Evaluation of Penicillin Antibiotic Resistance of Extended Beta Lactamase Producing *E.Coli*

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**Abstract:** In this research evaluate on the biofilm-forming capabilities of *E.coli*, from oral samples and their resistance against Penicillin. The study indicates the virulence of *E.coli* and their biofilm forming ability against penicillin has exhibited 35% at 250 mg/ml to 85% at 1000 mg/ml. Lipid peroxidation was found to be 17%, indicating a moderate level of oxidative damage to the bacterial membranes. At 500 mg, lipid peroxidation decreased to 4%, suggesting a reduced response to oxidative stress at this intermediate concentration. Interestingly, no lipid peroxidation was observed at 750 mg and 1g indicating that higher concentrations of the agent did not induce oxidative damage or that the bacteria had developed mechanisms to resist such stress at these higher levels. Biofilm formation in ESBL-producing *E.coli* contributes significantly to their antibiotic resistance and persistence in clinical settings. These findings suggest that while penicillin alone may not be effective for killing these strains, it could play a role in biofilm modulation, potentially enhancing the activity of combination therapies or disrupting colonization on surfaces

**Keywords:** Penicillin; ESBL-*E.coli*; Biofilm; Lipid peroxidation

# Introduction

Biofilm formation plays a crucial role in the resistance of bacteria to antibiotic treatments. In particular, the ability of bacteria to form biofilms is regulated by quorum sensing (QS), a system that controls gene expression based on population density. Blocking this QS system can disrupt the biofilm structure, rendering bacterial pathogens more susceptible to antibiotic treatment [(Wasfi et al., 2020)](https://paperpile.com/c/8GkGA1/YwlNm). Biofilms undergo five distinct stages of formation: bacterial dispersion, microcolony development, biofilm maturation, and both reversible and irreversible adhesion [(Czerwonka et al., 2016)](https://paperpile.com/c/8GkGA1/Oq41Z). The early stages of biofilm formation, including reversible and irreversible adhesion, are particularly important in enabling bacteria to survive antibiotic treatments [(Khatoon et al., 2018)](https://paperpile.com/c/8GkGA1/jJi0J). Studies have shown that strains with more negative zeta potential values tend to produce larger amounts of biofilm, indicating the strong influence of electrostatic forces on biofilm formation [(Kashef et al., 2020)](https://paperpile.com/c/8GkGA1/l4UoO). A complex biofilm structure, composed of surface-attached bacterial cells surrounded by a polymeric matrix that includes water, extracellular polymeric substances, and noncellular elements. These biofilms protect the bacteria from environmental stressors, including antibiotics, making biofilm-associated bacteria significantly harder to treat [(Flemming et al., 2023; Kumar et al., 2020)](https://paperpile.com/c/8GkGA1/ln69a+ZUzcV). Biofilms are frequently found in chronic wounds, with up to 75% of such wounds harboring biofilm-forming bacteria, which extends the wound healing process and complicates infection management [(Kwiecińska-Piróg et al., 2020)](https://paperpile.com/c/8GkGA1/UR2MH). Biofilm formation can delay tissue granulation and increase bacterial resistance to antimicrobial agents. [(Muthuswamy Pandian et al., 2022; Ramakrishnan et al., 2023)](https://paperpile.com/c/8GkGA1/JtiXZ+xWxSx), [(Merchant et al., 2022)](https://paperpile.com/c/8GkGA1/oYGOo), [(Sreevarun et al., 2023)](https://paperpile.com/c/8GkGA1/taMLT)*Escherichia coli*, a Gram-negative bacterium, is commonly associated with urinary tract infections (UTIs), particularly those involving catheterization. It is also known for its ability to produce biofilms, which contribute to its pathogenicity [(Clinical, n.d.)](https://paperpile.com/c/8GkGA1/JXRBW). The biofilm-forming capacity of *E. coli* contributes to its persistence in various infections, making it more difficult to treat with standard antibiotics. Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* strains are of particular concern due to their resistance to a broad range of beta-lactam antibiotics, including penicillin. In addition to *E. coli*, other bacterial species like *Proteus mirabilis* also exhibit biofilm-forming capabilities, contributing to their virulence in urinary tract infections [(Armbruster et al., 2018)](https://paperpile.com/c/8GkGA1/GUns0). These bacteria utilize various virulence factors such as lipopolysaccharides, urease enzymes, adhesion proteins, and quorum sensing molecules to facilitate biofilm formation [(Wasfi et al., 2020)](https://paperpile.com/c/8GkGA1/YwlNm). *Proteus mirabilis*, in particular, has been shown to form biofilms on urinary tract surfaces, which contribute to chronic infections and the formation of encrusted catheters, complicating treatment further [(Brien, 2021; Kour et al., 2020)](https://paperpile.com/c/8GkGA1/PSw0c+GC3H1). The ability of *E. coli* and other bacteria to form biofilms is closely linked to their resistance mechanisms. Biofilms provide a protective environment that enhances bacterial survival against antimicrobial agents, including penicillin, by preventing drug penetration and facilitating the exchange of resistance genes.[(Adel et al., 2023)](https://paperpile.com/c/8GkGA1/8Iw8K), [(Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/8GkGA1/GBKOj), [(Solanki et al., 2023)](https://paperpile.com/c/8GkGA1/PUoAm) This resistance is further compounded by the production of beta-lactamases, enzymes that degrade beta-lactam antibiotics and prevent their efficacy. [(Chokkattu et al., 2023)](https://paperpile.com/c/8GkGA1/c1KB4), [(Laghari et al., 2023; Ramakrishnan et al., 2023)](https://paperpile.com/c/8GkGA1/xWxSx+kuxvt), [(Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/8GkGA1/JtiXZ) Penicillin, a member of the Actinobacteria class, has historically been an effective antibiotic, but its use is limited by the emergence of resistant bacterial strains, particularly those producing extended beta-lactamase. *Penicillium* species, often associated with antibiotic production, exhibit varying degrees of resistance and sensitivity to penicillin, complicating therapeutic strategies [(Gomaa et al., 2019; Traxler et al., 2022)](https://paperpile.com/c/8GkGA1/wFShv+YBi8A). The increasing prevalence of novel penicillin-resistant strains underscores the importance of continued research into new therapeutic agents and strategies to combat these resistant pathogens.In this study, the focus was on evaluating the resistance of *ESBL*-producing *E. coli* to penicillin and its ability to form biofilms. The research also investigated the bioactive compounds from *Penicillium* species for their potential to inhibit biofilm formation in *E. coli* and to enhance the effectiveness of existing antibiotics. By examining the biofilm activity of *Penicillium* compounds, the study aimed to explore novel approaches to mitigating the resistance of *E. coli* to penicillin and other antibiotics. These bioactive compounds may offer new avenues for disrupting bacterial biofilms and improving treatment outcomes for infections caused by multidrug-resistant strains of *E. coli*.

# Materials and Methods

Oral samples taken from the obtained samples were stored in a plastic sterile container and placed in an ice box until reaching the laboratory. Collected samples were diluted and plated on LB agar. Following that, the plates were stored for 24 hours at 37°C in an incubator. Poly taxonomical identification of isolated bacterial strains involved a comprehensive approach to the morphological, biochemical analysis [(Tavares-Carreón et al., 2020)](https://paperpile.com/c/8GkGA1/90Z95).Overall, 17 isolates were isolated from 25 oral samples, among them multidrug resistant isolate was characterized using a morphological and biochemical test, including Gram staining, motility, and a range of biochemical assays such as Indole, Methyl Red (MR), Voges-Proskauer (VP), Citrate utilization, and various sugar fermentation tests. These tests were performed according to standard microbiological procedures.The extract of *E.coli* was evaluated for its antioxidant potential using the DPPH assay, nitrous oxide scavenging activity, and Total antioxidant activity. This assessment was conducted following the methodology outlined by [(Sivaperumal et al., 2023)](https://paperpile.com/c/8GkGA1/oUMkI), with minor adjustments.The biofilm development was screened using six-well plate techniques. Test organisms that had developed overnight were added to 10 millilitres of nutrition broth intended for bacterial pathogens. They were then incubated for 24 hours at 37˚C. The contents of the six well plates were then decanted, rinsed in PBS, and dried before stained with 0.1% acridine orange and propidium iodide. The extra discoloration was removed by washing with deionized H2O. Six well plates were dried and checked for biofilm growth using a confocal microscope.

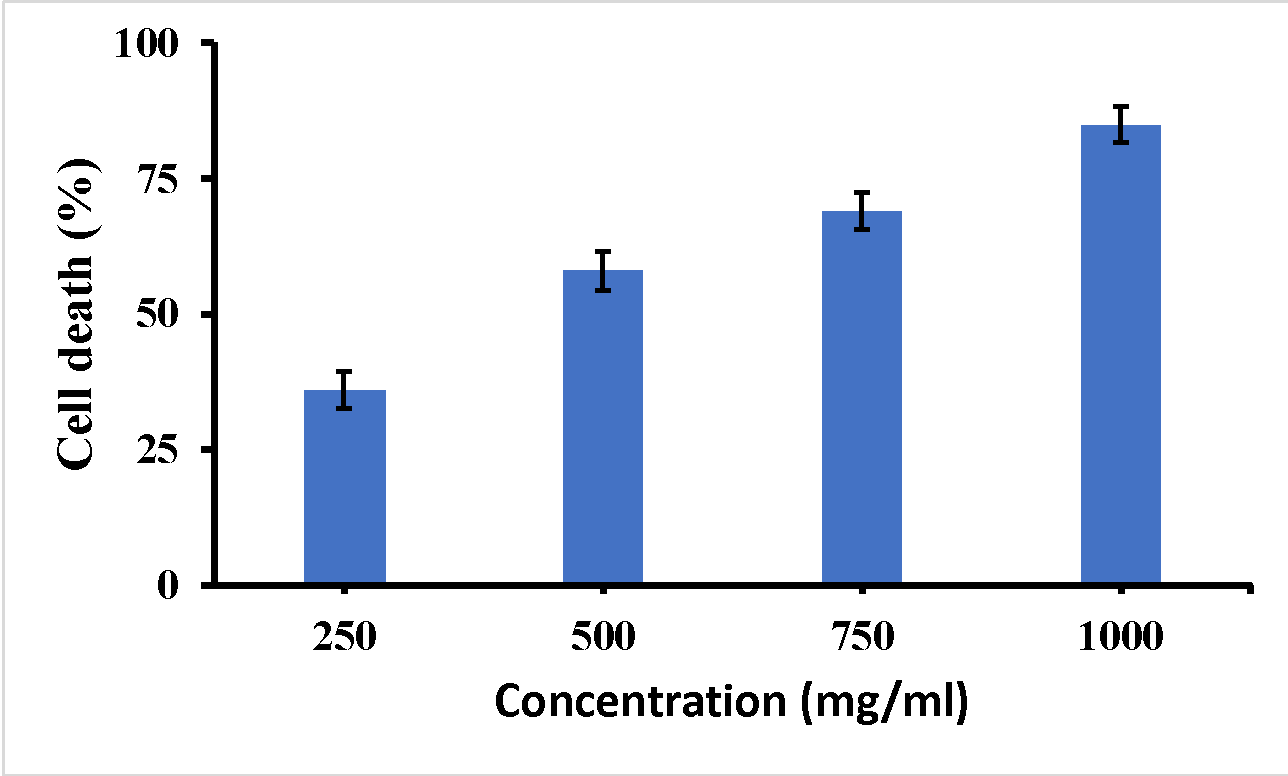
# Results

Overall, 17 isolates were isolated from 25 oral samples, among them multidrug resistant isolates were characterized using morphological and biochemical tests and other standard microbiological procedures [(Engelbrecht et al., 2021)](https://paperpile.com/c/8GkGA1/aBEBl). The isolate was identified as a Gram-negative**,**rod-shaped, and motile bacterium. It exhibited positive reactions for the Indole**,**Methyl Red (MR)**,** and Voges-Proskauer (VP) tests, indicating its ability to produce stable acid end-products from glucose fermentation and acetoin. The organism also showed positive catalase activity**,**starch hydrolysis**,** and was capable of fermenting both lactoseandmaltose, confirming its fermentative metabolism typical of enteric bacteria. However, it gave negative resultsfor citrate utilization**,**urease production**,**oxidase activity**,**inositol utilization**,** and triple sugar iron (TSI) reactions**,** suggesting an inability to utilize citrate as a sole carbon source, absence of urease and cytochrome oxidase enzymes, and limited ability to produce hydrogen sulfide or gas from multiple sugars. Variable reactions were observed in the fermentation of sucroseand xylose**,** indicating partial utilization of these carbohydrates. Based on this comprehensive biochemical profile, the organism was identified as belonging to the genus *Escherichia*, consistent with the characteristics of *Escherichia coli* (Table 1).

**Table 1.** Biochemical characteristics of multidrug resistant E.coli from oral samples

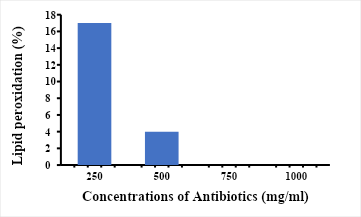
|  |  |
| --- | --- |
| **Gram stain** | **Negative** |
| Shape | Rod |
| Motility | Motile |
| Indole | Positive |
| MR | Positive |
| VP | Positive |
| Citrate | Negative |
| TSI | Negative |
| Oxidase | Negative |
| Catalase | Positive |
| Urease | Negative |
| Lactose | Positive |
| Maltose | Positive |
| Sucrose | Variable |
| Xylose | Variable |
| Starch | Positive |
| Inosital | Negative |
| Genus | *Escherichia* |

The cytotoxic activity of the tested compound was evaluated at different concentrations (250, 500, 750, and 1000 mg/ml), and the percentage of cell death increased in a dose-dependent manner (Fig. 1). At 250 mg/ml, approximately 35% of cells underwent death, whereas increasing the concentration to 500 mg/ml resulted in nearly 58% cell death. Further elevation to 750 mg/ml led to approximately 70% cell death, and the highest tested concentration of 1 g/ml showed a maximum cytotoxic effect with around 85% cell death. The data suggest a clear positive correlation between compound concentration and cytotoxicity, indicating its potent dose-dependent antimicrobial or antiproliferative activity. Error bars in the graph indicate standard deviation, confirming reproducibility and statistical reliability of the results.



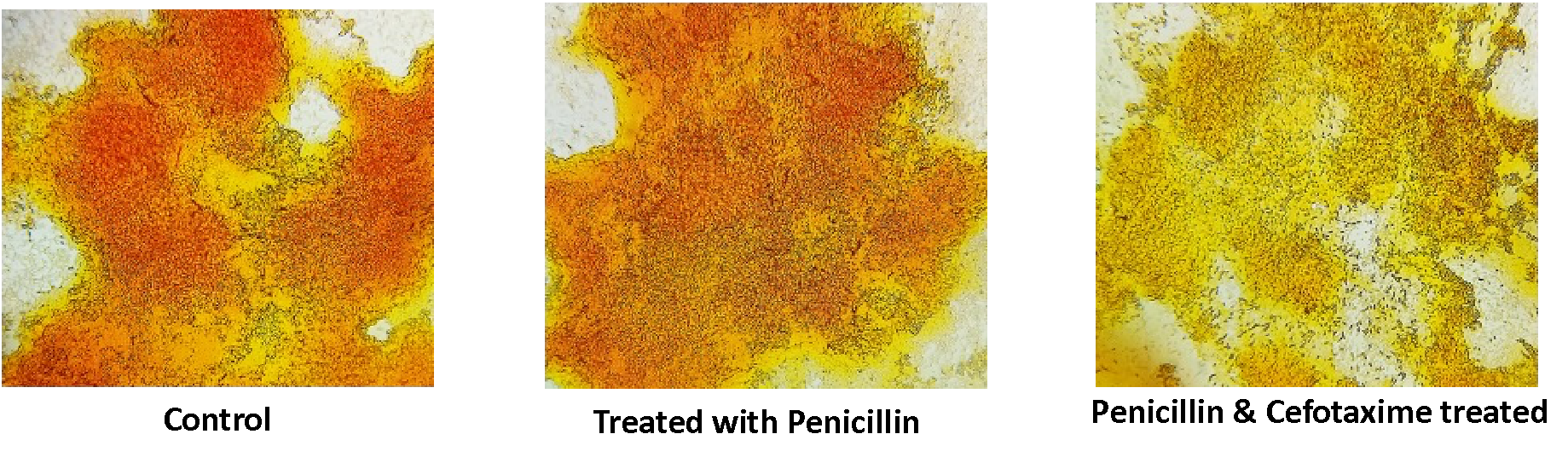
**Figure 1**. Antibiotic susceptibility of *E.coli* against the antibiotic at various concentrations

The lipid peroxidation levels in *beta-lactamase*-producing *Escherichia coli* strains, isolated from oral samples, were measured at varying concentrations of the experimental agent (Fig. 2). At a concentration of 250 mg, lipid peroxidation was found to be 17%, indicating a moderate level of oxidative damage to the bacterial membranes. At 500 mg, lipid peroxidation decreased to 4%, suggesting a reduced response to oxidative stress at this intermediate concentration. Interestingly, no lipid peroxidation was observed at 750 mg and 1g indicating that higher concentrations of the agent did not induce oxidative damage or that the bacteria had developed mechanisms to resist such stress at these higher levels.



**Figure 2.** lipid peroxidation levels in *beta-lactamase*-producing *Escherichia coli* strains

The results of this study suggest a complex relationship between oxidative stress and the resistance mechanisms of *beta-lactamase*-producing *E. coli* strains. The highest level of lipid peroxidation (17%) observed at the 250mg concentration suggests that at lower concentrations, the bacteria are more susceptible to oxidative damage, potentially due to insufficient activation of their defensive systems. This level of peroxidation indicates that the experimental agent is effective at inducing oxidative stress at lower concentrations, likely compromising bacterial cell membrane integrity and functioning. However, at 500mg, lipid peroxidation was significantly reduced to 4%, indicating a partial adaptation or an enhanced antioxidant defense mechanism in the bacteria. This may reflect an increased activation of protective systems, including *beta-lactamase*, which could serve a dual role in both antibiotic resistance and resistance to oxidative stress. This reduction in oxidative damage suggests that the bacteria are capable of mitigating oxidative stress when exposed to moderate concentrations of the agent.The most striking observation is the absence of lipid peroxidation at higher concentrations (750mg and 1g), which implies that the bacteria possess robust mechanisms to cope with oxidative stress at these levels. One possible explanation for this lack of lipid peroxidation is that at high concentrations, the bacteria have evolved resistance mechanisms, such as enhanced antioxidant activity or membrane repair systems, which prevent oxidative damage. Alternatively, the experimental agent may have reached a saturation point beyond which it no longer induces lipid peroxidation, potentially due to the bacteria's ability to withstand extreme oxidative conditions. These findings underscore the importance of understanding bacterial defense mechanisms, especially in the context of beta-lactamase producing strains, which already exhibit resistance to beta-lactam antibiotics(Nikalje et al., 2024) (Chehelgerdi et al., 2023). The ability of these bacteria to resist oxidative stress could be an additional layer of resistance that complicates treatment strategies. Further studies are needed to explore the specific mechanisms involved in this oxidative stress resistance, such as the role of antioxidant systems and the potential for synergistic effects when combined with other antimicrobial agents. Moreover, future research should focus on testing other *E. coli* strains with varying resistance profiles to assess the generalizability of these findings and their implications for clinical treatments.The ability of extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* to form biofilms was visually assessed in the presence of penicillin. The images show varying degrees of biofilm development (Fig. 3). In the absence of antibiotic stress (control), *E. coli* formed a dense, strongly stained biofilm layer, indicating robust adhesion and extracellular matrix production. Upon exposure to increasing concentrations of penicillin, a gradual reduction in biofilm intensity was observed. Moderate antibiotic levels resulted in partial inhibition of biofilm formation, while higher concentrations showed significantly weaker staining, suggesting disruption or reduced biofilm biomass.



**Figure 3.** Biofilm activity of bioactive flavonoids against *E.coli*

# Discussion

The results demonstrated a clear dose-dependent increase in cell death, with cytotoxicity rising from 35% at 250 mg/ml to 85% at 1000 mg/ml. This trend indicates that the test compound possesses significant biological activity that intensifies with concentration [(Wang et al., 2022)](https://paperpile.com/c/8GkGA1/x1x8F). Such a pattern suggests disruption of vital cellular functions, possibly through interference with membrane integrity, metabolic pathways, or induction of oxidative stress. If the compound is of natural origin, such as a polyphenol from marine actinobacteria, its activity may involve the generation of reactive oxygen species (ROS), leading to oxidative damage and cell death. These compounds are known for their antimicrobial and antioxidant properties, often targeting multiple cellular processes simultaneously [(Noman et al., 2021)](https://paperpile.com/c/8GkGA1/aM1EZ). The consistency of the results, indicated by low standard deviations, reinforces the reliability of the data. Given the potent cytotoxic effect at higher concentrations, the compound shows promise as an antimicrobial agent, particularly against resistant pathogens [(Nafie et al., 2021)](https://paperpile.com/c/8GkGA1/dFWKR) like Shiga toxin-producing *Escherichia coli*. [(Marya et al., 2022)](https://paperpile.com/c/8GkGA1/shofT), [(Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/8GkGA1/shofT+Dh2Nl), [(Wadhwani et al., 2022)](https://paperpile.com/c/8GkGA1/zd42N)The results indicate that penicillin exerts a measurable effect on the biofilm-forming capacity of ESBL-producing *E. coli*. Although β-lactam antibiotics like penicillin are typically ineffective against ESBL strains due to enzymatic degradation, the observed decrease in biofilm formation at higher penicillin concentrations suggests that the antibiotic still exerts sub-inhibitory effects on bacterial physiology [(Rajivgandhi et al., 2018)](https://paperpile.com/c/8GkGA1/GPaNP). This may involve interference with cell wall synthesis, leading to impaired adherence and extracellular matrix production, even if not completely bactericidal.[(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/8GkGA1/GOPa7+EFkwc+aEgJH), [(Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/8GkGA1/5XxTZ+oYGOo), [(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/8GkGA1/qptQ7+HX5nz)Biofilm formation in ESBL-producing strains contributes significantly to their antibiotic resistance and persistence in clinical settings [(Alonso-Español et al., 2023)](https://paperpile.com/c/8GkGA1/WncoZ). These findings suggest that while penicillin alone may not be effective for killing these strains, it could play a role in biofilm modulation, potentially enhancing the activity of combination therapies or disrupting colonization on surfaces [(Amer et al., 2022)](https://paperpile.com/c/8GkGA1/fTtuK). Further quantitative analysis, such as crystal violet absorbance measurements and CFU counting, would provide more precise insight into the biofilm inhibition levels [(Allkja et al., 2023)](https://paperpile.com/c/8GkGA1/HJOqW). Additionally, gene expression studies targeting biofilm regulatory genes(e.g., *csgA*, *bssS*, *fimH*) could clarify the mechanism of action.

# Conclusions

This study highlights the varying responses of *beta-lactamase*-producing *Escherichia coli* to oxidative stress induced by lipid peroxidation at different concentrations. The results demonstrate that oxidative damage is most pronounced at lower concentrations (250mg), with a significant reduction in peroxidation at intermediate (500mg) and no peroxidation at higher concentrations (750mg and 1g). These findings suggest that *E. coli* strains possess adaptive mechanisms, possibly involving *beta-lactamase* and other antioxidant defenses, which mitigate oxidative stress at higher concentrations. The ability of these strains to resist oxidative damage may represent an additional challenge in overcoming antimicrobial resistance. Further research is needed to better understand the underlying mechanisms of oxidative stress resistance in these bacteria and to explore potential therapeutic strategies to target both their antimicrobial and oxidative stress defense systems.

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