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*Abstract*

# Antibiofilm Activity of Ciprofloxacin and Sulfadiazine Combination Against *Escherichia coli* Biofilms: A Scanning Electron Microscopy Analysis †

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**Abstract:** Bacteria within biofilms display remarkable antibiotic resistance compared to planktonic bacteria. Given their implications in infectious diseases and multidrug resistance, exploring effective antimicrobial strategies to regulate biofilm formation is urgent. Eradicating bacteria within biofilms is challenging, needing combination therapy to combat persistent biofilm-related infections. In previous studies, we demonstrated the synergistic and partially synergistic effects of Ciprofloxacin (CIP) combined with antibacterial sulfonamides (SA) against *Escherichia coli* reference and clinical strains with intermediate quinolone resistance (*E. coli* IRQ). Notably, the CIP+ sulfadiazine (SDZ) combination exhibited superior efficacy. In this study, we assessed the antibiofilm activity of CIP and SDZ combinations on mature biofilms formed on glass discs using scanning electron microscopy (SEM), focusing on the *E. coli* IRQ strain. Mature biofilms (48-hour growth) were treated with individual antibiotics (CIP and SDZ) and their combinations (CIP+SDZ), considering minimal fractional inhibitory concentrations (FIC) from previous studies. The experiment was performed in triplicate. Treatments included: CIP (FICx100) + SDZ (FICx10), CIP (FICx100), and SDZ (FICx10). SEM micrographs highlighted an enhanced antibiofilm effect of CIP+SDZ combinations compared to individual drugs. Specifically, CIP (FICx100) + SDZ (FICx10) significantly reduced biofilm formation, caused disorganization, reduced extracellular matrix, and induced bacterial cell destruction, outperforming untreated and individually treated biofilms. These findings provide insights into the partially synergistic effect of this combination on *E. coli* IRQ, attributed to cooperative actions targeting diverse stages of DNA synthesis. The study underscores CIP+SDZ as a promising combination for treating biofilm-related infections.

**Keywords:** Ciprofloxacin; Sulfadiazine; Synergism; Biofilm; SEM

**Supplementary Materials:**

**Author Contributions:** Conceptualization, R.A.G.; M.C.B.; G.P.V.; methodology, R.A.G.; validation, R.A.G.; M.C.B.; G.P.V.; formal analysis, R.A.G.; G.P.V.; investigation, R.A.G.; resources, G.P.V.; writing—original draft preparation, R.A.G.; writing—review and editing, R.A.G.; G.P.V.; visualization,

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