

# Fighting the Emerging Antibiotic Resistance in Wastewater

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The development and spread of antibiotic resistance in wastewater poses a significant threat to both the environment and human health. Biofilm is a key factor that promotes antibiotic resistance in bacteria by increasing their tolerance to antibiotics and facilitating the transfer of antibiotic resistance genes. This project aims to isolate and identify antibiotic-resistant bacteria from a wastewater treatment plant (WWTP) and discover natural molecules that can inhibit biofilm formation. The hypotheses are (1) wastewater bacteria can develop multiple antibiotic resistances through biofilm formation, and (2) specific natural compounds can hinder bacterial biofilm formation, thus reducing the spread of antibiotic resistance among wastewater microbes. To test both hypotheses, LB agar plates containing different antibiotics were used to isolate antibiotic-resistant bacteria from the Logan Regional WWTP effluent. Two streptomycin-resistant (S2-1 and S2-2), four carbenicillin-resistant (Cb2-1 to Cb2-4) and four kanamycin-resistant (K2-1 to K2-4) bacteria were isolated. Notably, five strains (S2-1, Cb2-2, Cb2-4, K2-2 and K2-4) are double-antibiotic-resistant and one (Cb2-3) is resistant to three antibiotics. These strains were identified using 16S rRNA sequencing. Next, eleven natural compounds were screened for the ability to inhibit biofilm formation. It was found that curcumin, quercetin, and resveratrol effectively inhibited biofilm formation in *Pseudomonas aeruginosa* PAO1 and two biofilm-forming double/triple-antibiotic-resistant strains Cb2-2 and Cb2-3 at a concentration of 26.67 µg/mL. These findings show that natural products could be an effective tool in reducing the spread of antibiotic resistance and preventing the development of superbugs in wastewater.