TITLE: SEMIQUANTITATIVE STUDY OF INFLUENCE OF AMINULEVULINIC ACID ON BIOFILM PRODUCTION BY *Corynebacterium diphtheriae* SAMPLES.

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Corynebacterium diphtheriae strains continue to circulate worldwide causing diphtheria and invasive diseases, such as endocarditis, osteomyelitis, pneumonia and catheter-related infections. Therefore, it is extremely important to do studies focused on virulence factors that may contribute to elucidate the pathogenicity of this species. The relevance of biofilm formation in development of nosocomial infections and effects of antimicrobial agents on these surface-attached communities need further investigation. In the present study, we investigated qualitative and semi quantitative influence of aminolevulinic acid on biofilm production by *Corynebacterium diphtheriae* samples. Biofilm formation on negatively charged polystyrene surfaces was determined in 96-well flat-bottomed microtitre plates according to previously described methods. Aliquots of 200 µL of bacterial suspensions [0.2 optical density (OD) at λ=570 nm] were added to the microplate wells, containing TSB medium with aminolevulinic acid (0.38 mmol/L – Sigma Aldrich). Other microplates wells, without aminolevulinic acid was also prepared. TSB medium without bacteria were used as negative controls. After incubation at 37°C for 24 h, the contents of each well were aspirated and washed three times with 200 µL phosphate-buffered saline (0.01 mol/L, pH 7.2). The remaining attached bacteria stained with 2% crystal violet. The negative controls contained TSB only. The bound dye was then solubilized with 200 µL of 33% glacial acetic acid and the OD of the solution was measured at λ = 570 nm using an enzyme immunosorbent assay reader (BioRad, model 550). The results showed that the addition of aminolevulinic acid caused an increase in biofilm production in 4 of the 5 samples studied. Aminolevulinic acid is a porphyrin precursor. Previous studies, described in the literature, have demonstrated the influence of porphyrins in the production of biofilm. Additional studies are needed to discover the role of porphyrins in this mechanism of virulence.

KEYWORDS: *Corynebacterium diphtheriae*, biofilm, porphyrin, aminolevulinic acid, virulence factors.

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