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Efficacy of Indolicidin, Cecropin A (1-7)-Melittin (CAMA) and Their Combination Against Biofilm-Forming Multidrug-Resistant Enteroaggregative Escherichia coli

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The present study examined the anti-biofilm efficacy of two short-chain antimicrobial peptides (AMPs), namely, indolicidin and cecropin A (1-7)-melittin (CAMA) against biofilm-forming multidrug-resistant enteroaggregative Escherichia coli (MDR-EAEC) isolates. The typical EAEC isolates re-validated by PCR and confirmed using HEp-2 cell adherence assay was subjected to antibiotic susceptibility testing to confirm its MDR status. The biofilm-forming ability of MDR-EAEC isolates was assessed by Congo red binding, microtitre plate assays and hydrophobicity index; broth microdilution technique was employed to determine minimum inhibitory concentrations (MICs) and minimum biofilm eradication concentrations (MBECs). The obtained MIC and MBEC values for both AMPs were evaluated alone and in combination against MDR-EAEC biofilms using crystal violet (CV) staining and confocal microscopy-based live/dead cell quantification methods. All the three MDR-EAEC strains revealed weak to strong biofilm-forming ability and were found to be electron-donating and weakly electron-accepting (hydrophobicity index). Also, highly significant ($P < 0.001$) time-dependent hydrodynamic growth of the three MDR-EAEC strains was observed at 48 h of incubation in Dulbecco's modified Eagle's medium (DMEM) containing 0.45% D-glucose. AMPs and their combination were able to inhibit the initial biofilm formation at 24 h and 48 h as evidenced by CV staining and confocal quantification. Further, the application of AMPs (individually and combination) against the preformed MDR-EAEC biofilms resulted in highly significant eradication ($P < 0.001$) at 24 h post treatment. However, significant differences were not observed between AMP treatments (individually or in combination). The AMPs seem to be an effective candidates for further investigations such as safety, stability and appropriate biofilm-forming MDR-EAEC animal models. Collapse