Virulence of Drug-Resistant Pathogen Staphylococcus Aureus Against Cefpodoxime

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**Abstract:***S.aureus* is a Gram-positive pathogen that causes a variety of illnesses, from superficial skin infections to life-threatening conditions such as septicemia. This study investigates the effects of cefpodoxime, a third-generation cephalosporin, on the cell viability and beta-hexosaminidase release of S. aureus. Biochemical analyses confirmed *S.aureus* characteristic metabolic traits, including positive tests for catalase, urease, and Voges-Proskauer, while the Gram stain and cell morphology aligned with known descriptions of the organism. Cell viability assays revealed a dose-dependent reduction in *S. aureus* viability following cefpodoxime treatment, with concentrations of 750 µg/ml and above leading to complete bacterial eradication. Additionally, beta-hexosaminidase release assays demonstrated increased enzyme release with escalating concentrations of a test compound, peaking at 98.5% at 1000 µg/ml, indicating significant cellular damage or degranulation.

**Keywords:** *Staphylococcus aureus;* Cefpodoxime; Cephalosporin; Biochemical analyses; Beta-hexosaminidase

# Introduction

*Staphylococcus aureus* is a significant human pathogen associated with a wide range of infections, from simple skin disorders to life-threatening illnesses such as *pneumonia* & bacteremia [(Guo et al., 2020; Lavanya & Paramasivam, 2023)](https://paperpile.com/c/bzUpBx/Us3q+TTfk). The prevalence of antibiotic-resistant *S. aureus* is a concern globally, with a high incidence of resistance to common antibiotics such as penicillin G and tetracycline observed in various studies[(Zhang et al., 2022)](https://paperpile.com/c/bzUpBx/K8zgm). Methicillin-resistant *S. aureus* is a notable example, with resistance mechanisms including drug efflux and mutation of target proteins [(Assis et al., 2017; Shyam et al., 2023)](https://paperpile.com/c/bzUpBx/eur1W+gA1Ja). The emergence of drug-resistant strains, particularlyMRSA, poses a critical challenge to public health due to the limited therapeutic options available[(Abebe & Birhanu, 2023)](https://paperpile.com/c/bzUpBx/LDRPL) [(Pranati et al., 2021; Sakthi et al., 2021)](https://paperpile.com/c/bzUpBx/tkYl+5WPP). The pathogenicity of *Staphylococcus aureus* is primarily due to its capacity to produce a wide range of virulence factors, such as toxins and enzymes, and its propensity to form biofilms on both abiotic and biotic surfaces, which protect the bacteria from antimicrobial agents [(Madhumitha & Muralidharan, 2021; Sued-Karam et al., 2024)](https://paperpile.com/c/bzUpBx/AqecZ+fC3g9). Moreover, the emergence of MRSA has further complicated treatment options, as these strains exhibit resistance to multiple classes of antibiotics[(Nikolic & Mudgil, 2023)](https://paperpile.com/c/bzUpBx/EuPZB).Cefpodoxime, a third-generation cephalosporin antibiotic, operates by suppressing bacterial cell wall formation and selectively targeting penicillin-binding proteins. This action disrupts the cell wall, leading to bacterial lysis and death [(Ali & Jat, 2017)](https://paperpile.com/c/bzUpBx/AZutv). Clinically, cefpodoxime proxetil, the prodrug of cefpodoxime, is used to treat a variety of infections due to its broad-spectrum activity against gram-positive and gram-negative organisms[(G. & Ganapathy, 2022; Kumar & Ramesh, 2021)](https://paperpile.com/c/bzUpBx/l5hil+aQYh2)). It is effective against pathogens responsible for respiratory tract infections, UTI, gonorrhea & skin infections[(Castle, 2007)](https://paperpile.com/c/bzUpBx/gOfNb). It is particularly noted for its efficacy against respiratory pathogens, including *Staphylococcus aureus* [(Stetsiouk et al., 2023)](https://paperpile.com/c/bzUpBx/BlkDb).

The emergence of cefpodoxime-resistant *S.aureus* strains is a growing concern, contributing to the broader challenge of antibiotic resistance in clinical settings[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/bzUpBx/KbXDy+NAJXC+gdAxW)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/bzUpBx/KbXDy+NAJXC+gdAxW+Jf4dM). While specific studies on cefpodoxime resistance are lacking, research highlights the prevalence of multi-drug resistant (MDR) *S. aureus* strains across various environments, including hospitals, communities, and food sources [(Shil et al., 2021)](https://paperpile.com/c/bzUpBx/v1qNi). These strains often exhibit resistance to beta-lactams, including cefpodoxime, particularly when the mecA gene is present, conferring resistance to methicillin and other beta-lactams[(Poniatovskyi et al., 2020)](https://paperpile.com/c/bzUpBx/4xm0z). Individuals with chronic diseases, such as HIV or diabetes, may be at higher risk of infections by resistant strains[(Mohamadou et al., 2020)](https://paperpile.com/c/bzUpBx/SqUXk).Cefpodoxime resistance is primarily driven by several molecular mechanisms, including the production of beta-lactamase enzymes, modifications in penicillin-binding proteins, and the presence of efflux pumps that reduce the intracellular concentration of the antibiotic[(Sivajothi, 2016)](https://paperpile.com/c/bzUpBx/16AyX). In MRSA, the acquisition of the mecA gene, encoding an altered PBP (PBP2a) with a low affinity for beta-lactams, plays a crucial role in resistance, further complicating the efficacy of cefpodoxime [(Guo et al., 2020)](https://paperpile.com/c/bzUpBx/Us3q).

# Materials and methods

*Staphylococcus sp.* was collected from hospitals and The bacteria were cultured on Salt Mannitol agar for 24 hours at 37°C. After incubation, the colonial morphology was examined under a microscope. For biochemical identification, the pathogen was streaked on Salt Mannitol agar, and various biochemical tests were conducted according to[(S. et al., 1975)](https://paperpile.com/c/bzUpBx/xXhFC).The cell viability of *Staphylococcus sp.* was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay, following the procedure outlined by [(Larsson & Parris, 2023)](https://paperpile.com/c/bzUpBx/zFrHE). Briefly, log-phase cultures of *Staphylococcus sp.* were grown in nutrient-rich broth and then exposed to different concentrations of cefpodoxime for specified durations. After the exposure period, the MTT reagent was added to the samples, and absorbance was measured spectrophotometrically at a designated wavelength. Cell viability was determined by comparing the absorbance of the treated samples to that of untreated control samples.The inhibitory effects on beta-hexosaminidase activity from *Staphylococcus aureus* cell lysates were assessed using a protocol by [(Shahari et al., 2017)](https://paperpile.com/c/bzUpBx/NTi4x). Cultures of *S. aureus* were grown to mid-log phase, collected by centrifugation, and lysed through sonication. The clarified lysates were then used for the enzyme activity assay. For each test, 50 µL of the cell lysate at varying concentrations (250, 500, 750, & 1000 µg/ml) was mixed with 50 µl of the substrate p-nitrophenyl-N-acetyl-β-D-glucosaminide (1 mM in 0.1 M citrate buffer, pH 4.5). The reaction mixture was incubated at 37°C for 1 hour. After incubation, the reaction was halted by adding 200 µl of 0.1 M glycine buffer (pH 10.0), and absorbance was measured at 405 nm using a microplate reader. The percentage inhibition of beta-hexosaminidase activity was calculated by comparing the absorbance of test samples to that of the controls.

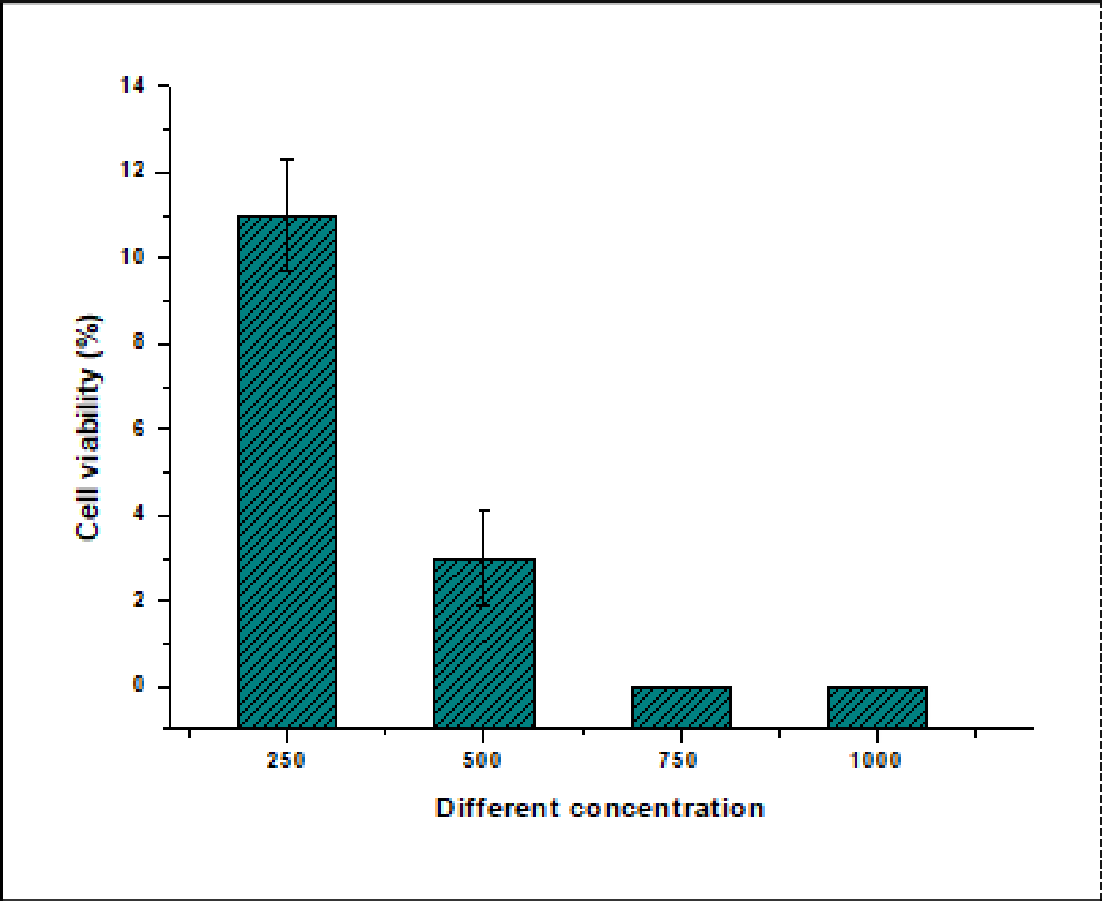
# Results

*Staphylococcus*  is a genus of Gram-positive, non-spore-forming bacteria. It is widely known for causing various infections, including skin infections and more severe conditions like septicemia. The biochemical analysis of this organism reveals several key characteristics (Table 1). The Gram stain result is positive, indicating a thick peptidoglycan cell wall, and the cells are cocci in shape. Although *Staphylococcus* is generally non-motile, a positive motility test in this case suggests a potential variant or contamination. The indole test is negative, indicating the absence of tryptophan degradation, while the methyl red (MR) test is also negative, confirming that mixed acid fermentation does not occur. A positive Voges-Proskauer (VP) test confirms the production of acetoin through the butylene glycol fermentation pathway, typical of *Staphylococcus aureus*. The organism does not utilize citrate as a sole carbon source, as shown by the negative citrate test, while the triple sugar iron (TSI) test is positive, indicating fermentation of glucose, lactose, and sucrose. The oxidase test is negative, showing the absence of cytochrome c oxidase. A positive catalase test confirms the presence of catalase enzyme, which breaks down hydrogen peroxide. The urease test is positive, showing the ability to hydrolyze urea. The organism also ferments lactose, maltose, and sucrose, but not xylose. A positive starch hydrolysis test suggests the presence of amylase, and inositol fermentation further highlights the organism's metabolic versatility.

**Table 1**. Biochemical Identification of *Staphylococcus aureus*

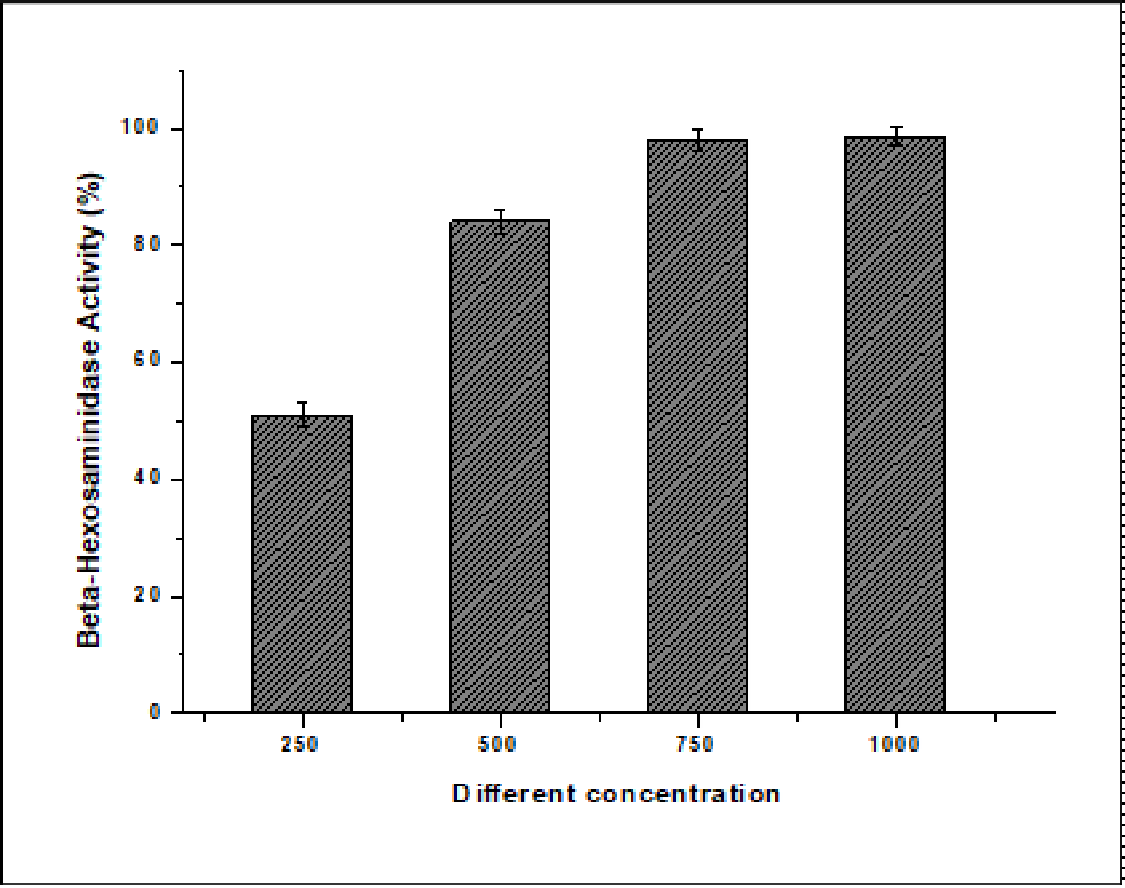
|  |  |
| --- | --- |
| **Biochemical characteristics** | **Results** |
| Gram stain | Positive |
| Shape | Cocci |
| Motility | Positive |
| Indole | Negative |
| MR | Negative |
| VP | Positive |
| Citrate | Negative |
| TSI | Positive |
| Oxidase | Negative |
| Catalase | Positive |
| Urease | Positive |
| Lactose | Positive |
| Maltose | Positive |
| Sucrose | Positive |
| Xylose | Negative |
| Starch | Positive |
| Inosital | Positive |

The cell viability of *Staphylococcus aureus* following treatment with cefpodoxime was examined at different concentrations (250, 500, 750, & 1000 µg/ml), showing a progressive, dose-dependent reduction in bacterial viability (Fig. 1). At low concentration (250 µg/ml), *S. aureus* exhibited a cell viability of 11%, indicating that cefpodoxime partially inhibited bacterial growth but allowed a small population of cells to survive. When the concentration was increased to 500 µg/ml, cell viability dropped significantly to 3%, demonstrating a stronger antimicrobial effect at this dose. At 750 µg/ml, no viable bacterial cells were detected, with a recorded 0% cell viability, indicating complete inhibition of bacterial growth. This suggests that cefpodoxime effectively eradicates *S. aureus* at this concentration. Similarly, at the highest concentration tested, 1000 µg/ml, 0% cell viability was observed, confirming the bactericidal nature of cefpodoxime at concentrations of 750 µg/ml and above.



**Figure 1.** Cell viability of Staphylococcus aureus treated with cefpodoxime at different concentrations.

The beta-hexosaminidase release assay was utilized to evaluate the effect of various concentrations of a test compound on the release of beta-hexosaminidase from *Staphylococcus aureus* cell lysates (Fig. 2). At a concentration of 250 µg/ml, the assay recorded a beta-hexosaminidase release of 51%, indicating moderate cellular disruption or degranulation. As the concentration increased to 500 µg/ml, the enzyme release rose significantly to 84%, reflecting a more pronounced effect on cellular integrity and suggesting that the compound has a stronger impact at this intermediate concentration(Nikalje et al., 2024). At 750 µg/ml, the release of beta-hexosaminidase was measured at 98%, demonstrating that the compound nearly maximizes enzyme release, indicating a high degree of cellular damage or degranulation (Chehelgerdi et al., 2023). At the highest tested concentration of 1000 µg/ml, the beta-hexosaminidase release reached 98.5%, showing that the release effect has plateaued, with only a marginal increase over the 750 µg/ml concentration.



**Figure 2.** Beta-hexosaminidase release from *Staphylococcus aureus* cell lysates treated with 250, 500, 750, and 1000 µg/ml of the test compound.

# Discussion

Cefpodoxime, a third-generation cephalosporin, has shown significant clinical efficacy against *S. aureus*, including methicillin-resistant strains[(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/bzUpBx/VAAXL+sma6A+2ZMIn). Studies confirm that cefpodoxime plasma concentrations exceed the minimum inhibitory concentration of 2-4 µg/ml, which inhibits 90% of skin infections, including *Staphylococcus species* [(Farooq et al., 2024)](https://paperpile.com/c/bzUpBx/5PXTA). However, biofilm formation can reduce the efficiency of antibiotics such as cefpodoxime. Biofilms provide a protective environment that restricts antibiotic penetration, lowering their potency[(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/bzUpBx/EN6SE+4KLVf+I5BIt). According to studies, younger biofilms (6 h) are more sensitive to antibiotics than older biofilms (48 h), highlighting the necessity of early intervention in maximising antibiotic efficacy[(Amorena et al., 1999)](https://paperpile.com/c/bzUpBx/lceNm). This shows that cefpodoxime treatment may be more successful against S. aureus infections if given before biofilm development[(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/bzUpBx/0O7ev+mKz47+7dMRa). In addition to cefpodoxime, various techniques for reducing *S. aureus* viability have been investigated. For example, thermosonication, which combines heat and ultrasound, has been found to effectively reduce bacterial viability in human milk[(Gomes et al., 2021)](https://paperpile.com/c/bzUpBx/vXkgq). Similarly, inhibiting the WalKR two-component system causes cell wall thickening and aberrant septa formation, compromising bacterial survival [(Delaune et al., 2011)](https://paperpile.com/c/bzUpBx/8PZd2). Such techniques may provide alternate or complementary strategies for the treatment of *S. aureus* infections*.  S. aureus* has adaptive mechanisms in response to different drugs. For example, sub-MIC levels of florfenicol produced cell wall thickening and decreased viability, with 20% of cells showing structural damage[(Blickwede et al., 2004)](https://paperpile.com/c/bzUpBx/dZRMU). In contrast, nafcillin and oxacillin exposure has been associated to increased AbcA transporter expression, which promotes bacterial survival [(Truong-Bolduc et al., 2024)](https://paperpile.com/c/bzUpBx/Gf02Q).*S. aureus* is known to secrete various virulence factors, such as toxins and enzymes, through specific secretion systems, beta-hexosaminidase release may reflect a more general indicator of cell damage rather than a specific secretion pathway[(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/bzUpBx/GuZmR+1hTD4+Dww7b). Membrane integrity, controlled by lipids like lysyl-phosphatidylglycerol (LPG) and lipoteichoic acid (LTA), plays a crucial role in maintaining cell structure and function [(Zheng et al., 2021)](https://paperpile.com/c/bzUpBx/9k8P9). Disruption of these membranes could lead to the observed increase in enzyme release, indicating that the test compound may interfere with these vital components. Extracellular vesicles (EVs) of S. aureus may transport virulence factors like β-lactamases and contribute to the bacterial response to membrane injury [(Silva Rosa da Luz et al., 2021)](https://paperpile.com/c/bzUpBx/mlreY).

# Conclusion

This study demonstrated the efficacy of cefpodoxime in inhibiting *Staphylococcus aureus* at concentrations of 750 µg/ml and above, resulting in complete bacterial death. The observed dose-dependent reduction in cell viability underscores the bactericidal nature of cefpodoxime, particularly when administered early in infection to prevent biofilm formation. Beta-hexosaminidase release further confirmed substantial cellular damage induced by a test compound, suggesting that it may act by disrupting membrane integrity, potentially through interference with key membrane lipids. This disruption could enhance cefpodoxime's bactericidal effects, offering a promising strategy for tackling resistant *S. aureus* strains. Future studies should explore the mechanisms underlying these effects and assess the potential for combining cefpodoxime with other therapeutic interventions, such as agents targeting bacterial secretion systems or cell wall synthesis pathways, to improve treatment outcomes in severe infections.

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