

# PCR Restriction Fragment Length Polymorphism Analyses of *V. Parahaemolyticus* MAM-7 Virulence Gene in Clinical and Environmental Strains

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## Abstract

Virulent and non-virulent *Vibrio parahaemolyticus* (*V. parahaemolyticus*) strains coexist together in seawater. A PCR–restriction fragment length polymorphism (PCR-RFLP) technique could differentiate between clinical (virulent) and environmental *V. parahaemolyticus* strains. MAM-7 corresponds to a virulence gene described in *V. parahaemolyticus* and that participates in initial stages of pathogen gut colonization. The objective of our study is to evaluate if PCR-RFLP analyses of MAM-7 gene can discriminate between clinical and environmental *V. parahaemolyticus* strains.

Ten *V. parahaemolyticus* clinical isolates and nine *V. parahaemolyticus* environmental isolates were used to obtain genomic DNA. A 2619 bp PCR product from MAM-7 gene was digested with *HindIII* and *AclI* restriction enzymes revealing a characteristic common pattern in 100% of *V. parahaemolyticus* clinical isolates. These patterns were absolutely different of those obtained from environmental isolates. PCR of toxin related genes (*tdh* and *trh*) showed that only clinical isolates were *tdh+*.

As a conclusion, PCR-RFLP of *V. parahaemolyticus* MAM-7 gene could discriminate between clinical *tdh+* isolates and environmental ones and could complement other diagnostic tools to detect and classify virulent strains. However, it is still necessary to analyze more samples of *V. parahaemolyticus*. Thus, while these results are promising, this study corresponds to preliminary work.

**Keywords:** *Vibrio parahaemolyticus*; RFLP; MAM-7.

## 1. Introduction

Gastrointestinal tract infections (GTIs) are one of the most common infectious diseases, affecting 1.7 billion people per year worldwide (WHO, 2014). In addition, GTIs cause approximately 760.000 deaths per year in children under five years old (WHO, 2014). GTIs are caused by diverse microorganisms such as viruses, parasites and bacteria that are found in contaminated water or food. Examples of bacteria that cause GTI are *Escherichia coli*, *Clostridium difficile*, *Campylobacter*, *Salmonella*, *Vibrio* among others [1]. *Vibrio sp.* grouped aquatic Gram negative bacteria and are commonly founded in marine and estuarine environment. From 12 pathogenic *Vibrio* species that infect humans, *V. parahaemolyticus* causes acute gastroenteritis (vibriosis) by consuming of undercooked seafood. Gastroenteritis is characterized by watery diarrhea, nausea, vomiting and abdominal pain [2,3].

The pandemic *V. parahaemolyticus* O3:K6 strain was first detected in Osaka (Japan) in 1950, and since 1996 this serotype has been spread throughout India, Europe, Africa, North, Central and South America [4,5]. Clinical *V. parahaemolyticus* strains isolated from human with vibriosis are usually differentiated from environmental strains by detection of *tdh* and *trh* genes and by their capability of hydrolyzing urea and inducing hemolysis in Wagatsuma blood agar (Kanagawa positive phenotype) [6,7].

Most environmental *V. parahaemolyticus* strains are considered non-virulent due to low detection of *tdh* and *trh* genes [7,8]. However, other studies show that most of *V. parahaemolyticus* strains carry one or more toxin genes [9]. In addition, there are strains