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Research paper

Copper (II) complexes with some antibiotics: synthesis, FT-IR study and in vitro antibacterial activity

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Abstract

Copper (II) complexes with commercial antibiotics, amoxicillin (AMX), azithromycin (AZT) and ciprofloxacin (CFL) were synthesized and isolated as solids. Structures of the isolated products were determined by FTIR spectroscopy. Antibacterial activities were determined on reference bacterial strains from the ATCC collection by diffusion technique. The results show that AMX and CFL coordinate Cu (II) ion as bidentate O-donor ligand. AZT coordinates metal center as bidentate NO-donor ligand. A difference in the morphology of antibiotic crystals and the synthesized complexes was found. Complex of Cu (AMX)2 show complete absence of antibacterial activity, while the other com-plexes show the same or even lower activity than the parent ligands.

Keywords: *Amoxicillin; Antimicrobial Activity; Azithromycin; Ciprofloxacin; Cooper Complexes.*

1. Introduction

Metal complexes are a promising source as new antibiotics. Some metal complexes are currently in clinical development for the treatment of cancer, malaria and neurodegenerative diseases. However, only little attention has been paid to their application as potential antimicrobial compounds. The group of authors have analyzed 906 metal-containing compounds for their antibacterial and antifungal activity and found an impressive success rate when compared to purely organic compounds (Frei et al. 2020). The variety of structures found in this analysis highlights the many possibilities that metal complexes can bring to drug discovery. The investigation of the interaction of biometals with O, N and/or S donor ligands used as drugs is interesting for several important reasons: for the purpose of achieving a uniform dose and biological distribution of the drug, monitoring of its pharmacokinetics and excretion, better antimicrobial, antitumor and antiulcer activities and reducing side effects of the drug (Cipurkovic et al. 2017). Amoxicillin (AMX) is a broad-spectrum semi-synthetic beta-lactam antibiotic with proven effective against a wide range of infections. It has a stronger effect against Gram-positive than against Gram-negative microorganisms and is more effective than penicillin. The mechanism of AMX activity is based on the inhibition of mucopeptide biosynthesis, which is important for the resistance and stability of the microbial cell wall during bacterial propagation (Kaur et al. 2011, Fernandez-Torres et al. 2010, Amin et al. 1994).

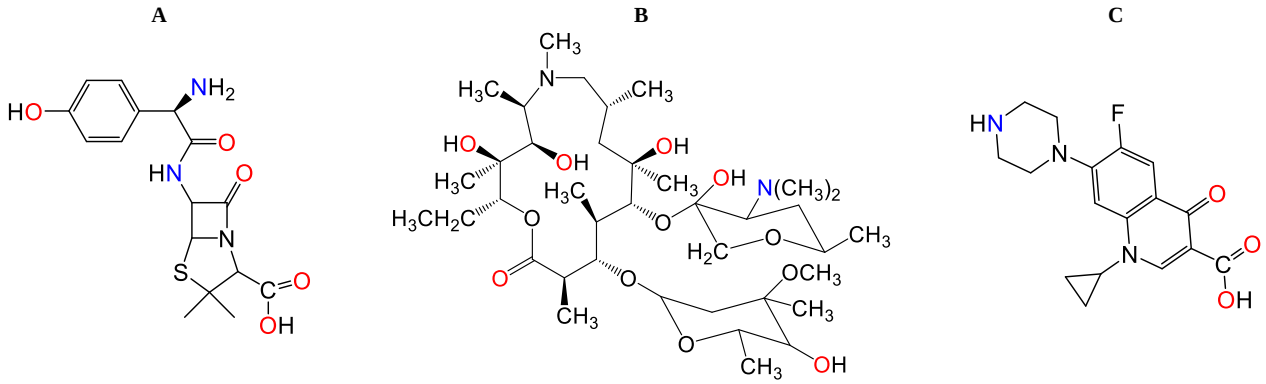

Fig. 1: Structure of Antibiotics Used As Ligands: (A) AMX; (B) AZT; (C) CFL.

Azithromycin (AZT) is a macrolide antibiotic structurally modified from erythromycin with an expanded spectrum of activity and improved pharmacokinetic properties (Dunn et al. 1996, Penezić et al. 2015). It differs from classical macrolides, showing a longer elimination half-life (t1/2) and better tissue distribution (Foulds et al. 1990). Studies have shown that azithromycin has high clinical efficacy and low side effects in the treatment of children with respiratory tract infections (Kolumbić-Lakoš et al. 2011). Ciprofloxacin (CFL) is a antimicrobial

fluoroquinolone commercially available as the monohydrate phase of its hydrochloride. The mechanism of CFL action is based on the inhibition of the activity of enzymes of DNA gyrase (topoisomerase II) and topoisomerase IV which are required for bacterial DNA replication, transcription, repair, strand supercoiling repair, and recombination (Horozić et al. 2018). Structures of these antibiotics are showed on Fig. 1.

2. Materials and methods

Most chemicals were obtained from commercial sources and used without further purification. A Nicolet iS10 FITR spectrophotometer was used to record FTIR spectra. Leica DM 2500P binocular microscope was used to investigate the morphology of ligands and Cu(II) complexes crystals.

2.1. Synthesis of complexes

Synthesis of Cu(II) complexes with AMOX and CFL: Ethanol-water mixture of volume ratio 50/50 (v/v) was used as the solvent for $CuSO_4$ x $5H_2O$ and antibiotics. The antibiotics were dissolved in hot solvent and then mixed with the metal in a M : L = 1: 2 molar ratio. Cu(II) and ligand solutions were mixed in a beaker and stirred on a magnetic stirrer without heating, adjusting the pH value to 5.6 with 1,0 mol/L solution of sodium hydroxide. After adjusting the pH values, the prepared solutions were stirred for half an hour on a magnetic stirrer and then put off in the dark area for ten days in order to precipitate the complexes. The products were filtered, dried at room temperature and stored in a desiccator (Cipurković et al. 2019, Horozić et al. 2019).
Synthesis of Cu(II) complex with AZT: The mass of 1.1984 g of AZT were dissolved in 40 mL of hot aqueous-ethanol solvent (v/v 20:20) and then the AZT solution was mixed with 40 mL of aqueous-ethanol $CuSO_4$ x $5H_2O$ solution of 0.02 mol/L concentration. The obtained solution was stirred for 3 hours, with the pH value adjusted to 5.6 with 1,0 mol/L sodium hydroxide and slightly warmed. After stirring, the solution was left in the dark area for 10 days to allow precipitation of the complex, followed by filtration and drying of the solid product.

2.2. Spectral and morphological characterization

The ATR technique was used to recording FTIR spectra. Samples were recorded in the range of 650-4000 cm^{-1} wavenumbers. The synthesized Cu(II) complexes and parent ligands were microscopically analysed to compare the color, texture and size of the crystals. The preparation was made by treating a solid sample with a few drops of dimethyl sulfoxide. Samples were taken after several hours.

2.3. In vitro antibacterial activity assay

Antibacterial activity was tested by agar-well diffusion method on reference bacterial strains from ATCC collection (E. coli ATCC 8739, E. faecalis ATCC 19433, S. aureus ATCC 25923 and S. enterica ATCC 13076). From the overnight cultures of microorganisms strains, suspensions of 0.5 McFarland turbidity were prepared (density 10^7-10^8 CFU/mL). The strains were then placed on the surface of the nutrient substrate-Mueller-Hinton agar, dispersed in sterile Petri dishes. Substrate thickness was 4 mm. Sterile drill-shaped holes were made ("wells") in the agar, and then 50 µL of complex solutions were added in it. Concentrations of AMX, CFL and their complexes were 1 mg/mL while concentrations of AZT and Cu $(AZT)_2$ complex were 7.6 mg/mL. After the plates were left at room temperature for 15 minutes, the substance was diffused into agar, plates were incubated at 37°C/24 h.

3. Results and discussion

The yield of the reaction of AMX and Cu (II) was 46.7%, while the yields of the reactions of AZT and CFL with copper were slightly higher, amounting to 65.6 and 69.2%, respectively. The synthesized complexes are insoluble in ethanol, methanol and water, and poorly soluble in dimethyl formamide. The solubility is good in dimethyl sulfoxide.

3.1. Structure of complexes

Based on the FTIR spectra of AMX and it´s Cu(II) complex, it is concluded that AMX coordinates the Cu(II) ion as a bidentate O-donor ligand. The oxygen atom of the O-H group (from -COOH) and the oxygen atom of the carbonyl group are involved in the formation of the bond, whereby a complex of square-planar geometry is formed. The reaction scheme and proposed structure of the Cu(II) complex with AMX are shown in Figure 2.

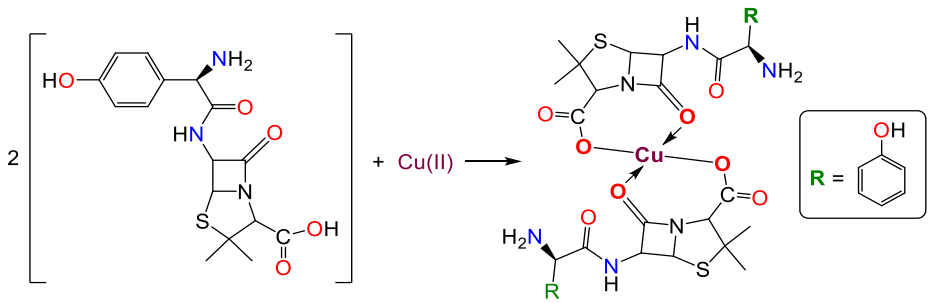

Fig. 2: Reaction Scheme and Proposed Structure of the Complex with AMX.

Figure 3. shows the reaction scheme and proposed structure of the $Cu(AZT)_2$ complex. The assumption is that AZT coordinates the metal ion as a bidentate N, O donor ligand. An oxygen atom of the hydroxyl group and a tertiary amine nitrogen atom are involved in forming the bond with the metal center. Considering that the metal center is also surrounded by two water molecules (conclusion based on the FTIR

spectra of the synthesized product), it is concluded that an octahedral geometry complex is formed. Compared to the white color of parent ligand, the copper complex is extremely blue, which originates from the presence of Cu(II) ions.
The reaction scheme and proposed structure of Cu(II) complex with CFL are shown in Figure 4. CFL coordinates copper(II) ion as a bidentate O-donor ligand. As in the case of the AMX complex, the oxygen atom of the O-H group (from -COOH) and the oxygen atom of the carbonyl group are involved in the reaction of the metal with CFL.

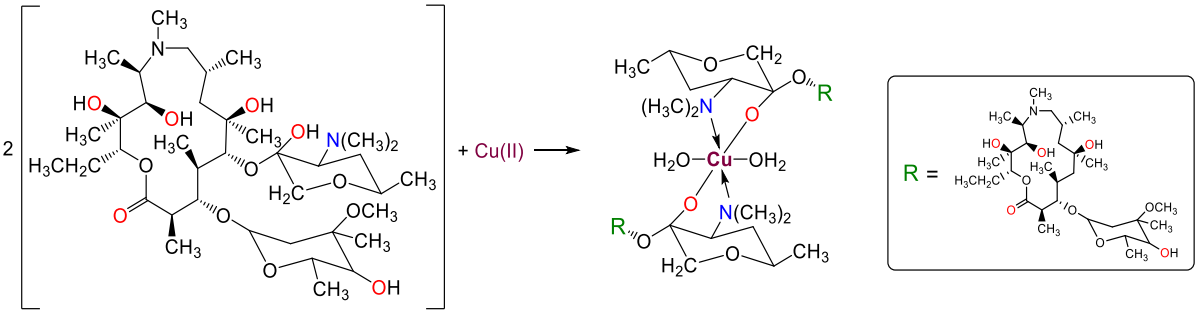

Fig. 3: Reaction Scheme and Proposed Structure of the Complex with AZT.

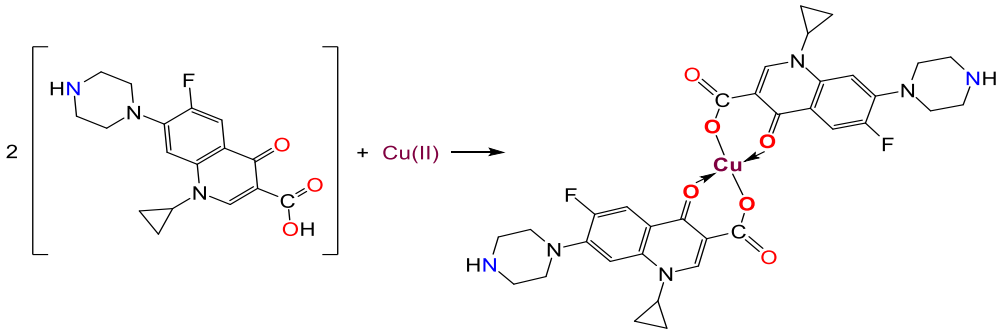

Fig. 4: Reaction Scheme and Proposed Structure of the Complex with CFL.

3.2. Spectral characterization

Figure 5 shows FTIR spectra of AMX and it´s synthesized Cu(II) complex. On the FTIR spectra of AMX, intense bands at 1772 and 1684 cm^{-1} are observed, which originate from the absorption of carbonyl groups present in the AMX structure. In the range of 3136-3522 cm^{-1}, broad bands of lower intensity are observed, belonging to the O-H and N-H groups. The vibrations of C-S and C-N bonds recorded in the FTIR spectra of AMX are also found in the spectra of complex in the very close range, at 695 and 1248 cm^{-1}. When comparing the FTIR spectra of Cu(II) complex with the spectra of the parent ligand, deviations in the position and intensity of the bands are observed. The most noticeable absence of bands on the spectra of the complex is observed at 1772 and 1684 cm^{-1}, indicating the interactions of Cu(II) ions with AMX via the O-donor atom of the C=O group. The differences in the spectra are also noticeable in the area characteristic of O-H bond elongation. The absence of the band characteristic of the O-H group was recorded in the complex spectra.

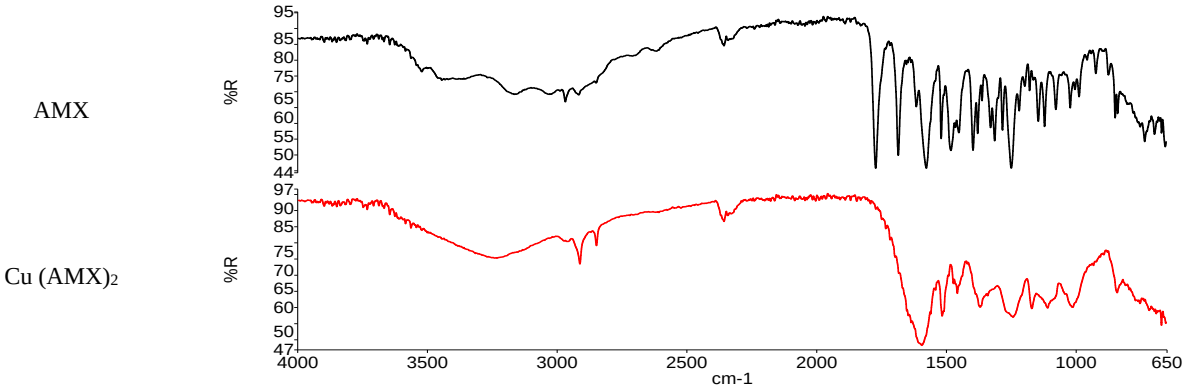

Fig. 5: FTIR Spectra of AMX and $Cu(AMX)_2$.

Two bands of lower intensity in the region of 3557 and 3484 cm^{-1} vibrations of the O-H group are visible on the FTIR spectra of AZT (Fig. 6). The intense band corresponding to C=O stretching vibrations was recorded at 1719 cm^{-1}. The band characteristic of this tertiary amine is observed at 1377 cm^{-1}. Compared to the AZT spectra, the FTIR spectra of Cu (II) complex is characterized by three bands of high intensity in the region characteristic of the vibrations of the O-H group (bands at 3516, 3434, and 3330 cm^{-1}). The band characteristic of the carbonyl group in the spectra of the complex occurs at 1585 cm^{-1}.
Figure 7 shows the FTIR spectra of CFL and $Cu(CFL)_2$. The wide peak which corresponds to the N-H bond vibrations was detected at 3376 cm^{-1}. Also, the wide peak of the O-H group at 3529 cm^{-1} is present in the ligand spectra. The strong peaks at 1622 and 1443 cm^{-1} confirm the presence of double C=C bond. Vibrations of the C-F bond cause the appearance of a strong, sharp peak at 1023 cm^{-1} and the weak peak between 3085-3013 cm^{-1} is most likely due to the vibration of the Ar-H bond. Low or medium peaks observed at 1106 and 1143

cm^{-1} were caused by stretching of the C-N bond. Due to the appearance of a strong intensity peak at 1268 cm^{-1}, the presence of the C-O group was confirmed.

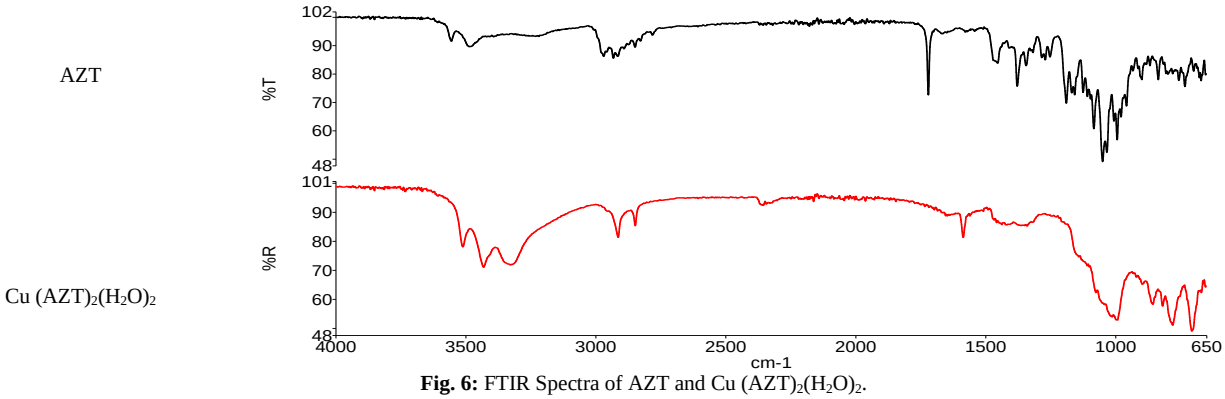

Fig. 6: FTIR Spectra of AZT and Cu $(AZT)_2(H_2O)_2$.

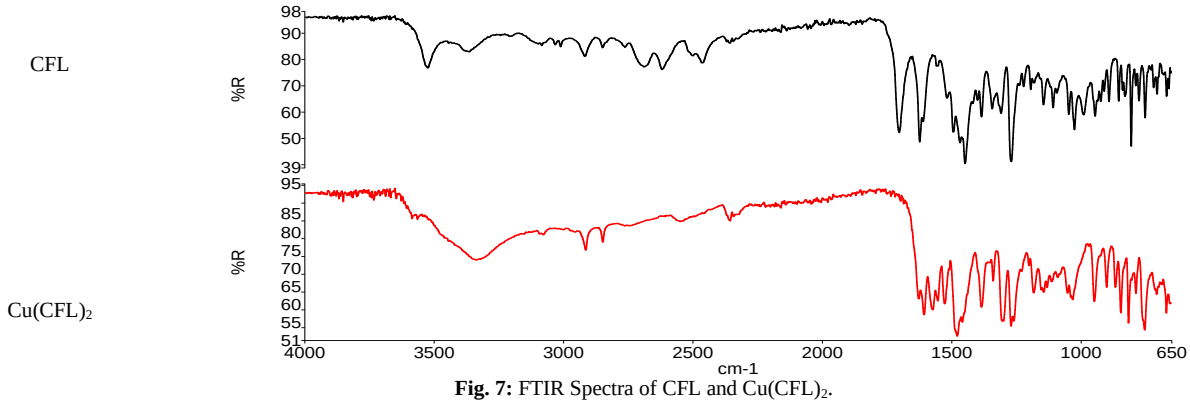

Fig. 7: FTIR Spectra of CFL and $Cu(CFL)_2$.

3.3. Crystal morphology

The AMOX crystals are characterized by an oval-shaped structure with a diameter of 0.7-0.9 mm. For Cu $(AMOX)_2$ complex, crystals of polygonal forms, interference colours of the first order. Crystal size ranges from 0.4-0.6 mm.

There are three crystalline forms in azithromycin: dendritic, radially radiant, and spherical. Based on the size, all AZT crystals belong to small crystals (below 1 mm). The interference colours are vibrant and of the first order. The crystals of Cu $(AZT)_2(H_2O)_2$ complex also belong to small crystals. The interference colours are poorly expressed so that the crystals are generally transparent. Crystal morphology indicates prismatic forms.

Regarding the size of the crystals of the CFL, they are divided into small crystals (below 1 mm) and medium crystals (1-10 mm). Interferential colours of CFL crystals are vibrant and of the first order. These crystals are characterized with radially shape originated from the same centre and prismatic forms. Crystals of Cu $(CFL)_2$ are small. Interferential colours are poorly expressed and crystals are mostly transparent. The morphology of crystals points to prismatic forms.

3.4. In vitro antibacterial activity

For scientific researchers working in the field of bioinorganic chemistry, the copper represents one of the most interesting biometals for the preparation of new metal-based drugs with strong potential for therapeutic applications (Duncan and White 2012, Čongradyová et al. 2014). In the last years, many scientific publications have reported antimicrobial properties of copper complexes (Sovari and Zobi, 2020). The results of our antimicrobial activity of antibiotics and synthesized Cu (II) complexes are presented in Table 1. The obtained results show that the Cu $(AMX)_2$ complex has no antibacterial activity in the case of the tested strains. It seems to be caused by a newly formed bond with the metal ions, which causes the ligand to lose its chemical identity and thus the ability to inhibit the synthesis of biomolecules responsible for the stability of the bacterial cell wall. By comparing the parent ligand inhibition zones of the strains tested, it was found that AMX had the highest activity against Enterococcus faecalis (inhibition zone is 33 mm), while the lowest activity was recorded in Staphylococcus aureus (19 mm). The results of Imran et al. (Imran et al. 2006), who conducted similar study, showed a higher antimicrobial activity of the $Cu(AMX)_2$ complex in the case of E. coli and S. aureus. The inhibition zones of the Cu(II) complex are larger than the inhibition zones of the parent antibiotic for E. coli and S. aureus.

AZT, as a broad-spectrum antibiotic, exhibits pronounced antimicrobial activity on both Gram positive and Gram negative bacteria. The results of the in vitro analysis of the antimicrobial activity of this antibiotic confirmed its pronounced antimicrobial effect, and the bacterial strains tested showed maximum sensitivity with a very pronounced inhibitory zone exceeding 30 mm. The copper complex with AZT has a noticeably weaker antimicrobial effect on the strains tested then a AZT as ligand. Mechanism of action of the tested antibiotic is such that, like other antibiotics belonging to the macrolide group, it binds to the 50S component of the 70S subunit of the bacterial ribosome, and thus inhibits protein synthesis in bacteria. It is assumed that forming a complex with copper, binding affinity with the ribosomal unit is reduced, which ultimately results in a reduced antimicrobial effect. Đokic et al. 1995. reported that divalent metal complexes with AZT, including the Cu(II) complex, have the same biological activity as parent antibiotic which is not in accordance of our results (Saeed Arayne et al. 2014). published the results of their study of AZT complexes with many metals. In this paper, synthesized $Cu(AZT)_2(H_2O)_2$ complex showed decrease antibacterial activity against E. coli and inactivity against S. aureus.

As already mentioned, the mechanism of CFL action is based on the inhibition of DNA enzyme activity, topoisomerase II) and topoisomerase IV, which are necessary for DNA replication. Before binding to these enzymes, CFL is chelated with metal ions (most commonly Mg^{2+}). Only after the chelation, CFL can bind to any of these two enzymes and block DNA chains synthesis. Results of antimicrobial in vitro screening test show that the tested $Cu(CFL)_2$ complex have almost the same antimicrobial activity as the antibiotic itself. It is very important that formation of copper and iron complexes does not reduce antimicrobial effect of CFL, since the forming of complexes with some of biometals is an indispensable process of antibiotic.

Table 1: Antimicrobial Activities of Antibiotics and It´S Cu (II) Complexes

Microorganism	Inhibition zone of antibiotics/Cu(II) complexes [mm]					
	$Cu(AMOX)_2$	AMOX	$Cu(AZT)_2(H_2O)_2$	AZT	$Cu(CFL)_2$	CFL
E. coli	-	28	25	35	40	40
S. aureus	-	19	24	37	35	37
S. enterica	-	26	22	38	39	39
E. faecalis	-	33	NA	NA	26	27

* NA - Not analyzed.

4. Conclusion

The interaction of AMX and CFL with Cu(II) ions forms a complexes of square-planar geometry. Complexation of AMX results in a product that has no antibacterial effect on the tested bacterial strains. In the case of $Cu(CFL)_2$ complex, no significant deviations were observed in antibacterial activity relative to the CFL. It is assumed that the complex with AZT has an octahedral geometry whereby the oxygen atom of the hydroxyl group and the nitrogen atom of the tertiary amino group of AZT are involved in forming the bond with metal ion. Less antibacterial activity was observed for $Cu(AZT)_2(H_2O)_2$ complex than for parent antibiotic.

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