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6. SUMMARY

With increasingly high shrimp consumption, the decline of wild harvests has forced domestication to become the major source of shrimp production. However, the farming industry has been deteriorating due to several factors, in particular the outbreak of disease.

Vibriosis is one of the most important serious infectious bacterial diseases that affect different kinds of marine fishes, mollusks and crustacean over the world.

At this study, diseased cultured shrimp samples were collected from farms at Damietta governorate and apparently healthy marine shrimp samples were collected from fishing ships at Alexandria governorate, Egypt.

The toxR sequence variation could differentiate *V. harveyi* from closely related Vibrio species. A PCR protocol amplifying a 390-bp fragment of the *V. harveyi* toxR was established and could be useful in the specific and rapid detection of the species. Similarly **(Conejero & Hedreyda 2003)** identified *V. harveyi* accurately on the basis of species-specific primers designed from toxR gene.

Although our PCR assay involves primer sets of V. harveyi haemolysin (vhh-F,vhh-R), no specific amplicons of the predicted size were obtained for 12 of isolated strains of *V. harveyi*(n=14).

The same results were obtained by **(Al-Shimaa 2015)** for all isolated strains of *V. harveyi*. These results may be due to genotypic diversity as explained by **(Zhang & Austin 2000)** who characterized a haemolytic strain of *V. harveyi* (VIB 645) as highly pathogenic to salmonids and found two identical haemolysin genes (vhhA and vhhB), **(Zhang et al. 2001)** who detected other less pathogenic strains possessed only a single gene or alternatively, no vvh gene was detected and **(Conejero & Hedreyda 2004)** suggested that the vhh gene is

present in all *V. harveyi* strains and it may be suitable for species specific detection by PCR, with only 85.6% gene identity with the haemolysin gene (tl) from the closely related species *V. parahaemolyticus*.

On contarary, **(Ruwandeepika et al. 2010)** found that all the virulence genes that are typical for the Harveyi clade vibrios, including (luxR, toxRVh (toxR V. harveyi), vhpA (metalloprotease), chiA (chitinase), gene for serine protease, and vhh (V. harveyi haemolysin) were present in all the tested forty-eight bacterial isolates belonging to *V. harveyi*. However, the presence of a typical virulence genes in isolates belongs to Harveyi clade vibrios suggesting horizontal gene transfer.

Cano-Gomez et al. (2009) reported that nevertheless, the pathogenicity of strongly haemolytic *V. harveyi* strains suggests that the vhh gene is not suitable as a species identification marker. This is because species-specific markers should be stable in the genome, and at the same time the involvement of vhh in virulence makes this gene susceptible to horizontal gene transfer as reported by **(Waldor & Mekalanos 1996).**

Moreover, an amplification product using the set of partial haemolysin gene primers confirmed the presence of amplifiable bacterial DNA of the investigated isolates in the PCR samples with a band of 647-bp as described by **(Haldar et al. 2010a)** was used to reconfirm the 16S rRNA gene-based identification of *V. harveyi* using the designed primer partial hly gene . There further analysis revealed probably a single copy of hemolysin gene, a potential virulence factor was encoded in all isolates of V. harveyi, although not in the same locus in the genome.