# INVESTIGATION OF GENE-GENE INTERACTIONS IN THE CONTEXT OF ANTIBIOTIC RESISTANCE

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## AIM:

To investigate the basis for aquired antibiotic resistance in the Gram-negative bacteria *Pseudomonas aueruginosa* .

## OBJECTIVES

1. Develop a computational model to identify key gene-gene interactions in *P. aeruginosa*.
2. Investigate synergistic mutations that lead to acquired antibiotic resistance in clinical isolates.

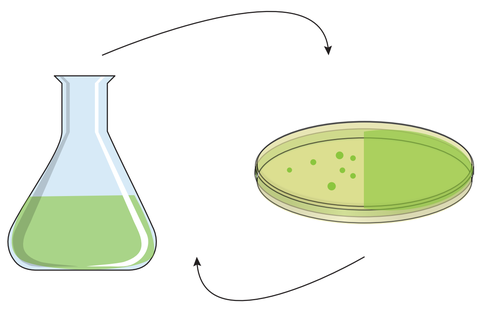
## INTRODUCTION

Pseudomonas aeruginosa is a multidrug-resistant causative agent of both acute and chronic infections. In this study, isolates of *P. aeruginosa* were evolved under the selective pressure of antibiotics to identify specific genes that contribute to resistance. After whole-genome sequencing, genes found to be mutated became the basis for further investigation.

A computational method was developed to identify key alleles that synergistically contribute to antibiotic resistance in 380 clinical and environmental isolates (with known resistance profiles). Further testing and expansion of this model could lead to a tool capable of quickly and effectively processing clinical isolates of *P. aeruginosa* to determine the antibiotic resistance profiles, and hence the best course of action for effective treatment.

## METHODS

### 1.



#### EVOVLING ANTIBIOTIC RESISTANCE MUTANTS

Experimental evolution of mutants to increasing concentrations of antibiotics using the antibiotic gradient plate method and measurement of their minimum inhibitory concentration (MIC) to determine the fitness score.

### 2.

#### GENE-GENE INTERACTION MODELLING

The data were fitted to a linear model (no pairwise or higher-order interactions) estimating the contribution of each gene to the MIC. Due to the high variance in these estimates, the model implied that gene-gene interactions contributed to MIC in a significant way.

The data were then fitted to multiple interaction models to infer gene-gene interactions. The models were compared using Akaike's Information Criterion (AIC).

$$\begin{pmatrix} w_{000} \\ w_{100} \\ w_{010} \\ w_{001} \\ w_{110} \\ w_{101} \\ w_{011} \\ w_{111} \end{pmatrix} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 1 & 1 & 1 & 0 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 1 & 0 & 0 & 1 & 0 \\ 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \end{pmatrix} \begin{pmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \\ \beta_3 \\ \beta_{12} \\ \beta_{13} \\ \beta_{23} \\ \beta_{123} \end{pmatrix}$$

The additive model with three genes showing that there are 23 = 8 possible com- binations of the genes and 8 parameters (one intercept, three main effects, three pairwise interactions and one three way interaction).

The model was only able to identify a possible 24 main effects and 19 pairwise interactions to be determined. In the hope to expand the data, a new dataset was generated using an additional ~340 clinical isolates that had been whole-genome sequenced and MIC tested.

### 3.

#### DETERMINIG THE IMPORTANCE OF ALLELES

Alleles of clinical isolates were identified using the variant calling tool Nesoni. These were filtered and converted to 0 or 1 depending on the presence or absence of a variant in comparison to the total dataset. Resulting in a matrix whose dimensioning were 340 x 2010.

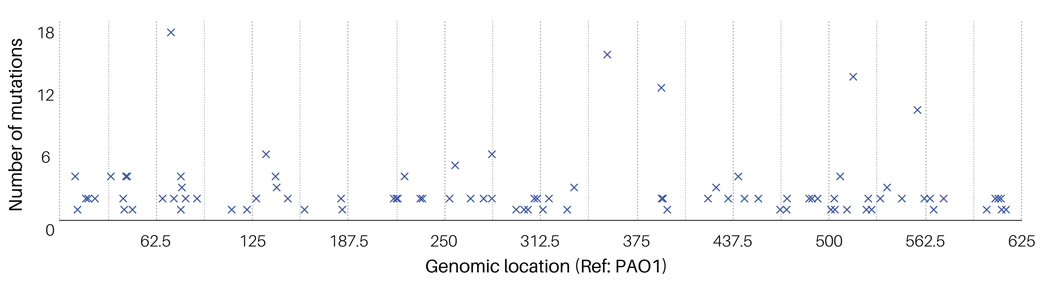
$$\begin{array}{l} \quad\ \beta_0\ \ \beta_1\ \ \beta\ldots \\ \text{Alleles: } \begin{matrix} 1 & 2 & 3 & \ldots \\ 0 & 1 & 1 & \ldots \\ 1 & 1 & 0 & \ldots \\ 1 & 0 & 0 & \ldots \end{matrix} \end{array} \quad \begin{array}{l} \text{MIC} \\ 0.625 \\ 0.94 \\ 2 \end{array} = \begin{pmatrix} \beta_0 & \beta_1 & \beta_{0+1} \\ \cdot & & \\ \cdot & & \end{pmatrix}$$

Possible gene-gene interactions were tested using the Lasso tool.

## RESULTS

#### EVOVLING ANTIBIOTIC RESISTANCE MUTANTS

**Figure 1.** Thirteen isolates were evolved independtly from the reference strain to increasing concentrations of antibiotic. The graph represents the genomic location of idividual mutations that occured after seven generations.




#### EXAMINING THE CONTRIBUTION OF GENES

**Figure 2A.** The percent of mutations occuring in different Ciprofloxacin evolved isolates with the same antibiotic resistance. Antibiotic resistance is shown as MIC values ranging from 0.06125 mg/L to 128 mg/L each represented by a colour.

**Figure 2B.** The contributing percent of mutations occuring in clinical isolates of *P. aeruginosa.* Antibiotic resistance is shown as MIC values ranging from 0.065 mg/L to >1.5 mg/L each represented by a colour.




#### EXAMINING THE CONTRIBUTION OF ALLELES

**Figure 3.** The number of variants identified in a representation of clinical isolates. Five of 340 isolates are represented here.




## SUMMARY

Aquired genetic mutations of clinical isolates of *P. aeruginosa* contribute to increased antibiotic resistace. This work develops a strategy to examine genomic alleles using computational models that predict gene-gene interactions

## FUTURE DIRECTIONS:

To develop a robust computational model that allows predictions of alleles that contribute to aquired resistance in *P. aeruginosa*.