Proceedings of the 3rd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/89/2025.20458

Antimicrobial Resistance Gene Profiles in Communities Selected with Novel and Previously Encountered Antibiotics

Huajie Cheng[1,a,*]

[1]*Institute of Science and Engineering, The University of Edinburgh, EH9 3BF, United Kingdom*
a. huajiecheng@outlook.com
**corresponding author*

Abstract: Antibiotics are extensively employed worldwide for therapeutic purposes, often leading to overuse, which constitutes a contributing factor to the emergence of antibiotic resistance (ABR). This phenomenon arises from diminished effectiveness of antibiotics in treating bacterial infections due to resistance development. However, limitations in experimental conditions and equipment making it challenging to pinpoint the precise sources of antibiotic resistance, consequently constraining the breadth of research on ABR. Genomic analysis of antibiotic-resistant bacteria assists in unveiling the origins and pathways of resistance dissemination. This study employs Nanopore-based metagenomic sequencing, enabling direct sequencing of microbial DNA within macrogenomic samples, thereby yielding comprehensive sequence data. Coupled with qPCR and 16S rRNA gene datasets, this approach facilitates the exploration of antibiotic resistance gene distribution and prevalence within microbial communities across different environments Focusing on microbial communities within Scottish farmlands, isolated colonies were exposed to antibiotics (sulfamonomethoxine and erythromycin) for several weeks. By comparing them to untreated control group microorganisms, the identification of antibiotic resistance genes (ABRGs), along with their variations in prevalence and abundance, aims to analyse the impact of these two antibiotics on these species.

Keywords: Antibiotic resistance (ABR), Antibiotic resistance genes (ARGs), Nanopore sequencing, Environmental antibiotic contamination.

1. Introduction

Antibiotics have made significant contributions to human and animal medicine by treating and preventing bacterial infections. However, the overuse and misuse of antibiotic use has led to a global crisis of antibiotic resistance, posing a severe threat to public health. The rise of resistant bacteria has progressively diminished the effectiveness of antibiotic therapies, with the World Health Organization highlighting that antibiotic resistance (ABR) not only increases mortality rates and healthcare costs but also negatively impacts economic development [1]. The emergence and spread of antibiotic-resistant bacteria have made treating infections more challenging, resulting in significant health and economic burdens.

A primary driver of antibiotic resistance is the transmission of resistance genes. Studies show that bacteria can spread antibiotic resistance genes (ARGs) through horizontal gene transfer (HGT) to other bacteria, even across species, accelerating the spread of resistance [2]. Furthermore, the

© 2025 The Authors. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

DOI: 10.54254/2753-8818/89/2025.20458

widespread presence of antibiotics in agriculture, aquaculture, and environmental settings has amplified the distribution of resistance genes [3]. In the environment, resistance genes not only circulate within bacterial communities but also spread through water systems, soil, and the food chain, ultimately reaching humans and exacerbating the complexity of the ABR issue. Furthermore, fresh produce, due to its consumption raw or with minimal processing, may serve as a major vehicle for the transmission of antimicrobial-resistant bacteria and genes. Additionally, it is subjected to selective pressures from antimicrobial agents and biocides throughout the food chain, making it a potential hotspot for the development and dissemination of antimicrobial resistance [4].

Antimicrobial resistance (AMR) refers to the ability of microorganisms such as bacteria, fungi, viruses, and parasites to evolve over time and resist the effects of antibiotics that were originally effective against them. This has become an increasingly serious global health challenge [5]. Microorganisms acquire resistance through various mechanisms, including spontaneous mutations in their genetic composition, which alter the antibiotic target, rendering the antibiotic ineffective. In addition, microorganisms can obtain antibiotic resistance genes through horizontal gene transfer (HGT) processes, such as conjugation, transformation, and transduction, from other organisms. The escalation of AMR is driven by these mechanisms, which promote microbial reproduction, enhance their resistance to antimicrobial agents, and allow resistant microorganisms to transfer resistance genes, thereby enabling non-resistant microorganisms to acquire resistance [6]. Despite extensive research on antibiotic resistance, gaps remain in understanding the environmental mechanisms of resistance gene dissemination, particularly regarding the evolution and spread of antimicrobial resistance genes (ARGs) within complex microbial communities. Existing studies indicate that ARGs are not limited to clinical environments but are also widespread in natural environments [7]. Therefore, studying the distribution and transmission routes of resistance genes within microbial communities is crucial for understanding the ecological impact of antibiotic contamination and providing scientific information for antibiotic management policies.

This study focuses on assessing the impact of antibiotic contamination on microorganisms in natural environments, particularly how antibiotic accumulation in soil and water promotes the spread of resistance genes. Specifically, it will use molecular tools to examine the distribution and fluctuations of ARGs within agricultural soils in Scotland, aiming to uncover the ecological risks posed by environmental antibiotic pollution and the response mechanisms of microbial communities.

2. Methods

To examine the effects of antibiotic contamination on freshwater sediment bacterial communities, we conducted a study using microcosms, metagenomic sequencing, and quantitative polymerase chain reaction (qPCR) to assess antibiotic resistance genes (ARGs).

2.1. Study Design

Freshwater sediment and water samples were collected from the upper and lower catchments of Tulloch Burn, Aberdeenshire, Scotland, in July 2021. The upstream samples, collected in forested areas, were treated with erythromycin (ERYT), commonly used in aquaculture. Downstream samples, closer to agricultural land, were treated with sulfamethoxazole (SFMX), widely used in livestock farming. Microcosms were prepared in sterile 50 mL falcon tubes with 15 g sediment and 25 mL water, amended with antibiotics at 0.1 μg/L and 10 μg/L. Each treatment was set up in triplicate, including untreated controls, and incubated at 25°C.

DOI: 10.54254/2753-8818/89/2025.20458

2.2. Sampling and DNA Extraction

Samples were collected from microcosms on days 0, 1, 7, 14, 21, 29, and 63 using autoclaved glass cores and stored at -20°C. DNA was extracted from 250 mg of sediment using the DNeasy PowerSoil Pro kit (QIAGEN), following the manufacturer's instructions. DNA was eluted in 50 µL for downstream analysis, and selected samples were stored at 4°C for long-read sequencing.

2.3. Nanopore Sequencing

Six selected DNA samples underwent metagenomic sequencing using the Oxford Nanopore Technology (ONT) kit. Library preparation included DNA end repair, barcode ligation, and adapter ligation, with ProNex beads used for purification. Sequencing was performed using an ONT MinION device, and fast5 files were converted to fastq files using Guppy software.

2.4. qPCR for ARG Quantification

Samples were analyzed for ARGs using Resistomap's SmartChip qPCR technology. DNA concentrations were adjusted to 10 ng/µL, allowing for the quantification of target ARGs and mobile genetic elements in each microcosm.

2.5. Data Processing and Analysis

Sequencing reads were assembled using Metaflye, followed by EPI2ME AMR workflow for antibiotic resistance profiling. Further filtering of sequences by length was done using the GALAXY platform, and BLAST searches against the CARD database were used to identify ARGs.

3. Results

3.1. Nanopore Data

The 6 samples were sequenced (Table 1). The results showed that day 14 had the highest amount of resistance genes, so we chose to Nanopore with both antibiotic concentrations on day 14 and day 1. It is important to note that due to the average sequence lengths of Bc1, Bc2, and Bc5 being less than 1 kb, DNA assembly can be challenging. The sequencing results were as follows: Estimated N50: 2.57 kb, estimated bases: 7.06 Gb, data produced: 95.39 GB, and reads generated: 5.4 million.

Table 1: Nanopore sequencing data

Barcode	Sample	Read Analysed	Total Yield	AVG SEQUENCE LENGTH
Bc1	D01-M (0µg/L SFMX)	988380	880.1 Mb	890 bp
Bc2	D14-A (0.1µg/L SFMX)	955713	841.7 Mb	880 bp
Bc3	D14-I (10µg/L SFMX)	663691	704.9 Mb	1,062 bp
Bc4	D01-13 (0µg/L ERYT)	640013	713.5 Mb	1,114 bp
Bc5	D14-5 (0.1µg/L ERYT)	17229	16.7 Mb	968 bp
Bc6	D14-9 (10µg/L ERYT)	903,062	942.9 Mb	1,004 bp

3.2. CARD Database Download

We used the Comprehensive Antibiotic Resistance Database (CARD; http://arpcard.mcmaster.ca) to analyze antibiotic resistance genes (ARGs). CARD provides extensive annotated data on ARGs, facilitating research on antibiotic resistance mechanisms and associated genes. For our analysis, we

DOI: 10.54254/2753-8818/89/2025.20458

focused on the Protein Homolog Model within CARD, which identifies protein sequences based on their similarity to reference sequences, covering genes conferring antibiotic resistance in variou organisms.

Samples treated with sulfamethoxazole (SFMX) and erythromycin (ERYT) were evaluated for resistance drug classes and mechanisms. In SFMX-treated samples, 13 drug classes were identified, with multidrug resistance being most prevalent. For resistance mechanisms, antibiotic efflux was the most common. In ERYT-treated samples, 11 drug classes were observed, also dominated by multidrug resistance, similar to SFMX.

3.3. Blast Results.

Sequencing of six samples revealed a total of 11 potential antibiotic resistance genes (Table 2). BLAST analysis was conducted for verification, with and no exact matches were found in the database, suggesting these genes may not be present in current records. Red-bordered cells in Table 2 indicate genes identified by EPI2ME, while dual verification through BLAST and EPI2ME is marked for genes identified by both. The gene intl1, which facilitates horizontal gene transfer (HGT) of resistance genes, aligned with three ABRG databases. Consequently, analysis focused on the top ten genes: sul1, sul2, drfB, oleC, ermA, ereA, mefA, mdtA, mexB, and int1.

Table 2: Blast and EP12ME Results

Gene	Target antibiotics (major)	EPI2ME	BLAST_N
sul1_1	Sulfonamide	NO	NO
sul2_1	Sulfonamide	NO	YES
dfrB	Trimethoprim	YES	YES
oleC	MLSB	YES	NO
ermA	MLSB	NO	NO
ereA	MLSB	YES	NO
mefA	MLSB	NO	YES
mdtA	MDR	YES	YES
mexB	MDR	YES	YES
intI1_1	Integrons	NO	NO
intI2_2	Integrons	NO	NO
16S rRNA_1	16S rRNA	YES	YES

3.4. Comparison of the ability of two antibiotics to stimulate nine resistance genes

Given that mdtA and mexB both fall within the Multidrug ABRG classification, they were grouped together for comparative analysis, focusing on their stimulation by SFMX and ERYT (Figure 1). Overall, ERYT resulted in greater induction of these two resistance genes, with mdtA exhibiting relatively higher expression levels compared to mexB across both antibiotic groups. Subsequently, a comparison was drawn among genes belonging to the MLSB ABRG group, namely ereA, ermA, mefA, and oleC. Notably, ereA was found to be the most readily induced gene within this group. Similarly, to the Multidrug ABRG category, ERYT displayed a greater propensity for stimulating the emergence of the ereA gene. In contrast, while oleC was the second most easily stimulated gene within the MLSB ABRG group, the SFMX had a greater impact on it than ERYT. T-tests indicated that, except for sul1, the remaining four genes (mdtA, ereA and mefA) had higher susceptibility to ERYT-induced stimulation. All calculated P-values were below 0.05, indicating statistically significant differences between the effects of SFMX and ERYT.

DOI: 10.54254/2753-8818/89/2025.20458

Table 3: UNPAIRED T-TEST

ABRG	ERYT(E) or SFMX(S)	P-value
SUL1	S>E	0.0016
MDTA	E>S	0.0001
EREA	E>S	0.0040
MEFA	E>S	0.0385

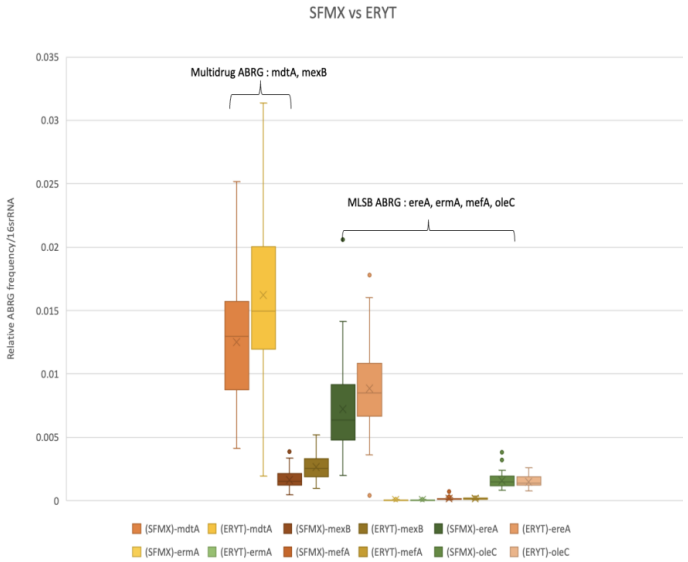


Figure 1: (A) Mean CT value of ABRG from 16s rRNA. MLSB ABRG includes macrolides, lincosamides and streptogramins-B(MLSB) resistance genes, which have similar mechanisms of bacterial inhibition. (B) T-test for resistance genes

3.5. Content of antibiotic-driven microbial resistance genes (key genes) produced at different SFMX concentrations.

3.5.1. Sulfonamide Resistance Genes (mexB, sul1, sul2)

Figure 2 shows that mexB displayed the highest expression levels across three repeated experiments over 63 days, while sul1 had the lowest content but showed significant statistical relevance.

DOI: 10.54254/2753-8818/89/2025.20458

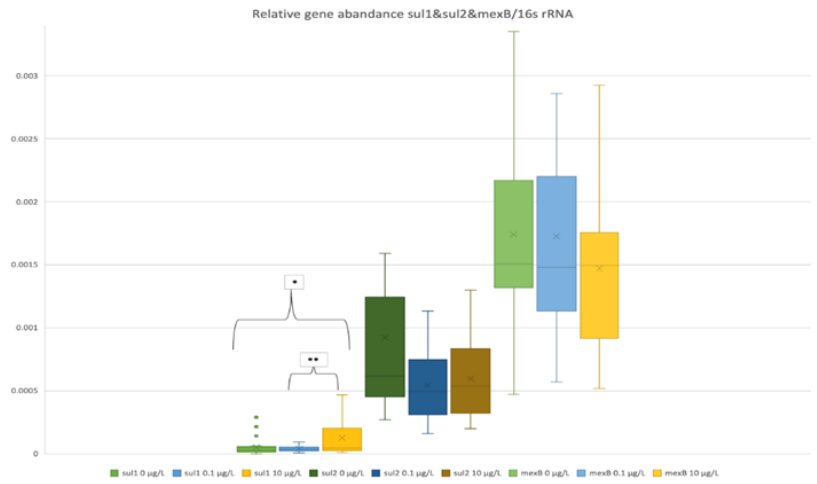


Figure 2: Relative abundance of sul1, sul2, and mexB / 16S rRNA. Y-axis: Gene frequency (=2^ (average CT of 16S rRNA - average CT of ABRG)). *: $p<0.05$, **: $p\leq0.005$.)

3.5.2. Non-SFMX-Related Resistance Genes

Among five other resistance genes (ermA, mefA, oleC, ereA, mdtA), mdtA exhibited the highest content, followed by ereA. Conversely, ermA showed the lowest expression level, but it still showed notable statistical significance (Figure 3).

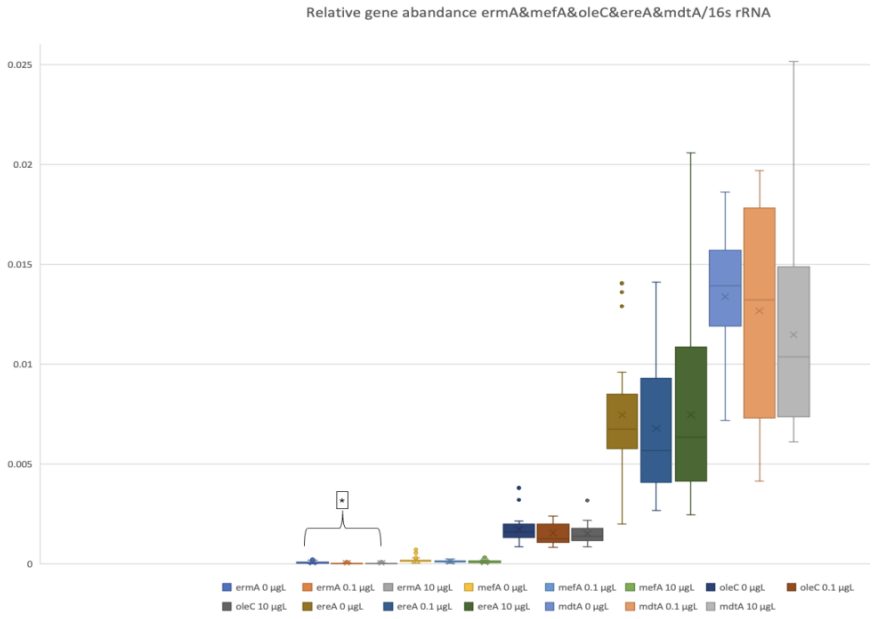


Figure 3: Relative abundance of ermA, mefA, oleC, ereA, and mdtA 16s rRNA.

3.5.3. ERYT-Related Resistance Genes (macrolide class)

Within the macrolide class (ermA, mefA, oleC, mexB, ereA, mdtA), mdtA had the highest expression, while ermA showed the lowest (Figure 4). At an ERYT concentration of 0.1 μg/L, mdtA, ereA, oleC, and ermA showed increased expression, which decreased to minimum levels at 0 μg/L for mdtA, oleC, ermA, and mefA.

DOI: 10.54254/2753-8818/89/2025.20458

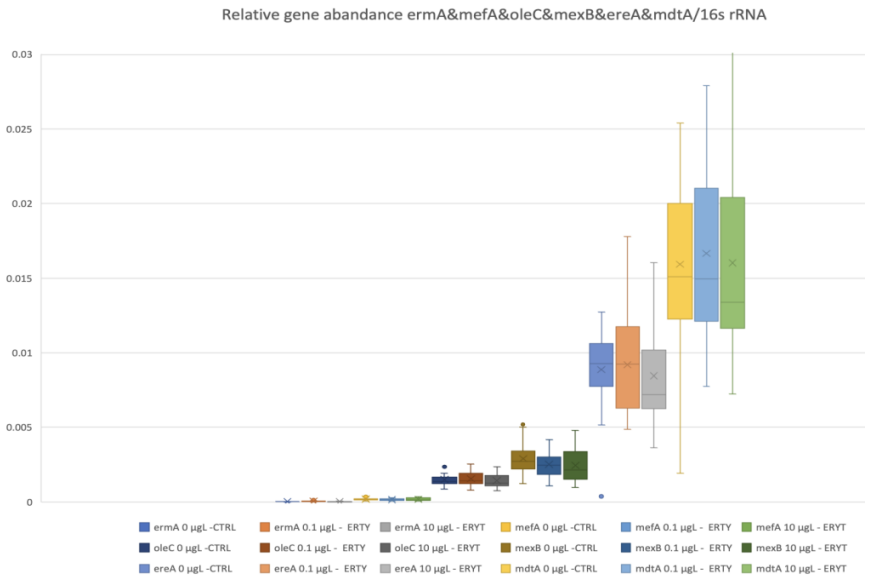


Figure 4: Relative gene abandance of Eram, mesa, Olek, ere A, a.4 Non-ERYT-Related Resistance Genes

Figure 5 compares sul1 and sul2 at varying ERYT concentrations, where sul2 displayed notably higher expression levels, peaking at 10 µg/L ERYT. In the absence of ERYT (0 µg/L), sul2 expression was lowest.

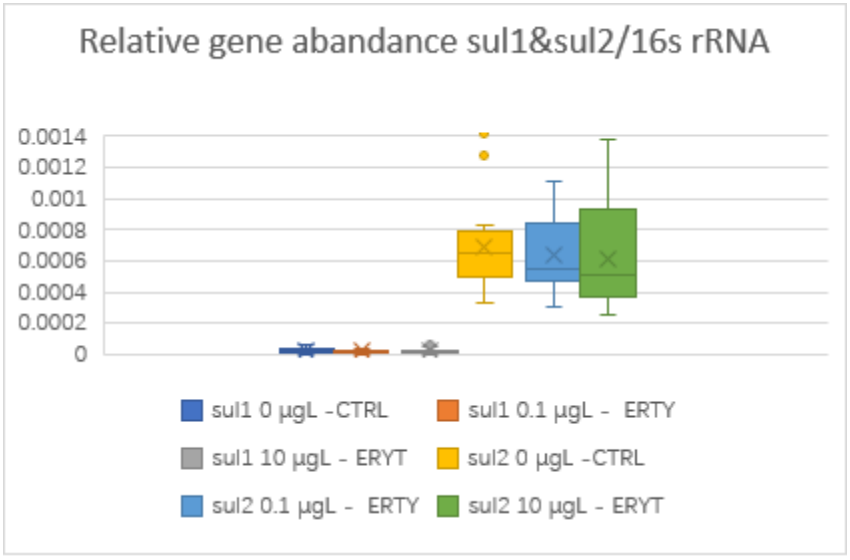


Figure 5: Relative gene abundance of sul1 and sul2/16s rRNA.

4. Discussion

4.1. Impact of Antibiotics on Resistance Genes (SFMX and ERYT)

4.1.1. Effect of SFMX on Resistance Genes

In the presence of SFMX, nine resistance genes were analyzed, revealing distinct expression patterns. The mexB gene consistently exhibited the highest expression across SFMX concentrations, while sul1 was the lowest (Figure 2). MexB is an outer membrane channel protein that plays a crucial role

DOI: 10.54254/2753-8818/89/2025.20458

in multidrug efflux, actively expelling antibiotics to support bacterial resistance. In contrast, sul1 is linked to integrons and facilitates horizontal gene transfer under sulfonamide pressure, potentially accelerating the spread of resistance genes, with notable expression variation across different SFMX concentrations. Despite sharing similar mechanisms, sul2 was consistently more abundant than sul1.

4.1.2. Effect of ERYT on Resistance Genes

In ERYT environments, six resistance genes were analyzed (ermA, mefA, oleC, ereA, mexB, and mdtA) (Figure 4). Among these, mdtA showed the highest expression, while ermA had the lowest. MdtA, a multidrug efflux pump, plays a significant role in antibiotic resistance under ERYT exposure. The lower activity of ermA, which utilizes ribosomal methylation as a resistance mechanism, may be attributed to the limited ERYT contamination observed, possibly explaining its reduced expression.

4.2. Comparison of Resistance Gene Frequencies between ERYT and SFMX

The resistance gene frequencies varied between ERYT and SFMX depending on their drug classes and mechanisms (Figure 1). T-test results revealed that mdtA, drfB, ereA, and mefA were more abundant in ERYT-treated environments compared to SFMX, likely due to their association with macrolide antibiotics, which promotes induction in ERYT-specific settings.

4.3. Changes in Expression of Individual Genes with Antibiotic Concentration and Over Time

Genes like mexB and mdtA showed the most significant expression changes over time under varying concentrations. These results align with the efflux pump mechanisms of these genes, suggesting that antibiotic exposure increases the prevalence of specific resistance genes within microbial communities. However, antibiotic persistence is typically limited in natural environments over time.

4.4. Limitations and Suggestions for Experimental Optimization

This study provides valuable insights into the dynamics of antibiotic resistance genes under antibiotic exposure but has certain limitations. Unexpectedly, some genes exhibited higher frequencies in environments without antibiotics than in those with exposure (Figure 2). This discrepancy could arise from experimental variability, including handling or data analysis errors. Despite strict protocols, unforeseen experimental fluctuations may have contributed to these findings.

Furthermore, while the study comprehensively assessed gene dynamics, certain underlying mechanisms influencing gene expression might not have been fully captured. Microbial communities may possess unknown mechanisms that impact gene frequency, highlighting the complex nature of microbial responses to their environment.

Further research is essential to validate and better understand these trends. Future studies focused on minimizing experimental variability and investigating additional mechanisms that drive gene dynamics within microbial communities.

5. Conclusion

In conclusion, the outcomes of this study have unveiled two pivotal aspects within antibiotic-resistant microbial communities. Firstly, the influence of varying concentrations of the same type of antibiotic on relevant resistance genes seems to be relatively subtle. This suggests that changes in the dosage of a specific antibiotic might not significantly impact the prevalence of associated resistance genes. Secondly, prolonged exposure of microorganisms to antibiotic-rich environments seems to promote the emergence of resistance genes. This phenomenon underscores the potential role of antibiotics in

DOI: 10.54254/2753-8818/89/2025.20458

driving the development of resistance genes within microbial populations. The observed interplay between antibiotic concentration and gene expression reinforces ongoing research and exploration into antibiotic resistance. As we advance in understanding microbial responses to antibiotics, these insights emphasize the significance of employing holistic methodologies to tackle the evolving challenge of antibiotic resistance.

References

[1] *Tellez-Isaias, G. (2022). The global antimicrobial resistance epidemic : Innovative Approaches and Cutting-Edge Solutions. London: IntechOpen.*

[2] *Blair, J. MA, et al. (2015). Molecular mechanisms of antibiotic resistance. Nature reviews microbiology. 13.1, pp. 42-51.*

[3] *Kümmerer, K. (2009). Antibiotics in the aquatic environment–a review-part I. Chemosphere. 75.4 pp. 417-434.*

[4] *Kläui, Anita, et al. (2024). Fresh produce as a reservoir of antimicrobial resistance genes: A case study of Switzerland. Science of the Total Environment. 907, 167671.*

[5] *WHO, UNEP. (2021).Antimicrobial resistance and the United Nations sustainable development cooperation framework.*

[6] *Ifedinezi, Onyinye, et al.(2024). Environmental antimicrobial resistance: implications for food safety and public health.Antibiotics. 13.11,pp. 1087.*

[7] *Baquero F, J L, Cantón R.(2008). Antibiotics and antibiotic resistance in water environments[J]. Current opinion in biotechnology. 19(3): 260-265.*