Amplification of *tlh* gene in other *Vibrionaceae* by specie-specific multiplex PCR of *Vibrio parahaemolyticus*.

Katherine García[1], Romina Yáñez[2], Roberto Bastías[2], Oscar Salgado[1]

1. Instituto de Ciencias Biomédicas, Universidad Autónoma de Chile. Av. El Llano Subercaseaux 2801, San Miguel, Santiago de Chile.
2. Laboratorio de Microbiología, Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Chile.

The surveillance of *V. parahaemolyticus* in the environment has been mainly performed by multiplex PCR amplification of three different hemolysin genes, which are specie-specific virulence factors. These genes are also employed in the determination of *V. parahaemolyticus* pathogenic load in seafood, and in the characterization of pathogenic strains associated to diarrhea cases in human. During environmental assessment that we performed every summer, we observed a *tlh* amplicon of a size slightly smaller than expected. This observation was coincident with low loads of *Vibrio parahaemolyticus* in the environment. By this reason, we probed the specificity of *tlh* primers for detection of *V. parahaemolyticus* at different loads.

Primers used for detection of the *Vibrio parahaemolyticus* specific thermolabil hemolysin (*tlh*) amplified a slightly smaller *tlh* gene, which is found in *Vibrio alginolyticus* and other related strains. These amplicons were observed when *Vibrio parahaemolyticus* was absent or in undetectable loads.

The amplification of a *V. parahaemolyticus* specific virulence factor in *Vibrio alginolyticus* and other related strains complicates potentially the estimation of bacterial load in seafood, because do not ensure the correct identification of *V. parahaemolyticus* during surveillance. Additionally, it could complicate the tracking of outbreaks of *V. parahaemolyticus* infections, considering that the genetic markers used would not be specie-specific.

Keywords: *Vibrio parahaemolyticus*, surveillance, hemolysin, virulence factor, multiplex PCR.

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