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Antibacterial and antibiofilm effect of copper complexes associated with imine ligands on Campylobacter strains in planktonic and sessile forms

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Campylobacter spp. is one of the main agents involved in human foodborne gastroenteritis worldwide. There is extensive concern about resistance to the drugs of choice, macrolides and fluoroquinolones. In view of this, new copper-based compounds have emerged as a strategy for this control due to their bactericidal effect. This study aimed to synthesize new copper(II) complexes associated with imine ligands and evaluate their effect against strains of *C. jejuni* (CJ) and *C. coli* (CC) in planktonic and sessile forms. The Clmp and memp ligands were obtained and combined with the copper(II) ion in methanol to form two complexes: [Cu(Clmp)(mftpy)](PF6)2 CL1 and [Cu(memp)(mftpy)](PF6)2 CL2. The complexes were characterized by proton NMR, UV-Vis, FTIR, EPR and high-resolution mass spectrometry. The antibacterial activity was evaluated on six strains of CJ and CC in planktonic and sessile form, four of which were isolated from chicken carcasses from the Ministry of Agriculture and Livestock (MAPA) and two from standard culture (ATCC). The minimum inhibitory concentration (MIC) of both compounds was determined by microdilution, at concentrations between 3.125 and 400 µg/ml. Antibiofilm activity was assessed for both compounds at a concentration of 800 µg/ml qualitatively and quantitatively, using the crystal violet and plate count methods, respectively, on 2 wild CJ and 2 CC. In these tests, 5% chicken juice was used to mimic industrial conditions. The biofilm tests were compared with a commercial sanitizer (peracetic acid - APA, 800 µg/ml). Scanning electron microscopy (SEM) image analyses were carried out on glass beads to evaluate the 3D structure of the treated and control biofilms. The concentrations of copper absorbed by the sessile forms were obtained by atomic absorption spectrometry. All data was subjected to statistical analysis. Both complexes were effective in reducing the viability of free *Campylobacter* strains, but the ATCC strains were more susceptible. We observed that CL1 and CL2 promoted a reduction of 3.19 and 3.68 log CFU of bacteria compared to the control, respectively. Compared to the control group, CL2 showed a significant reduction in biomass intensity, equivalent to 1.24 and 12.5, for CJ and CC, respectively. The reduction in sessile cell count was significant in both CL1 and CL2. However, CL1 proved to be more effective in controlling sessile CJ, with a reduction of 3.54 log. The greater activity of CL1 in sessile CJ is justified by the 2.18 times greater absorption of copper compared to CC and the 2.54 times greater absorption of copper compared to CL2. In the presence of APA, the biofilm showed a three-dimensional structure with an evident matrix, more expanded and spongy than in the control group. CL1 demonstrated an ability to compact the biofilm\'s extracellular matrix, but less densely than that observed

in APA, while CL2 promoted more intense fragmentation and destruction, resulting in a more unstable structure. The logarithmic reduction confirms that both complexes are biologically active and trigger processes that allow the control of pathogens, therefore classified as promising against the planktonic and sessile forms of CJ and CC.

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