TITLE: QUANTIFICATION OF *Vibrio* spp. IN OYSTERS USING QUANTITATIVE REAL-TIME PCR

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ABSTRACT:

Species of *Vibrio* spp. are naturally occurring estuarine bacteria. The most prevalent *Vibrio* spp. causing foodborne diseases worldwide are *V. cholerae*, *V. parahaemolyticus* and *V.vulnificus*. Thus, quantitative real time polymerase chain reactions (qPCRs) of these species can be an important tool for quantitative microbial risk assessment, which requires numerical data to determine the level of contamination at a specific stage of food production. The aim of this work was to develop a qPCR for quantification of *Vibrio parahaemolyticus* (VP), *Vibrio cholerae* (VC) and *Vibrio vulnificus* (VV) in oysters from four different locations on the southern coast of Bahia in northeastern Brazil. Each sample was composed of a pool of 40 oysters totaling 1,120 oysters in 28 separate collections during one year. The qPCR was standardized for quantification of specific fragments of target genes (*tlh*, *ct* and *vvh*) corresponding to each one of the mentioned bacteria. Standard curves showed $R^2 > 0.99$; the inter- and intra-experiment reproducibility presented a low coefficient of variation in all trials. The melting temperature was 78º C (VP), 76º C (VC) and 77º C (VC). Five samples (17.85%) presented an average of 2.8 log of *tlh* gene copy numbers. Three of them were from the natural habitat in urban areas, the banks of the estuary, and two were from long-line oyster farming systems, which were considered as preserved environments. The results showed the presence of *V. parahemolyticus* in these estuarine environments.

Keywords: Quality control; Food safety; Foodborne diseases; Molecular diagnosis.