

**TITLE:** IN VITRO EVALUATION OF THE BACTERICIDAL ACTIVITY OF LYSOZYME ON BACTERIA OF CLINICAL INTEREST IN VETERINARY MEDICINE

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### **ABSTRACT**

Pathogens commonly known in the clinical field still represent a therapeutic challenge due to the progressive increase of antimicrobial resistance, stimulating the search for natural substances. Lysozyme or 1,4- $\beta$ -N-acetylmuramidase is a natural enzyme, present in fluids such as saliva, tear and urine. It is found in abundance in egg albumin. Lysozyme has application in the food and pharmaceutical industry as an antimicrobial agent because of its anti-inflammatory, immunological, bactericidal and bacteriostatic activities. Gram-positive bacteria are sensitive to the action of the enzyme that causes bacterial wall lysis, as a result of the connection between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan. In gram-negative bacteria its function is limited due to the constitution of the cell wall. New technologies in the industry have increased the spectrum of action of lysozyme, allowing it to also act against Gram negative. The goal of this research was to evaluate the minimum inhibitory concentration (MIC) of lysozyme in pathogens. The strains were analysed at the concentration of 106, and belong to the Culture Collection of the Laboratory of Avian Medicine of FMVZ – USP. A solution of lysozyme on agar LB (Luria Bertani) without salt (pH 4.31) was distributed 96-well plates at the initial concentration of 2000 ppm, followed by serial dilution at the concentrations 1000, 500, 250, 125, 62 and 31 ppm. *Micrococcus luteus* was used as a positive control. *Staphylococcus aureus*, *S. intermedius* and *Enterococcus faecalis* showed a MIC of 31 ppm. For Gram negative bacteria the following cutoff points were observed: *Bordetella avium* 62 ppm, *B. hinzii* 62 ppm, *E. coli* Avian Pathogen - APEC 250 ppm, *E. coli* O157H7 250 ppm, *E. coli* enteroaggregative 250 ppm, *E. albertii* 125 ppm, *E. fergusonii* 62 ppm, *Klebsiella pneumoniae* 500 ppm, *Proteus mirabilis* 62 ppm, *Salmonella* Enteritidis 500 ppm, *S. Typhimurium* 125 ppm, *S. Heidelberg* 31 ppm and *Pseudomonas aeruginosa* > 2000 ppm. In gram negative, MIC values were higher, showing less efficacy. *K. pneumoniae*, *S. Enteritidis* and *P. aeruginosa* presented a higher resistance. The MIC results below  $\leq 250$  suggest that lysozyme may be an alternative for infection control by *B. avium*, *B. hinzii*, APEC, *E. coli* O157H7, EAEC, *E. albertii*, *E. fergusonii*, *P. mirabilis*, *S. Typhimurium* and *S. Heidelberg*. Different formulations of the product can be evaluated for in vivo efficacy determination to control these infections.

**Keywords:** lysozyme, natural enzyme, resistance

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