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Review

Non-Cholera Vibrios: The Microbial Barometer of Climate Change

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There is a growing interest in the role of climate change in driving the spread of waterborne infectious diseases, such as those caused by bacterial pathogens. One particular group of pathogenic bacteria – vibrios – are a globally important cause of diseases in humans and aquatic animals. These Gram-negative bacteria, including the species *Vibrio vulnificus, Vibrio parahaemolyticus* and *Vibrio cholerae*, grow in warm, low-salinity waters, and their abundance in the natural environment mirrors ambient environmental temperatures. In a rapidly warming marine environment, there are greater numbers of human infections, and most notably outbreaks linked to extreme weather events such as heatwaves in temperate regions such as Northern Europe. Because the growth of pathogenic vibrios in the natural environment is largely dictated by temperature, we argue that this group of pathogens represents an important and tangible barometer of climate change in marine systems. We provide a number of specific examples of the impacts of climate change on this group of bacteria and their associated diseases, and discuss advanced strategies to improve our understanding of these emerging waterborne diseases through the integration of microbiological, genomic, epidemiological, climatic, and ocean sciences.

Trends

Advances in strain typing techniques, such as those facilitated by whole-genome sequencing, now allow researchers to answer fundamental questions regarding the source, transmission, and dynamics of *Vibrio* outbreaks.

The development of complementary molecular techniques in environmental studies have provided fascinating insights into the role of climate warming on these pathogens in the environment.

Remote sensing-based techniques can provide a highly useful alternative approach to study when and where infections have been reported.

Increasing understanding of climate change impacts on marine ecosystems is providing more robust and reliable approaches for risk assessment. The ability to predict when and where different 'at risk' systems are undergoing rapid warming using process-driven climate models will greatly improve our understanding of future risk.

Increasing *Vibrio* Infections

Vibrios are Gram-negative rod-shaped bacteria that are natural constituents of estuarine and marine environments. Although a wide range of different bacterial species contain multiple chromosomes, *Vibrio* species are notable in that they possess two circular chromosomes. The genus *Vibrio* contains over 100 described species, and around a dozen of these have been demonstrated to cause infections in humans [1]. Infection is usually initiated from exposure to seawater or consumption of raw or undercooked seafood [2,3]. Cases of *Vibrio* infections have a marked seasonal distribution – most occur during summer and early autumn, corresponding to the period of warmer temperatures [4]. Several reports have recently indicated that human *Vibrio* illnesses are increasing worldwide, including fatal acute diarrheal diseases, such as gastroenteritis, and wound infections and septicemia [5,6]. A number of significant factors underpin the need for a greater understanding of these foodborne pathogens within an international context: compared to other major foodborne pathogens, the number of *Vibrio* infections is steadily increasing [7]. Indeed, the Centers for Disease Control and Prevention (CDC) estimates that the average annual incidence of all *Vibrio* infections increased by 41% between 1996 and 2005 in the USA [8]. Notably, of all the major bacterial foodborne pathogens (e.g., *Salmonella*, *Listeria*, *Escherichia coli* O157, and *Camplyobacter*), vibrios are the only group that are currently increasing in incidence in the USA [9]. Strikingly, infections from these pathogens are now being reported in areas with no previous incidence. These include outbreaks in Israel [10] and

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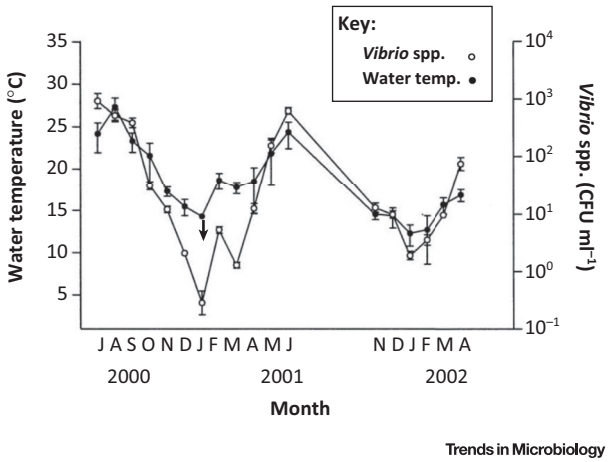

Figure 1. Relationship Between Total *Vibrio* Abundance and Ambient Environmental Temperatures. The close correlation between bacterial counts and environmental temperature make vibrios an exceptional microbial group to study the interaction between microbiology, climate, and infectious diseases. Figure kindly reproduced from Pfeffer *et al*. [14].

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Northern Europe [5,11,12] and *Vibrio parahaemolyticus* outbreaks in Western Europe and the Northeast USA [13]. The factors driving this increase are likely to be complex and multifactorial. Climate warming, in particular in temperate regions, appears to be playing a significant role in mediating the expansion of pathogenic vibrios [5]. Indeed, pathogenic vibrios grow well in low-salinity warm water (Figure 1), with their growth patterns ostensibly mirroring ambient temperature regimes [14]. Future climate scenarios, based on climate modelling, suggest that these bacterial pathogens are likely to continue to pose a significant and sustained public health threat. Other factors could also contribute to the observed increasing rates of vibriosis: an increase in consumption of shellfish (particularly raw oysters), an increase in population density in coastal regions, and improvements in epidemiology, diagnosis, and reporting.

We provide here a brief overview of the ecology, epidemiology, and public health relevance of these pathogens, and provide numerous relevant case studies regarding these bacteria as well as discuss the significance of these emerging pathogens in the context of a changing climate system.

Pathogenic Vibrios of Human Health Relevance: 'The Big Four'

Around a dozen *Vibrio* species can cause infections in humans. Human infections can be acquired from more than one route of exposure (e.g., consumption of seafood and exposure to contaminated water). We focus here on what we consider (from an epidemiological and microbiological context), as the 'big four' (*V. cholerae, V. vulnificus, V. parahaemolyticus* and *V. alginolyticus*). Globally, these four pathogens disproportionately dominate human infection reports associated with vibrios, although it should be noted that a wide range of other *Vibrio* species, including *V. damsela, V. hollisae, V. mimicus* and *V. metshnikovii*, have also been implicated in human infections.

V. vulnificus

V. vulnificus is a naturally occurring and common inhabitant of estuarine and coastal environments. Globally, *V. vulnificus* is a significant foodborne pathogen capable of causing necrotizing wound infections and primary septicemia, and is a leading cause of seafood-related mortality. Indeed, 95% of fatalities linked to seafood consumption in the USA are caused by this bacterium, underlying its importance as a key foodborne pathogen [15]. Most cases occur in

immunocompromised males or patients with underlying conditions resulting in elevated serum iron levels, primarily alcohol-associated liver cirrhosis [16]. *V. vulnificus* is currently subdivided into three biotypes based on genetic, biochemical, and serological features, as well as host range [17,18]. The molecular basis responsible for virulence in *V. vulnificus* is becoming more clearly defined, and includes mechanisms encompassing acid neutralization pathways, expression of capsular polysaccharides, use of iron-acquisition systems, cytotoxicity, motility, and expression of proteins involved in attachment and adhesion [15]. *V. vulnificus* is a rare cause of infection (generally around 100 cases a year in the USA, and sporadically in Europe and the Far East), but there has been a range of published studies that demonstrate a recent and discernible increase in reported infections [5,20]. Infections are characterised by a short incubation period between exposure and the onset of symptoms [21], typically within 24 h of exposure [16,22]. Typically, *V. vulnificus* wound infections are characterised by swelling, erythema, and intense pain. The infection progresses into visible lesions, which frequently evolve into vesicles or fluid-filled bullae that can become necrotic. *V. vulnificus* wound infections are of concern because of the significant mortality rate associated with this pathogen, the rapidity of disease onset, and the fact that there appears to be an increasing number of cases reported around the world. The high mortality rate associated with this pathogen through wound infection and food-borne infection underlines its importance from a public health perspective.

V. parahaemolyticus

V. parahaemolyticus is the most prevalent food-poisoning bacterium associated with seafood consumption and typically causes acute gastroenteritis. *V. parahaemolyticus* grows preferentially in warm (>15 °C), low-salinity marine water (<25 ppt NaCl) [23]. Common clinical characteristics of *V. parahaemolyticus* infections include abdominal cramps, nausea, headaches, diarrhoea, fever, and chills. *V. parahaemolyticus* has a well-established and characterised basis for virulence and pathogenicity. The vast majority of strains associated with human disease carry one (or more rarely two) haemolysin genes. These encode the thermostable direct hemolysin (TDH) [24,25], responsible for the Kanagawa hemolysis, and the TDH-related hemolysin (TRH) [26]. Both genes appear to be highly important in the initation of disease.

In the past two decades, numerous large-scale foodborne *V. parahaemolyticus* outbreaks have been reported around the world. These outbreaks have largely been driven by one specific clone of *V. parahaemolyticus* strains, the so-called pandemic clone which emerged in the Far East in the mid-1990s [27] and has subsequently disseminated around the world [28–30]. Most strains from this clonal complex (CC3) are sequence type (ST) group 3 [30,31]. However, recent studies utilising whole-genome sequencing have revealed that other variants of other clonal complexes (e.g., CC345 – ST189, ST88; CC120 – ST120; and CC8 – ST8), all of Asiatic origin, have caused outbreaks in other part of the world [32–34]. This interesting observation has suggested that pathogenic *V. parahaemolyticus* strains have, on numerous occasions, radiated away from their endemic sources to cause outbreaks in geographically distant areas. Examples of this include Peru in 2009, caused by CC120 strains [32], and an outbreak of CC8 strains in Maryland, USA, in 2010 [33]. More recently a highly pathogenic variant belonging to yet another clonal complex (CC36), and termed the Pacific Northwest complex [35,36], emerged on the west coast of the United States during an unusually warm spring [13,37]. This highly pathogenic variant has subsequently disseminated along the East Coast of the USA and caused significant foodborne outbreaks in the USA in recent years (2012); it was also observed in Europe in 2012 [13,37]. However, the clone that has spread to the East Coast of the USA belongs to a new ST36 variant within the CC36 clonal complex [38]. Some of these outbreaks (e.g., in Peru) have been linked to the occurrence of El Nino events [39]. As with *V. vulnificus*, several recent reports suggest that the number of *V. parahaemolyticus* infections appear to be increasing in Europe [23] and the USA [19,37]. Because *V. parahaemolyticus* infections are generally self-limiting (with typical gastroenteritis-like symptoms resolving within a few days), there is a large factor of

under-reporting, even in countries such as the USA that have dedicated surveillance and monitoring systems in place for these pathogens [40]. *V. parahaemolyticus* wound infections are considered more seriously than shellfish-associated infections, and frequently require intervention with appropriate antibiotics. A study by Weis identified that almost 40% of *V. parahaemolyticus* infections reported in Florida from 1998 to 2007 were wound-associated cases, with one reported fatality [20].

V. alginolyticus

An often overlooked bacterium, *V. alginolyticus* is increasingly recognised as an emerging human pathogen, and as with other vibrios the incidence of infection significantly increases during summer months [5,11]. *V. alginolyticus* is ubiquitous in seawater and tends to cause superficial wound and ear infections (otitis media and otitis externa). Most reports of *V. alginolyticus* wound infections result from exposure of cuts or abrasions to contaminated seawater. *V. alginolyticus*-associated infections may be resolved using appropriate antibiotics; however, very rarely these infections can progress to bacteraemia and necrotising fasciitis, particularly in the immune compromised. A study of vibriosis in Florida, USA (1998–2007), identified *V. alginolyticus* as a significant cause of infection, with 131 cases (almost 20% of all vibriosis infections) reported during this time period [20]. Recent epidemiological data suggest a rapid increase in the incidence of *V. alginolyticus* infections reported in the USA [19]. Numerous sporadic reports of *V. alginolyticus* have also been documented elsewhere, including recently in Europe [41].

Non-O1 *V. cholerae*

There is wide interest in the role of climate change on the dynamic of cholera (which will not be discussed further here, please refer to excellent articles addressing this issue [42,43]). There is less attention paid to non-O1/O139 variants of this bacterium. *V. cholerae* non-O1/non-O139 are the causative agents of sporadic, yet significant, gastrointestinal and extraintestinal infections [44] and, compared to cholera, are relatively understudied as a group of human pathogens. Most nontoxigenic cases involve self-limiting gastroenteritis or ear and wound infections in immunocompetent patients [45]. Non-O1/O139 infections (as with other vibrios) are often underdiagnosed, partly due to the inexperience of clinicians and microbiologists in suspecting vibrios, and to the fact that many diagnostic and clinical laboratories do not use the appropriate enrichment and culture media, such as thiosulfate-citrate-bile salt-sucrose (TCBS) agar, to isolate these bacteria. Numerous studies have shown that non-O1 *V. cholerae* strains are an important and potentially life-threatening cause of infections, and there has also been an increase in the number of reports of infections involving non-O1/non-O139 *V. cholerae* [45]. Data gathered in Northern Europe (1980–2010) identified a plethora of wound-associated non-O1 *V. cholerae* infections, most of which were linked to recreational exposure to seawater [5], and have included numerous reported fatalities. Indeed, a large increase in *V. cholerae* wound infections in 2014 corresponded both temporally and spatially with the largest and most intense heatwave reported in Northern Europe [11]. A notable observation was that over 75% of reported infections were caused by non-toxigenic *V. cholerae*.

How a Changing Climate Will Modulate Risk

A number of physical manifestations of climate change are likely to play a significant role in increasing risks associated with pathogenic vibrios [5], and in particular non-cholera vibrios such as *V. vulnificus*, *V. parahaemolyticus*, and non-O1 *V. cholerae*. These include generalised warming patterns, heatwaves, and extreme precipitation events. More robust global climate models (GCMs) allow us to more accurately predict which areas are likely to change over time, potentially providing a framework for determining future risks. We will briefly present some relevant case studies highlighting some of the factors that we believe will modulate risk with regard to these pathogens.

Warming of Marine and Coastal Regions

The recent change in sea surface temperature (SST) is considered as the most pervasive and severe impact in coastal ecosystems worldwide [46], particularly in light of recent observations demonstrating significant warming in over 70% of the world's coastlines [47]. Climate change can have direct impacts on marine ecosystems, such as through the warming of oceans; however, few studies have systematically assessed the role of warming in the marine environment to the abundance of *Vibrio* bacteria. Vezzulli *et al*. [48] scrutinised long-term plankton datasets from the continuous plankton recorder (CPR) from the 1960s onwards, using a set of novel molecular methods. They identified a significant increase in *Vibrio* abundance in the North Sea during the 1980s onwards, which corresponded both temporally and spatially with an increase in SST in the area. This study is unique in that it provides long-term molecular microbiological data with regard to these pathogens, but within the framework of a changing climate system. Extreme localised warming of coastal areas has been associated with seafood-related outbreaks in mid- and high-latitude areas, including *V. parahaemolyticus* outbreaks in Alaska [49] and Northern Spain [13,50]. It is striking that many outbreaks have taken place in areas that have recently undergone warming over the past three decades, with several incidents occurring in these temperate and high-latitude areas undergoing rapid warming trends (Figure 2).

Extreme Climatic Events

Increasing evidence suggests that climate change has led to changes in extreme climatic events, including heatwaves, and, in many regions, heavy precipitation, particularly in the past half century [51]. It is very likely that hot extremes, heat waves, and heavy precipitation events will continue to become more frequent in the future [51]. Several recent studies have pinpointed the role of extreme weather events in greatly increasing risks associated with pathogenic vibrios. Heatwaves in Northern Europe over the past three decades, in particular in 1994, 2003, 2006, and 2010, led to increased reports of *Vibrio* wound infections in the region [5]. The authors noted that extreme heatwaves and, where surface seawater temperatures exceeded 18 °C, were statistically associated with a significant increase in reported infections. More recently, the most intense heatwave ever experiences in Scandinavia, in 2014, corresponded with the highest yearly total of *Vibrio* infections in Finland and Sweden [11]. This study is

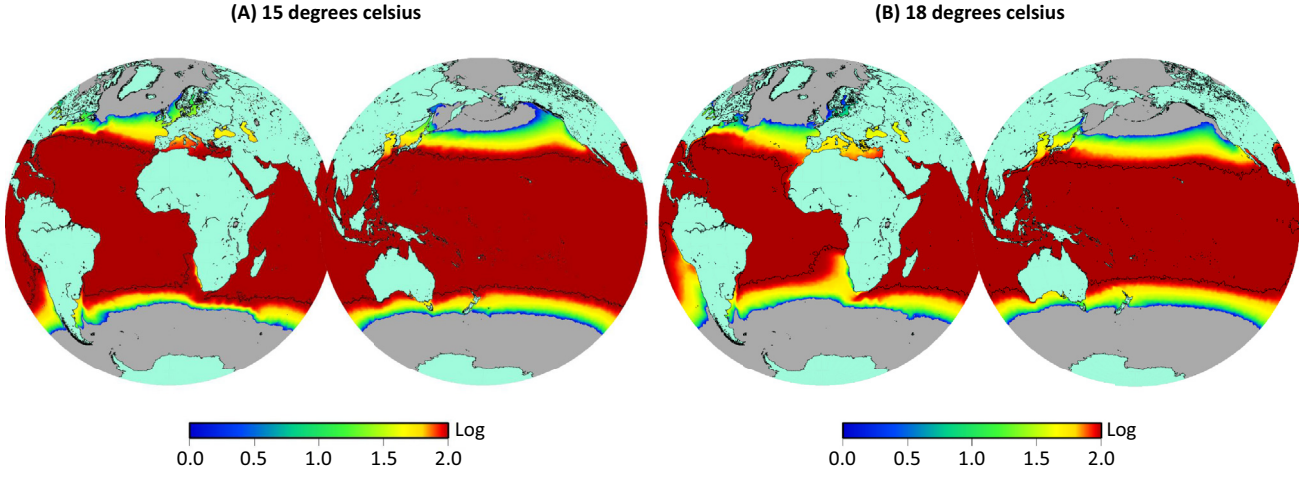

Figure 2. Living in a *Vibrio* World. Percentage of days (log transformed) where sea surface temperatures (SST) exceed 15 (A) and 18 (B) degrees Celsius for the 10-year period between 2006 and 2015. Recent outbreaks of vibriosis in temperate regions, including the Baltic Sea [5,11], Alaska [7,49], North East USA [13,37], Chile [7,28], and Northern Spain [23], highlight the poleward spread of reported vibriosis. Warming in these regions is likely to have contributed to these events. The figure was created from remote sensing sea surface temperature data (NOAA 1/4° daily Optimum Interpolation Sea Surface Temperature dataset, see https://www.ncdc.noaa.gov/oisst).

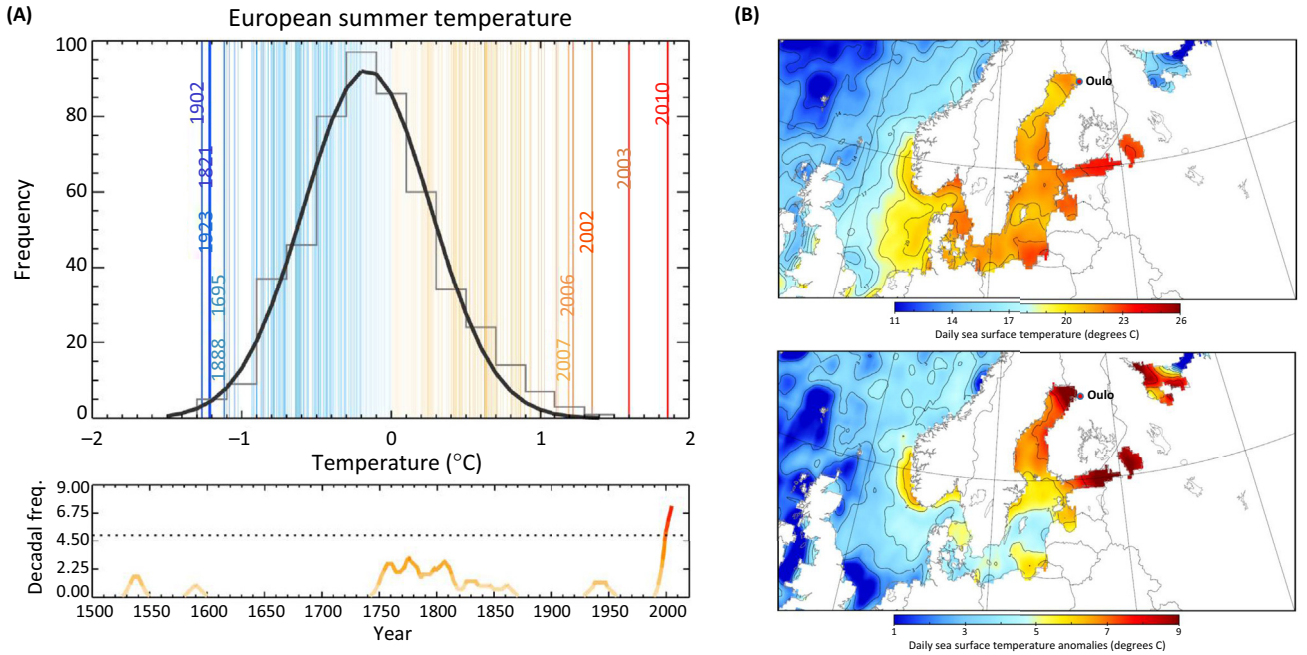

Figure 3. Distribution of Extreme Weather Events in Europe. (A) The increased frequency and severity of heatwave events, particularly over the last two decades. An extreme heatwave experienced in the Baltic Sea region in July 2014 (B) corresponded both temporally and spatially with a significant increase in *Vibrio* wound infections reported in Sweden and Finland. Infections were reported as northerly at Oulu (~65°N). Panel (A) kindly adapted from Barriopedro *et al*. [57].

particularly noteworthy because of the latitude of infections, with cases reported as far north as 65°N, less than 100 miles from the Arctic Circle. As with previous heatwaves, cases tended to be reported in areas with highly anomalous warm waters, irrespective of latitude (Figure 3). Increased *V. vulnificus* wound infections observed in Israel in the late 1990s closely corresponded with ambient temperatures [10], and, as with examples in Northern Europe [5,11], appear to be linked to heatwave events. It is very likely that under a warming climate system, significant heatwaves are likely to be experienced more regularly (Figure 3). Indeed, heatwave events in Europe that would occur twice a century in the early 2000s are now expected to occur twice a decade [52].

Of interest, other extreme climatic events have also been linked to an increase in reported infections. Data from the CDC [53] indicated a sharp increase in *Vibrio* wound infections following Hurricane Katrina making landfall on the Gulf coast of the USA in August 2005. Although precise exposure histories were not available for all patients, the infections caused by vibrios likely resulted from wounds exposed to flood waters among persons with medical conditions that predisposed them to *Vibrio* infections. Of 18 *Vibrio* wound infections reported in the region following Katrina, five (28%) patients with wound-associated *Vibrio* infections died; three deaths were associated with *V. vulnificus* infection, and two were associated with *V. parahaemolyticus* infection [53]. A spate of *V. vulnificus* infections were reported in New Caledonia in 2008 (including three fatalities) following heavy precipitation in the area [54]. These incidents are noteworthy because *V. vulnificus* infections are rare in the South Pacific. It is believed that the heavy precipitation experienced in New Caledonia in early 2008 significantly reduced salinity in the local area and provided fertile conditions for this bacterium to thrive, and may well have been a contributory factor for these infections. These examples suggest that an increase in extreme climatological events may increase risk.

Using Vibrios as Barometers of Change

Accurately assessing the role of climate in contributing to disease burden is one of the most important challenges facing clinical, public health, and regulatory sectors. We believe that vibrios possess a number of important characteristics that allow them to be used effectively as a microbial 'bellwether' for climate change – that is, an indirect measure of impacts in different coastal and marine systems, particularly in mid-latitude and temperature regions. These characteristics include the following:

1. Sensitivity to Temperature

All of the 'big four' pathogens highlighted here (*V. vulnificus, V. parahaemolyticus, V. cholerae*, and *V. alginolyticus*) grow in warm, low-salinity waters. Their growth is proportional to ambient environmental temperatures, and as a rule of thumb, these pathogens grow extremely well above 15 °C [11]. Infections reported in the community mirror this temperature-driven abundance, with a gamut of studies showing peak numbers of cases reported during summer months [5]. Numerous detailed multiyear studies augment the clinical and epidemiological information, providing incredible seasonal abundance data to suggest that temperature drives the presence of these pathogens (Figure 1).

2. Rapid Replication

Vibrios have some of the fastest replication times of all known and studied bacteria, and as such are highly responsive to favourable environmental stimuli. Studies have shown that *V. parahemolyticus* and other *Vibrio* species have replication times as little as 8–9 minutes [55,56], making this group of pathogens some of the most reactive and adaptable bacteria on the planet. This rapid response to external environmental stimuli, such as ambient temperature, allows the use of vibrios as an effective measure of change.

3. Unwelcome Visitors?

Conclusive evidence linking the emergence of infections with climate change remains a contentious area of science [5]. However, the reporting of *Vibrio* infections in temperate and cooler regions potentially allows us to attribute warming trends to the emergence of infectious diseases driven by climate change. For instance, outbreaks in Northern Europe [5], Alaska [49], Chile [28], and the Northeast USA [37] are highly unusual, particularly as these pathogens have historically been associated with tropical and subtropical environments such as the Gulf Coast of the USA. The recent reporting of infections in sub-Arctic waters further reinforces this observation [11]. The emergence of these pathogens into new areas may allow us to pinpoint localised changes in disease transmission caused by these bacteria, potentially driven by environmental change.

4. Exposure – When and Where

Vibrios are unusual as bacterial pathogens in that they have more than one portal of entry prior to the initiation of diseases (e.g., the consumption of shellfish produce, and exposure to contaminated water). The water exposure element is especially useful in tracking when and where individuals have come into contact with contaminated waters. Even rudimentary epidemiological analysis can then be used to attribute risk and inform future risk-assessment models, in particular as many cases are reported close to the source of exposure.

Concluding Remarks

Non-cholera vibrios are an increasingly important cause of human diseases. Because of their affinity for warm, low-salinity waters, rapid growth, and pathogenicity profile, they represent a

Outstanding Questions

Globally, systems of epidemiology associated with these pathogens are poor. Transnational improvements in diagnosing, reporting, and collating epidemiology data are urgently required. How can we improve monitoring and surveillance systems that allow for the sharing of data?

Where are we likely to see these important pathogens emerge in the future, in the context of a warming climate system?

How can we accurately attribute changing environmental factors and reliably demarcate the role that this plays in increasing clinical risk?

How can we provide a framework for sharing data between different stakeholders (e.g., clinicians, scientists, microbiologists, risk assessors, etc.) with regard to these important emerging pathogens?

How can the wealth of next-generation sequencing data being produced at the moment be used most appropriately in outbreak investigations and for understanding the emergence of these pathogens?

How important is remote-sensing-based analyses in understanding the environmental conditions prior to, during, and after *Vibrio* outbreaks?

useful barometer of change in the marine environment. Unfortunately, reliable quantitative projections of future climate-sensitive waterborne risks are difficult due to the complex interplay between climate, climate-sensitive disease, and interaction with the human host. Irrespective, vibrios represent an important and emerging waterborne threat, particularly in temperate and mid-latitude areas that are undergoing rapid warming. The mechanisms underlying the apparent invasion of these bacteria, as well as others, into new regions is an area of increasing interest [58]. Indeed, the emergence of non-cholera *Vibrio* diseases, particularly in geographical regions with a lack of long-term epidemiological datasets, provides startling practical challenges to the *Vibrio* research community. A major challenge is to foster cooperation between fundamental and applied research in order to answer basic questions regarding these emerging pathogens [59]. Improvements in the surveillance and reporting of these pathogens is absolutely paramount, particularly in regions such as Europe that lack a centralised and coordinated monitoring and epidemiological surveillance system for these emerging pathogens.

Update

Vezzulli *et al*. [60] recently provided further evidence that SST warming is strongly associated with the spread of vibrio pathogens in the environment. In this study, which utilised formalin-preserved plankton samples collected in the past half-century from the temperate North Atlantic, the authors noted a concomitant increase in *Vibrio* abundance as evidenced through molecular approaches. A notable increase in *Vibrio*-specific signals have coincided with an unprecedented occurrence of environmentally acquired *Vibrio* infections in the human population of Northern Europe and the Atlantic coast of the United States in recent years. These data build on a similar, albeit geogeographically restricted, study from the North Sea [48].

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