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EFFECT OF ANTIBIOTIC-LOADED MONTMORILLONITE NANOCLAY IN DEGRADING SELECTED BACTERIAL BIOFILMS

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Bacterial biofilms pose significant challenges in healthcare and industry due to enhanced resistance to antibiotics, which arise from their protective extracellular matrix and cellular structure, leading to persistent infections and development of antimicrobial resistance. This study explored the efficacy of ciprofloxacin (CIP) and tetracycline (TC) loaded montmorillonite (MMT) nanoclay in combating biofilm-forming bacteria. Antibiotic-loaded MMT nanocomposites were synthesised at three ratios of antibiotics. The synthesised composites were characterised using Fourier transformed infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). Drug loading and entrapment efficiency analysis revealed that 1:2 ratio exhibited the highest loading efficiency (CIP: 83.5±0.9%; TC: 55.3±0.8%) for both antibiotics, while the 1:1 ratio achieved the best entrapment efficiency (CIP: 55.2±1.7%; TC: 46.0±1.4%) ($p < 0.05$). Release studies showed both composites exhibit slow and controlled release at pH 7.4. Antibacterial activity was assessed using agar well diffusion method, where MMT-CIP (1:2) showed significantly larger inhibition zones (*E. coli*: 21.3±1.2 mm; *S. aureus*: 19.8±0.9 mm; *P. aeruginosa*: 18.6±1.1 mm) compared to free CIP ($p < 0.05$). Biofilm formation was quantified by microtiter plate crystal violet staining, and anti-biofilm efficacy was evaluated by determining the minimum biofilm inhibitory concentration (MBIC) and minimum biofilm eradication concentration (MBEC). MMT-CIP composites inhibited biofilm formation at 16 µg mL^{-1} (MBIC for *E. coli* and *S. aureus*) and eradicated established biofilms at 32 µg mL^{-1} (MBEC), values significantly lower than free CIP (MBIC 64 µg mL^{-1}; MBEC 128 µg mL^{-1}) ($p < 0.05$). While, MMT-TC composites displayed moderate efficacy, with MBIC values of 64 – 128 µg mL^{-1} and MBEC value of 256 µg mL^{-1}, and particularly less effective against methicillin-resistant *S. aureus* and clinical isolates of *P. aeruginosa*. These findings highlight the potential of MMT as a carrier for antibiotics, offering enhanced efficacy, controlled release, and reduced resistance development.

Keywords: Antibiotic resistance, Biofilm, Ciprofloxacin, Montmorillonite, Tetracycline