Activity of Copper (Cu(TTA)(phen)NO3) and Nickel (NiBTA) Inorganic Compounds in Caco-2 Cells and Control of Campylobacter jejuni

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Campylobacter jejuni is a leading cause of foodborne gastroenteritis and poses a significant public health challenge, often associated with the consumption of raw or undercooked chicken. Excessive use of antimicrobials in animal production increases the resistance of *Campylobacter*, particularly to the drugs used for treatment. *C. jejuni* also forms biofilms in industrial settings, complicating sanitary control. The search for new antimicrobials, such as metal complexes, represents an innovative strategy to address these challenges.The study evaluated the effectiveness of nickel (NiBTA) and copper (Cu(TTA)(phen)NO3) complexes against *C. jejuni* resistant to macrolides and fluoroquinolones, both in planktonic forms and biofilms. Cytotoxicity of the compounds was also assessed to ensure their safety and potential for future in vivo testing. Five *C. jejuni* strains from the Molecular Epidemiology Laboratory at the Federal University of Uberlândia, collected between October 2017 and July 2018 from chicken carcasses at federally inspected slaughterhouses provided by LANAGRO-RS, were used. These strains were resistant to erythromycin and ciprofloxacin. After reactivation in broth and selective agar (Bolton and CCDA, respectively), confirmed colonies were tested for susceptibility to nickel and copper metal compounds. Minimum Bactericidal Concentration (MBC) was determined by microdilution in adjusted Mueller-Hinton broth, with concentrations ranging from 0.78 to 100 µg/mL. Following 48 hours of incubation at 37°C under microaerophilic conditions, MBC was analyzed on CCDA agar. For biofilm tests, strains were cultured in broth with 5% chicken juice, and compounds were tested at concentrations of 3.12 to 400 µg/mL for 2 hours, with peracetic acid (300 to 4000 µg/mL) used as a comparison. Cell viability was measured with resazurin in Caco-2 cells. All tests were performed in triplicate, and data were statistically analyzed using GraphPad Prism. Copper showed significant bactericidal activity, inhibiting 1/5 strains at 1.56 µg/mL, 3/5 at 6.25 µg/mL, and 1/5 at 12.5 µg/mL. In biofilms, it was effective at 50 µg/mL (3/5) and 100 µg/mL (2/5 strains). Nickel exhibited lower effectiveness in planktonic forms, inhibiting 2/5 strains at 25 µg/mL and 1/5 at 12.5 µg/mL. In biofilms, it was effective at 200 µg/mL (3/5) and 400 µg/mL (2/5). Cell viability tests showed no significant statistical differences compared to controls. In cell culture, copper maintained 72.67% viability at 12.5 µg/mL after 24 hours and 100% at the lowest concentration. After 48 hours, viability was 25% at 6.25 µg/mL and 84% at 0.78 µg/mL. Nickel maintained 74.85% viability at 100 µg/mL after 24 hours and 67.35% after 48 hours. Results showed that copper was more effective in eliminating the microorganism, with an average of 6.56 µg/mL in planktonic form and 70 µg/mL in sessile form, compared to nickel, which had 14.06 µg/mL and 280 µg/mL, respectively. Copper, at lower concentrations,

demonstrated better performance in maintaining Caco-2 cell integrity. Both copper and nickel outperformed peracetic acid (2000 µg/mL) in antibiofilm activity. Thus, copper and nickel exhibit strong antimicrobial and antibiofilm properties against *C. jejuni* without significant alterations in Caco-2 cells, making them promising options for bacterial and biofilm control in industrial settings and potential treatment pathways.

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