The Infection of *Vibrio parahaemolyticus* in Shrimp and Human

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ABSTRACT

Vibrio parahaemolyticus is an aquatic zoonotic agent that can threaten human and aquaculture animal health. Humans can be infected by consuming contaminated raw seafood or wound-related infections. Generally infection of *V. parahemolyticus* is orally transmitted and causes gastroenteritis in humans while in aquaculture animals especially shrimp can cause Acute Hepatopancreatic Necrosis Disease (AHPND) or Early Mortality Syndrome (EMS) with a very high mortality rate and cause economic losses. Shrimp species susceptible to infection are *Litopenaeus vannamei, Penaeus monodon,* and *P. chinensis*. *V. parahaemolyticus* produces several toxins in human disease such as thermostable direct hemolysin (TDH), TDH-related haemolysin (TRH), and thermolabile hemolysin (TLH). Meanwhile, Photorabdus insect-related (Pir) toxins consisting of $PirA^{vp}$ and $PirB^{vp}$ are the toxins associated with AHPND in shrimp. The genes that encode the toxin are used as targets to diagnose *V. parahaemolyticus* pathogens molecularly. Until now the treatment of *V. parahaemolyticus* infection is using antibiotics and fluid therapy, but there were *V. parahaemolyticus* isolates from aquaculture that have been resistant to antibiotics so that the use of antibiotics in aquaculture must be controlled and the use of alternative therapy are very important to be developed to control *V. parahaemolyticus* infection.

Keywords: *V. parahaemolyticus*, zoonotic, gastroenteritis, Acute Hepatopancreatic Necrosis Disease (AHPND), Early Mortality Syndrome (EMS).

INTRODUCTION

Vibrio parahaemolyticus is a seafoodborne pathogen derived from the marine environment (Kanjanasopa et al., 2011). This bacterium include aquatic zoonotic agent that can cause Vibriosis in many species of fish, shellfish, and other aquatic animals. In humans it can cause gastroenteritis, sepsis, and wound infection (Zhang et al., 2016). Gastroenteritis caused by *V. parahaemolyticus* is usually characterized by reddish diarrhea with blood, stomach cramps, nausea, vomiting, dizzy, and fever (Yeung and Boor, 2004; Shimohata and Takahashi, 2010).

Infection caused by *V. parahaemolyticus* has been reported in different parts of the world. This bacterium can infect humans and aquatic animals (FAO and WHO, 2011). Transmission generally results from consuming raw seafood (Wang et al., 2015). In addition there are several reports of wound-related infections (Zhang et al., 2016).

The disease caused by *V. parahaemolyticus* is one of the most important diseases because it can infect both humans and aquatic animals. Economically, this disease causes economic losses in the aquaculture industry worldwide (Pui et al., 2014). Given this disease is one of the zoonotic diseases affecting the aquaculture industry and humans, so that a review of *V. parahaemolyticus* infection is very interesting to discuss.

MICROBIOLOGICAL ASPECTS

Vibrio parahaemolyticus is a Gram negative bacterium that was first isolated in Japan in the case of mass poisoning in 1950 (Broberg et al., 2011). In general, this bacterium is found in coastal areas and estuaries (Fabbro et al., 2010). *V. parahaemolyticus* is a halophilic bacterium that produces capsules with 12 variations of somatic antigens (O) and over 70 different capsular antigens (K) (Ceccarelli et al., 2013; Xu et al., 2014). Among these serotypes, serotype O3:K6, O4:K68, and

O1:$K_{untypeable}$ are the most pathogenic serotype and are contributing factors to foodborne disease (Jones et al., 2012).

In causing the disease, *V. parahaemolyticus* has several virulence factors including *toxR*, thermostable direct hemolysin (TDH), TDH-related hemolysin (TRH), and thermolabile hemolysin (TLH) (Kim et al., 1999; Ceccarelli et al., 2013; Ragunath, 2014; Stones and Krachler, 2015). The *toxR* gene was first identified in *V. cholerae* that serves as a positive transcription factor to *cholerae toxin* (ctx) (Lin et al., 1993). Furthermore, in *V. parahaemolyticus*, the *toxR* gene plays a role in stimulating the expression of the *tdh* gene which is a major virulence factor owned by *V. parahaemolyticus*. The *toxR* gene homology between *V. cholerae* and *V. parahaemolyticus* was 52% (Kim et al., 1999).

V. parahaemolyticus isolates considered as virulent strain are isolates carrying thermostable direct hemolysin (TDH) encoded by *tdh* gene or TDH-related haemolysin (TRH) encoded by the *trh* gene or both (Marlina et al., 2007; Ceccarelli et al., 2013). *V. parahaemolyticus* which is *tdh*-positive will show β-hemolytic properties on a special medium Wagatsuma Agar, this event is known as Kanagawa Phenomenon (KP). The primary target of TDH toxin is epithelial cells and intestinal cells. According to Hiyoshi et al. (2010) TDH has cytotoxicity, enterotoxicity, and lethal activity in mice. The effects of TDH on these cells will cause diarrhea during infection (Shimohata and Takahasi, 2010).

Clinical samples derived from outbreaks in the Republic of Maldives show a KP-negative which means not producing TDH. However, the strain is known to produce a new type of hemolysin, known as TRH. Immunologically there are similarities between TDH and TRH, both toxins are equally capable of causing

hemolysis in red blood cells, based on the results of genetic analysis there was a 70% homology between the two genes (Ham and Orth, 2012; Raghunath, 2014).

In some clinical cases sometimes there is no presence of *tdh* or *trh* genes. The absence of either of these genes suggested that there are other genes that play a role in causing the disease (Raghunath, 2014). *V. parahaemolyticus* is capable of producing additional toxins known as thermolabile hemolysin (TLH). This toxin is encoded by the *tlh* gene and has phospholipase activity and is able to lyse the erythrocytes (Stones et al., 2015).

INFECTION IN SHRIMP

Vibrio parahaemolyticus is one of the pathogenic agents that threatens the viability of aquaculture industry especially shrimp (Pui et al., 2014). *V. parahaemolyticus* is the cause of Acute Hepatopancreatic Necrosis Disease (AHPND) or Early Mortality Syndrome (EMS) in shrimp which causes great losses in the shrimp industry (Li et al., 2017). It affects much of the aquaculture industry in Mexico and Asia (Flegel, 2012). AHPND was first reported in China in 2009, followed by Malaysia in 2010, Vitenam in 2011, Thailand in 2012 and Mexico in 2013 (Pui et al., 2014). The mortality rate due to AHPND is very high at around 40-100% (De Schryver et al., 2014; Chu et al., 2016). Species of shrimps susceptible to infection of *V. parahaemolyticus* are *Litopenaeus vannamei*, *Penaeus monodon*, and *P. chinensis* (Tinwongger et al., 2014; Chu et al., 2016)

The AHPND is caused by a unique strain of *V. parahaemolyticus*. The genome analysis of *V. parahaemolyticus* isolated from Thailand and Mexico showed that the bacteria had plasmids carrying virulence factors of type IV pili proteins and conjugal transfer proteins (Gomez-Gil et al., 2014). In addition, the plasmid *V. parahaemolyticus* cause AHPND has a homologous region with Photorabdhus Pir toxins which is an

insecticidal toxin (Waterfield et al., 2005). Lai et al. (2015) and Lee et al. (2015) shows that *V. parahaemolyticus* AHPND secretes Photorabdus insect-related (Pir) toxins consisting of $PirA^{vp}$ and $PirB^{vp}$ into the medium. Based on the results of research Khimmakthong and Sukkarun (2017) reported that after 6 hours post infection, the spread of *V. parahaemolyticus* on infected shrimp can be found in gills, hepatopancreas, intestine, muscles, and hemolymph. Lai et al. (2015) added that $PirA^{vp}$ and $PirB^{vp}$ toxins were detected in the hepatopancreas post infection.

Shrimps infected with AHPND exhibits an array of clinical signs including lethargy, soft shells, whitish muscle, empty stomach and midgut, slow growth, and pale atrophied hepatopancreas that often have black streaks (Chu et al., 2016). According to Soto-Rodriguez et al. (2014) there are 3 stages AHPND that are initial, acute, and terminal. In the initial stage, the epithelial cells are elongated into tubular lumen, reduction of vacuole size and there is also an increased desquamation of tubular epithelial cells. In the acute stage of the disease, the tubular epithelium is necrotic. In the terminal stage of the disease, the intermediate tissue from the hepatopancreas tubules shows a severe inflammatory response, and the tubular epithelium becomes entirely necrotic, with dead cells at different phases of lysis with a severe desquamation of the cells, which accumulates in the lumen as dead cells.

A research report by Pui et al. (2014) in Sarawak Malaysia found a prevalence of *V. parahaemolyticus* infection of 41% in shrimp in aquaculture farms and potentially infectious to humans. From 3-year study, the annual prevalence rates of AHPND were 50%, 26% and 73% in 2011, 2012 and 2013 respectively (Chu et al., 2016). Anjay et al. (2014) detected *tdh* and *trh* gene of 55.3% and 0.59% in isolates derived from fish and shellfish in India. Abd-Elghany and Sallam (2013) found 33.3% contamination of *V. parahaemolyticus* in seafood. The results of Lai et al. (2015) showed that *V.*

parahaemolyticus AHPND was resistant to some antibiotics. Xu et al. (2016) added that the isolate *V. parahaemolyticus* obtained from aquatic product sellers in northern China showed antibiotic resistance properties to streptomycin (86.2%), while fewer were resistant to ampicillin (49.6%), cefazolin (43.5%), cephalothin (35.9%), and kanamycin (22.1%). All of the examined isolates were susceptible to azithromycin and chloramphenicol. Similar results were also reported by Pazhani et al. (2014) generally *V. parahaemolyticus* isolate exhibited resistance to streptomycin and ampicillin. Oh et al. (2011) reported that the prevalence of resistance in *V. parahaemolyticus* isolates to ampicillin was highest (57.8%), followed by resistance to rifampin (11.9%), streptomycin (8.7%), and trimethoprim (6.4%). With the discovery of resistance isolates derived from aquaculture, it is necessary to monitor the use of antibiotics in aquaculture (Lai et al., 2015; Xu et al., 2016).

INFECTION IN HUMAN

Transmission in humans is usually the result of consuming raw seafood or wounds exposed to marine or marine animals commonly occurring in Southeast Asian countries (Wong et al., 2000; Austin, 2010; Rahimi et al., 2010; Nelapati et al., 2012). *V. parahaemolyticus* causes for about 25% of cases of foodborne disease compare with other Vibrios (Feldhusen, 2000). *V. parahaemolyticus* can be found in various types of seafood worldwide (Teplitski et al., 2009; Zhang et al., 2016).

Outbreak cases of *V. parahemolyticus* infection have been reported in different parts of the world. Reports of events were commonly found in Asian, European, African and American countries (DePaola et al., 2000; Lozano-León et al., 2003; Drake et al., 2007; Su and Liu, 2007; Chao et al., 2009; FAO and WHO, 2011). During 1997 and 1998 there were more than 700 cases of illness due to *V. parahaemolyticus* in the United States of America, the majority of which were associated with the consumption of raw

oysters. In two of the 1998 outbreaks a serotype of *V. parahaemolyticus* (O3:K6), reported previously only in Asia, emerged for the first time as a principal cause of illness (FAO and WHO, 2011).

Various types of aquatic animals can be infected and act as a source of transmission of *V. parahaemolyticus* (Anjay et al., 2014). Every year, in Japan is reported about 500-800 people infected with *V. parahaemolyticus* due to consume seafood in raw conditions. Sashimi and sushi were the main sources of transmission 26% and 23% respectively (FAO and WHO, 2011). In China, 803 times have been reported that caused 17,426 people infected (Wang et al., 2011). In Denmark Hornstrup and Gahrnhansen (1993) reported that *V. parahaemolyticus* was found in 13 patients with wound and ear infections. This case was reported to be related to the marine environment. Cho et al. (2008) stated that there was no age-related infection in *V. parahaemolyticus* infection based on research conducted in Korea. Cases of infection caused by *V. parahaemolyticus* generally increase in the summer (June to October) associated with consumption of various types of seafood such as crab, shrimp, shellfish, lobster, fish, and oysters (FAO and WHO, 2011; Nelapati et al., 2012).

In humans, *V. parahaemolyticus* causes acute gastroenteritis after consuming contaminated seafood. After an incubation period of 12-24 hours, nausea and vomiting, abdominal cramps, fever, and watery diarrhea may occur. Occasionally found fecal leucocyte (Brooks et al., 2013). Broberg et al. (2011) added that sometimes blood pressure drops to shock. In severe events, it causes loss of consciousness, cyanosis, even death (Nair et al., 2007). Pathologic changes are commonly found like erosion of the jejunum and ileum, inflammation of the stomach, and damage to several internal organs such as liver, spleen and lung congestion (Wang et al., 2015). Treatment using antibiotics such as the use of doxycycline, ciprofloxacin, or erythromicin and fluid

therapy are a common therapy used to treat *V. parahaemolyticus* infection (Qadri et al., 2003; Wang et al., 2015).

LABORATORY ASPECTS

Isolation and identification of *V. parahaemolyticus* may be performed from clinical case samples as well as samples from the environment. The use of conventional methods such as culture methods and biochemical tests is still a standard method of detecting *V. parahaemolyticus*. The most commonly enrichment medium used is Alkaline Peptone Water (APW) or Alkaline Peptone Salt Broth (APS) with 3% NaCl addition, then grown on TCBS media characterized by the growth of 1-2mm and green colored colonies with the blue center colonies (Nelapati and Krishnaiah, 2010). Hara-Kudo (2003) developed a selective medium for growing *Vibrio sp.* named CHROMagar^{TM} Vibrio, the growth of *V. parahaemolyticus* colonies on this medium will be purple or violet. Isolation of bacteria using culture method and biochemical test is gold standard in detection and diagnosis of *V. parahaemolyticus*, but this method takes a long time to 3 days (Changchai and Saunjit, 2014), besides, there is similarity of biochemical properties between *V. parahaemolyticus* and *V. vulnificus*, so now commonly used molecular methods (Nelapati and Krishnaiah, 2010; Kanjanasopa et al., 2011; Yamazaki et al., 2011).

One of the most common molecular techniques used in the diagnosis of *V. parahaemolyticus* is Ploymerase Chain Reaction (PCR). There is a PCR technique developed by Kim et al. (1999) that has been validated by targeting the *toxR* gene encoding the ToxR Transmembrane which is a specific species marker belonging to *V. parahaemolyticus*, of 373 strains of *V. parahaemolyticus* tested using the *toxR* gene all showing positive results with 368 bp amplicon. In addition to the *toxR* gene, the *gyrB* gene may also be used for the detection of *V. parahaemolyticus* because it is conserved

in each isolate (Venkateswaran et al., 1998). The latest molecular diagnostic technique developed by Wang et al. (2016) in differentiating *V. parahaemolyticus* and *V. vulnificus* using multiple-endonuclease restriction real-time loop-mediated isothermal amplification technology (MERT-LAMP). This technique uses the *toxR* gene to detect *V. parahamolyticus* and *rpoS* gene for *V. vulnificus*. *V. parahaemolyticus* consists of various strains, to distinguish the various strains used serologic test (Honda et al., 2008; Caburlotto et al., 2010). It has been known that *V. parahaemolyticus* causing AHPND in shrimp is unique because it carries a special plasmid. Tinwongger et al. (2014) developed PCR with 100% accuracy to detect *V. parahaemolyticus* causing AHPND with TUMSAT-Vp3 primers with 360 bp amplicon.

CONTROL AND PREVENTION

Antibiotic therapy in the aquaculture industry is a common strategy that is often done. There are now many reported cases of antibiotic resistance, so it needs to be developed alternative therapy (Alagappan et al., 2010). Phage therapy is an alternative to control the effects of pathogenic bacteria. Based on research conducted by Lomelí-Ortega and Martínez-Díaz (2015) the use of A3S and Vpms1 phages during experimental infection was effective to prevent the mortality and to reduce the signs of Vibriosis in the larvae. Pattukumar et al. (2010) used probiotics *(Bacillus)* and successfully suppressed the number of pathogenic *V. parahaemolyticus* that influenced the shrimp health.

To reduce the risk of infection of *V. parahaemolyticus* associated with seafood, various physical and chemical methods have been developed. Depuration is one of the most commonly used ways to reduce the amount of contaminant bacteria in aquatic products (Croci et al., 2002). In addition to depuration, it is known that *V. parahaemolyticus* is very sensitive to heat (Yeung and Boor, 2004) so that cooking

seafood until cooked can prevent infection. Further Andrews et al. (2000) and Johnston and Brown (2002) suggest that a low temperature pasteurization treatment (e.g., 50°C for up to 15 min) can reduce the risk of Vibrio infections from raw oyster consumption. Freezing at -18ºC or -24ºC for 10 minutes proved effective for killing *V. parahaemolyticus* (Andrews et al., 2000). Several chemical reagents including electrolyzed oxidizing water, chlorine, and iodophors can reduce the amount of contaminants in seafood (Croci et al., 2002; Ren and Su, 2006).

CONCLUSION

Vibrio parahaemolyticus is an infectious agent that can infect aquaculture animal and human. This agent cause major economic losses in the shrimp industry and cause serious illness in humans. Although the mechanism of the occurrence of the disease and the mode of diagnosis have been known, but further efforts are needed in controlling infection of *V. parahaemolyticus* because there have been many reports related to antibiotic resistance.

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