

# **Overview of Antibiotic Resistance in the Environment**

**PACCARB September 26, 2018**

Jo Handelsman

Wisconsin Institute for Discovery

University of Wisconsin-Madison

# Environmental Resistome

Studies in unmanaged environments with no anthropogenic antibiotic exposure

- **Spatial isolation**—are there antibiotic resistance genes from antibiotic production and use?
- **Temporal isolation**—were there antibiotic resistance genes in the pre-antibiotic era?
- **Similarity to clinical resistance genes**—are the environmental genes similar to those found in clinical settings?

# Approaches to Discovery of Environmental Resistome

- **Functional metagenomics**—cloning and expression of DNA from environment; seek functionality independent of sequence
- **Sequence-based metagenomics**—massive sequencing; seek genes with sequence similarity to known resistance genes

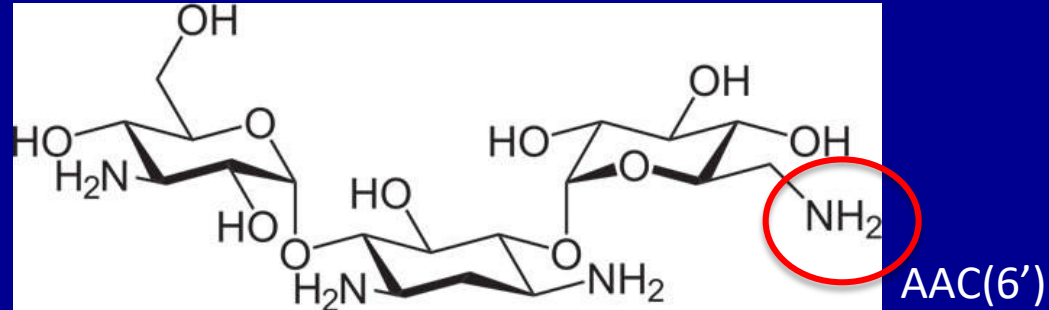
# Natural Environments Distant from Antibiotic Use



# The Environmental Resistome

- Geographic distribution of new clades of resistance genes
- Bifunctional proteins
- Inferred inter-domain gene transfer

# Phylogenetic Analysis of AAC(6') Genes

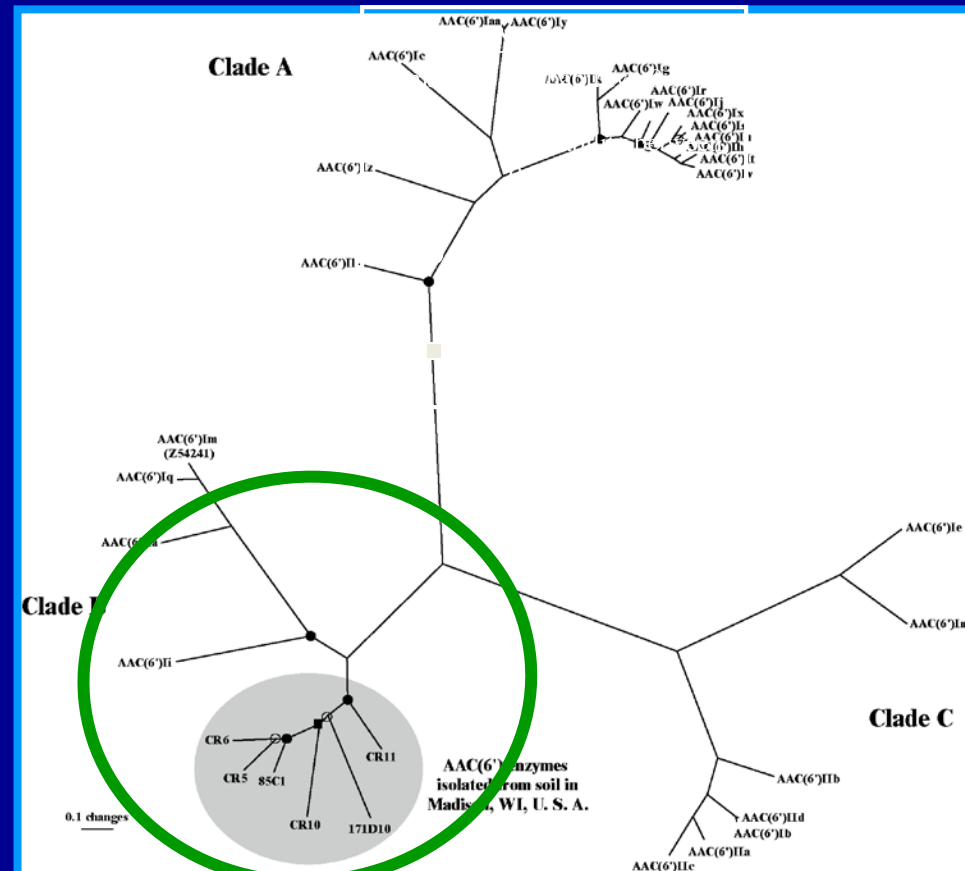


Resistance genes from **West Madison soil** form a new clade of acetyltransferases.

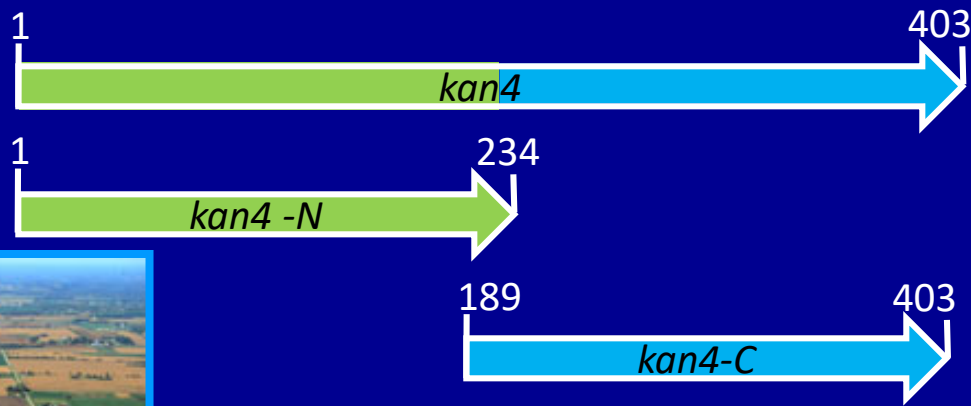
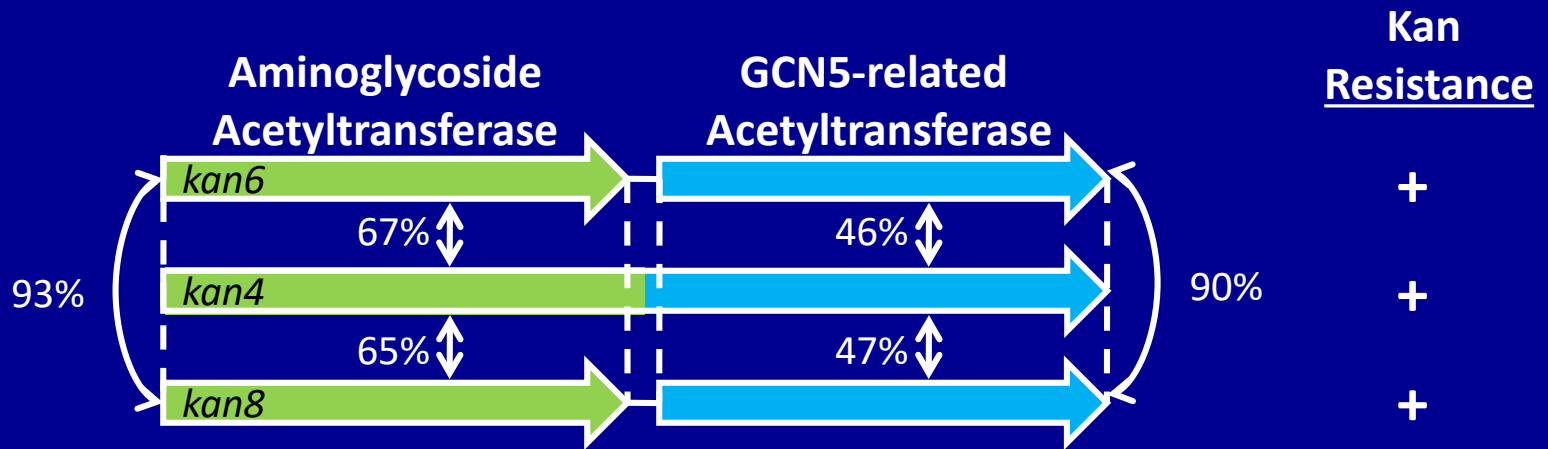
Found same gene cluster in **Alaska soil**.

Not in clinical samples.

Riesenfeld, et al. (2004)  
*Environ Micro.* 6(9), 981–989



# “Bifunctional” Kanamycin Resistance Proteins

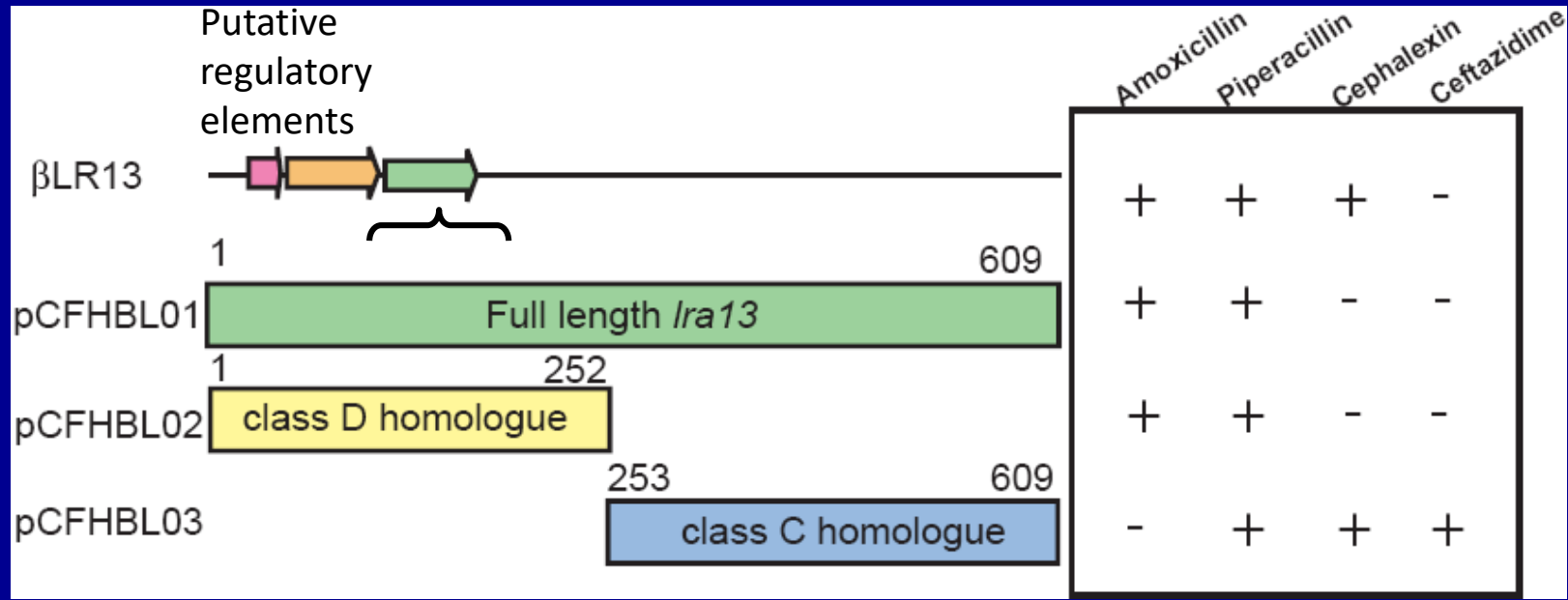


Empty vector



-

# Alaskan soil harbors a bifunctional $\beta$ -lactamase



56% aa identity to class D

54% aa identity to class C

*First reported  
bifunctional  $\beta$ -lactamase*





# The Environmental Resistome

- Geographic distribution of new clades of resistance genes
- Bifunctional proteins
- Inferred inter-domain gene transfer

# Key Findings in the Environmental Resistome

## Related genes over vast geographic space

- Kanamycin-resistance genes from soil are similar to each other (over wide geographic range), and diverge deeply from resistance genes found in clinical isolates

## Bifunctional resistance proteins

- Found apparent fusion of aminoglycoside resistance genes
- First bifunctional *B*-lactamase

## Possible inter-domain gene transfer

- Methyltransferase from microbial mat confers Km resistance on *E. coli*, but appears to be most recently from an Archaea

## Key Findings in the Environmental Resistome

Selection for multi-drug resistant bacteria from soil and PCR amplification yielded resistance genes with high sequence identity to genes found in clinical settings

*Forsberg et al., (2012) Science 337:1107*

Functional metagenomic analysis of resistance genes from 30,000-year-old permafrost identified  $\beta$ -lactamases with 53 to 84% identity to previously known genes (similar results with *tetM* and *vanA* homologs)

*D'Costa et al., (2011) Nature 477:457*

Cave-dwelling *Paenibacillus* sp. isolated from surface for 4M years contains multi-drug resistance; 5 new resistance determinants without modern homologs

*Pawlowski et al., (2016) Nature Comm. 7:13803.*

## Key Conclusions

- The environment contains copious antibiotic-resistance determinants
- Many are identical or similar to genes known in clinical settings
- Many are different and contain new motifs (i.e., bifunctional proteins)
- Antibiotic resistance occurs in the environment in the absence of anthropogenic influences

# Thanks to....

**Pat Schloss**

**Amy Klimowicz**

**Gabriel Lozano**

**Carlos Rios**

**David Relman**

**Patricia McManus**

**Lindsay Rusnak**

**Christian Riesenfeld**

**Heather Allen**

**Justin Donato**

**Luke Moe**