Evolution of Resistant Mutants in Antibiotic Treated Bacterial Cultures

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BACKGROUND

- Persistence is a rare non-genetic, non-heritable adaptive strategy that comes from phenotypic variants of bacteria, which enter a dormant-like state (2).
- Persister cells are an important health concern because they underlie the proclivity of recurrent infections to relapse, and they can serve as a reservoir from which drug resistance mutants can emerge (1).
- Despite reduced physiological activity, persister cells maintain their viability by sustaining a minimum adenylate energy charge, synthesizing spontaneously denatured or degraded proteins, and repairing antibiotic-induced DNA/protein/lipid damage.

OBJECTIVES

- We approach the problem of enriching cultures with antibiotic resistant bacteria by treating the samples with antibiotics for 22 consecutive days.
- When Ofloxacin is introduced into the bacterial culture, the bacterial cells' DNA is damaged. Bacterial cells then have to respond to this damage by inducing SOS response genes (3).
- DNA repair causes random mutations in the bacterial DNA (3).
- The non growing population of ofloxacin treated resistant mutants was monitored through flow cytometry.
- The multi drug tolerance of ofloxacin resistant mutants was assessed too.

APPROACH & PROCEDURE

Mutant Enrichment Assays

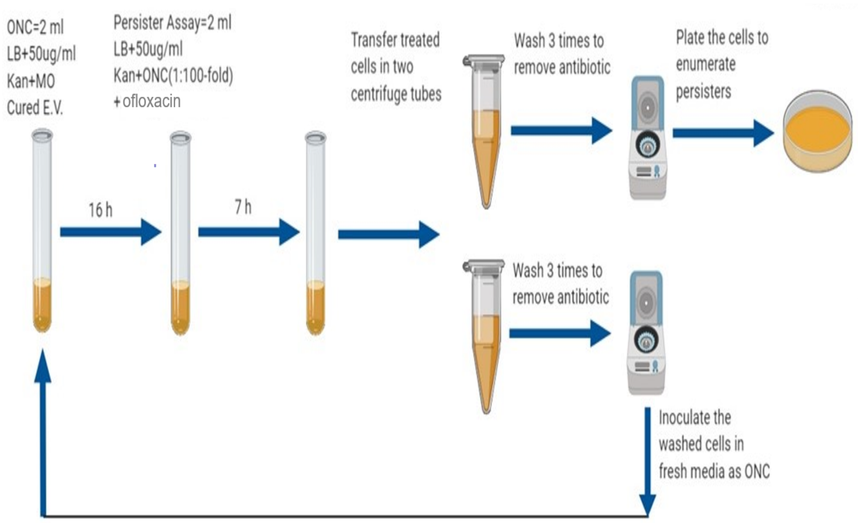

Figure 1. **Schematic Diagram of Mutant Enrichment Assay**

Multi Drug Resistance Assays

- Each ofloxacin resistant mutant was treated with gentamicin and ampicillin for 7 hours to asses the multi drug tolerance.

OD600 Growth Curves

The growth (OD600) of those resistant mutants was observed through a plate reader at designated time points.

Flow Cytometry

- Flow Cytometric measurements were taken at T=0, T=2, and T=3 of overnight cultures.
- This was used to see the independent growth of each sample by measuring the concentration of mCherry light emission.

RESULTS & DISCUSSION

- During the 22 day experiment we had 8 out of 10 sample survive the reoccurring antibiotic treatment. (Figure 2)
- All of the samples gained resistance to high concentrations of ofloxacin. (Figure 3)
- 5 samples, (F-J), have multi resistance to both ofloxacin and ampicillin. Sample I is resistant to all three antibiotics that were tested: Ofloxacin, ampicillin, and gentamicin. (Figure 4)

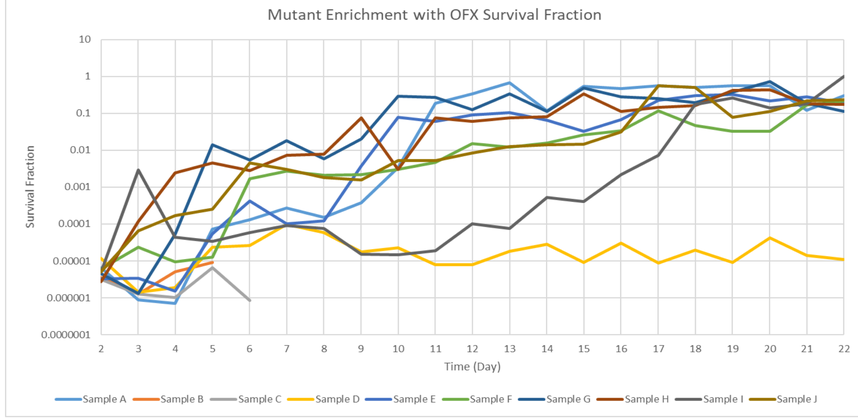

Figure 2. **Survival Fraction of resistant mutants emerging from persisters after being treated with Ofloxacin for 22 days.**

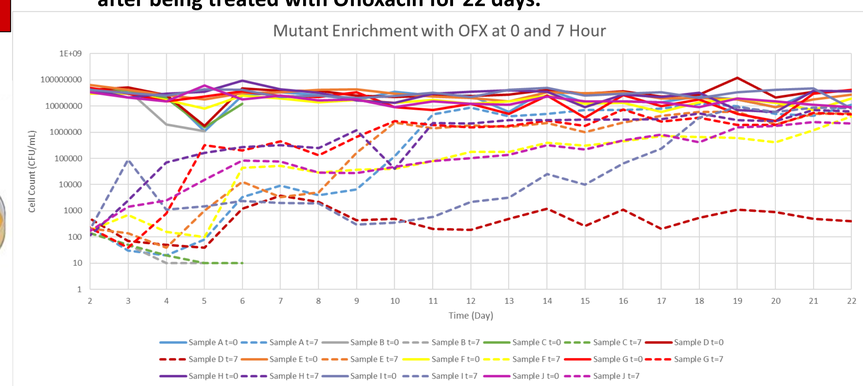

Figure 3. **Cell count of resistant mutants emerging from persisters after being treated with Ofloxacin for 22 days at 0th hour (Filled line) and 7th hour (Dashed Line)**

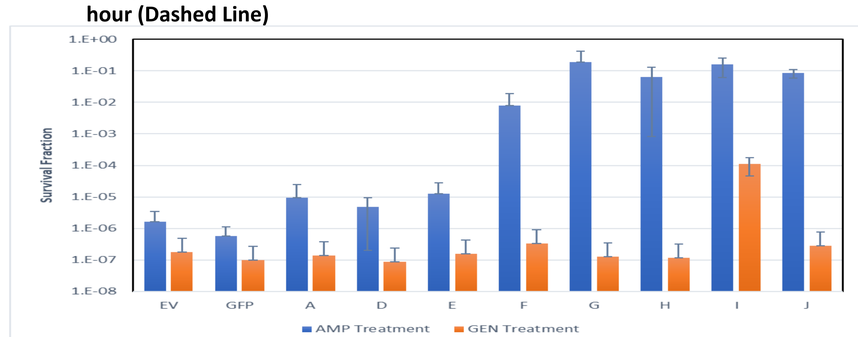

Figure 4. **The Data represents the survival fraction of 4 biological replicates for Ampicillin and 3 biological replicates for Gentamicin of 7 hour antibiotic treatment.**

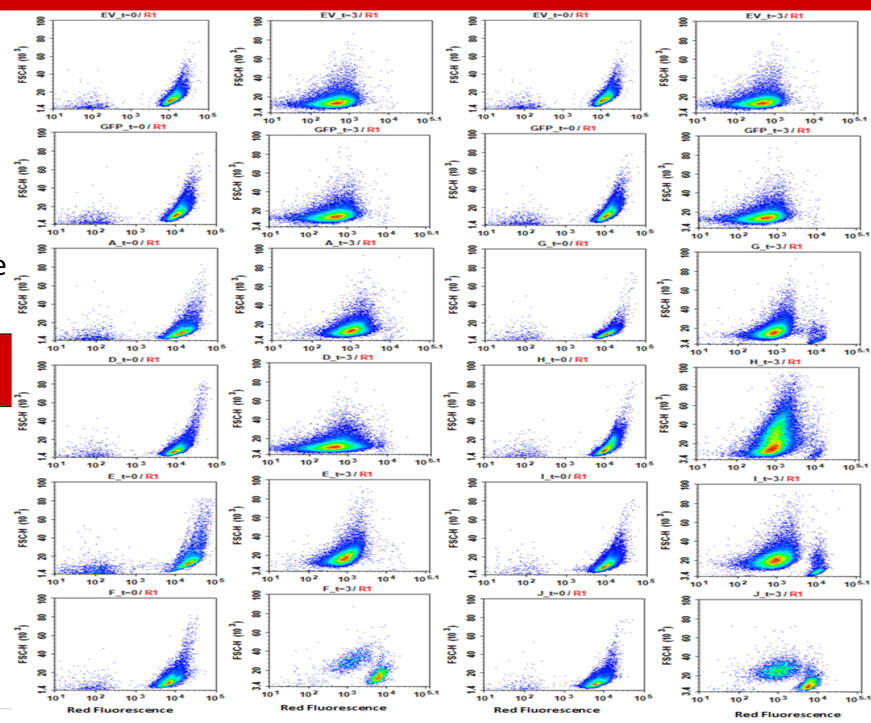

Figure 5. **The data represents one of the three biological replicates of the non growing cell assay.**

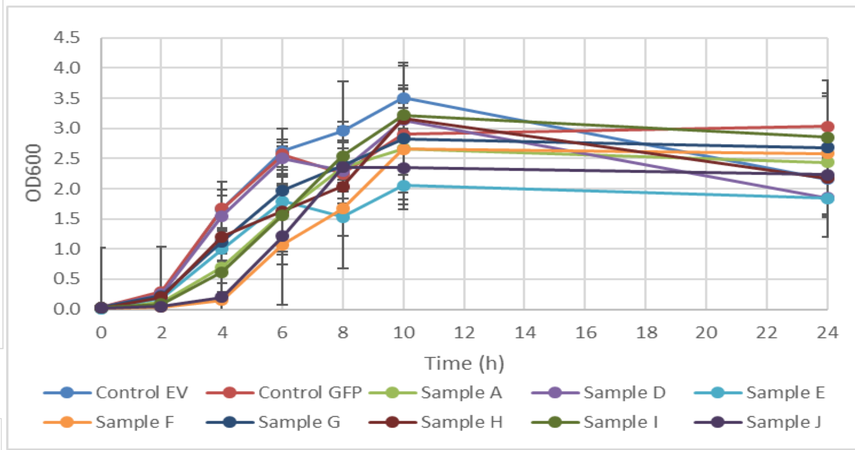

Figure 6. **The data represents three biological replicates of 24 hour optic density measurements of two controls and mutant samples.**

FUTURE DIRECTION

Sequencing the genomic DNA of the resistant mutants to identify the possible random mutations that are causing the antibiotic resistance.

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