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Antimicrobial efficacy of Cecropin A (1–7)- Melittin and Lactoferricin (17–30) against multi-drug resistant *Salmonella* Enteritidis

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ABSTRACT

The present study evaluated intracellular antibacterial efficacy of two short-chain cationic antimicrobial peptides (AMPs) namely, Cecropin A (1–7)-Melittin and lactoferricin (17–30) against three field strains of multi-drug resistant *Salmonella* Enteritidis. Initially, antimicrobial ability of both the AMPs was evaluated for their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against multi-drug resistant *S.* Enteritidis strains. Besides, the AMPs were evaluated for its *in vitro* stability (high-end temperatures, proteases, physiological concentrations of cationic salts and pH) and safety (haemolytic assay in sheep erythrocytes; cytotoxicity assay in murine macrophage RAW 264.7 cell line and human epithelioma HEp-2 cell line and beneficial gut lactobacilli). Later, a time-kill assay was performed to assess the intracellular antibacterial activity of Cecropin A (1–7)-Melittin and lactoferricin (17–30) against multi-drug resistant *S.* Enteritidis in RAW 264.7 and HEp-2 cells. The observed MBC values of Cecropin A (1–7)-Melittin and lactoferricin (17–30) against multi-drug resistant *S.* Enteritidis (128 μM; 256 μM) were generally twice or four-fold greater than the MIC values (64 μM). Further, both the AMPs were found variably stable after exposure at high-end temperatures (70 °C and 90 °C), protease treatment (trypsin, proteinase K, lysozyme), higher concentration of physiological salts (150 mM NaCl and 2 mM $MgCl_2$) and hydrogen ion concentrations (pH 4.0 to 8.0). Both the AMPs were found non-haemolytic on sheep erythrocytes, revealed minimal cytotoxicity in RAW 264.7 and HEp-2 cells, and was tested safe against beneficial gut lactobacilli (*L. acidophilus* and *L. rhamnosus*). Intracellular bacteriostatic effect with both cationic AMPs against multi-drug resistant *S.* Enteritidis was evident in RAW 264.7 cells; however, in both the cell lines, the significant bactericidal effect was not observed ($P > 0.05$) with both cationic AMPs understudy against multi-drug resistant *S.* Enteritidis. Based on the results of the present study, both the cationic AMPs understudy may not be useful for the intracellular elimination of multi-drug resistant *S.* Enteritidis; hence, further studies such as conjugation of these AMPs with either cell-penetrating peptides (CPP) and/or nanoparticles (NPs) are warranted.

1. Introduction

Salmonellosis has been reported as one of the most common foodborne illnesses across the globe with 550 million cases annually [1–3]. Further, the poultry sector has been projected as one of the most important reservoirs of *Salmonellae*, particularly non-typhoidal *Salmonella* (NTS), that can be transmitted to humans through a wide variety of food sources, thereby enabling the food chain unsafe from farm to fork [4,5]. Globally, *Salmonella enterica* subspecies *enterica* serovar Enteritidis and *Salmonella enterica* subspecies *enterica* serovar Typhimurium are considered to be the major zoonotic NTS serovars [6,7]. In India, the poultry industry is evolving and emerging as the world's second largest market, and often chicken meat has been reported as a potential source of NTS serotypes [7–9].

Salmonellosis in humans is routinely characterized by acute gastroenteritis with symptoms of fever, chills, nausea, vomiting, abdominal

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