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Occurrence and Characterization of Potentially Pathogenic *Vibrio* Species in Seafood Products and Mari culture Systems

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Abstract: Vibrios are associated with live seafood as they form part of the indigenous micro flora of the marine environment. Results also implied that if *V. cholerae* is the concern for the abstraction of seawater for keeping live seafood, both the site whereas the water is abstracted and the *E. coli* count are important parameters to be considered. Good manufacturing practices should always be observed by the trade to minimise the risk of *vibrio* food poisoning associated with the consumption of seafood products. Hygienic quality of fish tank water in particular the source of water for keeping live seafood is also important. Processed and ready-to-eat products were contaminated with at least one of the potentially pathogenic vibrios at significant frequencies (25 and 17.5 % of samples, respectively), with the level as high as 103 to 104 per gram in some samples.

Key words: *Vibrio* species · Seafood · Bacterial Pathogens · Marine Fish

INTRODUCTION

Vibrio species, Gram negative rod-(or curved rod-) shaped bacteria, are known to occur naturally in marine and freshwater environments and thus are commonly associated with seafood and/or food of freshwater origin [1-3]. Many species can cause gastrointestinal diseases. *V. parahaemolyticus* has been frequently involved in outbreaks of food borne diseases worldwide [4-5]. *V. cholerae* also constitutes a very important risk. The serogroups O1, O139 and O141 cause cholera, while other serogroups can cause less severe diarrhea [6-7]. *V. vulnificus* is another organism of great concern in seafood safety due to the severity of the disease and the high mortality rate it can cause [8-9]. Other species that have been increasingly recognized as food pathogens in recent years are *V. mimicus* and *V. alginolyticus*. *V. mimicus* has genetic and many biochemical similarities to *V. cholerae* (Figure 1.) [10-11] and its pathogenicity involves several toxins including that of *V. cholerae* [12]. Many food borne outbreak cases involving *V. mimicus* have been reported [13-15]. *V. alginolyticus* is one of the most common *Vibrio* species occurring in marine environments and seafood [16-19]. This species is an opportunistic pathogen [20-21] and its pathogenicity is thought to be similar to that of *V. parahaemolyticus* [22].

Fig. 1: Vibrio cholera

The occurrence of *Vibrio* spp. in raw seafood is common, especially seafood from regions with temperate climates around the world, from both natural and farm environments and in seafood of all types [23-27]. However, most surveys are qualitative, which causes difficulties in evaluating the risks relating to *Vibrio* spp. in raw seafood. The level of *Vibrio* spp. in raw seafood can also affect survival of the organisms through processing. For processed and ready-to-eat seafood (including ready-to-eat products that are intended for raw consumption, such as raw oyster [28-29], the presence and level of *Vibrio* spp. has a direct impact on food safety. Cases of food borne outbreaks resulting from consumption of ready-to-eat seafood dishes, especially those supplied by food catering/food service establishments, continually occur [30-34]. Nevertheless, reports on occurrence of *Vibrio* spp. in processed and

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ready-to-eat seafood are scarcely available. A few examples are the incidence of *V. parahaemolyticus* in smoked fish [35] and cooked crayfish [36].

Vibrio parahaemolyticus and Other Vibrios: *V. parahaemolyticus* is a marine organism, which can cause human gastroenteritis. It is generally undetectable in marine water below 19 °C but may grow in culture at temperatures as low as 5°C and on food at 10°C. Only about 1% of marine isolates produce a thermostable haemolysin, which is believed to be required for virulence. Generally, only shellfish are associated with the disease and no reported cases by the Center for Diseases Control, USA, were associated with finfish between 1978 and 1998 [37]. The first report of the organism being a food borne agent was with shirasu, a Japanese boiled and semi-dried sardine dish, which was probably contaminated from an uncooked food source or an excreting food handler. In Japan, the majority of outbreaks are caused by consumption of raw fish products (e.g. sushi) [38], due to the cultural preference for raw fish dishes. Since 1996, *V. parahaemolyticus* cases have increased across the world. A unique clone of *V. parahaemolyticus* O3:K6 is responsible for many of the recent *V. parahaemolyticus* outbreaks, including epidemics in India, France, Russia, Southeast Asia, Japan and North America. This strain has been responsible for 50 to 80% of all *V. parahaemolyticus* infections since 1996 and is referred to as the pandemic strain [39]. Currently, there is no specific guideline that describes a minimum level of *V. parahaemolyticus* in sea water fish and shellfish that could potentially be hazardous to humans. Proper chilling, use of post harvest treatments and/or cooking of fresh seafood will reduce risks. *V. vulnificus* (Figure 2, a and b) is associated with warm marine and estuarine waters. Human disease caused by the organism has only been observed in conjunction with marine bivalve (mostly oysters) and some crustacean consumption and when in contact with contaminated water (no CDC reported cases from fish, 1978-2005). The bacterium causes primary septicaemia, especially in individuals with underlying diseases (patients suffer from immune and liver diseases or blood disorders), which is often fatal (>50%). There is no risk associated with the consumption of properly chilled and cooked fresh fish. *V. cholerae* O1, the causative agent of cholera, is historically associated with faecally contaminated water, but the bacterium is known to survive and grow in the shallow marine and especially estuarine environment. It is particularly associated with disease following consumption of raw oysters from warm sewage polluted waters. Again, there is no risk from the consumption of properly handled and cooked fresh fish.

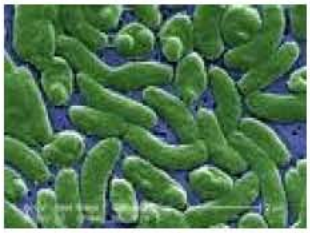

Fig. 2.a: Vibrio vulnificus

Fig. 2b: Vibrio vulnificus-Gram-negative, motile, curved rod-shaped bacteria (prokaryote).

Vibriosis: Within the Vibrionaceae, the species causing the most economically serious diseases in marine culture are *Listonella (Vibrio) anguillarum, Vibrio ordalii, V. salmonicida and V. vulnificus* biotype 2. L. anguillarum, aetiological agent of classical vibriosis, possesses a wide distribution causing a typical haemorrhagic septicaemia in a wide variety of warm and cold water fish species of economic importance, including Pacific and Atlantic salmon (Oncorhynchus spp. and S. salar), rainbow trout (Oncorhynchus mykiss), turbot (S. maximus), sea bass (D. labrax), seabream (S. aurata), striped bass (Morone saxatilis), cod (Gadus morhua), Japanese and European eel (Anguilla japonica and Anguilla anguilla) and ayu (P. altivelis). Fish affected by this classical vibriosis show typical signs of a generalized septicaemia with haemorrhage on the base of fins, exophthalmia and corneal opacity. Moribund fish are frequently anorexic with pale gills which reflects a severe anaemia. Oedematous lesions, predominantly centered on the hypodermis, are often observed [40].

Vibrio spp and seafood

Vibrios are abundant in the aquatic environment. Most of them require 2 to 3% NaCl or a seawater base for optimal growth. Vibrios are associated with live seafood as they form part of the indigenous micro flora of the environment at the time of seafood capture or harvest. Healthy live fish is protected by its immune system and therefore bacteria cannot grow in its flesh. When the fish dies, the immune system no longer functions and

the bacteria present are able to proliferate freely. In addition, bacteria may be found on the skin, chitinous shell, gills as well as the intestinal tracts of fish or shellfish [41-45]. If subsequent handling is improper and that there is no or inadequate pathogen reduction step (e.g. cooking) afterwards, the level of bacteria in the final product may increase to such an extent that may present a health risk to consumers. Molluscan bivalves are filter feeders and they tend to accumulate microorganisms in the surrounding waters which may also contain vibrios. They are usually grown and harvested in shallow, near-shore estuarine waters and are therefore likely to harbour high concentrations of pathogenic organisms including pathogenic vibrios. As they often are eaten raw or after a very mild heat treatment, they constitute a significant health risk to the consumers. Among the potentially pathogenic vibrios occurring naturally on fish and shellfish, *V. parahaemolyticus* is the most widespread. Endogenous marine species of *V. cholerae* can also be isolated from fish during cholera outbreaks. It has been suggested that vibrios are the most common bacterial causative agents in food poisoning resulting from the consumption of shellfish. Water temperature can greatly affect the vibrio levels in seafood. Vibrios can multiply rapidly between 20 and 40°C. Growth at the optimum temperature (37°C) can be very rapid and generation times of 9 to 10 minutes have been reported.6 *V. parahaemolyticus* is primarily associated with coastal inshore waters rather than the open sea. It is rarely isolated from water with temperatures below 15°C [46].

Factors Affecting Growth and Survival of Vibrios in Seafood

Effect of Temperature: Growth of pathogenic vibrios occurs optimally at around 37°C although the maximum and minimum growth temperatures are 43 and 5°C, respectively.23 All vibrios are sensitive to heat. In shellfish, heating to produce an internal temperature of at least 60°C for several minutes appears sufficient to eliminate the pathogenic vibrios.17 Chilling and refrigeration are critical control measures to prevent growth of these microorganisms.

Effect of pH and Other Factors: Vibrios are acid sensitive and grow best at pH values slightly above neutrality, i.e. 7.5 to 8.5.23. They are also sensitive to drying. While *V. parahaemolyticus* has an absolute Na+ ion requirement and shows optimal growth at about 2 to 4% NaCl, freshwater inactivates this organism [47].

DISCUSSION

The qualitative (presence-absence test) and quantitative (plate count) methods were used in parallel in order to obtain the advantages offered by both. The presence-absence test is more sensitive in revealing the presence of the organisms, while the plate count method gives the level of contamination which is more closely related to illnesses potentially caused by *Vibrio* species. The analysis of seafood or seafood-containing samples by the presence-absence test and/or plate count revealed contamination of different potentially pathogenic *Vibrio* spp. in all seafood categories (raw, processed and ready-to-eat). Contamination of raw seafood by *V. alginolyticus* was most frequent (61.5 %), followed by *V. parahaemolyticus* (43.6 %), *V. cholerae* (35.9 %), *V. mimicus* (23.1 %) and *V. vulnificus* (2.6 %). The level of contamination ranged from 50 to 4.5×10^4 per gram. Since *Vibrio* spp. can occur naturally in an aquatic environment, the presence of these organisms in raw seafood may be expected [48-49]. However, the high level (103-104 per gram) of *Vibrio* spp. in some samples of raw seafood may indicate inadequate control in storage temperature from the time of harvesting and this level is regarded as unsatisfactory by some food criteria. Furthermore, the high level (up to 104 per gram) of *V. parahaemolyticus* is regarded as potentially hazardous, considering the possibility of the contaminant strain(s) being pathogenic. These potentially pathogenic vibrios would also have an impact on safety of processed/cooked ready-to-eat food if they survive insufficient processing/cooking conditions, or they could be an important source of recontamination after processing. Contamination of pathogenic *Vibrio* spp. in industrially processed seafood products and ready to-eat seafood dishes. Industrially processed and ready-to-eat seafood samples were contaminated with the potentially pathogenic *Vibrio* spp. at significant frequencies. The presence of *V. cholerae* and *V. parahaemolyticus* (as well as other vibrios) in industrially processed and ready-to-eat foods is contrary to what is expected [50]. This also applies to other vibrios. Industrially processed seafood products, such as fish/shrimp balls and sea sticks, generally are passed through a pasteurization process, which should eliminate all non-spore-forming microorganisms. As for the ready-to-eat seafood dishes examined, most were cooked dishes, except one that was intended for raw consumption (prawn in fish sauce). The occurrence of *Vibrio* spp. in processed and cooked ready-to eat seafood indicated insufficient

processing or post-process contamination. Since contamination of vibrios in seafood is a problem worldwide (as reviewed above) and the same is assumed for processed and ready-to-eat seafood products, the results of this study are therefore believed to be implicative also for geographical areas other than Thailand. The occurrence or level of the potentially pathogenic *Vibrio* species presented here indicates risks in consumption of undercooked or re-contaminated processed and ready-to-eat (including uncooked) seafood and reaffirms the need to enhance their safety quality. The safety of industrially processed seafood and ready-to-eat seafood available at markets/supermarkets or food catering establishments can be of great significance for public health [51]. Preventive measures such as proper handling and storage of raw seafood, effective reduction of *Vibrio* spp. in seafood used as raw material and strict control of safety quality along food processing and food preparation processes (especially in the food industry and food catering unit) should be urgently applied. To promote the safety of ready-to-eat food, of which quality monitoring cannot be carried out routinely, educating food service personnel seems to be the most promising solution.

In conclusion, if pathogenic *Vibrio* species are present in shrimp pond water, it is likely that they could also be present in the shrimp itself, with the implicit consumer health risk, particularly in regions whereas raw shrimp is consumed. They can also become a major vector for cross contamination when not properly handled. However, in order to prevent possible adverse effects of microorganisms living in polluted waters, necessary hygienic measurements in all production steps (harvesting, transporting, processing, etc.) should be taken. In addition, heat treatment during cooking process should be efficiently done in order to minimize food borne diseases.

Consumers Have to View below Items

- Consume seafood as soon as possible.
- If seafood is not consumed immediately, they should be:

- Packed and stored at 4°C or below.
- Separated from raw food.
- Consumed within 1 to 2 days.
- Avoid consuming the internal organs of shellfish.
- The elderly, children, pregnant women and persons with lowered immunity should be careful when choosing food especially high risk food, such as sashimi and oysters to be eaten raw.

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