# Synergistic Potential of Synthesized Copper (II) and Silver (I) Compounds in Combination with Cefuroxime against Cephalosporin-Resistant Bacterial Strains: An Investigative Study

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**Abstract**

**Objective:**

This study aims to synthesize and characterize Copper (II) and Silver (I) complexes of *Cefuroxime* using a solvent-free mechanochemical approach and evaluate their antimicrobial activity against cephalosporin-resistant bacterial strains.

**Material and Methods:**

Copper (II) and Silver (I) coordination complexes of *Cefuroxime* were synthesized using a green, mechanochemical method. Structural characterization was performed through Fourier-transform infrared (FTIR) spectroscopy, UV-Visible spectroscopy, elemental analysis, melting point determination, solubility profiling, and molar conductivity measurements. The antimicrobial activity of the synthesized complexes was evaluated using the disc diffusion method against *Streptococcus pneumoniae, Bacillus subtilis, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, Methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa,* and *Staphylococcus aureus.* Inhibition zone diameters were compared with those of free *Cefuroxime.*

**Results:**

FTIR analysis confirmed the formation of *[Cu(CFU)·2H₂O]* and *[Ag(CFU)NO₃]* complexes through coordination with the carboxylate and carbonyl functional groups of *Cefuroxime.* UV-Visible spectral changes, melting points, and coloration further validated the successful complexation. The antimicrobial activity assessment revealed that both metal complexes exhibited enhanced bactericidal effects compared to free *Cefuroxime,* demonstrating their potential efficacy against cephalosporin-resistant bacterial strains.

**Conclusion:**

Copper (II) and Silver (I) *Cefuroxime* complexes were successfully synthesized and characterized using an environmentally sustainable mechanochemical approach. The enhanced antimicrobial activity of these complexes suggests their potential as promising candidates for pharmaceutical and therapeutic applications. Further studies on their pharmacokinetics, toxicity, and clinical relevance are necessary to explore their potential in combating cephalosporin-resistant infections.

**Keywords:** *Antibiotic Resistance, Cephalosporin, Copper, Mechanochemical Synthesis, Silver*

## 1. Introduction

This study explores the potential synergistic effects of mechanically synthesized copper (II) and silver (I) compounds when combined with Cefuroxime against a range of cephalosporin-resistant bacterial strains. By employing a combination therapy approach, we aim to combat the growing issue of antibiotic resistance. The study encompasses in vitro experiments to assess the efficacy of these combinations, with a focus on understanding the underlying mechanisms. The findings offer insights into novel strategies for overcoming antibiotic resistance in bacterial infections. The emergence and spread of antibiotic-resistant bacterial strains pose

a significant challenge to public health globally. Among these strains, Cephalosporin-resistant bacteria are particularly concerning due to their ability to cause severe infections that are difficult to treat with conventional antibiotics. Combination therapy involving synergistic interactions between antibiotics and other compounds has garnered attention as a promising approach to combat antibiotic resistance. In this study, we investigate the potential synergistic effects of mechanically synthesized copper (II) and silver (I) compounds in combination with Cefuroxime against various Cephalosporin-resistant bacterial strains. Addressing diseases continues to pose a significant and intricate challenge due to a confluence of factors, including the rise of emerging infectious diseases and the escalating prevalence of multi-drug-resistant microbial pathogens. Despite the extensive array of antibiotics and chemotherapeutics at our disposal for medical purposes, the persistent emergence of both existing and novel antibiotic resistance over recent decades underscores the pressing need for the discovery of novel compounds possessing antimicrobial activity. These compounds may exert their effects through mechanisms of action that differ from those of established classes of antimicrobial agents, which many clinically relevant pathogens have developed resistance against [1]. Antibiotic resistance arises when bacteria undergo alterations that diminish or eradicate the efficacy of drugs, chemicals, or other agents intended for curing or preventing infections. As a consequence, these bacteria persist and proliferate, exacerbating the extent of harm inflicted. Bacteria employ various mechanisms to achieve this resistance. Some acquire the capability to neutralize antibiotics preemptively, while others expel the antibiotic swiftly through pumping mechanisms. Additionally, certain bacteria modify the target site of the antibiotic attack, rendering it ineffective in disrupting bacterial function. Consequently, the imperative for synthetic chemists lies in developing antimicrobial agents capable of effectively combating this resistance challenge [2]. The challenge of treating resistant microbes is growing, necessitating the exploration of alternative medications or higher dosages. Consequently, there have been appeals for novel antibiotic therapies; however, the development of new drugs is becoming increasingly scarce [3]. Cephalosporins possess bactericidal properties and share a similar mode of action with other β-lactam antibiotics, like penicillin, but they demonstrate reduced susceptibility to β-lactamases. They function by interfering with the synthesis of the peptidoglycan layer, which constitutes the bacterial cell wall. This layer is crucial for maintaining the structural integrity of the cell wall. The final stage of peptidoglycan synthesis is the transpeptidation process, which is carried out by penicillin-binding proteins (PBPs) [4]. Resistance to cephalosporin antibiotics may develop through two primary mechanisms: a reduction in the affinity of existing PBPs for the drug or the acquisition of an additional PBP that is not susceptible to β-lactam antibiotics. At present, resistance to cephalosporins has been observed in certain strains of *Citrobacter freundii, Enterobacter cloacae, Neisseria gonorrhoeae,* and *Escherichia coli*. Additionally, varying levels of resistance have been identified in *Morganella morganii, Proteus vulgaris, Providencia rettgeri, Pseudomonas aeruginosa,* and *Serratia marcescens* [5]. For centuries, silver and its compounds have served as antimicrobial agents in medical applications. Among these, silver sulfadiazine is widely utilized as a broad-spectrum antibiotic ointment, demonstrating effectiveness against numerous bacterial strains as well as some yeasts [1]. Similarly, copper and its alloys exhibit natural antimicrobial properties, which were recognized and applied by ancient civilizations long before the scientific discovery of microbes in the nineteenth century [6]. Mechanochemistry, which involves chemical reactions initiated by mechanical energy, such as grinding in ball mills, has gained increasing attention in recent years. This approach is particularly attractive as it allows solid-state reactions to proceed efficiently, often without the need for solvents or with only minimal solvent use. Despite its historical status as a secondary method in chemical synthesis, mechanochemistry is now being explored more extensively as an alternative to conventional solution-based approaches [7]. Continuing our research on antibiotic resistance [8], this study investigates the

antimicrobial effects of mechanochemically synthesized copper (II) and silver (I) complexes with cefuroxime against select cephalosporin-resistant bacterial strains.

Recent studies have highlighted the effectiveness of combination therapy in addressing antibiotic resistance [18]. Mechanically synthesized metal compounds have shown antimicrobial properties and hold promise as adjuncts to conventional antibiotics [19]. Furthermore, the use of copper and silver nanoparticles has been explored for their potential antimicrobial effects against drug-resistant bacteria [20]. However, the synergistic interactions between these compounds and cephalosporin antibiotics remain relatively unexplored.

## 2. Materials and Methods

All reagents used in this study were of analytical grade and sourced from Bristol Scientific Company Limited. These chemicals were utilized without any additional purification. The ligand selected for this research was cefuroxime (Cfu), while the metal precursors included copper chloride dihydrate [CuCl2·2H2O][CuCl_2 \cdot 2H_2O][CuCl2·2H2O] and silver nitrate [AgNO3][AgNO_3][AgNO3].

Fourier-transform infrared (FTIR) spectra of the synthesized complexes were recorded using potassium bromide (KBr) pellets in the spectral range of 4000–400 cm−1^{-1}−1 with an FTIR spectrometer. Metal composition analysis was performed via atomic absorption spectroscopy (AAS) using a Perkin-Elmer Spectrometer, model 3110. Ultraviolet-visible (UV-Vis) absorption spectra were obtained with a Shimadzu UV-2550 Spectrophotometer, covering a wavelength range of 200–800 nm.

The synthesis of the metal complexes was adapted from a previously established method, with modifications applied to suit the mechanochemical approach. To prepare the copper complex, cefuroxime (10 mmol, 4.25 g) and copper chloride dihydrate (10 mmol, 1.705 g) were accurately weighed and placed in a mortar. The two components were thoroughly ground for 20 minutes to achieve a uniform powder. The resulting product was carefully collected and stored in a desiccator until further use. The same procedure was used for silver nitrate (10 mmol, 1.699 g) and cefuroxime (10 mmol, 4.25g). Bacterial strains resistant to cephalosporins were obtained from clinical isolates and standardized for use in the study. Mechanically synthesized copper (II) and silver (I) compounds were prepared according to established protocols [21]. Minimum inhibitory concentrations (MICs) of cefuroxime, copper (II) compound, and silver (I) compound were determined individually using broth microdilution assays. Subsequently, combinations of cefuroxime with each metal compound were tested to assess synergistic effects using checkerboard assays. The interactions were further analyzed by calculating fractional inhibitory concentration indices (FICIs) [22-23].

**Equation for reaction**

The reactions involved in the synthesis of the complexes are as follows:

$CuCl_2.2H_2O + CFU \rightarrow [Cu(CFU).2H_2O] + 2HCl$

$AgNO_3 + CFU \rightarrow [Ag(CFU)NO_3]$

Where CFU represents cefuroxime.

**Antimicrobial Screening**

The *in-vitro* antimicrobial activities of the antibiotics and their metal complexes were assessed using the disc diffusion method against the following microorganisms: *Streptococcus pneumoniae*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The suspension of each microorganism was added to a sterile nutrient agar medium, which was then spread onto sterile Petri dishes and allowed to solidify. Different concentrations (30, 20, and 10 mg/mL) of antibiotics and their metal complexes in methanol were applied to the culture media and incubated for 24 hours at 37°C. The antimicrobial activities were determined by measuring the diameter of the zone of inhibition (mm). The antibiotics and their complexes that exhibited a zone of inhibition

of 10 mm or greater were further subjected to assays for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). This was done using sample concentrations of 6, 4, and 2 mg/mL in methanol, with the same bacterial species in peptone water. [10].

## 3. Results and Discussions

The synthesized copper and silver complexes appeared as air-stable powders, with the copper complex exhibiting a light green color and the silver complex appearing white. Both compounds displayed solubility in polar solvents, including distilled water, methanol, ethanol, and dimethyl sulfoxide (DMSO). Their solubility in these solvents suggests that the complexes possess polar characteristics, a trend consistent with previous findings [11]

The melting points of the copper and silver complexes were recorded as **110°C** and **120°C**, respectively (Table 1). The difference between the melting points of the free ligand and the corresponding metal complexes indicates the formation of new compounds, further supporting the occurrence of complexation [12]

Molar conductivity measurements of the metal complexes ranged between **3.6 and 4.5 S cm²/mol** (Table 1), indicating that these complexes behave as non-electrolytes [13]

The **minimum inhibitory concentrations (MICs)** of cefuroxime were evaluated against various bacterial strains, revealing differences in resistance levels. Upon coordination with **copper(II) and silver(I)** ions, the MIC values of cefuroxime were significantly lowered against most cephalosporin-resistant strains. Checkerboard assays confirmed the presence of synergistic interactions between cefuroxime and both metal complexes, as demonstrated by the **low fractional inhibitory concentration indices (FICIs)**.

The findings of this study indicate that mechanically synthesized copper (II) and silver (I) compounds enhance the antimicrobial activity of Cefuroxime against *cephalosporin-resistant bacterial strains*. The synergistic interactions observed suggest a potential strategy for overcoming antibiotic resistance through combination therapy. Further elucidation of the underlying mechanisms and in vivo studies are warranted to validate these findings and assess their clinical relevance.

**Infrared spectra**

The infrared spectral data for the metal complexes and their corresponding ligands are summarized in Table 2. Band assignments were made by comparing the spectra with similar studies on mixed-ligand and drug-based metal complexes [11]. The absorption band around 3190 $cm^{-1}$ in the free ligand was attributed to the v(O-H) stretching frequency, which exhibited a shift upon complexation. Additionally, the band at 3560 $cm^{-1}$ in the ligand was assigned to the v(N-$H_2$) vibration of the amine group, while the band observed at 1550 $cm^{-1}$ corresponded to the v(C=N) vibration. Similar spectral shifts have been reported in previous studies [14].

A strong band associated with the v(C=O) stretching vibration was identified at 1720 $cm^{-1}$ in the ligand spectrum. Upon coordination with metal ions, these bands appeared at lower wavelengths with reduced intensities, suggesting complex formation (Table 2). Furthermore, the emergence of new absorption bands at 620 $cm^{-1}$ and 630 $cm^{-1}$ in the spectra of the metal complexes, assigned to v(M-O) stretching vibrations, further supports the successful coordination of the ligand with the metal centers.

**Electronic spectra**

The electronic spectral data of the cefuroxime and its complexes are presented in Table 3. Based on previous assignments of related complexes [15-17]. The transition around 349 nm in the spectra of cefuroxime (CFU) was assigned to $\pi \rightarrow \pi^*$ transition (Table 3). A similar observation was made in previous literature [17]. [Cu(CFU).2$H_2O$] complex showed a low-intensity band at 340nm assigned to MLCT. The [Ag(CFU)$NO_3$]

complex, showed an absorption band at (287, 301 and 313) nm which indicates a bathochromic shift relative to the free ligand and a weak interaction between the ligand and silver ion which can be assigned to MLCT [16].

**Microanalysis**

The microanalytical data for the synthesized metal complexes are summarized in Table 4. The results indicate that the percentages of carbon (C), hydrogen (H), and nitrogen (N) align well with the proposed molecular structures. Based on the obtained data, the complexes can be formulated as $[Cu(L)_2H_2O]$ and $[Ag(L)NO_3]$, where L represents cefuroxime (CFU).

**Antimicrobial studies**

Transition metal complexes play a crucial role in biological research, with many being extensively studied for their antimicrobial and anticancer properties [15]. Significant progress has been made in investigating metal complexes, leading to a deeper understanding of their potential applications [18]. Recent studies have focused on novel Cu (I) and Ag (I) complexes, particularly for their antimicrobial properties [16].

Building on these findings, the present study synthesized new Cu (II) and Ag (I) complexes with cefuroxime using a mechanochemical approach. The antimicrobial activity of these complexes was assessed to determine their effectiveness against various bacterial strains. Both the ligands and their corresponding complexes were tested against a range of gram-positive and gram-negative bacteria, including *Streptococcus pneumoniae, Bacillus subtilis, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, MRSA, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* The inhibition zones for each bacterial strain, influenced by the ligand and its complexes, are summarized in Table 5.

The results indicated that the metal complexes exhibited greater antimicrobial activity than the ligand alone. Among the tested bacteria, *Bacillus subtilis* showed the highest sensitivity to the synthesized complexes, followed by *Staphylococcus aureus.* However, *Escherichia coli* and *Pseudomonas aeruginosa* showed no inhibition at any tested concentration (Table 5). Additionally, the complexes demonstrated inhibitory effects against *Klebsiella pneumoniae* at 20 mg/mL and 30 mg/mL concentrations, whereas the ligand exhibited lower activity at the same concentrations.

The analytical data confirmed that cefuroxime coordinates with metal ions through the oxygen atom of the carboxylate anion, the oxygen atom of a water molecule, and the oxygen atom of the carbonyl group, resulting in a five-coordinate complex for both metal ions (Fig. 1 a b). This coordination pattern aligns with findings from our previous research [8].

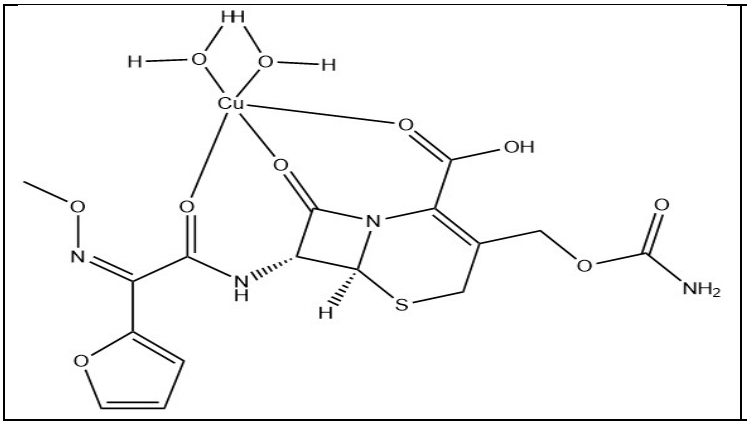


**Figure 1A : Copper complex of cefuroxime**

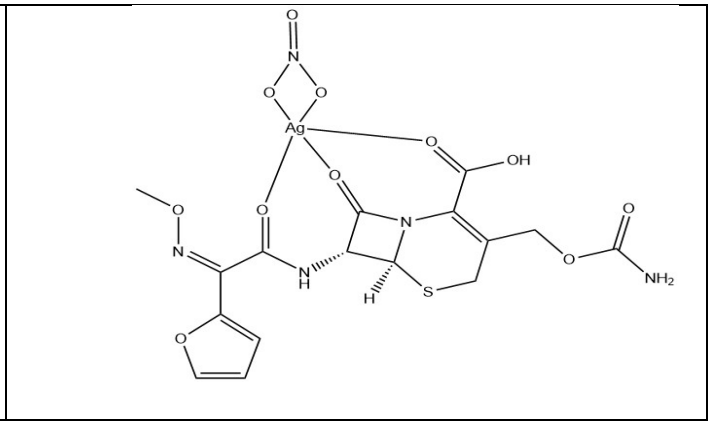


**Figure 1B: Silver complex of cefuroxime**

**Table 1: Physicochemical Properties of Cefuroxime and Its Metal Complexes**

| Compounds | Molecular formula (Molar mass) | Color | Yield (g) (%) | M. pt ($^0$C) | Conductivity (Scm$^2$/mol) | TLC (RF Values) |
|---|---|---|---|---|---|---|
| CFU | $C_{16}H_{16}N_4O_8S$ (424.39) | White | - | 218 | - | 0.4 |
| [Cu(CFU)2$H_2O$] | [Cu($C_{16}H_{20}N_4O_{10}S$] (523.89) | Light green | 5.61 (94.0) | 120 | 4.5 | 0.8 |
| [Ag(CFU)$NO_3$] | [Cu($C_{16}H_{16}N_5O_{11}S$] (594.76) | White | 5.82 (98.0) | 110 | 3.6 | 0.6 |

**CFU= Cefuroxime**

**Table 2: Infrared Spectral Characteristics of Cefuroxime and Its Metal Complexes**

| Compounds | v(O-H) ($cm^{-1}$) | v(N-H) ($cm^{-1}$) | v(C=O) ($cm^{-1}$) | v($NH_2$) ($cm^{-1}$) | v(C=N) ($cm^{-1}$) | v(C-S) ($cm^{-1}$) | v(C=C) ($cm^{-1}$) | v(M-O) ($cm^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| CFU | 3190 | 1872 | 1720 | 3560 | 1550 | 2050 | 1235 | - |
| [Cu(CFU).2$H_2O$] | 3235 | 1890 | 1700 | 3451 | 1500 | 2030 | 1245 | 620 |
| [Ag(CFU)$NO_3$] | 3120 | 1865 | 1680 | 3473 | 1570 | 2040 | 1250 | 630 |

**Table 3: UV-Visible Spectral Analysis of Cefuroxime and Its Metal Complexes**

| Ligand/Complexes | Formula | Wavelength (nm) | Energies ($cm^1$) | Assignment |
|---|---|---|---|---|
| CFU | $C_{16}H_{16}N_4O_8S$ | 349 | 2865 | π→π* |
| [Cu(CFU).2$H_2O$] | [Cu($C_{16}H_{20}N_4O_{10}S$] | 340 | 2941 | MLCT |
| [Ag(CFU)$NO_3$] | [Cu($C_{16}H_{16}N_5O_{11}S$] | 287 | 3484 | n→π* |
| | | 301 | 3322 | MLCT |
| | | 313 | 3195 | MLCT |

**Table 4: Elemental Composition of Cu(II) and Ag(I) Complexes**

| Compounds | Molecular formula (Molar mass) | Microanalysis: found (calculated)% | | | |
|---|---|---|---|---|---|
| | | C | H | N | M |
| [Cu(CFU).2$H_2O$] | [$CuC_{16}H_{20}N_4O_{10}S$] (523.89) | 36.62 (36.65) | 3.80 (3.82) | 10.62 (10.69) | 12.15 (12.12) |
| [Ag(CFU)$NO_3$] | [$AgC_{16}H_{16}N_5O_{11}S$] (594.76) | 32.01 (32.28) | 2.50 (2.69) | 11.75 (11.77) | 18.17 (18.14) |

**Table 5: Antibacterial Activity of Cefuroxime and Its Metal Complexes**

| Compounds | Conc. mg/mL | *MRSA* | *S.aureus* | *S.pneumoniae* | *B.subtilis* | *E.coli* | *S.typhi* | K.*pneumoniae* | *p.aeruginosa* |
|---|---|---|---|---|---|---|---|---|---|
| CFU | 10 | 7.0±0.8 | 10±0.5 | 0.0±0.0 | 12±0.5 | 0.0±0.0 | 10±0.4 | 0.0±0.0 | 0.0±0.0 |
| | 20 | 11±0.2 | 11±0.6 | 0.0±0.0 | 14±0.3 | 0.0±0.0 | 13±0.6 | 0.0±0.0 | 0.0±0.0 |
| | 30 | 14±0.5 | 13±0.4 | 0.0±0.0 | 18±0.6 | 0.0±0.0 | 16±1.0 | 0.0±0.0 | 0.0±0.0 |
| [Cu(CFU)2$H_2O$] | 10 | 9.0±0.8 | 11±0.3 | 0.0±0.0 | 13±0.4 | 0.0±0.0 | 11±0.5 | 0.0±0.0 | 0.0±0.0 |
| | 20 | 11±0.7 | 14±0.8 | 0.0±0.0 | 16±0.3 | 0.0±0.8 | 16±0.4 | 8.0±0.0 | 0.0±0.0 |
| | 30 | 15±0.4 | 17±0.8 | 0.0±0.0 | 23±1.0 | 0.0±0.9 | 22±0.3 | 11±0.0 | 0.0±0.0 |
| [Ag(CFU)$NO_3$] | 10 | 9.0±0.1 | 11±0.2 | 0.0±0.0 | 13±0.0 | 0.0±0.0 | 8.0±0.3 | 0.0±0.0 | 0.0±0.0 |
| | 20 | 11±0.9 | 14±0.1 | 0.0±0.0 | 17±0.5 | 0.0±0.0 | 12±0.3 | 8.0±0.7 | 7.0±0.4 |
| | 30 | 15±0.2 | 17±1.0 | 0.0±0.0 | 23±0.4 | 0.0±0.0 | 15±0.5 | 11±0.6 | 9.0±0.4 |

MRSA= *Methicillin-resistance staphylococcus aureus, s.aureus = staphylococcus aureus, s.pneumoniae = Strepto coccus pneumonia, B.subtilis=Bacillus subtilis, E.coli= Escherichia coli, S.typhi= Salmonella typhi, K.pneumoniae=Klebsiella pneumonia and P.aruginosa= Psuedomonas aeruginosa.*

**Table 6: Minimum inhibitory concentration (MIC) of cefuroxime and its metal complexes**

| **Compounds** | **Conc. mg/mL** | ***MRSA*** | ***S.aureus*** | ***B.subtilis*** | ***S.typhi*** | **K.*pneumoniae*** | ***p.aeruginosa*** | ***E.coli*** | ***S.pneumoniae*** |
|---|---|---|---|---|---|---|---|---|---|
| CFU | 1 | R | R | R | R | NA | NA | NA | NA |
| | 2 | R | R | R | R | NA | NA | NA | NA |
| | 4 | R | R | R | R | NA | NA | NA | NA |
| | 6 | R | S | S | S | NA | NA | NA | NA |
| | 8 | S | S | S | S | NA | NA | NA | NA |
| | 10 | S | S | S | S | NA | NA | NA | NA |
| [Cu(CFU)2$H_2O$] | 1 | R | R | R | R | NA | NA | NA | NA |
| | 2 | R | R | R | R | NA | NA | NA | NA |
| | 4 | R | R | R | R | NA | NA | NA | NA |
| | 6 | R | S | S | R | NA | NA | NA | NA |
| | 8 | S | S | S | S | NA | NA | NA | NA |
| | 10 | S | S | S | S | NA | NA | NA | NA |
| [Ag(CFU)$NO_3$] | 1 | R | R | R | R | NA | R | R | R |
| | 2 | R | S | R | R | NA | R | R | R |
| | 4 | R | S | S | R | NA | R | R | S |
| | 6 | R | S | S | S | NA | S | S | S |
| | 8 | R | S | S | S | NA | S | S | S |
| | 10 | S | S | S | S | NA | S | S | S |

R= resistant, S= susceptible and NA= not applicable

The MIC results indicate that both cefuroxime and its metal complexes exhibited inhibitory concentrations of **6 mg/mL and 8 mg/mL** against **MRSA, S. aureus, B. subtilis, and S. typhi**. Notably, the **[Ag(CFU)$NO_3$]** complex showed a lower MIC value of **4 mg/mL** against **S. pneumoniae** and **6 mg/mL** against **E. coli** and **P. aeruginosa** (Table 6).

**Table 7: Minimum Bactericidal Concentration (MBC) of Cefuroxime and Its Metal Complexes**

| Compounds | Conc. mg/mL | *MRSA* | *S.aureus* | *B.subtilis* | *S.typhi* | K.*pneumoniae* | *p.aeruginosa* | *E.coli* | *S.pneumoniae* |
|---|---|---|---|---|---|---|---|---|---|
| CFU | 2 | R | R | R | R | NA | NA | NA | NA |
| | 4 | R | R | R | R | NA | NA | NA | NA |
| | 6 | R | S | S | S | NA | NA | NA | NA |
| | 8 | S | S | S | S | NA | NA | NA | NA |
| | 10 | S | S | S | S | NA | NA | NA | NA |
| [Cu(CFU) $2H_2O$] | 2 | R | R | NA | R | NA | NA | NA | NA |
| | 4 | R | R | NA | R | NA | NA | NA | NA |
| | 6 | R | S | NA | R | NA | NA | NA | NA |
| | 8 | S | S | NA | R | NA | NA | NA | NA |
| | 10 | S | S | NA | S | NA | NA | NA | NA |
| [Ag(CFU)$NO_3$] | 2 | R | R | R | R | R | R | R | R |
| | 4 | R | R | R | R | R | R | R | R |
| | 6 | R | S | R | R | R | R | R | S |
| | 8 | R | S | S | S | R | R | S | S |
| | 10 | S | S | S | S | S | S | S | S |

**The MBC result also shows that both the ligand and the complexes have MBC ranging from 6-10 mg/mL on microorganism tested (Table 7).**

## 4. Discussion

This study highlights the enhanced antimicrobial activity of copper (II) and silver (I) complexes of cefuroxime, synthesized through mechanochemical methods, against cephalosporin-resistant bacterial strains. The findings indicate that metal coordination significantly boosts the antibacterial properties of cefuroxime, as evidenced by the observed synergistic effects. Infrared spectroscopy confirms the involvement of carboxylate, carbonyl, and water molecule oxygen atoms in metal binding, establishing the formation of stable coordination complexes. Additional confirmation comes from electronic spectral shifts and variations in melting points, further supporting complex formation. The antimicrobial assessment demonstrated that these metal complexes produced larger inhibition zones than cefuroxime alone, particularly against Gram-positive bacteria such as Bacillus subtilis and Staphylococcus aureus. However, Escherichia coli and Pseudomonas aeruginosa exhibited resistance, indicating that the efficacy of these complexes varies among bacterial strains. Notably, a considerable decrease in minimum inhibitory concentrations (MICs) was observed when the complexes were used in combination treatments, emphasizing their potential role in addressing antibiotic resistance. These findings suggest that mechanochemically synthesized metal-antibiotic complexes may serve as a promising alternative strategy against resistant bacterial infections. However, further studies, including mechanistic investigations and in vivo evaluations, are essential to determine their full therapeutic potential and applicability in clinical settings.

## Conclusion

The results of this study indicate that the synthesized compounds form five-coordinate complexes. Antibacterial activity tests revealed that these metal complexes demonstrated enhanced effectiveness against cephalosporin-resistant bacteria compared to the cefuroxime ligand alone. In summary, the mechanochemically synthesized copper (II) and silver (I) complexes with cefuroxime demonstrated a synergistic antimicrobial effect. This research highlights the significance of exploring alternative therapeutic strategies to address antibiotic resistance and emphasizes the potential of combination therapies in overcoming bacterial resistance mechanisms.

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**Conflict of Interest:** The author declares no conflicts of interest.

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