

Potential Seafood Safety Hazard: *Vibrio vulnificus*

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Abstract

Vibrio vulnificus is one of about a dozen species of *Vibrio* bacteria that can cause human illness, called vibriosis. *Vibrio* naturally live in certain coastal waters and are present in higher concentrations when water temperatures are warmer. People most often become infected with *Vibrio vulnificus* by eating raw or undercooked shellfish. *Vibrio vulnificus* can cause primary septicemia, a bloodstream infection with including fever, dangerously low blood pressure, and blistering skin lesions when taken with food. This review summarizes the general characteristics, survival in the environment and food, risk assessment and Control of *Vibrio vulnificus*

Keywords: *Vibrio, food pathogen, seafood, food safety*

Potansiyel Deniz Ürünleri Güvenliği Tehlikesi: *Vibrio vulnificus*

Öz

Vibrio vulnificus, insanlarda vibriosis olarak adlandırılan bir hastalığa neden olabilen yaklaşık bir düzine *Vibrio* bakteri türünden biridir. Vibriolar doğal olarak kıyıya yakın sularda bulunur ve ve su sıcaklıkları arttığında çok yüksek sayılara ulaşabilir. İnsanlar çoğunlukla ham veya az pişmiş kabuklu deniz ürünleri yiyerek *Vibrio vulnificus* ile enfekte olurlar. Gıdalarla birlikte alındığında *Vibrio vulnificus*, kana karışarak yüksek teş ile seyreden septisemiye, tehlikeli seviyede düşük kan basıncına ve cilt lezyonlarının kabarmasına neden olabilir. Bu makalede *Vibrio vulnificus*'un genel özellikleri, çevre ve gıdalardaki canlılığı, risk yönetimi ve kontrolü ile ilgili bilgiler özetlenmiştir

Anahtar Kelimeler: *Vibrio, gıda patojeni, deniz ürünleri, gıda güvenliği*

INTRODUCTION

Seafood, although can be cheapest source of quality animal protein compared to the local food requirement has a world market of more than US\$70 billion (Anonymous, 2005). These organisms are harmless as well as pathogenic *Vibrio* species. Seafood has responsible to convey a variety of diseases, illness and food poisoning to humans (Scoging, 1991). The human pathogens found in marine life, either naturally transferring to human during eating of shellfishes harvested from waters. Besides, human diseases contributed with *V. vulnificus* are still a big problem to the oyster factories and human healthy. Nowadays, outbreaks including *Vibrio* spp. figured a certain threat to the seafood industry (Scoging, 1991; Starks et al., 2000). Traditional techniques recently ready to indicate and enumerate these microorganisms are mainly taking long time, expensive, and complicated; and always needs more affirmation by additional assays, such as biochemical and immunological techniques. Today, the FDA suggested utilizing of postharvest treatment of oysters to lower the numbers of *V. vulnificus*. Indeed, water is considered as the most vital vehicle for vibrios prevalence. In immune compromised people, especially carrying with chronic liver disease, *V. vulnificus* can leak into the blood stream, causing a disease and life threatening described by fever and chills, decreased blood pressure, septic shock and blistering skin lesions. The contagion of *V. vulnificus* is quite combined with consumers of raw and undercooked oysters. Moreover, *V. vulnificus* can cause infection by directly infiltrate open wounds during swimming, shellfish cleaning and other marine activities, as the bacterium is highly invasive (Lee and Younger, 2002).

Classification and Pathogenicity of *Vibrio vulnificus*

As part of the family *Vibrionaceae* are characterised as gram negative, nonspore-forming rods, 0.5 to 0.8 μm in width and 1.4 to 2.6 μm in length (Drake, 2008), optionally anaerobic and usually halophilic bacteria. All of the pathogenic vibrios are reportable to cause foodborne illness, although *V. vulnificus* is taken into account the foremost important agents (Yesmin, 2016). Most species produce oxidase and catalase and ferment glucose while not producing gas (Drake, 2008). This foodborne microorganism is found throughout coastal waters. Contamination of an open wound with salt water harboring the organism is the main reason behind wound infections. Gastroenteritis is rare and will occur in healthy people when ingesting raw or undercooked shellfish contaminated with *V. vulnificus*. Primary blood poisoning results primarily from eating raw oysters (happens in immune patients; or those with liver disease, cancer, diabetes or hemochromatosis) though the infectious dose of *V. vulnificus* is unknown, previous studies have shown that even one organism is enough to cause illness in animal models with iron-storage disease (Starks et al., 2000). In addition, this bacterium is often isolated from stool, blood, and wound samples of infected persons. Patients affected by primary septicemia have a higher mortality (> 50%) than those suffering from wound infections (about 20-25%). The importance of *V. vulnificus* to the seafood manufacturing is primarily a consequence of the high mortality, and *V. vulnificus* illness is the leading reason for seafood-related deaths in this country (Shapiro, 1998). *V. vulnificus* it thrives within the marine environment,

and has been isolated from oysters, eels, sediments, and the water column. *V. vulnificus* is most frequently recognized because of its pathogenicity towards the human. A distinction has been made between numerous strains of *V. vulnificus* by classified similar strains together into totally different biotypes initially supported differing phenotypes, serological properties, and host range (Jones and Oliver, 2009). Moreover, *V. vulnificus* isolated from human wound exudates was distinguishable from each a forementioned biotypes (Bisharat, 1999), disposal to the recognition of biotype 3. Two primary routes of *V. vulnificus* infections are through intake and also the exposure of open flesh wounds. The consequent effects are septicemia and wound infections typically resulting in necrotizing fasciitis (Jones and Oliver, 2009; Bisharat, 1999). Septicemia, or blood poisoning, happens when toxin-producing bacteria proliferate within the bloodstream. The highest percentage of people who experience septicemia suffer from chronic liver disease (Horseman and Surani, 2011), followed by folks with compromised immune systems and alternative chronic diseases like diabetes (Horseman and Surani, 2011; Phippen and Oliver, 2017 & Kuhnt-Lenz et al., 2004). Historically, *V. vulnificus* strains are classified by biotyping, an approach supported a mixture of various phenotypic, serologic, and host range characteristics. Biotype 1 may be found in warm marine waters and was initially thought to be the only biotype related to human infection. Continuously, biotype 1 strains are pathogenic to humans, have totally different immunologically distinct lipopolysaccharide (LPS) varieties, and are indole positive (Hamdan et al., 2017). Biotype 2 was initial

thought to be pathogenic only to eels (Jones and Oliver, 2009), however this was later disputed supported human clinical evidence (Hamdan et al., 2017). In 1996, *V. vulnificus* biotype 3 was first represented when it was related to an outbreak involving 62 Israeli patients with either wound infection or blood poisoning (Bisharat, 1999, Hamdan et al., 2017). In other hand, infections will occur as a result of consumption of raw or undercooked seafood, primarily molluscan shellfish like oysters (DePaola et al., 1994). Uptake of the bacterium in healthy people is sometimes harmless but will cause delicate symptoms like gastroenteritis. Nevertheless, life threatening symptoms like blood poisoning can occur in the chronically sick, particularly those with liver disease. Mortality rates in these cases will be as high as 50% (Strom and Paranjpye, 2000; Tison et al., 1982). Wound infections are potential due to trauma related to handling contaminated seafood or the contact of open wounds with water containing *V. vulnificus*. Wound infections will become therefore severe that amputation is critical to prevent the spread of infection (DePaola et al., 1994). Millions of people throughout the globe consumed raw oysters and come into contact with waters containing *V. vulnificus*, however the rate of infection is extremely low, and less than 1 person in a million without predisposing diseases. Predisposing diseases embrace liver cirrhosis, hemochromatosis and alternative immune-suppressing illnesses like AIDS (Gholami et al., 1998). In deep knowledge, a large number of antibiotics are reportable as effective for infection treatment supported in vitro susceptibility tests. These embody tetracycline (Chiang and Chuang, 2003), ampicillin and broad spectrum

cephalosporin (**Chuang et al., 1992**) and a mixture of cefotaxime and minocycline. In last decade, treatment with doxycycline has been advocated (**Haq and Dayal, 2005**). There is many factors that have shown potential for contributory to the virulence of *V. vulnificus*. Surface factors include the presence of a capsular polysaccharide (CPS) that surrounds the cell. This provides the cells with protection from bactericidal mammalian serum also as phagocytosis by macrophages (**Gomez-Gil et al., 2014; Haq and Dayal, 2005**). Lipopolysaccharides may additionally contribute to virulence through the induction of inflammation, tissue injury, and septicemia shock (**Strom and Paranjpye, 2000**). The presence of pili permits for biofilm formation and attachment of *V. vulnificus* cells to epithelial surfaces, probably contributing to virulence as well (**Strom and Paranjpye, 2000**). Extracellular toxins are considered to be robust virulence factors. Extracellular cytolysin is a hemolysin that acts as a vascular porosity factor that ultimately causes host cell death and virulence has been correlated with the quantity of cytolysin produced throughout cell growth (**Heasman and Ridley, 2008**).

Epidemiology

While the quantity of cases of *V. vulnificus* primary blood poisoning is low, the rate is high and might exceed 50% (**Tison et al., 1982**). Wound infections are liable for 20-25% mortality rate and typically acquire surgical removal of affected tissue or amputation. Peoples who are both immune compromised and have high serum iron levels (from hemochromatosis) or chronic liver damage are at the highest risks for infection by this organism (**Tison et al., 1982**). Clinical

symptoms with primary septicemia include sudden chills, nausea, fevers, and skin lesions usually characterize illness caused by this microorganism. Development of the sepsis is often fast and may cause a lot of complications like septic shock, which has an extremely high morbidity rate (**Kumamoto and Vukich, 1998**). In fact, virulence factors like the polysaccharide capsule of *V. vulnificus*, and host factors such as high iron levels. There are three major clinical manifestations of vibrio infection: wound infection, primary blood poisoning, and gastroenteritis. In addition, *V. vulnificus* case occur sporadically, the previous are nearly always sporadic whereas the latter may occur in outbreak settings. Another case control study conducted by **Hlady and Klontz (1996)** noticed that illness manifestation proportions of 51%, 24%, and 77% for gastroenteritis, wound infection, and septicemia, respectively. Mortality rates were 1% for gastroenteritis, however were 5% for wound infection and 44% for septic illness. 68% % of gastroenteritis and 83% of primary blood poisoning cases were related to raw oyster eating (**Hlady and Klontz, 1996**). Hence, a fatal case of *V. vulnificus* associated meningoencephalitis occurred during a patient who consumed raw fish and had a chronic liver disease (**Nazir et al., 2016**). **Penland et al. (2000)** noticed 17 cases of trauma-associated ocular infections, as well as 7 caused by *V. vulnificus*, 5 by *V. alginolyticus*, 3 by *V. parahaemolyticus*, and 1 each by *V. albensis* and *V. fluvialis*. The first case of a *V. vulnificus* optical infection not related to shock was recently reported in Korea and connected to raw fish consumption (**Jung et al., 2005**). In several studies proves that primary blood poisoning caused by *V.*

vulnificus is typically contributing with the consumption of raw shellfish and is named as a general illness characterized by fever and shock and in which *V. vulnificus* is isolated from blood or an otherwise sterile site (**Strom and Paranjpye, 2000**). In a previous study, male and female rats were injected with a high dosage of refined lipopolysaccharide (LPS). Most of the male rats (82%) died when being injected, however only 21% of the female rats died. However, females with depleted estrogen levels had twice the mortality rate (75%) of females with more than estrogen (**Merkel et al., 2001**).

Ecology

Vibrios are found in aquatic environments like estuaries, marine coastal waters and sediments, and aquatic culture settings in temperate and tropical climates around the world (**Thompson et al., 2004**). *V. vulnificus* is widely distributed in warm coastal environments throughout the globe however detailed ecological studies are done only in a few countries. This organism will be isolated from coastal marine and estuarine waters, sediment, plankton, numerous shellfish (both molluscan and crustacean), and finfish species. The abundance varies despite the role of salinity and temperature most likely has the foremost vital effect on the prevalence and levels of the pathogenic vibrios. In fact, the growth of *V. vulnificus* is favored by comparatively high temperatures and the organism has an optimum growth temperature of 37°C (**Poirier et al., 2017**). **Wright et al., (1993)** were ready to culture *V. vulnificus* from estuarine waters of Chesapeake Bay collected at temperatures as low as 8°C; but, *V. vulnificus* wasn't recovered at temperatures

less than 12.5°C from Gulf of Mexico waters. In general, *V. vulnificus* is isolated sometimes from surface water samples from the Gulf of Mexico in January through March, once water temperatures are below 20°C (**Poirier et al., 2017**). Peak recovery of *V. vulnificus* happens in September, and there is substantial seasonal variation in prevalence and levels of the organism. Studies have demonstrated that during the summer months, *V. vulnificus* levels were similar (about 10⁴ CFU/g) in oysters harvested from the Gulf of Mexico and Mid-Atlantic states, but the levels were considerably higher in the Gulf of Mexico for other seasons (**Di et al., 2017**). Two strains of *V. vulnificus* isolated from both oysters and bay water were identified in Galveston Bay, TX. *V. vulnificus* were not found during the coldest months, December through February when temperatures fell below 15°C (**Lin and Schwarz, 2003**). The strains varied in their temporal isolation, only one of which was isolated in March through May, and the other becoming dominant in September through the remainder of the year. The difference in the predominant strain could be attributed to the higher salinities experienced later in the season. Throughout the study, *V. vulnificus* was isolated from 74% of oyster samples, and only 45% of water samples (**Lin and Schwarz, 2003**). **Pfeffer and others (2003)** conducted their studies in the water column and sediments of a North Carolina estuary. They observed a slight negative correlation with dissolved oxygen concentration in the water column with the isolation of *Vibrio* species and *V. vulnificus*; however, dissolved oxygen did not contribute to the variability of *V. vulnificus* in the water column. Indeed, the correlation was in part attributed to the relationship of rising

temperatures to decreasing oxygen saturation in the water column. This study established a significant relationship between *V. vulnificus* and temperature, accounting for almost 50% of the variability. The range of salinity in which *V. vulnificus* were isolated from was 8-14, however no significant correlation determined (Pfeffer et al., 2003). Considerably, the organism survives in sediment during winter. In warm tropical environments, as in India, salinity is the major factor affecting the abundance of this organism. The influence of salinity can be seen even in warmer water. In US waters, numbers of *V. vulnificus* were high at salinity between 5‰ and 25‰, but dropped by 58–88% at salinities over 30‰. *V. vulnificus* can settle plankton and fish gut. *V. vulnificus* produces chitinase, which might help the organism to colonize zooplankton. 14.1% aquatic birds in Japan are positive for *V. vulnificus*. Finely, in Japan, highest densities of *V. vulnificus* were observed from fish in Lake Shinji. There is no correlation between the prevalence or occurrence of *V. vulnificus* and fecal contamination of waters, hence fecal coliforms and *Escherichia coli* cannot be used as an indicator organism for this pathogen (Pfeffer et al., 2003).

Survival in the Environment and Food

Vibrio vulnificus not rise in oysters at temperatures under 13 °C and refrigeration could lead to reduction in numbers. Some researchers reported that levels in refrigerated shellfish became non noticeable (< 3/g) in 14-21 days, others observed survival in artificially contaminated oysters for 14 days at 2 °C, supposing that refrigeration cannot be relied upon for minimizing of this pathogen in oysters. The average of drop in refrigerated

oyster shell stock has been conducted to be 0.041 log unit per day. 4-5 log₁₀ reduction in numbers of natural *V. vulnificus* portion in oysters occurs when frozen to 40 °C and stored for 3 weeks but cold adaptation at 15 °C may reduce the properties of freezing. A combination of vacuum packaging and freezing can bring down *V. vulnificus* counts by 3-4 log₁₀ units in 7 days and though numbers continue to decline till day 7, complete elimination cannot be achieved. In other hands, *V. vulnificus* resides inside vary tissues of oysters, hence depuration is ineffective in minimization of this pathogen, but relaying oysters in high-salinity (43‰) waters for 17-49 days caused a reduced in population from 10³ CFU/g to <10 MPN/g (Jones and Oliver, 2009; Yu et al., 2017).

Risk assessment

Nowadays, oyster gathering waters are labeled taking the coliform or fecal coliform index. Regrettably, since *Vibrio* species are pervasive to the aquatic environment, the scales of the traditional fecal indicators do not correspond to with the presence of environmental vibrios, and thus the fecal coliform index is not beneficial for controlling these microorganisms (Froelich and Noble, 2016). In focus pervious researches, the FAO/WHO risk assessment for *V. vulnificus* in raw oysters supposed by the US FDA to evaluate the risk of *V. vulnificus* primary blood poisoning in the USA, because fundamental data for *V. vulnificus* levels in oysters, at the point of eating and for the totally population available for the USA. This hazard assessment used data gained by weekly analysis of *V. vulnificus* levels in oysters from four Gulf States carrying out during 1994–1995. Postharvest training

(duration shellfishes in collecting from water, till to first refrigeration) utilize in the paradigm were depend on research findings were progress in Gulf Coast. Moreover, *V. vulnificus* development in oysters, the thrive ones during refrigeration, and levels at consumption were conducted rely on information from pervious findings in the US Gulf Coast. The highest indicated levels of *V. vulnificus* at harvest were 5.6×10^3 CFU/g during the hot season, and the lowest were 8.0×10^1 CFU/g in the wet season, for shellfishes harvested from waters with a salinity of below 30 ppt. Additionally, the hazard parameters are predicted the minimizing of diseases that could be obtained by postharvest counterparts to diminish *V. vulnificus* levels to suitable values such as 3, 30, or 300 /g. In the USA, there are three validated approaches to attain end-point norm of < 3 MPN/g. *V. vulnificus* and these include mild heat treatment (50 °C), freezing with extended frozen storage and high hydrostatic pressure. If all oysters are treated to attain target level of 3 /g., the paradigm expected that the quantity of cases could be diminishing from now 32 noted cases per year to one case every six years (Yu et al., 2017; Froelich and Noble, 2016). Simultaneous to frozen range of 0-20 h, the expected illness ranged from 17.7 till 59.3 noted cases, recommended that quick cooling of shellfishes separately is not enough to reach fundamental reduction in the number of *V. vulnificus* diseases. While *V. vulnificus* levels in oysters collecting from waters with a salinity of greater than 30‰ is more significantly minimized. Re-breeding shellfishes to high-salinity waters ($>32\%$) has been shown to reduce *V. vulnificus* levels by 3-4 log units (< 10 /g) within two weeks (Jones et al., 2017).

Control and Minimizing of *Vibrio vulnificus*

Freezing of oysters after collecting is very vital for avoiding of replication of *V. vulnificus*. Multiplication of *V. vulnificus* not happen at degrees under 13 °C, so this is the global evidence which used to stop and control of this pathogen. Levels of this pathogen in shell shellfishes carry without refrigeration for 3.5, 7.0, 10.5, and 14 h increased 0.75, 1.3, 1.74, and 1.94 log units, respectively. Belongs to the United States standards, while water temperature increased 27°C, commercial shellfish should be cooling within 10 h of harvest. There are additional control plans for shell associated with two or more *V. vulnificus* illness. Temperature control should be conducted during 14 h when water temperature is between 18 and 23°C; within 12 h if water temperature is 23-28 °C; within 6 h if water temperature increases 28 °C (Lin and Schwarz 2003; Jones et al., 2017). Basically, in United State of America, chilling contributed with frozen phase is desired as a control assay for *V. vulnificus*, however the process must be advocated and hazard analysis critical control point (HACCP) compliant. High hydrostatic pressure is a novel technique that is allowed to use in USA for postharvest handling of oysters. To achieve a 5 log₁₀ diminished of *V. vulnificus* levels, a treatment of 250 mega Pascal (MPa) for 120 s is adopted (Bross et al., 2017). In focus, commercial heat shock treatment is utilize in somewhere of the world as an approaches aid for oysters. The process includes immersing frozen shellfishes in drinkable water through temperature of 67 °C for roundly 5 min rely on oyster size and statements. Heat shocked oysters are chilled by spraying with potable water for one min before peeling and washing. 2-4 log₁₀ reduction in *V.*

vulnificus counts can be gained by heat shock process. Low temperature pasteurization (50 °C for 10 min) minimized *V. vulnificus* counts from 10⁵ MPN/g. to tiny levels in injected shell stock oysters. Moreover, depending on FAO/WHO risk assessment, Codex Committee on Food Hygiene invented a Code of Hygienic Practice for monitoring of *Vibrio* spp. in seafood with an annex on control measures for *V. vulnificus* in bivalve molluscs. This Code recommends assessment of validation of monitoring measure should be conducted together with the Codex Guidelines for the validation of food safety control measures (Anonymous, 2008). Finally, the interruption between Shellfish Sanitation Program has improves education materials and fact noted papers to declare to public about the join between *V. vulnificus* infections and diabetes, liver disease, and other immune compromised conditions.

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