TITLE: INVESTIGATION OF THE PRESENCE AND EXPRESSION OF SHIGA–LIKE TOXIN ENCODING GENES IN *Corynebacterium diphtheriae* STRAINS

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ABSTRACT:

The clinical manifestations observed in *Corynebacterium diphtheriae* infections, the main etiologic agent of diphtheria, are frequently associated with the action of diphtheria toxin (DT), its main virulence factor. However, non-producing strains of DT have been isolated from invasive infections, suggesting the involvement of other factors in the pathogenicity of this species. The Shiga toxin (Stx) blocks protein synthesis, thus causing cell death and may be found in different microorganisms. Several studies point out that the genes coding for Stx are transferable between species. Within the ribosomes and was first identified in *Corynebacterium ulcerans*. Bioinformatics analysis revealed that its tertiary structure is highly conserved and showed great similarity to the A chain of the Shiga toxin of *Escherichia coli* (SLT-1). Facing the discovery of the gene encoding a possible Shiga-like toxin in *C. ulcerans*, it is important to investigate not only the presence of similar genes in *C. diphtheriae*, but also their functions. The available genomes in the NCBI Chromosome Sequences database were used. The *in silico* analysis was performed by the following online applications: Blast (alignment), Primer-Blast (drawing of the primers), TMHMM 2 (prediction of the transmembrane portions), Signal P2.0 (signal peptide), Phyre[2] and I-TASSER (3D structure of the proteins analyzed). The quantification and differential expression of the Shiga-like toxin encoding gene in *C. diphtheriae* HC01 strain was performed by the RT-qPCR technique. The cytotoxic activity of *C. diphtheriae* HC01 strain was also evaluated in Vero cells. Moreover, different growth conditions (4°C and 37°C) were used in both techniques. *In silico* analysis confirmed the presence of a Shiga-like protein encoding gene in several strains of *C. diphtheriae*. The RT-qPCR technique confirmed the presence and expression of this gene in *C. diphtheriae* HC01 strain, as well as an increased expression when submitted to 4ºC. A similar behavior was also observed in the cytotoxicity assay in Vero cells. Finally, the construction of a mutant strain by the disruption of the Shiga-like protein encoding gene in *C. diphtheriae* is underway.

Keywords: *Corynebacterium diphtheriae*, Shiga-like toxin, real time PCR, virulence.

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