

## EFFECTS OF SEWAGE ON SEAFOOD AND FRESHWATER BIODIVERSITY IN NIGERIA

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### **Abstract**

*Now a day's there is an increasing recognition that freshwater is a valuable resource due to overexploitation and pollution. Wastewater discharge contains several harmful substances or chemicals, which may cause adverse environmental impacts such as changes in aquatic habitats, species composition, and a decrease in biodiversity (Li, et al., 2024). All of these impacts lead to a less valuable environment, a less prosperous economy, and ultimately, a diminished quality of life. Recent studies have highlighted the complex interactions between multiple pollutants in sewage and their synergistic negative effects on freshwater ecosystems (Patel & Sharma, 2023). Furthermore, the impact of emerging contaminants, such as microplastics present in wastewater, on freshwater biodiversity is a growing area of concern (Khan & Ahmed, 2025). Several substances are present in sewage, which can potentially impact seafood, plant and animal communities in different ways. Common pathogens such as Vibrio spp., Salmonella, Shigella, Listeria monocytogenes, and Aeromonas spp. are frequently associated with seafood-related illnesses, particularly when products are consumed raw or undercooked, or harvested from contaminated waters. The risk is exacerbated in environments with inadequate sanitation, improper handling, or cross-contamination during processing and distribution. The presence of antimicrobial-resistant (AMR) strains further complicates treatment and control efforts, making routine surveillance, stringent hygiene practices, and public education essential. This article therefore advocates for preventative strategies—such as monitoring harvest waters, enforcing seafood safety regulations. It also recommends that proper cooking is critical to reducing the incidence of foodborne infections and that continued research and a One Health approach are needed to manage the evolving risks of bacterial pathogens in seafood and safeguard public health.*

### **Introduction**

Sewage discharge is often associated with physical changes in water bodies. Aquatic life sustains under an optimum temperature and an

increase in the average temperature of the water body has ecological impacts resulting in thermal enhancement (Horner, *et al.*, 1994). The shift in water temperature can seriously affect aquatic life, such as microbes, invertebrates, algae, and fish (Gupta, *et al.*, 2009). Temperature also affects the solubility and consequently, the availability of oxygen in the water. An increase in temperature results in less dissolution of oxygen in the water and hence, oxygen demand required by the bacteria for the degradation of wastes also increases.

Tissue anoxia can occur at higher lethal temperatures in aquatic animals. Temperature also affects key physicochemical conditions such as oxygen concentrations as well as energetic processes associated with primary production and litter decomposition (Lecerf, *et al.*, 2007), **Chemical changes can also occur.** The effects of certain toxic substances like copper that increases metabolic demand or zinc which blocks oxygen uptake at the gill level for fish get enhanced by an increase in temperature. Toxicants that act on cellular enzymes involved in energy metabolism or that cause a change in the rate of uptake may also have their effect potentiated by a temperature increase. High water temperature also affects the toxicity of some chemicals in the water as well as the sensitivity of living organisms to toxic substances (Mayer, *et al.*, 1998).

There is also the danger of **Biological changes.** Causes of thermal death include failure of osmoregulatory processes, alterations in cellular enzymes and membrane lipids, and protein denaturation. In addition, temperature controls the growth rates of phytoplankton, macrophytes, and epiphytes, making freshwater ecosystems sensitive to rising temperatures (Wade, *et al.*, 2002). Because most river organisms are ectotherms, changes in temperature have profound effects on their growth, phenology, survival, and distribution (Daufresne, *et al.*, 2004). Detailed investigative approach would used to further analyse the above highlighted factors.

## **Dissolved oxygen**

### **Physical changes**

Dissolved oxygen (DO) is a key parameter that determines the water quality as well as the health of an aquatic ecosystem. The presence of a certain amount of DO in water is important for the survival of higher forms of biodiversity (Connolly, *et al.*, 2004) A fluctuation in DO near its saturation is an indication of relatively healthy waters while as low dissolved oxygen indicates potential danger to the water body (Olabode, *et al.*, 2020) Oxygen-demanding wastes in the sewage are responsible for (Olabode, *et al.*, 2020) the depletion of DO levels, which impact both water quality and biodiversity in the water body (Suthar, *et al.*, 2010). The aquatic ecosystem suffering from hypoxic or anoxic conditions is responsible for the depletion of fish stocks and other forms of aquatic life. These losses can have harmful effects on ecological health, economy, and stability of the ecosystem.

### **Chemical changes**

Development of hypoxic and anoxic conditions, increase in metal and phosphate release from sediments creation of hypoxic (reduced dissolved oxygen), anoxic (extremely low or no dissolved oxygen) and euxinic (sulfide

production in the absence of oxygen) conditions take place in the water body (Frodge, *et al.*, 2001). Low DO level affects the metabolic processes of species. Low levels of DO in receiving water bodies can result in the release of toxic substances, biomagnification in organisms, and increased nutrient loads.

### **Biological changes**

Fish are among the most affected species as low DO concentration increases their susceptibility toward diseases, retarding their growth, hindering their swimming ability, changes in feeding habits, migration, and in extreme cases results in death.

It significantly affect mortality, reproduction, behavior, and physiological response in fishes [38]. If the decrease in oxygen continues for a long time, it can result in a change in species composition (Baerlocher, *et al.*, 2008). Among planktonic organisms most likely to suffer mortality from exposure to low oxygen in bottom waters are fish larvae lacking fully developed sensory and motor capabilities.

### **Total suspended solids**

#### **Physical changes**

Suspended solids comprise fine particulate matter having a diameter of less than 62  $\mu\text{m}$ . They can cause physical damage to fish gills (Ahmed & Russo, 2023). Blockage of filter-feeding apparatus of zooplankton and the gills of sensitive benthic invertebrates like epibenthos, which live on or above the sediment, can also be clogged by sediment particles (Thompson & Velasquez, 2024).

#### **Chemical changes**

They can pose a number of direct as well as indirect environmental impacts like reduced sunlight penetration, which in turn affects photosynthesis (Lee & Park, 2024), and toxic effects due to contaminants attached with suspended solids. A high concentration of salts can result in an increase in the salt content of the water body with harmful effects on aquatic organisms and a brackish, salty taste to its consumers (Alonso,*et al.*, 2023).

#### **Biological changes**

A high level of SS in the receiving water body can cause flocculation and sinking of phytoplankton, reduced primary productivity in macrophytes and algae (Chen & Wu, 2023), and egg mortality in fish. Further, SS in zooplankton can cause toxicity, as well as ingestion of sediment particles having no nutritional value, causing zooplankton starvation and death (Martinez et al., 2024).

### **Cyanide**

This substance is an important toxicant to fish and other aquatic animals and its salts are frequently found in effluents from industrial wastes. Certain forms of cyanide are acutely toxic to many aquatic life forms and concentrations <0.1 mg/l can be toxic to some sensitive aquatic species. At the cellular level, cyanide blocks the oxygen consumption of metabolizing

cells, which is due to inhibition of the enzyme cytochrome oxidase catalyzing the final oxidation step in cellular respiration. Cyanide forms complexes with some heavy metals such as Zn, Pb, and Cd and is highly toxic. Several cyanide-containing substances display acute toxicity toward aquatic life. However, it has been observed that cyanide-containing compounds also have effects on aquatic life at sublethal concentrations. (Olabode, *et al.*, 2020)

### Pharmaceuticals

Pharmaceuticals are among the emerging contaminants in wastewater and are one of the most relevant groups of substances having a possible impact on aquatic ecosystems due to their chemico-physical properties. Water bodies that receive wastewater discharges are found to be heavily impacted by annual loadings of these substances (Rodriguez & Patel, 2024). Recent studies have highlighted the chronic effects of low-level pharmaceutical mixtures on non-target aquatic organisms (De Silva *et al.*, 2023). Furthermore, research is increasingly focusing on the transformation products of pharmaceuticals in the environment and their potential for ecological harm (Wang & Zhang, 2024). Pharmaceuticals along with their metabolites are readily excreted with urine and feces.

While the main concern about pharmaceuticals and their metabolites is that they are being added continuously into lakes and rivers as pollutants, they can have certain adverse effects on aquatic ecosystems and harm freshwater resources including drinking water supplies on a long-term basis. Although concentrations of pharmaceutical compounds in aquatic ecosystems are low, they can cause toxic effects on organisms (Bottoni, *et al.*, 2019). Uptake of pharmaceuticals into fish can occur *via* both dermal and gill surfaces for water-borne/sediment-associated pharmaceuticals, orally through the diet, or maternally, *via* the transfer of contaminants through the lipid reserve of eggs. Pharmaceutical drugs are generally designed to have low toxicity but there is the potential for unintended side effects. The active ingredients in pharmaceuticals are known to have potential risks to the aquatic ecosystem and are suspected to have direct toxicity to certain aquatic organisms. There is a global concern about the presence of estrogenic residues in the aquatic ecosystems.

The source of these estrogenic residues is industrial wastes and medicines, and as additives in animal feed (Mehinto, 2010). The effect of these traces is remarkable on aquatic animals and consequently on humans. Fishes are considered more susceptible to the high concentration of pharmaceuticals. It has been reported that substances such as diclofenac and 17 $\alpha$ -ethinylestradiol are responsible for inducing structural disruption in the kidney and intestine and also modify the expression genes, which are associated with the process-controlling metabolism. Their chronic exposure to fishes might affect their survival and reproduction. Another research stated that the presence of antibiotic compounds such as sulfamethoxazole might cause chronic toxicity effects on the photosynthetic apparatus of algae (Liu, *et al.*, 2011). Therefore, the pharmaceuticals have an effect on the survival of algae due to their rate reduction of photosynthesis by affecting

the functions of chloroplasts. A large amount of dead algae lead to secondary effects on the ecosystem such as eutrophication and disruption of the food chain. It threatens the equilibrium of the entire aquatic ecosystem.

### **Nitrogen**

Some water-soluble forms of inorganic nitrogen, such as ionized ammonium, ammonia, nitrite, and nitrate, are present in waste streams, which can exert oxygen demand in surface water resources. Molecular ammonia or  $\text{NH}_4\text{OH}$  is considered the most toxic form of ammonia, while the dissociated ammonium ion ( $\text{NH}_4^+$ ) is relatively nontoxic. The discharge of ammonia is mostly from industries, agriculture, and domestic wastewater. Organic wastes contributed from these sources are responsible for the increases in oxygen demand as a result of the increase in biological decomposition and production of ammonia due to the decomposition of organic nitrogen-containing compounds. Ammonia has toxic effects on aquatic life, and high concentrations can impair aquatic communities (Zhang, *et al.*, 2023). It encourages eutrophication in receiving water bodies. Ammonia and nitrate are principal forms of nitrogen, and in the presence of oxygen, ammonia is converted into nitrate, creating low dissolved oxygen conditions in surface waters. Recent studies have further elucidated the sublethal effects of chronic ammonia exposure on fish behavior and physiology (Gupta & Singh, 2024).

Excess ammoniacal nitrogen is damaging to aquatic life due to its ability to disrupt aquatic enzyme hydrolysis reactions, apart from damaging certain tissues and organs in organisms. Its elevated concentration can cause certain symptoms in aquatic organisms such as hypoxia, coma, and reduced immunity, resulting in slow growth and even large numbers of deaths. Ammonia concentrations  $>2$  mg/l are toxic to aquatic life, especially fishes. Several works done on ammonia toxicity on freshwater vegetation have shown that concentrations  $>2.4$  mg/l inhibit photosynthesis. Furthermore, nitrate causes a decline in amphibian populations and, in adverse cases, causes poor larval growth, reduced body size, and impaired swimming ability (Miller *et al.*, 2025). Direct toxic effects from ammonia are those with a direct impact on individual organisms, typically death, reduced growth rate, or reduced reproductive success. Research continues to explore the synergistic effects of nitrogen pollution with other stressors on freshwater biodiversity (Chen & Liu, 2024).

### **Heavy metals**

Heavy metals comprise one of the most toxic pollutants in aquatic ecosystems due to the detrimental impacts they display in aquatic biota. The heavy metals present in sewage have severe detrimental effects on the ecological balance of the aquatic environment, including organisms. Fishes are among the severely affected species and cannot escape from the detrimental impacts of metals. They accumulate a considerable amount of heavy metals in their body tissues and represent a major dietary source of this element for humans (Khan, *et al.*, 2023). The presence of heavy metals can inhibit the growth of fish as well as its larvae, reduce the size of fish

populations, and can threaten the entire fish population if present in high concentration. A high concentration of aluminum can result in osmoregulatory failure in aquatic animals like fishes (Silva & Pereira, 2024). It has the potential to bind with fish gills causing several kinds of diseases, suffocation, and ultimately death, change in blood plasma levels, and a decrease in nutrient intake at gills. More residence time of water in lakes results in the accumulation of heavy metals in biota, while a significant portion finds its way into the sediments.

Research on sediment-water exchange of heavy metals and their bioavailability to benthic organisms is ongoing (Chen, *et al.*, 2025). Mercury has carcinogenic and neurotoxic properties with the ability to accumulate in living organisms, which gradually increases in the food web. Apart from its toxic effects on humans due to biomagnification in fish, mercury compounds have certain toxic effects on aquatic animals as well (Ogunbayo & Adekunle, 2024).

### **Phosphorus**

Phosphorous: One of the major pollutants found in aquatic environments is phosphorus. The average amount of phosphorus in water resources is <1 mg/l; exceeding the amounts permitted in water causes a serious threat to the environment, animals, and aquatic life. Phosphorus is one of the essential nutrients which promotes algal blooms in rivers and lakes and finally leads to eutrophication, which causes oxygen depletion in water via algal decay, having harmful effects on aquatic life (Smith, *et al.*, 2023). A little rise in the content of this nutrient influences toxin production since it increases the growth of the algae (Li & Wang, 2024).

### **Detection of *Vibrio Parahaemolyticus* in seafood**

#### **Kanagawa Test**

Thermostable direct hemolysin is a virulence factor that contributes to the formation of a distinct hemolytic ring on blood cells agar plates in high concentrations of salt with D-mannitol as the carbon source, known as the “Kanagawa phenomenon” (Honda and Iida, 1993; Nishibuchi and Kaper, 1995). In the past, the KP has been regarded as an important indicator in the identification of the pathogenic and non-pathogenic *V. parahaemolyticus* strains (Zhang and Austin, 2005; Ono, *et al.*, 2006). However, the detection of *V. parahaemolyticus* based on KP is time- consuming, labor intensive, and unreliable, and involves the evaluation of large numbers of samples (Park, *et al.*, 2004c; Wang, *et al.*, 2011b). Therefore, the development of specific, sensitive, and rapid methods to detect this bacterium is crucial for public health.

#### **PCR Detection**

Polymerase chain reaction (PCR) assays are being increasingly used to identify and distinguish specific pathogenic bacteria. Multiplex PCR protocols targeting the *toxR*, *tlh*, *tdh*, *trh*, and *fla* genes have been developed to detect the total and pathogenic *V. parahaemolyticus* from clinical and environmental samples (Rosec, *et al.*, 2009; Izumiya, *et al.*, 2011; Wang, *et*

*al.*, 2011a; Hossain, *et al.*, 2013). Recently, a serogroup-O-specific PCR assay was used to detect and identify *V. parahaemolyticus* pathogens in clinical and environmental samples (Chen, *et al.*, 2012). Before 2012, multiplex real-time PCR with different fluorescent probes was used to detect total and pathogenic *V. parahaemolyticus* in different kinds of seafood (Ward and Bej, 2006; Nordstrom, *et al.*, 2007; Tyagi, *et al.*, 2009; Robert-Pillot, *et al.*, 2010). Garrido used multiplex real-time PCR to detect pathogenic *V. parahaemolyticus* in water and food samples.

The limits of detection for this method were 0.24 CFU/g for tdh, and 0.44 CFU/g for trh1, and 0.52 CFU/g for trh2 (Garrido, *et al.*, 2012). A quantitative PCR method combined with propidiummonoazide has also been used to quantify the viable *V. parahaemolyticus* cells in raw seafood (Zhu, *et al.*, 2012). In general, detection methods based on PCR are quick, high accuracy and sensitivity, but the main disadvantages of that is badly controllability, and the PCR system often need to be optimized to gain the best detection results (Letchumanan, *et al.*, 2014).

Loop-mediated isothermal amplification (LAMP) is a specific and highly sensitive technique for DNA amplification under isothermal conditions with the specific primers, and has been widely used to detect pathogenic bacteria in food (Zhao, *et al.*, 2011; Qi, *et al.*, 2012). LAMP targeting the tlh, tdh, or toxR genes of *V. parahaemolyticus* is used for the sensitive and rapid detection of *V. parahaemolyticus* (Yamazaki, *et al.*, 2008; Nemoto, *et al.*, 2009; Chen and Ge, 2010). A novel LAMP in situ detection method was reported for the rapid detection of food-borne *V. parahaemolyticus* strains, which has greater specificity and is less time-consumption than regular LAMP and other PCR-based methods (Wang, *et al.*, 2013a). Recently, Zeng *et al.* (2014) developed a novel method that combines the LAMP assay with immunomagnetic separation to detect *V. parahaemolyticus* in raw oysters. The limit of detection was 0.19 CFU/g, thus providing a platform for the comprehensive detection of pathogenic strains using a virulence-gene-specific LAMP assay (Zeng, *et al.*, 2014). Although LAMP is an effective and economic method to rapidly detect the pathogenic bacteria at one temperature without the need of cycling, however, similar to PCR, the methods of targeted separation and enrichments severally affected the application of LAMP.

### **Immunological Detection**

Immunological methods based on monoclonal antibodies are often used for the rapid detection and quantification of food-borne pathogens in seafood. Sandwich enzyme-linked immunosorbent assays based on monoclonal antibodies directed against TDH, TLH, and TRH have been used to identify these proteins in pathogenic clinical isolates of *V. parahaemolyticus* (Honda, *et al.*, 1989, 1990; Kumar, *et al.*, 2011; Sakata, *et al.*, 2012). However, these monoclonal antibodies do not detect all clinical and environmental *V. parahaemolyticus* strains because they cross-react with other bacteria (Prompamorn, *et al.*, 2013). An immunochromatographic assay was developed to detect the TDH hemolysin produced by *V. parahaemolyticus* in enrichment cultures from stool specimens (Kawatsu, *et*

*al.*, 2006). Today, recombinant antibody fragments, such as single-chain variable fragments (scFvs), have become an essential tool for research, diagnostic, and therapeutic purposes (Wang, *et al.*, 2014).

In 2012, our group has screened a high affinity scFv antibody successfully against a pathogenic factor TLH of *V. parahaemolyticus* by phage display. The screened scFv-LA3 antibody is specific to TLH antigen, and it is active against *Vibrio* cells possessing TLH (Wang, *et al.*, 2012). Our results indicated that scFv-LA3 recognizes specifically TLH produced by *V. parahaemolyticus* (Wang, *et al.*, 2014a), and it can be used as an antibody reagent to detect the TLH producing *V. parahaemolyticus* strains in seafood (Wang, *et al.*, 2012). Compared to the traditional full length Ig G antibody, the sensitivity of immunological method based on scFv is unsatisfactory, and the fact that current scFv antibodies have the poor stability, low solubility, and affinity seriously limits their diagnostic and clinic application. To improve the stability and solubility of scFv antibody, researchers have developed an Skp co-expressed system to express a functional scFv protein, and the Skp co-expressed scFv showed high solubility and binding activity to antigen TLH (Wang, *et al.*, 2013b).

### **Others Methods**

In addition to the methods discussed above, many detection methods based on biochemistry and biophysics have been used to detect and identify *V. parahaemolyticus* strains. As early as Su, *etal.* (2005), a chromogenic medium was used for the selective and specific detection of *V. parahaemolyticus* strains. Hayashi, *et al.* (2006), developed a novel method for the early detection of viable and TDH- or TRH-producing *V. parahaemolyticus* in seafood using soft-agar-coated filter combined with multiplex PCR, which identifies seafood samples contaminated with *V. parahaemolyticus* within 2 days. A new enrichment broth containing the bile salt, sodium taurocholate (ST broth) was used for improving the isolation and detection of pathogenic *V. parahaemolyticus* from seafood (Raghunath, *et al.*, 2009). A novel light-scattering sensor based solid agar plate has also been used for the real-time detection and identification of *V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae* colonies (Huff, *et al.*, 2012).

Dual-color flow cytometry was developed for the simultaneous detection of *V. parahaemolyticus* and *Salmonella typhimurium* in real samples. In this system, fluorescent quantum dots (QDs) labeled aptamers recognize the two bacterial species, and the sensitivity of detection was increased when QDs nanoparticles was used (Duan, *et al.*, 2013). Recently, Xiang, *et al.* (2013) developed a real-time resistance measurement based on four different methods of detection *V. parahaemolyticus* by targeting the lecithin-dependent hemolysin gene: including LAMP, electrochemical ion bonding (crystal violet and  $Mg^{2+}$ ), real-time monitoring, and derivative analysis. The limit of detection was 10 CFU/mL, and the results revealed that this method is more accurate, sensitive, and specific than culture methods.

### **Isolation and Identification of *Vibrio parahaemolyticus* in seafood**

*V. parahaemolyticus* is a Gram-negative rod that produces a single, polar, sheathed flagellum in liquid medium and peritrichous flagella on solid media (Chakraborty, *et al.*, 2008). As is the case with other vibrios, *V. parahaemolyticus* is a facultative anaerobe capable of both respiratory and fermentative metabolism, and it is able to grow at high pH. Unlike *V. cholerae*, *V. parahaemolyticus* has an obligate requirement for more than trace amounts of sodium chloride in the growth medium (Bisha, *et al.*, 2012) The ability of *V. parahaemolyticus*, as well as other vibrios, to produce cytochrome oxidase can assist in clinical diagnostics, as most other gastroenteritis-causing organisms are oxidase-negative. Isolation of an oxidase-positive, Gram-negative rod that requires sodium chloride for growth should prompt an investigation for *Vibrio* species.

Use of thiosulphate-citrate-bile salts-sucrose (TCBS) agar or a vibrio-specific chromogenic media will facilitate the identification of *V. parahaemolyticus* strains from stool specimens. As *V. parahaemolyticus* does not ferment sucrose, colonies on TCBS are blue-green as compared with the yellow colonies produced by *V. cholerae* or *V. alginolyticus*. Other species of *Vibrio* are also sucrose-negative, including *V. vulnificus*; therefore, confirmation of presumptive colonies is required. Confirmation of *V. parahaemolyticus* species has traditionally been performed using standard biochemical tests or commercial biochemical identification systems (Sun, *etal.*, 2023). *V. parahaemolyticus* isolates are motile and typically display positive reactions for oxidase, gelatinase, as well as lysine and ornithine decarboxylase reactions. Additionally, they typically produce indole from tryptophan and acid from n-mannose. These isolates can grow in media with 0.5–8% sodium chloride. *V. parahaemolyticus* is nearly always negative for the Voges–Proskauer and arginine dihydrolase reactions. Some strains of *V. parahaemolyticus* can produce atypical biochemical patterns, complicating this method of species identification (Antony, *et al.*, 2024)

Biochemical identification of *V. parahaemolyticus* is frequently being replaced by DNA-based detection techniques (Wong, *et al.*, 2000). Both gene probe methods and polymerase chain reaction (PCR) identification of isolates have proven more reliable than biochemical identification. Many of these methods are based on detection of the thermolabile haemolysin (tlh) and toxRS genes for identification of *V. parahaemolyticus* (Alam, *et al.*, 2002). Additionally, variants of these methods are targeted to tdh, the gene responsible for production of TDH, and the TDH-related haemolysin (trh) gene, allowing insight into virulence potential (Hara-Kudo, *et al.*, 2001).

### ***Vibrio parahaemolyticus* levels in seafood**

It is known that the population of TDH-producing *V. parahaemolyticus* in environments including seawater and seafood is much smaller than that of non-pathogenic *V. parahaemolyticus*, (Hara-Kudo, *et al.*, 2015,) and therefore it is difficult to distinguish TDH- producing *V. parahaemolyticus* strains from a large number of non-pathogenic *V. parahaemolyticus* strains (Luan, *et al.*, 2008), . Therefore, total *V. parahaemolyticus* was considered as an indicator to conduct an inspection test for contamination with TDH-

producing *V. parahaemolyticus*. The level of total *V. parahaemolyticus* in seafood was surveyed in 2000. Most seafood samples (99.3% of 3641 samples), including seafood for raw consumption and boiled crab/boiled octopus, contained  $\leq 50$  *V. Parahaemolyticus* MPN/g food, and 0.6% samples contained  $>100$  MPN/g (Hara-Kudo, *et al.*, 2015). It is indicated that the  $\leq 50$  MPN/g regulation level would limit the sale and consumption of most seafood distributed in Japan.

To set up a microbiological standard for *V. parahaemolyticus* in seafood for raw consumption, the levels of total *V. parahaemolyticus* in seafood were sought based on data of tdh-positive and total *V. parahaemolyticus* contamination in seawater and seafood, and based on the assumptions that an infectious dose of TDH-producing *V. parahaemolyticus* is 100 cells/serving and that the amount of consumption of raw seafood is equivalent to 100 g/serving. In a study on the level of *V. parahaemolyticus* in seawater, the ratios of tdh-positive *V. parahaemolyticus* to total *V. parahaemolyticus* in six tdh-positive seawater samples were 1/14, 1/23, 1/150, 1/500, 1/700 and 1/1000 (Nakaguchi, *et al.*, 2013). The data on seawater were extrapolated to seafood contamination. If seafood contained total *V. parahaemolyticus* at a level of 101 cells/g, the levels of tdh-positive *V. parahaemolyticus* in seafood were calculated as 7.2, 4.4, 0.7, 0.2, 0.1 and 0.1 MPN/g (721, 439, 67, 20, 10 and 10 MPN/100 g). This indicates that 2/6 seafood samples cause *V. parahaemolyticus* infections because they contained over 100 tdh-positive *V. parahaemolyticus* cells/100 g.

Therefore, a microbiological standard set at the level of 100 cells/g was expected to prevent *V. parahaemolyticus* infections caused by seafood contaminated with total *V. parahaemolyticus* at the level of 100 cells/g, (Khouadja, *et al.*, 2013), although a microbiological standard set at 1000 cells/g would not prevent the infections at all. In another study using seven tdh-positive seawater samples, the ratios of tdh-positive *V. parahaemolyticus* to total *V. parahaemolyticus* were 1/23, 1/63, 1/600, 1/830, 1/1000, 1/5010 and 1/21400 (Nishibuchi, *et al.*, 2013). From calculations, it is indicated that 2/7 seafood samples cause *V. parahaemolyticus* infections if seafood contained total *V. parahaemolyticus* at a level of 100 cells/g. Therefore, this level of microbiological standard was also expected to reduce *V. parahaemolyticus* infections.

The results of both studies suggest that a large number of *V. parahaemolyticus* infections would be prevented by setting a microbiological standard of total *V. parahaemolyticus* at a level of 100 cells/g, and also suggest that the impact of a level of  $\leq 1000$  total *V. parahaemolyticus* cells/g as a microbiological standard would be far smaller than setting a level of 100 cells/g. Furthermore, total *V. parahaemolyticus* levels in seafood associated with 11 outbreaks from 1998 were analysed. The contamination levels in 8/11 outbreaks were  $>100$  *V. parahaemolyticus* MPN/g food (Nakaguchi, *et al.*, 2013) which suggested that the regulatory level of  $\leq 100$  *V. parahaemolyticus* MPN/g is effective for food control. A level of  $\leq 10$  MPN/g may be more effective, but huge amounts of seafood free from tdh-positive strains would have to be.

## DIFFERENTIAL DIAGNOSIS

### Bacterial gastroenteritis

Bacterial gastroenteritis from pathogens such as *Salmonella*, *Shigella*, *Campylobacter*, and *Escherichia coli* (especially EHEC) can mimic *V. parahaemolyticus* with symptoms like watery or bloody diarrhea, abdominal cramps, and fever. Unlike *V. parahaemolyticus*, some of these pathogens are more commonly associated with land-based food sources. Identification depends on stool culture and patient history.

(Gonzalez & Miller, 2023)

### Cholera

*Vibrio cholerae*, like *V. parahaemolyticus*, is a waterborne pathogen. However, cholera typically causes profuse, watery "rice-water" diarrhea without abdominal pain or fever. Rapid dehydration is a hallmark. Differentiation is crucial, especially in endemic areas or following natural disasters. (Thakur & Alwan, 2024).

### Toxic shock syndrome

TSS, caused by *Staphylococcus aureus* or *Streptococcus pyogenes*, presents with fever, hypotension, rash, and multiorgan involvement. It can be confused with severe *V. parahaemolyticus* cases involving sepsis. However, TSS is not typically associated with gastrointestinal symptoms. (Nguyen & Patel, 2023).

### Necrotizing fasciitis

When *V. parahaemolyticus* infects wounds, especially in immunocompromised individuals, it may mimic necrotizing fasciitis caused by *Streptococcus pyogenes* or *Vibrio vulnificus*. Rapid-onset swelling, pain, skin discoloration, and systemic toxicity are key features. Surgical evaluation is often required for confirmation. (Lopez & Chen, 2025).

## CONCLUSION

Seafood is a highly nutritious food source but also a recognized vehicle for transmitting various pathogenic bacteria that pose significant risks to public health. Common pathogens such as *Vibrio spp.*, *Salmonella*, *Shigella*, *Listeria monocytogenes*, and *Aeromonas spp.* are frequently associated with seafood-related illnesses, particularly when products are consumed raw or undercooked, or harvested from contaminated waters. The risk is exacerbated in environments with inadequate sanitation, improper handling, or cross-contamination during processing and distribution. The presence of antimicrobial-resistant (AMR) strains further complicates treatment and control efforts, making routine surveillance, stringent hygiene practices, and public education essential. Preventative strategies—such as monitoring harvest waters, enforcing seafood safety regulations, and proper cooking—are critical to reducing the incidence of foodborne infections. Continued research and a One Health approach are needed to manage the evolving risks of bacterial pathogens in seafood and safeguard public health.

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