Food safety remained one of the most important global health issues and food-borne diseases caused by microbes were widespread public health problem. Ingestion of fish contaminated by *Vibrio parahaemolyticus* and *Aeromonas hydrophila* hemolytic can cause mild gastroenteritis with diarrhea and abdominal cramps. Conventional methods for pathogen detection based on biochemical and microbiological tests are time-consuming, labour-intensive and involve analysis of a large number of food samples. For this reason, an specie-specific PCR assay was developed increasing reproducibility and rapid detection of pathogens in St Peter’s fillets. The contamination by *Vibrio parahaemolyticus* and *Aeromonas hydrophila* hemolytic was tested in 20 samples collected in fish farming in Cachoeira de Macacu. DNA templates were prepared from fish fillets by DNeasy blood and tissue kit quantified by fluorimeter and were amplified by a duplex PCR targeting for *toxR* and *aerolysin* genes for detection of *Vibrio parahaemolyticus* and *Aeromonas hydrophila* hemolytic, respectively. Two samples were found contaminated by *Vibrio parahaemolyticus*, three of them, by *Aeromonas hydrophila* hemolytic and two by both pathogens. The two isolates of *Vibrio parahaemolyticus* were also tested for virulence factor thermostable direct hemolysin (tdh), but in none of them, virulence factors correlated to infection and diseases, were found. These results indicate that the isolates in the present study do not contain the virulence factor HDT, but the absence of this does not mean that the *Vibrio parahaemolyticus* cannot cause disease in humans, since the microorganisms may present other virulence factors, not tested. The presence of *Aeromonas hydrophila* hemolytic by itself indicates that those farm products should not be consumed.