Indoor Dust Microbes Contain Mobile Antibiotic Resistance Genes

Taylor Brown, Sarah Ben Maamar, Adam Glawe, Erica Hartmann
Northwestern University: McCormick School of Engineering

Objectives

This research project is being conducted to further understand the effects of antimicrobial substances in the built environment and the ways in which bacteria transfer antibiotic resistance genes.

Background

- Humans use antibiotics to clear bacterial infections
- Some bacteria carry genes that code for resistance to antibiotics
- Streptomycin is an antibiotic that is used to treat tuberculosis
- The *gidB* gene codes for streptomycin resistance
- A variety of resistance genes can be transferred horizontally (to other living bacteria)
- Current built environment antimicrobial chemicals could increase this phenomenon by providing selective pressure

Research Questions

- Where are these genes located in the bacteria?
- How are they transferred, and to which other bacteria?
- How can these findings inform design choices in the built environment?

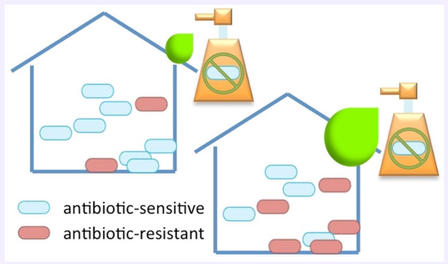


Diagram of the effects of antimicrobial substances on bacterial antibiotic resistance in the built environment. Retrieved from Hartmann, E. M., et al. (2016).

Methods

This project contained two areas of focus, but both relied on the same initial procedure:

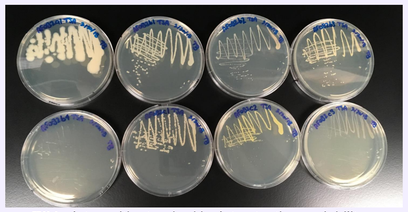
- Identify dust samples with *gidB* in the metagenomic database
- Grow all isolates from each sample on tryptic soy agar (TSA)
- Inoculate each viable culture into tryptic soy broth (TSB)

The first approach examined the resistance phenotypes of each species:

- Streak each culture onto a TSA plate with streptomycin
- Perform a Gram stain on each resistant culture to identify species
- Use a swab to grow a lawn culture onto Mueller Hinton Agar (MHA)
- Perform disc diffusion tests with Chloramphenicol (C30), Sulfamethoxazide Trimethoprim (SXT), Ciprofloxacin (CIP5), Meropenem (MEM10), Ampicillin (AM10), and Gentamicin (GM10)

The second approach aimed to identify the *gidB* gene and its location

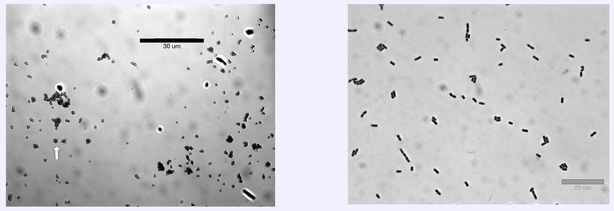
- Design primers for *gidB* from the metagenomic database
- Extract DNA of resistant cultures with Dneasy® PowerSoil® Kit (50)
- Extract plasmid DNA with QIAprep® Spin Miniprep Kit (50)
- Optimize Polymerase Chain Reaction (PCR) for *gidB* genes



TSA plates with streaked isolates testing variability.

Results

The first approach has revealed 10 resistant species from two of the samples. The DNA from each resistant species has been extracted, and seven have been imaged. Two of these images can be seen below.



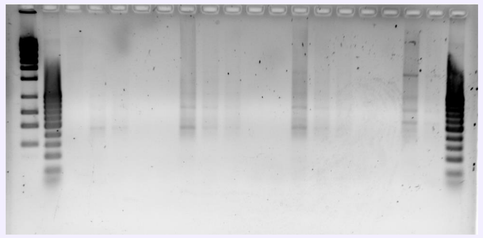
Two species imaged for identification. The first is from sample AF082b3 and the second is from AF091b6. Identification is ongoing, but the first is predicted to be *Staphylococcus equorum.*

The disc diffusion tests are an ongoing process, but the results for the first two samples tested are shown below.

The leftmost plate represents the streptomycin resistant phenotype. The center and rightmost plate display disc diffusion tests.

The second approach has led to optimization progress for the first primers designed. The optimized conditions were:

- 5.0 µL 5x Buffer, 0.5 µL dNTP's, 2 µL $MgCl_2$, 0.75 µL forward primer, 0.75 µL reverse primer, 0.125 µL Taq Polymerase, 10.875 µL nuclease-free water, and 5 µL of DNA
- Undiluted DNA led to increased amplification (1:5, 1:10, 1:50, and 1:100 dilutions were tested)
- The thermal cycler was used with a 51.5^{0C} gradient
- A known positive sample was used in the most successful trial, AF002b11, but tested samples were also examined during optimization



Electrophoresis gel imaged during PCR optimization

Conclusions

- Streptomycin-resistant colonies (formed from multiple species) reside in dust samples
- These species have diverse resistance phenotypes, allowing for future experimentation with conjugation assays
- Primers designed to amplify the *gidB* gene function best when mixed with Magnesium Chloride and higher concentrations of DNA
- Streptomycin cannot be prescribed for bacterial infections for resistant species
- These bacteria live in the environments where humans spend up to 90% of their time (Hartmann, E. M., et al., 2016)
- Transfer of streptomycin resistance genes could render streptomycin useless- the same could occur for *any antibiotic*

Future Directions

The progress with the disc diffusion test allows for future design of a conjugation assay. The donor and recipient species will be chosen as a result of their resistance phenotypes.

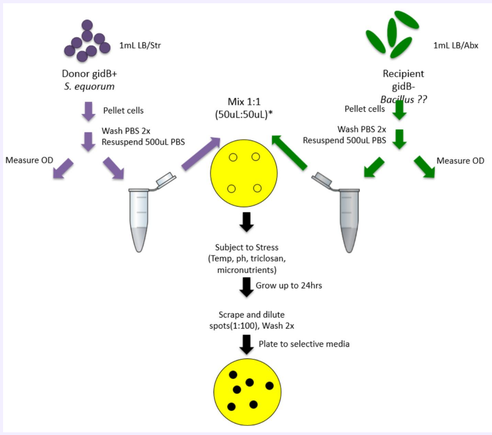


Diagram of conjugation assay procedure, illustration by Adam Glawe

References

Hartmann, E. M., R. Hickey, T. Hsu, C. M. Betancourt Román, J. Chen, R. Schwager, J. Kline, G. Z. Brown, R. U. Halden, C. Huttenhower, and J. L. Green. 2016. Antimicrobial chemicals are associated with elevated antibiotic resistance genes in the indoor dust microbiome. Environmental Science & Technology, 50:9807-9815.

Acknowledgements

This project is largely possible due to the support of Dr. Erica M. Hartmann (Principal Investigator), Sarah Ben Mamaar (project supervisor) Adam Glawe (laboratory technician), and the other undergraduate members of the Hartmann Lab. Special thanks is also given to the Biology and the Built Environment Center at the University of Oregon for their contribution to the dust samples used in this research. Funding for this project was provided by the Alfred P. Sloan Foundation and the Northwestern University Data Science Initiative. Photography recommendations and training given by Vlad Tchompalov.