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Bacterial Mutations for Antibiotic Resistance

Confer Fitness Costs

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**Introduction**

Antibiotic resistance is a growing problem in the medical field, posing a major threat to public health as pathogens are becoming more difficult to treat. Resistance is conferred by mutations within the bacterial genome that allow them to efflux toxic compounds, like drugs, at a rate high enough to maintain homeostasis (Praski et. Al 2017). Resistance mutations that cause structural and functional changes in the parts of the cell targeted by an antibiotic, such as genes that control DNA coiling, transcription, and protein synthesis, can also reduce bacterial replication (Reynolds 2000). This makes sense because if higher levels of resistance require a cell to utilize its resources to eliminate a drug or mutate to change their biological functions, there will be a phenotypic effect. In which case, we may expect to see a negative correlation in MIC (minimum inhibitory concentration) and fitness. Research focuses on eliminating or controlling antibiotic resistance, with the most obvious and successful strategy being to stop using antibiotics. This being easier said than done, the idea here is that mutations that confer resistance impose a fitness cost in the absence of antibiotics (Melnyk et. Al 2015). Genotypes without the resistance mutations that do not have to pay the fitness cost would therefore replace resistant strains at a rate proportional to the cost of resistance (Melnyk et. Al 2015).

However, not all bacteria suffer fitness costs from the resistance mutations in the absence of antibiotics. Some may develop second-site mutations that restore fitness, genetic linkage between a resistance gene and other selected mutations can exist, and lastly, the resistance mutation could simply have no effect on fitness. In this study, I investigated the relationship between resistance mutations in bacteria and their relative fitness, to determine if there were fitness costs associated with single mutations that confer resistance. My data includes a variety of studies that have both directly and indirectly evaluated fitness costs associated with the minimum inhibitory concentration of antibiotics needed to prevent the growth of a bacteria. Pathogenic bacteria that develop single or multiple mutations to resist higher concentrations of multiple antibiotics will have lower relative fitness in the absence of said antibiotic, as a result of fitness cost.

**Materials and Methods**

Minimum inhibitory concentration (MIC) represents the minimum concentration of an antibiotic that a bacterium can withstand, thus measuring its resistance capability. This value was determined by growing the mutant strains in increasing concentrations of the antibiotic, of which the ranges varied by study. MIC is measured as the concentration interval of upper limit growth and the first concentration that inhibits bacterial growth after 24 hours. Relative fitness was determined using competitive growth assays of the parent (non-mutant) strain and the mutant strain in the absence of antibiotic intervention. Relative fitness was calculated by W = 1 – b where b is the density of the mutant strain divided by the density of the ancestral strain. Therefore, any fitness value of less than 1 shows a fitness cost in the mutant strain. Studies that reported the fitness cost rather than the relative fitness were converted by subtracting their reported value from 1. Studies varied in which units MIC was reported, which I accounted for in each figure. R was used to graph correlations found throughout a variety of studies comparing relative fitness to minimum inhibitory concentration. A linear regression line was made for each study to allow for visualization of the relationship between relative fitness and mutational resistance, with the exception of Figure 1. Figure 1 did not show a clear linear regression, so I chose to use a curved regression line and a line showing what I predicted to happen within the dataset.

**Results**

The first study (Figure 1) was a compilation of data from several studies run under the same standards that I held my own studies to. This study contained a wide variety of datasets that corresponded to relative fitness and antibiotic resistance mutations over several species of bacteria with different antibiotics. They found that there was a significant fitness cost associated with resistance mutations. Rather than a smooth correlation, the relationship in this dataset was unique. The red dashed line shown in the graph (Figure 1) corresponds to a curved regression line that represents the dataset, while the blue line represents what I would predict to happen in the dataset. In conclusion, there is a drop-off in relative fitness at very high concentrations of antibiotic resistance.



**Figure 1.** Drug resistance as fold-increase minimum inhibitory concentration relative to the drug-sensitive ancestor plotted as a function of relative competitive fitness to the sensitive ancestor.

The second study (Figure 2) evaluated the fitness costs associated with rifampicin resistance in *Enterococcus faecium*. 12 mutants were assessed using competitive growth assays against their parent strain. Double mutants were found to be the least fit. Per generation fitness cost was observed to be anywhere between 2.5 and 10%. Fitness was measured in their study as a percentage of fitness cost, which I then converted to relative fitness by subtracting their value from 1 and dividing by 100.



**Figure 2.** Minimum inhibitory concentration of rifampicin needed to inhibit mutant *Enterococcus faecium* plotted against its relative fitness when the antibiotic is absent.

The third study (Figure 3) was focused mostly on selection bias of drug resistance in *marR* mutations to confer ciprofloxacin resistance in *Escherichia coli. MarR* is a repressor protein that functions in the multidrug efflux pump of *E. coli* (Praski et. Al). This study evaluated differences in inactivation patterns in clinical versus in vitro strains of *E. coli*. Because I sought to only evaluate the relative fitness in terms of resistance capabilities, I considered all patterns of inactivation to be resistance mutations and included all data points. Relative fitness in this case was determined using growth competition assays between the wild type and mutant strains.



**Figure 3.** Minimum inhibitory concentration(mg/L) of ciproflaxin needed to inhibit mutant *E. coli* plotted in terms of its relative fitness in the absence of the drug.

The fourth study (Figure 4) included a brief introduction of fitness costs of the mutations in *Salmonella* that allow for increased growth rate in the presence of rifampicin. The study itself was more about making discoveries of subsequent mutations that compensate for said fitness costs. To ensure that the mutations were not selected as an adaptation to the lab, but to rifampicin, they sequenced parallel evolved lineages from the same ancestral isolates. Their results indicated that adaptation to growth in the laboratory was only a minor contributing factor to selection for these particular mutations. The data included from this study consists of secondary mutations that occur to compensate for the fitness costs that *Salmonella* suffer from their initial rifampicin resistance mutations. As one might expect, the secondary mutations that compensate for fitness costs, flatten out the regression line. Relative fitness in this study was also determined using growth competitive assays between the wild type and mutant strains. 

**Figure 4**. Minimum inhibitory concentration(mg/L) of rifampicin needed to inhibit the growth of *Salmonella* with secondary compensatory mutations plotted in terms of its relative fitness in the absence of the drug.

The fifth study (Figure 5) indicated that there have been suggestions of a negative correlation between fitness costs of rifampicin resistance mutations in various bacteria. Their goal was to expand on this hypothesis by testing 122 different mutations in *Salmonella*. MICs and relative fitness costs were evaluated and recorded for each mutant strain, with and without the presence of the antibiotic. I have included the data for MIC and relative fitness without the presence of the antibiotic, as my investigation is focused on how well the strain will do if we cease administration of antibiotics. Their relative fitness was expressed as a percentage using competitive growth assays, which I also divided by 100 to convert to relative fitness with no units.



**Figure 5.** Minimum inhibitory concentration(mg/L) of rifampicin capable of inhibiting mutant *Salmonella* plotted against its relative fitness in the absence of the drug.

The final study (Figure 6) is from the same time period of which research was just beginning to evaluate fitness costs in terms of resistance mutations in bacteria. Resistance to rifampicin is conferred by mutations in the rpoB gene, which encodes the beta-subunit of RNA polymerase in *Mycobacterium tuberculosis* (Mariam et. Al). Mutations were identified within isolated mutant strains and resistance capabilities were determined in competitive growth assays with their susceptible parent. All mutants demonstrated a fitness cost in the absence of the drug.



**Figure 6.** Minimum inhibitory concentration(µg/mL) of rifampicin to inhibit the growth of mutant *Mycobacterium tuberculosis* in terms of its relative fitness in the absence of the drug.

**Discussion**

Through meta-analysis, I was able to determine that resistance mutations in bacteria that confer antibiotic resistance do have a fitness cost in the absence of the drug. This is what I expected to find, as other cellular processes are affected by the mutations that allow for antibiotic resistance. Each study shown has a different degree of negative correlation between resistance and relative fitness, indicating a wide variety among species of bacteria and antibiotics. Variation between strains and antibiotics indicates that there are no-cost mutations present in these studies. In addition to the variation, mutant strains with high MIC in each study show a range of fitness values from 0 to 1. This could mean that a variety of factors are playing a role in fitness costs.

Study 4 eliminated the possibility of fitness cost being a result of laboratory environment, yet still showed a wide range of relative fitness values at high minimum inhibitory concentrations. Further research pertaining to the variation between values of relative fitness of high MICs is a possibility in determining the mechanism behind this phenomenon. From the data available in this study, we can still confidently conclude that resistance in the absence of the antibiotics could eliminate selection for resistance mutations.

Secondary mutations, in this study, have been shown to flatten the negative correlation between resistance and relative fitness, which is exactly what we would expect, as that is what they are selected to do. I would predict eliminating resistant strains with secondary compensatory mutations would pose a greater challenge than simply eliminating single mutational strains. In addition to compensatory mutations, other discrepancies in the dataset may include epistasis between resistance and other mutations, the environment, and genetic linkage between resistance mutation and other mutations that are also under selection (Melnyk et. Al). Future research should focus on understanding the components that confer fitness cost on a genetic level, particularly compensatory mutations and other mutations that are linked to resistance mutations.

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