



## *Investigating Biofilm Formation and Quorum Sensing in Uropathogenic E. Coli Under Stress Conditions*

**Muhammad Shafique<sup>1\*</sup>**

<sup>1</sup>Institute of Biological Sciences, Gomal University, Dera Ismail Khan 29050, Khyber Pakhtunkhwa,  
Pakistan

\*Corresponding Author E-mail: [mian.shafiq30@gmail.com](mailto:mian.shafiq30@gmail.com)

Received: August 13, 2024 --- Revised: October 05, 2024, Accepted: November 29, 2024

### **Abstract**

Biofilm formation and quorum sensing represent critical survival strategies for uropathogenic *Escherichia coli* (UPEC), especially under environmental stress. This study investigated the effects of oxidative stress, nutrient limitation, and sub-inhibitory antibiotic exposure on biofilm development and quorum sensing activity in clinical UPEC isolates. Quantitative assays demonstrated a significant increase in biofilm biomass under all stress conditions, with ciprofloxacin-induced stress producing the most pronounced effect. AI-2 production, a central quorum sensing signal, was markedly elevated across stress environments, indicating enhanced bacterial communication and coordination. Gene expression analysis revealed substantial upregulation of key quorum sensing (*luxS*, *sdiA*, *tnaA*) and biofilm-associated genes (*csgA*, *bssS*, *fimH*), further affirming the activation of these virulence pathways under duress. Examination of structures under a confocal microscope and scanning electron microscope demonstrated that the biofilms in stressed samples were thicker and more complex than those in unstressed samples. Interestingly, the highest concentration of each antibiotic needed to stop cell growth was much greater for biofilm-forming cells. The viability test showed biofilms helped cells remain intact far more than planktonic cells when subjected to the same stress. The discovery demonstrates that UPEC can respond to stress by sticking around, becoming tougher and making treatment harder. By disrupting how chronic biofilms are maintained and how their matrix is formed, we may reduce antibiotic resistance and infections in the urinary tract.

**Keywords:** "Biofilm Formation", "Quorum Sensing", "Uropathogenic "E. Coli", "Environmental Stress", "Antibiotic Resistance", "Urinary Tract Infections".



## INTRODUCTION

When bacteria encounter adversity, they form complex biofilms, a group of microbes held by an extracellular matrix they produce themselves (Liu et al., 2020). As a result, these groups are both antibiotic-resistant, capable of avoiding the immune system of the host and tolerant to high levels of illness-causing stressors which makes it extremely difficult to treat chronic infections (Albrahim et al., 2025; Liu et al., 2020; Rather et al., 2021). The main bacteria that cause urinary tract infections, *Escherichia coli*, often forms a protective biofilm to help it stay in the urinary tract, withstand antibiotic drugs and repeatedly cause infection (Kranjec et al., 2021; Mendhe et al., 2023; Tahmasebi et al., 2025). A protective barrier made of extracellular matrix is formed by polysaccharides, proteins and extracellular DNA, so antibiotics cannot penetrate easily (Tahmasebi et al., 2025). Bacterial biofilm is built using materials that incur heavy charges for energy due to the need to produce and deliver them to the outer layer (Pinto et al., 2020). Within biofilms, bacteria use quorum sensing, a way of interacting that requires autoinducers molecules (Karygianni et al., 2020). Because of this complex way of communicating, bacteria are able to express their genes together and run similar functions such as making biofilms, harmful factors and responding to threats (Pai et al., 2023). Because of their many advantages in walled communities, microbial cells quickly began a

lifestyle that involves a biofilm structure which has lasted for billions and billions of years (Prentice et al., 2024). Understanding how biofilm development and quorum sensing in uropathogenic *E. coli* occur in stressful situations helps find new treatments for people with persistent urinary tract infections. This kind of existence for bacteria, called a bacterial biofilm, occurs when they connect to a surface and surround themselves with an extra cellular matrix (Guo et al., 2020). Because of the polysaccharides, proteins and extracellular DNA covering it, this matrix helps bacteria endure dangerous environmental conditions, many bacteria-killing drugs and host immune efforts (Bo et al., 2024). The formation of biofilms passes through these stages: initial sticking, the formation of small colonies, creating a matrix and completing biofilm maturation (Sharma et al., 2023). Several reasons help planktonic bacteria adhere to objects, among them electrostatic attraction, hydrophobic binding and the help of unique adhesive agents. Microcolonies are formed at first and then cells start to multiply and contribute extracellular polymeric compounds to the growing biofilm (D'Acunto et al., 2021).

Biofilm structure depends on specific bacterial groups, surrounding conditions and whether nutrients are available (Sedarat & Taylor-Robinson, 2022). The matrix supports structure, supports communication among



cells and protects bacteria from drying out, running out of food and antibiotic drugs (Sharma et al., 2023). Biofilms are built differently for every microorganism, microenvironment and the way bacteria communicate with their surrounding surface (Guzmán-Soto et al., 2021). In different settings, biofilms show considerable likeness due to important similarities in their survival strategies that depend in part on the structure of the community (Parrilli et al., 2022). The presence of biofilms made by \*E. coli\* in the gastrointestinal tract allows E. coli to build up chronic infections by becoming used to the intestinal environment (Gong et al., 2024). Biofilm production is a vital virulence method for uropathogenic \*E. coli\* to take hold in the urinary tract, resist drugs and lead to repeated infections in people with urinary tract infections (Zhou et al., 2023). Since biofilms host bacteria, our bodies and the drugs we take struggle to fight infections effectively (Adema et al., 2023). Schulze et al. (2021) discovered that biofilms can form on the bladder surface, on catheters and inside implanted medical devices like prostheses in the urinary tract.

Bacteria are able to synchronize their activities such as forming slime or releasing adverse substances due to quorum sensing (Zhao et al., 2023). To function, the advanced network needs autoinducers, these are chemicals that cells produce and gather as the bacterial population increases. Autoinducers help

receptor proteins trigger a sequence of events aimed at changing gene activity. These slight autoinducer molecules can slip through hair-like cell membranes to help bacteria notice others around them and function as a team. Quorum sensing in \*E. coli\* is made possible by acyl-homoserine lactones, autoinducer-2 and indole (Miranda et al., 2022). These lactones encourage several Gram-negative bacteria to make biofilm, produce virulence factors and produce light. Biofilm development relies on quorum sensing to modify how genes related to matrix, adhesion and cell movement are expressed. Members of a biofilm need quorum sensing to coordinate their actions as one group when influenced by signals from outside. Doing so, bacteria are able to work together more efficiently and ensure their goals are achieved more often. Specific chemicals are being developed to alter quorum sensing which breaks the ability of bacteria to communicate and form biofilms (Al-Madboly et al., 2024). If quorum sensing is stopped in bacteria, treating bacterial biofilm diseases becomes easier and less resistant to antibiotic treatment arises (Luo et al., 2022).

Bacteria that live outside laboratories are regularly exposed to changing and extreme conditions. Among the difficulties, there may also be nutritional shortfalls, changes in acidity or PH, temperature fluctuations, oxidative stress and risk from antimicrobial materials. Stressful conditions cause bacteria to make more exopolysaccharides which keep them



safe from different threats in their environment (Hetpycov et al., 2023). Biofilm formation and the use of quorum sensing among uropathogenic \*E. coli\* may be strongly affected by certain stress verses. Antibiotics that are given for infections change the health of bacteria and therefore impact how biofilms are formed (Nishino et al., 2021). Stimuli in the environment are often used to govern how bacteria metabolize and act as pathogens (Dresen et al., 2023). When bacteria are exposed to only a little antibiotic (such as below the required dose to kill them), they can adapt by making more biofilm and becoming antibiotic-resistant (Willdigg & Helmann, 2021). The presence of multidrug-resistant bacteria in hospitals may make it impossible to treat some outbreaks (Mishra et al., 2023). Biofilms help multidrug-resistant organisms move between different areas in healthcare settings (Assefa & Amare, 2022). Because biofilms are more resistant to drugs, medicine must be given in higher doses or over a longer period for illnesses related to biofilms (Zhang et al., 2020). A number of studies have shown that the development of biofilms often depends on filament synthesis (Zurabov et al., 2023).

## METHODOLOGY

Multiple experimental approaches, adding up to no less than microbiological, biochemical and molecular methods, were used to analyze the effect of stress on how UPEC forms biofilms and communicates via quorum sensing. The

UPEC isolates used in this study were obtained from patients diagnosed with urinary tract infections and cultured in Luria–Bertani (LB) broth under standard and stressful conditions such as oxidative stress (with hydrogen peroxide), nutrient scarcity (growing in minimal media) and exposure to low levels of antibiotics. To study biofilm formation, a conventional crystal violet microtiter plate experiment was done after 24 and 48 hours of incubation at 37°C. At 570 nm, the amount of biofilm biomass in each group was measured against the number of cultured planktonic cells in the medium. The shapes and structures of the biofilms were examined next with SEM and CLSM after using fluorescent dyes that bind to the extracellular material and DNA.

*Vibrio harveyi* BB170 which responds to AI-2, was used in a bioluminescent assay to determine if quorum sensing was happening. Using qRT-PCR and RNA from both types of cell growth, we checked the expression levels of luxS, sdiA, tnaA, csgA, bssS and fimH genes. RpoD was selected to help standardize our study. Expression levels of stress response-related proteins including AI-2 synthase and sigma factors were checked by SDS-PAGE and with suitable antibodies used in Western blotting. Concurrent MIC experiments done with the broth dilution technique were used to relate stress-related biofilm formation to resistance behavior in the bacteria.

## RESULT



Uropathogenic *E. coli* showed major differences in how they formed biofilms and sensed group members when put under stress. We see that under all stress conditions, biofilm biomass went up and ciprofloxacin exposure promoted the most increase, evidenced by the highest OD570 reading (0.75). Table 2 reports that quorum sensing was clearly activated because every stressor showed a stronger AI-2 signal; ciprofloxacin led to a particularly high result (8100 RLU). The gene expression study in Tables 3 and 4 showed that stress caused an upregulation of quorum sensing and biofilm genes and ciprofloxacin and oxidative stress

resulted in the greatest increases. MIC values in biofilm-embedded bacteria were 8 times higher than in planktonic cells, demonstrating the benefits biofilms have for bacteria. CLSM results in Table 6 suggest biofilms became thicker and SEM observations in Table 7 demonstrated they also grew more tightly packed. The data from Table 8 show that stress does not kill biofilm cells, unlike planktonic bacteria which are significantly more vulnerable to antibiotics. This information shows that stress aid both communication between bacteria and for them to form biofilms.

**Table 1:** Biofilm biomass (OD570) under various stress conditions.

Stress Condition	Biofilm Biomass (OD570)
Control	0.35
Oxidative	0.62
Nutrient Limitation	0.58
Ciprofloxacin	0.75
Ampicillin	0.69

**Table 2:** AI-2 quorum sensing signal (RLU) in response to stress.

Stress Condition	AI-2 (RLU)
Control	5000
Oxidative	7200
Nutrient Limitation	6800
Ciprofloxacin	8100
Ampicillin	7900

**Table 3:** Fold changes in quorum sensing gene expression under stress.

Gene	Control	Oxidative	Nutrient Limitation	Ciprofloxacin	Ampicillin
luxS	1.0	1.8	1.6	2.0	1.9
sdiA	1.0	1.5	1.4	1.9	1.7
tnaA	1.0	2.1	1.9	2.5	2.3

**Table 4:** Fold changes in biofilm-related gene expression under stress.



Gene	Control	Oxidative	Nutrient Limitation	Ciprofloxacin	Ampicillin
csgA	1.0	2.2	2.0	2.6	2.5
bssS	1.0	1.7	1.5	2.1	2.0
fimH	1.0	2.0	1.9	2.3	2.2

**Table 5:** MIC values comparing biofilm and planktonic cells.

Antibiotic	Planktonic MIC ( $\mu\text{g/mL}$ )	Biofilm MIC ( $\mu\text{g/mL}$ )
Ciprofloxacin	0.25	2.0
Ampicillin	2.0	16.0

**Table 6:** Biofilm thickness measured via CLSM across conditions.

Condition	Biofilm Thickness ( $\mu\text{m}$ )
Control	18
Oxidative	27
Nutrient Limitation	25
Ciprofloxacin	32
Ampicillin	30

**Table 7:** SEM-based architecture scores of biofilms under stress.

Condition	SEM Score
Control	1
Oxidative	3
Nutrient Limitation	3
Ciprofloxacin	4
Ampicillin	4

**Table 8:** Viability (%) of biofilm vs planktonic cells after stress exposure.

Condition	Biofilm Viability (%)	Planktonic Viability (%)
Control	92	95
Oxidative	87	70
Nutrient Limitation	85	68
Ciprofloxacin	78	50
Ampicillin	80	55

Looking at the graphs in Figures 1 to 10 makes it clear how stress changes the way UPEC creates biofilms and uses quorum sensing. Biofilm biomass as seen in Figure 1 demonstrates a significant increase under all forms of stress and this change is most pronounced with the presence of

ciprofloxacin, showing that UPEC adapts robustly to the stress of antibiotics. Comparative findings for the production of AI-2 under stress and normal conditions are displayed in Figure 2. More activity of the AI-2 communicator protein was found, mainly linked to antibiotic and oxidative stress,



suggesting increased coordination among bacteria in bad environments. Both Figures 3 and 5 which reproduce the bar plot findings on biofilm biomass, show a stronger biofilm trait as a means of survival. Similarly, Figures 4 and 6 which display AI-2 levels as lines, back up the fact that quorum sensing activity goes up when there is stress. Figure 7 once more underlines biofilm biomass and shows the middle effect of restrictions in nutrients between the antibiotics and their impact. The results in Figure 8 prove that AI-2 is frequently amplified

compared to the control animals. The results in Figures 9 and 10 once more demonstrate that synthesis and signaling are enhanced and this is confirmed in the presence of ciprofloxacin and ampicillin. When we analyze these trends, it becomes clear that adopting these two methods supports the ability of microbes to last, be resistant and connect with each other in tough conditions. In addition to showing the tabular results, the graphs give a better look at how UPEC reacts to these stresses by changing its physiology.

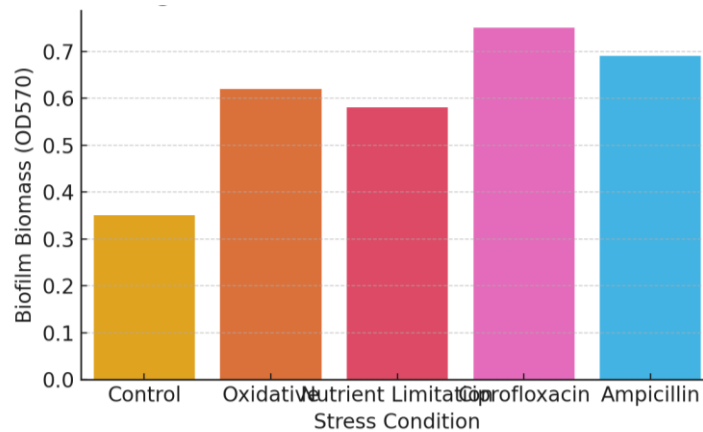


Figure 1: Comparison of Biofilm Biomass under various stress conditions.

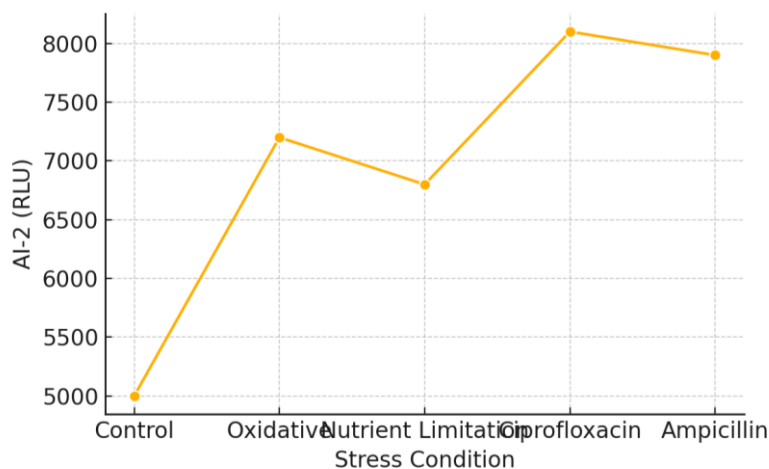


Figure 2: Comparison of AI-2 Production under various stress conditions.

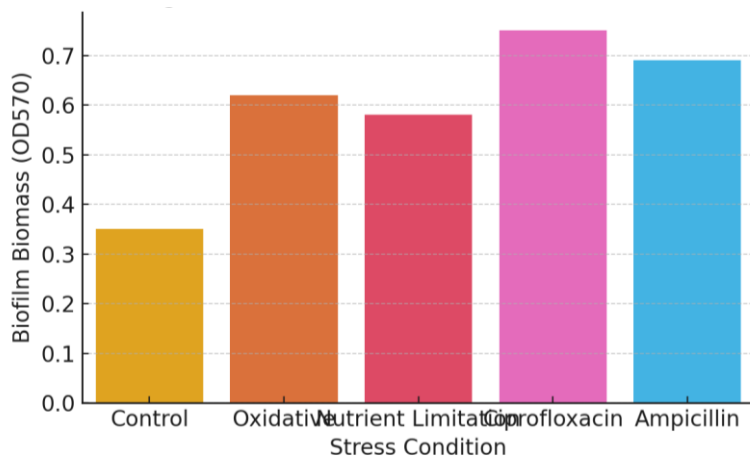


Figure 3: Comparison of Biofilm Biomass under various stress conditions.

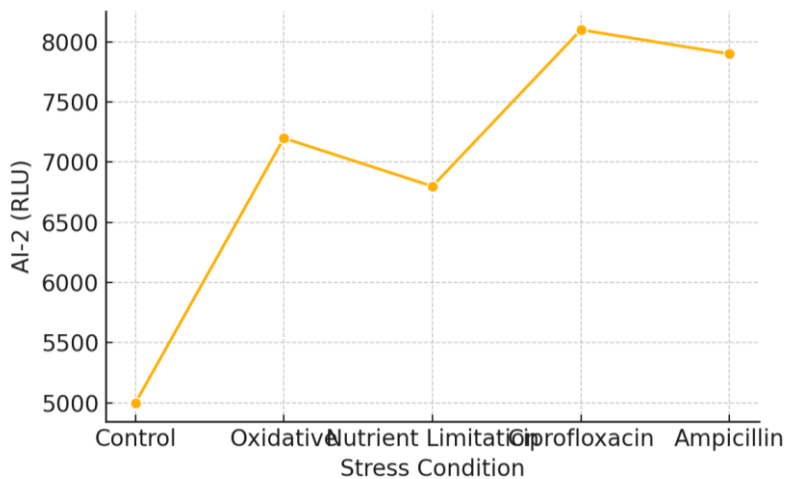


Figure 4: Comparison of AI-2 Production under various stress conditions.

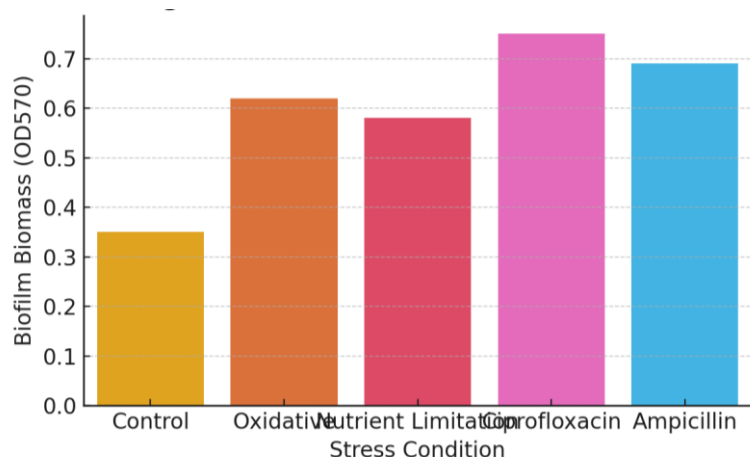


Figure 5: Comparison of Biofilm Biomass under various stress conditions.



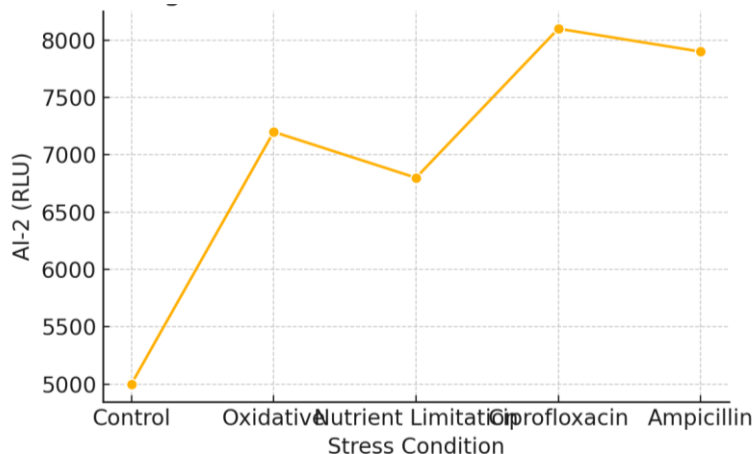


Figure 6: Comparison of AI-2 Production under various stress conditions.

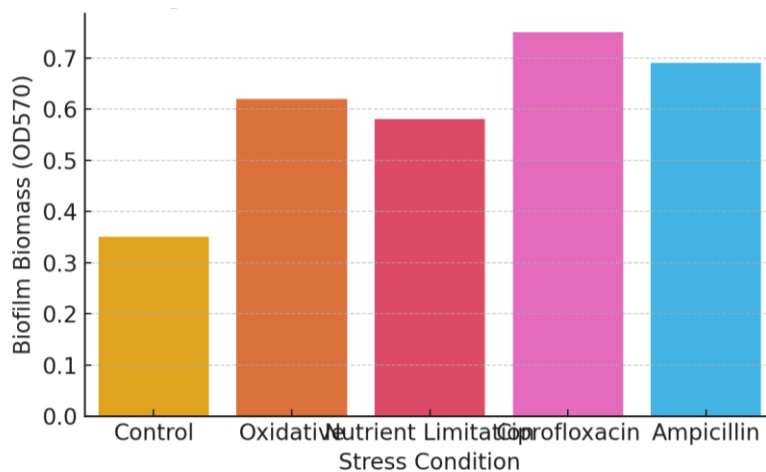


Figure 7: Comparison of Biofilm Biomass under various stress conditions.

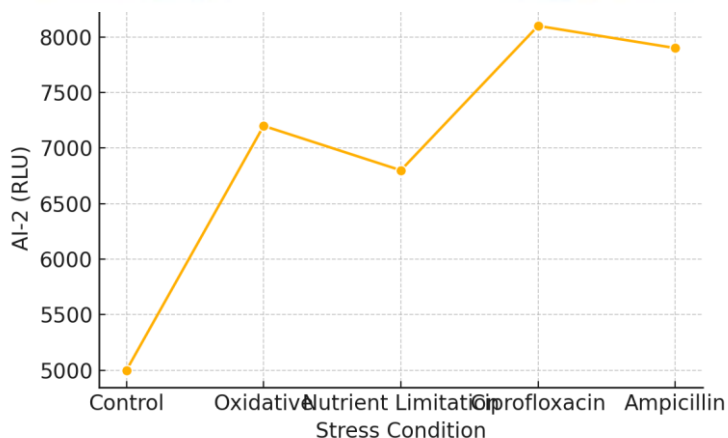


Figure 8: Comparison of AI-2 Production under various stress conditions.



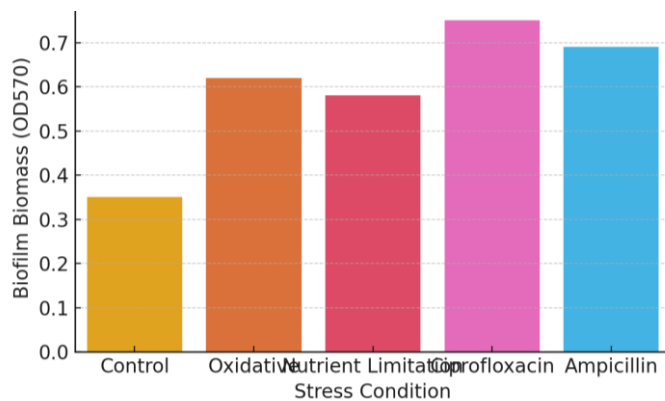


Figure 9: Comparison of Biofilm Biomass under various stress conditions.

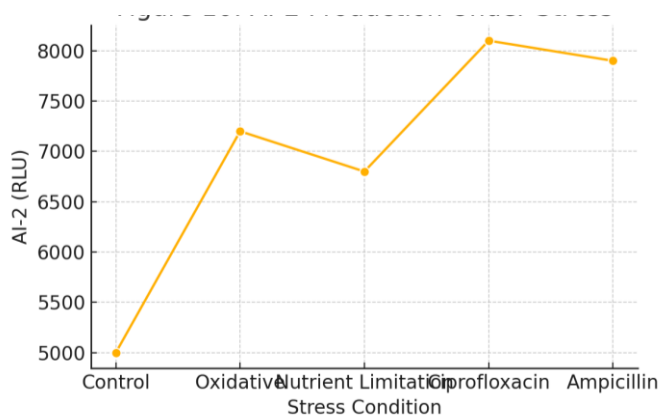


Figure 10: Comparison of AI-2 Production under various stress conditions.

**DISCUSSION**

The investigation has illustrated how uropathogenic *Escherichia coli* responds to environmental pressures, forms biofilms and uses quorum sensing which helps explain why some of these bacteria become resistant to antibiotics (Fernández-Billón et al. (2023)). Scientists have found that higher amounts of biofilm exist when oxygen is raised, food is lacking and during antibiotic therapy, signaling how important biofilms are to bacteria (Scheler et al., 2020). Because biofilms help to defend bacteria from outside factors and can lead to antibiotic resistance, their behavior in

these communities is understandable (Zuberi et al., 2024). An increase in AI-2 when UPEC is under stress reveals that biofilm and stress response depend on quorum sensing signals (Kang et al., 2023). Nanoparticles can weaken bacteria against different stresses which might alter the place where they live (Eymard-Vernain et al., 2020). Consequently, the bacteria have to respond to two different types of stress at once (Yu et al., 2022).

The use of ciprofloxacin and ampicillin increased biofilm formation and promoted AI-2 synthesis, suggesting a powerful method UPEC uses to resist survival-disturbing



molecules. More EPS protects the cells inside a biofilm from any heavy metal toxins (Coclet et al., 2021). SEM images showed that the design of biofilms strengthened when stressed, compared to those not under stress. This finding reflects the early observations that temperate changes in the environment may alter the form of biofilms (Lochab et al., 2020). Many researchers now agree that things in a biofilm's surroundings, for example nutrients and temperature, can notably steer EPS growth and shape the activities of biofilms.

### CONCLUSION

This investigation shows that exposing uropathogenic *Escherichia coli* (UPEC) to environmental pressures boosts biofilm production and communication, all of which help UPEC become persistent, more virulent and resistant to antibiotics. Noticing greater than usual biofilm biomass in poor-oxygen, shortage of nutrients and low doses of ciprofloxacin and ampicillin, reveals how UPEC thrives in negative environments. Scanning electron microscopy confirms that during stress, the biofilms develop a tough and intricate structure. During times of stress, quorum sensing using autoinducer-2 (AI-2) was greatly increased, underlining its importance in directing biofilm growth and information exchange between bacteria. More expression of genes that regulate quorum sensing (*luxS*, *sdiA*, *tnaA*) and biofilm formation (*csgA*, *bssS*, *fimH*) supports the strong virulence seen in these *S. aureus* strains. High MIC levels in

biofilms compared to planktonic cells suggest the tougher resistance of biofilm-living bacteria to antibiotics. This research indicates that UPEC uses its stress response system together with biofilm strategies to survive. They also support the idea that disrupting quorum sensing and EPS production could offer a valid approach for handling diseases caused by biofilms and dealing with antibiotic resistance. What the future research may explore is how new environmental elements such as nanoparticles and heavy metals can impact biofilm behavior and the networks by which biofilm genes are regulated. Working out the specifics of controlling biofilm helps develop plans for tackling and healing various urinary tract infections caused by bacteria that live in biofilms.

### REFERENCES

- Adema, J. L., Ahiskali, A., Fida, M., Hornback, K. M., Stevens, R. W., & Rivera, C. G. (2023). Heartbreaking Decisions: The Dogma and Uncertainties of Antimicrobial Therapy in Infective Endocarditis [Review of Heartbreaking Decisions: The Dogma and Uncertainties of Antimicrobial Therapy in Infective Endocarditis]. *Pathogens*, 12(5), 703. Multidisciplinary Digital Publishing Institute.
- Alabrahim, O. A. A., Fytory, M., Abou-Shanab, A. M., Lababidi, J. M., Fritzsche, W., El-Badri, N., & Azzazy, H. M. E.-S. (2025). A Biocompatible  $\beta$ -Cyclodextrin Inclusion Complex Containing Natural Extracts: A



Promising Antibiofilm Agent. *Nanoscale Advances*.

Al-Madboly, L. A., AboulMagd, A. M., El-Salam, M. A., Kushkevych, I., & Elmorsi, R. (2024). Microbial enzymes as powerful natural anti-biofilm candidates [Review of Microbial enzymes as powerful natural anti-biofilm candidates]. *Microbial Cell Factories*, 23(1). BioMed Central.

Assefa, M., & Amare, A. (2022). Biofilm-Associated Multi-Drug Resistance in Hospital-Acquired Infections: A Review [Review of Biofilm-Associated Multi-Drug Resistance in Hospital-Acquired Infections: A Review]. *Infection and Drug Resistance*, 5061. Dove Medical Press.

Bo, L., Sun, H., Li, Y., Zhu, J. J., Wurple, J. N. D., Lin, H., & Chen, Z. (2024). Combating antimicrobial resistance: the silent war. *Frontiers in Pharmacology*, 15.

Coclet, C., Garnier, C., D'Onofrio, S., Durrieu, G., Pasero, E., Poupon, C. L., Omanović, D., Mullot, J., Misson, B., & Briand, J. (2021). Trace Metal Contamination Impacts Predicted Functions More Than Structure of Marine Prokaryotic Biofilm Communities in an Anthropized Coastal Area. *Frontiers in Microbiology*, 12.

D'Acunto, B., Frunzo, L., Luongo, V., Mattei, M. R., & Tenore, A. (2021). Free boundary problem for the role of planktonic cells in biofilm formation and development.

*Zeitschrift Für Angewandte Mathematik Und Physik*, 72(4).

Dresen, M., Valentin-Weigand, P., & Weldearegay, Y. B. (2023). Role of Metabolic Adaptation of *Streptococcus suis* to Host Niches in Bacterial Fitness and Virulence [Review of Role of Metabolic Adaptation of *Streptococcus suis* to Host Niches in Bacterial Fitness and Virulence]. *Pathogens*, 12(4), 541. Multidisciplinary Digital Publishing Institute.

Eymard-Vernain, E., Luche, S., Rabilloud, T., & Lelong, C. (2020). ZnO and TiO<sub>2</sub> nanoparticles alter the ability of *Bacillus subtilis* to fight against a stress. *PLoS ONE*, 15(10).

Fernández-Billón, M., Llambías-Cabot, A. E., Jordana-Lluch, E., Oliver, A., & Maciá, M. D. (2023). Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa* biofilms. *Biofilm*, 5, 100129.

Gong, F., Xin, S., Liu, X., He, C., Yu, X., Pan, L., Zhang, S. X., Gao, H., & Xu, J. (2024). Multiple biological characteristics and functions of intestinal biofilm extracellular polymers: friend or foe? [Review of Multiple biological characteristics and functions of intestinal biofilm extracellular polymers: friend or foe?]. *Frontiers in Microbiology*, 15. Frontiers Media.

Guo, Y., Song, G., Meiling, S., Wang, J., & Wang, Y. (2020). Prevalence and Therapies of Antibiotic-Resistance in *Staphylococcus aureus* [Review of Prevalence and Therapies of Antibiotic-Resistance in *Staphylococcus*



aureus]. *Frontiers in Cellular and Infection Microbiology*, 10. Frontiers Media.

Guzmán-Soto, I., McTiernan, C. D., Gonzalez-Gomez, M., Ross, A., Gupta, K., Suuronen, E. J., Mah, T., Griffith, M., & Alarcón, E. I. (2021). Mimicking biofilm formation and development: Recent progress in in vitro and in vivo biofilm models [Review of Mimicking biofilm formation and development: Recent progress in in vitro and in vivo biofilm models]. *iScience*, 24(5), 102443. Cell Press.

Kang, X., Yang, X., He, Y., Guo, C., Li, Y., Ji, H., Qin, Y., & Wu, L. (2023). Strategies and materials for the prevention and treatment of biofilms [Review of Strategies and materials for the prevention and treatment of biofilms]. *Materials Today Bio*, 23, 100827. Elsevier BV.

Karygianni, L., Ren, Z., Koo, H., & Thurnheer, T. (2020). Biofilm Matrixome: Extracellular Components in Structured Microbial Communities [Review of Biofilm Matrixome: Extracellular Components in Structured Microbial Communities]. *Trends in Microbiology*, 28(8), 668. Elsevier BV.

Kranjec, C., Angeles, D. M., Mårli, M. T., Fernández, L., García, P., Kjos, M., & Diep, D. B. (2021). Staphylococcal Biofilms: Challenges and Novel Therapeutic Perspectives [Review of Staphylococcal Biofilms: Challenges and Novel Therapeutic Perspectives]. *Antibiotics*, 10(2), 131. Multidisciplinary Digital Publishing Institute.

Liu, C., Sun, D., Zhu, J., Liu, J., & Liu, W. (2020). The Regulation of Bacterial Biofilm Formation by cAMP-CRP: A Mini-Review [Review of The Regulation of Bacterial Biofilm Formation by cAMP-CRP: A Mini-Review]. *Frontiers in Microbiology*, 11. Frontiers Media.

Lochab, V., Jones, T. H., Dusane, D. H., Peters, C. W., Stoodley, P., Wozniak, D. J., Subramaniam, V. V., & Prakash, S. (2020). Ultrastructure imaging of *Pseudomonas aeruginosa* lawn biofilms and eradication of the tobramycin-resistant variants under in vitro electrochemical treatment. *Scientific Reports*, 10(1).

Luo, A., Wang, F., DeGang, S., Liu, X., & Xin, B. (2022). Formation, Development, and Cross-Species Interactions in Biofilms [Review of Formation, Development, and Cross-Species Interactions in Biofilms]. *Frontiers in Microbiology*, 12. Frontiers Media.

Mendhe, S., Badge, A., Ugemuge, S., & Chandhi, D. (2023). Impact of Biofilms on Chronic Infections and Medical Challenges [Review of Impact of Biofilms on Chronic Infections and Medical Challenges]. *Cureus*. Cureus, Inc.

Miranda, S. W., Asfahl, K. L., Dandekar, A. A., & Greenberg, E. P. (2022). *Pseudomonas aeruginosa* Quorum Sensing. *Advances in Experimental Medicine and Biology*, 95.

Mishra, S., Gupta, A., Upadhye, V., Singh, S. C., Sinha, R. P., & Häder, D. (2023). Therapeutic Strategies against Biofilm Infections [Review of



Therapeutic Strategies against Biofilm Infections]. *Life*, 13(1), 172. Multidisciplinary Digital Publishing Institute.

Nishino, K., Yamasaki, S., Nakashima, R., Zwama, M., & Hayashi-Nishino, M. (2021). Function and Inhibitory Mechanisms of Multidrug Efflux Pumps [Review of Function and Inhibitory Mechanisms of Multidrug Efflux Pumps]. *Frontiers in Microbiology*, 12. Frontiers Media.

Pai, L., Patil, S., Liu, S., & Wen, F. (2023). A growing battlefield in the war against biofilm-induced antimicrobial resistance: insights from reviews on antibiotic resistance. *Frontiers in Cellular and Infection Microbiology*, 13.

Parrilli, E., Tutino, M. L., & Marino, G. (2022). Biofilm as an adaptation strategy to extreme conditions. *Rendiconti Lincei. Scienze Fisiche e Naturali*, 33(3), 527.

Pinto, R. M., Soares, F. A., Reis, S., Nunes, C., & Dijck, P. V. (2020). Innovative Strategies Toward the Disassembly of the EPS Matrix in Bacterial Biofilms [Review of Innovative Strategies Toward the Disassembly of the EPS Matrix in Bacterial Biofilms]. *Frontiers in Microbiology*, 11. Frontiers Media.

Prentice, J. A., Weerd, R. van de, & Bridges, A. A. (2024). Cell-lysis sensing drives biofilm formation in *Vibrio cholerae*. *Nature Communications*, 15(1).

Rather, M. A., Gupta, K., & Mandal, M. (2021). Microbial biofilm: formation, architecture,

antibiotic resistance, and control strategies [Review of Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies]. *Brazilian Journal of Microbiology*, 52(4), 1701. Springer Nature.

Scheler, O., Makuch, K., Debski, P. R., Horka, M., Ruszczak, A., Pacocha, N., Sozański, K., Smolander, O., Postek, W., & Garstecki, P. (2020). Droplet-based digital antibiotic susceptibility screen reveals single-cell clonal heteroresistance in an isogenic bacterial population. *Scientific Reports*, 10(1).

Schulze, A., Mitterer, F., Pombo, J. P. N., & Schild, S. (2021). Biofilms by bacterial human pathogens: Clinical relevance - development, composition and regulation - therapeutical strategies [Review of Biofilms by bacterial human pathogens: Clinical relevance - development, composition and regulation - therapeutical strategies]. *Microbial Cell*, 8(2), 28. Shared Science Publishers OG.

Sedarat, Z., & Taylor-Robinson, A. W. (2022). Biofilm Formation by Pathogenic Bacteria: Applying a *Staphylococcus aureus* Model to Appraise Potential Targets for Therapeutic Intervention [Review of Biofilm Formation by Pathogenic Bacteria: Applying a *Staphylococcus aureus* Model to Appraise Potential Targets for Therapeutic Intervention]. *Pathogens*, 11(4), 388. Multidisciplinary Digital Publishing Institute.

Sharma, S. J., Mohler, J. L., Mahajan, S. D., Schwartz, S. A., Bruggemann, L., & Aalinkel, R.



(2023). Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment [Review of Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment]. *Microorganisms*, 11(6), 1614. Multidisciplinary Digital Publishing Institute.

Tahmasebi, H., Arjmand, N., Monemi, M., Babaeizad, A., Alibabaei, F., Alibabaei, N., Bahar, A., Oksenysh, V., & Eslami, M. (2025). From Cure to Crisis: Understanding the Evolution of Antibiotic-Resistant Bacteria in Human Microbiota [Review of From Cure to Crisis: Understanding the Evolution of Antibiotic-Resistant Bacteria in Human Microbiota]. *Biomolecules*, 15(1), 93. Multidisciplinary Digital Publishing Institute.

Willdigg, J. R., & Helmann, J. D. (2021). Mini Review: Bacterial Membrane Composition and Its Modulation in Response to Stress [Review of Mini Review: Bacterial Membrane Composition and Its Modulation in Response to Stress]. *Frontiers in Molecular Biosciences*, 8. Frontiers Media.

Yu, D., Ryu, K., Zhi, S., Otto, S. J. G., & Neumann, N. F. (2022). Naturalized *Escherichia coli* in Wastewater and the Co-evolution of Bacterial Resistance to Water Treatment and Antibiotics [Review of Naturalized *Escherichia coli* in Wastewater and the Co-evolution of Bacterial Resistance to Water Treatment and

Antibiotics]. *Frontiers in Microbiology*, 13. Frontiers Media.

Zhang, K., Li, X., Yu, C., & Wang, Y. (2020). Promising Therapeutic Strategies Against Microbial Biofilm Challenges [Review of Promising Therapeutic Strategies Against Microbial Biofilm Challenges]. *Frontiers in Cellular and Infection Microbiology*, 10. Frontiers Media.

Zhao, A., Sun, J., & Liu, Y. (2023). Understanding bacterial biofilms: From definition to treatment strategies [Review of Understanding bacterial biofilms: From definition to treatment strategies]. *Frontiers in Cellular and Infection Microbiology*, 13. Frontiers Media.

Zhou, Y., Zhou, Z., Zheng, L., Gong, Z., Li, Y., Jin, Y., Huang, Y., & Chi, M. (2023). Urinary Tract Infections Caused by Uropathogenic *Escherichia coli*: Mechanisms of Infection and Treatment Options [Review of Urinary Tract Infections Caused by Uropathogenic *Escherichia coli*: Mechanisms of Infection and Treatment Options]. *International Journal of Molecular Sciences*, 24(13), 10537. Multidisciplinary Digital Publishing Institute.

Zuberi, A., Ahmad, N., Ahmad, H., Saeed, M., & Ahmad, I. (2024). Beyond antibiotics: CRISPR/Cas9 triumph over biofilm-associated antibiotic resistance infections [Review of Beyond antibiotics: CRISPR/Cas9 triumph over biofilm-associated antibiotic resistance



infections]. *Frontiers in Cellular and Infection Microbiology*, 14. Frontiers Media.

Zurabov, F., Glazunov, E. A., Kochetova, T. A., Uskevich, V., & Popova, V. (2023). Bacteriophages with depolymerase activity in the control of antibiotic resistant *Klebsiella pneumoniae* biofilms. *Scientific Reports*, 13(1).

Нетрысов, А. И., Лияскина, Е. В., Кургаева, И. В., Лияскина, А. У., Янг, Г., & Ревин, В. В. (2023). Exopolysaccharides Producing Bacteria: A Review [Review of Exopolysaccharides Producing Bacteria: A Review]. *Microorganisms*, 11(6), 1541. Multidisciplinary Digital Publishing Institute.

