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Review Article

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Review on Pathogenic Vibrios from Marine Food Resources

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Abstract

This present study focused on the isolation of *Vibrio* species from marine food resources such as fish and crustacean. Also to study the resistant pattern of isolated vibrios against various antibiotics to unravel their resistance pattern in antibiogram and study the impact of ecological factors on its survivability.

Introduction

Seafood especially shellfish is a food substrate for some zoonotic Vibrios of which these microorganisms, cause food poisoning and diarrhea in human (Merwad *et al.*, 2011). Sea foods are prone to bacterial contamination and could cause health risk to consumers (Wafaa *et al.*, 2011). Vibrios are associated with live seafood as they form part of the indigenous microflora of the marine environment (Adeleye *et al.*, 2010). The aquatic ecosystem is the natural habitat of microorganisms belonging to the *Vibrio* genus and the most known is *Vibrio cholerae*, the causative agent of cholera. Otherwise, many species are able to transmit intestinal or extra intestinal diseases to human (Rodrigues *et al.*, 2001).

Pathogenic *Vibrios* have been a public health concern for seafood consumers and have been cause of import bans, detentions and rejections in international fish trade (Wafaa *et al.*, 2011). *V. parahaemolyticus* and *V. vulnificus* are part of the natural flora of estuarine and coastal marine environments worldwide and have been isolated from sea and brackish water of both tropical and temperate regions, sediments and a variety of seafood especially shellfish and bivalve mollusks (Kirs *et al.*, 2010).

Vibrio infections are generally acquired either through ingestion of foods and water contaminated with human faeces or sewage, raw fish and seafood, or they are associated with the exposure of skin lesions, such as cuts, open wounds and abrasions, to aquatic environments and marine animals (Toti *et al.*, 1996; Lee and Younger, 2002). Food borne hazards are still of

great concern for human health and in particular the risks connected with shellfish and seafood consumption continue to be important both in developing and developed countries despite the advances in technology, changes in food processing and packaging (Feldhusen, 2000; Huss *et al.,* 2000; Egli *et al.,* 2002).

The members of Vibrionaceae are the natural pathogens of the aquatic environment, which is also inhabited by shellfish, shrimp and other aquatic organisms (Colakoglu *et al*., 2006; Mahbub *et al*., 2011; Ebrahimzadeh Mousavi *et al*., 2011; Raissy *et al.,* 2011). Vibrios are among the most important bacterial pathogens of cultured shrimp responsible for a number of diseases, and mortalities up to 100% have been reported due to this disease. *Vibrio* species are a normal part of the bacterial flora in aquatic environments and formerly considered to be mostly opportunistic pathogens (Mahbub *et al.,* 2011; Raissy *et al.,* 2011).

Vibrio is a genus of bacteria indigenous to the aquatic environment also contaminant of raw or under cooked seafood. Some bacterial species of this genus are now considered as emerging pathogens, involved in food-borne infections in humans (Gopal *et al.,* 2005; China *et al.,* 2003). The genus *Vibrio* are Gram negative, curved, rod shaped, halophilic, non-spore forming bacteria, autochthonous inhabitants of the marine and estuarine environment. They occur in saline aquatic environments, both free in the water and bound to animate and inanimate surfaces (Huq *et al.,* 1983; Montanaria *et al.,* 1999).

The members of the family Vibrionaceae are a significant component of the microflora which includes more than 64 species (Thompson *et al.,* 2005). Few of these reports however, have looked at the likely risks from a microbiological food safety point of view (Okonko *et al.,* 2009). In the last 20 years, many halophilic *Vibrio* species such as *V.parahaemolyticus, V.alginolyticus, V.vulnificus, V. hollisae, V. fluvialis V.mimicus, V. furnissii* and *V. damsella,* have been implicated inhuman enteric infections, wound infections and septicemia due to the consumption of shellfish and exposure to seawater (Thompson *et al.,* 2004; Merward *et al.,* 2011).

They account for a significant proportion of human infections such as gastroenteritis usually associated with consumption of raw or undercooked seafood, wound infections, septicemia and ear infections (Adeleye *et al.,* 2010). Most of these vibrios secrete enterotoxins in foods, water or in the gastrointestinal tract (Nishibuchi and DePaola, 2005). Antibiotics and other chemotherapeutic agents are commonly used in fish farms either as feed additives or immersion baths to achieve either prophylaxis or therapy. It was observed that individual and multiple antibiotic resistances were associated with antimicrobial use. Acquired antibiotic resistance in bacteria is generally mediated by extra-chromosomal plasmids and are transmitted to the next generation (vertical gene transfer) and also exchanged among different bacterial population (horizontal gene transfer). Extensive use of these antibiotics has resulted in an increase of drug-resistant bacteria as well as R-plasmids (Son *et al.,* 1997).

Marine *Vibrio* species are known to produce various extracellular products, some of which are known pathogenicity factors (Hasegawa *et al.,* 2008). These toxic proteins include cytolysins, proteases, lipases, siderophores, exopolysaccharides, and effectors delivered via Type III secretion systems (Hasegawa *et al.,* 2008). Secades and Guijarro (1999) reported that stress environmental conditions could play an important role in the induction or repression of the enzyme by specific compounds. Production of extracellular proteases has been shown to be sensitive to repression by different carbohydrate and nitrogen sources (Haulon *et al.,* 1982). Faced with starvation conditions in seawater, *V. alginolyticus* cells have been shown to undergo strong structural and metabolic modifications under laboratory conditions (Ben Kahla-Nakbi *et al.,* 2006).

V. parahaemolyticus has been frequently involved in outbreaks of food borne diseases worldwide (Crump *et al.,* 2003; Dalsgaard *et al.,* 1999).

V.parahaemolyticus is often isolated from seawater, sediment and a variety of seafood including shrimp, crab, oyster and clam due to its halophilic characteristics (Merwad, *et al.*, 2011). This bacterium is one of the leading causes of food borne gastroenteritis associated with ingestion of undercooked shellfish throughout the world including the United States, China, Japan and Korea (Merwad *et al.*, 2011). Also, this microbial infection is characterized by diarrhea, vomiting, nausea, abdominal cramps and low grade fever (Pinto *et al.*, 2008).

V. alginolyticus is a halophilic *Vibrio* first recognized as being pathogenic in humans in 1973 (Zen-Yoji *et al.*, 1973). Wound infections account for 71% of *V. alginolyticus* infections. Ear infections are also seen with this organism. Gastroenteritis was thought to be a rare presentation of *V.alginolyticus* infection, but it accounted for 12% of infections in one series (Hlady and Klontz, 1996). Other clinical syndromes reported in association with *V. alginolyticus* infection include chronic diarrhea in a patient with AIDS (Caccemese and Rastegar, 1999), conjunctivitis (Lessner *et al.*, 1985) and post-traumatic intracranial Infection (Opal and Saxon, 1986). Resistance to tetracycline and chloramphenicol has been reported in a few isolates of *V.alginolyticus*, but all strains appear to be sensitive to ciprofloxacin (French, 1990).

V. alginolyticus is considered one of the most frequent species living freely in water and sediment and can survive in sea water even in famine conditions while maintaining their virulence (Kahla-Nakbia *et al.*, 2007). The first reports identifying *V. alginolyticus* as possessing the *trh* gene occurred in Alaska and in Tunisia. In addition, it has been shown that strains of *V.alginolyticus* carry the *trh* gene and the pathogenic *V.alginolyticus* strains is recognised as a potential reservoir of many known virulence genes of other *Vibrio* species in the aquatic environment which have been demonstrated to contributes to the onset of wound infections, enteric pathologies, septicaemia and peritonitis in humans by exposure to seawater (Masini *et al.*, 2007).

V.alginolyticus is a Gram negative, asporogenous rod that is either straight or has a single rigid curve. Cells are motile, mostly having singular polar flagellum when grown in liquid medium. Most members of the genus *Vibrio* produce oxidase and catalase enzymes and ferment glucose without producing gas, besides being pathogenic (Kaysner and DePaola, 2001). *V.alginolyticus* is an opportunistic pathogen to human and marine animals (Zhao *et al.*, 2010; Reilly *et al.*, 2011). The outbreaks of *V. alginolyticus* infections increase rapidly during summer seasons (Sganga *et al.*, 2009). Most of these infections stemmed from contact of cuts to contaminated seawater (Reilly *et al.*, 2011). Infection by *V. alginolyticus* causes otitis, endophthalmitis and wound infections (Austin, 2010; Reilly *et al.*, 2011).

V. alginolyticus is considered to be a part of normal marine flora (Austin *et al.*, 1995; Vandenberghe *et al.*, 1998). However, some studies have shown its virulence to aquatic animals (Lighter, 1993). Despite the boom of fish farming in Guangdong, China, the whole industry was badly hampered by the fish mortality because of vibrosis, where *V. alginolyticus* is the dominant causative species. 16S genes and part of the toxR sequence of *V.alginolyticus* and *V. parahaemolyticus* are highly homogeneous (Osorio and Klose, 2000). Previous studies have shown that *V. parahaemolyticus* is closely related to either tdh or trh or both genes (Iida *et al.*, 1998), but no results have been reported yet clarifying which virulence genes of *V.alginolyticus* are critical for its pathogenicity. Only a few studies were conducted to search *V. cholerae* virulence genes in *V. alginolyticus* in Sardinia, Italy (Sechi *et al.*, 2000), but none in Guangdong. No information is available on the presence of homologous *V. parahaemolyticus* virulence genes in *V. alginolyticus*. Therefore, it is necessary to investigate whether *V.parahaemolyticus* and *V.cholerae* virulence genes present in *V.alginolyticus* in Guangdong. Additionally, we have to assess whether *V.alginolyticus* pathogenic strains have arisen, and if there is a correlation between the virulence

and possession of *V. parahaemolyticus* and/ or *V.cholera* virulence genes in *V. alginolyticus*.

As a representative of the halophilic vibrios, *V. alginolyticus* is isolated from coastal waters and sediments all over the world (Uchiyama, 2000; Hsieh *et al.*, 2007) and is considered to be part of the normal marine microflora. However, *V. alginolyticus* is an important bacterial pathogen of humans, causing wound infections, otitis media, otitis externa, endophthalmitis and gastrointestinal infection (Reina *et al.*, 1995; Li *et al.*, 2010). This bacterium also belongs to the most important opportunistic pathogens of aquatic animals, including fish, shellfish, crustaceans, coral and echinoids, causing serious disease and damage in cultured fish and important economic losses (Liu *et al.*, 2004). Several virulence factors, including the iron uptake system (Litwin and Calderwood, 1993), extracellular haemolysin (Lee *et al.*, 1996) and proteases are suggested as the major contributors to pathogenicity in this species.

These bacteria have been found in water samples from marine environment, mussels, fish and their products. Since the end of the 80s, the presence of these microorganisms was correlated to human infections. A large number of *Vibrio* species are pathogenic to humans, marine vertebrates and invertebrates, by producing and secreting various virulence factors such as enterotoxin, haemolysin, cytotoxin, protease, lipase, phospholipase, siderophore, adhesive onto surfaces and haemagglutinins (Austin and Austin, 1999; Shinoda, 1999). The most common type of virulence factors among *Vibrio* species is a haemolysin, an enterotoxin (Lida and Honda, 1997; Shinoda, 1999) which strikes the erythrocyte membranes causing them to lyse and leading to emancipation of iron-binding proteins (haemoglobin) as well as lysing of the blood cells (Lida and Honda, 1997; Shinoda, 1999). The infection sites (the medium ear, conjunctive membrane, open wounds) were hosting a complex microbial flora. Infections caused by *V. alginolyticus* can present themselves as septicemic related to the ingestion of contaminated seawater or seafood products especially in patients as elders, children, carriers of diabetes mellitus or chronic hepatic pathologies (Carli *et al.*, 1993).

Bacterial resistance to antibiotics has become an emerging medical issue threatening the public health because of wide availability of antibiotics and sometimes misuse of drugs without proper prescription (Davies and Amabiles-Cuevas, 2003). More and more pathogenic bacteria have shown resistance to one or many of the antibiotics. It has been observed that antibiotic susceptibility of *Vibrio* species is dynamic and varies with the environment (Ottaviani *et al.*, 2001; Jun *et al.*, 2003). According to Nambabi *et al.* (2010), the ability of *V. alginolyticus* to survive and grow in treated and disinfected seawater, preceded to successive contamination of hake fish, henceforth, be symbolic of related *V. parahaemolyticus*, should this pathogen be present. It is therefore, significant that this center of contamination be controlled to diminish the potential health hazard to consumers.

Vibrios come on the top list of pathogens with direct jeopardy to mariculture development due to high mortalities associated with their invasion to fishes. It is crucial to know that, Vibrios are ubiquitous to marine environment, while clinical disease outbreaks only occur when a sharply stressed fish get exposed to the flaring up infectious agent (Austin and Austin, 2012). Septicemia induced by Vibriosis is characterized by haemorrhages on the base of pectoral fins, exophthalmia, loss of appetite and edematous lesions on the body surface (Toranzo *et al.*, 2005).

V. alginolyticus and *V.parahemolyticus* are responsible for mass mortalities among fish stocks in many marine fish farms throughout the Mediterranean area and severe economic losses in aquaculture worldwide (Snoussi *et al.*, 2008). *V.alginolyticus* causes many epizootic outbreaks among the Gilthead seabream and European sea bass populations, which possess high economic value at the Mediterranean communities (Zorrilla *et al.*, 2003).

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