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Evolution Under Thermal Stress Affects Escherichia coli’s Resistance to Antibiotics

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Evolution Under Thermal Stress Affects *Escherichia coli*'s Resistance to Antibiotics

A thesis submitted in partial satisfaction

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Master of Science in Biology

by

Austin Bullivant

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ABSTRACT OF THE THESIS

Evolution Under Thermal Stress Affects *Escherichia coli*'s Resistance to Antibiotics

by

Austin Bullivant

Masters in Biology

University of California, Los Angeles, 2022

Professor Pamela Yeh, Chair

Exposure to both antibiotics and changes in temperature can induce similar physiological responses in bacteria. Thus, changes in growth temperature could affect resistance to antibiotics. Previous studies have found that evolution under antibiotic stress causes shifts in optimal growth temperature of bacteria, but little is known if evolution under thermal stress affects antibiotic resistance. Examining 114 heat-adapted strains, we asked if evolution under thermal stress affects optimal growth temperature, if there are any correlations between heat adaptation and antibiotic resistance, and if antibiotic efficacy for these strains change depending on the local environment's temperature. We found that: (1) most of the heat-adapted strains displayed a decrease in optimal growth temperature relative to the ancestor strain, (2) there were complex patterns of changes in antibiotic resistance when comparing the heat-adapted strains to the ancestor strain, and (3) there were no significant correlations between antibiotic resistance and changes in optimal growth temperature.

The thesis of Austin Bullivant is approved.

Colin Kremer

Alejandra Rodrigues-Verdugo

Pamela Yeh, Committee Chair

University California, Los Angeles

2022

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

Environmental stressors such as antibiotics and extreme temperatures can impact the survival and growth of organisms, altering the selection pressures in the environment (Lynch & Gabriel 1987; Huey & Kingsolver 1989; Savage et al. 2004; Bennett & Lenski 2007; Reed et al. 2011; Buckley et al. 2016). In bacteria, these selective pressures can drive the evolution of populations and influence bacterial capacity to withstand perturbations in their environment (Imhof & Schlötterer 2001; Tello et al. 2012; Van Boeckel et al. 2015; Donhauser et al. 2020).With recent decades seeing a rise in both access to life-saving antibiotics (O'Neill 2014; Van Boeckel et al. 2017; Klein et al. 2018) as well as shifting global temperature due to climate change (Parmesan et al. 2006; Cavicchioli et al. 2019), understanding the relationships between temperature change and antibiotic exposure is becoming more critical to predicting future trajectories of bacterial populations.

Since the beginning of life, organisms have had to evolve mechanisms to survive changes in temperature that affect their biological processes (Lindquist 1986; Bada & Laczano 2002; Schwartzman & Lineweaver 2004; Dell et al. 2011; Rohr et al. 2018; Cavicchioli et al. 2019). Both hot and cold temperatures induce different physiological responses in bacteria which impacts their survival and propagation (Yamanaka 1999; Yura 2019). High temperatures typically result in the misfolding of cellular proteins as well as the formation of aggregates that interfere with essential functions (Richter et al. 2010; Vabulas et al. 2010). To withstand and cope with high temperature stress, cells have evolved a heat shock response (Ritossa 1962; Schlesinger et al. 1982), leading to increased expression of chaperone proteins to prevent misfolding as well as proteases to degrade aggregates (Arsène et al. 2010; Roncarati et al. 2017). Cold temperature responses, on the other hand, are not as thoroughly understood (Yamanaka

1999). One common response is the stiffening of DNA and RNA structures which results in an overall slower rate of DNA replication and protein synthesis (Phadtare & Inouye 2008). This stiffening response can also impact lipids, decreasing the efficiency of transport proteins as well as affinity for substrates associated with growth (Yamanaka 1999; Phadtare & Inouye 2008; Barria et al. 2013).

Compared to temperature, antibiotics are a more novel source of stress (Levy & Marshall 2004; Mlot 2009; Davies & Davies 2010; Nelson et al. 2010). The more recent widespread use of antibiotics through human activity can also alter bacterial growth and has forced them to adapt to survive in these stressed conditions (Wright 2005; Souli et al. 2008; Van Boeckel et al. 2017; Rodriguez-Verdugo et al. 2020). When evolving resistance to antibiotics, bacteria use one of three main mechanisms to assist in their survival and propagation: (a) *tolerance* allows bacteria to self-inhibit growth when exposed to antibiotics (Kester & Fortune 2014); (b) *persistence* occurs when a portion of the bacteria population is able to slow growth rates in high drug concentrations (Balaban et al. 2004; Wakamoto et al. 2013); (c) *resistance* is the accumulation of changes which allows bacteria to survive for extended durations in environments with antibiotics (Brauner et al. 2016; Rodriguez-Verdugo et al. 2020). By using these mechanisms, bacteria can often effectively respond to drug induced stress when faced with various antibiotics.

When an adaptation to one source of stress evolves, it can also alter how a population reacts to other stressors (Brooks & Crowe 2019; Cruz-Loya et al. 2021). With antibiotics and temperature having some overlap in what cellular functions they affect (Cruz-Loya et al. 2019), it has been suggested that the mechanisms of action between different types of antibiotics and varying temperature ranges are similar. Aminoglycosides, for instance, irreversibly associate to the ribosome, and introduce errors in protein translation causing aggregates to form (Mingeot-

Leclercq et al. 1999; Goltermann et al. 2013; Greulich et al. 2015). The mechanisms of action for these antibiotics impact similar cellular processes as high temperature conditions (Richter et al. 2010; Vabulas et al. 2010), resulting in non-functional proteins. Further investigation evaluated potential interactions between antibiotics and temperature stress for *E. coli* (Cruz-Loya et al. 2019). Results suggested that aminoglycosides interact with high temperature (46°C) environments to produce higher efficacy than expected from the antibiotics and temperature working alone (Cruz-Loya et al. 2019).

In Tenaillon et al. (2012), derivative strains of an *E. coli* ancestor were evolved under thermal stress for 2000 generations. It was found that some of these strains exhibited a resistance to rifampicin without any prior exposure to antibiotics (Rodriguez-Verdugo et al. 2013). It has been suggested that there may be some co-opted mechanisms between temperature tolerance and antibiotic resistance, with bacteria employing similar mechanisms to cope with with both stressors (Cruz-Loya et al. 2019). These possible co-opted mechanisms between the two types of stress reveals that both temperature change and antibiotic resistance might be more intertwined than most would initially believe.

Here we evaluate heat-adapted strains of *E. coli* to determine if there is a correlation between shifts in optimal growth temperature and changes in antibiotic resistance. Specifically, we ask the following questions: (1) how does evolution under thermal stress affect the optimal growth temperature of *E. coli*, (2) how does heat adaptation affect strength of antibiotic resistance, and (3) are there any relationships between a change in optimal growth temperature and a change in antibiotic resistance?

MATERIALS AND METHODS

Bacterial Strains

We examined the ancestor strain of *Escherichia coli* B genotype *REL1206,* a descendent of *REL606* (Tenaillon et al. 2012), using the *E. coli* strains generated in Rodriguez-Verdugo et al. (2013). A total of 114 replicate populations were descended from *REL1206* and independently evolved to a heat stress (42.2°C) for 2000 generations.

Generation of Heat-Response Curves

We extracted our bacteria strains from frozen stock and allowed them to grow 16 to 18 hours overnight at 37°C. We obtained optical density at 600nm (OD_{600}) from each of the overnight samples. We then diluted any strains that displayed an OD_{600} reading above 0.5 by 1:100 in a culture tube with 3mL of fresh lysogeny broth (LB). We allowed any strains below a 0.45 OD_{600} reading to continue growing. After we ensured OD_{600} measurements are consistent across all strains, 200µL of bacteria are transferred into each well of a 96-well plate. We divided the 96-well plate in half to provide six replicates of thirteen strains for more accurate results. We then pin transferred the bacteria into 96-well plates containing 200µL of LB per well before being covered by a porous seal to allow for gas exchange. We placed the plates in incubators with sixteen staggered temperatures ranging from 12°C to 50°C for approximately 18 to 22 hours. We collected the OD_{600} measurements after the incubation period. We used six replicates of LB as a negative control and subtracted from the OD_{600} of the other strains to only measure bacterial growth.

To generate heat-response curves for each strain, we used a modified Briere model to fit the experimental data, defining the temperature dependence of growth as g(T) (Briere et al. 2019). The reparametrized extended Briere model is as follows:

$$g(T) = g_{max}\left[\left(\frac{T - T_{min}}{\alpha}\right)^{\alpha}\left(\frac{T_{max} - T}{1-\alpha}\right)^{1-\alpha}\left(\frac{1}{T_{max} - T_{min}}\right)\right]^{s}$$

where the value of g_{max}, the highest measured growth of the examined strain, and values of α and s are given by parameters a and b which alter the shape of the bacterial growth curve such that: $\alpha = \frac{a}{(a+b)}$ and $s = a + b$ (Briere et al. 2019). In this reparametrized equation, the first term within the brackets denotes the growth of the strain relative to the minimum temperature where growth occurs, the second term denotes the growth relative to the maximum temperature where growth occurs, and the third term accounts for the entire range of growth for a given strain of *E. coli*. We calculated the optimal growth temperature for each strain using:

$$T_{opt} = \alpha T_{max} + (1 - \alpha)T_{min}$$ (Briere et al. 1999).

A few examples of fitting this model to our data set can be found in Figure 2.

Determining Strength of Antibiotic Resistance

To test for antibiotic resistance, we ran a series of serial dilutions for each antibiotic in 96-well plates. Specifically, we diluted the antibiotics twenty times with each dilution being one half of the previous concentration. We used twelve antibiotics for our experiments which span the major classifications of antibiotics used in medical settings (Table 1). We extracted antibiotics from a stock solution and diluted in LB to a concentration of 4000µg/mL. We added antibiotics into a 96-well plate containing 100µL of LB and serially diluted wells in the plate, establishing a range of concentrations from 2000µg/mL to approximately 0.008µg/mL. We adjusted the starting concentration values for antibiotics that required greater resolution to determine antibiotic resistance. We sealed the plates and placed them in a 37°C incubator for 22–24 hours before we collected OD_{600} measurements. We determined and compared IC_{50} values,

the minimum amount of antibiotic needed to kill 50% of the bacteria population, to evaluate antibiotic resistance. The IC_{50}s for all heat-adapted strains were determined when plates were incubated at 37°C, a non-stressed environment. We also took a small subsample of fifteen random strains to determine the IC_{50} data for each antibiotic at 42°C, the adapted environment, to assess how this may change antibiotic resistance.

We determined the $\log_2$ mean fold change of the IC_{50} to evaluate how heat adaptation potentially changed the IC_{50} value and thus antibiotic resistance levels.

$$\text{IC}_{50}\text{ fold change} = \frac{(IC_{50})_{heat\ adapted}}{(IC_{50})_{ancestor}}$$

Values greater than one indicate that heat adaptation also conferred resistance to the antibiotic and values less than one indicate heat adaptation increased sensitivities to the antibiotic.

RESULTS

Heat-Adapted Strains Display a Decreased Optimal Growth Temperature

We found optimal growth temperature decreased for the heat adapted strains averaging at 35.13°C (std. dev. = 1.9°C). This is a significant decrease compared to the optimal growth temperature of ancestral strain (38.09°C) (two-tailed, one-sample t-test, $\mu = 38.09$, $p = 2.2 \times 10^{-16}$). Eight strains had a higher optimal growth temperature relative to the ancestor (Fig. 1).

Strength of Antibiotic Resistance Changed between Different Temperatures

In general, heat-adaptation changed the strains' IC_{50} values to multiple antibiotics. We found a significant increase of resistance to gentamycin and levofloxacin when IC_{50} values were

determined at 37°C. However, only levofloxacin showed a significant increase in IC_{50} value at both 37°C and 42°C (two-tailed, one-sample t-test, $\mu = 1, p_{37°C} = 9.47 \times 10^{-4}$, $p_{42°C} = 1.14 \times 10^{-7}$) (Table 2 and Table 3). We also found a significant increase in sensitivity to ampicillin, ciprofloxacin, cefoxitin, clindamycin, tetracycline, and trimethoprim (two-tailed, one-sample t-test, $\mu = 1, p < 2.73 \times 10^{-4}$, Table 2) when IC_{50} values were determined at 37°C. This significant increase in sensitivity was also found in ampicillin, clindamycin, ciprofloxacin, and tetracycline when IC_{50} values were determined at 42°C (two-tailed, one-sample t-test, $\mu = 1, p < 1.54 \times 10^{-7}$, Table 3).

We compared the IC_{50} of each antibiotic at 37°C and 42°C to see if there were any differences in antibiotic resistance between the heat-adapted strains and the ancestral strain (Fig. 3). We found that three of twelve antibiotics (cefoxitin, clindamycin, and levofloxacin) showed significantly different IC_{50} values at 42°C compared to 37°C (Table 4). Levofloxacin and cefoxitin showed significantly higher IC_{50} values at 42°C (two-tailed, two-sample t-test, $p < 0.003$). IC_{50} values for clindamycin was significantly higher at 37°C (two-tailed, two-sample t-test, $p= 0.002$). A full listing of p-values and test statistics can be found in table 4.

No Significant Relationships between Optimal Growth Temperature and Antibiotic Resistance

We investigated if we could find any relationships between changes in optimal growth temperature and corresponding IC_{50} values. We observed that at 37°C and at 42°C, no significant correlations between IC_{50} values and optimal growth temperature changes for any antibiotic (Spearman correlation, $p > 0.05$, Table 5). The relationship between the gentamycin IC_{50} values determined at 42°C and the change in optimal growth temperature was the strongest relationship found a nearly significant (Spearman correlation, $R= 0.48$, $p= 0.073$) (Figure 4).

DISCUSSION

We asked how the adaptation to a higher thermal stress on *E.* coli may affect their optimal growth temperature, antibiotic resistance, and how these two factors may be correlated. We found that optimal growth temperature varied among the 114 strains, with most showing a lower optimal growth temperature than the ancestor strain's optimum temperature. No strains exhibited a significant relationship between antibiotic resistance and changes in optimum growth temperature. We did see significant changes in antibiotic resistance between the heat-adapted strains and the ancestor strain.

We hypothesized that the evolved strains examined would have an increased optimal growth temperature relative to the ancestral strain. Heat shock responses are a highly conserved physiological response among prokaryotes and eukaryotes (Richter et al. 2010; Hug & Gaut 2015). This response promotes increased synthesis of heat-shock proteins and chaperones to degrade any formed aggregates and prevent further protein misfolding (Vabulas et al. 2010; Mondal et al. 2014). When exposed to high temperatures for long periods of time, individuals that show increased expression of this heat-shock response will likely be favored in this stressed environment (Bennett et al. 2017). Experimental evolution of bacteria under thermal stress can adjust in response to stress through two possible strategies. In the short-term, phenotypic plasticity can help bacteria acclimate whereas genetic changes help in longer-term scenarios (Hug & Gaut 2015). With our heat-adapted strains experiencing high heat stress for 2000 generations, we expected to see an increased optimal growth temperature. However, our results did not match our initial hypothesis. We were surprised to find that the vast majority (96%) of our heat-adapted strains displayed a lower optimal growth temperature than the ancestral strain.

One possible explanation for the decreased optimal growth temperatures seen in some of the heat-adapted strains could be due to an interaction between adaptation and acclimation to the bacteria's local environment (Hug & Gaut 2015). A previous study investigated how the phenotypes of heat-adapted *E. coli* would respond when placed in different temperature conditions (Hug & Gaut 2015). It was found that the majority of the heat-adapted strains' phenotypes displayed a return to the unstressed phenotypic state (Hug & Gaut 2015). Our observations of lower optimal growth temperatures for *E. coli* support previous studies, that suggest *E. coli* can employ adaptive strategies in an attempt to return the bacterium cell to its unstressed physiological state found at 37°C (Hug & Gaut 2015; Lambros et al. 2021). An attempt to return to an unstressed physiological state may enable bacteria to increase their fitness in stressful environments (Lambros et al. 2021).

Another possible explanation for an observed decrease in optimal growth temperatures is that these heat-adapted have high phenotypic plasticity for temperature stress. (Miller et al. 2020). A previous study focusing on how cyanobacteria respond to extreme temperature argued that plasticity allows for innovation to arise in stressed populations (Miller et al. 2020; Levis & Pfennig 2020). It was found that under extreme temperature conditions some cyanobacteria populations developed a novel form of a cell wall that is less permeable allowing them to survive in these high temperatures (Miller et al. 2020). It is thought that this plasticity allows the bacteria to "buy time" in novel environments and helps them to persist in conditions that may not be optimal (Fox et al. 2019).

We also hypothesized that antibiotic resistance for our heat-adapted strains should change depending on the temperature conditions they are grown under. Cruz-Loya et al. (2019) highlighted a stressor network between temperature and antibiotics. This is in part seen due to

the how “hot-like” antibiotics, such as aminoglycosides, can negatively affect the same cellular mechanisms that hot temperatures do (Goltermann et al. 2013; Cruz-Loya et al. 2019). We observed significant increases in antibiotic resistance (IC_{50}) for multiple antibiotics.

The heat adapted strains had significantly different levels of resistance to two-thirds (8 of the 12) of the antibiotics we tested at 37°C. Among these eight, five of them had significantly different resistances at 42°C. This suggests that heat-adaptation affected resistance levels to these antibiotics. Interestingly, one of the drugs that our strains were most resistant to at 37°C and 42°C was levofloxacin (LVX) which is associated with greater performance at cold temperatures (Cruz-Loya et al. 2019). Also, despite having similar mechanisms of action, not every antibiotic of the same class responded to temperature in the same manner. We encourage future studies to further investigate if these patterns are consistent across multiple antibiotics that possess the same mechanism of action.

We hypothesized that for heat-adapted *E. coli* strains, there is a positive relationship between changes in optimal growth temperature and strength of antibiotic resistance. However, our results did not support our initial hypothesis. With some antibiotic classes and temperature overlapping in the cellular mechanisms they affect, an adaptation to temperature may confer increased fitness against antibiotics (Cruz-Loya et al. 2019). Prior studies have shown that, if possible, cells will attempt to evolve a co-opted response that is able to function when exposed to various types of stressors (Dragosits et al. 2013; Święciło 2016). However, we found no significant positive correlations between optimal growth temperature and antibiotic resistance.

One possible explanation as to why we do not see significant relationships between optimal growth temperature and antibiotic resistance may be due to their novel traits not providing an immediate fitness benefit (Karve et al. 2015; Toll-Riera et al. 2016). Novel

mutations that are not the primary focus of experimental evolution can arise in bacterial populations over time (Karve & Wagner 2022). These novel mutations can appear as a byproduct of the evolution of other adaptive traits and may become beneficial once the environment changes (Karve & Wagner 2022). Fitness trade-offs have been observed when bacteria are evolved under antibiotic stress before exposed to novel temperatures (Herren & Baym 2022). Strains that evolved resistance to antibiotics were found to have reduced growth at extreme temperature ranges and relatively normal growth at optimal growth temperature conditions (Herren & Baym 2022). Our results suggest that in unstressed temperature environments, changes in antibiotic resistance might not be directly due to shifts in optimal growth temperature.

Other studies that have focused on the effects of temperature stress have examined temperature niche breadth, focusing on a range of temperatures where bacteria can grow effectively (MacFadden et al. 2018; Herren & Baym 2022). Examining a range of temperatures may provide useful information for examining heat-adaptation, such as any phenotypic plasticity bacteria can exhibit in response to temperature stress (Payne & Wagner 2019). While we examined a range of temperatures to determine the optimal growth temperature of the heat-adapted *E. coli,* a more thorough examination of niche temperature breadth would provide a more complete understanding of how different dimensions of heat adaptation affects antibiotic resistance.

With increases in temperature forecast in many parts of the world as a result of global climate change, it is becoming more crucial to understand temperature-antibiotic interactions. Despite numerous studies on how climate change can affect disease vectors (Campbell et al. 2015; Ogden & Lindsay 2016; Mordecai et al. 2019), few have examined how the pathogens themselves would be affected by shifts in temperature (Rodriguez-Verdugo et al. 2020). Novel

temperature environments can affect bacteria by shifting antibiotic resistant populations across different geographic regions (MacFadden et al. 2018) and also result in increased rates of antibiotic resistance (Ratkowsky et al. 1982; McGough et al. 2018). Our work further examines how long-term exposure to heat stress can affect *E. coli*'s responses to both temperature and antibiotic stressors. Approaching this problem with an evolutionary lens and examining the relationships between different stressors may assist future researchers in identifying crucial interactions among stressors.

TABLES

Table 1. List of Antibiotics examined in this study.

Identification			
Drug/Chemical	Abbreviation	Main Mechanism	Molecular Weight (g/mol)
Ampicillin	AMP	Cell wall	371.4
Cefoxitin	FOX	Cell wall	449.4
Levofloxacin	LVX	DNA gyrase	361.3
Ciprofloxacin	CPR	DNA gyrase	367.8
Nitrofurantoin	NTR	DNA	238.2
Trimethroprim	TMP	Folic Acid	290.3
Tobramycin	TOB	Aminoglycoside	467.5
Gentamycin	GEN	Aminoglycoside	1488.8
Streptomycin	STR	Aminoglycoside	728.7
Clindamycin	CLI	Protein Synthesis	479.5
Erythromycin	ERY	Protein Synthesis	733.9
Tetracycline	TET	Protein Synthesis	444.4

Table 2. The fold change of IC_{50} values determined at 37°C after heat adaptation. Comparisons in **bold** indicate a significant difference after performing a Bonferroni multiple comparison correction (Bonferroni corrected $\alpha = 0.002$).

Drug	estimate	t-statistic	p-value	sample size (n)	95% Confidence Interval	
					2.50%	97.50%
AMP	**0.340**	**-5.855**	**5.62E-08**	**104**	**0.116**	**0.564**
CLI	**0.521**	**-16.225**	**2.95E-30**	**104**	**0.462**	**0.579**
CPR	**0.283**	**-7.196**	**9.98E-11**	**104**	**0.085**	**0.481**
ERY	0.938	-1.316	0.191	103	0.845	1.031
FOX	**0.575**	**-13.485**	**1.73E-24**	**103**	**0.513**	**0.638**
GEN	**1.416**	**6.139**	**1.54E-08**	**104**	**1.281**	**1.550**
LVX	**11.469**	**4.762**	**6.23E-06**	**104**	**7.109**	**15.829**
NTR	3.719E+15	1	0.320	104	-3.656E+15	1.109E+16
STR	2.63E+106	1.423	0.159	85	-1.05E+106	6.32E+106
TET	**0.298**	**-44.755**	**7.24E-69**	**102**	**0.266**	**0.329**
TMP	**0.789**	**-3.770**	**2.73E-04**	**103**	**0.678**	**0.900**
TOB	1.019	0.449	0.654	104	0.936	1.101

Table 3. The fold change of IC_{50} values determined at 42°C after heat adaptation. Comparisons in **bold** indicate a significant difference after performing a Bonferroni multiple comparison correction (Bonferroni corrected $\alpha = 0.002$).

Drug	estimate	t-statistic	p-value	sample size (n)	95% Confidence Interval	
					2.50%	97.50%
AMP	**0.137**	**-51.157**	**2.54E-17**	**14**	**0.101**	**0.173**
CLI	**0.199**	**-21.199**	**4.88E-12**	**14**	**0.117**	**0.280**
TET	**0.311**	**-25.497**	**8.06E-12**	**12**	**0.253**	**0.370**
CPR	**0.400**	**-9.608**	**1.53E-07**	**14**	**0.267**	**0.534**
FOX	0.749	-3.260	0.006	14	0.583	0.914
TMP	1.028	0.163	0.873	14	0.662	1.393
NTR	1.122	1.010	0.330	14	0.863	1.380
GEN	1.557	2.300	0.037	14	1.038	2.077
ERY	1.563	2.999	0.010	14	1.160	1.965
TOB	1.636	1.717	0.108	14	0.842	2.430
STR	1.922	1.608	0.130	14	0.693	3.151
LVX	**19.925**	**9.837**	**1.14E-07**	**14**	**15.798**	**24.051**

Table 4. Comparing mean fold change for antibiotics showed that eight of the twelve drugs examined demonstrated a significant change in IC_{50} values between the two temperatures. A negative t-statistic indicates that the IC_{50} values for that drug were higher at 42°C. Comparisons in bold indicate a significant difference after performing a Bonferroni multiple comparison correction (Bonferroni corrected $\alpha = 0.004$)

Drug	37°C Mean Fold Change	42°C Mean Fold Change	t-statistic	P-value
AMP	0.1973	0.1366	2.062	0.0498
CLI	**0.4304**	**0.1985**	**3.478**	**0.0019**
CPR	0.4287	0.4003	0.082	0.9358
ERY	0.9104	1.5625	-3.041	0.0061
FOX	**0.4188**	**0.7487**	**-3.400**	**0.0022**
GEN	1.3967	1.5573	-0.502	0.6198
LVX	**6.3127**	**19.924**	**-5.900**	**4.17E-06**

NTR	0.6983	1.1216	-2.786	0.0098
STR	4.5607	1.9217	1.326	0.20578
TET	0.2238	0.3114	-2.207	0.0367
TMP	0.5926	1.0277	-2.130	0.0436
TOB	1.0303	1.6357	-1.600	0.1303

Table 5. No significant relationships between IC_{50} values and changes in optimal growth temperature were found at 37°C or 42°C. A Spearman correlation was conducted to determine strength of any possible relationship and the p-value.

Drug	Temperature (°C)	# of Strains	Spearman Correlation (R)	P-value
AMP	37	114	-0.097	0.32
CLI	37	114	0.19	0.052
CPR	37	114	0.068	0.49
ERY	37	114	0.011	0.91
FOX	37	114	0.041	0.68
GEN	37	114	-0.0084	0.93
LVX	37	114	-0.051	0.6
NTR	37	114	0.027	0.79
STR	37	114	-0.029	0.79
TET	37	114	0.09	0.37
TMP	37	114	0.041	0.68
TOB	37	114	0.067	0.5
AMP	42	15	-0.45	0.091
CLI	42	15	-0.13	0.64
CPR	42	15	0.35	0.2
ERY	42	15	0.16	0.58
FOX	42	15	0.036	0.9
GEN	42	15	0.48	0.073
LVX	42	15	0.19	0.51
NTR	42	15	0.24	0.4
STR	42	15	0.0036	0.99
TET	42	15	-0.011	0.98
TMP	42	15	0.22	0.43
TOB	42	15	0.42	0.12

FIGURES

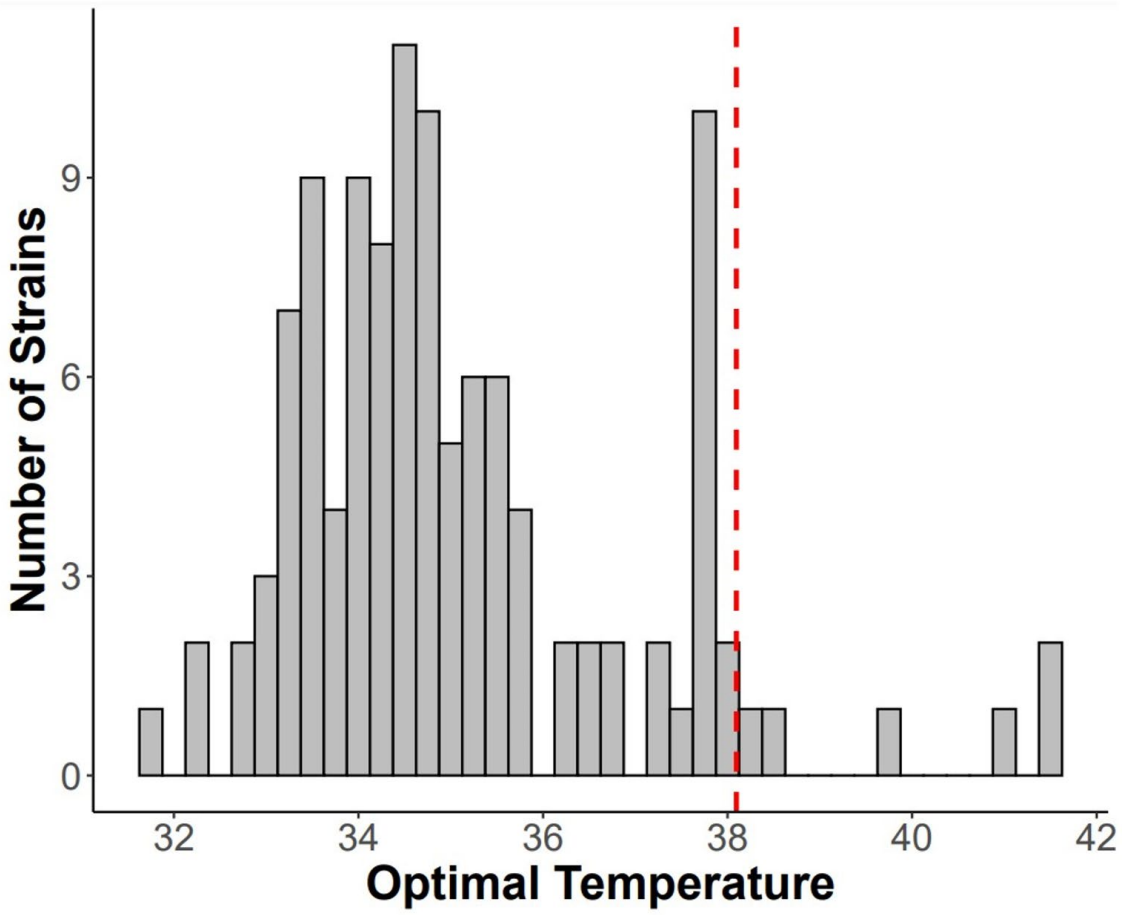

Fig. 1 Distribution of optimal growth temperatures shows that majority of strains (~96%) had a lower optimum temperature compared to the ancestor (38.09°C). The vertical, dashed red line represents the ancestor's optimal growth temperature.

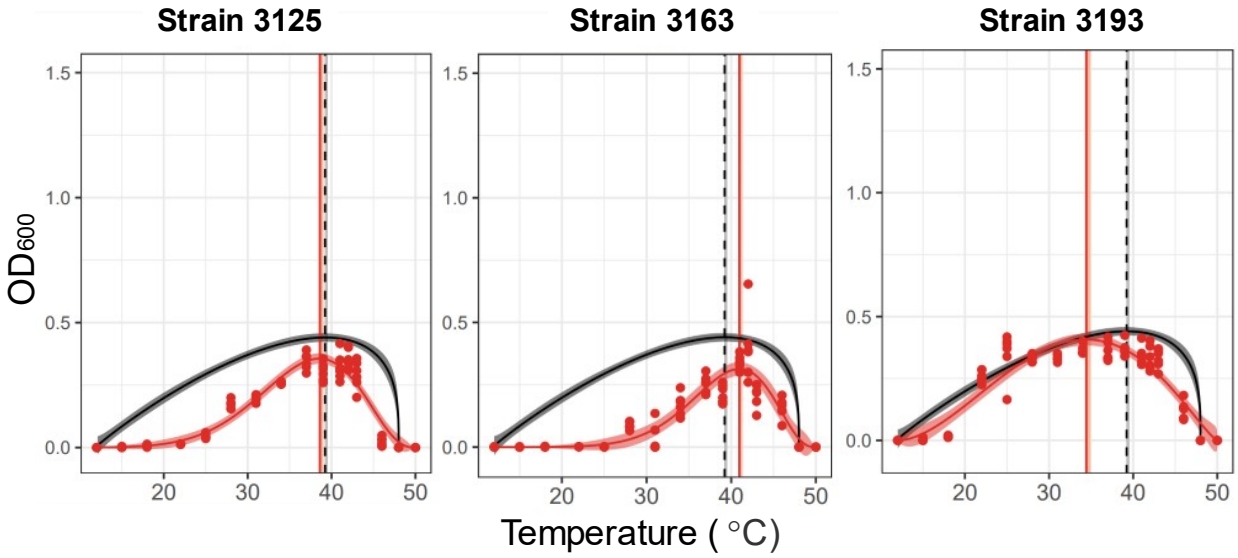

Fig. 2 Heat-response curves for the strains displayed a variety of responses in optimal growth temperature. Solid black line represents the growth of our ancestral strain of *E. coli* with the vertical, dashed black line depicts the ancestor's optimal growth temperature. The vertical, solid red line denotes the optimal growth temperature of our heat-adapted strains with the non-vertical, red line representing the heat-adapted strain's growth response curve. These strains are representatives of the optimal growth temperature shifts we observed in our bacteria with few strains showing either no change (Strain 3125) or an increased optimal growth temperature (Strain 3163) relative to the ancestor strain and most showing a decreased optimal growth temperature (Strain 3193).

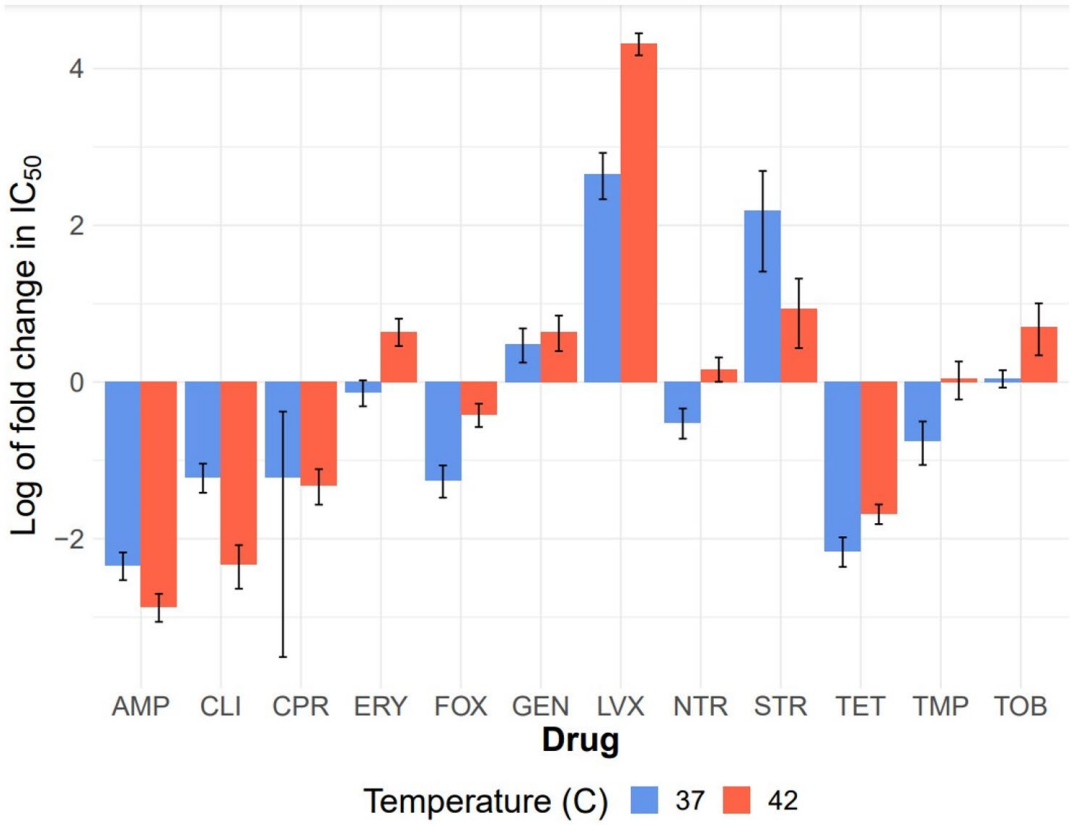

Fig. 3 *E. coli* strains had either varied or lower resistance to antibiotics relative to the ancestor after evolving under heat stress for most of the tested antibiotics. Log_2 of fold change was used to compare the heat-adapted strains between the two temperature conditions with the ancestor strain's antibiotic resistance. We used standard error as our error bars.

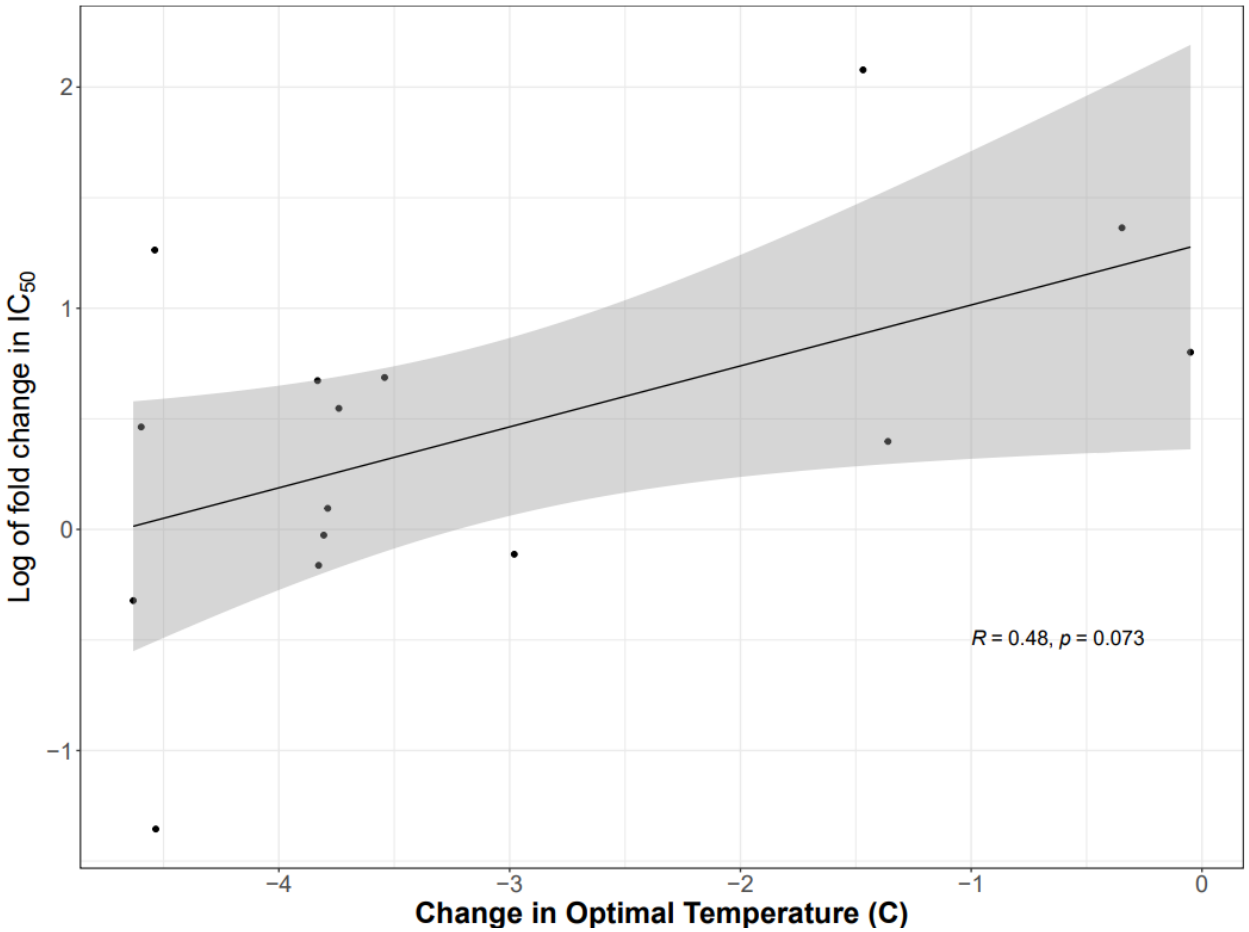

Fig. 4. No significant relationships were found during comparisons of IC_{50} values and changes in optimal growth temperature. This figure of gentamycin at 42°C acts as a representative for our other antibiotics. Spearman correlation (R) and p-value are provided on the figure.

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