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REVIEW ARTICLE

VIBRIO ALGINOLYTICUS EMERGING FOOD BORN PATHOGEN

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ABSTRACT

Vibrioinfections are becoming increasingly common worldwide. Pathogenic Vibrio's cause 3 major syndromes of clinical illness: gastroenteritis, wound infection and septicemia. *Vibrio alginolyticus* is a natural host estuarine and coastal waters as well as sea food and cannot be eradicated in these niches. This bacterium capable of carrying the pathogenic gene trh is a threat to public health. Epidemiologic data suggest that the majority of these infections are foodborne and associated with consumption of raw or undercooked shellfish. In addition, it is increasingly recognized as a potential threat to humans by causing food poisoning, intestinal inflammation, and wound infections. The aim of this paper is to summarize the literature on *Vibrio alginolyticus* responsible of enteric and other diseases, its ecology, pathogenicity and visibility with description methods for identification and give summary of preventive measurements to fight against food borne illness associated with *Vibrio alginolyticus.*

INTRODUCTION

Among the three major habitats of the biosphere, the marine realm which covers 70% of the earth's surface provides the largest inhabitable space for living organisms, particularly microbes. The terms "Marine Microbes" covers a diversity of microorganisms, including microalgae, bacteria and archaea, protozoa, fungi and virus. These can be prokaryotes and eukaryotes. Disease outbreaks in marine organisms appear to be escalating worldwide and a growing number of human bacterial infection have been associated with recreational and commercial uses of marine resources (Baffone *et al*., 2005; Ben kahla-Nakbi *et al*., 2007). A surprising number of vibrio species have been reported from marine environments (Gomez-Leon *et al*., 2005; Hidalgo *et al*., 2008; Belcazar *et al*., 2010) and the probability of their transmission to humans is correlated with abiotic factors that affect their distribution, especially the temperature of seawater during the summer (Croci *et al*., 2001; Thomson *et al*., 2006). In other hand, *Vibrio alginolyticus* is considered as marine fish and shellfish pathogen (Gomez leon *et al*., 2005). This bacterium is a common inhabitant of the marine environment in both temperate and tropical waters and associated with high mortality in aquaculture systems. *Vibrio alginolyticus* is associated with human infections related to consumption of raw or undercooked sea products causing severe gastroenteritis and extra-intestinal disease (wound, intracranial infection in immunocompromised and cirrhotic patients). This illnessoccurs frequently during the summer related to an increase in the water temperature (Croci *et al*., 2001; Thompson *et al*., 2004). This microorganism produces many extracellular proteases responsible for interaction between the bacterium and cell host and play an important role in human infection and fish pathology (Ottaviani *et al*., 2001; Thomson *et al*., 2004). The mechanism of pathogenicity is still complex and related to several factors including cytotoxin, enterotoxins, lytic enzymes (Ottaviani *et al*., 2001; Rim lajnef et al., 2012).

Vibrio alginolyticus

*Vibrio alginolyticus*is a halophilic (salt-tolerant) Gram-negative bacterium found naturally in temperate marine and estuarine environments. This species is recognised as a human pathogen and the incidence of infection significantly increases during summer months (Morris *et al*., 1985). *Vibrio alginolyticus* is the most commonly isolated vibrio species in marine environments from all over the world. Its numbers correlate with increase in temperature (Oliver and Kaper, 1997). It has been isolated from both fin fish and shell fish (Shikongo-Nambabi *et al*., 2012).

Isolation and Identification of Vibrio alginolyticus

Vibrio species are non-fastidious and grow readily on basic laboratory media, but some need supplementation of vitamins,

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amino acids and minerals. They grow better at alkaline pH (7.5 to 8.5) and require added NaCl. The optimum growth temperature ranges from 15 to 30°C (Thompson *et al*., 2004). Most Vibrio species grows on Mac Conkey agar, but do not ferment lactose. Isolation of Vibrio species from environmental sources is usually done by pre-enrichment step in Alkaline Peptone Water (APW), pH 8.6 supplemented with 1to2% NaCl, followed by plating on solid medium such as Thiosulphate Citrate Bile Salts Sucrose (TCBS) agar. Enrichment media are normally incubated at room temperature (18 to 22°C), while solid media are incubated at 25°C. TCBS is a selective differential media that incorporates bile salts, alkaline pH (8.6) and 1% NaCl as selective agents, sucrose as a fermentable sugar and bromothymol blue as the pH indicator. On TCBS, sucrose fermenters form yellow colonies, while non-sucrose fermenters are green (Shikongo - Nambati *et al*., 2012).

Table 1. Biochemical Characterization-Typical morphology of Vibrio colonies on TCBS agar

Organisms	Growth	Color of colonies
Vibrio alginolyticus	+++	Large yellow colonies
Vibrio parahaemolyticus	+++	Blue colonies with green centers
Vibrio cholerae	+++	Large yellow colonies
Vibrio fluvialis	+++	Yellow colonies
Vibrio vulvificus	+/++	Yellow greenish yellow colonies
Vibrioharveyi / Vibrio fischeri	+/++	Grey to bluish green colonies

Table 2. Biochemical reactions of Vibrio alginolyticus

Test	Reactions
Growth on sheep blood agar (Swarming)	+
Growth on Mac Conkey agar	+
Growth in nutrient broth with	
0% Nacl	_
1% Nacl	+
6% Nacl	+
8% Nacl	_
10% Nacl	_
Motility	+
Lysine decarboxylase	+
Ornithine decarboxylase	+
Cytochrome oxidase	+
Lipase	+
Catalase	+
Carbohydrate fermentation	
Glucose	+
Mannose	+
Mannitol	+
Maltose	+
Sucrose	+
Glycerol	+
Galactose	+
Inositol	_
Sorbitol	_
Rhamnose	_
Gelatinase	+
Nitrate Reduction	+
Simmons citrate	+
Indole	+
Jordan tartrate	_
Voges - Proskauer -	_
Phenylaline deaminase -	_
Tryptophan deaminase -	_
Beta-galactosidade -	_
Arginine dihydrolase -	_
Hydrogen sulfide	_
Sodium malonate	_
Sodium acetate	_
Melibioze	_
Amygdalin	_
Lactose	_
Raffinose	_
Xylose	_
Cellobiose	_
Erythritol	_
Esculin	_
	_

Table 3. Phenotypic traits used to differentiate between *V. parahaemolyticus* and *V. alginolyticus* are (Farmer *et al*., 2004 and Oliver and Kaper, 1997)

Phenotypic test	*V. parahaemolyticus* (%)	*V. alginolyticus* (%)
Voges – Proskauer (VP) test in 1% NaCl	80 - 95	0
Urea hydrolysis	15	0
Cellobiose	5	3
Dulcitol	3	0
Sucrose	1	99
ONPG	5	0
L-Arabinose	80 - 89	0 – 1
Growth in nutrient broth with		
10 %	0 - 2	69 – 100
12%	0 - 1	17 - 100

Different Methods Used for Bacterial Identification

An array of molecular techniques is gaining popularity for the identification of different aquaculture-related bacterial pathogens. Suitable genetic fingerprinting methods are essential for rapid and accurate tracking of different marine vibrio's. Among DNA sequence-based identification, analysis of 16S rRNA and other housekeeping gene sequences are the most popular and precise methods currently used to identify closely related Vibrio. Among other methods, ribo typing and PCR-based techniques, e.g., Amplified Fragment Length Polymorphism (AFLP), Fluorescence In Situ Hybridization (FISH), Random Amplified Polymorphic DNA (RAPD), repetitive extra genic palindrome–PCR (rep-PCR), and Restriction Fragment Length Polymorphism (RFLP) have yielded the most valuable information and new insights into the identification of closely related marine bacteria. Below we discuss some of these methods commonly used for identification.

PCR-based identification

Although there are a handful of methods for the identification of marine Vibrioas described above, the majority require two or more step approaches like PCR and sequencing (16S rRNA and MLST), PCR and digestion with restriction enzymes (PCR-RFLP, AFLP), or the use of radioisotope labelled probes which are expensive, time-consuming and hazardous for health. A simple and rapid identification method of Vibrio-related disease to aquaculture settings is essential for taking preventive and curative measures in aquaculture. PCR-based identification is a suitable alternative because it is comparatively easy, less expensive and can be completed within several hours. However, success of this method depends on the selection of target gene, which should be species-specific, widely distributed and also stable in the genome (Shruti Chatterjee and Soumya Haldar, 2012).

Virulence factors and Pathogenicity of Vibrio alginolyticus

Vibrio alginolyticus is one of the commonest infectious pathogen affecting marine vertebrates and invertebrates which includes fish, shrimp and mollusk. Studies have shown that strains of *V. alginolyticus* are potential reservoir of many virulence genes in the aquatic environment that may contribute to the development of wound infections, enteric diseases and sepsis in humans by exposure to seawater. Previous study indicated that there are several major virulence genes contributing to the virulence of the pathogens such as

Table 4. Cycling condition for PCR of 16S rRNA and virulence genes of *V. alginolyticus*

Genes	Initial denaturation	Denaturation	Annealing	Extension	Cycles
16S rRNA	94 ℃ for 3 min	94 ℃ for 1 min	55 ℃ for 1 min	72 ℃ for 1 min	30
Collagenase	95 ℃ for 5 min	94 ℃ for 30 sec	57 ℃ for 30 sec	72 ℃ for 50 sec	34
ToxR	94 ℃ for 4 min	94 ℃ for 1 min	54 ℃ for 1 min	72 ℃ for 1 min	35
OmpK	94 ℃ for 4 min	94 ℃ for 1 min	58.1 ℃ for 1 min	72 ℃ for 1 min	35

Table 5. Primers used for amplication of virulence genes of *V. alginolyticus*

Target genes	PCR primer sequences (5'-3')	Product sizes	References
Collagenase	VA-f:CGAGTACAGTCACTTGAAAGCC VA-r;CACAACAGAACTCGCGTTACC	737bp	Di pinto et al.,2004
Tox R	f:GATTAGGAAGCAACGAAAG r:GCAATCACTTCCACTGGTAAC	658	Xie et al.,2005
Ompk	f:GGCGGTCGCTCTGGTATT 319Cai et al.,2009 r:TTGCCATCGTAAGTGCTGTA		

Tox R codes for Toxin R, Omp K codes for outer membrane protein K.

Outer membrane protein (OMP), *thermolabile hemolysin (TLH), collagenase, toxR, toxRS* and also cholera toxin. For instance, *OMP* is believed to play important roles in infection and pathogenicity to the host and the expression of this gene is controlled by *tox R* gene. *Collagenase* has been widely utilized as biomarker in molecular identification of *V. alginoyticus* as well as capable of degrading conjunctive tissue, basal epithelial membrane that leads to extra intestinal pathology and dissemination to blood stream. Although the mechanism by which the organism infects humans has yet to be comprehensively determined, *collagenase, ompK,* and *toxR* has been recognized as primary virulence factors in *V. alginolyticus*. The illness occurs frequently during the summer related to an increase in the seawater temperature. This microorganism produces many extra cellular proteases responsible for interaction between the bacterium and cell hosts (human and animals) and plays an important role in human infections and fish pathologies (Lee 1995). The mechanism of pathogenicity induced by vibrio infections is still complex and related to several factors including cytotoxins, enterotoxins and lytic enzymes (Lyer *et al*., 2000, Ottaviani *et al*., 2001). The adhesive properties of vibrio species are a key factor of bacterial pathogenicity. This ability may represent potential infection risk for human and aquatic stressed animals (Mejdi snonssi *et al*., 2008).

The mode of infection and transmission of this species remains to be studied, a transmission paths are probably sea water. Studies have considered strains of *Vibrio alginolyticus* as a potential reservoir of many virulence genes known in other vibrio species in the aquatic environment which may contribute to the development of wound infections, enteric diseases and sepsis in humans by exposure to sea water (Lajnef *et al*., 2008; Majdi snoussi *et al* 2008). The first reports showed that identification of *Vibrio alginolyticus* has the trh gene occurred in Alaska (Narjol *et al*., 2006) and Tunisia (Ben Kahla-Nakbi *et al*., 2006). In addition, it has been shown that in Morocco, strains of *Vibrio alginolyticus* carry the trh gene is a virulence gene associated with a positive kanagawa phenomenon (Sabir *et al*., 2012). Indeed, in human, *Vibrio alginolyticus* can be isolated from skin infections, often as a result of contact with sea water (Scheftel *et al*., 2006). Similarly, *Vibrio alginolyticus* was isolated from the pus of ear and spitting, while being responsible for conjunctivitis infection and tissue necrosis and opacification of the sphenoid sinus (Lopes *et al*., 1993). In addition, it has been shown responsible for gastroenteritis and peritonitis in human. Studies have report infection with *Vibrio alginolyticus* have caused mortality in immunocompromised patients. The first case of septic shock due to *Vibrio alginolyticus* in a cirrhotic patient has been reported in Korea after eating sea food (Sabir Mustapha *et al*., 2013). *Vibrio alginolyticus* is associated with white spot in shrimp in India and Taiwan while the zoonotic hazard of this pathogen has been implicated in ear, soft tissue and wound infections in human (Adebayo-Tayo *et al*., 2011). *Vibrio alginolyticus* is largely opportunistic pathogen causing systemic infections in persons with underlying diseases such as the immune compromised individuals, these with severe burns, cancers or with a history of alcohol abuse (Oliver and Kaper, 1997) though it has occasionally been associated with cases of gastroenteritis and diarrhoea. In healthy individuals *Vibrio alginolyticus* associated with extra intestinal infection such as wound or ear infection (Novotny *et al*., 2004). The bacterium was also isolated from the blood of a leukaemia patients alongside *Pseudomonas aeruginosa* (Oliver and Kaper 1997). *Vibrio alginolyticus* is also important food spoilage organism producing histamine by the decarboxylation of histidine and is responsible for scombroid poisoning characterised by nausea, vomiting, abdominal cramps, neurological disorders and skin irritations (Novotny *et al*., 2004; Ray and Bhunia, 2008).

Case studies

The species *Vibrio alginolyticus* was isolated in 1997 from patients during an outbreak of acute enteric illness in Vladivostock, Russia (Smolikova *et al*., 2001). Some foodborne illness cause by *Vibrio alginolyticus* were identified in 96 cases after eating brine shrimp in Chifeng Hongsthar China in 2004 (Xie *et al*., 2005). Reporting 47-year-old female patient who has sea bathing history, left ear discharge complained was present for nearly three months. In Otoscopic

examination, left modified radical Mastoidectomy cavity was realized. Ear discharge sample in stuart transport medium was inoculated in BAP and EMB agar medium and incubated at 37ºC overnight. This resulted in a pure growth of a Gram-negative bacterium. The colonies had smooth, convex morphology and were creamy in consistency and gray with full edges on BAP agar. Based Biochemical reaction and Microscopic observation this strain was initially identified as *Vibrio alginolyticus* by the Phoemix 100 automated microbial system (Becton Dickinson Diagnostics, USA) (Burak Ekrem citil *et al*., 2015). The Monastir-Sayada seacoast is characterised by an algal bloom distribution during all the year especially during the three months of the summer related to an increase in the temperature with a rare faction of fish capture. This bathing area is characterised by a traditional fishing activities practised especially by old fisherman. Near this area a fish form is installed and the *Vibrio alginolyticus* is always isolated from seawater, fish larvae and older specimens and is associated with mass mortality during the summer (Snoussi *et al*., 2008). Wound infections account for 71% of *Vibrio alginolyticus* infections. Ear infections are also seen with this organism. Gastroenteritis was thought to be a rare presentation of *Vibrio alginolyticus* infection, but it accounted for 12% of infections in one series. Other clinical syndromes reported in association with *Vibrio alginolyticus* infection include chronic diarrhoea in a patient with AIDS, conjunctivitis, and post-traumatic intracranial infection (Nicholas and Daniels *et al*., 2000).

There was a case report in July 2011, a woman in her 70s presented to the dermatology clinic in Guernsey in the channel Isles, British Isles, with a non-healing infected wound on her lower leg. The patient was not receiving any medication, was otherwise healthy and had no past history of diabetes or other chronic conditions. Two weeks previously the patients had injured her leg on a plant pot in the garden. The patient continued her habit of swimming regularly in the sea off Guernsey and also cleaned the wound with a salt water solution made up at home and applied a seaweed dressing. On examination crusing erythema surrounding the wound was noted, indicative of an infection. A charcosal swab was taken for bacteriological culture and this resulted in the pure growth of gram-ve bacterium. Based on the Physiochemical analysis, Molecular characterisation and other test it was conformed as *Vibrio alginolyticus* (Reilly *et al*., 2011). *Vibrio alginolyticus* wound infections are rare in India with sporadic cases previously reported in the UK (Hartley *et al*., 1991) The Netherland (Schets *et al*.,2010) and Denmark (Holf *et al*., 2006). *Vibrio vulnificus* is the most virulent species of non-cholera vibrio's. Actually, *Vibrio alginolyticus* is considered comparatively non-pathogenic in human in contrast to *Vibrio vulnificus*. It has been rarely reported that *Vibrio alginolyticus* causes infections. Most clinical isolates are recovered from superficial wound or external ear infection. Conjunctivitis, acute gastroenteritis, bacteraemia and necrolising fasciitis caused by *Vibrio alginolyticus* have been reported (Burak Ekrem citil *et al*., 2015).

Vibrio species as food-borne pathogens

Vibrio's are responsible for a number of clinical conditions such cholera, gastroenteritis, septicaemia and wound infections (Jay *et al*., 2005; Oliver and Kaper, 1997; Thompson *et al*; 2004). Twelve Vibrio species have been documented as potential food-borne disease agents in human: *Vibrio cholera, Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio alginolyticus, Vibrio funissii, Vibrio fluvialis, Vibrio damsela, Vibrio mimicus, Vibrio hollisae, Vibrio cincinatiencis, Vibrio harveyi* and *Vibrio metchnikovii* (Adams and Moss, 2008; ICMSF, 1996; Thomson and swings, 2006). A few species are pathogens of fish while some other species are involved in coral bleaching (Thomson *et al*., 2004). Vibrio species are transmitted to humans mostly via sewage contaminated water or sea food (finfish, molluscus and crustaceans) when consumed raw or partially cooked (De paola *et al*., 2000; ICMSF, 1996; Oliver and Kaper, 1997). Though vibrio species have been isolated from marine environments, poor processing practises are regarded as the major cause of the food contamination (Kaysuer *et al*., 1992). The bacteria may persist in the food depending on storage temperatures, pH and the product water activity (ICMSF, 1996) until the food is consumed, thereby causing disease. The level of vibrio's in sea water is generally high during summer months, but very low or undetectable during winter when cultural methods of detection are used due to the ability of these organisms to revert into a dormant unculturable state (White house *et al*., 2010).

Table 6. Association of vibrio species with different clinical syndrome. (Nicholas and Daniels *et al*., 2000)

Organism	Gastroenteritis	Wound infection	Primary Septicaemia
Vibrio alginolyticus	+	++	-
Vibrio cholerae non-01	++	+	+
Vibrio cholera 01	++	-	-
Virbio cincinnatiensis	-	-	-
Vibrio damsela	-	++	-
Vibrio fluvialis	++	(+)	(+)
Vibrio furnissii	++	-	-
Vibrio hollisae	++	(+)	(+)
Vibrio metachnikovii	(+)	-	-
Vibrio mimicus	++	(+)	(+)
Vibrio parahaemolyticus	++	+	(+)
Vibrio vulnificus	+	++	++

+, less common presentation; ++, common presentation; (+), rare presentation; -, No presentation.

Table 7. Recommended antimicrobial therapy for Vibrio infections (Nicholasand Daniels *et al*., 2000)

Vibrio species	Recommended Antimicrobial Agent
Vibrio cholerae 01 or 0139	No antimicrobial therapy, oral rehydration only
Mild Moderate to severe	Doxycycline, 300mg (Single dose), or Ciprofloxacin, 1g (Single dose), or Norfloxacim, 400mg bid for 3days.
Non-choleraeVibrio	No antimicrobial therapy, oral rehydration only
Gastroenteritis Mild	Ciprofloxacin, 500mg PO bid for 3days, or Deoxycycline, 100mg PO bid for 3days, or Norfloxacin, 400mg bid for 3days
Moderate to severe	Ceftazidime, 2g IV tid, orCefotaxime, 2g IV tid, and / or Doxycycline, 100mg IV bid, or Ciprofloxacin, 400mg IV bid.
Wound infection/cellulitis Septicemia	Ceftazidime, 2g IV tid, or Cefotaxime, 2g IV tid, and /or doxycycline, 100mg IV bid, or Ciprofloxacin, 400mg IV bid.

Treatment

Most report of *Vibrio alginolyticus* wound infections results from exposure of cuts or abrasions to contaminated seawater.

Vibrio alginolyticus associated infections may be resolved using appropriate antibiotics, however, very rarely these infections can progress to bacteraemia and necrotising fasciitis, particularly in the immunocompromised (Reilly *et al*., 2011; Sabir Mustapha *et al.,* 2013). *Vibrio alginolyticus* is resistance to tetracycline and chloramphenicol has been reported in a few isolates of *Vibrio alginolyticus*, but all strains appear to be sensitive to ciprofloxacin (Nicholas and Daniels *et al*., 2000). Strikingly, as with other pathogenic vibrio species, cases appear to be correlated with warm surface seawater temperature, and it has been suggested that the number of infections may increase with warming of coastal regions attributed to climate changes. Recent reports have suggested that the number of bathing water-associated vibrio cases in northern Europe are increasing (Baker-Austinc *et al*., 2010).

Prevention

Patients who admit to have otorrhea and skin infection symptoms especially from temperate region should be questioned for sea bathing history. To identify the phenotype and to interpret the antibiotic susceptibility profiles of the microorganism, bacteriological culture samples must be taken before start of treatment. This property in the medical history should be shared with the microbiology laboratory in order to be guiding to define pathogen. Given that *Vibrio alginolyticus*is a bacterial pathogen, prevention is the only way to avoid food poisoning incidents or following contact with a contaminated environment. Indeed, the water and seafood monitoring should allow the detection of potentially pathogenic isolates, especially during the warmer months when the bacterial concentration is high (Sabir Mustapha *et al*., 2013).

Conclusion

The route of transmission from the environment to human includes marine accidents and deep contact between fishermen and saline seawater. The emergence of *V. alginolyticus* as a pathogen responsible for food poisoning in human after consumption of contaminated seafood and swimming in contaminated area. It is therefore necessary to integrate *V. alginolyticus* in the list of pathogenic Vibrio sought in samples of marine environment and fishery products to prevent food poisoning collective. To reduce the risk of Vibrioinfections, consumers should avoid eating raw or undercooked Shellfish during the warmer months. The risk of Vibrio infections is higher during the Spring – Summer months due to the intensive fishing activities and extra intestinal infection related to Vibriostrains may arise after entry of the organism through wounds in the skin.

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