Functional Microbiomics: Identifying and Characterizing Novel Reservoirs of Antibiotic Resistance

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Infectious Disease and Antibiotic Resistance: not a matter of *IF*, just *WHEN*

Methicillin Resistant *Staphylococcus aureus* (MRSA) infections reported in the US [1000’s]

US mortality, 2006: MRSA kills more people than AIDS
18,964  14,627

*Agency for healthcare research and quality, 2006*

*Fischbach & Walsh, Science, 2009*
How do pathogens evolve resistance?

**mutation**

Types of mutation:
- Deletion
- Duplication
- Inversion
- Insertion
- Translocation

**horizontal gene transfer**
Who are the resistance gene DONORS?

Aminoglycoside Antibiotic-Inactivating Enzymes in Actinomycetes Similar to Those Present in Clinical Isolates of Antibiotic-Resistant Bacteria

(streptomyces/origin of R-factors/gentamicin-acetate)

RAOUL BENVENISTE* AND JULIAN DAVIES†


Resistance gene origin hypothesis: antibiotic resistance genes originated as producer self immunity and was transferred to human pathogens
Project 1: GENERAL GOAL
Understand role of environmental microbes as reservoirs of antibiotic resistance machinery for pathogens

HYPOTHESIS

• Majority of antibiotics are produced by soil microbes
• Microbial machinery for further processing must also exist
  • Microbes that can degrade antibiotics may be a significant reservoir of antibiotic resistance for pathogens

SPECIFIC IDEA
Assay whether microbes from diverse soils that can utilize antibiotics as sole source of carbon
Experimental Design

SOIL

- resuspend in minimal media
- 4x Filter
- 500uL Innoculum

11 soils X 18 antibiotics

Toothpick passage 1

Toothpick passage 2

Plate on solid (100, 1, 0.01uL, no carbon)

16s Profiling of CLONES

Liquid passage of CLONES

Restreak

Expts. by G. Dantas, M. Sommer and R. Oluwasegun
Bacteria Subsisting on Antibiotics

Widely distributed in the environment

Extremely antibiotic resistant

Penicillin catabolism involves enzymes that encode resistance in pathogenic bacteria

LIQUID CHROMATOGRAPHY / MASS SPECTROMETRY

1. Chromatography of spent media
2. Mass spec of new peaks
3. Putative pathway

Bacteria subsisting on antibiotics are phylogenetically diverse and closely related to pathogens

- Comprise 3 of 4 phyla of the human microbiome
- 27% of nearest neighbors are human pathogens
- A reservoir of resistance for pathogens?

How do pathogens evolve resistance?

**mutation**

- **Types of mutation**
  - Deletion
  - Duplication
  - Inversion

- **Insertion**
  - Chromosome 20
  - Chromosome 4

- **Translocation**
  - Chromosome 20
  - Derivative Chromosome 20

**horizontal gene transfer**
The human-associated microbiome: our extended genotype

- Human genotype and phenotype are defined by interactions with the environment:
  - Commensal microflora must contribute to human health (microbiome)
  - Invasive microbes
  - Immune system: Communication and defense
  - Nutrients, antibiotics, probiotics
Project 2: GENERAL GOAL

Understand role of the human microflora as a reservoir of antibiotic resistance for pathogens

HYPOTHESIS

• ‘normal’ human microbiome has been repeatedly exposed to antibiotics through therapy and diet
• may have accumulated reservoir of antibiotic resistance determinants that are accessible to pathogens

SPECIFIC IDEA

Functionally characterize antibiotic resistance genes from microbiomes of healthy individuals
Sampling aerobic CULTURED bacteria from microflora of healthy humans

Healthy humans free of antibiotic exposure > 1 year

Fecal samples at multiple time points

Plate to obtain single colonies

determination of antibiotic resistance profiles
High levels of resistance in CULTURED aerobic isolates from healthy humans

Functional characterization of microbiome resistance genes: EXPERIMENTAL DESIGN

1. Microbe isolation
2. (meta)Genomic DNA extraction
3. Ligation
4. Transformation
5. Selection

- Human fecal sample
  - no antibiotic therapy >1 year

Expts. by G. Dantas and M. Sommer
Resistance genes from aerobic CULTURED bacteria are highly similar to resistance genes in pathogens.

### TEM-1 beta lactamase from *Neisseria meningitidis*

- **PATHOGEN**
  - ATGAGTATTCATTTTGCTGTCGCCTTAATCCCTTTTTGCGGATT 50
  - ATGAGTATTCATTTTGCTGTCGCCTTAATCCCTTTTTGCGGATT 50
- **COMMENSAL**
  - TTGGCTCCCTTGTGCTACCCAGAAAAGCCTGGTAAAGTAAAAGATG 100
  - TTGGCTCCCTTGTGCTACCCAGAAAAGCCTGGTAAAGTAAAAGATG 100

### CTX-M-15 beta lactamase from *Klebsiella pneumoniae*

- **PATHOGEN**
  - ATGTTTAAAACTACGGCCAGTTACCCGCTGATGCGCCACAGCTG 50
  - ATGTTTAAAACTACGGCCAGTTACCCGCTGATGCGCCACAGCTG 50
- **COMMENSAL**
  - CAGCTGTGTTAGGAAGTGTGCGCTGTATGGCAGACGACGTAC 100
  - CAGCTGTGTTAGGAAGTGTGCGCTGTATGGCAGACGACGTAC 100

Resistance genes from UNCULTURED bacteria are mostly novel, but fully functional in cultured host

95 METAGENOMIC Resistance Genes

![Bar graph showing the percentage sequence identity at nucleotide level for NCBI and Pathogen datasets](image)

The human microflora is a substantial reservoir of antibiotic resistance genes

105 AEROBIC CULTURED Resistance Genes

- Resistance genes from aerobic CULTURED Proteobacteria are highly similar to pathogenic resistance genes

95 METAGENOMIC Resistance Genes

- Resistance genes from UNCUULTURED bacteria are mostly novel, but fully functional in cultured host

β-lactamases from human microflora illustrate diversity of microbiome resistance genes

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