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Vibrio parahaemolyticus: A review on the prevalence, biofilm formation and method of preservation of seafood

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Vibrio parahaemolyticus are human foodborne pathogen linked to the consumption of contaminated raw and undercooked seafood. Seafood are highly perishable and prone to contamination. Therefore, it is important to maintain its safety for public health. *V. parahaemolyticus* is responsible for several foodborne outbreak in Asian countries including Japan, China and Taiwan and has been acknowledged as the major cause of human gastroenteritis in the United States. This review aims to provide an insight on *V. parahaemolyticus* food poisoning, prevalence in seafood, biofilm formation ability and several methods of preserving seafood.

Keywords: *Vibrio parahaemolyticus*, seafood, foodborne disease, biofilm formation, preservation method.

INTRODUCTION

Vibrio parahaemolyticus belongs to the *Vibrionaceae* family. They are Gram negative, ubiquitous, halophilic facultative anaerobic bacteria found in marine, estuarine environments that are positive to the biochemical test catalase and oxidase. They can survive at a temperature between 5ºC and 43ºC and are grown at 37ºC. The *Vibrio* genus consists of 142 species that are mainly found in the marine environment and its taxonomy is been consistently revised as a result of discovering new species (Sawabe et al. 2013). *V. parahaemolyticus* is an important member of the *Vibrio* spp. capable of causing infection to human. *V. parahaemolyticus* are bacteria that possess the flagella and can move freely underwater or when fixed to an animate object such as a shellfish (Gode-potratz et al. 2011). *V. parahaemolyticus* possess two flagella which help them adapt to a different environment. The polar flagella help with movement while the lateral flagella are linked to biofilm formation (Broberg et al. 2011).

The bacterial *V. parahaemolyticus* is reported as the most prevalent pathogen associated with seafood and this is because *V. parahaemolyticus* outbreak has occurred vigorously worldwide as a result of ingestion of raw and undercooked seafood which has led to the inflammation of the bowel. The availability of this pathogenic bacteria in the marine habitat should be a great concern to humans due to the consistent outbreak of the disease (Ceccarelli et al. 2013). *V. parahaemolyticus* causes infection by attaching

itself to the fibronectin and phosphatidic acid on the host cell thereby unleashing different toxins into the cytoplasm of the host cell and this will lead to a life-threatening illness (Gode-Potratz et al. 2011).

V. parahaemolyticus was first discovered in the 1950s in Japan as a foodborne disease with a huge outbreak originating from the prevalent serotype O3: K6 from 1997 to 2001 (Hara-Kudo et al. 2012). *V. parahaemolyticus* has been reported as the major causative agent of seafood associated gastroenteritis in several countries such as the United States and Asian countries (Scallan et al. 2011).

PREVALENCE OF *V. parahaemolyticus* IN SEAFOOD

Seafood and seafood products are vulnerable to foodborne bacteria and are capable of causing diseases when they are consumed by human. Seafood is a highly nutritious food and easily digestible food (Yagoub and Ahmed, 2013). Despite the high protein content in them, seafood is implicated in the transfer of foodborne disease globally. The World Health Organization (WHO) defined foodborne disease as a disease caused by consuming food contaminated with bacteria (Velusamy et al. 2010). Bacteria such as the *Vibrio* spp., *Listeria monocytogenes, Campylobacter*, *Salmonella* are reported as the main cause of foodborne disease globally (Velusamy et al. 2010). *Vibrio* spp. has been reported as the major cause of foodborne outbreaks in the Asian countries and this includes Japan, China, India, Taiwan (Hara-Kudo et al. 2003) Korea (Lee et al. 2008) and Malaysia (Tunung et al. 2010). The bacteria can multiply in the human system thereby causing foodborne diseases or food poisoning which are detrimental to one's health and in some cases can lead to death (Letchumanan et al. 2015).

V. parahaemolyticus is an important foodborne pathogen that causes gastroenteritis when raw or semi-cooked seafood are consumed (Letchumanan et al. 2015) and it is one of the main agents causing food poisoning in countries where seafood are being consumed. The existence of *Vibrio* spp. in seafood also give a clue about the condition of the wet market because the seafood sold in the market are usually placed in an open-ice tray and the ice melts faster leaving the fish at ambient temperature. Thus, the bacteria in the seafood will be able to multiply faster if no fresh ice cubes are placed on them (Nelapati et al. 2012). Furthermore, Yang et al. (2008) reported that the environment temperature also plays an important role in the rate *V. parahaemolyticus* contaminates raw fish. Toxigenic *V. parahaemolyticus* isolated from raw seafood in Malaysia (Sujeewa et al. 2009; Syamimi Hanim and Tang, 2019).

The occurrence of *V. parahaemolyticus* is dependent on different factors such as the water temperature, salt and oxygen concentration, availability of sediments and aquatic organisms (Letchumanan et al. 2015). *V. parahaemolyticus* usually inhabit and multiply rapidly in the gut of filter-feeding shellfish like oysters, mussels and clams (Sumner, 2011)

The pathogen *V. parahaemolyticus* has been isolated from seafood such as shrimp in the Asian countries (Deepanjali et al. 2005) and has been linked to several foodborne diseases in Japan (Hara-Kudo et al. 2012), Taiwan (Yu et al. 2013), China (Li et al. 2014) and Bangladesh (Bhuiyan et al. 2002). Yano et al. (2014) reported the isolation of pathogenic *V. parahaemolyticus* from shrimps in Thailand which possess the antimicrobial resistance strain. In addition, Al-Othrubi et al. (2011) also reported the presence of pathogenic and antimicrobial resistance *V. parahaemolyticus* from shrimps in Malaysia. According to Chen et al. (2013), *V. parahaemolyticus* is seen as the main cause of foodborne disease in China which has been linked to the consumption of shrimps contaminated with *V. parahaemolyticus.* Also, Peng et al. (2010) reported an outbreak of *V. parahaemolyticus* in China caused as a result of consumption of contaminated shrimps. In addition, pathogenic *trh V. parahaemolyticus* was detected in shrimps with the bacterial densities less than 100MPN/g in samples (Xu et al. 2014). Also, Rahimi et al. (2010) isolated 9.3% *V. parahaemolyticus* in shrimps in Iran.

In addition, Letchumanan et al. (2015) detected and isolated pathogenic *V. parahaemolyticus* containing the *tox*R gene from shellfish samples in Malaysia. Also, Tran et al. (2018) reported an outbreak of *V. parahaemolyticus* in Vietnam caused as a result of consumption of contaminated shellfish. *V. parahaemolyticus* is also seen as the main cause of foodborne diseases in Iran (Rahimi et al. 2010). In addition, Baffone et al. (2000) detected *V. parahaemolyticus* from several finfish such as the anchovies, grey mullet, red mullet, sardines and the Atlantic mackerel using the selective medium and biochemical test. Pathogenic *V. parahaemolytivus* containing the *tdh* virulent gene was also detected in horse mackerel obtained

from Japan markets (Hara-Kudo et al. 2003).

Aside the antibiotic resistant *V. parahaemolyticus*, Reyhanath and Kutty (2014) detected multidrug resistant strains of *V. parahaemolyticus* from a fishing land in South India. Also, multi-drug resistant strains of *V. parahaemolyticus* was isolated from a marine environment in South India of which most of the strains are resistant to ampicillin (Sudha et al. 2014).

In Taiwan, *V. parahaemolyticus* isolated from oyster and clam possess the hemolytic activities and the presence of *tdh, trh* and T3SS (Yu et al. 2013). Qadri et al. (2005) reported several cases of *V. parahaemolyticus* gastroenteritis in Spain, Greece, Britain, Turkey, Denmark and Yugoslavia. In addition, an outbreak of *V. parahaemolyticus* was reported after the consumption of contaminated oysters collected In Washington and British Columbia (CDC, 2006).

BIOFILM FORMATION ABILITY OF *V. parahaemolyticus*

Biofilms are a structurally complex group of microorganisms that are designed in such a way that they bind to biotic or abiotic surfaces and are attached within a matrix of extracellular polymeric substances (Mizan et al. 2015). Biofilm formation involves several processes which begin with microbial fixation followed by the accumulation of an extracellular matrix made of polymeric substances like proteins, polysaccharides, humic substances, extracellular DNA (Flemming and Wingender, 2010). Biofilm formation is essential because it serves as protection to the bacteria from harsh environmental condition (King et al. 2008). At first, the cells assemble as a micro-colonies and then undergo cell division, they grow and encase themselves in an extracellular matrix thereby leading to the formation of a complex and differentiated associations thus ease nutrient uptake (Toutain et al. 2004). The formation of biofilms occur in stages and they are: (1) bacteria colonize a surface. (2) bacteria form micro-colonies and (3) micro-colonies form biofilms (Johnson, 2008) The ability of bacteria to possess biofilm makes them more resistant to environmental stress in about 1000 folds than the free-living bacteria. Thus, *V. parahaemolyticus* has the ability to form biofilm thereby producing adherence factors which makes it easier to bind to surfaces (Donlan, 2002). Bacteria are mostly resistant to antimicrobial agents but the ability to form biofilms makes them more resistant to the same antimicrobial agents. The biofilm-forming bacteria possess the antibiotic resistance gene because of the presence of the extracellular polymeric substance (EPS) matrix which makes it difficult for antibiotics to effect the bacteria. Thus, the ability of the pathogen to survive in its habitat, cause infection and its transmission increases with its ability to form a biofilm (Kadam et al. 2013). Biofilm is capable of protecting the bacteria by providing firm three-dimensional multicellular, complex, self-assembled structures that contain exopolymeric substances (Costa et al. 2013) and are mainly grouped based on their genotypic and phenotypic properties (Nadell et al. 2013). The pathogen *V. parahaemolyticus* are capable of forming biofilms on different surfaces and this includes the chitin of diatoms (Frischkorn et al. 2013), oysters (Aagesen et al. 2013) and stainless steel (Vezzulli et al. 2008). They are able to form a strong biofilm in a liquid medium. The production of biofilm entails; transportation and attachment of the free-living bacteria to a fixed place, cell multiplication, tiny colonies are formed, the daughter cells produced are disseminated into the water column (Matin et al. 2011). The attachment stage is the first and most important phase of biofilm formation. Several factors can affect *V. parahaemolyticus* attachment and they are physiochemical agents like temperature, pH, salinity (Cai et al. 2013) and surface conditions like the substrate type, surface roughness and chemical compositions (AlAbbas et al. 2012). Since biofilms are important for balancing nitrogen and carbon cycles in aquaculture, they help to increase the production of shrimp by growing on submerged substrates thereby seeving as a good source of protein (Pandey et al. 2014). Biofilm exhibit various features and they include; assembling of biofilm with cells, exchange of resistance plasmids with cell, ability to produce endo-toxins, resistance to antimicrobials and host defense system clearance (Guiton et al. 2010). *V. parahaemolyticus* demonstrate two cell types: the cell emerges as a short rod with a single-sheathed polar flagellum when cultured in a liquid medium. This flagellum serves as a tool for its movement. However, once the pathogen is grown on a solid surface it appears as a swarmer cell type. The swarming motility and the ability to form biofilms are linked to the pathogenic potential of *V. parahaemolyticus* (Overhage et al. 2008).

METHODS TO KILL *V. parahaemolyticus* AND PRESERVE SEAFOOD

Seafood is nutritious food with high amount of protein, fatty acids, minerals and vitamins.

However, they get spoilt easily because their shelf life is less than a day without proper means of preservation. They are subjected to oxidation and the taste and texture changes when stored inappropriately. *V. parahaemolyticus* has been found to survive in fish product over extended period of time when incubated at room temperature (Tang et al. 2014; Tang et al. 2017). Several methods have been developed to minimize or eradicate the risk of *V. parahaemolyticus* infections implicated with seafood consumption. The use of heat to deactivate *V. parahaemolyticus* in seafood is the most frequently used method (Su and Liu, 2007). Low temperature freezing (-18ºC or 24ºC) or high temperature treatment (>55ºC) for 10min will completely kill *V. parahaemolyticus* in oysters (Andrews et al. 2000). An excellent method used in destroying pathogenic *V. parahaemolyticus* in seafood is the High-pressure processing (HPP) method (Cook et al. 2002). In addition, chemicals such as chlorine, electrolyzed oxidizing water and iodophors are also used effectively in the reduction of *V. parahaemolyticus* in seafood (Ren and Su, 2006).

Cooling

Seafood are generally placed on ice flakes in the market. This cooling techniques can retain the freshness of the seafood but will not stop microbial growth or enzymatic actions. Cooling must be done as soon as the seafood is dead. It is essential that proper refrigeration is done during transporting the seafoods to maintain its shelf life (Bunka et al. 2013). Aside from the normal ice flakes used, mechanically refrigerated seawater (RSW) has been developed and it is more efficient in slowing down microbial spoilage better than normal ice. A disadvantage of this cooling system is that when spoilage eventually occurs, the spoilage microorganism will be evenly distributed to the entire seafood. Furthermore, ice slurries are also used (Garcia-Soto et al. 2011) because it prevents the seafood from undergoing oxidation and dehydration as the entire seafood is completely covered in the ice water system. Thus, reducing spoilage and less physical damage to the seafood (Pineiro et al. 2005). The ice slurries are made more effective by adding natural antioxidants or organic acids such as ascorbic acid, citric acid and lactic acid mixtures to them and this reduces oxidation and slows down bacterial growth (Garcia-Soto et al. 2011).

Deep chilling

Deep chilling is used to freeze seafood till it gets to or below the standard freezing point which is usually between -0.5 and -2.8C (Kaale et al. 2011). Deep chilling is used efficiently to stop microbial growth, extend its shelf life and also prevent drip loss which is a common occurrence during seafood thawing (Fukuma et al. 2012). However, this method can increase protein degradation and lipid oxidation due to incomplete freezing of the seafood. Since a portion of the seafood is not properly frozen, an increase in enzymatic activity, muscle protein denaturation and membrane damage will occur (Dunn and Rustad, 2007).

Freezing

Freezing is an ancient technique used in preserving seafood over a long period of time. It reduces the growth of microorganisms and also alters the rate of enzymatic activity thereby maintaining the taste, smell, texture and nutritional properties of the seafood better than cooling and deep chilling (Alizadeh et al. 2007). However, the ice crystals formed during freezing is dependent on whether the freezing occurs rapidly or slowly. When freezing occurs slowly, large ice crystals are formed which increases the rate of decomposition, texture damage and membrane disruption (Alizadeh et al. 2007). However, when freezing occurs rapidly, small ice crystals are formed and this reduces the rate of decomposition (Li and Sun, 2002). The conventional freezer is an example of a slow freezing method while cryogenic freezing, high-pressure freezing, liquid immersion freezing and air blast freezing, plate freezing are examples of a rapid freezing technique (Hall, 2011). Freezing can also have a negative impact on the structural and chemical properties of muscle protein by increasing its fatty acids content and oxidation process (Leygonie et al. 2012).

High-pressure processing

This method has been effectively used to kill microorganisms in 1899 and has been successfully used in preserving food and seafood (Rastogi et al. 2007). The high-pressure processing does not make use of heat but effectively destroy pathogenic bacteria in seafood and lengthen its shelf life without changing its nutritional value, taste and physical appearance. The use of high-pressure treatment is almost the same as using high temperatures. Although, the high-pressure deform the cell of bacteria and

causes cell and structural damage to bacteria (Rastogi et al. 2007). This method also increases protein denaturation, affects muscle enzymes, myofibrillar protein and proteolysis. The high-pressure treatment is reported to increase the shelf life of red mullet (*Mullus surmuletus*) from 12 to 15 days (Erkan et al. 2010). This high-pressure treatment applied to mackerel (*Scomber scombrus*) also enhance its sensory and functional properties (Aubourg et al. 2013). The treatment of 300MPa for 180s effectively reduced *V. parahaemolyticus* including the O3: K6 strains in oysters (Cook, 2003). Furthermore, an increase in the pressure and processing time will lead to an increase in the reduction of *V. parahaemolyticus,* because of HPP effective against *V. parahaemolyticus* at a lower temperature (Phuvasate and Su, 2015). Once the temperature is reduced to 1.5ºC, the processing time is changed to 5min from 10min and pressure lowered to 250MPa (Phuvasate and Su, 2015).

Relaying and depuration

Relaying and depuration are often used to minimize bacterial contamination in shellfish. During relaying, the shellfish is moved from a contaminated area to a non-contaminated area before harvesting to purify the shellfish naturally. However, the discharge of human waste into the marine habitat leads to an increase in pollution thereby limiting clean environment for the growth of the shellfish because the human waste contains several microbial pathogens which can result in contamination of bivalve molluscan shellfish (Geoghegan et al. 2016) The depurative process enables the shellfish eject sand and grit from its gut into clean seawater thereby reducing bacterial contamination and lengthening the shelf life of refrigerated seafood. Depurative is most effective in reducing *V. parahaemolyticus* when carried out at a low temperature and done consistently (Phuvasate et al. 2012). The UV light treatment is mainly used during depuration and relaying process to disinfect shellfish before its been marketed because it doesn't alter the organoleptic features of the shellfish (Lees, 2010). Besides, two phage groups have been effectively used in minimizing the numbers of *V. parahaemolyticus* in the raw oyster: a Siphoviridae phage pVp-1 (Jun et al. 2014) and VPp1a phage isolated from *V. parahaemolyticus* (Peng et al. 2013)

Irradiation

Irradiation is a non-thermal technique used to eliminate pathogenic bacterial in seafood. It is often used to improve the safety and shelf life of several kinds of food. It involves the use of gamma irradiation and in recent times X-rays to destroy pathogenic bacteria such as *Vibrios* in live oysters (Mahmoud, 2009). It was reported that live oysters can survive 0.5 – 3.0 kGy dose of gamma irradiation without altering its sensory attributes. However, a huge decrease in *V. parahaemolyticus* count was recorded when a low dose of gamma rays of 1.0kGy was used (Jakabi et al. 2003). Furthermore, a ready to eat shrimp inoculated with *V. para*haemolyticus treated with 0.1 – 4kGy X-ray showed a 6-log reduction in CFU at 3kGy (Mahmoud, 2009).

Natural organic treatments

The use of essential oils, tea polyphenols and organic acids to seafood have been reported to lengthen its shelf life, reduce the multiplication of bacteria and also enhance the flavour of seafood thereby increasing its marketing status. Essential oils like thyme, oregano, rosemary, turmeric and shallots are reported to reduce the amount of non-pathogenic spoilage bacteria in seafood (Li et al. 2012). Several polyphenols like catechins, epigallocatechin, epicatechin gallate and epicatechin obtained from tea are high in antioxidants and antimicrobial properties. In addition, when shrimps are submerged in a 0.01% catechin solution for 15min, the growth of the bacteria reduces and there is a reduction in lipid oxidation and melanosis (Nirmal and Benjakul, 2009). Immersing dried-seasoned jumbo squid in a tea phenol solution also protects the seafood from bacterial spoilage, moisture loss and lipid degradation (Dong et al. 2013).

CONCLUSION

V. parahaemolyticus is mostly found naturally occurring in marine and coastal environment worldwide and it has been implicated as the main cause of gastroenteritis linked to the consumption of seafood. *V. parahaemolyticus* also can form biofilm on different contact surfaces in the food industry which can be a source of food contamination and a threat to public health. The consumption of seafood is increasing rapidly across the globe thus the contamination of seafood with *V. parahaemolyticus* possess a major threat to public health. Therefore, it is crucial to preserve seafood from *Vibrio* contamination. Different methods have been discussed in this review to effectively preserve seafood and kill *V. parahaemolyticus.*

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

JYHT, AAG, NY and SR conceived of the presented idea. NKA performed the experiments and wrote the manuscript. JYHT and AAG reviewed the manuscript. All authors read and approved the final version.

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