Pairwise antagonism assay of clinical Acinetobacter isolates

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Many bacteria antagonize each other in nature; laboratory evidence of these interactions dates from 1925 (Gratia). However, antagonism assays are typically performed between a small number of 'sensitive' strains screening a range of potential 'killers' in the hunt for natural antibiotics, or a single 'killer' against a broad range of 'pathogens.' By contrast, several recent studies have performed pairwise assays across a range of organisms; including marine *Vibrio* (Cordero et al 2012) and fresh water *Bacillus* (Perez-Gutierrez et al 2012) strains. These studies have revealed some critical new microbial ecology by using the complete suite of antagonisms among strains. Environmental strains are often viewed as more microdiverse than clinical strains, in part because clinical strains experience purifying selection by the immune system and rapid population expansions. To explore the frequency and distribution of antagonism in clinical isolates, a pairwise antagonism dataset is being collected from clinical *Acinetobacter*. Significant method development has gone into finding a method robust and sensitive. Overnight cultures, grown in trypticase soy broth at 37°C, are spread on trypticase soy agar to create a confluent bacterial lawn. Using a 96-well pin replicator, 94 isolates of *Acinetobacter* and two negative control checks are inoculated on the lawn. The plates are incubated at 37°C for 18 to 24 hours and the assay scored for the presence or absence of a colony growing on the bacterial lawn and the presence or absence of a clearing around the inoculation points. The revealed suite of antagonisms is rich and diverse. 8,836 interactions were scored; of them, at least 5,640 are suggestive of antagonism. In a preliminary dataset, almost every strain already participates as an antagonist against some other strain. This is much higher than the frequencies reported in other environmental collections; and does not suggest a single ecological niche for the clinical strains. This data is collected in the context of antimicrobial susceptibilities and limited clinical metadata, providing a contrast to the environmental data which has been generated for the environmental strains. As we continue to collect antagonism data on clinical *Acinetobacter* isolates, we will learn more about the naturally occurring antimicrobial chemicals that these organisms produce and secrete.

Pairwise antagonism assay of clinical *Acinetobacter* isolates

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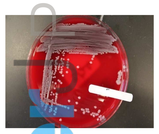
Background

- Many bacteria antagonize each other
- Antagonism assays are typically performed between a small number of 'sensitive' strains screening a range of potential 'killers' in the hunt for natural antibiotics, or a single 'killer' against a broad range of 'pathogens.
- Studies have revealed some critical new microbial ecology by using the complete suite of antagonisms among strains. Environmental strains are often viewed as more microdiverse than clinical strains, in part because clinical strains experience purifying selection by the immune system and rapid population expansions.
- To explore the frequency and distribution of antagonism in clinical isolates, a pairwise antagonism dataset is being collected from clinical *Acinetobacter*.
- Significant method development has gone into finding a method robust and sensitive.

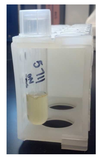
Methods

94 clinical *Acinetobacter* isolates are received from Walter Reed Army Medical Center and stored at -80°C

Each isolate is subbed and incubated at 37°C overnight

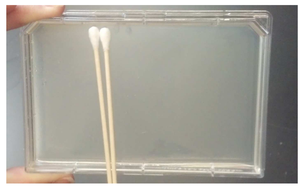


Isolates are grown in TSB overnight at 37°C

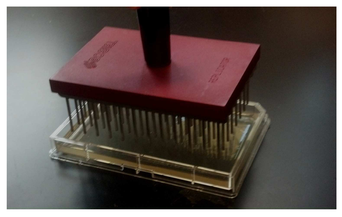


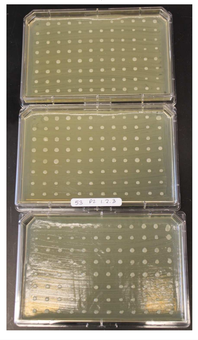
100 freezer plates are made with all 94 isolates randomized and duplicated. 2 full 96 well plates are needed to accommodate all isolates and 2 standard blank wells for negative controls. They are stored at -80°C.

The overnight broth is spread onto 3 sterile TSA plates, per isolate, in three directions to create a confluent lawn of growth.

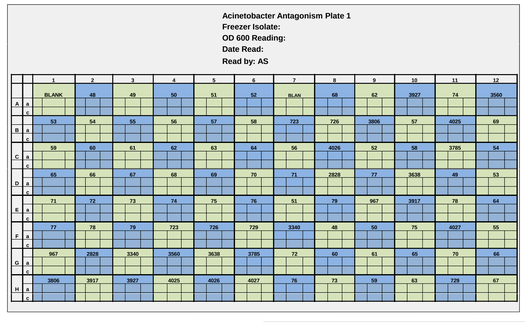


Freezer plates are thawed and a 96 well pin replicator is used to transfer the isolates onto the inoculated lawn. Incubate overnight at 37°C





All isolates are scored in triplicate as positive or negative with respect to colony growth and antagonism and recorded on the data collections sheet.



Results

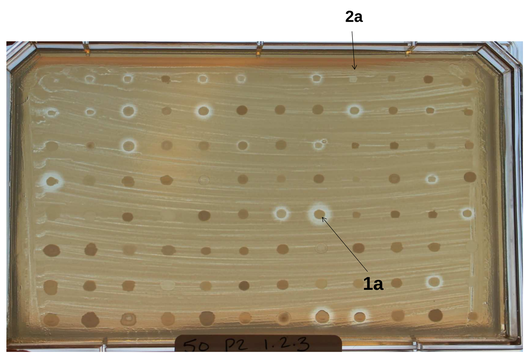


Plate showing many antagonistic reactions, both the large, obvious halo around the colony (**1a**) and the more subtle clearing of growth under the colony (**2a**)

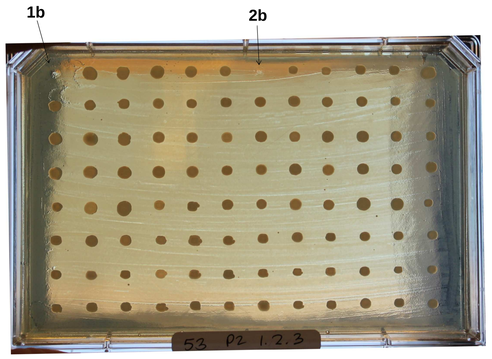
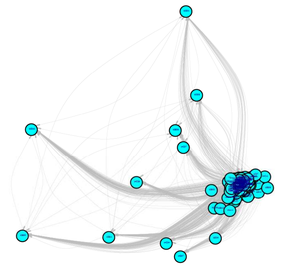
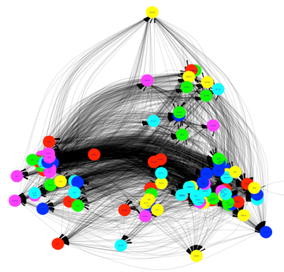


Plate showing no antagonistic reactions. Note (**1b**) and (**2b**) are negative growth controls.

Interaction maps: Each circle represents an isolate tested.



Vibrio to *Vibrio* interactions

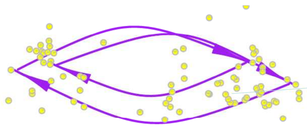


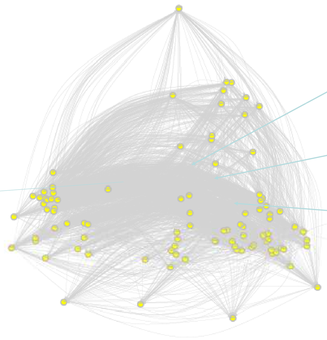
Acinetobacter to *Acinetobacter* interactions

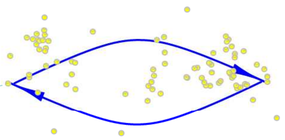
Cordero, 2012 (1)

4 reflexive cycles

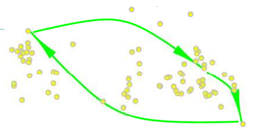
28,656 four-member cycles



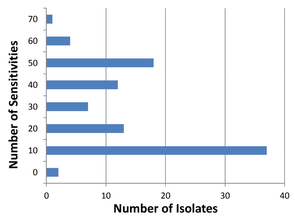


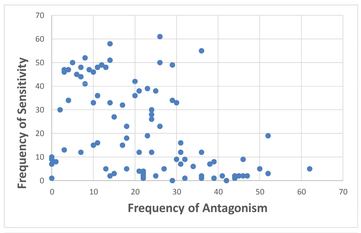


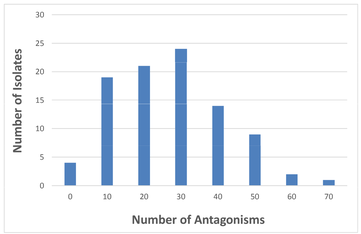
224 mutual cycles

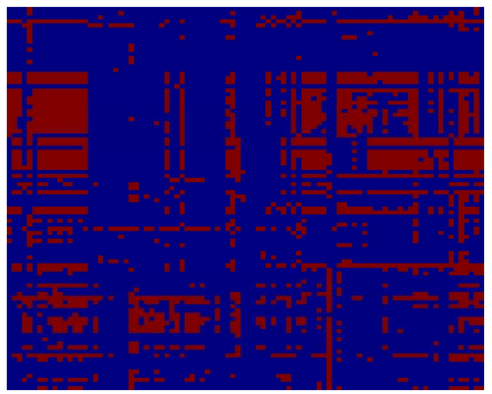


1,344 rock-paper-scissors cycles









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(1) Ecological Poplualtions of Bacteria Act as Socially Cohesive Unites of Antibiotic Production and Resistance. Cordero, et al. *Science 337, 1228 (2012)*