# Evaluating the Evolution of Antibiotic Resistance Across increasing Concentrations of Trimethoprim

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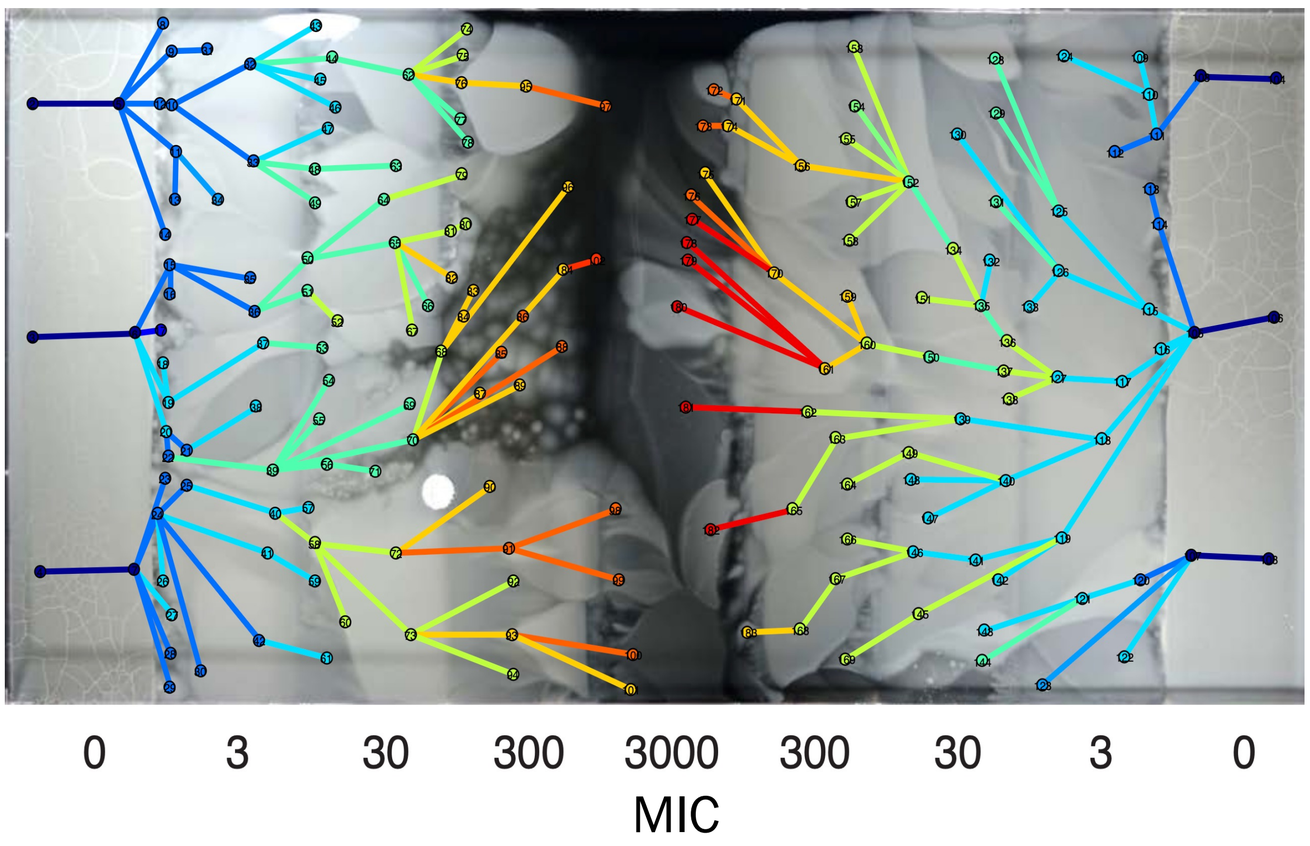


**Figure 1.** Antibiotic Landscape With Sampled Bacterial Colonies. MIC denoting antibiotic concentration.

## Methods

Whole Genome Sequencing (WGS) samples from a Microbial Evolution and Growth Arena (MEGA) plate were retrieved from the Sequence Read Archive[1] from an Escherichia coli strain exposed to selected areas that had increasing amounts of trimethoprim (0, 3, 30, 300, 3000 – Minimum Inhibitory Concentrations)[2].

A reference genome was assembled at BV-BRC[3] using Flye [4] and annotated using RASTtk [5] from the isolates that were exposed to 0 MIC of trimethoprim.

WGS reads from all other isolates (3, 30, 300, 3000 MIC) were aligned using BWA [6] to the reference genome. Single Nucleotide Polymorphisms (SNPs) between the reads and the sequence of the reference genome were detected using BCF[7]. SNPs that occurred in more than 80% of the reads were examined to look for potential changes in protein structure or in the upstream region of a gene.

SNPs resulting in an amino acid change may affect the shape of the resulting protein, possibly resulting in antibiotic binding loss. The locations of shared SNPs among isolates were investigated. Their location in protein domains[8] of interest and their corresponding protein structure[9] was noted. These were mapped and visualized at the BV-BRC.

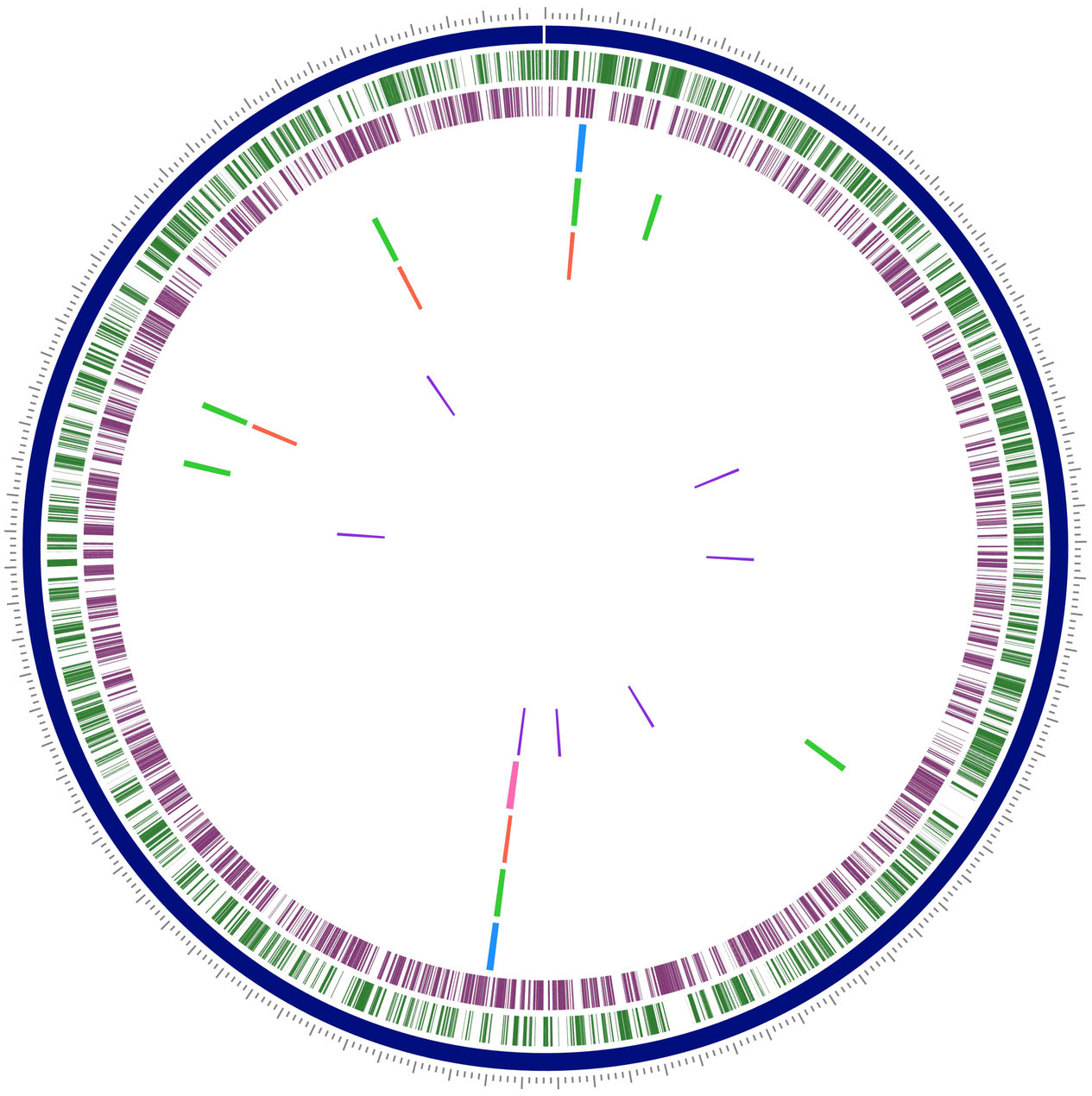

**Figure 3.** Circular view of filtered SNPs passed down across MIC concentrations. The tracks from outer to inner: Forward strain, Reverse Strain, 0 MIC SNPs, 3 MIC SNPs, 30 MIC SNPs, 300 MIC SNPs, and 3000 MIC SNPs

## Background

Antimicrobial resistance (AMR) is of increasing concern on a global scale, and predictions are that by 2050, AMR will take ten million lives annually and cost 100 trillion USD [1]. A recent study documented the response of Escherichia coli to the antibiotic trimethoprim in real time, using both a unique visualization method combining with genome sequencing[2]. We conducted a detailed examination of the genomic changes in response to increased antimicrobial pressure and document where these changes occurred within proteins.

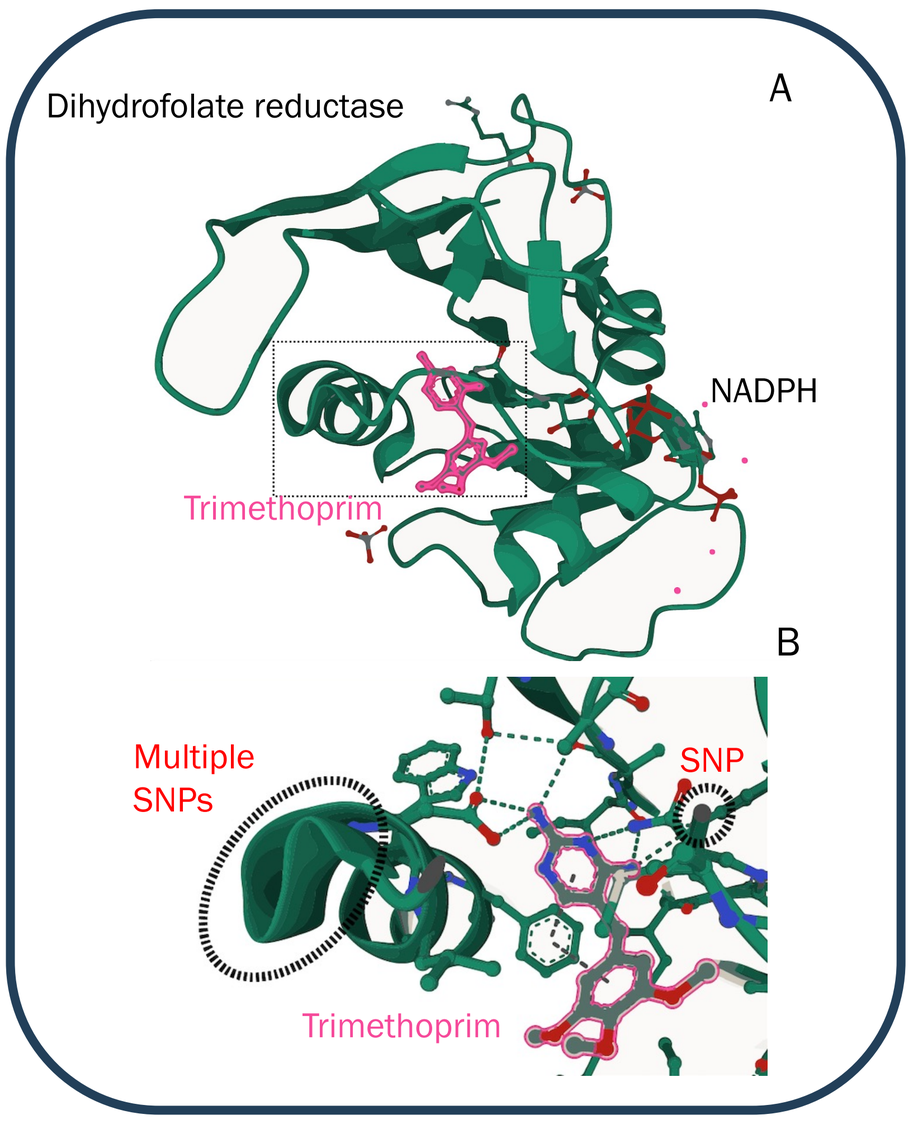


**Figure 2.** (A) A crystal structure[10] of the *Escherichia coli* dihydrofolate reductase protein in complex with the antimicrobial trimethoprim and NADPH (B) denoting the location of the SNPs identified in the analysis.

## Results

- 306 SNPs were found among all isolates that were compared to the reference genome. 13 genes were found to have SNPs that were shared either among isolates within the same MIC, or across multiple MICs (Fig 3).
- Of these, three genes had SNP changes occurring across multiple MIC exposures: Multiple antibiotic resistance protein (marR), dihydrofolate reductase (DHFR) and a low-affinity inorganic phosphate transporter. The marR and DHFR genes both had multiple SNPs in several locations across the gene.
- DHFR has been described as being important in antimicrobial resistance[10]. The SNP locations in DHFR were mapped onto a determined structure that shows the interaction between this protein and trimethoprim (Fig 2).

## References

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