**Supplemental Information for**

**Adaptive Landscapes of Variant Mutant Alleles Change as Concentration of Antibiotic Change**

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**Figure 1:** Adaptive Landscapes for Cefprozil (CPR) at various concentrations: A) 80μg/mL, B) 100μg/mL, C) 128μg/mL. Forward arrows signify new substitutions and backward arrows signify reversions. Red arrows represent significance with a p-value ≤ 0.05. Black arrows represent non-significance, p-value ≥ 0.05. The global optimum allele is highlighted in red D) Composite of all concentrations, showing only the arrows that remain in the same direction throughout the three concentrations. We see a triple mutant is the optimum at the highest concentration and double mutants as optima at the two lower concentrations. We also observed the largest number of significant differences in growth rates at the middle concentration of 100 μg/mL, which was also the concentration where we observed the greatest number of significant improvements resulting from reversions.

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| **C)Macintosh HD:Users:portia:Dropbox:TEM.50:TEM-50 Landscapes:CAZ:CAZ1.25Landscape copy.pdf** | **D)Macintosh HD:Users:portia:Dropbox:TEM.50:TEM-50 Landscapes:Ceph. Composites:CAZComposite copy.pdf** |

**Figure 2:** Adaptive Landscapes for Ceftazidime (CAZ) at various concentrations: A) 0.0625μg/mL, B) 0.1μg/mL, C) 1.25μg/mL. Forward arrows signify new substitutions and backward arrows signify reversions. Red arrows represent significance with a p-value ≤ 0.05. Black arrows represent non-significance, p-value ≥ 0.05. The global optimum allele is highlighted in red D) Composite of all concentrations, showing only the arrows that remain in the same direction throughout the three concentrations. At the highest concentration of 0.125 μg/mL, a triple mutant is selected as the global optimum; however, at lower concentrations double and single mutants become the global optima. We also observed the largest number of significant differences in growth rates at the highest concentration of 0.125 μg/mL.

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| C)Macintosh HD:Users:portia:Dropbox:TEM.50:TEM-50 Landscapes:TZP:IRM Landscapes:TZP8.512LandscapeIRM copy.pdf | D) |

**Figure 3:** Adaptive landscapes for Piperacillin + Tazobactam (TZP) inhibitor showing only selectivity for the inhibitor resistant mutations (bold). The global optimum is highlighted in red. Red arrows represent significance with a p-value ≤ 0.05. A) Landscape with Tazobactam at 8 μg/mL and Penicillin 128 μg/mL, B) Landscape with Tazobactam at 8 μg/mL Penicillin 256 μg/mL, and C) Landscape with Tazobactam at 8 μg/mL 512 μg/mL. The global optima contained at least one IRM in each of the different adaptive landscapes, with a double mutant being selected at the two lowest concentrations of 128 μg/mL and 256 μg/mL and a triple mutant was selected as the global optimum at the highest concentration of 512 μg/mL. In all three adaptive landscapes, we observed just two to three significantly different growth rates among the new substitutions.

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**Figure 4:** Adaptive landscapes for Amoxicillin + Clavulanic Acid (AMC) inhibitor showing only selectivity for the inhibitor resistant mutations (bold). The global optimum is highlighted in red. Red arrows represent significance with a p-value ≤ 0.05. A) Landscape with Clavulanic Acid at 8 μg/mL and Amoxicillin at 512 μg/mL, B) Landscape with Clavulanic Acid at 8 μg/mL and Amoxicillin at 1024 μg/mL. A triple mutant was selected as global optimum at the lower concentration of 512 μg/mL and a double mutant was selected as global optimum at the higher concentration of 1024 μg/mL. In both adaptive landscapes, we observed the global optimum containing at least on IRM.

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| C)Macintosh HD:Users:portia:Dropbox:TEM.50:TEM-50 Landscapes:SAM:IRM Landscapes:SAM8.32.LandscapeIRM copy.pdf | D)Macintosh HD:Users:portia:Dropbox:TEM.50:TEM-50 Landscapes:SAM:IRM Landscapes:SAM8.64LandscapeIRM copy.pdf |

**Figure 5:** Adaptive landscapes for Amoxicillin + Sulbactam (SAM) inhibitor showing only selectivity for the inhibitor resistant mutations (bold). The global optimum is highlighted in red. Red arrows represent significance with a p-value ≤ 0.05. A) Landscape with Sulbactam at 8 μg/mL and Amoxicillin 8 μg/mL, B) Landscape with Sulbactam at 8 μg/mL Amoxicillin 16 μg/mL, and C) Landscape with Sulbactam at 8 μg/mL and Amoxicillin at 32 μg/mL. D) Landscape with Sulbactam at 8 μg/mL and Amoxicillin at 64 μg. The global optima contained at least one IRM in each of the different adaptive landscapes, with the triple mutant 1101 being selected twice at the two highest concentrations; 64 μg/mL and 32 μg/mL. TEM-50 was selected as global optimum at the lowest concentration of 8 μg/mL, and a double mutant being selected at 16 μg/mL. The concentration that had the most significantly different growth rates among the new substitutions selecting for inhibitor resistance was the highest at 64 μg/mL.

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**Figure 6:** Adaptive Landscapes for Ampicillin (AMP) at various concentrations: A) 2,048μg/mL, B) 1,024μg/mL, C) 512μg/mL. Forward arrows signify new substitutions and backward arrows signify reversions. Red arrows represent significance with a p-value ≤ 0.05. Black arrows represent non-significance, p-value ≥ 0.05. The global optimum allele is highlighted in red. TEM-50 was selected as the global optimum at the highest concentration of 2,048 μg/mL, a single mutant was selected as global optimum at 1,024 μg/mL, and a triple mutant was selected as global optimum at 512 μg/mL. We noticed at the highest concentration of 2,048μg/mL there were the most significantly different growth rates.

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**Figure 7:** Adaptive Landscapes for Amoxicillin (AM) at various concentrations: A) 1,024μg/mL, B) 512μg/mL, C) 256μg/mL. Forward arrows signify new substitutions and backward arrows signify reversions. Red arrows represent significance with a p-value ≤ 0.05. Black arrows represent non-significance, p-value ≥ 0.05. The global optimum allele is highlighted in red. A double mutant was selected as the global optimum at the highest concentration at 1024 μg/mL a triple mutant was selected at 512 μg/mL, and a single mutant was selected at the lowest concentration of Amoxicillin 256 μg/mL. The concentration in which the most new substitutions are favored is at 512 μg/mL. However, we observed the most significantly different growth rates at the highest concentration of 1024 μg/mL.

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**Figure 8:** Adaptive Landscapes for Cefepime (FEP) at various concentrations: A) 0.0312μg/mL, B) 0.0156μg/mL. Forward arrows signify new substitutions and backward arrows signify reversions. Red arrows represent significance with a p-value ≤ 0.05. Black arrows represent non-significance, p-value ≥ 0.05. The global optimum allele is highlighted in red. TEM-50 was selected as the global optimum for the lower concentration and a triple mutant was selected as global optimum at the higher concentration. There are more significantly different growth rates at the higher concentration of 0.0312 μg/mL.

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**Figure 9:** Adaptive Landscapes for Cefotaxime (CTX) at various concentrations: A) 0.123 μg/mL B) 0.06 μg/mL, C) 0.05 μg/mL, D) 0.04 μg/mL. Forward arrows signify new substitutions and backward arrows signify reversions. Red arrows represent significance with a p-value ≤ 0.05. Black arrows represent non-significance, p-value ≥ 0.05. The global optimum allele is highlighted in red. TEM-50 was selected as the global optima for the two lowest concentrations of 0.04 μg/mL and 0.05 μg/mL, a double mutant was selected as the global optimum at 0.06 μg/mL, and a triple mutant was selected at the highest concentration of 0.123 μg/mL. The concentration that contains the highest number of significantly different growth rates was 0.05 μg/mL. As the concentration decreases with Cefotaxime, the number of new substitutions selected also decreases.

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| C)Macintosh HD:Users:portia:Dropbox:TEM.50:TEM-50 Landscapes:CTT:CTT0.0312Landscape copy.pdf | D)Macintosh HD:Users:portia:Dropbox:TEM.50:TEM-50 Landscapes:Ceph. Composites:CTTComposite copy.pdf |

**Figure 10:** Adaptive Landscapes for Cefotetan (CTT) at various concentrations: A) 0.125 μg/mL B) 0.0625 μg/mL, C) 0.0312 μg/mL.Forward arrows signify new substitutions and backward arrows signify reversions. Red arrows represent significance with a p-value ≤ 0.05. Black arrows represent non-significance, p-value ≥ 0.05. The global optimum allele is highlighted in red. , D) Composite of all concentrations, showing only the arrows that remain in the same direction throughout the three concentrations. Triple mutants were selected as global optima at the two lower concentrations of 0.0312 μg/mL and 0.0625 μg/mL, and a double mutant was selected at the highest concentration of 0.125 μg/mL. We observed the highest number of significant differences in growth rates occurring at the highest concentration of Cefotetan at 0.125 μg/mL. The number of new substitutions seems more favored at the middle concentrations of 0.0625 μg/mL.

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| **Penicillins** | **Concentration (μg/mL)** | **F: B** |
| **Amoxicillin** | 1024 | 10:22 |
|  | 512 | 17:15 |
|  | 256 | 13:19 |
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| **Ampicillin 8X** | 256 | 22:10 |
|  | 128 | 18:14 |
|  | 64 | 20:12 |
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| **Pen + Inhibitors** | **Concentration (μg/mL)** | **F: B** |
| **Piperacillin + Tazobactam** | 8/512 | 15:17 |
|  | 8/256 | 13:19 |
|  | 8/128 | 12:20 |
|  |  |  |
| **Amoxicillin + Clavulanic Acid** | 8/1024 | 16:16 |
|  | 8/512 | 16:16 |
|  |  |  |
| **Ampicillin + Sulbactam** | 8/64 | 17:15 |
|  | 8/32 | 18:14 |
|  | 8/16 | 13:19 |
|  | 8/8 | 24:8 |
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| **Cephalosporins** | **Concentration (μg/mL)** | **F: B** |
| **Cefprozil** | 12.5 | 17:15 |
|  | 10 | 15:17 |
|  | 8 | 21:11 |
|  |  |  |
| **Cefotetan** | 0.125 | 14:18 |
|  | 0.0625 | 21:11 |
|  | 0.0312 | 18:14 |
|  |  |  |
| **Cefotaxime** | 0.123 | 19:13 |
|  | 0.06 | 17:15 |
|  | 0.05 | 18:14 |
|  | 0.04 | 14:18 |
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| **Ceftazidime** | 0.125 | 18:14 |
|  | 0.1 | 19:13 |
|  | 0.0625 | 19:13 |
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| **Cefepime** | 0.0312 | 22:10 |
|  | 0.0156 | 22:10 |

Table 2: List of the ratios, new substitutions: reversion, for each antibiotic treatment and concentration used. Antibiotics in first column, concentration in μg/mL in the second column, and ratio in third column.