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Exploiting Lactoferricin (17–30) as a Potential Antimicrobial and Antibiofilm Candidate Against Multi-Drug-Resistant Enteroaggregative *Escherichia coli*

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The study evaluated the *in vitro* antimicrobial and antibiofilm efficacy of an antimicrobial peptide (AMP), lactoferricin (17–30) [Lfcin (17–30)], against biofilm-forming multi-drug-resistant (MDR) strains of enteroaggregative *Escherichia coli* (EAEC), and subsequently, the *in vivo* antimicrobial efficacy was assessed in a *Galleria mellonella* larval model. Initially, minimum inhibitory concentration (MIC; 32 μM), minimum bactericidal concentration (MBC; 32 μM), and minimum biofilm eradication concentration (MBEC; 32 μM) of Lfcin (17–30) were determined against MDR-EAEC field isolates (n = 3). Lfcin (17–30) was tested stable against high-end temperatures (70 and 90°C), physiological concentration of cationic salts (150 mM NaCl and 2 mM $MgCl_2$), and proteases (proteinase-K and lysozyme). Further, at lower MIC, Lfcin (17–30) proved to be safe for sheep RBCs, secondary cell lines (HEp-2 and RAW 264.7), and beneficial gut lactobacilli. In the *in vitro* time-kill assay, Lfcin (17–30) inhibited the MDR-EAEC strains 3 h post-incubation, and the antibacterial effect was due to membrane permeation of Lfcin (17–30) in the inner and outer membranes of MDR-EAEC. Furthermore, in the *in vivo* experiments, *G. mellonella* larvae treated with Lfcin (17–30) exhibited an increased survival rate, lower MDR-EAEC counts ($P < 0.001$), mild to moderate histopathological changes, and enhanced immunomodulatory effect and were safe to larval cells when compared with infection control. Besides, Lfcin (17–30) proved to be an effective antibiofilm agent, as it inhibited and eradicated the preformed biofilm formed by MDR-EAEC strains in a significant ($P < 0.05$) manner both by microtiter plate assay and live/dead bacterial quantification-based confocal microscopy. We recommend further investigation of Lfcin (17–30) in an appropriate animal model before its application in target host against MDR-EAEC strains.

Keywords: antimicrobial peptide, biofilm, confocal microscopy, enteroaggregative *E. coli*, *Galleria mellonella*, lactoferricin (17–30)