Pan Genomes of Pathogenic Bacteria

Authors: Austin Temple, Haley Riley, Dr. Akhilesh Ramachandran, and Dr. Wouter Hoff*

Abstract: Antibiotics play a big role in today's society, but with the ever growing antibiotic resistance of bacteria, the effectiveness of this type of treatment is diminishing. Researchers everywhere have been trying to understand the specific genes associated with antibiotic resistance in the hope of finding a better way to combat this threat. Our team decided to join in the search--exploring the resistance and genes of *Pseudomonas* and *Enterococcus* bacteria. Our hypothesis is that there will be a relationship between the resistance of the bacteria against certain antibiotics and the presence of a set or one particular gene. We plan on isolating bacterial samples, extracting their DNA, and testing them against a range of antibiotics. The data we gain from this exploration could greatly aid in the understanding of resistance in pathogenic bacteria.

Keywords: Pathogens, Bacteria, Antibiotic, Resistance

Introduction

Pathogenic bacteria are becoming increasingly resistant to antibiotics; growing antibiotic resistance is a major problem considering antibiotics are the primary way we treat many diseases today. Because this issue is so prominent, it has prompted many questions from the scientific community, the main one being, "What is the causing the resistance?" Bacteria have become antibiotic resistant in many ways such as: their enzymes can inactivate antibiotic molecules, mutations in their genes, the bypass of a metabolic pathway, and their proteins can help protect the targeted site (Hrabak et al. 2013). When antibiotics were first introduced in the 1930s, there were little to no problems with antibiotic resistance because few antibiotics had been used in the medical field; treatment could kill a large percentage of the bacteria—curing the patient. However, the small percentage of bacteria that went untreated would continue to reproduce, creating a bacterium with a high tolerance against antibiotics.

Thanks to discoveries made by various scientists, we now know that some pathogenic bacteria have genes which aid in antibiotic resistance (Joshi et al. 2013, Salipante et al. 2015, Rouli et al. 2015). These genes are the result of a mutation of normal cellular genes, the addition of foreign resistance genes, or a combination of the two (Harbottle et al. 2006). While we have a general idea of the type of gene or genes responsible, they have yet to be identified in many bacteria. In our research, we plan to explore the antibiotic resistance genes in *Pseudomonas* and *Anthracosis* across multiple species.

The most common *Pseudomonas* infection found in humans is called *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa*, an opportunistic bacteria, takes advantage of individuals with weak immune systems--cancer patients, burn victims, and those with cystic fibrosis or HIV. The bacteria creates an infection and produces tissue-damaging toxins. The severity of the case and the symptoms of the patient usually vary depending on the location of the

* Faculty Mentor, Department of Microbiology and Molecular Genetics, Oklahoma State University

infection; skin infections are typically more mild than those that occur in the blood or lungs (Lister et al. 2009). However, the fatality rate is approaching fifty percent, and the CDC estimates that roughly 51,000 infections occur in the United States each year with more than 6,000 (13%) cases being multidrug-resistant (MDR). The CDC has since classified multidrug-resistant *Pseudomonas* as a serious threat.

Another bacteria that has been deemed a serious threat by the CDC is the Vancomycin-resistant *Enterococcus*. *Enterococci* are currently considered the second leading cause of healthcare-associated bacteremia. The most common type of *Enterococci* infection occurs in the urinary tract of men. This type of infection is commonly found in hospitalized patients or individuals that have been in long-term care settings. The bacteria's ability to survive in such an environment highlights the amplitude of its antibiotic resistance. *Enterococcus faecium*, one of the more prevalent species in relation to humans, does not express the typical traits associated with pathogenic bacteria and have shown to be resistant even against drugs of last resort (Huycke et al. 1998).

Salipante et al. (2015) performed their research on extraintestinal pathogenic *Escherichia coli* strains; the researchers sequenced 380 isolates of their pathogen and used a microbial genome-wide-association study (GWAS) to identify the genes associated with antibiotic resistance. While the GWAS was successful, the team's experimental assays were unable to distinguish any novel antibiotic resistance determinants. The researchers noted in their conclusion that polygenic factors may exist, or even that resistance could be multifactorial; they were simply afraid of the complexity of dissecting such information considering it was out of their range of study.

Rouli et al. (2015) experimented on pathogenic bacteria with the goal to classify bacteria into different species by their pan-genomes. Looking at the core genomes, they concluded that *Escherichia coli* and *Shigella* are very similar . To find the bacterial genomes, they used next-generation sequencing technologies (NGS). They were then able to make comparisons of the bacteria's genome with a software that constructs multiple genome alignments in the presence of large-scale evolutionary events (MAUVE). When studying the bacteria's resistance genes, the researchers used databases such as the Antibiotic Resistance Genes DataBase (ARDB). They could find 412 multi-resistant bacteria among four cultivable grounds. These strains were tested against 23 antibiotics, and a decrease in gene number was apparent in ten strains, specifically in those related to transcription and the amino acid metabolism.

Joshi et al. (2013) "retrieved 1,221 resistant genes from ARDB, which are responsible for resistance to first and second line antibiotics that were used to cure the Mycobacterium tuberculosis infection". The scientists found that the genes they gathered shared fifty-three common genes. After performing a phylogenetic analysis, they found that over sixty percent of the genes coded for acetyltransferase. The acetyltransferase is proven to detoxify antibiotics by acetylation, which causes it to be antibiotic resistant. We hypothesize that there will be a connection between the bacteria's gene expression and antibiotic resistance based on the success of previous experiments with similar methods.

Methods

We plan to obtain 15 isolates of both *Pseudomonas* and *Anthracosis* from clinical specimen sent in to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) for diagnostic testing. We will perform the bacterial culture on blood agar plates (BAP), MacConkey agar plates (MAC) and phenylethyl alcohol agar plates (PEA). BAP are used as a general purpose media for bacterial culture. MAC and PEA media are selective for gram positive and gram negative bacteria respectively. We will then plate the clinical specimen onto culture plates to be incubated overnight at 37ºC and 5% CO2. After overnight culture, we will isolate individual bacterial colonies for generation of pure cultures. We will use matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) to identify the bacteria. Stock cultures of the pure isolates will be made and stored at -70ºC for use in downstream genomic sequencing studies.

The antibiotic susceptibility profile will be based on minimum inhibitory concentration (MIC) we collect by using a commercial platform (TREK Sensititre, Thermofisher). The manufacturer's recommended protocols will be followed. A 0.5 McFarland suspension of the bacterial isolate will be made in sterile water, while 10uL of this suspension will be added to cation adjusted Mueller Hinton Broth. We will then use 50uL of this suspension to be added to individual wells of a 96 well plate containing different dilutions of antibiotics and incubated at 37ºC overnight. We will determine the MIC values, the lowest concentration of antibiotic solution that completely inhibits bacterial growth, based on bacterial growth in the 96 well plate manually or using an automated plate reader.

When isolating the bacterial genome, we will use a column based commercial DNA extraction kit (eg: Qiagen DNeasy kit) following manufacturer recommendation. Fresh overnight bacterial cultures will be used for the DNA extraction. The quality of our DNA samples will be determined spectrophotometrically by measuring the absorbance at 260nm, 230nm and 280nm. The isolates we collect will be sent to Professor Youssef's undergraduate and graduate students at Oklahoma State University (OSU) for DNA sequencing. The sequence will be analyzed for the presence of antibiotic resistance genes using published resistome databases.

Expected Results

We expect to find a correlation between antibiotic resistance and the resistomes within the pathogens, and our hope is to further analyze the resistance genes we acquire.

Discussion

If our hypothesis is supported, then we will be able to pinpoint the resistomes in *Pseudomonas* and *Anthracosis* and find that certain species of bacteria share similar resistomes. If our hypothesis is unsupported, then we will find no correlation of resistomes among the multiple species of bacteria being tested. Similar to Salipante et al. (2015) research that found no significant antibiotic resistance genes and they believed that there may be many factors that contribute to antibiotic resistance. If our research is supported and with the ability to compare bacteria's core genomes, our research will contribute to the existing

theories that pathogens share common genes that have resistance to antibiotics. We will pinpoint the resistant genes and any other factors that contribute to the antibiotic resistance of the pathogenic bacteria.

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