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Vibrio bacteria in raw oysters: managing risks to human health

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The human-pathogenic marine bacteria *Vibrio vulnificus* and *V. parahaemolyticus* are strongly correlated with water temperature, with concentrations increasing as waters warm seasonally. Both of these bacteria can be concentrated in filter-feeding shellfish, especially oysters. Because oysters are often consumed raw, this exposes people to large doses of potentially harmful bacteria. Various models are used to predict the abundance of these bacteria in oysters, which guide shellfish harvest policy meant to reduce human health risk. *Vibrio* abundance and behaviour varies from site to site, suggesting that location-specific studies are needed to establish targeted risk reduction strategies. Moreover, virulence potential, rather than simple abundance, should be also be included in future modeling efforts.

1. Introduction

All coastal waters and estuaries contain *Vibrio* bacteria. Although many *Vibrio* species are harmless, several can cause serious disease in humans or animals. *Vibrio vulnificus* and *V. parahaemolyticus* are the most common types of sometimes deadly foodborne and wound *Vibrio* infections. Recognized infections from *Vibrio* species are on the rise, and although there is some uncertainty, most researchers predict that climate change will increase cases [1–5]. Research on microbial ecology, food safety and ecology of shellfish reservoirs has revealed insights into *Vibrio* ecology and led to tools for predicting risky periods or locations. Nonetheless, regional and strain variation complicate predictions. *Vibrio vulnificus* and *V. parahaemolyticus* are detectable in coastal waters and estuaries with salinities greater than 5‰ [6]. These bacteria are 'particle-lovers' and will attach to particulates and other organisms in the water column. This attachment facilitates their uptake by filter-feeding molluscs, such as oysters, that share similar habitats. Through this substrate filtration, oysters can concentrate these bacteria, including the pathogenic forms of these bacteria, by up to 100 times the concentration found in the surrounding waters [7]. Because oysters are often eaten raw, this can pose an infection risk for people consuming them [8]. Most infections are unreported, and it is estimated that in the USA approximately 84 000 people contract a food-borne infection from *Vibrio* spp. every year, the highest rate since nationwide reporting began [9]. *V. parahaemolyticus* is, by far, the most common infective agent of the two species. Infections with *V. parahaemolyticus* produce symptoms that include diarrhoea accompanied by abdominal cramps, nausea, vomiting, headache, chills and low-grade fever [10]. Some strains with increased virulence have been implicated in recent outbreaks, with serotype O4:K12 garnering lots of recent attention [11–13]. Foodborne *V. vulnificus* infections are less common than *V. parahaemolyticus*, but the morbidity associated with these infections is far more severe. With this species, the initial symptoms can be the same as *V. parahaemolyticus*, but the infection can progress rapidly (within hours) to primary septicaemia [14,15]. Here the symptoms are grave, including hypotension and secondary lesions that develop on the extremities. When this occurs, the incubation period is often very short, and death can occur 24–72 h after eating a single oyster [16]. *V. vulnificus* is the single most fatal foodborne pathogen in the USA, where it comprises 95% of all seafood-related deaths and boasts a fatality rate nearing 50%, even with aggressive medical treatment. Rarely seen in most bacteria, these bacteria also have a second route of infection. They can

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enter the body via wounds, either preexisting or obtained through coast-related activities such as fishing, swimming, oyster harvesting or handling seafood. Wound infections from these bacteria progress rapidly, and lead to necrotizing fasciitis, also known as 'flesh-eating disease', at the site of infection. Jones & Oliver [16] and Letchumanan *et al.* [17] describe details on pathogenesis and diseases caused by *V. vulnificus* and *V. parahaemolyticus*, respectively.

Almost all (90%) *V. vulnificus* and *V. parahaemolyticus* infections stem from eating undercooked or raw oysters, and there are in place several regulations and tools that reduce risk. The Interstate Shellfish Sanitation Conference (ISSC) was formed in 1982 with the goal of promoting a safe shellfish product by addressing sanitation and cooperating with state and federal agencies, industry and academia. The ISSC issues a National Shellfish Sanitation Program guide for the control of molluscan shellfish (NSSP) recognized by the US Food and Drug Administration (FDA). Included in the guide is a Model Ordinance with provisions that ensure shellfish-producing states are in compliance with safety guidelines [18]. Contained within the model ordinance are outlines for shellfish dealer certification, shellfish handling and processing and plant inspection procedures. Furthermore, there are sections that specifically consider how to control *V. parahaemolyticus* and *V. vulnificus*.

When a threshold of laboratory-confirmed *V. parahaemolyticus* cases is exceeded, the shellfish harvest areas that are implicated are closed for at least 7–21 days. After the minimum time has passed and prior to re-opening, shellfish samples must be collected from the harvesting area to ensure fewer than 10 pathogenic bacteria are counted per gram of oyster meat. Although the specific requirement to quantify pathogenic *V. parahaemolyticus* via *tdh* or *trh* (see below for detail on these genes) is commendable, the correlation between these markers and infections is not straightforward.

To control for *V. vulnificus*, the NSSP requires that oysters for raw consumption must meet minimum time-to-temperature requirements during harvest periods, where average monthly water temperatures exceed 26.6°C (80°F). Neither of these, nor any of the other regulations set forth by the NSSP, are arbitrary. Rather, they were carefully conceived using an extensive risk-per-serving model for *V. parahaemolyticus* and the assessment that less than 30 *V. vulnificus* cells per gram of oyster is of little risk [19,20]. Additionally, the NSSP contains a model for *V. parahaemolyticus* growth rate inside oysters, which is used to determine control measures. States are required to institute control plans for *V. vulnificus* if there have been two infections within the last 10 years. *V. parahaemolyticus* control plans are initiated based on two infections in a 3 year period, an outbreak having occurred within 5 years, or if average water temperatures during harvest exceed 27.2°C (81°F) [18]. Although these regulations reduce infection risk, the number of infections is still increasing.

To further reduce infections during autumn 2009, the FDA announced that Gulf Coast oysters harvested during warm water months could not be served raw without first undergoing post-harvest processing (PHP) [21]. Through PHP, oysters are treated using such techniques as high pressure, irradiation, pasteurization-like heating or flash freezing to reduce the number of harmful bacteria. PHP leaves the oyster raw, but no longer alive. Treatment could have lowered the infection rate from uncooked or raw shellfish, and such regulations have already existed in California since 2003. Nevertheless, the response to this ban was overwhelmingly negative. State officials, local legislators, the shellfishing industry and the public raised such an outcry over increased production costs, altered taste of oysters, and even the 'right' to eat whatever one chooses that the FDA suspended the ban a month later [22]. A similar attempt at a warm-weather Gulf Coast oyster ban failed in 1995, when the ISSC rejected the FDA-proposed action, instead enacting strict time-to-temperature rules [23].

Harvest restrictions, while effective at reducing infections, do not eliminate the problem. Furthermore, outright bans on marketing raw shellfish severely affect the industry and are unwelcome by some consumers. There is a need for tiered regulation that simultaneously reduces infection risk without ceasing to provide raw shellfish altogether. A further complication is that overstating risk can cause desensitization to risk-based statements. Desensitization can be reduced by educating shellfish growers and the shellfish consuming public. For example, California was the first state, in 1991, to require that those locations serving Gulf Coast oysters warn customers about infection risk [23]. The warning has since been modified from the original wording and now reads, in both English and Spanish, 'This facility offers raw oysters from the Gulf of Mexico. Eating these oysters may cause severe illness and even death in persons who have liver disease (for example alcoholic cirrhosis), cancer or other chronic illnesses that weaken the immune system. If you eat raw oysters and become ill, you should seek immediate medical attention. If you are unsure if you are at risk, you should consult your physician' [24, p. 2]. Although many states now have similar warnings, Mouzin *et al.* [23] concluded that a warning fails to reduce infections.

Even though the *Vibrio* models referenced by the NSSP are high quality and robust, they have limitations. For *V. vulnificus*, most studies find that temperature drives bacterial concentrations [25–34], *V. vulnificus* most easily being isolated in oyster tissues with a temperature range from 15°C to 17°C [26,27,29,32]. Exceptions include Parvathi *et al.* [35], Jones *et al.* [36] and Givens *et al.* [37], though a common limitation to these studies is a lack of variation in temperature over the sampling period. Models assume that salinity does not play a factor in *V. vulnificus* abundance, but reported effect of salinity on *V. vulnificus* varies from location to location, including insignificant [25,30,32,37], positive [29], inhibitory [26,28,31,38] or nonlinear [27] associations. Finally, both regulations are 'reactive' rather than 'proactive', requiring cases to be noted, or concentrations of bacteria in warmer waters to be high prior to action. Thus, there is vast room for improvement in monitoring and modelling pathogenic forms of vibrios, and data gaps that can be filled. Furthermore, the FDA *V. parahaemolyticus* model predicts bacterial abundance based on water temperature [20], but it is becoming obvious that models should consider other environmental factors. For example, in the Gulf Coast region, Zimmerman *et al.* [39] found significant, but site-dependent, relationships between *V. parahaemolyticus* and turbidity and salinity, but not chlorophyll *a*.

Observed regional variation in *Vibrio* ecology suggests a need for site-specific models. A study based outside of the Gulf Coast region, in the Chesapeake area, by Parveen *et al.* [39] found that unlike along the Gulf Coast, in the Chesapeake area neither salinity nor chlorophyll *a* were significant factors, but rather that *V. parahaemolyticus* abundance was driven by

water temperature, turbidity and dissolved oxygen [40]. An ambitious project by Johnson *et al.* [25] incorporated data from multiple regions, including the Gulf Coast, Northwest and Northeast regions of the USA. They noted that total suspended solids, dissolved organic carbon and salinity, in addition to water temperature, were significant controlling factors of *V. parahaemolyticus* in oysters, while salinity and chlorophyll *a* were not [25]. They also concluded that state-by-state modelling should be considered, based on the variation that they observed at each site they conducted experimentation. When multiple sites in South Carolina were sampled, the combinatory impacts of salinity and temperature appeared to be complicated, with some sites showing that both salinity and temperature correlated with *V. parahaemolyticus* abundance, some sites neither, or some with only temperature [41]. In a recent North Carolina-specific study, no correlation with salinity was observed [26]. This study also found 10-fold fewer *V. parahaemolyticus* cells per gram of shellfish than has been reported on average for similar water temperatures in oysters from the Gulf Coast, further highlighting the importance of region-specific studies. Outside of the USA, a study on shellfish in Mexico found that turbidity actually had a negative effect on *V. parahaemolyticus* concentrations in oysters [42]. While vibrios are often particle attached, Lopez-Hernandez *et al.* [42] hypothesized that non-digestible particles led to downshifts in filter-feeding in oysters. In Brazil, *V. parahaemolyticus* was found to be negatively correlated with salinity, and studies in New Zealand have demonstrated that salinity is unrelated or negatively correlated to *V. parahaemolyticus* shellfish abundances, depending on the study [43,44].

For *V. vulnificus*, the story is less convoluted but still variable. Nearly all studies that examine the number of *V. vulnificus* cells in oysters find that temperature is one of the major driving forces in determining bacterial concentrations [25–34]. Exceptions are by Parvathi *et al.* [35], Jones *et al.* [36] and Givens *et al.* [37], and a common limitation to these studies was the lack of variation in temperature over the sampling period. Of those that have shown positive correlations to temperature, there was tight agreement on a temperature threshold for when *V. vulnificus* is most easily isolated in oyster tissues. This temperature range is from 15°C to 17°C [26,27,29,32]. Conversely, reports on salinity for *V. vulnificus* are more variable from location to location. The effect of salinity on *V. vulnificus* oyster populations has been calculated to be insignificant by some [25,30,32,37], positive by others [29], inhibitory by yet others [26,28,31,38] or in the case of the study by Motes *et al.* [27], nonlinear.

One important note for correlation assessments for both *V. vulnificus* and *V. parahaemolyticus* is that even when environmental parameters have been important driving factors of abundance, there are still 'missing factors'. Regional differences in both the bacterial communities and their reactions to environmental stimuli make clear the need for multiple, focused studies of the *Vibrio* species. When predictive models or risk assessments are generated, these tools appear to only be relevant to the local area at best. In fact, even when a single region is examined, there are often site-by-site differences in prediction capabilities making even regional management of risk a challenge. It is not possible to manage *Vibrio*-associated infection risk under a single modelling system, and any attempt to do this would be difficult and inaccurate. Each oyster-producing state would need to develop their own dataset to increase confidence in our ability to forecast, now-cast or hindcast these bacteria in oysters. But these spatial aberrations are only the first of several obstacles.

Strains of *V. vulnificus* and *V. parahaemolyticus* vary in virulence, with some being pathogenic and others seemingly harmless [10,16]. The FDA and ISSC acknowledge that strain virulence should be considered, but at the time the NSSP was created there was not enough data to implement such fine detail. It seems evident that simple quantification of *V. vulnificus* and *V. parahaemolyticus* does have merit, and has been successfully used in regulation and in other microbial risk assessment tools [18–20,45,46]. Still, evidence continues to mount that the quantity of a particular *Vibrio* species inside an oyster may not be as important as the number of pathogenic cells.

For *V. parahaemolyticus*, potential pathogenicity is indicated by the presence of genes for thermostable direct haemolysin (*tdh*) and tdh-related haemolysin, *trh*. Briefly, the strains that possess at least one of these genes are often associated with cytotoxicity within the host [47,48]. However, there are other virulence-related factors, and there are also *tdh*- or *trh*-positive strains found in environmental samples and clinical isolates that contain neither gene [17]. Most studies that attempt to correlate *tdh*- and/or *trh*-positive *V. parahaemolyticus* strains in oysters to environmental parameters have had little success. Often, there are not enough of these strains above the limit of detection to make statistically significant comparisons [7,37,40,44,49,50]. Occasionally, when there are sufficient data on pathogenic strains, correlations with other measured data are still non-significant [51]. Though admittedly variable, Johnson *et al.* [32] and Zimmerman *et al.* [39] found that turbidity was correlated with *trh*- and *tdh*-positive strains and not with concentrations of total *V. parahaemolyticus*. Intriguingly, temperature was not significantly related to concentrations of pathogenic strains [32,39]. By contrast, Jones *et al.* [36] observed that when temperature did not affect total concentrations of *V. parahaemolyticus*, it did have an effect on *tdh*- and *trh*-positive strains.

Several different methods are available for detecting potentially pathogenic *V. vulnificus* strains. Most oyster studies use genetic differences found in either the virulence-correlated gene (*vcg*) or in the 16S rDNA gene [52–56]. Clinically isolated *V. vulnificus* strains are associated with the 'C' genotype of *vcg* and the 'B' type of the 16S rRNA gene, whereas strains isolated from the environment correlate with the 'E' and 'A' types of *vcg* and 16S, respectively. For simplicity, the potentially pathogenic strains, determined by either method, will be termed 'P' while those less likely to cause septicaemia will be called 'NP' (non-pathogenic). There are only a handful of studies that examine the prevalence of virulent *V. vulnificus* in oysters. As with *V. parahaemolyticus*, the relatively low occurrence of these strains, especially outside of the Gulf Coast region, makes even targeted analyses difficult [26,37]. Several authors agree that the P strains of *V. vulnificus* are more temperature-sensitive than NP strains, where the P strains in oysters increase in relative abundance to NP strains as water temperatures increase [31,57,58]. A similar reaction has been observed in *V. vulnificus* as has been noted in *V. parahaemolyticus*, where the normal drivers of abundance, temperature and salinity in the case of *V. vulnificus*, are not the factors that appear to influence the concentration of pathogenic strains. Factors such as dissolved oxygen and pH correlate with P strains while not seeming to affect total *V. vulnificus* (B. A. Froelich and R. T. Noble 2016, unpublished data). Thus, it

appears that the pathogenic strains of both *V. vulnificus* and *V. parahaemolyticus* have a niche different from that of the species as a whole, hence deserving further attention.

Neither total *V. parahaemolyticus* nor total *V. vulnificus* concentration appears to be related to the observed concentration of pathogenic strains in oysters [26,31,32,37,58]. One notable exception is presented by Han *et al.* [51], who found significant correlations between total and pathogenic *V. parahaemolyticus* in oysters, despite a ratio between the two that varied widely. However, most pathogenic strains in that study were obtained from Chinese markets, rather than directly from the environment, and the authors remark that handling practices could alter pathogenic *V. parahaemolyticus* levels [51].

Although it seems obvious to link water quality with oyster quality, most studies that examine environmental effects on *V. vulnificus* or *V. parahaemolyticus* find less variation in the water column than in oysters. In fact, it is not uncommon for bacterial concentrations inside oysters to be quite different from the water that they were harvested from, even when sampling is tightly controlled temporally and spatially [26,31]. Furthermore, variation in *Vibrio* concentrations from oyster to oyster can be orders of magnitude, even when oysters are collected from the exact same clutch. It seems that while *Vibrio* spp. in the water column react quickly to changing conditions, once they colonize the interior of the oysters they remain relatively stable. It is hypothesized that the *Vibrio* colonization of oysters occurs at the larval settlement stage, and exogenous bacteria in adult oysters are merely transient [59]. Major ecological disruptions, such as large upshifts in salinity, can disrupt these resident populations, but nominal conditions may not have a large intra-oyster effect [38,59–61]. This independence from the surrounding water makes it difficult to predict pathogenic *Vibrio* concentrations within oysters from environmental parameters.

In the face of likely changing *Vibrio* concentrations with impending climate change and warming estuarine and coastal waters, we are left with hurdles to improving regulations that aim to reduce food-borne infections. Current US regulations are based on obsolete data, are relevant mostly to a specific region of the USA, and make simplifying assumptions. Moreover, current risk assessment does not take into account the differences in growth rates of different strains among different oyster species. Simultaneously, outbreaks of illness are expanding latitudinally to locations never suspected of *Vibrio*-associated risk, while oyster growers and commercial markets are increasing with the burgeoning boutique oyster business [62,63]. The current rules are protective and reduce risk, yet infections are still increasing. Current guidelines might not be appropriate for every state or region, as differences in *Vibrio* population structure and abundance vary from location to location. Underprotection leads to health risks, while overprotection increases costs. Increasing the number of location-specific studies would help strengthen the understanding of these dynamic naturally occurring pathogens. Future studies should include specific quantification of pathogenic forms of each species, as it is clear that concentrations at the species level do not necessarily indicate health risks. For *V. vulnificus*, this can include either C/E genotyping, *pilF* polymorphism to determine serum resistance potential, or AB ribotyping [54,55,64]. For *V. parahaemolyticus*, at the minimum, research should include detection and quantification of the *tdh* and *trh* genes. Newer types of equipment, such as digital PCR, and advanced techniques, including next-generation sequencing, are becoming cheaper and more available. Sequencing environmental samples and using digital PCR to detect rare sequences can help overcome the problem of pathogen rarity and improve pathogen-specific modelling. Region-specific, hydrodynamic models that take into account environmental and biogeochemical factors might also improve understanding. Including environmental factors, such as salinity, temperature, pH, dissolved oxygen and chlorophyll *a*, along with novel factors, such as oyster ploidy, oyster condition, particulates, chitin or strain pathogenicity might further the understanding of this complex and important problem.

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