**[Microevolution of Biofilms and the Effects on Antibiotic Resistance]**

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**Antibiotic resistance is a growing field of research due to the fact that bacterial cells are mutating to be able to use different cellular mechanisms, such as the production of biofilms, in order to resist being affected by antibiotics. It is an important field to research because there are already some pathogens that are highly resistant and the overall rate of resistance is rising due to multiple factors. Recent progress includes assessing changes in antibiotic susceptibility by comparing the minimum inhibitory concentration of a broth microdilution assay and a biofilm formation assay from the bacteria *Acinetobacter baumannii* against both Tetracycline and Ciprofloxacin. Currently there are many preventative measures in place that are trying to slow the rate of resistance from rising such as WHO’s Global Action Plan on Microbial Resistance; however, these measures will only be able to slow the rate of resistance and will not change the bacteria’s ability to quickly adapt to the changing environments.**

**Introduction**

Antibiotic resistance is defined as the growth of bacterial cells in a concentration of an antibiotic that would normally stop growth or kill the cell and has been an important field of study since the first case of Penicillin resistant bacteria was observed in 1947. Currently there are six pathogens that are notorious for being resistant to antibiotics that are known as the ESKAPE pathogens, one of them being *Acinetobacter baumannii*. These pathogens all have different mechanisms that they use to block antibiotics from working. There are four basic mechanisms that are used by bacterial cells for resistance and those are efflux pumps, porin modification, target modification, and modification or destruction of the antibiotic. Resistance can also fall into two different categories: genetic and non-genetic. Genetic (intrinsic) resistance occurs when an organism can endure typically stressful or lethal conditions compared to individuals of the same species that don’t have that adaption. Non-genetic (acquired) resistance occurs when a bacterium that was initially sensitive to an antibiotic is no longer sensitive and a resistant bacterial population begins to increase. The primary focus of “Rapid Microevolution of Biofilm Cells in Response to Antibiotics” is biofilm formation in *A. baumannii*, which is an acquired form of antibiotic resistance. A biofilm is defined as a self-produced extracellular matrix that is made up of DNA, protein, and polysaccharides that have their own specific mechanisms for resistance such as persister cells that reside deep within the matrix and by blocking the diffusion of antibiotics. It is important for antibiotic resistance to be studied because mutations in bacterial DNA are constantly happening and as a result the risk of resistance is always growing. For instance, the antibiotic Colistin is used as a last resort antibiotic in farm animals to stop the spread of disease among them, however the gene of resistance to Colistin, mcr-1, has now been found around the world. “In some places, nearly 100% of farm animals carry mcr-1, and an increasing number of people do as well (Reardon, 2017).”

**Recent Progress**

The primary goal of the experiment conducted in “Rapid Microevolution of Biofilm Cells in Response to Antibiotics” was to understand how genetic and phenotypic diversity is generated within biofilms of a highly virulent strain of *A. baumannii* and how these genes evolve in favor of resistance. *A. baumannii* was chosen as the subject for the experiment because it is intrinsically resistant to most antibiotics already, but it is still susceptible to a few antibiotics. For this experiment two different antibiotics, Tetracycline and Ciprofloxacin, were chosen because of the low level of resistance from *A. baumannii*. These belong to two different classes of antibiotics and due to this, they have different mechanisms of how they work; Tetracycline belongs to the class of Tetracyclines and works by inhibiting protein synthesis by binding to the 30S subunit of bacterial ribosomes, while Ciprofloxacin belongs to the class of Fluoroquinolones that function by inhibiting DNA gyrase and topoisomerases involved in transcription thereby disrupting cell division. In order to provide valid data and allow for the proper analysis of their results, the group took a multipronged approach that allowed for them to simultaneously observe processes occurring in biofilm communities and processes reflected in differential gene expression, while monitoring changes in the genome of cells that are dispersed from biofilms. The additional results from this approach help to supplement the results that were obtained during their primary testing procedures which aim to fully understand how resistant biofilms have become. Biofilm formation was assessed through the use of spectrophotometric quantification of biofilms stained with crystal violet (CV) compared to the initial planktonic isolates and biofilm effluent isolates. The evolution of antibiotic resistance was measured by comparing the initial MIC of the cell to the MICs of cells recovered from antibiotic-exposed biofilms. Cells that are dispersed from biofilms typically have high rates of phenotypic variation so genome sequencing was used to sequence the genomes of 30 random isolates from each treatment type and the initial inoculum. RNA extraction and transcriptomics was performed on whole biofilms that were harvested as soon as effluent isolates were collected from each biofilm sample in order to identify genes whose transcription was up- or down-regulated in each treatment.

**Discussion**

The results from this experiment intend to address how biofilm formation has evolved within bacterial species and the resulting effects that this phenotype can have on antibiotic resistance. Assessment of the biofilm formation showed that many biofilm effluent isolates that were exposed to Tetracycline showed an increase in biofilm formation. When measuring the evolution of antibiotic resistance, it was noted that the biofilm cells showed a consistent increase in their resistance to antibiotics after being exposed to both Ciprofloxacin and Tetracycline. From the genome sequencing they were able to view multiple mutations that had occurred, however the results of some factors such as doubling time and mutation rate are still unclear. From the mutations that were observed, there were correlations that were strong enough to directly link specific mutations with sample origins and/or phenotypes. After RNA extraction and transcriptomics, it was discovered that nearly half of the genes of *A. baumannii* were significantly up- or down-regulated. Among the most up-regulated genes in biofilms were genes involved in the synthesis of ribosomal proteins and the genes/gene clusters involved in the biosynthesis of proteins for type VI secretion systems, efflux, cell surface modification, and pili. Overall, from the results it can be interpreted that the phenotype for biofilm formation has become increasingly more frequent, especially when biofilm producing cells are exposed to concentrations of antibiotics. Although this experiment has offered deep insight into how the biofilm produced by *A. baumannii* has evolved, the biofilm mode of life is still largely underexplored which leaves many unanswered questions about how resistant biofilm mechanisms behave in other bacteria species when exposed to antibiotics. While the results from the experiment are valid and display the genomic flexibility of bacteria, they only give insight into how the biofilm cells from one strain of bacteria respond to two different antibiotics. However, they do set the stage for a greater understanding of control strategies that could be developed and used to help monitor the evolution of resistance and demonstrate how large-scale genome sequencing and transcriptomics can be used identify mutations with potential roles in biofilm formation and antibiotic resistance. Although these control strategies are not fully understood right now, there are still other forms of preventative measures that have been put into place by other organizations such as the World Health Organization to hopefully help slow the rate of resistance. Although the rate of resistance is rising, scientists are working hard to combat it. There are multiple different approaches being taken to experiment with restoring antibiotic sensitivity in different bacteria. One of these even includes the use of a MarR inhibitor in combination with an efflux pump inhibitor in order to restore the sensitivity to Colistin in Colistin-Resistant E. coli cells (Sundaramoorthy, 2019). Along with trying to restore antibiotic sensitivity, scientists are also searching for new kinds of antibiotics using methods such as artificial intelligence to find new antibiotics completely from scratch (Marchant, 2020).

**References**

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