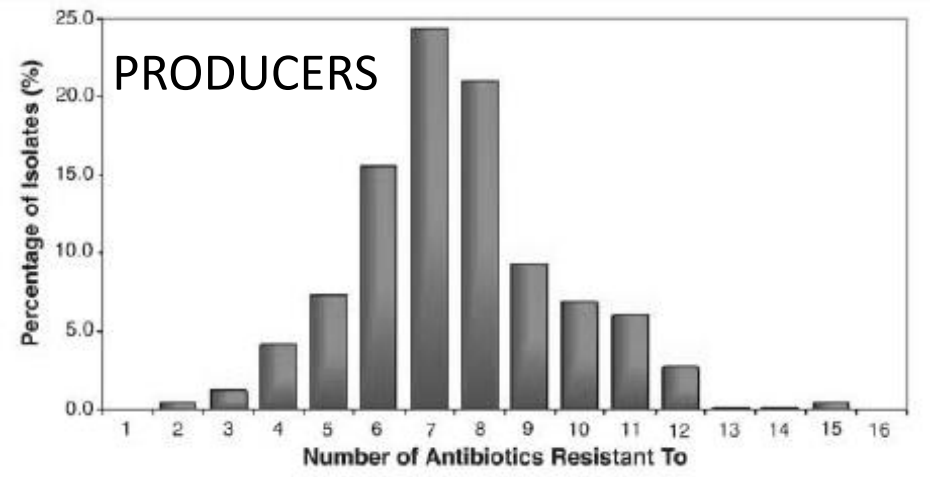
Abx Passage 7 days

Abx Passage 7 days

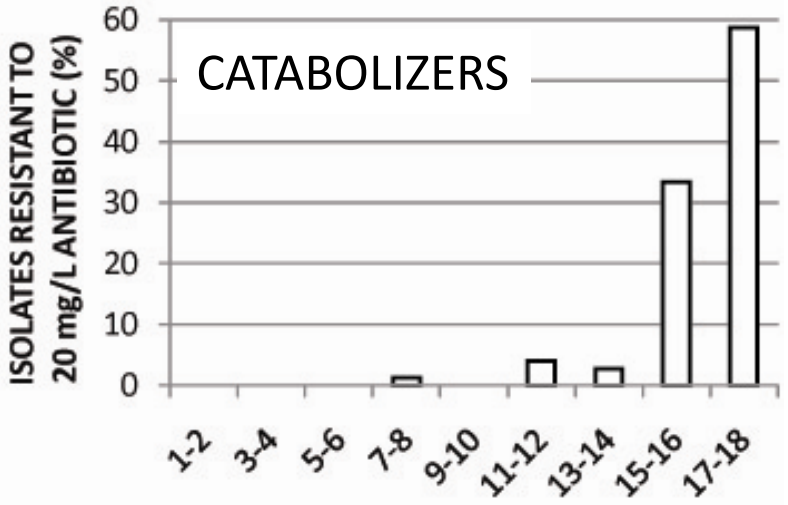
Test abx resistance



Resistant Susceptible



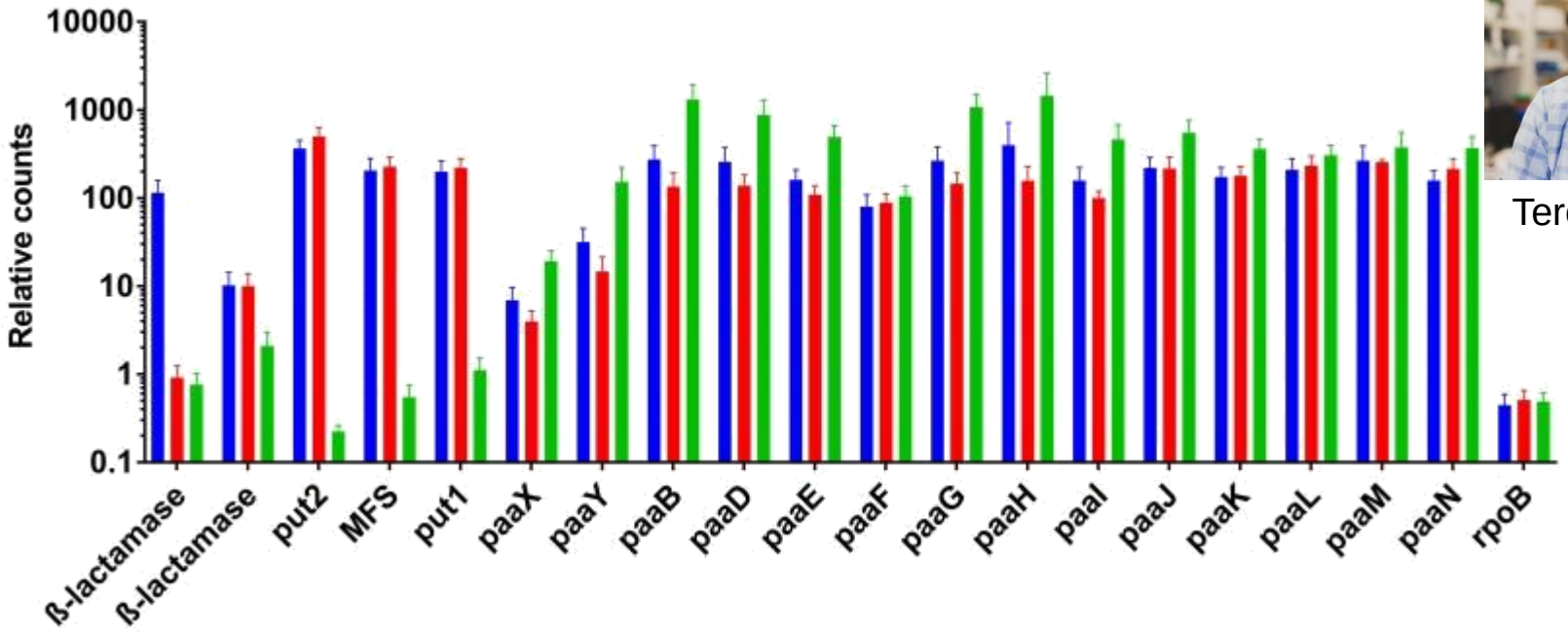
D'Costa et al,, ***Science*** *(2006)*

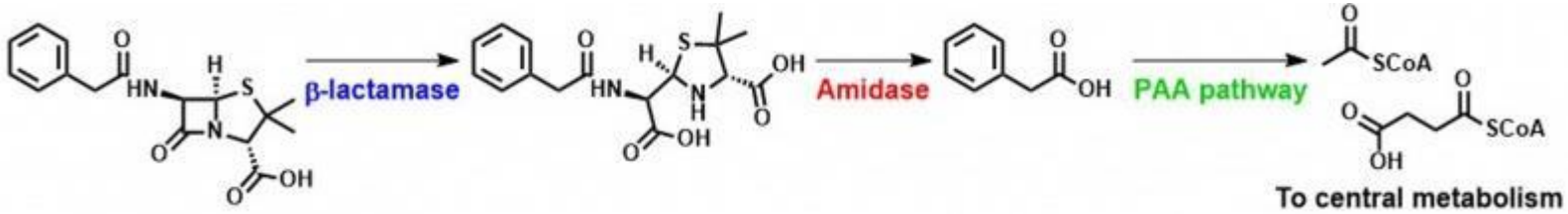


Dantas, Sommer, et al,, ***Science*** *(2008)*

Characterizing the mechanism of β-lactam catabolism by soil isolates:
ABC07 consumes penicillin *via* β-lactamase, amidase, and phenylacetic acid operon

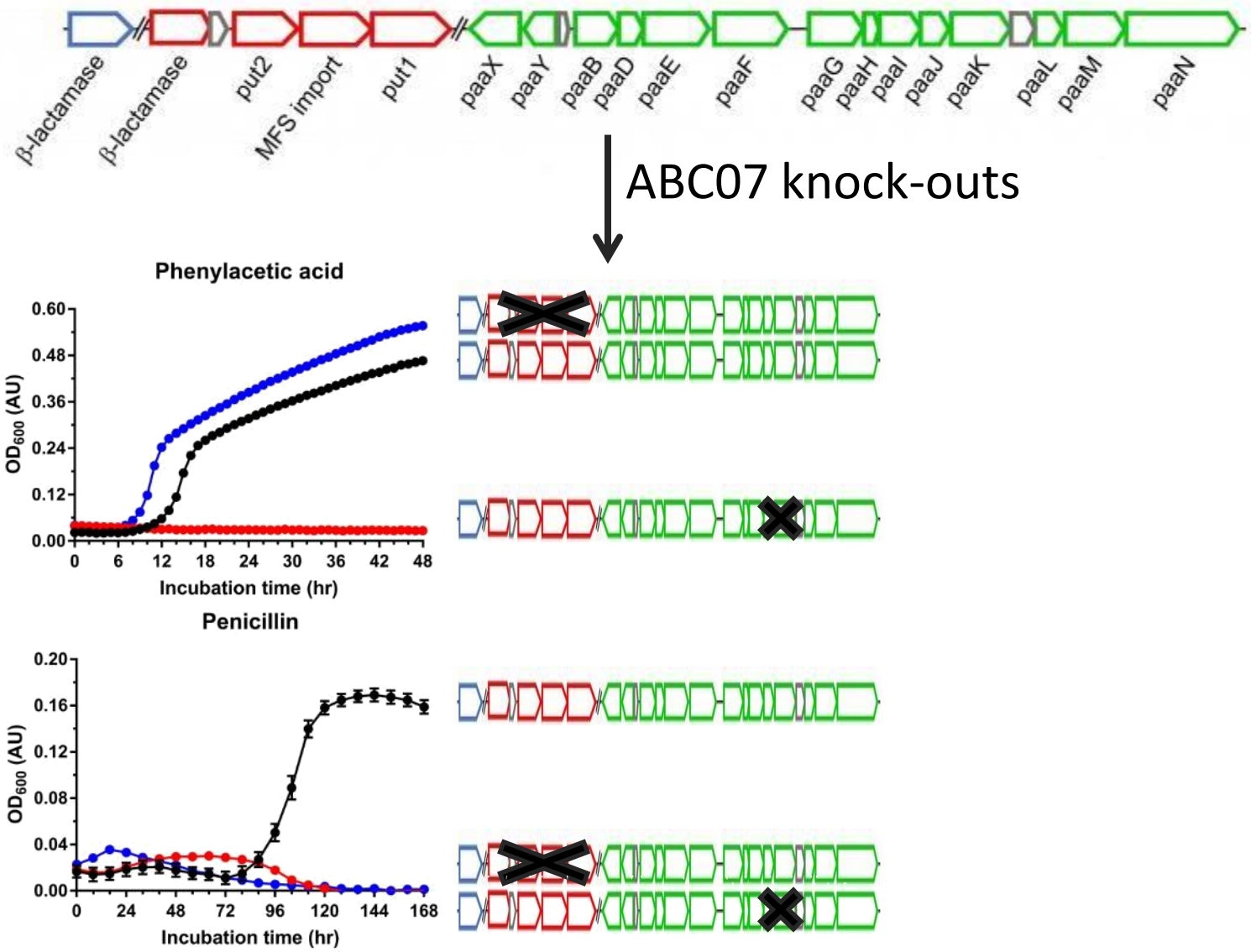
Terence Crofts





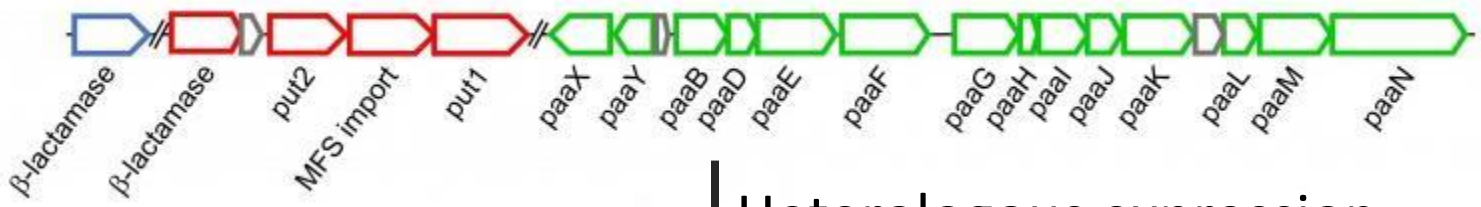
Crofts, Wang, Spivak, Gianoulis, Forsberg, Gibson, Johnsky, Broomall, Rosenzweig, Skowronski, Gibbons, Sommer, Dantas. ***Nature Chemical Biology,*** *(2018)*

Phenylacetic acid and penicillin utilization (*put*) operons are **necessary** for growth on penicillin in ABC07



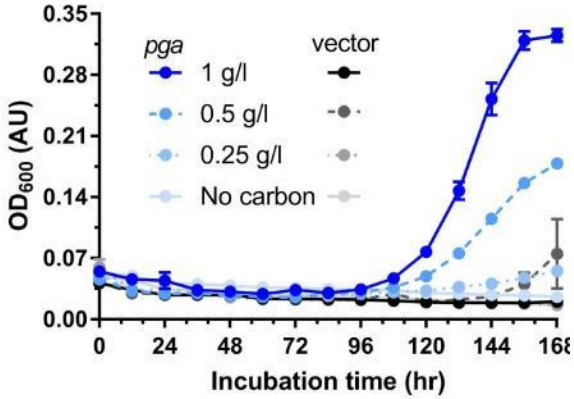
Crofts et al. ***Nature Chemical Biology,*** (*2018*)

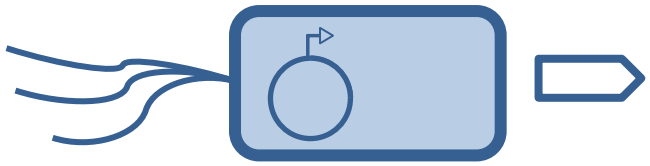
put operon or penicillin amidase (*pga*) expression are **sufficient** for improved growth on penicillin or benzylpenicilloic acid in *E. coli*



Heterologous expression

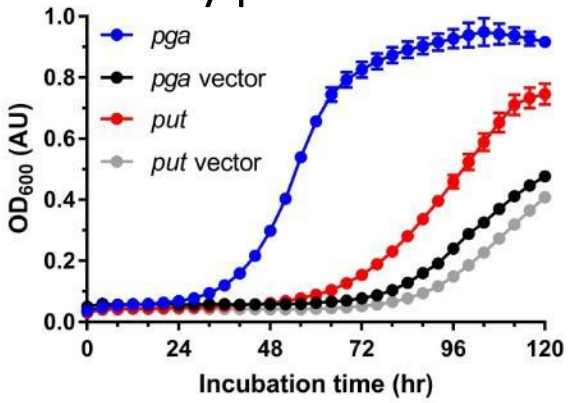
Penicillin

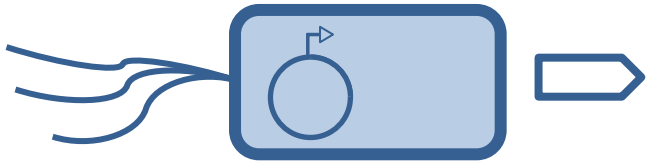




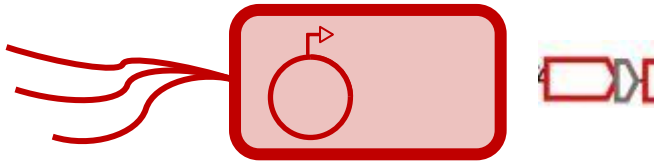
E. coli w/ ***paa*** **operon**
+ ***NATIVE*** **penicillin amidase**

Benzylpenicilloic acid





E. coli w/ ***paa*** **operon**
+ ***NATIVE*** **penicillin amidase**



+ ***put*** **operon**

Crofts et al. ***Nature Chemical Biology,*** *(2018)*

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

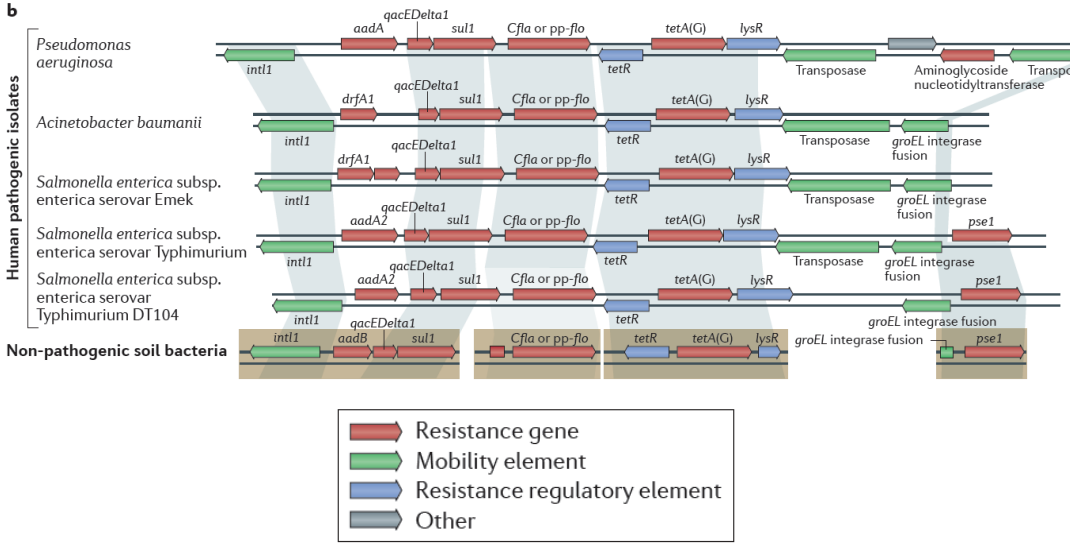
The Shared Antibiotic Resistome of Soil Bacteria and Human Pathogens

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

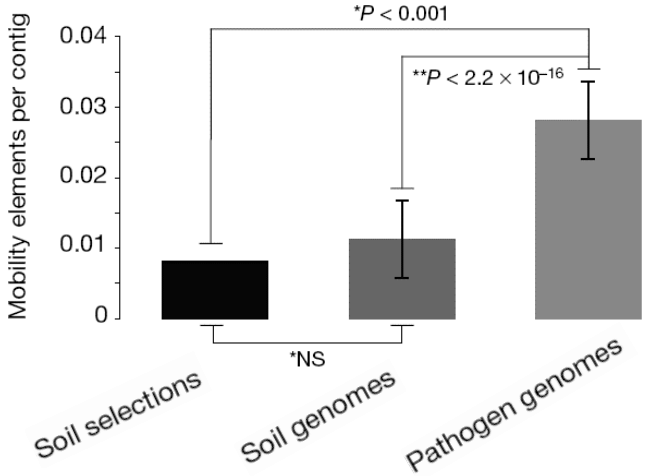
Bacterial phylogeny structures soil resistomes across habitats

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

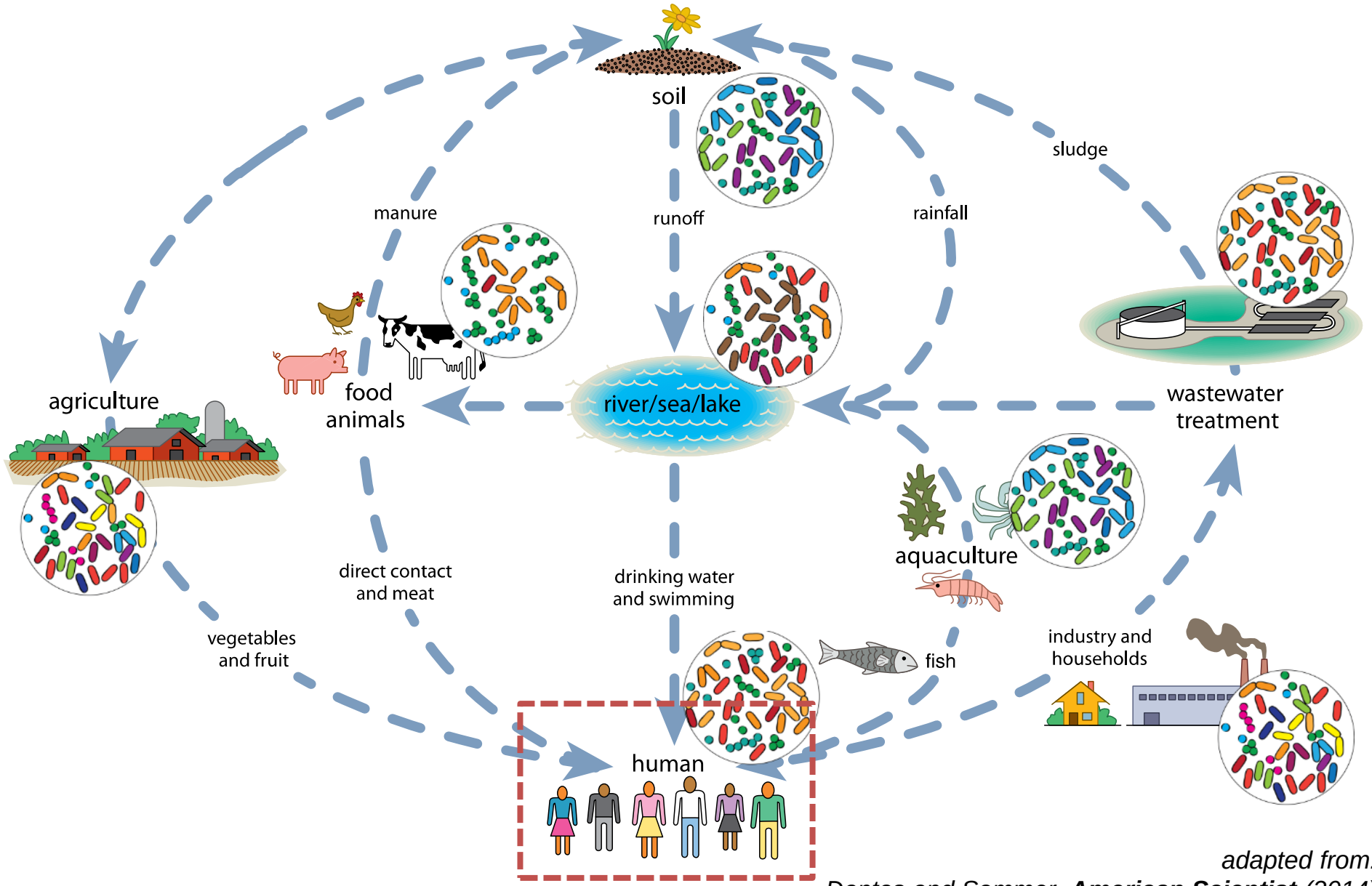
Kevin Forsberg

Sanket Patel



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

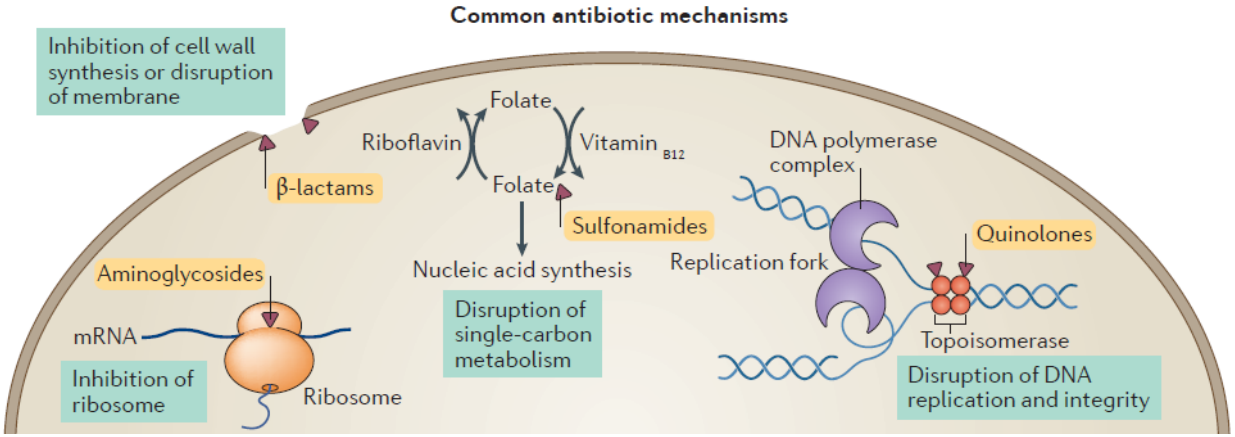
Transmission networks of microbiomes and resistomes across habitats



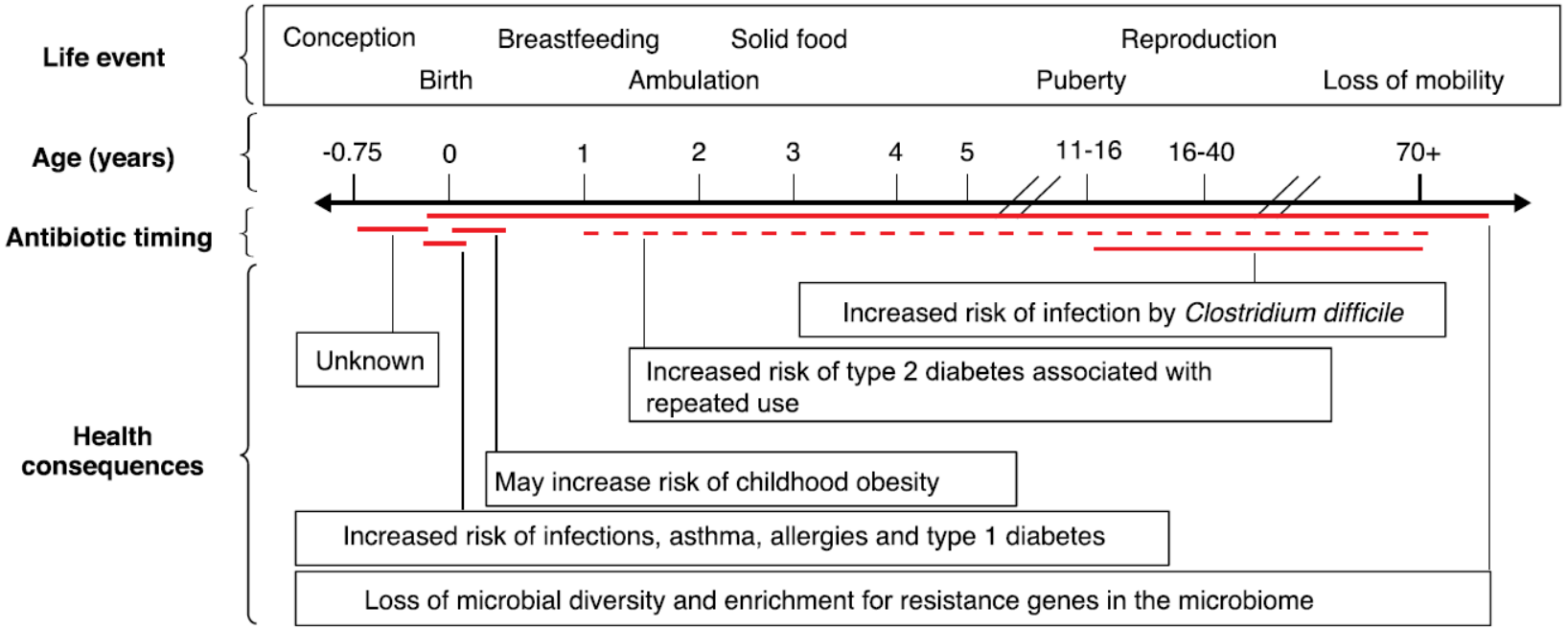
adapted from:
Dantas and Sommer, ***American Scientist*** *(2014)*

Resistance spreads across habitats

Antibiotic perturbation of the human microbiome can be dysbiotic

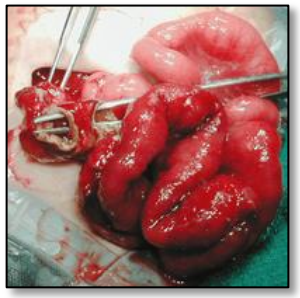


Crofts, Gasparrini, Dantas. **Nature Reviews Micro** *(2017)*

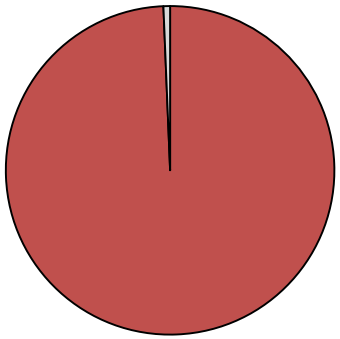


Langdon, Crook, Dantas. **Genome Medicine** *(2016)*

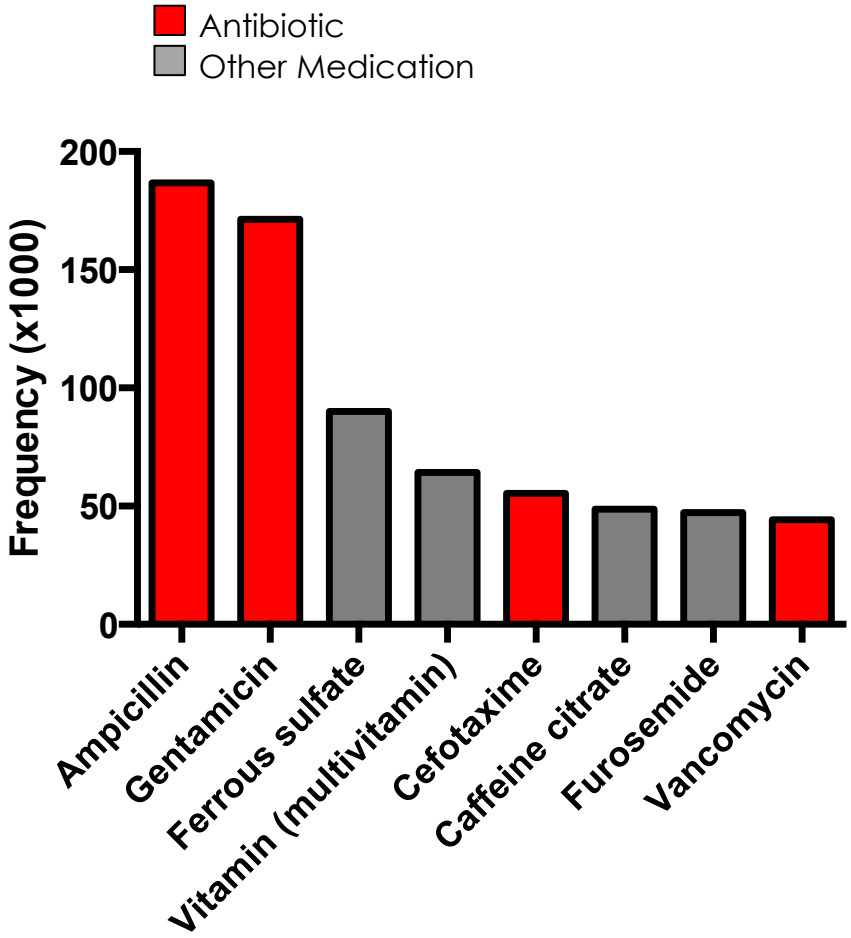
Antibiotics are the most prescribed medication for preterm infants



Preterm birth is **leading cause of infant death**
Preterm infants are highly susceptible to infections



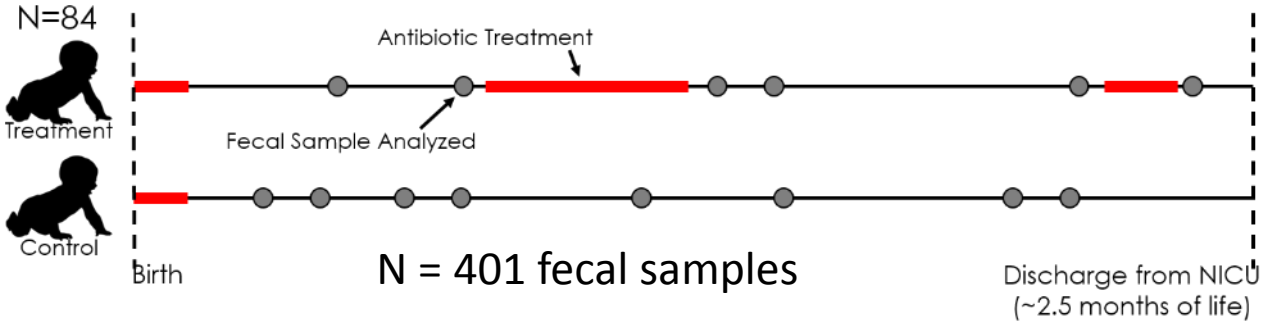
99%
of VLBW infants receive
antibiotics
in the
1st two days of life

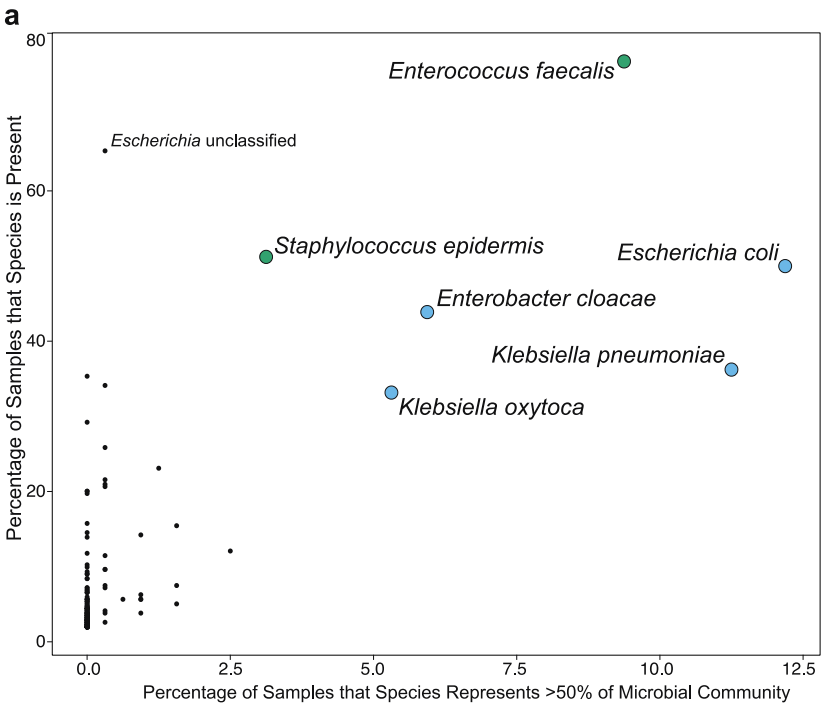


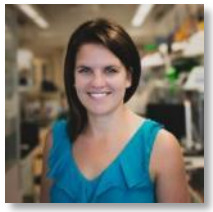
Gasparrini, Crofts*, Gibson, Tarr, Warner, Dantas.* ***Gut Microbes****, (2016)*

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

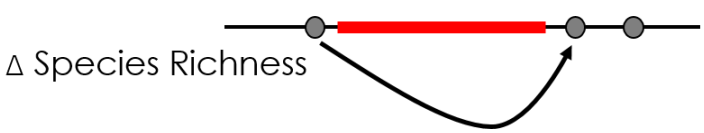
Preterm birth is **leading cause of infant death**

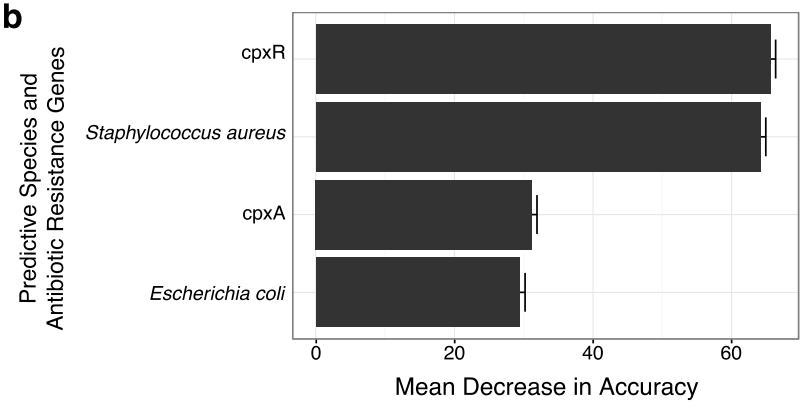






Molly Gibson

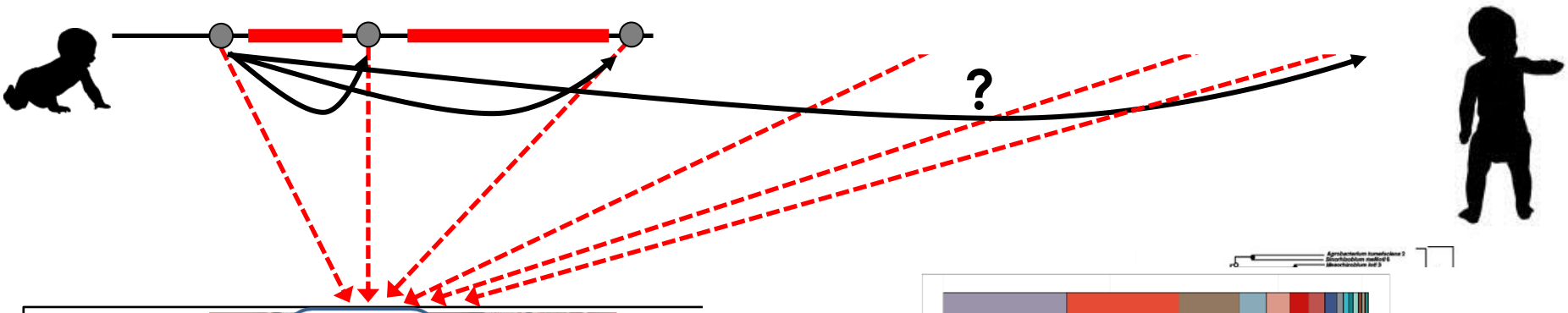


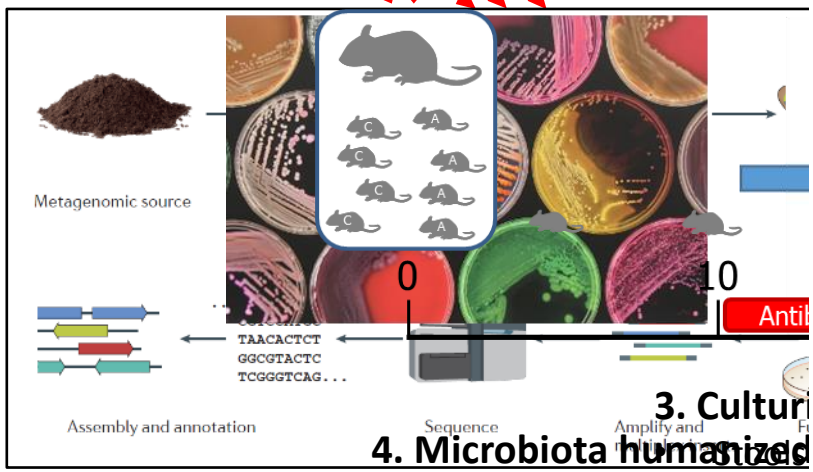


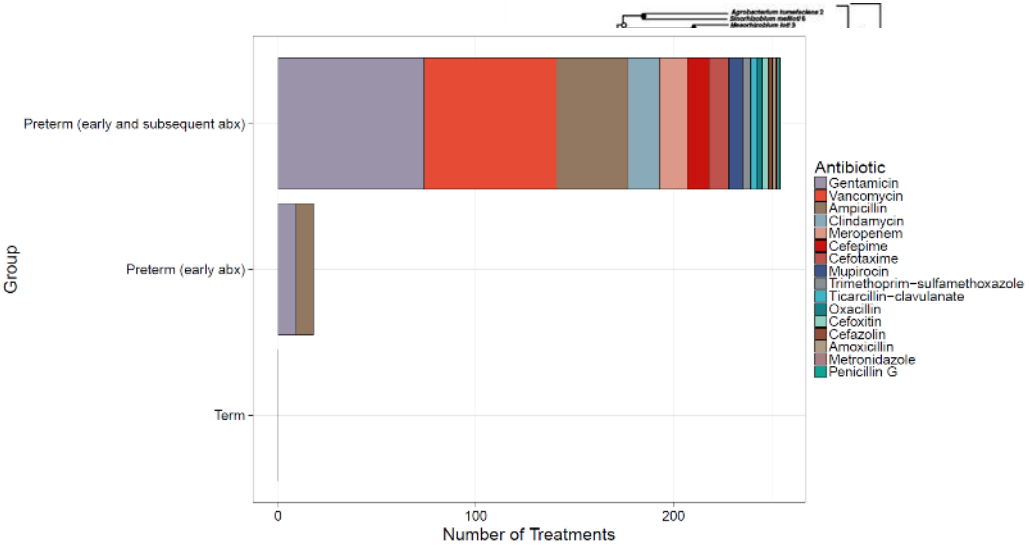
85% prediction accuracy based on 4 variables

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

Next steps: Persistent perturbations of preterm infant gut microbiome and resistome?







3. Culturi

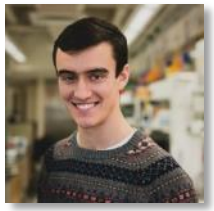
4. Microbiota humanized

2. Functional metagenomics

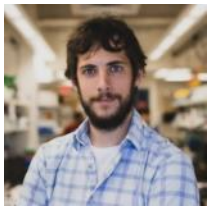
- 22 libraries from 448 stools (308 Gb bacterial DNA)
- Screened on 16 antibiotics and sequenced
- Resistance gene assembly and annotation in progress

- 558 isolates sequenced and assembled (*E. cloacae*, *E. faecalis*, *E. coli*, *K. pneumoniae*, *K. oxytoca*, *S. epidermidis*)

1. Microbiome composition
2. Resistome composition
3. MDRO carriage
4. Validate findings in animal model

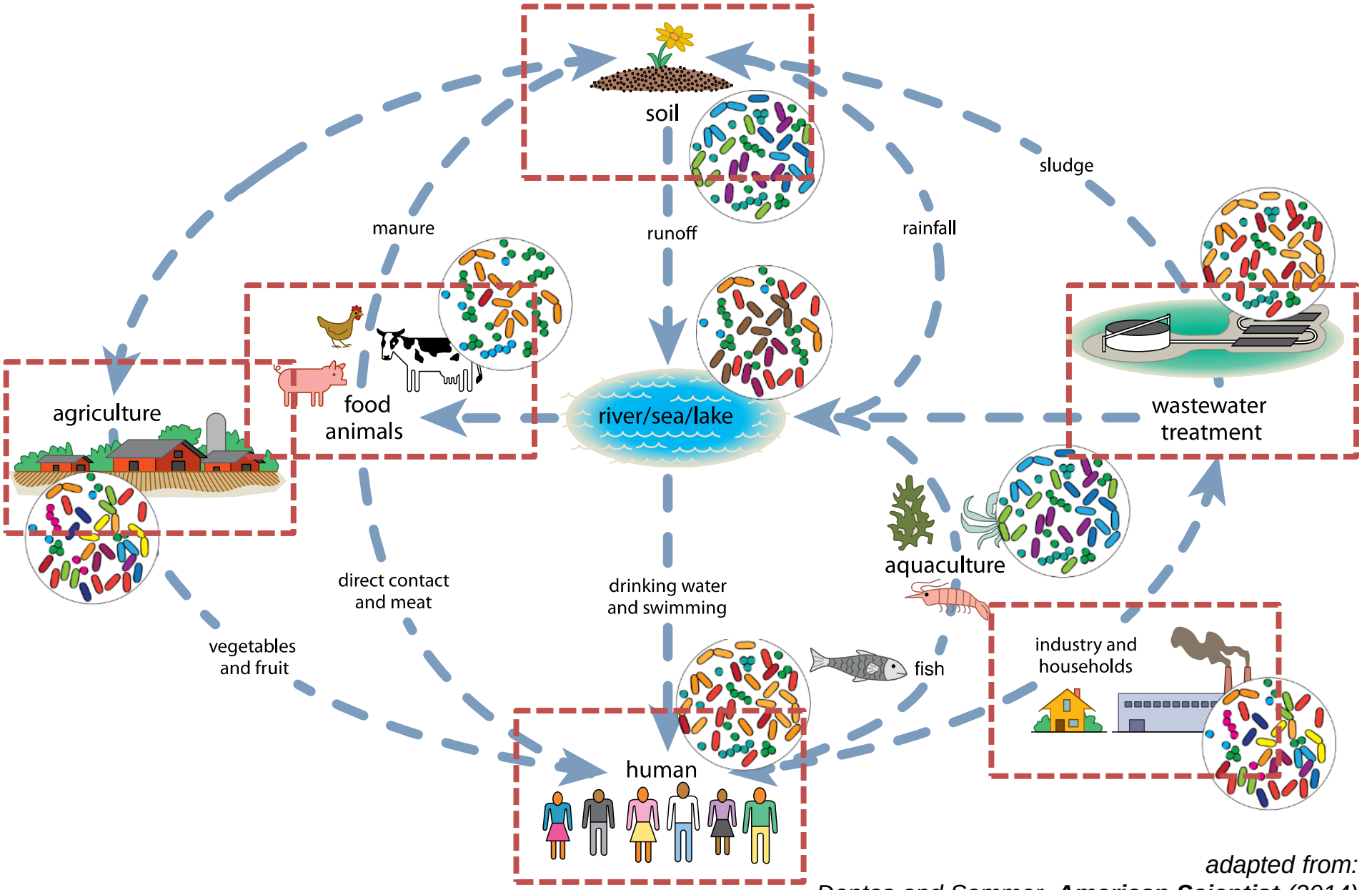


Drew Gasparrini



Terence Crofts

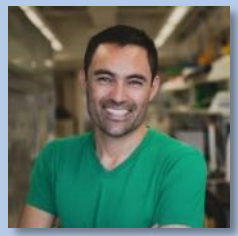
Transmission networks of microbiomes and resistomes across habitats



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

Cross-Habitat Resistome Dynamics in Resource-Poor Human Environments in Central and South America

Erica Pehrsson

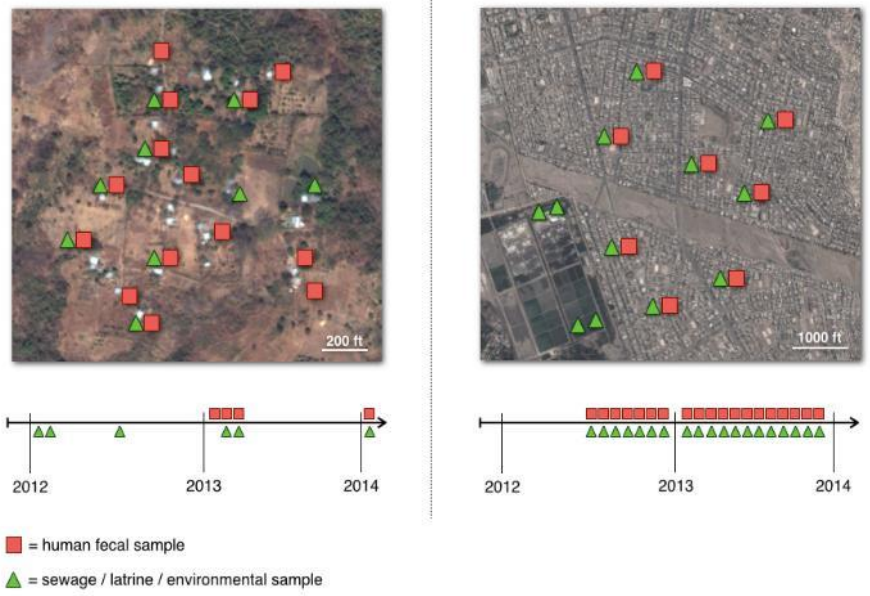


Pablo Tsukayama

Dissemination of bacteria and antibiotic resistance genes across interconnected habitats in low-income settings in Latin America

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

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



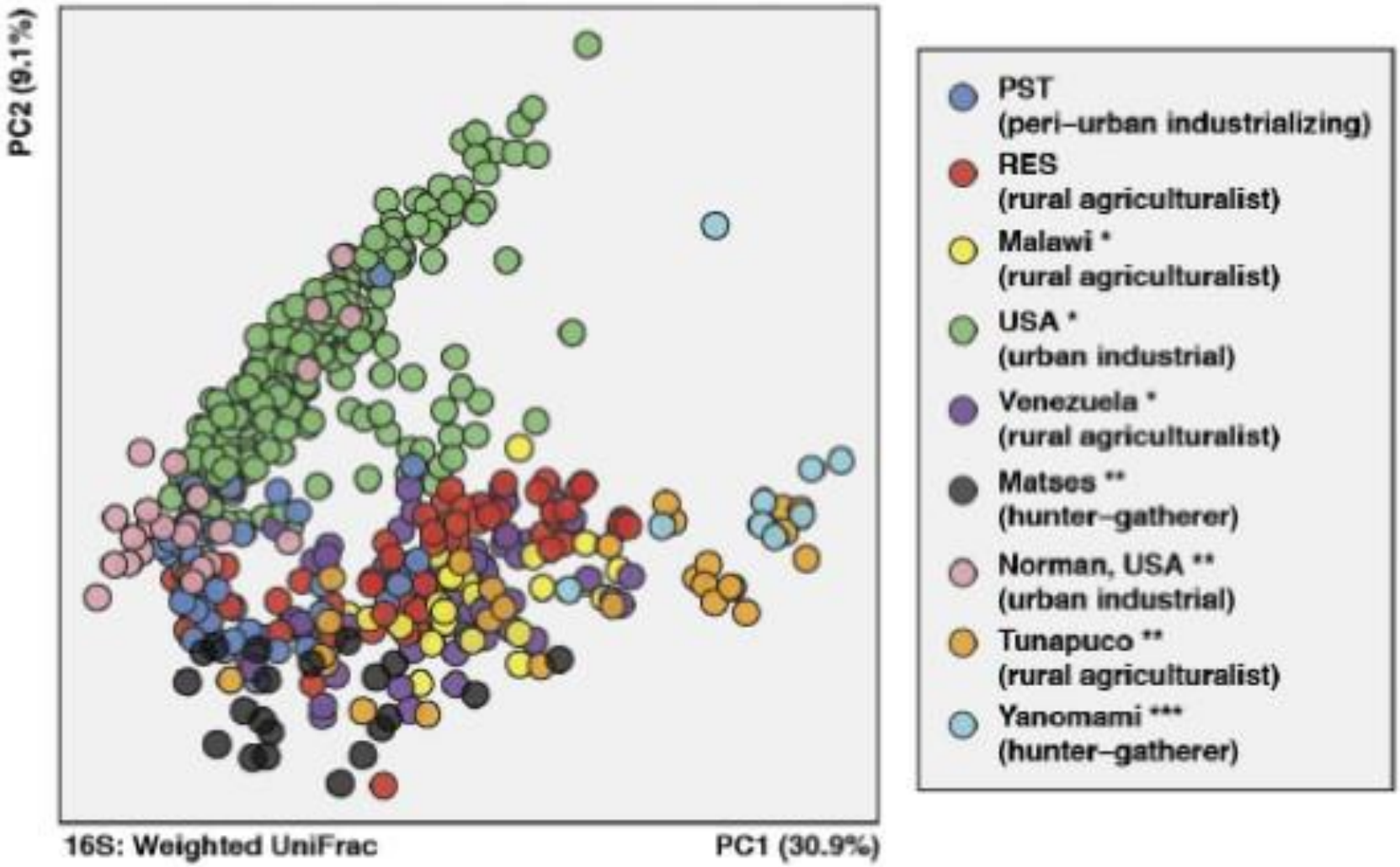
- **Most microbiome studies from extremes of global population**: industrialized nations or remote hunter-gatherers
- **Low-income, resource-poor** settings represent **2/3rd of global population**
- **Models** for **global population growth** (pop. density, living conditions)
- **Lower hygiene standards = higher rates** of **infectious disease** and **bacterial exchange**
- Frequent **misuse of antibiotics**

SAMPLES ANALYZED FROM RES and PST:

- **263 fecal samples** from 115 individuals from 27 houses
- **209 environmental samples** from animal feces, soils, water, sewage

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

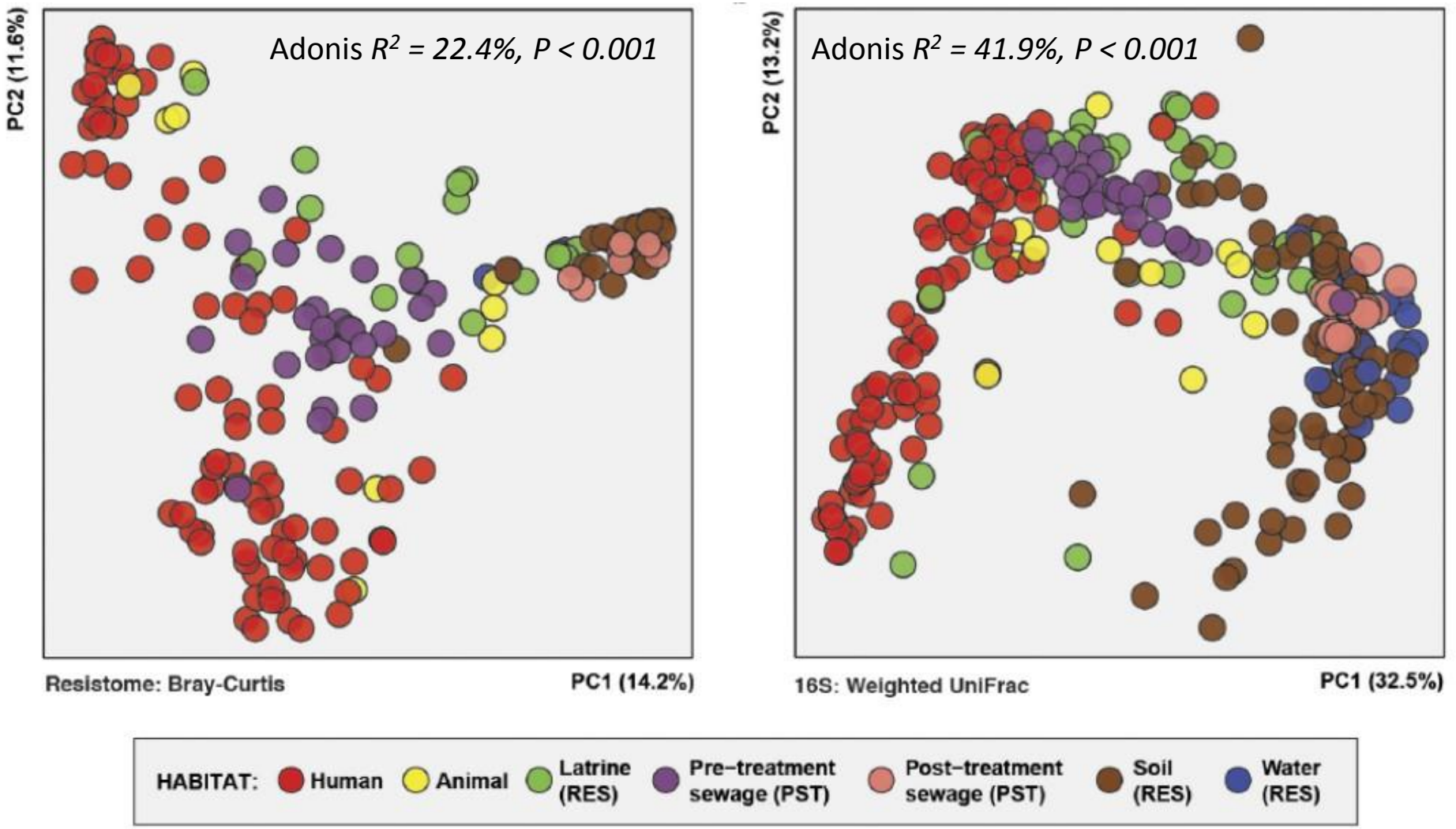
El Salvador and Peru cluster with other human gut microbiota by lifestyle across industrializing gradient



Adonis R^2 = 37.6%, $P < 0.001$

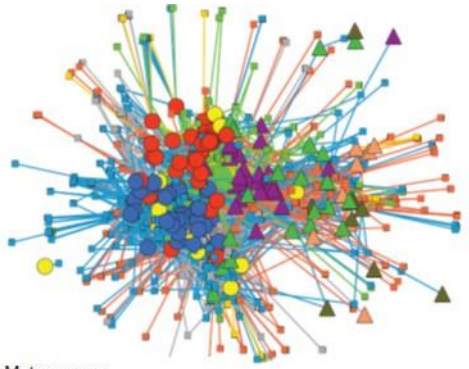
Pehrsson, Tsukayama*, et al.* ***Nature*** *(2016)*

El Salvador and Peru microbiomes and resistomes cluster by habitat



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

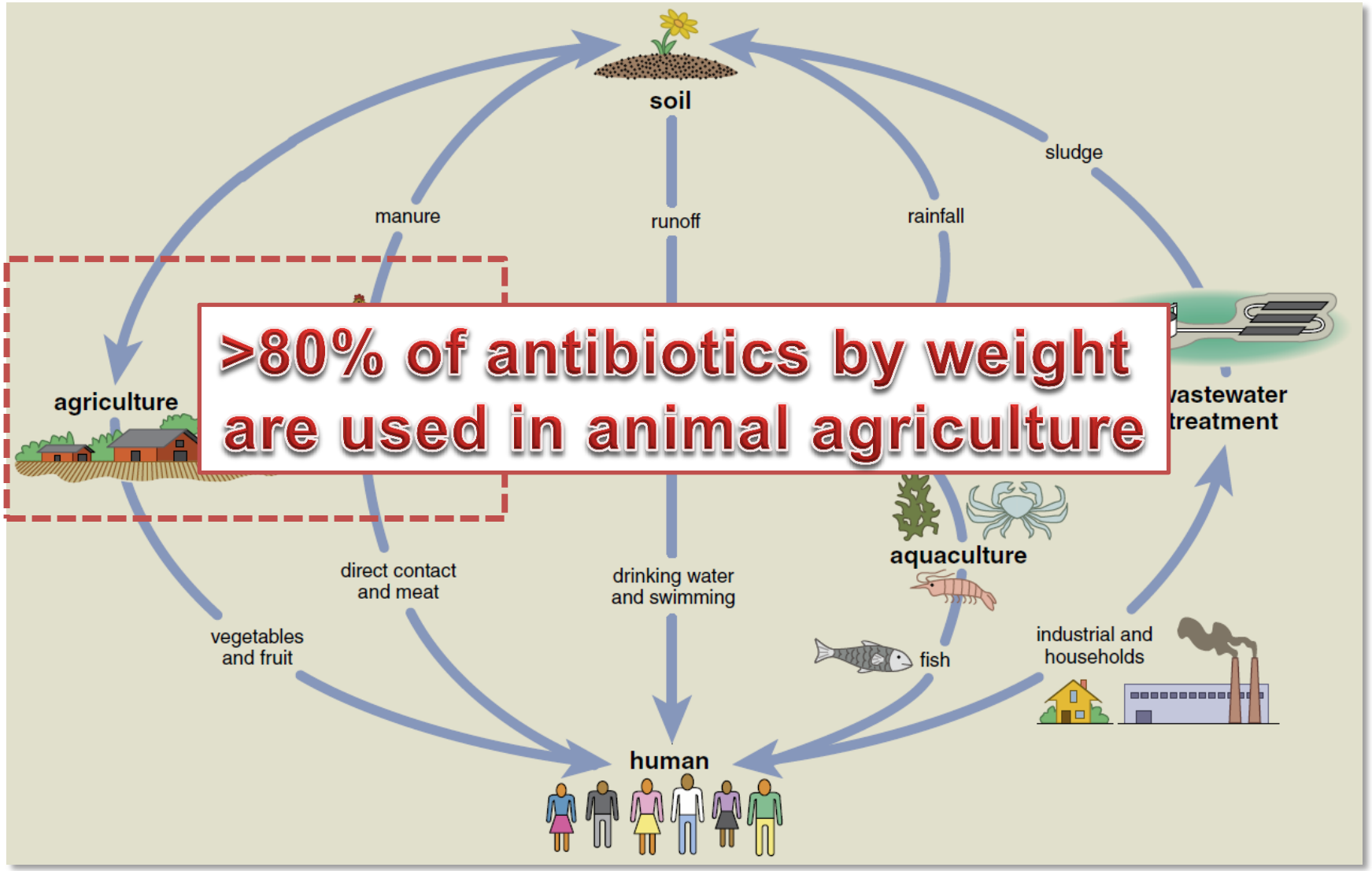
Identification of resistome dissemination hotspots may help with surveillance



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

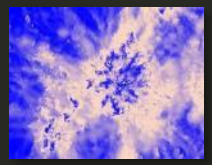
Pehrsson, Tsukayama*, et al.* ***Nature*** *(2016)*

Antibiotic resistance is an ECOLOGICAL problem

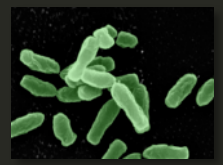


Dantas & Sommer, ***American Scientist*** *(2014)*


RIGHT TO ASSEMBLE
Dantas Lab



Acknowledgements



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External:

- Saadia Andleeb (NUST)
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- William Hoyos (UJMD)
- Sanjay Shukla (Marshfield)
- Mark Simons (US NMRC)

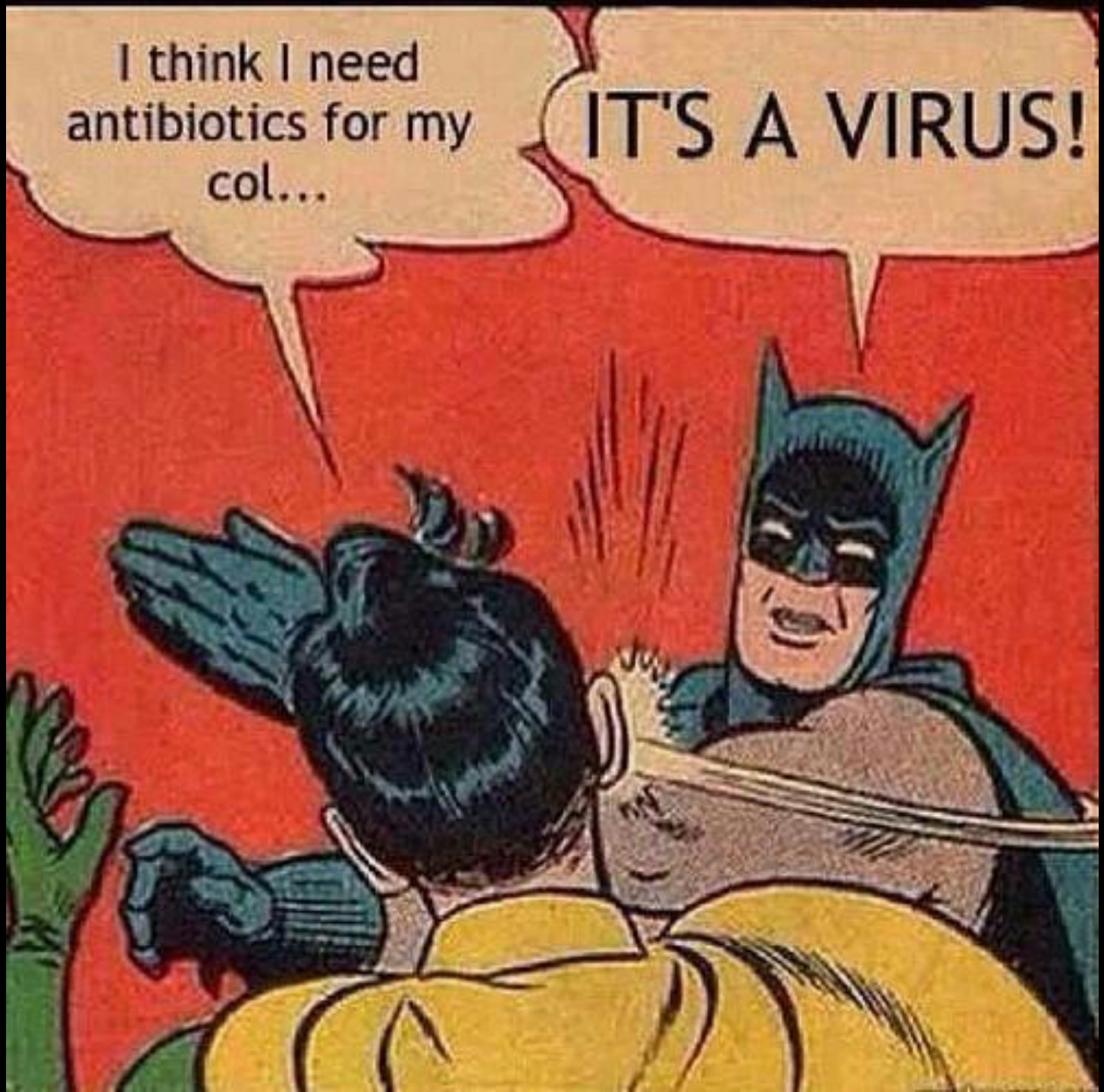
Financial Support

Current:

- CDC Prevention Epicenter
- CDC OADS BAA
- DOE Biosys-Design (BER)
- Mallinckrodt Foundation
- Gates Foundation (GCE)
- NIH R01 (NIAID)
- NIH R01 (NICHD)
- USAID/NAS Pak-US

Completed:

- BJHF-CTS
- CDI (IRI)
- I-CARES
- Kenneth Rainin Foundation
- March of Dimes Foundation
- MSF Epicentre
- National Academies KFI
- NIH R01 (NIGMS)
- NIH Director's New Innovator Award (NIDDK)
- DOE SysBio (BER)


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