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

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Clinical resistance rapidly follows deployment for ALL antibiotics
Antibiotic Resistant Infections Are A Leading Cause of Death

700,000 deaths in 2014

10 Million estimated deaths in 2050

(Treatment of Antibiotic Resistant Infections Is Expensive)

$55 Billion cost to the US economy in 2013

$100 Trillion estimated cost to global economy by 2050

(Resistant Infections Are Increasing BUT New Antibiotic Discovery Is Decreasing)

(UK Prime Minister’s AMR Report, 2014)

$55 Billion (US CDC, 2013)

$100 Trillion (UK Prime Minister’s AMR Report, 2014)

(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

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

**Antibiotic Resistance Reservoir (RESISTOME)**

- Known, Readily Cultured
- Known, Not Readily Cultured
- Unknown
Functional metagenomic selections identify novel antibiotic resistance genes in microbial communities

Increasing functional metagenomic throughput via next-gen sequencing


Transmission networks of microbiomes and resistomes across habitats

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

Soil Proteobacteria share MULTIDRUG resistance gene clusters with human pathogens

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

Adapted from Hillen (2002)

BUT cryptic soil resistance genes are still clinically-relevant *e.g. tetracycline resistance*
Target- and Resistance-Based Mechanistic Studies with TP-434, a Novel Fluorocycline Antibiotic

Trudy H. Grossman, Agata L. Starosta, Corey Fyfe, William O’Brien, David M. Rothstein, Aleksandra Mikolajka, Daniel N. Wilson, and Joyce A. Sutcliffe

Gene Center, Department of Biochemistry, and Center for Integrated Protein Science Munich (CIPSM), University of Munich, Germany, and Tetraphase Pharmaceuticals, Inc., Watertown, Massachusetts, USA

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 [IC₅₀] = 0.29 ± 0.09 μg/ml) and [³H]tetracycline ribosome-binding competition (IC₅₀ = 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**”

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

Tetracycline destructases are widespread in diverse metagenomes and pathogens:

69 additional potential tetracycline destructases were computationally predicted from diverse metagenomes:
- Soil
- Gut
- Latrine
- Previously described

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

Tet(X) identified in MDR pathogens:
- E. faecium
- S. aureus
- K. pneumoniae (Leski et al. 2013)
- A. baumanii (Deng et al. 2014)
- P. aeruginosa (Leski et al. 2013)
- Enterobacter spp. (Leski et al. 2013)

Drew Gasparrini

Gasparrini et al., unpublished
Biochemical and structural elucidation of novel mechanism of resistance
(in collaboration with Tim Wencewicz and Niraj Tolia)

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

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
Inhibiting tetracycline destructase activity rescues tetracycline efficacy

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

![Image of petri dishes showing inhibition zones with and without anhydrotetracycline (aTC)]

**Graph**: Theoretical additivity vs. *E. coli* + *tet*(56)

Tet(56) FICI = 0.1875

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

Common antibiotic mechanisms:
- Inhibition of cell wall synthesis or disruption of membrane
- β-lactams
- Aminoglycosides
- mRNA
- Inhibition of ribosome
- Ribosome

Disruption of metabolism
- Riboflavin
- Folate
- Vitamin B12
- Sulfonamides
- Nucleic acid synthesis
- Disruption of single-carbon metabolism
- Replication fork
- DNA polymerase complex
- Topoisomerase
- Quinolones
- Disruption of DNA replication and integrity

Life event:
- Conception
- Birth
- Breastfeeding
- Ambulation
- Solid food
- Reproduction
- Puberty
- 16-40
- Loss of mobility

Age (years):
- -0.75
- 0
- 1
- 2
- 3
- 4
- 5
- 11-16
- 16-40
- 70+

Antibiotic timing:
- Increased risk of infection by *Clostridium difficile*
- Increased risk of type 2 diabetes associated with repeated use
- May increase risk of childhood obesity
- Increased risk of infections, asthma, allergies and type 1 diabetes
- Loss of microbial diversity and enrichment for resistance genes in the microbiome


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**

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

Preterm birth is the leading cause of infant death.

N = 401 fecal samples

85% prediction accuracy based on 4 variables

Transmission networks of microbiomes and resistomes across habitats

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

263 fecal samples from 115 individuals from 27 houses
209 environmental samples from animals, soils, sewage

Erica Pehrsson
Pablo Tsukayama

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

Antibiotic resistance is an ECOLOGICAL problem

>80% of antibiotics by weight are used in animal agriculture

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I think I need antibiotics for my col...

IT'S A VIRUS!