

TITLE: CHLORHEXIDINE DIGLUCONATE SUBINHIBITORY CONCENTRATIONS CAN INDUCE *IN VITRO* THE PHENOTYPIC EXPRESSION OF ANTIMICROBIALS RESISTANCE IN MICROORGANISMS RELATED TO PULMONARY INFECTIONS FOUND IN THE ORAL CAVITY ICU PATIENTS.

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ABSTRACT

Oral hygiene with chlorhexidine digluconate (CHX) is one of the preventive measures against microorganisms associated with pneumonia present in the oral biofilm of hospitalized patients. However, sub-inhibitory concentrations of CHX may induce *in vitro* the phenotypic expression of antimicrobial resistance. The objective of the study was to evaluate *in vitro* phenotypic changes in the susceptibility of Gram-negative bacteria to antimicrobials through selective pressure by exposure to chlorhexidine digluconate sub-MICs. The Matrix Associated Laser Desorption-Ionization (MALDI-TOF) system was used to identify the prevalent species in nosocomial respiratory infections, *P.aeruginosa*, *K. pneumoniae* and *A.baumannii* isolated from the dental biofilm. Antimicrobial susceptibility profile was determined by the melting disk technique according to the Clinical & Laboratory Standards Institute (CLSI) and minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of chlorhexidine were determined according to the microdilution method on plate. The susceptibility test showed that 33.3% of *K. pneumoniae* and 100% of *P. aeruginosa* were sensitive to all antimicrobials, and that 100% of *A.baumannii* and 66.6% of *P. pneumoniae* were resistant to more than one agent in three or more antimicrobial classes. The MIC for chlorhexidine ranged from 0.0000315% to 0.001% and the CBM from 0.00025% to 0.0040%. One sample of *K.pneumoniae* and two of *P.aeruginosa* were exposed to dilution of chlorhexidine (0,00056 a 1,159 g/L) during ten consecutive days. At every 24 hours, the samples were re-exposed to antiseptic, using visible growth at the highest CHX concentration. Before and after induction, disc diffusion test was used to establish the susceptible profile to several antibiotics. Data analysis of the induction of sub-inhibitory concentrations of CHX revealed that there was a significant increase in MIC of 66.6% of the isolated *P.aeruginosa* and 100% of the *K.pneumoniae* tested between day 1 and 10 ($p < 0.05$). Only 33.3% of the isolated *P.aeruginosa* samples had a statistically significant sensitivity profile variation between day 1 and 10. A reduction of the inhibition halo of all tested strains was observed. The study concluded that rapid colonization of oral biofilm by multiresistant Gram-negative bacteria can occur. Sub-inhibitory concentrations of CHX may induce an increase in phenotypic expression of antimicrobial resistance *in vitro*.

Keywords: Chlorhexidine; pneumonia; oral health; intensive care units; antimicrobials resistance;

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