

Antibiotic Resistance in the Human Microbiome

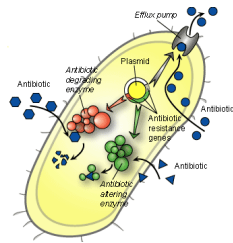
By Pradheep Vemula

Article: "Functional Characterization of the Antibiotic Resistance Reservoir in the Human Microflora"
http://www.bio.tju.edu.cn/struggles/Genomics_09/lectures_04/antibio_humanagut_09.pdf

The Perils of Antibiotic Resistance

- **Bacteria that readily become resistant to antibiotics:**

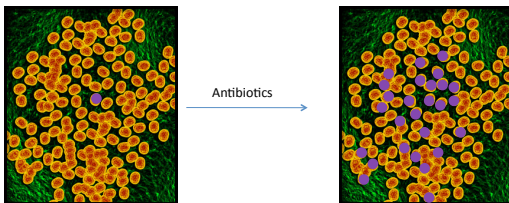
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Salmonella* and *E. coli*



Hospital acquired Staph infections caused 94,000 life threatening infections and caused 18,650 deaths in 2005 (Source: CDC)

In the same time span, only 16,000 people died from AIDS

Development of Antibiotic Resistance



How does that first mutant develop?

Random Genetic Mutations:
1 in 10^8 Chance

What about lateral gene transfer?

Bacteria:

- Outnumber human cells by at least 10:1
- Some estimates say that there 1000s of species of bacteria within a normal human gut
- Provide many benefits
 - Metabolize energy sources that go unused by humans
 - Produce vitamins (like biotin)
 - Prevent the growth of harmful species
 - Help train the host immune system
 - Perhaps even interact with host through hormones and regulatory factors

Could these bacteria already have antibiotic resistance and be a source of the gene to pathogens?

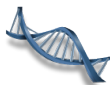
Human Microflora

- Is exposed to antibiotics via:
 - Drugs that an individual takes
 - Drugs are often misused and/or overprescribed
 - The food that an individual eats
 - Almost all animal farms give large doses of antibiotics to the animals to increase profit margins
 - Water
 - Drinking water in some areas has been shown to be contaminated with small amounts of antibiotics

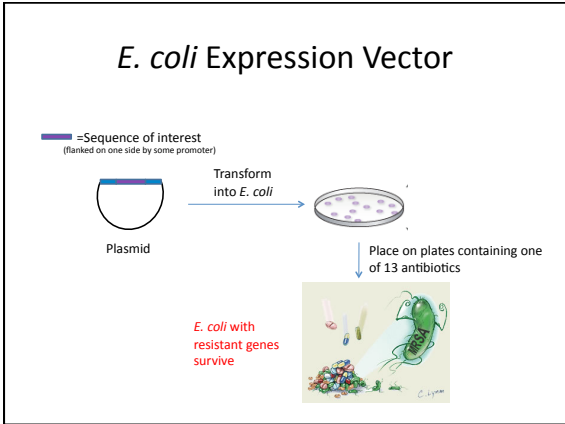
Sommer *et al.* used two separate methods to study the antibiotic resistance genes in the human microflora



Metagenomic Study



- Collected DNA from saliva and fecal matter of 2 human subjects
 - Total of about 9.3Gbp (with very poor coverage)
- Mechanically sheared into 1-3kbp segments
- Expressed in *E. coli*



- ### Antibiotics Used
- | | |
|---------------------|---------------------|
| 1. D-Cycloserine | 8. Penicilling |
| 2. Amikacin | 9. Piperacillin |
| 3. Gentamicin | 10. Cefdinir |
| 4. Sisomicin | 11. Minocycline* |
| 5. Chloramphenicol* | 12. Oxytetracycline |
| 6. Amoxicillin | 13. Tetracycline |
| 7. Carbenicillin | |

- ### Results of Metagenomic Study
- 95 unique inserts found that confer some type of antibiotic resistance
 - Average similarity to the closest known gene:
 - 69.5% similarity at nucleotide level
 - 65.3% similarity at amino acid level
 - 20% of inserts had higher than 90% amino acid identity to some known protein
 - In several cases, inserts were 100% identical to hypothetical proteins in GenBank

What does this mean?

- The genes with high homology are probably a result of lateral gene transfer to or from pathogens (20% of the resistance genes)
- The majority of the genes probably evolved via convergent evolution—and are somehow not available for gene transfer
- Also, most of these genes have not previously been characterized; it is possible that there are many more such genes that are as of yet unknown

Second Study: DNA of Bacterial Isolates

- 572 Bacterial strains were isolated from fecal matter
 - Most of these belonged to proteobacteria, a phylum of bacteria
 - Normally, less than 1% of the gut is composed of proteobacteria
- These were grown on a rich media and then functionally examined for antibiotic resistance genes

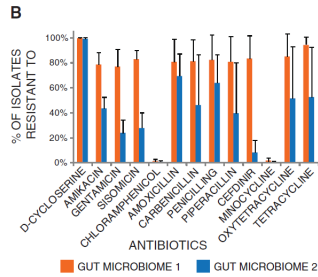


Results of Bacterial Isolates

- 115 unique inserts
- 95% of these inserts were 90+% identical at the nucleotide level to known resistance genes
- Almost 50% of these inserts were 100% identical to known resistance genes



More Results of Bacterial Isolates



Conclusions

- While a vast number of antibiotic resistance genes exist in the gut, most of them are not being transferred to pathogens.
 - If these genes become transposable, the results could be dire
- The resistance genes in bacterial isolates are essentially the same as those found in pathogens.
 - This could mean:
 - These bacterial isolates are dormant pathogens, or
 - These bacterial isolates form a vast reservoir of antibiotic resistance genes that pathogens can make use of, or
 - A combination of both
