Environmental Science and Ecotechnology 22 (2024) 100475

Contents lists available at ScienceDirect

Environmental Science and Ecotechnology

journal homepage: www.journals.elsevier.com/environmental-science-and-ecotechnology/

Short Communication

Nonlinear impacts of temperature on antibiotic resistance in *Escherichia coli*

Wenya Zhao [a, b], Shikan Zheng [a, b], Chengsong Ye [a, b], Jianguo Li [a, b], Xin Yu [a, b, *]

[a] College of the Environment and Ecology, Xiamen University, Xiamen, 361102, China
[b] Fujian Key Laboratory of Coastal Pollution Prevention and Control, Xiamen, 361102, China

ARTICLE INFO

Article history:
Received 13 February 2024
Received in revised form
1 August 2024
Accepted 2 August 2024

Keywords:
Antibiotic resistance
Temperature
Gatifloxacin
Resistance evolution
Fitness costs

ABSTRACT

The increase in bacterial antibiotic resistance poses a significant threat to the effectiveness of antibiotics, and there is growing evidence suggesting that global warming may speed up this process. However, the direct influence of temperature on the development of antibiotic resistance and the underlying mechanisms is not yet fully understood. Here we show that antibiotic resistance exhibits a nonlinear response to elevated temperatures under the combined stress of temperatures and antibiotics. We find that the effectiveness of gatifloxacin against *Escherichia coli* significantly diminishes at 42 °C, while resistance increases 256-fold at 27 °C. Additionally, the increased transcription levels of genes such as *marA*, *ygfA*, and *ibpB* with rising temperatures, along with gene mutations at different sites, explain the observed variability in resistance patterns. These findings highlight the complexity of antibiotic resistance evolution and the urgent need for comprehensive studies to understand and mitigate the effects of global warming on antibiotic resistance.

1. Introduction

Global warming has escalated environmental problems, such as high temperatures, droughts, wildfires, and torrential rainfall. It has also amplified human health risks, such as increased bacterial resistance, regarded as one of the greatest threats to human health in the 21st century [1,2]. The selective pressure of antibiotics is generally considered the main reason for disseminating and conserving bacterial resistance in the environment [3].

In addition to the selective effect of antibiotics, many physicochemical factors have been reported to play a role in the occurrence of bacterial antibiotic resistance in the environment, such as heavy metals [4], salinity [5], mutagenic compounds [6], and temperature changes [7]. Big data analysis has shown a 1.14-fold increase in carbapenem-resistant *Klebsiella pneumoniae* for every 1 °C rise in average temperature [3]. Keba stated that temperature contributes more to antibiotic resistance than dosage [8]. Linear regression is often used to analyze the relationship between temperature rise and antibiotic resistance. However, this pattern is influenced by multiple factors, including antibiotic usage, population density, economic development, and corruption levels [1,3]. The specific impact of temperature remains unclear. Given the current rise in global temperatures, it is crucial to determine the role of temperature in this context.

Temperature plays an important role in bacterial survival, transmission, and infection [9]. It can persistently stress bacteria regarding general physio-biochemical characteristics, such as growth and intracellular chemical reaction rates, and characteristics closely related to resistance, such as gene transfer [10–12]. Some researchers have found that *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* have distinct sensitivities to various antibiotics at different temperatures [13,14]. For example, high-temperature adapted *E. coli* has increased resistance to sulfonamide and nitrofuran antibiotics and may also acquire resistance to rifampicin [15].

However, it is unclear how bacterial antibiotic resistance and its mechanisms evolve when antibiotic selective pressure coexists with the stress of raised temperature, a widespread scenario in climate change. To shed light on this, the present study explored the resistance evolution in wild-type *E. coli* under the combined effect of temperature (five temperatures varying from 22 to 42 °C) and antibiotic exposure.

* Corresponding author. College of the Environment and Ecology, Xiamen University, Xiamen,361102, China.
E-mail address: xyu@xmu.edu.cn (X. Yu).

https://doi.org/10.1016/j.ese.2024.100475

2. Materials and methods

2.1. Strain, medium, and antibiotics

The ancestor *E. coli* K12 was stored at −80 °C and later revived and cultured using Luria-Bertani (LB) broth and a solid medium. The antibiotics mentioned in the text were sourced from Macklin, and their corresponding Chemical Abstracts Service (CAS) numbers are: gatifloxacin (112811-59-3), ampicillin (69-53-4), methicillin (132-92-3), erythromycin (114-07-8), rifampicin (13292-46-1), streptomycin (3810-74-0), tetracycline (64-75-5), and chloramphenicol (56-75-7).

2.2. Experimental evolution

The evolution experiment was conducted in a 24-well plate with 2 mL of liquid LB. The strains were first passaged twice without exposure to antibiotics at temperature in evolutionary experiments. The strains were passaged (BMG Labtech, Germany) after the microplate reader determined that the bacterial solution had an optical density at 600 nm (OD_{600}) greater than 0.8. Subsequently, 20 μL of bacterial solution and gradient-increased gatifloxacin solution were continuously transferred to liquid LB, with a total volume of 2 mL. Four replicates were prepared. The concentrations of the antibiotics were increased in a 1, 2, 5, 10, 20, 50, 100, 200, 500, and 1000 μg L^{-1} gradient at different temperatures (22, 27, 32, 37, and 42 °C) (schematic, Supplementary Material Fig. S1). The passage was repeated three times at each antibiotic concentration. The last passage of the population was saved and designated as the antibiotic-evolved population at different temperatures (denoted as "temperature-G″ when evolved under gatifloxacin and "temperature-A″ if the antibiotic is ampicillin). Meanwhile, the ancestor strains that evolved continuously in LB without antibiotics, maintaining the same frequency of passages, were considered the populations that evolved at different temperatures as controls, referred to in the text as "temperature-0." The evolved strains obtained at different stages were stored at −80 °C in 25% glycerol.

Before each passage, it was ensured that at least one of the four replicates had an OD_{600} greater than 0.8. The passage interval of the temperature-only populations was consistent with those exposed to antibiotics at different temperatures. *E. coli* was grown at 180 rpm at a constant temperature. OD_{600} was measured and used to construct growth curves at hourly intervals throughout the incubation. The *E. coli* grown at 17 °C stopped growing when the antibiotic concentration reached 50 μg L^{-1}, and the treatment stopped.

2.3. Phenotypic characterization

The phenotypic characterization of the *E. coli* evolved from gatifloxacin exposure at different temperatures was determined, along with the minimum inhibitory concentration (MICs, by referring to CLSI), the heritability of antibiotic resistance and the tolerance of the evolved strains, the intracellular reactive oxygen species (ROS) through 2′,7′-dichlorodihydrofluorescein diacetate, the cell membrane formation ability using the crystal violet staining method, and the mutation rate of the evolved strains using the fluctuation assay method. More experimental details can be found in the Methods section of the Supplementary Materials.

2.4. Fitness costs

According to the data model based on the growth curve [16], the growth data of the last evolutionary stage of *E. coli* were fitted by curveball (http://curveball.yoavram.com), including four different parallels and the optimal model was selected to simulate the competition experiment. The growth of the evolved strain and wild-type *E. coli* under antibiotic-free conditions at 37 °C was predicted to obtain the fitness costs of the strain. The lag times of the strains at 22 °C were too long to compare, and it was assumed that the strains' adaptability was poor.

2.5. DNA extraction, whole-genome sequencing, and bioinformatics analysis

The whole-genome DNA of the strain exposed to gatifloxacin at different temperatures was extracted. According to the kit instructions, total bacterial DNA was extracted using a Tiangen kit. The reference genome was the National Center of Biotechnology Information (NCBI) Reference Sequence NZ_CP071521.1, sequencing the evolved bacterial genome (Novogene Bioinformatics Technology Co., Ltd., Tianjin, China). The original image data file obtained through Illumina sequencing was transformed into the original sequenced reads via base calling analysis. The experimental details, sequencing, and bioinformatic analysis data can be found in the Methods section of the Supplementary Materials.

2.6. Ribonucleic acid extraction and bioinformatics analysis

Ribonucleic acid (RNA) was extracted from the evolved endpoint strains exposed to gatifloxacin at different temperatures. The process was carried out at 4 °C (Tiangen kit). Immediately after extraction, complementary deoxyribonucleic acid (cDNA) was reverse transcribed as described, and the concentrations were recorded with Nanodrop (B-500 BioPhotometer, Shanghai) and stored at −20 °C. The expression levels of the 43 target genes were then quantified, including stress response, biofilm formation, heat shock, antibiotic resistance, and redox. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed according to the manufacturer's instructions (TransGen Biotech, China) using the ABI Q6 qPCR system (Life Technology, Singapore). The experiments set up three parallels, and the primers are shown in Supplementary Materials.

2.7. Molecular docking

To further understand the contribution of mutations at different temperatures to quinolone resistance at the molecular structure level, molecular docking of the protein DNA gyrase subunit A (GyrA) and gatifloxacin was performed. First, protein homology modeling of the mutant was performed in SWISS-MODEL (https://swissmodel.expasy.org/interactive# sequence) based on the *E. coli* K12 protein (PDB ID: P0AES5), and GROMACS was used to calculate the optimal structure of the mutant protein and ligand. UCSF DOCK 6.10 [17] was used for the molecular docking of proteins and ligands, and the grid fraction (binding energy [kcal mol^{-1}]) was calculated to screen for the optimal binding state. PyMol was used to visualize the binding process of GyrA and the ligand.

3. Results and discussion

In the evolutionary experiment, wild-type *E. coli* K12 was exposed to a gradient of gatifloxacin concentrations (1 μg L^{-1}–1 mg L^{-1}) at different temperatures. Each treatment was cultured for about 2000 generations. The MICs of the *E. coli* increased with antibiotic dosage, but this increase did not follow a simple positive or linear correlation with elevated temperature. Gatifloxacin resistance was the highest at 27 °C, with a 256-fold increase, and the lowest at 42 °C, with an eight-fold increase. Resistance to antibiotics other than gatifloxacin was also induced at

various temperatures (Fig. 1a). The MICs of the *E. coli* were essentially stable over the temperature control without antibiotics. In addition, gatifloxacin resistance in 27-G and 42-G could be heritable under antibiotic-free conditions (Fig. 1b). We also conducted experiments with increasing concentrations of antibiotics at 17 °C. The growth of *E. coli* ceased when the antibiotic level reached 50 μg L^{-1}. We propose that 17 °C is not an optimal growth condition. Additionally, the strategies for resistance evolution at lower temperatures may differ from those at higher temperatures.

Optimizing the lag time is considered the primary adaptive change produced by bacteria in the face of stress [18] and can provide the basis for acquiring multiple resistance [19]. In the present study, the lag time of *E. coli* at different temperatures increased with the gatifloxacin concentration gradient, but it was more pronounced at lower temperatures. For example, the lag time at 22 °C increased from 6 h (under antibiotic-free conditions) to 28.2 h (1 mg L^{-1}) (Fig. 1c). The *E. coli* grown at all temperatures exhibited "hormesis" during the stationary phase and was least inhibited at 42 °C (Fig. 1d). It was also least inhibited at 42 °C when another antibiotic ampicillin was used (Supplementary Materials S4). Furthermore, the biofilm formation ability of the evolved *E. coli* was generally enhanced (Fig. 1j), and the intracellular ROS levels and mutation rates increased, contributing to the development of resistance (Fig. 1k and l). Differences in the growth response of

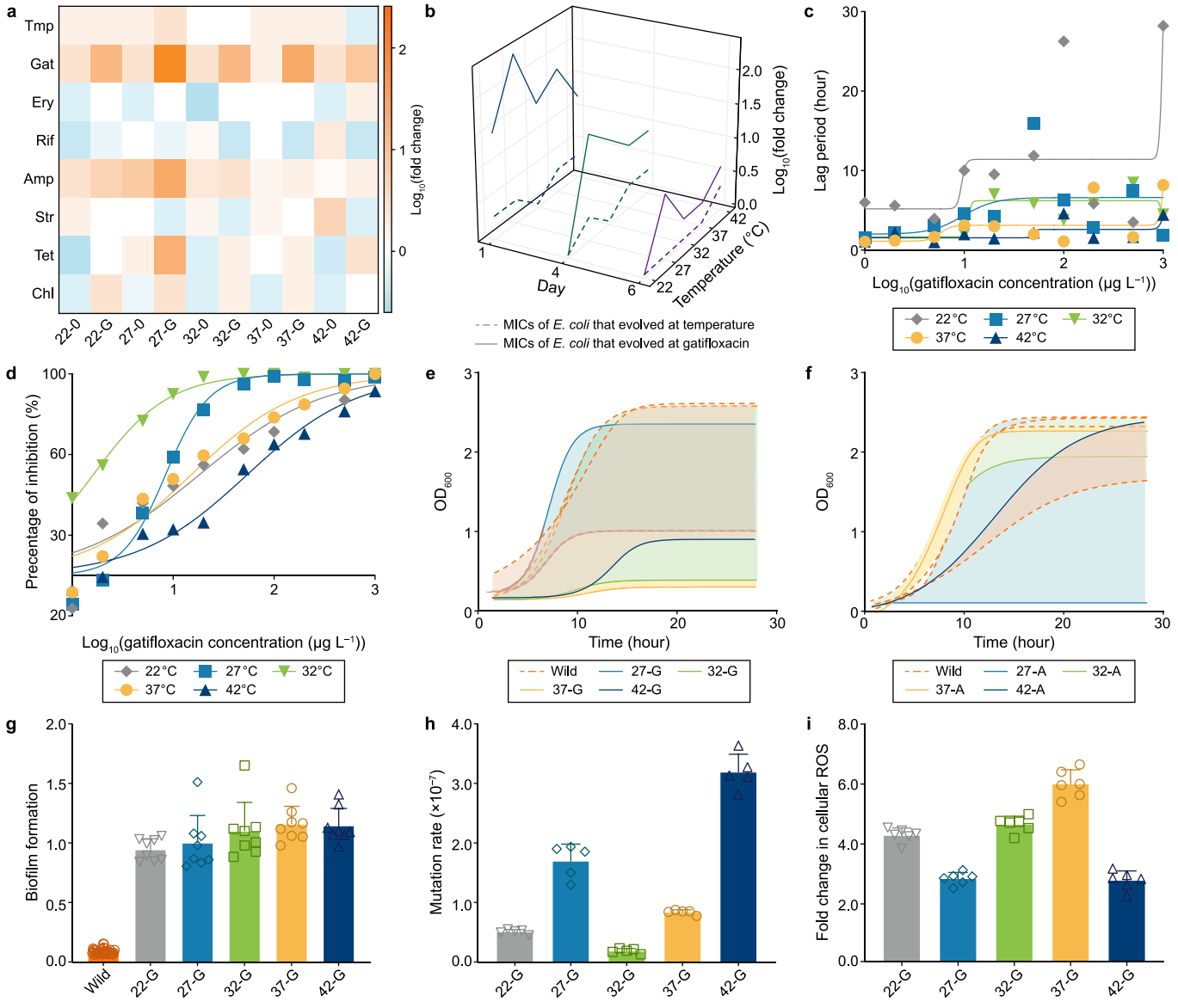

Fig. 1. Antibiotic resistance and phenotype variations of *Escherichia coli* after antibiotic exposure at different temperatures. **a**, Fold changes in the MICs of eight antibiotics in *E. coli* evolved with and without gatifloxacin at different temperatures compared with wild-type *E. coli*. **b**, Gatifloxacin-evolved *E. coli* exhibit heritable antibiotic resistance in the absence of antibiotics at 37 °C. The heritability of resistance in *E. coli* that evolved with (solid line) and without (dashed line) gatifloxacin at different temperatures is indicated by changes in MICs during the cultivation of these strains at 37 °C without antibiotics. **c**, Changes in the lag phase time in *E. coli* during exposure to elevated gatifloxacin at different temperatures. **d**, Change in the inhibition rate of *E. coli* during exposure to elevated gatifloxacin at different temperatures. **e**–**f**, The fitness costs of evolved strains in gatifloxacin (**e**) and ampicillin (**f**) under different temperature treatments compared to wild-type strains. The solid and dashed lines indicate the position of the growth curves of the evolved *E. coli* and wild-type *E. coli* when co-cultured in competition. A lower position signifies a poorer competitive ability, i.e., a higher fitness cost. **g**–**i**, Phenotypic characterization of *E. coli* after evolving in gatifloxacin at different temperatures: biofilm forming ability (**g**); mutation rate (**h**); cellular ROS (**i**). The significance level was $P < 0.01$. Tmp: trimethoprim, Gat: gatifloxacin, Ery: erythromycin, Rif: rifampicin, Amp: ampicillin, Str: streptomycin, Tet: tetracycline, Chl: chloramphenicol.

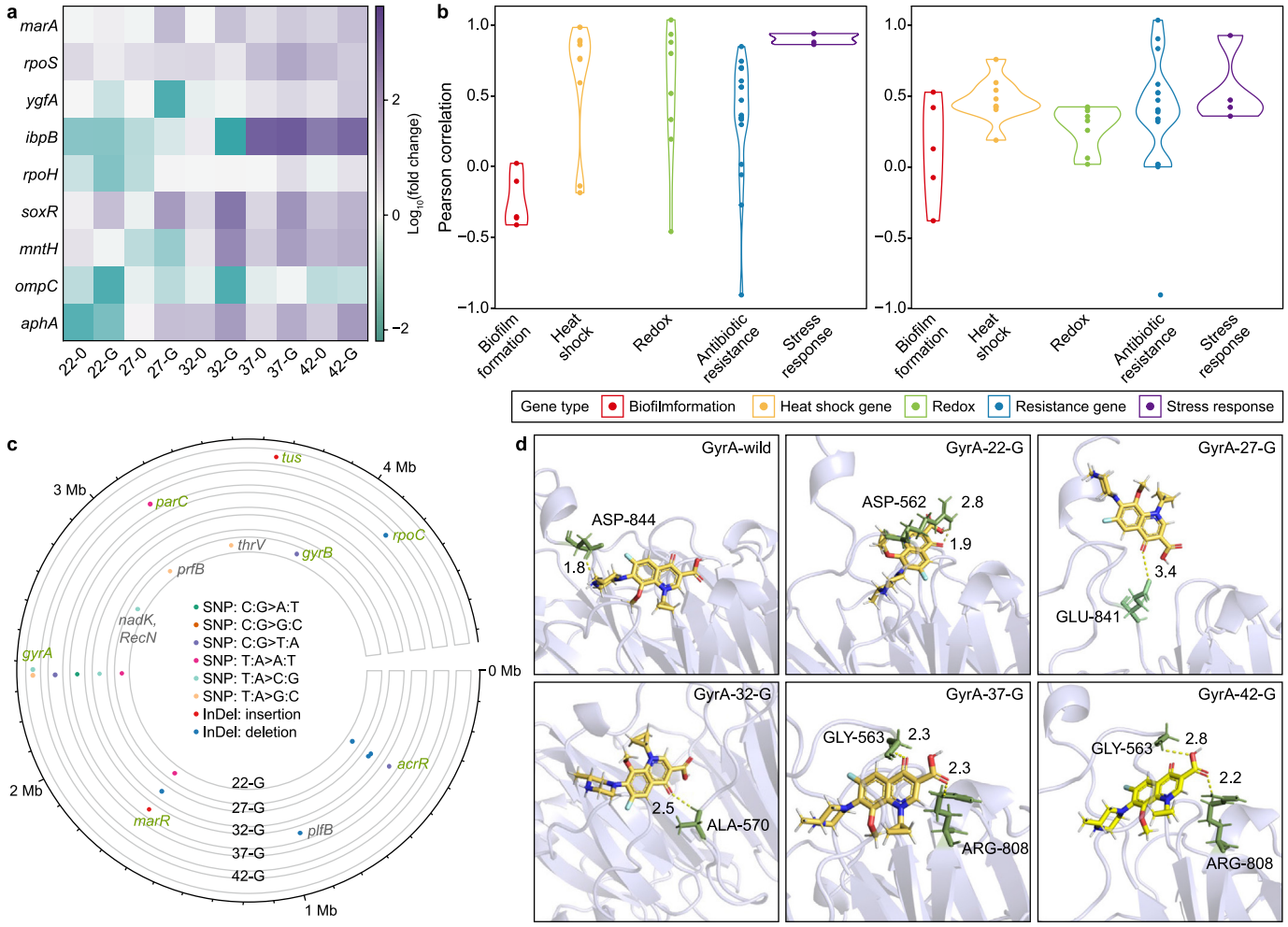

Fig. 2. Gene expression, gene mutation, and molecular docking of mutant GyrA in *Escherichia coli* after evolving in gatifloxacin at different temperatures. **a**, The expression levels of some *E. coli* genes were changed after evolution. 16 S rRNA gene was used as the reference. The abbreviation consists of *E. coli* evolved in temperature-treated conditions: the number represents the temperature during evolution, and the following "0" and "G" indicate *E. coli* evolved without and with gatifloxacin. **b**, With (G) or without (0) gatifloxacin treatment, the increased temperature was correlated with the upregulation of several types of gene expression levels. **c**, Whole genome sequencing revealed effective genetic variation in the *E. coli* genome after evolution. The outer circle represents the size of the reference genome. Different colored dots represent the types of variation in the genomic response sites. Green indicates that the gene is a confirmed antibiotic-resistance mutation, and grey indicates that it has not been identified. **d**, Molecular docking was performed between the GyrA of both wild-type and evolved mutant strains with the antibiotic gatifloxacin. The mutant strains were those that evolved under different temperatures and increasing gradients of gatifloxacin. GyrA-22-G represents the docking structure of gatifloxacin with mutated *E. coli* GyrA that evolved under 22 °C and gatifloxacin. By analogy, GyrA-27-G, GyrA-32-G, GyrA-37-G, and GyrA-42-G represent the docking structures of the gatifloxacin and mutated *E. coli* GyrA that evolved with gatifloxacin at 27, 32, 37, and 42 °C, respectively.

E. coli to antibiotic stress at various temperatures were observed, such as pronounced mutation rates in 27-G and 42-G (Fig. 1k). These differences may underlie the evolution of distinct levels of antibiotic resistance in *E. coli*.

Fitness costs are essential factors limiting the dissemination of antibiotic resistance [20]. We simulated the growth of strains that evolved separately in two antibiotics at 37 °C in an antibiotic-free environment. Except for 27-G, which was exposed to gatifloxacin, and 42-A, which was exposed to ampicillin, *E. coli* showed significant fitness costs (Fig. 1e and f). The two exceptions above are consistent with the results of the resistance heritable test (Fig. 1b). The mechanism may be that stepwise mutation and compensatory mutation at the evolutionary temperature reduce fitness costs. Mutations in *E. coli* can improve fitness and dramatically lower antibiotic sensitivity in acquiring quinolone resistance [21]; this may be analogous.

We investigated the underlying causes of the varying resistance phenotypes observed at different temperatures. First, the transcript levels of most genes generally increase with temperature. The expression of 43 genes in evolved *E. coli* was determined by RT-qPCR. The proportion of upregulated genes increased significantly as the temperature rose to 37 and 42 °C (Fig. 2b), and some genes, such as the heat shock gene *ibpB*, were upregulated almost a thousandfold. The upregulation of efflux pumps and global transcriptional regulators (*marA*, *rpoS*, and *ygfA*) was beneficial for *E. coli* in conferring multiple resistance and adaptability. There are also individual genes whose expression is negatively correlated with temperature, such as *ompC*, which tends to reduce the uptake of external substances at low temperatures, making bacteria more tolerant to antibiotics [22].

Second, mutation is evolution's main driver, indicating that resistance is likely to change significantly. By comparing the gene sequences of wild-type and evolved *E. coli*, a total of 12 genes were found to be mutated, with seven genes undergoing resistance mutations (Fig. 2c). The genes involved in the mutation were *acrR* and *marR*, which regulate the overall resistance of *E. coli* and are

part of the negative regulation of the multidrug efflux pump regulator [23]. There were also mutations in the target genes *gyrA*, *gyrB*, and *parC*, which can confer high levels of quinolone resistance by reducing antibiotic affinity [24]. The *gyrA* mutation occurred at any temperature of gatifloxacin exposure and twice in 42-G. The *Tus* and *rpoC* mutations in 42-G and 37-G indicated the acquisition of β-lactam and rifampicin resistance [25]. Intriguingly, we identified several genetic variations in the 22-0 and 42-0 controls, where no antibiotics were added. These metabolic genes associated with zinc ion transport, movement, and adhesion will likely confer a greater selective advantage (Supplementary Material Table S3), easing the metabolic constraints on growth [25]. The unique gene mutations and different mutation sites at 22 and 42 °C indicated specific responses at different temperatures.

Temperature affects how resistance mutations occur in bacteria and their strength. The molecular docking model evaluated the affinity between GyrA with different mutation sites and gatifloxacin at the structural level [26]. The binding sites of GyrA and gatifloxacin were found to shift at various exposure temperatures. Compared to the binding energy of −35.209 kcal mol^{-1} in the wild type, the evolved strains in each treatment showed varying degrees of reduced binding energy, with the most significant reduction observed in 27-G. The double mutation of Asp87Gly and IIe798Ser in 42-G also reduced the electrostatic force, but it increased the polarity effect and ranked it backward. This shows that the induction mechanism at different temperatures is distinct but may have some preference.

4. Conclusion

In summary, global warming has been widely reported to contribute to increasing bacterial resistance to antibiotics [8] and to the spreading and maintaining of ARGs [3]. However, the results of the present study show that the role of temperature in the emergence and development of resistance is uncertain. Global warming may primarily drive the development of antibiotic resistance by escalating antibiotic usage. Higher temperatures are likely to increase the incidence, frequency, and duration of epidemics, inevitably leading to more antibiotic use [2,3]. Additionally, other issues arising from global warming induce or keep bacterial resistance, including the increased use of heavy metals and pesticides, as well as frequent natural disasters [2,4].

CRediT authorship contribution statement

Wenya Zhao: Writing - Review & Editing, Writing - Original Draft, Formal Analysis, Data Curation, Conceptualization. **Shikan Zheng:** Methodology, Data Curation. **Chengsong Ye:** Validation, Supervision, Investigation, Data Curation. **Jianguo Li:** Supervision, Data Curation, Conceptualization. **Xin Yu:** Validation, Supervision, Funding Acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (U2005206) and the National Key R&D Program of China (2023YFE0112100).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ese.2024.100475.

References

[1] D.R. MacFadden, S.F. McGough, D. Fisman, M. Santillana, J.S. Brownstein, Antibiotic resistance increases with local temperature, Nat. Clim. Change 8 (2018) 510.
[2] M. Romanello, et al., The 2022 report of the Lancet Countdown on health and climate change: health at the mercy of fossil fuels, Lancet 400 (2022), 6736(22)01540-9.
[3] W. Li, et al., Association between antibiotic resistance and increasing ambient temperature in China: an ecological study with nationwide panel data, Lancet Reg Health West Pac 30 (2023) 100628.
[4] L.G. Li, Y. Xia, T. Zhang, Co-occurrence of antibiotic and metal resistance genes revealed in complete genome collection, ISME J. 11 (2017) 651–662.
[5] L. Hou, et al., Urban ponds as hotspots of antibiotic resistome in the urban environment, J. Hazard Mater. 403 (2021) 124008.
[6] D. Li, S. Zeng, M. He, A.Z. Gu, Water disinfection byproducts induce antibiotic resistance-role of environmental pollutants in resistance phenomena, Environ. Sci. Technol. 50 (2016) 3193–3201.
[7] M. Cruz-Loya, R. Martínez, J. Smith, A. Johnson, P. Kim, T. Lee, J. Thompson, Stressor interaction networks suggest antibiotic resistance co-opted from stress responses to temperature, ISME J. 13 (2019).
[8] H.E.J. Kaba, E. Kuhlmann, S. Scheithauer, Thinking outside the box: association of antimicrobial resistance with climate warming in Europe – a 30 country observational study, Int. J. Hyg Environ. Health 223 (2020) 151–158.
[9] F.C. Fang, E.R. Frawley, T. Tapscott, A. Vázquez-Torres, Discrimination and integration of stress signals by pathogenic bacteria, Cell Host Microbe 20 (2016) 144–153.
[10] J.L. Bada, A. Lazcano, Some like it hot, but not the first biomolecules, Science 296 (2002) 1982–1983.
[11] D. Braun, A. Libchaber, Thermal force approach to molecular evolution, Phys. Biol. 1 (2004) P1.
[12] D.J. Dwyer, M.A. Kohanski, J.J. Collins, Role of reactive oxygen species in antibiotic action and resistance, Curr. Opin. Microbiol. 12 (2009) 482–489.
[13] P. Mira, N. Lozano-Huntelman, A. Johnson, V.M. Savage, P. Yeh, Evolution of antibiotic resistance impacts optimal temperature and growth rate in *Escherichia coli* and *Staphylococcus epidermidis*, J. Appl. Microbiol. 133 (2022) 2655–2667.
[14] A.A. Al-Nabulsi, R.H. Osaili, I.A. Shaker, T.M. Olaimat, M.H. Jaradat, N.A. Zain Elabedeen, M.M. Holley, Effects of osmotic pressure, acid, or cold stresses on antibiotic susceptibility of *Listeria monocytogenes*, Food Microbiol. 46 (2015) 154–160.
[15] A. Rodríguez-Verdugo, B.S. Gaut, O. Tenaillon, Evolution of *Escherichia coli* rifampicin resistance in an antibiotic-free environment during thermal stress, BMC Evol. Biol. 13 (2013) 1–11.
[16] Y. Ram, N. Dellus-Gur, T. Bibi, O. Karkar, I. Obolski, R. Hadany, M. Borenstein, Predicting microbial growth in a mixed culture from growth curve data, Proc. Natl. Acad. Sci. U.S.A. 116 (2019) 14698–14707.
[17] S. Mukherjee, T.E. Balius, R.C. Rizzo, Docking validation resources: protein family and ligand flexibility experiments, J. Chem. Inf. Model. 50 (2010) 1986–2000.
[18] O. Fridman, A. Goldberg, I. Ronin, N. Shoresh, N.Q. Balaban, Optimization of lag time underlies antibiotic tolerance in evolved bacterial populations, Nature 513 (2014) 418.
[19] I. Levin-Reisman, N. Fridman, I. Ronin, O. Shinar, A. Kamerer, D. Kishony, N.Q. Balaban, Antibiotic tolerance facilitates the evolution of resistance, Science 355 (2017) 826–830.
[20] C.M. Herren, M. Baym, Decreased thermal niche breadth as a trade-off of antibiotic resistance, ISME J. 16 (2022) 1843–1852.
[21] L.L. Marcusson, N. Frimodt-Moller, D. Hughes, Interplay in the selection of fluoroquinolone resistance and bacterial fitness, PLoS Pathog. 5 (2009).
[22] M. Masi, E. Pages, M. Damblin, R. Dupont, A. Nicolas, L. Meunier, J.M. Bolla, Cephalosporin translocation across enterobacterial OmpF and OmpC channels, a filter across the outer membrane, Commun. Biol. 5 (2022) 1059.
[23] J.L. Martinez, A. Fajardo, L. Garmendia, A. Hernandez, J.F. Linares, L. Martinez-Solano, C. Alvarez-Ortega, A global view of antibiotic resistance, FEMS Microbiol. Rev. 33 (2009) 44–65.
[24] K. Mehla, J. Ramana, Structural signature of Ser83Leu and Asp87Asn mutations in DNA gyrase from enterotoxigenic *Escherichia coli* and impact on quinolone resistance, Gene 576 (2016) 28–35.
[25] T.M. Conrad, A. Frazier, C. Joyce, B.L. Cho, D. Knight, S. Lewis, B.O. Palsson, RNA polymerase mutants found through adaptive evolution reprogram *Escherichia coli* for optimal growth in minimal media, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 20500–20505.
[26] Y. Shenhar, A. Rasouly, D. Biran, E.Z. Ron, Adaptation of *Escherichia coli* to elevated temperatures involves a change in stability of heat shock gene transcripts, Environ. Microbiol. 11 (2009) 2989–2997.