

Public health aspects of *Vibrio* spp. related to the consumption of seafood in the EU

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Abstract

Vibrio parahaemolyticus, *Vibrio vulnificus* and non-O1/non-O139 *Vibrio cholerae* are the *Vibrio* spp. of highest relevance for public health in the EU through seafood consumption. Infection with *V. parahaemolyticus* is associated with the haemolysins thermostable direct haemolysin (TDH) and TDH-related haemolysin (TRH) and mainly leads to acute gastroenteritis. *V. vulnificus* infections can lead to sepsis and death in susceptible individuals. *V. cholerae* non-O1/non-O139 can cause mild gastroenteritis or lead to severe infections, including sepsis, in susceptible individuals. The pooled prevalence estimate in seafood is 19.6% (95% CI 13.7–27.4), 6.1% (95% CI 3.0–11.8) and 4.1% (95% CI 2.4–6.9) for *V. parahaemolyticus*, *V. vulnificus* and non-cholerae *V. cholerae*, respectively. Approximately one out of five *V. parahaemolyticus*-positive samples contain pathogenic strains. A large spectrum of antimicrobial resistances, some of which are intrinsic, has been found in vibrios isolated from seafood or food-borne infections in Europe. Genes conferring resistance to medically important antimicrobials and associated with mobile genetic elements are increasingly detected in vibrios. Temperature and salinity are the most relevant drivers for *Vibrio* abundance in the aquatic environment. It is anticipated that the occurrence and levels of the relevant *Vibrio* spp. in seafood will increase in response to coastal warming and extreme weather events, especially in low-salinity/brackish waters. While some measures, like high-pressure processing, irradiation or depuration reduce the levels of *Vibrio* spp. in seafood, maintaining the cold chain is important to prevent their growth. Available risk assessments addressed *V. parahaemolyticus* in various types of seafood and *V. vulnificus* in raw oysters and octopus. A quantitative microbiological risk assessment relevant in an EU context would be *V. parahaemolyticus* in bivalve molluscs (oysters), evaluating the effect of mitigations, especially in a climate change scenario. Knowledge gaps related to *Vibrio* spp. in seafood and aquatic environments are identified and future research needs are prioritised.

KEYWORDS

analytical methods, antimicrobial resistance, climate change, interventions, public health, risk assessment modelling, virulence

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Biological Hazards (BIOHAZ) to deliver a scientific opinion on the public health (PH) aspects of *Vibrio* spp. related to the consumption of seafood in the EU. Infections deriving from environmental, recreational and/or occupational exposure to vibrios were not considered. The assessment had to cover *Vibrio parahaemolyticus*, *Vibrio vulnificus* and non-O1/non-O139 *Vibrio cholerae*; other species had to be considered as well, when relevant.

In **ToR1**, EFSA was requested to review, for the relevant *Vibrio* spp., the existing information on occurrence and concentration in seafood, available analytical methods, pathogenicity to humans and virulence factors, as well as antimicrobial resistance (AMR) and persistence mechanisms in different environments. It was decided that the relevant *Vibrio* spp. to be considered in the current opinion are *V. parahaemolyticus*, *V. vulnificus* and non-O1/non-O139 *V. cholerae*. Other species as *Vibrio alginolyticus*, *Vibrio fluvialis* and *Vibrio mimicus* may occasionally lead, particularly in individuals with underlying health conditions, to seafood-associated infection, but their PH relevance is minor compared to those three species.

Based on the available data on seafood placed or intended to be placed on the EU market, it was concluded that, across seafood categories, the *V. parahaemolyticus* pooled prevalence estimate was 19.6% (95% CI 13.7–27.4), with the highest estimates for bivalve molluscs (27.8%; 95% CI 18.6–39.5) and gastropods (28.8%; 95% CI 10.5–58.3). About one out of five (18.4%; 95% CI 11.1–29.01) of *V. parahaemolyticus*-positive samples contained pathogenic (i.e. *tdh*+ and/or *trh*+) strains. *V. parahaemolyticus* pooled mean concentration in bivalve molluscs was 1.91 log₁₀ CFU/g or MPN/g (95% CI 0.68–3.14), while in crustaceans, was 3.33 log₁₀ CFU/g (single study). The *V. vulnificus* pooled prevalence estimate was 6.1% (95% CI 3.0–11.8) and was highest for bivalve molluscs with 9.9% (95% CI 4.0–22.60); with a pooled mean concentration of 1.84 log₁₀ CFU/g or MPN/g (95% CI –2.33 to 6.01). Non-cholerae *V. cholerae* pooled prevalence estimate was 4.1% (95% CI 2.4–6.9) and the mean concentration was 1.34 MPN/g (single study). No data were retrieved in either tunicates or echinoderms.

Standardised microbiological methods are available for the detection of the relevant *Vibrio* spp. in seafood and for the enumeration of *V. parahaemolyticus* and *V. vulnificus*. These methods rely on culturing and include molecular tests for species identification and/or for the detection of genes associated with pathogenicity. Alternative approaches are available for the detection, enumeration and identification (e.g. loop-mediated isothermal amplification (LAMP)-PCR, quantitative, digital and viability PCR, matrix-assisted laser desorption ionisation–time of flight mass spectrometry or MALDI–TOF MS). For characterisation, serotyping and pulsed-field gel electrophoresis (PFGE) have been progressively replaced by sequencing technologies [e.g. (core genome) multi-locus sequence typing or (cg)MLST schemes]. Whole genome sequencing (WGS) is progressively more applied for full characterisation of clinical *Vibrio* isolates and in outbreak investigation but currently has limited use in official food control activities in the EU.

Pathogenic *Vibrio* species possess a wide array of virulence factors (VFs) that allow the colonisation and spread in the hosts. Their virulence is multifactorial; although with different impact, some VFs may be present in all species (i.e. capsule, flagellar mobility and pili), while other VFs are specific to certain species or strains within one species. The outcome of an infection with *Vibrio* species is determined both by bacterial and host factors. *V. parahaemolyticus* mainly leads to acute gastroenteritis (GE), also in healthy individuals. Its pathogenicity is significantly associated with the haemolysins TDH (thermostable direct haemolysin) and TRH (TDH-related haemolysin), which are well-established pathogenicity markers. The type 3 secretion system 2 (T3SS2) has been also associated with pathogenic strains. *V. vulnificus* infections are relatively rare and affect mainly individuals with underlying health conditions, possibly leading to sepsis and death. This species possesses several VFs but, as reliable discrimination of pathogenic from non-pathogenic strains is lacking, to date all *V. vulnificus* strains are considered potentially pathogenic. *V. cholerae* non-O1/non-O139 is generally associated with self-limited GE or mild extraintestinal symptoms. However, in susceptible individuals, strains can cause more severe infections, sepsis and death. For this species, no single pathogenicity marker has been identified so far.

A large spectrum of AMRs, of which some are known as intrinsic resistances, has been reported in the few studies on *Vibrio* isolates from seafood or food-borne infections in Europe. The AMRs most frequently detected were ampicillin (70%–100%; seven studies) and streptomycin (30%–70%; six studies) for *V. parahaemolyticus*, and colistin (87%–100%; four studies), ampicillin (4%–75%; five studies) and streptomycin (11%–68%; four studies) for non-O1/non-O139 *V. cholerae*. Antimicrobial resistance genes (ARGs) associated with mobile genetic elements, conferring resistance to various β -lactam types, quinolones, sulfonamides, aminoglycosides, tetracyclines, folate pathway inhibitors and phenicols, have been detected in *Vibrio* spp. Resistances against medically important antimicrobials (MIAs) like carbapenems (authorised only for humans) and 3rd/4th-generation cephalosporins (categorised as highly important antimicrobials) associated with mobile elements are increasingly found in the relevant *Vibrio* spp., and detection in imported seafood isolates has been reported.

The main persistence mechanisms of the relevant *Vibrio* species in the aquatic environment include the VBNC state, biofilm formation on biotic and abiotic surfaces, persister cells, anti-grazing strategies to avoid protozoan predation and association with other aquatic organisms acting as reservoirs.

In **ToR2**, EFSA was requested to identify the factors in the aquatic environments and in food that influence occurrence and growth of the relevant *Vibrio* spp. and affect transmission of their virulence and resistance determinants. It was concluded that the impacting factors are temperature, (sea)water salinity, solar and UV radiation (extrinsic factors); pH, water activity of food, nutrient content (intrinsic factors) and predation, parasitism and commensalism (implicit factors). Temperature is the most relevant driver for *Vibrio* abundance in the aquatic environment and in food, followed, in the environment, by salinity. Complex interactions among environmental factors make region-specific environmental models and validations required. Transmission of virulence or resistance determinants is affected by the presence of chitin (triggering

natural competence) and phages (acting as reservoirs of these genetic determinants), and contaminants of the aquatic ecosystem such as antimicrobials and heavy metals.

In **ToR3**, EFSA was requested to review the evidence on the impact of climate change on the occurrence and levels of the relevant *Vibrio* spp. in water environments and seafood. Climate change induces a shift towards conditions in the aquatic environment suitable and more conducive for *Vibrio* growth and persistence. It affects: (i) the geographical distribution of the coastal areas suitable for *Vibrio* spp., with areas characterised by brackish/low-salinity waters (e.g. Baltic Sea, transitional waters of the Baltic and the North Sea, the Black Sea) and coastal areas with large riverine inputs at higher risk; (ii) the temporal distribution of conditions suitable for *Vibrio* spp.; (iii) the frequency, distribution and potentially intensity of extreme weather events which may provide conditions favourable to *Vibrio* spp. Climate change may also affect the structure (i.e. the composition) of the *Vibrio* populations in the aquatic environments or speed up the evolution or selection of new *Vibrio* variants. It is anticipated that the occurrence and levels of the relevant *Vibrio* spp. in seafood will increase both globally and in Europe in response to coastal warming and extreme weather events as heatwaves, especially in low-salinity/brackish waters.

In **ToR4**, EFSA was requested to list and review prevention and control measures along the food chain for the relevant *Vibrio* spp. It was concluded that maintaining the cold chain is important to prevent vibrios' growth in seafood. A mild thermal treatment of oysters in water at 50°C with or without thermal shock, (flash) freezing followed by long-term frozen storage, high-pressure processing using industrially feasible conditions or irradiation reduce vibrios in seafood. Depuration under controlled conditions, although with variable reductions, is a post-harvest processing treatments for the segment of the market preferring consumption of live oysters.

In **ToR5**, EFSA was requested to review risk assessment modelling options for *Vibrio* spp. in seafood and to identify the knowledge gaps and data needed to perform a risk assessment on the public health impact of the relevant *Vibrio* spp. in seafood at the EU level. The identified QMRA addressed *V. parahaemolyticus* in various types of seafood, and *V. vulnificus* in raw oysters and octopus, and no risk assessment addressed non-O1/non-O139 *V. cholerae*. The majority of QMRAs had a national or regional scope and two covered conditions in Europe. Most rely heavily on the first US FDA assessment of *V. parahaemolyticus* in raw oysters, which was also applied to *V. vulnificus*. Their scope ranged from harvest to consumption. Changes in numbers of *Vibrio* spp. in seafood are estimated from time–temperature at different stages in the food chain considering growth and sometimes inactivation and removal/washing. The beta-Poisson dose–response (DR) models for *V. parahaemolyticus* (based on *tdh+* strains) and *V. vulnificus* have limitations and the applicability to conditions in EU is unknown. The endpoints are the probability of illness for the general or susceptible populations and range from GE to severe illness for *V. parahaemolyticus* and septicaemia for *V. vulnificus*. Other endpoints or populations are evaluated by multiplying with the proportions of illness cases affected by these outcomes or the proportion of the population of interest to the whole population based on epidemiological and census data. Consumption data, dietary habits, food origin and food types are very specific to the country or region of interest and were based on several types of consumption surveys and/or additional consumer related data to estimate exposure. Mitigations during harvest or directly after included practices motivated by sea surface temperature (SST) – considering the effects of seasons and climate change – and by pathogen occurrence, such as time to refrigeration and depuration. In later stages, practices evaluated were time–temperature during storage and cooking/heating, the effect of setting different pathogen target levels on the number of illnesses averted and the subsequent percentage of non-compliant harvest lost (harvest above the target levels), as well as more specific mitigations (e.g. adding vinegars, washing of fish cavities). The impact of climate change and different mitigations was only evaluated for *V. parahaemolyticus* in bivalve molluscs, most notably oysters. A QMRA relevant in an EU context would be *V. parahaemolyticus* in bivalve molluscs (oysters), evaluating the baseline risk and the effect of mitigations, especially in a climate change scenario.

In **ToR6**, EFSA was requested to recommend areas for future research on *Vibrio* spp. in seafood and aquatic environments. A key priority for future research is to establish an EU-wide baseline survey for the relevant *Vibrio* spp. in relevant seafood products, including at the primary production and retail stages. This survey will support: (i) gathering of representative and harmonised data on the relevant *Vibrio* spp.; (ii) definition of sentinel sites at primary production level to investigate temporal trends in *Vibrio* spp. occurrence; (iii) obtaining a representative collection of food isolates for characterisation (detection of VFs and of AMR); (iv) establishing a baseline for future reference in relation to the assessment of the effect of climate change. Other research needs include to improve analytical methods (for detection, quantification and AMR testing) and their comparability, to improve understanding of *Vibrio* VFs and discrimination of pathogenic strains, to gather data on *Vibrio* abundance during specific climatic events, to reassess DR models, to develop/validate models on the occurrence of *Vibrio* spp. (particularly *V. parahaemolyticus*) and environmental factors in the EU context, and to develop an exploratory QMRA to identify the most important gaps and guide research.

It is recommended to develop a case definition for human 'vibriosis' at EU level and to consider vibriosis for compulsory reporting. It is also recommended to systematically report data on *Vibrio* spp. occurrence in seafood collected in national monitoring programmes in the EU monitoring and collection of information on zoonoses (Zoonoses Directive 2003/99/EC), to systematically characterise *V. parahaemolyticus* isolates of clinical and food origin for pathogenicity (*tdh* and *trh* genes) and *V. cholerae* isolates of clinical and food origin for serotype and/or presence of cholera toxin genetic determinants and to characterise by WGS a selection of isolates of *Vibrio* spp. of PH relevance of clinical, food and environmental origin to allow the implementation of genomic surveillance. Further, it is recommended to arrange computational resources to sustain the development and long-term operability of EU-wide *Vibrio* suitability maps operating with high-resolution data of SST and sea surface salinity. Finally, it is recommended to survey resistances against MIAs associated with mobile elements in *Vibrio* spp. isolates from seafood.

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

Vibrio spp. are a group of common, Gram-negative, rod-shaped bacteria that are part of the natural microbiome of fresh-water, estuarine and marine environments. A dozen of the over 160 recognised species¹ of *Vibrio* are potentially pathogenic to humans. *Vibrio cholerae* (O1 or O139 serogroups) is the aetiological agent of cholera while other pathogenic *Vibrio* species include *V. parahaemolyticus*, *V. vulnificus*, and non-O1/non-O139 *V. cholerae*. Vibrios grow in temperate and warm waters with moderate salinity [5–25 ppt (part per thousand)]. Under non-favourable conditions vibrios may enter the viable but non-culturable (VBNC) state, a state of reduced metabolic activity characterised by higher resistance to environmental stressors (Li et al., 2014).

Non-cholera *Vibrio* spp. cause vibriosis — infections normally acquired through exposure to sea water or consumption of raw/undercooked seafood. They may be occasionally detected also in other commodities, such as fermented foods, raw vegetables or ready-to-eat foods, but the factors influencing their transmission in the food chain is still a matter of discussion (Valero et al., 2021). The accumulation of vibrios in seafood, and in particular bivalves, followed by consumption of those products either raw or not fully cooked is an established route of human exposure. Other factors affecting exposure include storage and transportation at inappropriate temperatures, contamination by an infected food handler, or cross-contamination through contact with contaminated seafood or seawater (FAO and WHO, 2020). Further to this, *V. parahaemolyticus* displays the ability to form biofilms (Wang et al., 2022), underlying the need for cleaning and disinfection in seafood handling environments. Clinical manifestations are most commonly mild, self-limiting gastroenteritis (GE), except for *V. vulnificus*, an opportunistic pathogen whose infection in individuals with predisposing health conditions (e.g., chronic liver diseases, hemochromatosis, immunocompromised) can rapidly lead to fatal septicaemia (Baker-Austin et al., 2018). Several other *Vibrio* species and some strains of *V. parahaemolyticus* and *V. vulnificus* can lead to vibriosis in aquatic animals and negatively impact animal production (de Souza Valente & Wan, 2021).

Current predictions show that the warming of marine waters as a result of global climatic change may have an impact on the transmission of these pathogens. In fact, it has been argued that this group of pathogens represents an important and tangible barometer of climate change in marine systems (Baker-Austin et al., 2016). Indeed, the association between the increase of sea surface temperatures (SST) in the Baltic area between 1982 and 2010 and the emergence of *Vibrio* infections clustered around the Baltic Sea area was observed (Baker-Austin et al., 2013) and was confirmed in correspondence with an extreme heat wave in northern Scandinavia during summer 2014 (Baker-Austin et al., 2016). As a consequence, the European Centre for Disease Prevention and Control (ECDC) started to monitor *Vibrio* growth in the Baltic Sea during summer through a near real-time model (i.e., the *Vibrio* suitability tool in a *Vibrio* map viewer²) that uses daily updated remote sensing data to examine environmental conditions such as SST and salinity. Alongside, climate change as a driver of emerging risk of *Vibrio* infections through food has been raised by EFSA in the CLEFSA (CLimate change and Emerging risks for Food Safety) project. *Vibrio* spp., especially *V. parahaemolyticus* and *V. vulnificus*, were indicated among the 'biological hazards to human health' issues with a very high likelihood of emerging in Europe under a near-future climate scenario (EFSA, 2020a).

Odeyemi (2016) reviewed the occurrence of *V. parahaemolyticus* in seafood based on studies reported in the 2003–2015 period; European studies reported prevalences from 7.83% for mussels (Normanno et al., 2006) to 77.8% for shrimps (Copin et al., 2012). Concentrations were also variable depending on studies and methods: in Italy, an average concentration of 77 CFU/g (range $3\text{--}1.8 \times 10^3$) was reported in bivalve shellfish (Suffredini et al., 2014) and 400 most probable number (MPN)/g (range $3\text{--}1.6 \times 10^5$) in crustacea (Caburlotto et al., 2016). Outbreaks of *V. parahaemolyticus* infections following consumption of contaminated raw or undercooked seafood have been reported around the world. In the EU, seven strong-evidence foodborne outbreaks (FBOs) caused by *V. parahaemolyticus* with 'Crustaceans, shellfish, molluscs and products thereof' as the suspected vehicle have been reported (2010–2020 period), causing 127 cases, of which 50 were hospitalised. Only a minority of *V. parahaemolyticus* environmental isolates are pathogenic to humans, pathogenicity being mainly associated with the production of haemolysins (thermostable direct haemolysin, TDH, or TDH-related haemolysin, TRH). Recent studies, however, demonstrated the absence of the genes codifying for TDH and TRH in approximately 10% of clinical isolates (Raghunath, 2015), highlighting the relevance of other possible virulence factors (VFs), such as type 3 and type 6 secretion systems, adhesion and iron uptake systems.

Vibrio vulnificus is the leading cause of seafood-related deaths in the United States and displays the highest fatality rate of any foodborne pathogen (Scallan et al., 2011); 51.6% of cases reported to the Food and Drug Administration (FDA) between 1992 and 2007 died (Jones & Oliver, 2009). *V. vulnificus* optimum salinity lays between 10 and 18 ppt; it is rarely isolated from waters with salinities > 25 ppt, so occurrence and human infections are infrequent in such environments (e.g., the Mediterranean Sea). Although survival of *V. vulnificus* in cold water (< 10°C) is achieved by entering the VBNC state, it is rarely isolated when water temperatures are lower than 13°C, so that most cases occur in the warmer summer months. Attempts to identify a single genetic marker for *V. vulnificus* virulence have failed. Clinical and environmental strains are

¹<https://lpsn.dsmz.de/search?word=vibrio>.

²<https://geoportal.ecdc.europa.eu/vibriomapviewer/>. The model used for the *Vibrio* viewer has been calibrated to the Baltic Region in Northern Europe and might not apply to other worldwide settings prior to validation.

often divided according to polymorphisms of *pilF* (pilus type IV), *vcg* gene, 16S RNA gene, or capsular polysaccharide, however no single target has proved completely reliable (Roig et al., 2018).

Non-O1/non-O139 *V. cholerae* strains do not generally produce the cholera toxin typical of the O1/O139 serogroups but may be responsible for GE cases varying from mild to serious in severity in association with the production of a heat-stable enterotoxin or the expression of other VFs (e.g., the El Tor haemolysin or the RtxA cytotoxin). Similar pathogenicity may be expressed by non-cholera-toxin-producing *V. cholerae* O1 and O139 strains. Occurrence of non-O1/non-O139 *V. cholerae* in seafood is well documented, with a prevalence of 5.6% in different products collected in Italy (Ottaviani et al., 2009), 11% to 16% in mussels from German production areas (Huehn et al., 2014), and with a sporadic detection in water from shellfish-growing areas in France (Cantet et al., 2013).

The issue of the public health (PH) risk associated with *Vibrio* spp. in seafood was considered, to different levels, in several documents. In 2001 the Scientific Committee on Veterinary Measures relating to Public Health published an opinion on *V. vulnificus* and *V. parahaemolyticus* in raw and undercooked seafood, concluding that the incidence of infections by these two species in Europe could not be quantified. Concern was raised, however, that the trends in international trade, consumption of raw seafood and number of susceptible persons may lead to an increase of infections (SCVM, 2001). Following this, risk assessments were developed by FAO/WHO on *V. vulnificus* in raw oysters (FAO and WHO, 2005b), *V. cholerae* O1 and O139 in warm water shrimp (FAO and WHO, 2005a), and *V. parahaemolyticus* in seafood (FAO and WHO, 2011). However, as modelling in these risk assessments was mainly based on data gathered in the United States, their application in areas where initial *Vibrio* concentrations, environmental conditions, and harvesting/post-harvesting practices may differ significantly, should be considered with caution. With regard to the EU area, in 2022, the German Federal Institute for Risk Assessment published a health risk assessment of the occurrence of *Vibrio* spp. in food, which remarked that food investigation should concentrate on *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae*, and that the consumption of raw or insufficiently cooked food products is one of the most important factors for their transmission (BfR, 2022).

Risk assessments focused on the EU situation have not taken place since the previously mentioned opinion of the SCVM relating to Public Health published in 2001. This, together with the presence of pathogenic *Vibrio* spp. in European marine and freshwater and in seafood, the underreporting of human cases due to the lack of surveillance of non-cholerae *Vibrio* infections in the EU, the evidence of outbreaks of *Vibrio* infections following consumption of contaminated raw or undercooked seafood also in the EU, the absence of food safety criteria of *Vibrio* spp. in seafood, and the warming pattern of the SST in particular areas, underpin the need to propose a self-task mandate by the BIOHAZ panel on the PH aspects of *Vibrio* spp. related to the consumption of seafood in the EU.

Terms of reference

The BIOHAZ Panel is asked to issue a scientific opinion on the PH aspects of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and non-O1/non-O139 *Vibrio cholerae* (and other species whenever relevant) related to the consumption of seafood for the EU population. More specifically, EFSA is requested to address the following terms of reference (ToRs):

ToR1. To review, for the relevant *Vibrio* spp., the existing information on occurrence and concentration in seafood, available analytical methods, pathogenicity to humans and virulence factors, as well as antimicrobial resistance and persistence mechanisms in different environments.

ToR2. To identify the factors in the aquatic environments and in food (including during production and processing) that influence occurrence and growth of the relevant *Vibrio* spp., and affect transmission of their virulence and resistance determinants.

ToR3. To review the evidence on the impact of climate change on the occurrence and levels of the relevant *Vibrio* spp. in water environments and seafood.

ToR4. To list and review prevention and control measures along the food chain for the relevant *Vibrio* spp.

ToR5. To review risk assessment modelling options for *Vibrio* spp. in seafood and to identify the knowledge gaps and data needed to perform a risk assessment on the PH impact of the relevant *Vibrio* spp. in seafood at the EU level.

ToR6. To recommend areas for future research on *Vibrio* spp. in seafood and aquatic environments.

1.2 | Interpretation of the Terms of Reference

Seafood, for this assessment, was understood to be food of marine and animal origin. It encompasses a variety of food products, including finfish, crustaceans, molluscs (with the classes cephalopods, bivalve molluscs and gastropods), tunicates and echinoderms (e.g. sea urchins and holoturidae). The seafood groups characterised by an exoskeleton are often referred to as shellfish. A representation of these groups can be found in Figure 1. Food products from inland aquaculture settings reproducing the marine environment were included. Marine food of non-animal origin (e.g. seaweed) were not considered.

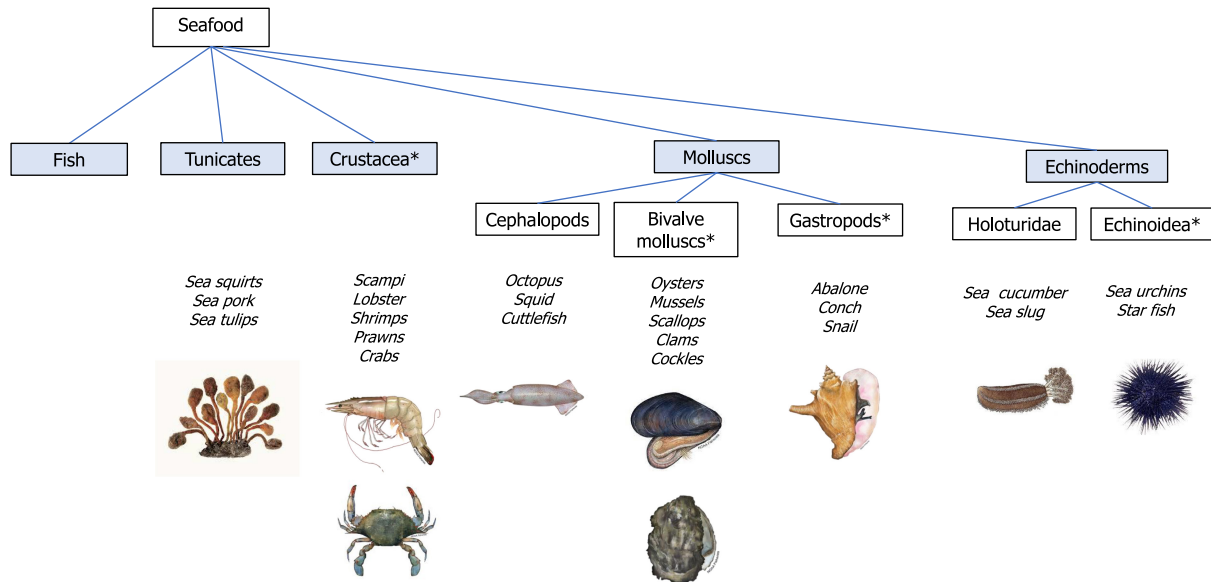


FIGURE 1 Classification of seafood as used in this assessment. Images are used from the fish and invertebrate photo gallery of the Alaska Fisheries Science Center, National Oceanic and Atmospheric Administration (NOAA) Fisheries. *Referred to as shellfish.

The assessment deals with *Vibrio* food-borne transmission through the consumption, by the EU population, of seafood as placed or intended to be placed on the EU market (thus including global trade). Hence, infections deriving from environmental, recreational and/or occupational exposure to vibrios were not considered. The consumer's part in the risk assessment [e.g. storage, cross-contamination or (under)cooking during food preparation] were addressed in ToR4 and 5.

The assessment covers *V. parahaemolyticus*, *V. vulnificus* and non-O1/non-O139 *V. cholerae*. Other species were to be covered as well when they were as relevant as these three species considering their presence in seafood placed or intended to be placed on the EU market and their reliable identification as the cause of illness in humans in the EU through seafood consumption.

Specifically, for **ToR1**, the occurrence and concentration of the relevant *Vibrio* spp. in seafood covered both the detection/enumeration of the aforementioned species and, where achievable, the specific detection/enumeration of their pathogenic strains or of strains with characteristic features associated with pathogenicity or virulence. Analytical methods covered those used for the detection and enumeration of the different relevant *Vibrio* spp. including, where available, methods targeting pathogenic strains within a species. Methods addressing the identification and characterisation of virulence profiles and closely related strains of the different relevant *Vibrio* spp. were also considered. Strengths and limitations of the described methods were addressed.

Pathogenicity was defined as the ability of the relevant *Vibrio* spp. (or of a subset of strains within the species) to cause disease in a human host and virulence as the degree to which a pathogen causes damage to the host. For the review of the pathogenicity and VFs, features differentiating *Vibrio* strains with pandemic potential were addressed. Antimicrobial resistance (AMR) was understood as the evidence of resistance to antimicrobial compounds in isolates of relevant *Vibrio* spp. taken from seafood or in clinical isolates associated with FBOs or cases. Clinical isolates with no clear link with food-borne transmission were excluded. Both phenotypic expression of resistance as well as genotypic information were considered. Emerging AMRs that are regarded as a possible threat for the public were also addressed. Persistence is understood as the ability of a given organism to establish itself and remain within a given environment for a long term (long-term survival in the specific environment).

For **ToR2**, factors referred to extrinsic and intrinsic factors that affect the presence or concentration (due to growth or inactivation), or that affect horizontal or vertical transmission of pathogenicity, virulence or resistance determinants in the relevant *Vibrio* spp. in aquatic environments and in food. Implicit factors affecting occurrence, growth and transmission of virulence/resistance determinants were also addressed. The aquatic environments were considered in their whole and not limited to areas associated with seafood production (aquaculture or fishing). Furthermore, both seawaters and brackish waters were considered. Seafood was covered in all its production stages, including the aquaculture process, harvesting/fishing, processing and transport up to retail level. Domestic handling and consumption practices were out of the scope.

For **ToR3**, the evidence on the impact of climate change was interpreted as the reporting of empirical observations or of modelling studies assessing the changes of the occurrence and/or concentration of the relevant *Vibrio* spp. (in water environments and/or in seafood) in correlation with one or more of the changes of the marine environment associated with global climate change (e.g. increase of SST associated with warming trends or extreme weather events).

For **ToR4**, control measures for vibrios in seafood that are already in place (either locally or globally), as well as those experimentally tested for possible implementation were included. The advantages and disadvantages of the prevention and control measures were listed and included, when relevant and feasible, a qualitative evaluation of their efficacy (effect on prevalence/concentration of the relevant *Vibrio* spp. in seafood). Economic or environmental impacts or user acceptance were not considered.

For **ToR5**, the risk assessment modelling options for *Vibrio* spp. in seafood were reviewed based on existing risk assessments, models and approaches and covered identifying the data needed to perform a risk assessment on the PH impact of the relevant *Vibrio* spp. in seafood at the EU level. The characteristics of the available models considered the methods, assumptions, data, model limitations, etc. Performing a risk assessment itself was not envisaged.

Each ToR were translated into an assessment question (AQ). These read as follows:

- **AQ1/**For the relevant *Vibrio* spp., what is their occurrence and concentration in seafood, available analytical methods, pathogenicity to humans and virulence factors, as well as antimicrobial resistance and persistence mechanisms in different environments?
 - SQ1.1/What are the *Vibrio* spp. of highest relevance for PH in the EU through consumption of seafood beyond *V. parahaemolyticus*, *V. vulnificus* and non-O1/non-O139 *V. cholerae*?
 - SQ1.2/Considering the relevant *Vibrio* spp. (from SQ1.1), what is their prevalence and concentration in seafood placed or intended to be placed on the EU market?
 - SQ1.3/Considering the relevant *Vibrio* spp. (from SQ1.1), what are the available analytical methods for the detection, enumeration and characterisation in seafood?
 - SQ1.4/Considering the available analytical methods (from SQ1.3), what are their characteristics and how do they contribute to risk assessment?
 - SQ1.5/Considering the relevant *Vibrio* spp. (from SQ1.1), what is their pathogenicity to humans and which are their virulence factors?
 - SQ1.6/Which are the most frequently detected antimicrobial resistances in the relevant *Vibrio* spp. (from SQ1.1) isolated from seafood and from seafood-borne infections?
 - SQ1.7/Which of the detected antimicrobial resistances (SQ1.6) are of concern due to their possible horizontal transmission to other bacteria or for their emerging in the EU?
 - SQ1.8/Considering the relevant *Vibrio* spp. (from SQ1.1), what are their persistence mechanisms in different environments?
- **AQ2/**What are the factors in the aquatic environments and in seafood that influence occurrence and growth of the relevant *Vibrio* spp., and affect transmission of their virulence and resistance determinants?
 - SQ2.1/What are the factors in the aquatic environments and in food, including during production and processing that affect the presence or concentration of the relevant *Vibrio* spp. (from SQ1.1)?
 - SQ2.2/What factors in the aquatic environments and in food are considered to affect transmission of *Vibrio* virulence and resistance determinants?
- **AQ3/**What is the impact of climate change on the occurrence and levels of the relevant *Vibrio* spp. in water environments and seafood?
 - SQ3.1/What data sources, tools and models are available or are under development to evaluate the impact of climate change on the relevant *Vibrio* spp. in water environments and seafood and what are their limitations?
 - SQ3.2/How is climate change affecting the occurrence and levels of the relevant *Vibrio* spp. in the aquatic environment at a global level and in Europe?
 - SQ3.3/How is climate change affecting the occurrence and levels of the relevant *Vibrio* spp. in seafood at global level and in Europe?
- **AQ4/**What are the prevention and control measures along the seafood chain for the relevant *Vibrio* spp.?
- **AQ5/**What are the risk assessment modelling options for *Vibrio* spp. in seafood and which are the knowledge gaps and data needed to perform a risk assessment on the PH impact of the relevant *Vibrio* spp. in seafood at the EU level?
 - SQ5.1/What are the available (semi-) quantitative risk assessments (QMRA) for the relevant *Vibrio* spp. (SQ1.1) in seafood or in specific seafood types/products?
 - SQ5.2/What are the characteristics of the available risk assessment models (listed in SQ5.1)?
 - SQ5.3/Which are the knowledge gaps and data needed to perform assessments addressing the PH impact of relevant *Vibrio* spp. in different types of seafood at the EU level?
- **AQ6/**What are areas for future research on *Vibrio* spp. in seafood and aquatic environments?

1.3 | Additional information

Regulation (EC) No 853/2004 sets out definitions and hygiene requirements for bivalve molluscs and fishery products. Bivalve molluscs are filter-feeding lamellibranch molluscs. Typical products in an EU context include mussels (*Mytilus*

edulis), oysters (*Crassostrea gigas*) and scallops (*Pecten maximus*). Fishery products mean all seawater or freshwater animals (except for live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods, and all mammals, reptiles and frogs) whether wild or farmed and including all edible forms, parts and products of such animals.

Implementing Regulation (EU) 2019/627 classifies production areas of live bivalve molluscs according to the levels of faecal indicators (*Escherichia coli*) in the mollusc flesh and intravalvular liquid in one of three categories, Classes A, B and C. According to Regulation (EU) 853/2004, Class A molluscs may be placed on the market directly for human consumption, while molluscs from Class B areas must be treated in a purification centre or relayed, while those from Class C areas must be relayed over a long period (at least 2 months); after treatment they must comply with the Class A health standards prior to being placed on the market. Molluscs from Class B and Class C areas may also be heat-treated using one of the legally prescribed methods. Bivalve molluscs should not be harvested from non-classified areas, i.e. areas with no data or exceeding the limit of the faecal indicator. These requirements also apply to echinoderms, tunicates and marine gastropods.

2 | DATA AND METHODOLOGIES

The approach to answer the ToRs was defined in advance and is described in the protocol (Annex A). It covers both the problem formulation (i.e. what the assessment aims to address) and which methods will be used for addressing the problem. The problem formulation ('what') includes the clarification of the mandate (see further refined in Section 1.2) and consists of the steps (1) translation of the mandate into scientifically answerable AOs, (2) definition of the sub-questions (SQs) of each AO, if needed, and their relationship (conceptual model) and (3) the selection of the approach for the assessment. The planning of the methods for conducting the assessment ('how') consists of (1) specifying the evidence needs and the methods for answering each AO/SQ, including uncertainty analysis and (2) the methods for integrating evidence across AOs/SQs and addressing the remaining and overall uncertainty. Protocol development followed the draft framework for protocol development for EFSA's scientific assessments (EFSA, 2020b).

2.1 | Data

2.1.1 | Data on food-borne outbreaks at EU/EEA level

Data on 'strong and weak evidence' FBOs with *Vibrio* spp. as causative agent were extracted from the EFSA zoonoses database on 2 February 2023. The datafile was filtered considering time (from 2010 to 2021) and relevant food vehicles [i.e. the food(stuff) suspected of causing human cases] considered as seafood or with evidence that it contained seafood. The available evidence was summarised by *Vibrio* spp., retrieving information on the number of outbreaks, number of cases, number of hospitalised cases and number of deaths. Reporting per year and per country was described. Further information from other free data fields, such as food vehicle information and contributory factors, was consulted and included, when relevant. More information about the reporting of FBOs can be found in (EFSA, 2022).

2.1.2 | Data of the Rapid Alert System for Food and Feed (RASFF)

Commission Regulation (EU) No 16/2011³ lays down the implementing measures for the requirements of Regulation (EC) No 178/2002 around the RASFF.⁴ This RASFF system facilitates the notification of food and feed safety alerts among the competent authorities of Member States. It is not an epidemiological surveillance system but is primarily a communication facility enabling food safety risks to be averted before they could be harmful to European consumers. RASFF data were used to investigate the *Vibrio* spp. found in seafood since 2010. Data were extracted from the RASFF database on 2 February 2023 for the notification type 'food' and the hazard category 'pathogenic microorganisms' (more specific the hazard *Vibrio*). The extracted data were filtered considering time (from 2010 onwards) and for the product categories 'bivalve molluscs and products thereof', 'cephalopods and products thereof', 'crustaceans and products thereof', 'farmed crustaceans and products thereof', 'fish and fish products', 'molluscs and products thereof' and 'wild caught crustaceans and products thereof'. The information was summarised using alert/notification per product category, *Vibrio* spp. and year. The occurrence of notification types associated with human disease ('food poisoning – alert' or 'consumer complaint – information') was checked specifically in notifications related to samples containing exclusively species other than *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus*. Information on detection of *Vibrio* spp. with specific VFs was checked.

³Commission Regulation (EU) No 16/2011 of 10 January 2011 laying down implementing measures for the Rapid alert system for food and feed. OJ L 6, 11.1.2011, pp. 7–10.

⁴http://ec.europa.eu/food/safety/rasff/index_en.htm.

2.1.3 | Zoonoses monitoring data

Reporting of monitoring data on zoonoses in animals, food and feed is mandatory for the EU Member States, in compliance with Directive 2003/99/EC.⁵ Data must be reported on a mandatory basis for eight zoonotic agents and based on the epidemiological situations in the Member State, and also on the agents and zoonoses spelled out in Annex I of that Directive. On the food-animal side there is no coordinated monitoring at EU level for *Vibrio* spp. Data on the occurrence of *Vibrio* spp. on food were extracted on 14 February 2023 from the zoonoses monitoring database without time restrictions (until 2021). The extracted data were filtered for matrix considering seafood. Data were summarised considering the units (single units and batches), stage, context (e.g. surveillance, monitoring) and strategy of sampling (e.g. 'census', 'convenience' and 'objective' vs. 'suspect', 'selective').

2.1.4 | Food Standards Australia New Zealand information request

Following Australia's largest *V. parahaemolyticus* outbreak in raw oysters,⁶ an information request from the Food Standards Australia New Zealand (FSANZ) was shared with the EFSA Microbiological Risk Assessment (MRA) Network and international partners in 2022. Replies to the following questions were received from Croatia, Cyprus, Estonia, France, Germany, Ireland, Norway, Sweden (through MRA representatives) and US Food and Drug Administration (US FDA), Canadian Food Inspection Agency (Canada), New Zealand NZ Ministry for Primary Industries (MPI, New Zealand), Food Safety Commission of Japan (Japan) and FSANZ (Australia) (through international collaboration) and can be found in Annex B.

- Is *V. parahaemolyticus* and/or *V. vulnificus* notifiable human diseases in your country? Have you witnessed an increase in *Vibrio* species illness over the past 3–5 years? If so, do you consider that this is climate change related? Is there any substantive correlative evidence for this?
- What *Vibrio*/environmental monitoring (e.g. sea water temperature, salinity) in shellfish-growing areas, if any, do you or your colleagues undertake? Has changes in domestic requirements in your country changed requirements for equivalence testing of imported seafood?
- What phenotypic methodologies (quantitative and presence/absence) are currently being used for routine identification and testing of vibrios in shellfish? Noting we've witnessed challenges with non-culturable *Vibrio* and issues with MPN methods.
- Are environmental or human isolates routinely sequenced? Are there specific genes (other than *tdh* and *trh* for *V. parahaemolyticus*) that are of interest from a risk perspective? Are sequences on a public or internal database and what metadata is uploaded associated with those sequences?

2.2 | Methodologies

2.2.1 | *Vibrio* spp. of highest PH relevance in the EU through consumption of seafood (SQ1.1)

A **general literature search** was conducted to provide a cross-sectoral data source of review papers, books or book chapters to be used for the various AQs and SQs; a study was considered eligible when the record included information on *V. parahaemolyticus*, *V. vulnificus*, non-O1/non-O139 *V. cholerae* or other relevant species with regards to the topics covered under the different ToRs. The search strategy and study selection can be found in the protocol (Annex A).

Specifically, for SQ1.1, a study was eligible when it informed on any *Vibrio* spp. of PH relevance in the EU through consumption of seafood. Also, recent reports, articles and reviews were consulted related to *Vibrio* FBOs/cases in the EU. Information on seafood transmission of *Vibrio* species other than *V. parahaemolyticus*, *V. vulnificus* or non-O1/non-O139 *V. cholerae* in the EU was retrieved through a literature search in Eurosurveillance⁷ (on 26 February 2023 with the keyword 'Vibrio' in all fields) and in Web of Science™ Core Collection⁸ (on 19 June 2023).

The archive of ProMED records⁹ was screened on 12 March 2023 using the keyword 'Vibrio' in posts' title or text; no time restrictions were applied (data from inception to 2023). All retrieved posts were screened for evidence of food-borne vibriosis occurring in the EU. In addition, the recent risk assessments undertaken by EU member states and international bodies on the topic were collected and screened for evidence of reported food-borne vibriosis. Furthermore, the results of the

⁵Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC OJ L 325, 12.12.2003, pp. 31–40.

⁶<https://www.foodstandards.gov.au/industry/FoodIncidents/Pages/Vibrio-parahaemolyticus-and-raw-Pacific-oysters.aspx>.

⁷<https://www.eurosurveillance.org/>.

⁸Including SCI-Expanded, SSCI, AHCI, CPCI-P, CPCI-SSH, ESCI, CCR, Expanded, IC. The search combined the records retrieved using the following two search strings: (a) ALL = (case*) AND TS = (alginolyticus OR fluvialis OR hollisae OR mimicus OR metschnikovii OR moetus OR cincinnatiensis OR furnissi); (b) TS = (vibrio OR vibrios OR Vibrionaceae OR vibriosis OR parahaemolyticus OR vulnificus OR cholerae) AND TS = (case* OR outbreak*) AND [search string #3 from Table A.4 of the protocol] AND [search string #4 from Table A.4 of the protocol].

⁹<https://promedmail.org/>.

FSANZ information request (Section 2.1.4) were considered. This information was supplemented by the knowledge/expertise of the Working Group (WG) and Panel members.

The data retrieved from the FBOs (Section 2.1.1), RASFF (Section 2.1.2) and EFSA zoonoses monitoring data (Section 2.1.3) were summarised as described above.

A list of *Vibrio* spp. that are found to be associated with seafood and with human disease in EU was prepared, obtained from the various sources of evidence, and the most relevant *Vibrio* species were identified based on the available data and expert judgement. These are referred to as relevant *Vibrio* species in the text.

2.2.2 | Occurrence of the relevant *Vibrio* spp. in seafood placed or intended to be placed on the EU market (SQ1.2)

A systematic review¹⁰ was used to identify and extract data on the occurrence (i.e. prevalence and concentration¹¹) of the relevant *Vibrio* spp. in seafood produced and/or commercialised in Europe. The search covered studies published between January 2010 and September 2023. The search strategy, study selection and data extraction can be found in the protocol.¹² Data were extracted for EU member states, countries that were EU Members in the period considered in the extensive literature search (ELS) (i.e. UK), EU accession candidates and members of the European single market. The evidence retrieved was synthesised by listing the data on the occurrence of the relevant *Vibrio* spp. in seafood on the EU market according to seafood category and type (animal species). The synthesis includes country of the study, year(s) of sampling, the sampling point (stage of the food chain), sampling size and number of positive units, concentration measure (for quantitative analysis), the tested amount, a reference to the method used. Where reported, data related to pathogenic strains (occurrence) or to the identification of features associated with pathogenicity/virulence (e.g. serotype, sequence type, toxin genes) were captured. For *V. cholerae*, due to the scarcity of data explicitly addressing non-O1/non-O139 *V. cholerae*, data were included in the synthesis when dealing with non-cholerae *V. cholerae*, i.e. when strains were tested using either serological (sero-agglutination) tests (i.e. non-O1, non-O139 or both) or molecular tests (i.e. no detection of cholera toxin-associated genes, *ctx*, *ctxA* and/or *ctxB*). Reporting pathogenic *V. parahaemolyticus* was highly heterogeneous among studies¹³ and results were harmonised to express proportion of samples containing *tdh+* and/or *trh+* *V. parahaemolyticus* upon *V. parahaemolyticus*-positive samples.

The literature search identified 234, 436, 404 and 15 bibliographic references in PubMed, Web of Science Core Collection, Scopus and SciELO, respectively. A total of 463 out of 1089 primary research studies were removed after deduplication. From the remaining 626 publications (primary research studies), 462 were discarded after screening of titles and abstracts. At the end of full text screening of 164 publications, 49 publications were considered eligible. Two publications concerned results already contained and extracted from other records and, therefore, the data from 47 primary studies were extracted using a standardised spreadsheet. Most of these publications included data on more than one *Vibrio* species relevant for PH, seafood category or species, sampling area or sampling periods, etc. Therefore, finally, a total of 389 data entries (sample size ≥ 3) were associated with the 47 primary research publications. One paper with detailed yearly analysis was redundant with a pluriannual synthesis paper and then removed for further analysis. Removing 14 duplicated information, the final number of data entries is $N=375$ for 46 primary research publications. Excluding entries not dealing with non-cholerae *V. cholerae* ($N=5$) reduced the database to 370 entries and 46 primary research publications. Extracted data are available at <https://zenodo.org/records/11640367> and will be incorporated in the 'Pathogens in Food (PIF) database'¹⁴ accessible through a web application (<https://pif.esa.ipb.pt/>) (Faria et al., 2022). A random-effect meta-analysis model was carried out, considering study as random effect and a logit transformation of the prevalence data (Gonzales-Barron et al., 2021; Xavier et al., 2014), using metafor package (v3.8-1) (Viechtbauer, 2010) environment of RStudio (v 2023.03.0). To estimate parameters and their confidence interval the restricted maximum likelihood estimator (RMLE) procedure was used. The I^2 statistics or intraclass correlation estimates the proportion of between study variance from the total variance. If I^2 is higher than 50%, the heterogeneity between studies is notable (Higgins & Thompson, 2002). Between-study variability can be considered as significant when it represents at least 25% of the total variability in the outcome measure (Gonçalves-Tenório et al., 2018; Higgins & Thompson, 2002). Samples sizes below 3 units were not considered in statistical analysis. Quantification data for *Vibrio* were scarce and heterogeneous (reporting being either based on positive or on positive and negative units). Only quantified data with available means and standard deviation, after standardisation in \log_{10} units, (positive or above LOQ), were used to

¹⁰Conducted under grant agreement GP/EFSA/BIOHAW/2023/05 (Pathogens in foods database: extension with *Vibrio* and parasites) with as beneficiaries Instituto Politécnico de Bragança (IPB) as coordinator and Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (Anses) as co-beneficiary.

¹¹Used as a synonym for cell count or cell density.

¹²<https://zenodo.org/records/10282514>.

¹³Results were expressed as (i) overall prevalence of samples containing *tdh/trh*-positive *V. parahaemolyticus* in all tested samples, (ii) proportion of samples containing *tdh/trh*-positive *V. parahaemolyticus* upon *V. parahaemolyticus*-positive samples, or (iii) proportion *tdh/trh*-positive isolates upon tested isolates of the *V. parahaemolyticus* species.

¹⁴Pathogens-in-Foods (PIF) is a database of systematically formatted occurrence data of the most important microbiological hazards in foods randomly surveyed in Europe. The database contains data extracted from peer-reviewed articles published since 2000, retrieved through systematic literature searches and using a publicly available protocol describing the search and screening process. The foodborne pathogens currently covered are: *Bacillus cereus*, *Campylobacter* spp., *Clostridium perfringens*, *Listeria monocytogenes*, *Salmonella* spp., Shiga toxin-producing *Escherichia coli*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Cryptosporidium* spp., *Giardia* spp., *Toxoplasma gondii*, Hepatitis A virus, Hepatitis E virus and Norovirus. In 2025, PIF database will be populated with data on the occurrence of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and non-O1/non-O139 *Vibrio cholerae* in seafood and of fish parasites of public health importance (including Anisakids) in fishery products.

estimate a pool mean and its confidence interval by meta-analysis using weights assigned to each sample based on the inverse of the overall error variance (i.e. 1/variance) with study as random effect.

2.2.3 | Available analytical methods for the detection, enumeration and characterisation of the relevant *Vibrio* spp. in seafood and their contribution to risk assessment (SQ1.3–1.4)

International Organization for Standardization (ISO)/European Committee for Standardization (CEN) catalogues were consulted as well as other methods from national bodies (e.g. FDA BAM, Ministry for Primary Industries NZ, Health Products and Food Branch Canada). Also, information shared by international bodies were consulted. Food and Agriculture Organization (FAO)/World Health Organization (WHO) reviews of methods were considered in the discussion of the characteristics of the available methods. The recent reviews collected through the general literature search (Section 2.2.1) as well as the results of the FSANZ information request (Section 2.1.4) were used. Finally, primary research studies were considered using the data collected through the ELS as described in Section 2.2.2, in which the method of detection (and also quantification) were extracted.

Data from included studies were extracted in relation to the general structure of the method, principle, scope, laboratory technique adopted, breadth of application, availability of performance characteristics [limit of detection (LOD), limit of quantification (LOQ), accuracy, specificity, sensitivity, etc.] and framework of their collection (in-house, ILS). Priority was given to official control and standardised methods; analytical methods only experimentally tested were only briefly summarised.

For SQ1.3, the evidence retrieved was synthesised by listing the methods used for the detection, enumeration and characterisation of the relevant *Vibrio* species, and describing their use on different seafood types and/or in different contexts and summarising (when available) their performance characteristics. For SQ1.4, the characteristics of the analytical methods identified in SQ1.3, grouped per types, were addressed, and summarised through a Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis.

2.2.4 | Pathogenicity to humans and virulence factors of the relevant *Vibrio* spp. (SQ1.5)

The virulence factor database (VFDB)¹⁵ was consulted to list the possible virulence genes of the most relevant *Vibrio* spp. in seafood. This list was used as a basis for bibliographic searches. Recent reviews collected through the general literature search (Section 2.2.1) were also used to gather information about the relevant *Vibrio* species with regards to their pathogenicity to humans and VFs. Furthermore, the literature searches performed on PubMed (Section 2.2.1) were screened to identify relevant studies to complete the data obtained previously. Comprehensive lists were created, including VFs important for all pathogenic vibrios and the specific determinants for each species.

2.2.5 | Antimicrobial resistances in the relevant *Vibrio* spp. isolated from seafood and from seafood-borne infections (SQ1.6) and those of concern due to their possible horizontal transmission to other bacteria or for their emerging in the EU (SQ1.7)

The AMR group of the Zoonoses Network was contacted to share available data, or the information on where to find them (e.g. National Reports, public databases, outbreak investigation) for the relevant *Vibrio* species, that stem from isolates from seafood (environmental samples, retail or production, e.g. primary production). Additionally, the recent reviews collected through the general literature search (Section 2.2.1) were used as well as the results of the FSANZ information request (Section 2.1.4). Furthermore, specific literature searches were performed on 10 January 2023 in PubMed using 'Vibrio AND Antimicrobial resistance' alone or combined with 'shellfish' as search terms. Records were screened to identify studies from Europe in which food isolates were examined.

Data were extracted and presented in a first table summarising phenotypic AMR data from the European consortium ASK,¹⁶ French data (CNR Vibrio et Choléra) and data extracted from primary research papers. As most papers do not contain information on the coding AMR genes, information on AMR genes is depicted in another table, presenting examples of the AMR determinants in food strains.

Dr Jens-Andre Hammerl, Head of the Consultant Laboratory for *Vibrio* spp. in Food of the German Federal Institute for Risk Assessment (BfR) attended the WG meeting on 10 January 2024 as hearing expert to summarise the BfR Opinion 'Bacterial foodborne Vibrio infections: health risk assessment of the occurrence of *Vibrio* spp. (non-cholera vibrios) in food' (BfR, 2022). Further, he gave some examples of studies on *Vibrio* of the BfR related to (i) *Vibrio* spp. occurrence in German aquaculture systems, (ii) *Vibrio* spp. ecological cut off values for different species, and (iii) emergence of multidrug-resistant *V. parahaemolyticus* in imported seafood – genetic basis and spread of resistance plasmids.

¹⁵The virulence factor database (VFDB – <http://www.mgc.ac.cn/VFs/>) is an integrated and comprehensive online resource for curating information about VFs of bacterial pathogens Chen, L., Yang, J., Yu, J., Yao, Z., Sun, L., Shen, Y., & Jin, Q. (2005). VFDB: a reference database for bacterial virulence factors. *Nucleic Acids Res*, 33(Database issue), D325-328. <https://doi.org/10.1093/nar/gki008>.

¹⁶<https://zenodo.org/record/3592267>.

The distribution of AMR genes for the EU and EFTA countries data was obtained from the global *V. parahaemolyticus* genomes based on a collection of nearly 10,000 genomes. Some of the strains are from the collection of prof. Jaime Martinez-Urtaza (Department of Genetics and Microbiology, Universitat Autònoma de Barcelona) or are part of ongoing studies to expand the genome collection; other genomes were retrieved from the European Nucleotide Archive (ENA)¹⁷ and the National Center for Biotechnology Information (NCBI).¹⁸ For detection of AMR genes, a resistance genes detection pipeline based on one of the standard databases (CARD database¹⁹) was used. The phylogenetic tree was prepared and includes the reference genome from Japan 'Osaka' as reference. The RIMD 2210633 strain has been added as the global reference strain which has been historically used for all the phylogenetic analysis of *V. parahaemolyticus*. The metadata includes the source of the strain, i.e. country, origin (clinical, environmental or unclear), date of isolation and subtype. The antibiotic-resistant genes are shown as present, absent or not applicable. To build the ARGs European *V. parahaemolyticus* tree, the Parsnp tool, a fast core-genome multi-aligner and SNP detector, from the Harvest suite was used (Treangen et al., 2014). Parsnp calculates the MUMi distances between the reference genome (RIMD_2210633) and each one of the 152 genomes used in this study. The resulting Newick formatted core genome SNP tree was then uploaded onto the webtool I-Tol (Letunic & Bork, 2021), midpoint rooted and the metadata of the samples was incorporated.

2.2.6 | Persistence mechanisms in different environments of the relevant *Vibrio* spp. (SQ1.8)

Information on the different strategies that the relevant *Vibrio* spp. use to persist in the environment, including the aquatic environment and environments associated with food production/processing/transport was mainly retrieved from review articles, books and book chapters as collected from the search strategy described in Section 2.2.1 and from reports. The primary research studies were collected through non-extensive searches and snowballing (or citation searching). The evidence retrieved was synthesised in a narrative way by describing all the bacterial mechanisms that allow the bacteria to persist in the different environments.

2.2.7 | Factors in the aquatic environments and in food, including during production and processing that affect the presence or concentration of the relevant *Vibrio* spp. (SQ2.1)

Information on the factors in the aquatic environments and in food, including during production and processing that influence presence or concentration (growth or inactivation) of the relevant *Vibrio* spp. was mainly retrieved from review articles, books and book chapters as collected from the search strategy described in Section 2.2.1 and from reports. The primary research studies were collected through snowballing through non-extensive searches in PubMed and Scopus using, as search terms, the three species relevant for PH, and the selected identified factors (temperature, salinity, pH, etc.). The evidence retrieved was synthesised in a narrative way by listing the factors under the various categories (extrinsic, intrinsic or implicit factors). Data on available growth or inactivation models within selected temperature ranges were extracted and summarised in tables.

2.2.8 | Factors in the aquatic environments and in food that are considered to affect transmission of *Vibrio* virulence and resistance determinants (SQ2.2)

Information on the different factors that affect transmission of VFs and/or resistance determinants of the relevant *Vibrio* spp. in the aquatic environment or in the food was mainly retrieved from review articles, books and book chapters as collected from the search strategy described in Section 2.2.1 and from reports. The primary research studies were collected through snowballing through non-extensive searches and snowballing. The evidence retrieved was synthesised in a narrative way by listing the factors that promote the transmission of VFs and resistance determinants in the different environments.

2.2.9 | Impact of climate change on the occurrence and levels of the relevant *Vibrio* spp. in seafood (AQ3)

At first, general information on the effect of climate change on *Vibrio* spp. was retrieved from review articles, books and book chapters as collected from the search strategy described in Section 2.2.1. Following, technical reports from international organisations [e.g. European Environmental Agency, Intergovernmental Panel on Climate Change (IPCC), etc.] and primary research papers on the topic were collected based on the knowledge of the WG members, through snowballing from the reviews, book chapters and reports retrieved in the cross-sectoral literature search, or through specific literature searches in the Web of Science™ Core Collection or in NCBI. Primary research studies specifically addressing

¹⁷<https://www.ebi.ac.uk/ena/browser/home>.

¹⁸GenBank – <https://www.ncbi.nlm.nih.gov/genbank/>; RefSeq – <https://www.ncbi.nlm.nih.gov/refseq/>; SRA – <https://www.ncbi.nlm.nih.gov/sra>.

¹⁹<https://card.mcmaster.ca/>.

seafood-associated *Vibrio* outbreaks with a strong climatic component of the event were collected through non-extensive searches and snowballing.

Available data sources [oceanographic data sets and websites, e.g. Copernicus, National Oceanic and Atmospheric Administration (NOAA), ECDC] and predictive models (mechanistic models, suitability models, etc.) were compiled based on technical reports and on experts' knowledge.

The evidence retrieved was synthesised visually, using *Vibrio* suitability projections for Europe (e.g. figures showing mapping of areas likely to sustain vibrios occurrence and growth in the future under different climate emission scenarios), and in a narrative way. In addition, specific case studies of past *Vibrio* outbreaks in Europe and throughout the world, either published or provided by the experts, were used to outline climatic/environmental conditions likely to drive risk.

2.2.10 | Prevention and control measures along the seafood chain for the relevant *Vibrio* spp. (AQ4)

Information on prevention and control measures along the seafood chain for the relevant *Vibrio* spp. focused on those measures already in place while methods that have only been tested experimentally were summarised briefly. Reviews, books and book chapters were first considered and will be collected from the search described in Section 2.2.1. In addition, recent risk assessments undertaken by EU member states and international bodies were considered. More recent papers describing primary research studies were collected, where needed, through non-extensive searches and snowballing. The measures were first listed based on the available information and a definition for each of the measures was provided. The evidence retrieved was synthesised in tables giving first an overview of the measures already in place and those measures that have only been tested experimentally. For those measures already in place, their advantages and disadvantages were provided and, if relevant and possible, a qualitative evaluation of their efficacy was provided.

2.2.11 | Risk assessment modelling options for *Vibrio* spp. in seafood and knowledge gaps and data needs (AQ5)

First, the available QMRAs covering the relevant *Vibrio* spp. in seafood or in specific seafood types/products were identified and listed based on non-extensive literature searches, snowballing, and based on the knowledge and expertise of the WG and Panel members. Further, the characteristics of each of the steps (hazard identification, exposure assessment, hazard characterisation and risk characterisation) in the identified QMRAs were summarised and evaluated. The evaluation addressed objectives, approaches, methods, assumptions, data and limitations. The outcome of the evaluation together with the information and conclusions presented in response to the other ToRs was the basis for an analysis to identify the data and knowledge needed to perform assessments addressing the PH impact of relevant *Vibrio* spp. in relevant types of seafood at the EU level. The analysis considered different factors impacting on the relevance and scope of such assessments (e.g. the number of food-borne cases, frequency of consumption, production volumes).

2.2.12 | Areas for future research on *Vibrio* spp. in seafood and aquatic environments (AQ6)

Based on the knowledge gaps identified through the outcome of the approach followed as described in previous sections, research needs were identified based on expert knowledge (WG and BIOHAZ Panel members) and prioritised based on expert group judgement.

2.2.13 | Uncertainty analysis

As recommended by the EFSA guidance and related principles and methods on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018a, 2018b), an uncertainty analysis was implemented. Given the nature and context of the ToRs of the mandate, the uncertainty analysis was restricted to an overview of the uncertainty sources affecting the different AQs (Table A.1 in Appendix A). They describe the strengths and weaknesses of the collected evidence and served as a source of information for the discussion on knowledge gaps and research needs.

3 | ASSESSMENT

3.1 | Relevant *Vibrio* spp. in seafood (SQ1.1)

3.1.1 | Introduction

The 2001 opinion of the Scientific Committee on Veterinary Measures relating to Public Health on *V. vulnificus* and *V. parahaemolyticus* in raw and undercooked seafood stated that three *Vibrio* species represent a serious and growing PH hazard:

V. cholerae (serogroups O1 and O139, causing cholera), *V. parahaemolyticus* and *V. vulnificus*. Furthermore, the opinion underlined that non-O1/non-O139 *V. cholerae* strains, and strains of *V. fluvialis*, *V. hollisae* and *V. mimicus* had been associated with infections (mostly GE) arising from seafood consumption, and that they should in future be considered (SCVM, 2001).

Except for cholera, vibriosis are not included among the food-borne and waterborne diseases monitored within the European Food- and Waterborne Diseases and Zoonoses Network (FWD-Net) managed by ECDC. Therefore, no harmonised surveillance of *Vibrio* infections is currently available in the EU.

Data from the US Centers for Disease Control and Prevention (CDC) Foodborne Diseases Active Surveillance Network (FoodNet) report an incidence of *Vibrio* infections of 0.99 per 100,000 inhabitants (223 cases) for 2022 and 5527 infections during 1996–2022. The largest part of these infections is represented by *V. parahaemolyticus* (52%), followed by *V. alginolyticus*, *V. vulnificus* and *V. cholerae* (12%, 11% and 8%, resp.) considering culture-confirmed infections.²⁰ Significantly, despite the availability in the US of data from long-standing active (FoodNet²¹) and passive (COVIS, Cholera and Other *Vibrio* Illness Surveillance²²) surveillance systems, overall underreporting (underdiagnosis plus underreporting stricto sensu) was estimated to exceed a factor 1.8 for *V. vulnificus*, 36 for toxigenic *V. cholerae* and 150 for *V. parahaemolyticus* and the other species. This leads to a yearly estimated number of food-borne vibriosis in the US of 52,408, with 278 hospitalisations and 48 deaths (Scallan et al., 2011). Most illnesses (70.8%–73.6%) are attributed to molluscs' consumption, followed by crustaceans (13.5%–18.9%) and fish (3.7%–7.5%) (Painter et al., 2013). It can be presumed that in the EU, in the absence of a harmonised surveillance system, an overall higher underestimation of incidence (including underdiagnosis and underreporting) occurs for vibriosis.

3.1.2 | Food-borne outbreaks at EU/EEA level

During 2010–2021, 32 strong and weak evidence FBOs associated with *Vibrio* species in seafood were reported. *V. parahaemolyticus* in the 'Crustaceans, shellfish, molluscs and products thereof' or 'Fish and fish products' food categories was responsible for 30 of the 32 FBOs, causing 221 cases and 57 hospitalisations. One outbreak (12 cases) was caused by an unspecified *Vibrio* spp. in the 'Crustaceans, shellfish, molluscs and products thereof' category and another one (47 cases in a residential institution) was reported for non-toxigenic *V. cholerae* in mixed food (Table 1), possibly in relation to cross-contamination. Outbreaks have been mainly reported by France ($N=23$), Spain ($N=8$) and Portugal ($N=1$). A yearly average of 2.7 FBOs were reported, with a maximum of 7 and 5 in 2016 and 2018, respectively.

TABLE 1 Summary of the food vehicles in the 'strong and weak evidence' FBO associated with *Vibrio* spp. in the EU/EEA as reported in EFSA's zoonoses database (2010–2021).

FBO agent	FBO vehicle	No of FBO	Cases	Hospitalisations
<i>V. parahaemolyticus</i>	Total	30	221	57
	Crustaceans, shellfish, molluscs and products thereof ^a	27 ^b	212	52
	Fish and fish products	3	9	5
Non-toxigenic <i>V. cholerae</i>	Total	1	47	1
	Mixed food ^c	1	47	1
<i>Vibrio</i> spp., unspecified	Total	1	12	0
	Crustaceans, shellfish, molluscs and products thereof ^a	1	12	0
All	Total	32	280	58

Abbreviation: FBO, food-borne outbreaks.

^aThis group includes crustaceans, molluscs, tunicates and echinoderms.

^bFBO vehicle information: cooked crustaceans (1), unavailable (26).

^cThe residents ate puree meals; one of the puree menus included hake; information provided by Mrs Carmen Varela Martínez by e-mail on 2 June 2023 (Varela Martínez, 2023).

3.1.3 | RASFF notifications

Considering the relevant food categories, 75 RASFF notifications related to *Vibrio* spp. were reported between 2010 and January 2023. Only four notifications were associated with food of EU origin. Most (66/75) notifications were for crustaceans and products thereof, five were for bivalve molluscs and products thereof, two for fish and fish products, and two for cephalopods and products thereof.

²⁰<http://www.cdc.gov/foodnetfast>.

²¹<https://www.cdc.gov/foodnet/index.html>.

²²<https://www.cdc.gov/vibrio/surveillance.html>.

The *Vibrio* spp. reported in the RASFF notifications are summarised in Table 2. In five notifications, more than one *Vibrio* spp. was detected. *V. parahaemolyticus* was present, alone or in combination with other species, in about one third of notifications (27%/75%–36%), followed by *V. vulnificus* (21%/75%–28%) and non-O1/non-O139 *V. cholerae* (6%/75%–8%), with further 28 notifications related to *V. cholerae* non-O1 or serologically uncharacterised *V. cholerae* (for a total of 34%/75%–45% notification associated with *V. cholerae*). Additionally, *Vibrio alginolyticus* (4 notifications) and *Vibrio mimicus* (one notification) were also notified, mostly in association with other *Vibrio* spp. Since official controls customarily rely on standardised or otherwise recognised analytical methods, species included in such methods – as *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* – are expected to be more readily reported.

Five notifications between 2018 and 2021 related to detection of *V. parahaemolyticus* in imported products included information on the presence of genes associated with pathogenicity (two notifications for *tdh+*, one for *trh+* and two for *tdh+/trh+* isolates; see Section 3.4 for information on *Vibrio* pathogenicity and virulence markers).

Only one notification was reported as stemming from food poisoning or consumer complaints and was associated with the occurrence of multiple species (non-O1/non-O139 *V. cholerae*, *V. alginolyticus*, *V. mimicus*).

TABLE 2 Number of RASFF notifications related to single or multiple *Vibrio* spp. detection in various seafood categories (2010–February 2023).

	<i>Vibrio</i> species								No of notifications		
	<i>V. parahaemolyticus</i>	<i>V. vulnificus</i>	<i>V. cholerae</i> non-O1/ non-O139	<i>V. cholerae</i> non-O1	<i>V. cholerae</i>	<i>V. alginolyticus</i>	<i>V. mimicus</i>	<i>Vibrio</i> unspecified	Total	From outside Europe	From EU
Crustaceans and products thereof		√							66	66	0
					√				19	19	0
	√								17	17	0
			√						15	15	0
					√				5	5	0
	√								4	4	0
	√	√							1	1	0
				√					1	1	0
						√			1	1	0
	√				√		√		1	1	0
	√	√			√				1	1	0
								√	1	1	0
Bivalve molluscs and products thereof									5	1	4
	√								2	0	2
					√				1	0	1
	√					√			1	1	0
			√			√	√		1 ^a	0	1
Cephalopods and products thereof									2	2	0
	√								1	1	0
					√				1	1	0
Fish and fish products									2	2	0
	√				√				1	1	0
					√				1	1	0
Total	27	21	6	1	27	4	1	1	75	71	4

^aNotification stemming from food poisoning or consumer complaints.

3.1.4 | Zoonoses monitoring data

The EU monitoring data for *Vibrio* spp. in seafood included data for the 2018–2021 period, mostly in the distribution stage, from three EU countries only. In total, 111 and 110 samples were found positive for *V. parahaemolyticus* and serologically uncharacterised *V. cholerae*, respectively. *V. vulnificus* was found in 9 samples (Table 3). As for RASFF notifications, also monitoring programmes mostly rely on recognised analytical methods and, therefore, it can be assumed that species covered by such methods are more easily detected.

TABLE 3 Number of seafood samples tested in various seafood categories for *Vibrio* spp. for monitoring purposes in the period 2018–2021 in the EU.^a

	Species	Total units positive	Total units tested
Crustaceans	<i>V. parahaemolyticus</i>	106	299
	<i>V. cholerae</i>	103	256
	<i>V. vulnificus</i>	9	20
	<i>Vibrio unspecified</i>	0	9
Fish	<i>V. parahaemolyticus</i>	5	205
	<i>V. cholerae</i>	7	577
Fishery products	<i>V. parahaemolyticus</i>	0	3
	<i>Vibrio unspecified</i>	0	6
Grand total		230	1375

^aSingle units (759 units) and batches (616 units) were sampled. The sampling context was mainly monitoring (1351 units) and for few samples surveillance (24 units). The sampling strategy consisted mainly of objective sampling (1361 units), the remainder (14 units) using selective sampling.

3.1.5 | Literature search

A total of 25 recent reviews dealing with *Vibrio* cases or outbreaks were retrieved through the general literature. Only one record (Ahmed et al., 2022) reported a case in an EU country (The Netherlands) attributed to a species other than *V. parahaemolyticus*, *V. vulnificus* or non-O1/non-O139 *V. cholerae*. The case was a sepsis episode in a 50-year-old man with a history of tuberculosis, chronic obstructive pulmonary disease and alcohol abuse. Species attribution was controversial due to the reporting of the isolate as *Vibrio albensis* in the review, as opposed to its identification as non-O1/non-O139 *V. cholerae* in the primary study (Engel et al., 2016). Both seawater contact and seafood consumption (ready-made tuna salad) were reported as possible source of infection, but no *Vibrio* spp. were detected in the suspected seafood product.

Among the 1046 records captured through the literature searches, five records reported human infections with *Vibrio* species other than *V. parahaemolyticus*, *V. vulnificus* or non-O1/non-O139 *V. cholerae* in EU countries with an identified or suspected food-borne origin. Amato et al. (2022), in a multi-country study on vibriosis in Norway, Sweden, Denmark, Finland, Poland and Estonia in 2018, reported 152 *V. alginolyticus* infections. However, information on exposure was available for a minority of cases (20/152), and only two infections were attributed to consumption of contaminated food or water. Similarly, Hoefler et al. (2022) reported 23 *V. alginolyticus* infections in Bay of Biscay (France) between 2001 and 2019, but only one characterised by GE, the others being associated with otitis, osteitis or pneumonia. Brehm et al. (2021), in a retrospective study on vibriosis in Germany (2018–2019) reported one GE case due to *V. fluvialis* lacking conclusive information on seafood consumption. Finally, two GE cases following seafood consumption (oysters and cockles) due to *Vibrio hollisae* were reported by (Edouard et al., 2009; Gras-Rouzet et al., 1996). Due to the reclassification of this species in the distinct *Grimontia* genus (Thompson et al., 2003), these cases were not further considered.

In comparison, a considerable number of *V. parahaemolyticus* and non-O1/non-O139 *V. cholerae* cases associated with seafood consumption were described in Europe since the 1990s (summarised in Annex C). These include large *V. parahaemolyticus* outbreaks or sporadic cases reported in four EU member states, and several non-O1/non-O139 *V. cholerae* sporadic cases in many countries. Two reports on seafood-borne *V. vulnificus* infections were retrieved for cases in Spain and French overseas territories, but several investigations of *V. vulnificus* septicaemia cases were inconclusive due to critical patients' conditions.

3.1.6 | ProMED records

In total, 13 of the 408 retrieved ProMED records reported non-cholera *Vibrio* cases in EU countries between 1997 and 2021. The cases were mostly related to wound infections or bacteraemia/septicaemia associated with non-O1/non-O139 *V. cholerae*, *V. vulnificus* and *V. alginolyticus*, following exposure to seawater. No report of food-borne infections with species different than *V. parahaemolyticus*, *V. vulnificus* or non-O1/non-O139 *V. cholerae* was retrieved.

3.1.7 | FSANZ data request

The FSANZ data request (Annex B) illustrated that human disease caused by *V. parahaemolyticus* and/or *V. vulnificus* are not notifiable in Croatia, Cyprus, Estonia, France, Ireland, but is notifiable in Sweden since 2004, in Norway since 2019 and in Germany since 2020.

There was no reporting of an increase of human illness caused by *Vibrio* species over the past 3–5 years in Croatia, Cyprus, Estonia, Germany, Ireland and Norway.

In Sweden, there was a slight increase in numbers over the last 10-year period; however, this may be due to an increased awareness among physicians. The maximum number of reported cases in Sweden (178) was in 2018, which was a warm summer. Most cases are swimming related (e.g. ear infections in children, wound and blood infections). The epidemiology of vibriosis cases in countries bordering the North Sea and Baltic Sea area has been fully described in Amato et al. (2022).

The number of non-cholera *Vibrio* infections is increasing in France (an average of 10 cases per year between 1995 and 2016, 25 cases in 2017, 59 in 2018, 67 in 2019, 47 in 2020, 46 in 2021). *V. parahaemolyticus* was the most frequently reported species since 2018, mainly in association with moderate forms of GE following seafood consumption, followed by *V. cholerae* non-O1/non-O139, but with which displays a significant number of imported cases. The emergence of *V. fluvialis* associated with GE in the last 5 years was also reported. Details are provided by the reports of the French National Reference Centre for *Vibrio* and cholera related to 2017–2020²³: excluding travel-associated cases, five *V. fluvialis* cases (three in 2018, two in 2019) and one *V. mimicus* case (2018) associated with seafood consumption were reported. In some of these cases, predisposing health conditions were documented. In comparison, in the same period 2017–2020, the reported number of *V. parahaemolyticus* cases associated with seafood consumption was 75, and the number of seafood-borne non-O1/non-O139 *V. cholerae* cases was 14.

3.1.8 | Previous risk assessments in EU countries

The 2012 ANSES risk assessment summarised the non-cholerae *Vibrio* spp. leading to human infections in the 1995–2009 period. In total, 15 cases of GE (potentially of food origin and linked to seafood consumption) were due to non-O1/non-O139 *V. cholerae* (2 cases contracted in France, 7 abroad) and *V. parahaemolyticus* (65 cases contracted in France, 1 abroad) (ANSES, 2012).

The 2022 BfR risk assessment reported that the annual number of infections with non-cholera vibrios in coastal areas in Germany ranged from 0 to 20 between 2002 and 2019. The majority involved wound and ear infections and only occasionally GE cases. BfR concluded that investigation of foods for potentially pathogenic *Vibrio* species should concentrate on *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae*, as these species are worldwide in the aquatic ecosystems, and can therefore be present in international trade and locally produced seafood. The hazard identification also considered *V. alginolyticus* and *V. metschnikovii*, as these species had often been detected in seafood samples in Germany between 2017 and 2020. Other *Vibrio* species (*V. mimicus*, *V. fluvialis*, *V. furnissii* and *V. harveyi*) are detected only occasionally in food products in Germany and were not further addressed (BfR, 2022).

3.1.9 | Concluding remarks

- No harmonised surveillance system for non-cholera vibriosis is currently available in the EU, and human disease by *V. parahaemolyticus* or *V. vulnificus* is notifiable in a few EU Member States. Based on this, it should be expected that the underestimation of non-cholera vibriosis in the EU is considerable.
- *Vibrio* infections acquired during travels abroad or through seawater exposure and leisure activities are often reported in the EU. Therefore, proper case description and investigation are crucial to define if infections are domestically acquired and if they should be attributed to food-borne transmission.
- *V. parahaemolyticus* is currently the species most frequently associated with human disease through seafood consumption in the EU, followed by non-O1/non-O139 *V. cholerae*. Food-borne transmission of *V. vulnificus* is rarely documented in the EU.
- Other *Vibrio* species as *V. alginolyticus*, *V. fluvialis* and *V. mimicus* may occasionally lead, particularly in individuals with underlying health conditions, to seafood-associated infections, but their PH relevance is minor compared to *V. parahaemolyticus*, *V. vulnificus* or non-O1/non-O139 *V. cholerae*.
- According to RASFF notifications, which are mostly associated with seafood imported to the EU, the species *V. parahaemolyticus*, non-O1/non-O139 *V. cholerae* and *V. vulnificus* are more often reported in seafood products placed or intended to be placed on the EU market compared to other *Vibrio* species. Other species reported are *V. alginolyticus* and *V. mimicus*.
- The *Vibrio* spp. considered in the current opinion are *V. parahaemolyticus*, *V. vulnificus* and non-O1/non-O139 *V. cholerae* as, other species are less frequently found in seafood placed or intended to be placed on the EU market and, to date, have been rarely identified as the cause of illness in humans in the EU through seafood consumption.

3.2 | Occurrence of the relevant *Vibrio* spp. in seafood placed or intended to be placed on the EU market (SQ1.2)

Overall, the 46 primary research publications retrieved through ELS covered 370 data entries on the occurrence (i.e. prevalence and/or concentration) of the relevant *Vibrio* spp. in seafood produced and/or commercialised in Europe. Of these, 224 entries concerned *V. parahaemolyticus*, 77 entries non-cholerae *V. cholerae* and 69 entries *V. vulnificus*, with studies

²³<https://www.pasteur.fr/fr/sante-publique/cnr/les-cnr/vibrions-cholera/rapports-d-activite>.

undertaken in 13 EU countries.²⁴ A further 9 studies were carried out in non-EU countries.²⁵ Publications concerned *V. parahaemolyticus* ($N=42$), non-cholerae *V. cholerae* ($N=21$) and *V. vulnificus* ($N=19$). The overall number of samples tested for detection (sometimes associated with enumeration) and for enumeration only was, respectively, 10,614 and 64 for *V. parahaemolyticus*, 2907 and 64 for *V. vulnificus*, and 2613 and 7 for non-cholerae *V. cholerae*.

The studies encompassed samplings performed between 2000 and 2022 lasting from 2 to 120 months (median: 16 months), for 16 studies being shorter than 1 year. Moreover, the sampling period could be restricted to some months; most of the time, the summer period was covered by studies conducting sampling at primary production stage. Less than one third of the publications summarised studies that began in the last decade (i.e. initiated in or after 2014). In 11 publications, it was not possible to ascertain the year of sampling.

The largely uneven distribution of the studies across countries is reflected in the FAO areas and subareas of origin of the products. Seafood products originated mostly from the Mediterranean and Black Sea²⁶ and, secondly, from the Northeast Atlantic,²⁷ while testing of products from the Pacific Ocean²⁸ was reported in a minority of cases.

Sampling at the primary production stage and at the distribution stage were roughly equally represented in the data set (154 and 189 entries, 27 and 19 publications, respectively), while sampling at the manufacturing stage was present in 27 entries and 4 publications. However, while entries dealing with the distribution or manufacturing stage addressed the different seafood categories (58 entries for molluscs, 53 for crustaceans, 85 for fish meat and 20 for other seafood types, like sushi or surimi), the data related to primary production were almost exclusively associated with molluscs (145 of 154 entries). Significantly, despite the high number of publications related to bivalves at primary production (21 publications), only two (Suffredini et al., 2014) reported information on the classification^{29,30} of the corresponding harvesting areas with separate results for each seafood category.

Based on the extracted data, the prevalence of *Vibrio* species of PH relevance in studies performed on seafood products in the European market is summarised in Figure 2. The result of random-effect meta-analysis of the prevalence data is included.

Most studies ($n=42$) and entries ($n=224$) reported data on the prevalence or concentration of *V. parahaemolyticus* (222 entries reported prevalence data). The type of seafood considered is given in Figure 2A. The entries dealing with bivalve molluscs mostly reported data on clams (e.g. *Ruditapes*, *Ensis*, *Meretrix*, *Chamelea*), mussels (*Mytilus* spp.), oysters (*Crassostrea* spp.) and scallops. The pooled prevalence estimate of *V. parahaemolyticus* was 19.63% (95% CI 13.68–27.37). The highest prevalence was found in bivalve molluscs (27.83%; 95% CI 18.56–39.49) and gastropods (abalones, winkles, conchs) (28.77%; 95% CI 10.46–58.27). Within the bivalve molluscs category, oysters and scallops had the lowest prevalence. The pooled prevalence estimates for mussels harvested in the Northeast Atlantic area was 55.89% (95% CI 23.64–83.84) ($I^2=45.99$), while for those harvested from the Mediterranean and Black Sea it was 23.17% (95% CI 8.40–49.79) ($I^2=51.79$). However, using random meta-regression, this difference is not significant by Moderator test QM test ($p>0.05$). Other factors such as the period of sampling, the species of mussels and environmental factors could interfere with the direct comparison. Least, the representativity of each fishing area for the comparison is not guaranteed. Overall, for most seafood categories, the heterogeneity between studies was found to be significant ($I^2>25%$) (Gonçalves-Tenório et al., 2018), indicating that variability between studies remains non negligible; this variability could be explained by some factors such as different sites, years, period of sampling, or methods of detection between studies.

²⁴Bulgaria $n=2$, Croatia $n=1$, Cyprus $n=1$, France $n=6$, Germany $n=1$, Greece $n=1$, Italy $n=15$, The Netherlands $n=1$, Poland $n=2$, Portugal $n=2$, Slovenia $n=1$, Spain $n=3$, and Sweden $n=1$.

²⁵Data from countries that were EU Members in the period (2010–2023) considered in the ELS, EU accession candidates, or members of the European single market were also extracted; Switzerland $n=1$, Türkiye $n=7$, and the United Kingdom $n=1$.

²⁶FAO area 37, with 26 publications and 160 entries, 115 of which related to the Adriatic Sea, Ionian Sea and Sardinia.

²⁷FAO area 27, with 8 publications and 32 entries, 17 of which related to Portuguese waters.

²⁸FAO areas 61, 71 and 87, with 5 publications and 9 entries.

²⁹Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products.

³⁰Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls.

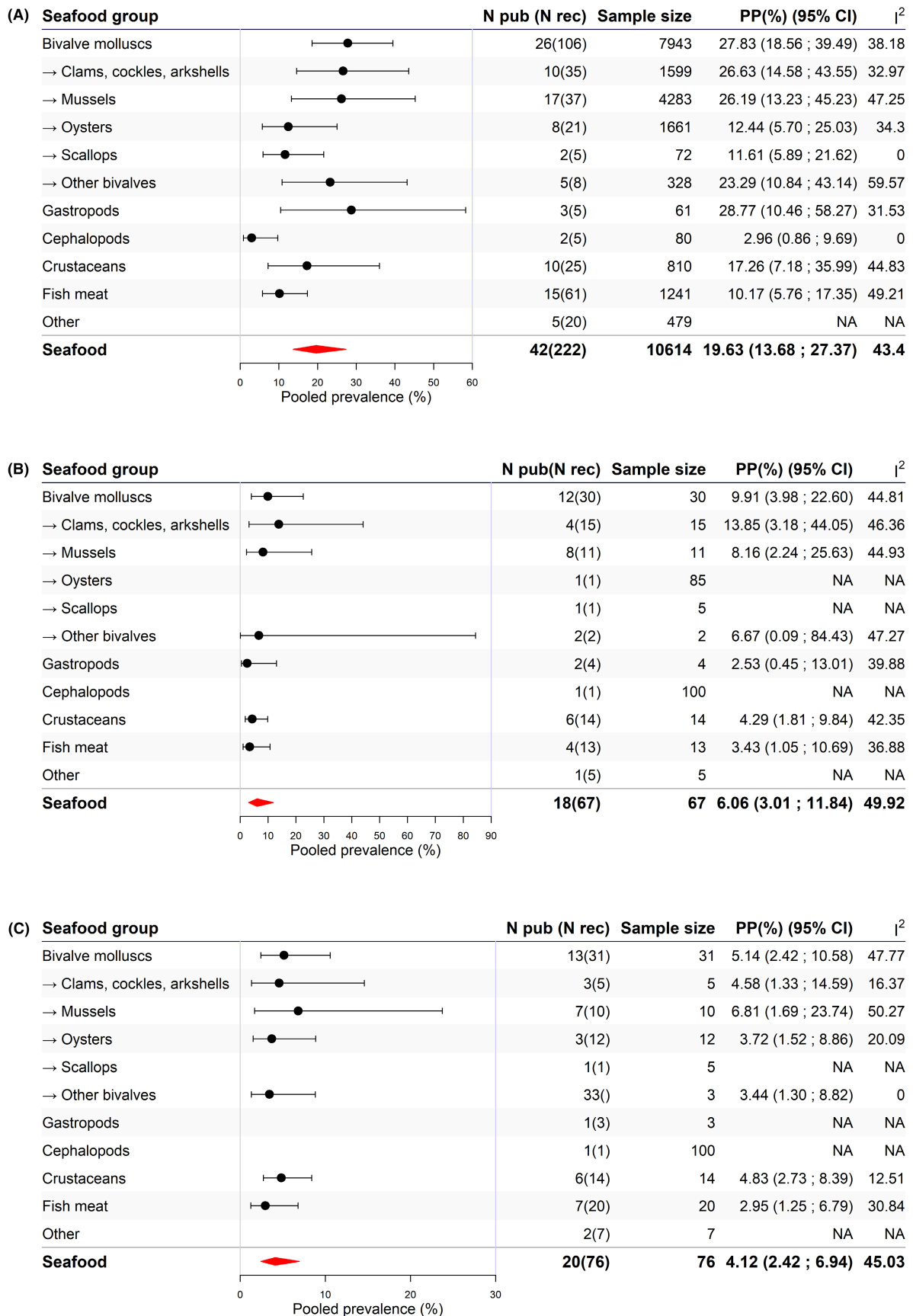


FIGURE 2 Pooled prevalence of *V. parahaemolyticus* (A), *V. vulnificus* (B) and non-choleraemic *V. cholerae* (C) in the different seafood groups based on the meta-analysis. I^2 , intraclass correlation estimates the proportion of variance, from the total variance, explained by differences between the studies. Whenever the number of studies is small and the variability inside studies is high, the I^2 can be uncertain; NA, not applicable. Non-choleraemic *V. cholerae* includes samples that were tested using either sero-agglutination tests (non-O1, non-O139 or both) or molecular tests (no detection of cholera toxin-associated genes, *ctx*, *ctxA* and/or *ctxB*). Meta-analysis results are not presented for those food groups with only a single data entry and for the category 'other'.

A significant proportion of the *V. parahaemolyticus* studies (19/42) included information on the detection of both genetic markers associated with *V. parahaemolyticus* pathogenicity, namely *tdh* and *trh* genes in positive samples (with sample size ≥ 3). The result of random-effect meta-analysis of the proportion of samples containing *tdh+* and/or *trh+* *V. parahaemolyticus* on *V. parahaemolyticus*-positive samples is given in Table 4. Considering all seafood categories, on average about one out of five (18.41%; 95% CI 11.06–29.05) of *V. parahaemolyticus*-positive samples contained pathogenic (i.e. *tdh+* and/or *trh+*) *V. parahaemolyticus*.

TABLE 4 Proportion of samples with *tdh+* and/or *trh+* *V. parahaemolyticus* on *V. parahaemolyticus*-positive samples.

Seafood group	N publications (N entries)	Sample size in the category	Proportion (%) [95% CI] and I^2
Bivalve molluscs	16 (36)	588	20.89 [11.65, 34.58] $I^2 = 32.61$
– Clams, cockles, arkshells	5 (12)	112	24.26 [16.73, 33.80] $I^2 = 0$
– Mussels	9 (17)	329	19.87 [8.07, 41.20] $I^2 = 33.71$
– Oysters	4 (5)	128	27.56 [20.12, 36.50] $I^2 = 0$
– Scallops	0	0	NA
– Other bivalves	2 (2)	19	4.90 [0.68, 27.78] $I^2 = 0$
Gastropods: abalones, winkles, conchs	2 (2)	14	10.09 [2.03, 37.77] $I^2 = 0$
Cephalopods	0	0	NA
Crustaceans	4 (8)	121	10.93 [6.13, 18.75] $I^2 = 0$
Fish meat	1 (3)	11	NA
Other	0	0	NA
Total	19 (49)	734	18.41 [11.06, 29.05] $I^2 = 34.08$

Abbreviations: CI, confidence interval; I^2 , intraclass correlation estimates the proportion of variance, from the total variance, explained by differences between the studies. Whenever the number of studies is small and the variability inside studies is high, the I^2 can be uncertain; NA, not applicable.

V. vulnificus prevalence was the object of 18 publications and 67 entries, the result of the random-effect meta-analysis of prevalence data is given in the Figure 2B. *V. vulnificus* pooled prevalence was 6.06% (95% CI 3.01–11.84) and was highest for bivalve molluscs (9.91%; 95% CI 3.98–22.60), especially in clams, cockles and arkshells.

The prevalence of non-choleraenic *V. cholerae* was the focus of 20 publications and 76 entries (Figure 2C). Most studies tested for cholera toxin-associated genes *ctxA* and/or *ctxB*, and few cases tested for O1/O139 sero-agglutination. The non-choleraenic *V. cholerae* pooled prevalence was 4.12% (95% CI 2.42–6.94).

Quantitative data on the concentration of the three relevant species were reported in only 8 publications, yielding 49 data entries dealing with *V. parahaemolyticus* (7 studies) in molluscs (23 entries) or crustaceans (10 entries), with non-choleraenic *V. cholerae* in bivalve molluscs (3 publications, 5 entries) or with *V. vulnificus* in bivalve molluscs (3 publications, 11 entries). Considering entries with mean and standard deviation reported (13 entries, 4 publications, mostly concerning bivalves) *V. parahaemolyticus* pooled mean in bivalve molluscs was 1.91 \log_{10} CFU/g or MPN/g (95% CI 0.68–3.14). The maximum mean concentration found in a survey was 4.99 \log_{10} CFU/g reported in farmed mussels at retail (Stratev et al., 2021). For crustaceans, a mean concentration was estimated in shrimp of 3.33 \log_{10} CFU/g (one entry). The maximum concentration in one sample was greater than 5.2 \log_{10} MPN/g (Caburlo et al., 2016). For *V. vulnificus*, the mean and standard deviation were only available in 3 of 11 entries (2 publications) concerning bivalves, with contrasting results. The pooled mean was approximately 1.84 \log_{10} CFU/g or MPN/g (95% CI –2.33 to 6.01). For non-choleraenic *V. cholerae*, only one entry quantified a mean of 1.34 MPN/g in cockles (Baron et al., 2017).

Several limitations were identified in the retrieved data, including (i) absence of relevant information on the sampling plan, (ii) absence of sampling period and the year of sampling, (iii) uneven seasonal distribution of sampling, (iv) uneven geographic distribution of sampling, (v) limited information on sample origin/strategy (at retail level) or on sanitary classification of production areas (for bivalve shellfish), (vi) heterogeneous reporting for pathogenicity testing and concentration estimates, (vii) lack of linkage between environmental parameters (temperature, salinity) and sample results in primary production, (viii) mixing of different seafood species or different origins in results, (ix) results presented, in particular for pathogenicity testing, as a mix of isolates from different samples, and (x) small sample size for each species or each type of seafood ($N < 30$).

3.2.1 | Concluding remarks

- Data on the prevalence and quantitative levels of the relevant *Vibrio* spp. in seafood produced and/or commercialised in Europe were extracted from 46 primary studies performed in 16 countries yielding 376 data entries (2000–2022 period).

When reported, most studies dealt with samples from the Mediterranean Sea and, to a minor extent, the Northeast Atlantic. Among them, 228 entries concerned *V. parahaemolyticus*, 78 entries non-cholerae *V. cholerae* and 70 entries *V. vulnificus*.

- The main aim of the studies was the detection of one or more of the relevant *Vibrio* species, and quantitative analysis was rarely performed (49 entries). In seafood placed or intended to be placed on the EU market:
 - Across seafood categories, the *V. parahaemolyticus* pooled prevalence estimate was 19.6% (95% CI 13.7–27.4). The highest prevalence was estimated for bivalve molluscs (27.8%; 95% CI 18.6–39.5) and gastropods (abalones, winkles, conchs) (28.8%; 95% CI 10.5–58.3). Within the bivalve molluscs category, oysters and scallops had the lowest prevalence.
 - Considering all seafood categories, about one out of five (18.4%; 95% CI 11.1–29.1) of *V. parahaemolyticus*-positive samples contained pathogenic (i.e. *tdh*+ and/or *trh*+) *V. parahaemolyticus*.
 - The *V. vulnificus* pooled prevalence was estimated as 6.1% (95% CI 3.0–11.8) and was highest for bivalve molluscs (9.9%; 95% CI 4.0–22.6), especially in clams, cockles and arkshells.
 - Non-cholerae *V. cholerae* pooled prevalence was estimated as 4.1% (95% CI 2.4–6.9).
 - For most seafood categories, the heterogeneity between studies was found to be significant which could be explained by factors such as different sites, years, period of sampling, or methods of detection.
 - Concentration data on the three *Vibrio* species were rarely reported. *V. parahaemolyticus* pooled mean concentration in bivalve molluscs was 1.91 log₁₀ CFU/g or MPN/g (95% CI 0.68–3.14), while for crustaceans only the mean from one study was available (3.33 log₁₀ CFU/g). For *V. vulnificus* the pooled mean concentration in bivalve molluscs was 1.84 log₁₀ CFU/g or MPN/g (95% CI –2.33 to 6.01), and for non-cholerae *V. cholerae*, the mean from one study on cockles was 1.34 MPN/g.
 - No data were retrieved on detection or quantification of *V. parahaemolyticus*, non-O1/non-O139 *V. cholerae* or *V. vulnificus* in tunicates or echinoderms.
- Several limitations were identified in the retrieved data, including the absence of essential sampling descriptors, uneven temporary and geographic distribution, heterogeneity of data reporting, data aggregation among different species, and limited number of tested samples for certain species.

3.3 | Analytical methods for the detection, enumeration and characterisation of the relevant *Vibrio* spp. in seafood and their contribution to risk assessment (SQ1.3–SQ1.4)

3.3.1 | Methods for the detection and enumeration

Well-established and standardised microbiological procedures are being used in routine monitoring of *Vibrio* spp. International and National Health Organisations and Authorities recommend the use of **standard microbiological methods** for the detection and enumeration of the relevant *Vibrio* spp. in seafood (Annex D). These procedures rely on cultural methods and the isolation of bacterial isolates occasionally combined with molecular identification/characterisation.

The available methods for the **detection** of the relevant *Vibrio* species rely on direct plating on selective or non-selective agar media or on conventional selective enrichment of a suspension of seafood homogenate in alkaline peptone water followed by plating onto one or two selective agar media for isolation of presumptive pathogenic *Vibrio* spp. Up to 10 characteristic colonies are then subjected to identification by biochemical testing, colony hybridisation and/or PCR and further characterisation by serotyping or detection of pathogenicity markers (e.g. TDH for *V. parahaemolyticus*) [ISO 21872-1:2017³¹; FDA-BAM (2004)³²; MFLP-37 (2019)³³ and MFLP-102 (2017)³⁴]. Serotyping of *V. cholerae* O1 and O139 is included in FDA-BAM (2004) but only suggested, with no technical information on the procedure, in ISO 21872-1:2017.

The available standardised methods for the **enumeration** of *V. parahaemolyticus* and/or *V. vulnificus* in seafood utilise direct plating of seafood homogenate suspensions on selective agar or on non-selective agar followed by colony

³¹ISO 21872-1:2017. Microbiology of the food chain – Horizontal method for the determination of *Vibrio* spp. Part 1: Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*. International Organization for Standardization, Geneva, Switzerland.

³²FDA BAM (2004). Bacteriological Analytical Manual Chapter 9: *Vibrio*. Food & Drug Administration, Bacteriological Analytical Manual, Washington DC. <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-9-vibrio>, accessed on the 14 May 2024.

³³MFLP-37 (2019). Detection, isolation, and enumeration of *Vibrio parahaemolyticus* and/or *Vibrio vulnificus* in Seafood. Laboratory Procedures for the Microbiological Analysis of Foods. Volume 3. Government of Canada.

³⁴MFLP-102 (2017). Identification of *Vibrio parahaemolyticus* colonies by real-time polymerase chain reaction. Laboratory Procedures for the Microbiological Analysis of Foods. Volume 3. Government of Canada.

hybridisation [ISO/TS 21872-2:2020,³⁵ FDA-BAM (2004)³⁶] or conventional MPN enrichment followed by colony isolation and species identification by biochemical testing, colony hybridisation or PCR [FDA-BAM (2004)³⁷, GB 4789.7-2013,³⁸ GB 4789.44-2020³⁹].

These detection and enumeration methods include the use of selective and/or differential agar media which provide discrimination between *Vibrio* species and other microbiota and, especially when chromogenic media are adopted, differentiation of *Vibrio* spp. of PH relevance from other *Vibrio* spp. Molecular confirmation methods markedly improve identification of *Vibrio* spp. of PH relevance and discrimination between pathogenic and non-pathogenic strains, e.g. of *V. parahaemolyticus*. However, if a species of PH interest or a pathogenic strain within the species is present in much lower concentrations than other *Vibrio* species or non-pathogenic strains, the isolation and confirmation of a larger number of colonies, or the application directly on enrichments of PCR methods for species/pathogenicity genes detection, may be required to achieve detection. Despite some limitations (see Section 3.3.3), the ISO methods are commonly used at the international level for the detection of *V. parahaemolyticus*, *V. vulnificus* and/or *V. cholerae* and the enumeration of total and potentially enteropathogenic *V. parahaemolyticus* in seafood (Hartnell et al., 2019; Suffredini et al., 2014). These methods and the FDA-BAM methods have been used in most studies reporting data on the occurrence of the relevant *Vibrio* spp. in seafood as presented in Section 3.2.

Further to the standardised methods, a variety of other methods combining culture-dependent and molecular methods have been developed and evaluated for optimised and rapid detection and enumeration of *Vibrio* in environmental and seafood samples. These include qualitative and quantitative (MPN) methods with reduced culture enrichment time combined with PCR detection approaches (Blanco-Abad et al., 2009; Luan et al., 2008; Machado & Bordalo, 2016; Robert-Pillot et al., 2014) and/or isolation of strains (Chen et al., 2012). Furthermore, quantitative PCR (qPCR) methods have been reported (Niu et al., 2018; Robert-Pillot et al., 2010), especially for the enumeration of total and pathogenic *V. parahaemolyticus*. Aside from already existing methods to confirm identification of vibrios, matrix-assisted laser desorption ionisation–time of flight (MALDI–TOF) mass spectrometry (MS) with databases built on environmental, clinical and reference strains; e.g. PVBase⁴⁰ (Liu et al., 2022) proved to be precise and cost-effective tools for accurate and rapid identification of a large number of *Vibrio* spp. Loop-mediated isothermal amplification (LAMP) assays have also been developed and successfully applied to detect *V. parahaemolyticus* and *V. vulnificus* from environmental water and aquatic products (Liu et al., 2017; Tian et al., 2022). Recent FBO investigations involving digital droplet PCR (Hu et al., 2022; Zhao et al., 2022; Zhou et al., 2023) or combining next generation sequencing (NGS) and digital PCR (Li et al., 2019) showed that these methods provide new fast tools for accurate detection and quantification of pathogenic *Vibrio* species. Finally, viability PCR showed to be a promising approach to overcome the underestimation of microorganisms in the VBNC state by culture-based methods, and different assays have been developed to detect and quantify VBNC *V. parahaemolyticus* and *V. cholerae*, although, to date, their application has been mostly on experimentally contaminated seafood (Liu et al., 2018; Wu et al., 2015).

3.3.2 | Methods for characterisation

Classical typing analyses of pathogenic *Vibrio* species have been based on **serotyping**, which specific schemes developed for the O- antigen of *V. cholerae* and the O- and K-antigens of *V. parahaemolyticus*. Serotyping was useful to discriminate strains from outbreaks in the earlier days and was afterwards enhanced by pulsed-field gel electrophoresis (**PFGE**), which allowed for a higher discriminatory power. These techniques showed some inconsistencies in the serotyping scheme particularly of *V. parahaemolyticus*, where identical serotypes showed different genetic profiles and extensive serotype diversity was present among isolates representing the same genetic types (Zhang et al., 2022).

With the introduction of sequencing technologies, multi-locus sequence typing (**MLST**) schemes based on PCR-amplification and Sanger sequencing of a limited number of housekeeping genes (from 5 to 10) were developed for non-O1/non-O139 *V. cholerae* (Octavia et al., 2013), *V. cholerae* O1/O139 (Kanampalliwar & Singh, 2020), *V. parahaemolyticus* (González-Escalona et al., 2008) and *V. vulnificus* (Bisharat et al., 2007) using a dedicated website to analyse and deposit genomic data and profiles.⁴¹

The emergence of genomic epidemiology approaches applying **WGS** and other **NGS** approaches, coupled to open access bioinformatic tools, has further changed the techniques applied for characterisation at the strain level. In particular, WGS replaced the PCR-Sanger approach for MLST with in silico analysis of variants for each genome locus.⁴² An extended

³⁵ISO/TS 21872-2:2020. Microbiology of the food chain – Horizontal method for the determination of *Vibrio* spp. Part 2: Enumeration of total and potentially enteropathogenic *Vibrio parahaemolyticus* in seafood using nucleic acid hybridization. International Organization for Standardization, Geneva, Switzerland.

³⁶FDA BAM (2004). Bacteriological Analytical Manual Chapter 9: *Vibrio*. Food & Drug Administration, Bacteriological Analytical Manual, Washington DC. <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-9-vibrio>, accessed on the 14 May 2024.

³⁷FDA BAM (2004). Bacteriological Analytical Manual Chapter 9: *Vibrio*. Food & Drug Administration, Bacteriological Analytical Manual, Washington DC. <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-9-vibrio>, accessed on the 14 May 2024.

³⁸GB 4789.7-2013. National Food Safety Standard – Microbiological Examination of Food Hygiene – Examination of *Vibrio parahaemolyticus*. National Standard of the People's Republic of China.

³⁹GB 4789.7-2020. National Food Safety Standard – Microbiological Examination of Food Hygiene – Examination of *Vibrio vulnificus*. National Standard of the People's Republic of China.

⁴⁰<https://www.frontiersin.org/articles/10.3389/fmicb.2022.872825/full#supplementary-material>.

⁴¹<https://pubmlst.org/>.

⁴²<https://pubmlst.org/organisms/vibrio-parahaemolyticus>; <https://pubmlst.org/organisms/vibrio-cholerae>.

core genome MLST (**cgMLST**) scheme using 2254 core genes of *V. parahaemolyticus* genome was developed by (Gonzalez-Escalona et al., 2017) and an analogous scheme is available for *V. cholerae* (Liang et al., 2020), both of which outperform previous methods in terms of resolution. WGS is today routinely applied in the investigation by public health agencies (e.g. CDC, FDA, China CDC, UK-PHE) of *Vibrio* FBOs to identify genetic variants of strains with particular epidemic potential, to follow ecological expansion and emergence of highly pathogenic *V. parahaemolyticus* clones⁴³ (Abanto et al., 2020), for clinical surveys, and for research purposes, but the use in food control activities (beyond outbreak investigations) has been limited. Most application of NGS in *Vibrio* studies reported to date have been focused on WGS of isolates obtained by culture. **Other omics techniques**, such as metabarcoding (based on 16S rRNA gene or other target sequences) (Zampieri et al., 2021) or metagenomics have been applied only in research contexts and rarely for food safety. Shotgun metagenomics, for instance, allowed the identification of markers in the intestinal microbiome of patients with cholera at the time of exposure to *V. cholerae* (Levade et al., 2021) and rare pathogens such as *V. vulnificus* in patients with skin and soft tissue infections (Wang et al., 2020). Metagenomic shotgun sequencing have been also applied in environmental investigations to determine the presence of pathogenic vibrios linked to some of extreme weather events, such as hurricanes (Brumfield et al., 2023).

3.3.3 | Contribution to risk assessment

The methods currently available for the analysis of *Vibrio* spp. of PH relevance display a variegated assortment of advantages and disadvantages, as well as notable areas for future development. The SWOT of these methods in regards of their contribution to risk assessment is provided in [Table 5](#).

⁴³As of January 2024, publicly available *V. parahaemolyticus* genomes have reached a total of 10,000 genomes (<https://www.ncbi.nlm.nih.gov/assembly/?term=vibrio%20parahaemolyticus>; NHI, National Library of Medicine, consulted on 15.1.2024).

TABLE 5 Characteristics of the available analytical methods for the detection and enumeration of potentially enteropathogenic *Vibrio* spp. in seafood and their contribution to risk assessment.

	Culture-based detection methods	Culture-based quantification methods	Conventional typing methods	WGS (from isolates)	Metagenomics (targeted and untargeted)
Strengths	<ul style="list-style-type: none"> A validated ISO standard is available for Vp, Vv and Vc Provide comparable data on prevalence for exposure assessment Provide isolates for further characterisation, including detection of pathogenicity markers Technically easy and affordable 	<ul style="list-style-type: none"> An ISO standard is available for Vp Provide data on contamination levels for exposure assessment Support the acquisition of data for modelling (including DR models, growth/inactivation models, effect of climate change, efficacy of control measures, etc.) May provide isolates for further characterisation Can provide both data on proportion of samples with pathogenic strains and proportion of pathogenic strains on total for that species within a sample 	<ul style="list-style-type: none"> Allow strain discrimination Allow easy identification of epidemic clones Support basic outbreak investigation Support basic investigation of strain introduction in an environment Affordable compared to WGS 	<ul style="list-style-type: none"> Robust for isolates characterisation High-level phylogenetic resolution for outbreak investigation and trace-back Provide information for source attribution and identification of transmission routes/pathways Data can be analysed (and re-analysed) for different aims Data sharing is technically easy 	<ul style="list-style-type: none"> Provide microbial population data (composition and variation, resistome, etc.) Bypass cultivation or isolation, therefore applicable also to non-cultivable, fastidious, or slow-growing <i>Vibrio</i> spp. of interest, even achieving the whole genome of some organisms
Weakness	<ul style="list-style-type: none"> Performance comparison between standard methods is not available In some methods pathogenicity characterisation (e.g. <i>tdh/trh</i> of Vp, serogroup of Vc) is optional Random isolation of a limited number of colonies may lead to underestimation of the proportion of samples containing pathogenic strains Time consuming 	<ul style="list-style-type: none"> No interlaboratory validation for currently available methods No ISO standard for quantitative analysis of Vv or Vc Not systematically coupled to strain isolation for further characterisation Time consuming and labour intensive 	<ul style="list-style-type: none"> Lack resolution to discriminate closely related strains Rapid obsolescence of techniques and databases due to advent of WGS Time consuming and labour intensive 	<ul style="list-style-type: none"> Metadata sharing is complex (legal framework, ethical issues, etc.) Insufficient standardisation of data analysis Data analysis requires specialised personnel Not yet affordable for all potential users 	<ul style="list-style-type: none"> Lack of sensitivity for detection species or sub-groups within species of interest Absence of isolates for further analysis Absence of phenotypic testing Insufficient standardisation of data analysis Not yet applicable for untargeted detection of species of PH interest and monitoring purposes Data analysis requires trained personnel Not yet affordable for all potential users

TABLE 5 (Continued)

	Culture-based detection methods	Culture-based quantification methods	Conventional typing methods	WGS (from isolates)	Metagenomics (targeted and untargeted)
Opportunities	<ul style="list-style-type: none"> • Easy to combine with molecular screening methods (e.g. conventional, real-time and viability PCR) and with detection of new molecular targets • Easy to couple with genomic characterisation of isolates • Support acquisition of prevalence data also in challenging areas (e.g. low-income countries, areas with basic laboratories) 	<ul style="list-style-type: none"> • May support the definition of quantitative microbiological criteria or trade standards 	<ul style="list-style-type: none"> • Support acquisition typing data also in challenging areas (e.g. low-income countries, areas with basic laboratories) 	<ul style="list-style-type: none"> • Potential for routine use in both diagnostic and food control • User-friendly bioinformatic pipelines can extend use and acquisition of data from non-specialised laboratories • Support hazard identification through subtype discrimination (beneficial for a more targeted risk assessment) • Identification of new sequences for microorganism detection and characterisation (prediction of virulence, host association, stress resistance, etc.) in association with other omics • Potential for new approaches integrating these data into risk assessment (hazard identification, characterisation and exposure assessment) to support decision making 	<ul style="list-style-type: none"> • May detect new or re-emerging infectious agents • Potential to study microorganisms dynamics within complex microbial communities
Threats	<ul style="list-style-type: none"> • The lack of VBNC detection may lead to underestimation of prevalence and to underestimation of exposure 	<ul style="list-style-type: none"> • The lack of VBNC detection may lead to underestimation of contamination levels and to underestimation of exposure 	<ul style="list-style-type: none"> • The lack of discriminative power may lead to false epidemiological associations 	<ul style="list-style-type: none"> • The insufficient standardisation in data analysis may lead to inconsistent results 	<ul style="list-style-type: none"> • May report signals from non-viable organisms, leading to an overall inaccurate interpretation of results

Abbreviations: DR, dose response; ISO, International Organization for Standardization; PCR, polymerase chain reaction; PH, public health; TDH, thermostable direct haemolysin; TRH, TDH-related haemolysin; VBNC, viable but non-culturable; Vc, *V. cholerae*; Vp, *V. parahaemolyticus*; Vv, *V. vulnificus*; WGS, whole genome sequencing.

In particular, conventional cultural methods provide the advantage of standardisation, which allow for an improved comparability of data in surveys on *Vibrio* occurrence and concentrations, though it should be noted that full harmonisation and intercomparison of *Vibrio* methods (including evaluation of LOD and LOQ estimates) is still needed for interpreting and aggregating occurrence data. Besides, quantitative approaches for routine application in monitoring are missing for some of the species of PH relevance, limiting the production of quantitative data to integrate in exposure estimates of classical QMRAs. It should also be considered that, while the ability to provide isolates for further characterisation is a relevant feature of cultural methods, the culture and isolation process may disadvantage less common strains, as the pathogenic ones, therefore leading to an underestimation of their risk. On the other side, molecular methods, and in particular WGS and NGS/WGS methods, may provide a significant and variegated contribution to risk assessment (extensively reviewed in EFSA BIOHAZ Panel, 2019). Beside their application in outbreak investigation and source attribution, they can contribute to the hazard characterisation by identifying molecular markers associated with specific phenotypes, or by supporting the definition of strain/virulence-related DR curves (Pouillot et al., 2024). Furthermore, the omics methodologies can be particularly helpful to investigate the variability of vibrios in relation with virulence/AMR, behaviour in food, in the environment and in animal reservoirs (Rantsiou et al., 2018) and may provide, with metagenomics, a shift from studying microorganisms dynamics as a standalone towards studying them within the dynamics of the microbial communities.

3.3.4 | Concluding remarks

- Standardised microbiological methods are available for the detection of *V. parahaemolyticus*, *V. cholerae*, *V. vulnificus* in seafood and for the quantification of *V. parahaemolyticus* and *V. vulnificus*. These methods rely on microorganisms' culture and include molecular tests for species identification and/or for the detection of genes associated with pathogenicity. Serological tests are included in some cases for the identification of *V. cholerae* O1 and O139.
- Performance characteristics of detection and quantification methods of *Vibrio* spp. are only reported for ISO methods. Most studies investigating environmental matrices and food samples report FDA-BAM and ISO methods. However, a comparison of the performance of these methods has not been performed.
- To address some of the limitations of the standardised microbiological methods, a variety of different methods have been developed, including alternative approaches for detection, quantification and identification (e.g. LAMP-PCR, quantitative, digital and viability PCR, MALDI-TOF MS).
- Among methods for the characterisation of *Vibrio* species, serotyping and PFGE, have been progressively replaced by sequencing technologies. MLST schemes are available for all *Vibrio* spp. of PH relevance while (cg)MLST schemes are available for *V. parahaemolyticus*, non-O1/non-O139 *V. cholerae*. WGS has been progressively more applied for full characterisation of *Vibrio* isolates in clinical surveys, research activities and outbreak investigations, but its use in official food control activities has been limited.
- The available analytical methods contribute to different elements of risk assessment:
 - culture-based methods provide harmonised data on occurrence and levels of *Vibrio* spp. in seafood products for exposure estimates, support the isolation of strains for further characterisation and, being easy and affordable, can be extensively applied in widely different contexts;
 - WGS, in association with other omics as transcriptomic, proteomic and metabolomic, contribute to hazard characterisation through the identification of molecular markers associated with phenotypic traits, the investigation of species variability in relation to virulence/AMR and behaviour of the microorganism in food and in the environment, and by supporting outbreak investigation and source-attribution studies;
 - metagenomics provides information on microbial populations (including non-culturable strains and/or difficult to cultivate species) and may support the study of microorganisms' dynamics within complex microbial communities.
- The main limitations of the current analytical methods which affect risk assessment are:
 - for culture-based methods: the lack of intercomparison of standard methods' performance, the absence of systematic characterisation of pathogenic strains within the methods, the possible underestimation of prevalence and contamination levels and thus, exposure due to underdetection of pathogenic strains and of microorganisms in the VBNC state;
 - for NGS/WGS approaches: the complexity of data production and metadata sharing, the insufficient standardisation in data analysis which may lead to inconsistencies and, for metagenomics, the absence of isolates for further characterisation, the absence of phenotypic information and the reporting of signals from non-viable microorganisms.

3.4 | Pathogenicity to humans and virulence factors of the relevant *Vibrio* spp. (SQ1.5)

V. parahaemolyticus can cause acute GE in humans, including in healthy individuals after consumption of raw or undercooked fish or seafood. The main symptoms include watery diarrhoea (in the most severe cases bloody diarrhoea), abdominal pain, fever, vomiting, nausea, fatigue and headache. In healthy individuals, infection is normally self-limiting, lasting

1–10 days. In subjects with underlying clinical conditions (e.g. immunocompromised), the infection can occasionally result in septicaemia (Ceccarelli et al., 2019). GE due to *V. parahaemolyticus* occurs as outbreaks or sporadic cases caused by different serotypes. However, two transcontinental expansions of the pathogen have been reported over the last two decades. The pandemic clonal complex 3 (CC3) [or sequence type 3 (ST3)], which was most often identified as serotype O3:K6, has caused most of the disease globally since 1996. The recent CC36 (or ST36, identified as serotype O4:K12), which was restricted to the Pacific Northwest region of the USA and Canada until its detection in the northwest of Spain in association with a large outbreak in August 2012 (Han et al., 2019; Martinez-Urtaza et al., 2017).

V. vulnificus can infect a wide variety of hosts, including humans and different fish species, and it is considered zoonotic as it can be transmitted from infected fish to humans. Humans can be infected with *V. vulnificus* by eating raw seafood or can develop severe wound infections after contact with seawater, both of which can lead to sepsis. The progression of the infection to sepsis depends on the infected patient's health status and gender (Baker-Austin & Oliver, 2018; Merkel Sandra et al., 2001). In subjects with no predisposing condition, the disease is usually self-limiting and rarely leads to septicaemia, while the infection can lead to death by sepsis in individuals with underlying conditions (Ceccarelli et al., 2019). The most frequently documented risk factors are chronic liver diseases, haemochromatosis, diabetes mellitus, malignancies and biliary tract diseases (Hernández-Cabanyero & Amaro, 2020). In the USA, the mortality rate associated with *V. vulnificus* infection is ~33%, and *V. vulnificus* causes > 95% of seafood-related deaths (Walter, 2023). CDC data suggest that, after eating raw oysters, people with liver disease are 80 times more likely to become ill with a *V. vulnificus* infection and 200 times more likely to die from this infection than people without liver disease (Hlady et al., 1993). The species is highly variable and is divided into 5 phylogenetic lineages plus one pathovar (pv. piscis), which groups all the strains harbouring the fish virulence plasmid (pFv) (Carmona-Salido et al., 2021). Regardless of lineage, however, a single strain or clone can be virulent or avirulent to human. A subcutaneously inoculated iron-dextran treated mouse model has been used to evaluate virulence potential (skin infection versus systemic infection and death) in *V. vulnificus* (Lydon et al., 2021). There have been several unsuccessful attempts to find a genetic marker for pathogenicity by comparing environmental and clinical strains. Analysing the genome of 80 isolates from different sources, nearly 90% of the virulence-related genes described are found in the core genome of the species. This suggests that all *V. vulnificus* strains should be considered potentially pathogenic for humans (Roig et al., 2018).

V. cholerae is classified according to its major surface antigen, the O antigen, into ~200 O-serogroups, however, only 2 serogroups, O1 and O139, are strongly associated with cholera toxin (CTX) and toxin-co-regulated pilus (TCP) production, related to pandemic cholera. Unlike O1 and O139 strains, non-O1/non-O139 *V. cholerae* strains are, with few exceptions, generally devoid of CTX and TCP and are not associated with epidemic cholera. Since both CTX and TCP are encoded within the genome of a filamentous bacteriophage, it has been hypothesised that non-O1/non-O139 *V. cholerae* strains can occasionally acquire genes for toxin production by transduction or other horizontal gene transfer (HGT) mechanisms (Vezzulli et al., 2020). Nevertheless, non-O1/non-O139 *V. cholerae* may produce other toxins and are generally associated with self-limited GE or mild extraintestinal symptoms (Ceccarelli et al., 2019). In susceptible host, non-O1/non-O139 *V. cholerae* members can cause exfoliated wound infections, tissue necrosis or sepsis, and high mortality (Igere et al., 2022).

Pathogenic *Vibrio* species possess a wide array of VFs that will allow the colonisation and spread in the host. The virulence of these pathogens is multifactorial, i.e. several of the VFs interact to produce damage in the host. Some factors may be present in all species, while others are specific to certain species or strains within one species. The VFDB was consulted (see Section 2.2.4) to extract a list of the main VFs of *V. cholerae* O395, *V. parahaemolyticus* RIMD 2210633 and *V. vulnificus* CMCP6 and YJ016,⁴⁴ and then complemented by literature search.

Among the **VFs present in all pathogenic species** are factors associated with motility, attachment and colonisation, such as the capsule, flagellum and adhesins. The **capsule** has been described as essential for a successful infection, colonisation of intestine cells or resistance to the phagocytosis (Johnson, 2013; Jones & Oliver, 2009; Letchumanan et al., 2014), and may characterise different impact within the species (Bian et al., 2020; Pettis & Mukerji, 2020). **Flagellar motility** is a highly regulated processes linked to host colonisation, chemotaxis, adhesion, biofilm formation and virulence in vibrios (Khan et al., 2020). Other types of appendages are the **pili**, among which a special subfamily of pili, termed Type IV pili, can bind to specific receptors of the epithelium and other types of cell tissue of different mammals, giving them a role in invasion and colonisation. Other factors required for infection are related with the nutrient acquisition as vibrios must produce iron uptake systems in iron limited environment as the human gut (Johnson, 2013; Roig et al., 2018). Numerous studies have been carried out to understand the regulatory systems controlling expression of virulence genes in *Vibrio* spp. These processes are regulated by well characterised bacterial regulatory systems like quorum sensing (QS), virulence regulators ToxR and ToxT or the ferric uptake regulator (Fur) among others (Hernández-Cabanyero & Amaro, 2020; Midgett & Kull, 2023).

⁴⁴Genome sequences available in VFDB, often corresponding to the first genome obtained for *V. cholerae* O395 (Feng, L., Reeves, P. R., Lan, R., Ren, Y., Gao, C., Zhou, Z., Ren, Y., Cheng, J., Wang, W., Wang, J., Qian, W., Li, D., & Wang, L. (2008). A recalibrated molecular clock and independent origins for the cholera pandemic clones. *PLOS ONE*, 3(12), e4053. <https://doi.org/10.1371/journal.pone.0004053>), *V. parahaemolyticus* RIMD 2210633 (Makino, K., Oshima, K., Kurokawa, K., Yokoyama, K., Uda, T., Tagomori, K., Iijima, Y., Najima, M., Nakano, M., Yamashita, A., Kubota, Y., Kimura, S., Yasunaga, T., Honda, T., Shinagawa, H., Hattori, M., & Iida, T. (2003). Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of *V. cholerae*. *Lancet*, 361(9359), 743–749. [https://doi.org/10.1016/s0140-6736\(03\)12659-1](https://doi.org/10.1016/s0140-6736(03)12659-1), *ibid.*) and *V. vulnificus* CMCP6 (Kim, Y. R., Lee, S. E., Kim, C. M., Kim, S. Y., Shin, E. K., Shin, D. H., Chung, S. S., Choy, H. E., Progulsk-Fox, A., Hillman, J. D., Handfield, M., & Rhee, J. H. (2003). Characterisation and pathogenic significance of *Vibrio vulnificus* antigens preferentially expressed in septicemic patients. *Infect Immun*, 71(10), 5461–5471. <https://doi.org/10.1128/iai.71.10.5461-5471.2003>, *ibid.*) and YJ016 (Chen, C. Y., Wu, K. M., Chang, Y. C., Chang, C. H., Tsai, H. C., Liao, T. L., Liu, Y. M., Chen, H. J., Shen, A. B., Li, J. C., Su, T. L., Shao, C. P., Lee, C. T., Hor, L. I., & Tsai, S. F. (2003). Comparative genome analysis of *Vibrio vulnificus*, a marine pathogen. *Genome Res*, 13(12), 2577–2587. <https://doi.org/10.1101/gr.1295503>).

The outcome of *Vibrio* infections is determined both by bacterial and host factors. This includes the specific virulence genes of the pathogen, the ability of the host to modulate an adequate immune response, and the ability of the host to eliminate the pathogen. This relationship between pathogen and host is especially important for *V. vulnificus* or *V. cholerae* infections since certain predisposing factors of the host are determining factors for the onset of symptoms.

Clinical strains of *Vibrio* isolated from patients have a variety of VFs. Only for *V. parahaemolyticus*, some genes are considered well-established pathogenicity markers. In fact, TDH and TRH are currently the most predictive indicators of pathogenicity, and most *V. parahaemolyticus* infections are associated with strains that possess these genes, although some papers reported the detection of *tdh*–/*trh*– strains among clinical isolates (Baker-Austin et al., 2020; Jones et al., 2012). However, in these cases, no direct evidence on the pathogenicity of these strains or their role in causing the infections was available. For *V. vulnificus*, several attempts to find a good genetic virulence marker were unsuccessful. Therefore, the WHO considers all *V. vulnificus* isolates as potentially pathogenic for humans (FAO and WHO, 2021). Non-O1/non-O139 *V. cholerae* is a very variable group of strains that continues to be very little studied, hence a genetic marker that can be used to determine the pathogenicity of an isolate has not yet been identified.

As mentioned, beyond the previously described VFs common to more than one species (i.e. capsule, flagella, etc.), vibrios have a large repertoire of VFs that include, among others, proteases, cytolysins and different toxins. A summary of the most relevant VFs specific of *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* non-O1/non-O139, their coding genes and mode of action is presented in [Table 6](#).

TABLE 6 Virulence factors specific of *V. parahaemolyticus*, *V. vulnificus* and non-O1/non-O139 *V. cholerae*.

Virulence factor	Presence	Gene(s)	Mode of action	Reference
<i>V. parahaemolyticus</i>				
Thermostable direct haemolysin (TDH)	Pathogenic strains	<i>tdh</i>	<p>This protein exhibits enterotoxigenic activity, cytotoxicity, cardiotoxicity and increased vascular permeability.</p> <p>Clinical strains of Vp isolated from ill patients usually produce a clear beta-type haemolysis on Wagatsuma agar due to production of TDH. This phenomenon is called the Kanagawa phenomenon (KP). Only a small proportion of the environmental strains show a positive KP (< 1%). Therefore, TDH encoded by the <i>tdh</i> gene has been considered a major VF of Vp.</p> <p>Five sequence variants of the <i>tdh</i> gene (<i>tdh1</i> to <i>tdh5</i>) can be distinguished, which are > 97% identical. Only <i>tdh4</i> is codified in a plasmid. All KP-positive Vp strains possess <i>tdh1</i> and <i>tdh2</i>, and both are encoded in the 80 kb pathogenicity island Vp-PAI. The expression level of <i>tdh2</i> is higher than the rest, this protein being the main responsible for the KP. Some KP-negative isolates are <i>tdh</i>-positive strains (around 48%) as they possess the chromosomal <i>tdh3</i> and <i>tdh5</i> genes, and the plasmid-borne <i>tdh4</i> genes. These strains produce TDH toxin at a lower level, so that the KP effect is not appreciated.</p>	Cai and Zhang (2018), Gavilan et al. (2021)
TDH-related haemolysin (TRH)	Pathogenic strains	<i>trh</i>	<p>Strains carrying the <i>trh</i> gene code for TRH, which is a heat labile toxin, with immunological, haemolytic and toxicity properties similar to TDH. TRH was judged to be associated with reported illnesses and thus considered to be another important VF of Vp. There are two sequence variants of the <i>trh</i> gene (<i>trh1</i>–<i>trh2</i>).</p>	Gavilan et al. (2021)
Type III Secretion System (T3SS)	T3SS2, located on Vp-PAI, pathogenic strains	Gene apparatus including translocon complex genes and effectors genes	<p>These secretion systems are multicomponent nanomachines that enable Gram-negative bacteria the delivery of proteins called ‘effector’ into the host cell and manipulate host cellular processes.</p> <p>Unlike T3SS1 which is present in all isolates of the species, the T3SS2 genes are present in pathogenic strains on pathogenicity island Vp-PAI on chromosome 2. T3SS2 can deliver up to 10 effector proteins that, in this case, are able to control the actin cytoskeleton dynamics and the innate inflammatory responses. Two distinct lineages (T3SS2α and T3SS2β) have been reported, correlating with the presence of <i>tdh</i> and <i>trh</i> genes, respectively.</p>	Caburlotto et al. (2009), Ceccarelli et al. (2019), Okada et al. (2009)
<i>V. vulnificus</i>				
Haemolysin/cytolysin	All strains	<i>vvhA</i>	<p>VvhA is a pore-forming toxin conferring strong haemolytic activity to Vv. It triggers apoptosis, necrotic cell death and autophagy by dysregulation of host cell signalling, leading to host tissue damage. Also, injection of purified VvhA induces proinflammatory cytokine and inflammatory chemokines in a mouse model, which presumably results in septic shock.</p>	Kaus et al. (2014), Yuan et al. (2020)

(Continues)

TABLE 6 (Continued)

Virulence factor	Presence	Gene(s)	Mode of action	Reference
Metalloproteinase	All strains	<i>vvp</i>	This protease has been shown to increase vascular permeability and oedema through the generation of bradykinin. Furthermore, activation of prothrombin and fibrinolysis by Vvp results in clotting and protects the bacteria from the host immune defences. Both activities support the dissemination of Vv from the intestines to the blood stream leading to sepsis. Vvp can also facilitate iron uptake by Vv by digesting haem proteins, transferrin and lactoferrin. Vvp was thought to be important in bacterial growth and disease development. Nevertheless, a Vvp-deficient mutant has been shown to be even more virulent than the wild-type strain, probably because of overexpression of the cytolysin in the absence of Vvp.	Li and Wang (2020), Shao and Hor (2001)
Multifunctional autoprocessing repeats-in-toxin (MARTX) toxin	All strains	<i>rtxABCD</i>	RtxA belongs to the MARTX toxins, which are modular proteins of high molecular weight with conserved external modules that form pores in the eukaryotic membrane, through which the toxin's central module composed by different effector domains is translocated. Seven distinct toxin variants have been identified in Vv according to the repertoire and organisation of the effector domains. The effector content can vary from 2 to 5 effectors selected from a total of nine known effector domains. RtxA has been involved in the programmed necrotic death, changes of cytoskeleton structure, actin aggregation of host cells, production of reactive oxygen species and pore formation in the host cell membrane. In the murine infection model is the primary virulence factor for Vv.	Gavin and Satchell (2015), Kling et al. (2022)
Neuraminidase	All strains	Nan locus	Vv can utilise <i>N</i> -acetylneuraminic acid as a nutrient during infection, adherence to epithelial cells and intestinal colonisation. Mutants in some of the genes of the nan locus have been proved to have an attenuated virulence in murine infection model	Jeong et al. (2009), Kim and Kim (2023)
Non-O1/non-O139 <i>V. cholerae</i>				
Type III Secretion System (T3SS)	Unknown/unclear ^a	Genes encoding the T3SS structural proteins (<i>VcsRTCNS2</i> , <i>VcsVUQ2</i> , <i>VcsJ2</i> and <i>VspD</i>), the translocated proteins (Vops) and the T3SS transcriptional regulators (<i>VttR_A</i> and <i>VttR_B</i>) (in strain AM-19226)	The Vc T3SS is most closely related to the Vp T3SS2. In strain AM-19226 (Vc O39) T3SS function is important for colonisation. The genome of this strain codifies 12 different effectors proteins, some of them with critical role in colonisation and others that could be essential for adherence to eukaryotic cells.	Chaand et al. (2015), Schirmeister et al. (2014), Shin et al. (2011)
Haemagglutinin protease ([HA]/protease)	Unknown/unclear	<i>hapA</i>	[HA]/protease is a versatile metalloprotease that can increase the severity of a cholera infection by multiple mechanisms; produce inflammation and caused morphological changes in the small intestine. It enhances fluid accumulation in the rabbit ileal loop model. When purified protein was injected in this animal model a dose-dependant haemorrhagic fluid response accompanied with significant tissue damage was observed.	Benitez and Silva (2016)

TABLE 6 (Continued)

Virulence factor	Presence	Gene(s)	Mode of action	Reference
MARTX toxin of <i>Vibrio cholerae</i> (MARTX _{Vc})	All strains	<i>rtxABCD</i>	All Vc strains have a <i>rtxA</i> gene except for classical strains. Vc has a MARTX toxin that delivers three effectors by autoprocessing: an actin crosslinking domain that introduces an isopeptide bond between actin molecules, making the actin unable to assemble an actin cytoskeleton; a Rho-inactivation domain that induces actin depolymerisation through loss of active RhoA; and an alpha-beta hydrolyse enzyme that has not yet been characterised. The MARTX _{Vc} effector domains simultaneously promote virulence and suppress inflammatory responses.	Dolores and Satchell (2013), Woida and Satchell (2020)
Neuraminidase	Unknown/unclear	Nan locus	Catabolism of <i>N</i> -acetylneuraminic acid. These genes provide the capacity to utilise sialic acid as a carbon and energy source, an advantage in the mucus-rich environment of the gut.	Ceccarelli et al., (2019)
Type VI Secretion System (T6SS)	Unknown/unclear	IAHP (IcmF associated homologous proteins) gene cluster, together with other virulence-associated secretion (VAS), Hcp and VgrG genes	T6SS is a specialised transport apparatus which is capable of translocating proteins to a target cell that could be eukaryotic or prokaryotic. In bacteria, immunity genes are the best-characterised defences, protecting against specific cognate effectors. In Vc the immunity to antibacterial T6SS effector function is necessary for host colonisation. Some non-O1/non-139 Vc strains possess a constitutively active T6SS and have been linked to outbreak of GE, although its contribution to disease still needs to be more systematically evaluated.	Ceccarelli et al. (2015), Fu et al. (2013), Ho et al. (2014)
Toxin-coregulated pilus	Unknown/unclear	<i>tcp</i>	TCP is a Type IV bacterial pilus that is required for colonisation of the intestinal tract. It is not associated with epidemic cholera.	Ghosh et al. (1997)
Haemolysin/cytolysin A	All strains	<i>hlyA</i>	Haemolysin A (HlyA) is an important virulence factor that belongs to the pore-forming toxin family. HlyA has vacuolar and cytotoxic activity in many cell lines and is associated with rapid mortality in mice.	Gutierrez et al. (2007)
Heat-stable enterotoxin (NAG-ST)	Unknown/unclear	<i>st (stn/sto)</i>	Heat-stable enterotoxin is a 17 amino acid peptide. ST acts in a manner similar to the <i>Escherichia coli</i> toxin STa; the toxin will increase in the intracellular concentrations of cyclic guanosine monophosphate. The intracellular increase in cyclic guanosine monophosphate results in a stimulation of chloride secretion and net fluid secretion, resulting in diarrhoea.	Hodges and Hecht (2012)

Abbreviations: [HA]/protease, haemagglutinin protease; GE, gastroenteritis; IAHP, IcmF-associated homologous proteins; KP, Kanagawa phenomenon; MARTX, multifunctional autoprocessing repeats-in-toxin; NAG-ST, heat-stable enterotoxin; PAI, pathogenicity island; PAI, pathogenicity island; RID, Rho-inactivation domain; TCP, Toxin-coregulated pilus; TDH, thermostable direct haemolysin; TRH, TDH-related haemolysin; VAS, virulence-associated secretion; Vc, *V. cholerae*; Vp, *V. parahaemolyticus*; Vv, *V. vulnificus*.

^aComparison of non-O1/non-O139 *V. cholerae* isolates from ear/wound infections and from diarrheal patients in Germany showed the presence of T3SS exclusively in the latter strains (Schirmeister et al., 2014).

3.4.1 | Concluding remarks

- Pathogenic species of the genus *Vibrio* possess a wide array of VFs that allow the colonisation and spread in the host. The virulence of these pathogens is multifactorial, i.e. several VFs interact to produce damage in the host. Although with different impact, some of these factors may be present in all species (i.e. the capsule, flagellar mobility and pili), while others are specific to certain species or strains within one species.
- The severity of infections with *Vibrio* species is determined both by bacterial (specific virulence genes) and host factors (predisposing conditions and immune response).
- *V. parahaemolyticus* mainly leads to acute GE, also in healthy individuals.
 - The pathogenicity in humans is significantly associated with two haemolysins, TDH and TRH, and these factors are considered well-established pathogenicity markers, but strains lacking their coding genes (*tdh* 1–5, *trh* 1–2) can be occasionally detected in clinical specimens.
 - The presence of a type 3 secretion system (T3SS2), located on pathogenicity island Vp-PAI, has been associated with pathogenic strains.
 - *V. parahaemolyticus* infections occur as either outbreaks or sporadic cases caused by different serotypes/sequence types. Two transcontinental expansions of specific serotypes/sequence types (ST3 and ST36) of *V. parahaemolyticus* have been reported from the late 1990s.
- *V. vulnificus* infections are relatively rare and affect mainly susceptible individuals with underlying conditions. In those patients the disease can lead to sepsis and possibly death.
 - This species possesses several VFs, including haemolysins (VvhA), protease (Vvp), multifunctional autoprocessing repeats-in-toxin (MARTX) toxin and neuraminidases.
 - Reliable discrimination of pathogenic from non-pathogenic strains has not yet been achieved and therefore, to date, all *V. vulnificus* strains are considered potentially pathogenic.
- Non-O1/non-O139 *V. cholerae* is generally associated with self-limited GE or mild extraintestinal symptoms. However, in susceptible individuals, strains can cause more severe infections, sepsis and death.
 - The most important VFs described for this group of microorganisms are T3SS, MARTX toxin, [HA]/protease and neuraminidase.
 - Despite the number of studies published for the *V. cholerae* species, few of them specifically addressed non-O1/non-O139 *V. cholerae*, and no reliable genetic marker associated with pathogenicity has been identified so far.

3.5 | Antimicrobial resistance in the relevant *Vibrio* spp. isolated from seafood and from seafood-borne infections (SQ1.6–1.7)

AMR is defined as the inability or reduced ability of an antimicrobial agent to inhibit the growth of a bacterium, which, in the case of a pathogenic organism, could lead to therapy failure (EFSA and ECDC, 2022). *Vibrio* bacteria are known to acquire AMR by mutations within the bacteria and by uptake of AMR genes through HGT. Various mobile/mobilisable genetic elements were found in vibrios that facilitate the dissemination of AMR genes, e.g. conjugative plasmids, transposons, integrative conjugative elements (ICE), integrons and insertion elements (Das et al., 2020; Dutta et al., 2021; Escudero & Mazel, 2017; Kaushik et al., 2019; Kitaoka et al., 2011).

The presence of antimicrobial-resistant potentially pathogenic bacteria in food can directly threaten consumers through infections that may be resistant to antibiotic treatment. Additionally, these bacteria may transfer AMR genes to the microbiota of consumers (Brisabois et al., 2019). In a recent EFSA opinion on the role of the food-producing environment in AMR transmission (EFSA BIOHAZ Panel, 2021), *Vibrio* were considered as Group 2 bacteria (i.e. environmental or commensal bacteria that could act as donor of resistance genes (e.g. to Gram-negative pathogenic bacteria) to last-resort antibiotics). It was noted that the human disease burden resulting from antimicrobial-resistant indigenous aquatic or fish bacteria has been insufficiently studied. Moreover, insufficient information on the lineages or serotypes able to cause infection and on the AMR profiles of aquatic and fish indigenous bacteria belonging to *V. parahaemolyticus* and *V. vulnificus* precludes their current inclusion in Group 1 (bacteria causing infection resistant to antimicrobials of choice for the treatment of serious infections or to last-resort antibiotics).

As gastrointestinal infections caused by *Vibrio* spp. are normally self-limiting (see Section 3.4), treatment with antimicrobials is not recommended (CDC⁴⁵). Only in cases of severe infections or in individuals with underlying medical conditions antimicrobial treatment should be applied. Long-term surveillance data from the US (period 1990 to 2010) shows that two

⁴⁵<https://www.cdc.gov/vibrio/diagnosis.html>.

thirds of patients with *Vibrio* spp. illnesses were prescribed antibiotics – in the order quinolones (56.1%), cephalosporins (24.1%), tetracyclines (23.5%) and penicillins (4.3%) (Wong et al., 2015).

Antimicrobials are intensively used in aquaculture systems (Deekshit et al., 2022) and several diseases of farmed animals are caused by *Vibrio* species, with *V. parahaemolyticus* and *V. vulnificus* as important pathogens (Sony et al., 2021). For this reason, *Vibrio* bacteria isolated from aquaculture systems show considerable AMR patterns (Vaiyapuri et al., 2021). Furthermore, as 70% of the EU demand of aquaculture products is met through import (EUMOFA, 2023), use of antimicrobials in aquaculture in countries exporting seafood to the EU pose a threat for the European consumer (Brisabois et al., 2019). Antimicrobial compounds in aquaculture systems worldwide were used in 11 of the 15 major producing countries between 2008 and 2018, with in total 67 compounds used, and oxytetracycline, sulfadiazine and florfenicol as the most frequently used (Lulijwa et al., 2020). In a meta-analysis on antibiotic-resistant bacteria from aquatic food animals of Asia, among the five most detected seafood bacterial species (one of which was *Vibrio* spp.), resistance was highest to penicillins (60.4%), macrolides (34.2%), sulfonamides (32.9%) and tetracyclines (21.5%). Furthermore, resistance to critically important antimicrobials for human medicine (CIAs⁴⁶) – now referred to as medically important antimicrobials (MIAs⁴⁷) (WHO, 2024) – was found in these five species (Schar et al., 2021). Resistance to some compounds in the reserve group of last-resort antimicrobials for human medicine was also observed and varied by pathogen, e.g. in case of *Vibrio* spp. polymyxin B resistance was 39.1% (95% CI: 33–44) and colistin resistance was 42.7% (95% CI: 38–47) (Schar et al., 2021).⁴⁸

3.5.1 | Reported antimicrobial resistances in *V. parahaemolyticus*, *V. vulnificus* and non-O1/non-O139 *V. cholerae* isolated from seafood and from seafood-borne infections in Europe

The EU monitoring of AMR in zoonotic and commensal bacteria from food-producing animals and food thereof does not include *Vibrio* spp. (EFSA and ECDC, 2022). EFSA (2019) proposed to perform baseline surveys (BLSs) on AMR in bacteria from aquaculture and/or (imported) seafood, targeting selected bacteria species depending on the matrix and objective, among which *Vibrio* spp. were listed. As a follow-up, EFSA proposed and provided technical and scientific support for the development of a BLS on the occurrence of AMR in bacteria isolated from EU/EFTA aquaculture productions that will target for marine aquaculture production: *Aeromonas* spp. from salmon and seabass; for freshwater aquaculture production: *Aeromonas* spp. from trout, and for shellfish production, *E. coli*, ESBL-producing *E. coli*, enterococci (*E. faecium* and *E. faecalis*) and *V. parahaemolyticus* and *V. alginolyticus* from mussels (EFSA, in press).

Annex E provides an overview of recent European studies on the AMR of the relevant *Vibrio* spp. Nearly all studies, both for clinical and food isolates, used the clinical breakpoints of the Clinical and Laboratory Standards Institute (CLSI). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has recently developed clinical breakpoints for certain antimicrobials for five *Vibrio* species, including *V. alginolyticus*, *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* (Karatuna et al., 2024). Epidemiological cut-off (ECOFF), which are used for epidemiological surveillance and monitoring resistance trends in bacterial populations are available, from EUCAST for certain antimicrobials for *V. cholerae*,⁴⁹ whereas they are under development for other *Vibrio* spp.

The AMR phenotype of ***Vibrio* clinical isolates** was investigated by the National Reference Laboratory (NRL) for vibrios in France. In total 72% ($N=72$) of the non-O1/non-O139 *V. cholerae* isolates were from gastrointestinal infections, of whom several were travel associated. No *V. vulnificus* isolates from food-borne infections were reported, while the overwhelming majority of *V. parahaemolyticus* isolates (96%; $N=106$) stemmed from gastrointestinal infections. In clinical isolates of *V. parahaemolyticus* and *V. cholerae* a high prevalence of resistance against polymyxin B (73% in *V. parahaemolyticus*, 89% in *V. cholerae*) and colistin (71% in *V. parahaemolyticus* and 91% in *V. cholerae*) was observed. Many clinical *V. parahaemolyticus* isolates also showed resistance to ampicillin (42%) and sulfonamides (31%). Clinical non-O1/non-O139 *V. cholerae* were also resistant to sulfonamides (39%), and sulfamethoxazole/trimethoprim (15%). An Italian study reporting phenotypic data on AMR of nine clinical *V. cholerae* isolates, showed that all isolates were resistant to colistin and 88% showed intermediate resistance to erythromycin. Resistance to nalidixic acid (22%), some aminoglycosides and β -lactams was also found (e.g. 55% of strains were resistant/intermediate to ampicillin) (Ottaviani et al., 2018).

Most AMR data on ***Vibrio* isolates from seafood** in the EU are on *V. parahaemolyticus*. In seven studies, the prevalence of ampicillin resistance of *V. parahaemolyticus* was between 70% and 100%, and resistance against streptomycin was

⁴⁶CIAs, according to WHO. (2019). *Critically important antimicrobials for human medicine, 6th rev.* https://cdn.who.int/media/docs/default-source/gcp/who-mia-list-2024-lv.pdf?sfvrsn=3320dd3d_2, are antimicrobial classes which meet both criteria C1 (the antimicrobial class is the sole, or one of limited available therapies, to treat serious bacterial infections in people) and C2 [the antimicrobial class is used to treat infections in people caused by either (1) bacteria that may be transmitted to humans from non-human sources or (2) bacteria that may acquire resistance genes from non-human sources].

⁴⁷Antimicrobial authorised for use in human medicine and therefore listed on the WHO CIA List. Medically important antimicrobials are categorised on the WHO CIA List, according to specific criteria, as either critically important, highly important or important for human medicine.

⁴⁸Resistance to polymyxin B/colistin is intrinsic in *V. vulnificus* and non-O1/non-O139 *V. cholerae* and has been used in an enrichment medium for these two species adding the two antimicrobials Massad, G., & Oliver, J. D. (1987b). New selective and differential medium for *Vibrio cholerae* and *Vibrio vulnificus*. *Appl Environ Microbiol*, 53(9), 2262–2264. <https://doi.org/10.1128/aem.53.9.2262-2264.1987>. The resistance is based on modification of the bacterial LPS decreasing its negatively charged surface and involves the *alm* operon (*almG* gene encoding a glycytransferase, Henderson, J. C., Herrera, C. M., & Trent, M. S. (2017). *AlmG*, responsible for polymyxin resistance in pandemic *Vibrio cholerae*, is a glycytransferase distantly related to lipid A late acyltransferases [Article]. *Journal of Biological Chemistry*, 292(51), 21205–21215. <https://doi.org/10.1074/jbc.RA117.000131>) and other LPS modifying genetic determinants Herrera, C. M., Henderson, J. C., Crofts, A. A., & Trent, M. S. (2017). Novel coordination of lipopolysaccharide modifications in *Vibrio cholerae* promotes CAMP resistance. *Mol Microbiol*, 106(4), 582–596. <https://doi.org/10.1111/mmi.13835>. Not all isolates express the resistance.

⁴⁹<https://mic.eucast.org/> accessed on 19 April 2024.

> 30% (up to 68%) in five studies. Colistin resistance of *V. parahaemolyticus* was only examined in two studies. In one study (Ottaviani et al., 2013), resistance was detected in > 90% of all isolates, whereas in the other (Castello et al., 2022) ~25% of the isolates showed increased tolerance to this compound. A genotypic study of *V. parahaemolyticus* isolates from retail prawns revealed the presence of resistance genes against aminoglycosides, β -lactams, folate pathway inhibitors, phenicols, quinolones, sulfonamides, tetracyclines and rifamycins (Janecko et al., 2021), but it remains unclear if the presence of these genes resulted in a reduced susceptibility of the isolates, as other studies reported that AMR phenotype and genotype often do not match (Lepuschitz et al., 2019; Lou et al., 2016; Zhang et al., 2023).

A meta-analysis comprising worldwide studies assessed the prevalence of AMR phenotypes of *V. parahaemolyticus* isolates from bivalves (Albini et al., 2022). Whereas for Asia and Africa enough studies were available to estimate the prevalence, for Europe such analysis was not possible. Hence, the genomes of 152 *V. parahaemolyticus* strains isolated in Europe from clinical, environmental and other sources were used for screening of AMR-related genes (available in <https://doi.org/10.5281/zenodo.12514500>). Besides genes present in all European *V. parahaemolyticus* strains (*tet35* and *txr*, genes encoding proteins of efflux pumps related to tetracycline resistance, *parE*, the gene encoding a penicillin-binding-protein related to PBP3 of *Haemophilus influenzae*), numerous β -lactamase encoding genes with varying frequencies were found (most frequently *bla*_{CARB-18} in 55% of all strains, *bla*_{CARB-22} in 18%, *bla*_{CARB-21} in 9%). Resistance genes against other antibiotic classes were present at lower frequencies, e.g. *fosX* (3%), *sul2* (2%), *dfrA31* (3%).

AMR data on *V. vulnificus* seafood isolates were available from only two Italian studies, one showing low levels of resistance to cefazolin (Serratore et al., 2017), while in the other the five isolates were streptomycin resistant (Castello et al., 2022).

Few data on AMR of food-borne strains of **non-O1/non-O139 *V. cholerae*** isolated in Europe were reported. The AMR phenotype pattern was similar to that of clinical strains investigated in the study by (Ottaviani et al., 2018). All strains were colistin resistant and showed reduced susceptibility to various aminoglycosides and β -lactams. Remarkably many Italian isolates from seafood at retail (92%) displayed an intermediate resistance to the macrolide erythromycin. In another Italian study (Castello et al., 2022), resistance against β -lactams and the combination trimethoprim/sulfamethoxazole (42%) was observed. Five non-O1/non-O139 *V. cholerae* isolates from prawns sold in the UK contained resistance genes against aminoglycosides, β -lactams, phenicols, quinolones and tetracyclines (Janecko et al., 2021). Resistance/intermediate resistance to colistin (87%), imipenem (78%) and ampicillin (11%) was most common in isolates from retail seafood in Germany (Zhang et al., 2022). AMR genes identified through WGS could explain some of the observed in vitro phenotypes, e.g. *bla*_{CARB} presence and ampicillin resistance, *almG* presence and colistin resistance, *qnrVCs* presence and quinolone (ciprofloxacin and nalidixic acid) resistance, *varG* imipenem resistance and *dfrA31* presence and trimethoprim resistance. The presence of genes encoding efflux pumps that may be able to exclude different antibiotics from the bacterial cell were identified in all 63 non-O1/non-O139 *V. cholerae* isolates, however their putative role in conferring resistance must be proven experimentally.

3.5.2 | Antimicrobial resistance of concern due to their possible horizontal transmission to other bacteria or for their emerging in the EU

Table 7 shows the genetic localisation of AMR genes observed in the relevant *Vibrio* spp. isolated from seafood. The emergence of multidrug-resistant bacteria that possess resistance to more than two different classes of antibiotics is mainly caused by HGT through mobile genetic elements (Das et al., 2020; Dutta et al., 2021). Dissemination of AMR genes (ARGs) through mobile genetic elements (MGEs) like plasmids, ICEs, integrons, transposable elements and insertion sequences has been described for vibrios. Many MGEs that have been found first in the toxigenic *V. cholerae* strains are also present in environmental and food-borne non-O1/non-O139 *V. cholerae* strains (Das et al., 2020) and in other *Vibrio* species. Conjugative *Vibrio* plasmids were the first identified MGE responsible for the horizontal transfer of ARGs. Plasmids of environmental *V. cholerae* were shown to be transferable to other Gram-negative bacteria (intergeneric transfer) or, with higher frequencies, to other strains of the same species (intraspecific transfer) (Amaro et al., 1988). Vibrios also contain integrons which are genetic elements that can capture ARGs and confer AMR to the recipient bacteria. Class 1 integrons that are present in many vibrios are a major player in the dissemination of ARGs across Gram-negative bacteria (Rapa & Labbate, 2013). In the case of *V. cholerae*, a large integron termed superintegron is located on the second chromosome. The superintegron carries ARGs but also additional other genes and turned out to be widespread in the genus *Vibrio* including *V. vulnificus* and *V. parahaemolyticus* (Escudero & Mazel, 2017; Hazen et al., 2010). ARGs of *Vibrio* spp. can be also located in transposons or just adjacent to insertion sequences. These MGEs are able to move themselves (and associated resistance genes) to new locations in the same or in different DNA molecules within a single cell (Partridge et al., 2018). The ICEs are large genomic islands and are self-transmissible by conjugation. ICEs can excise as a circular form and integrate into an attachment site of another host chromosome (Partridge et al., 2018). The first ICE was discovered in *V. cholerae* and was termed SXT as it was shown to carry ARGs conferring resistance to sulfamethoxazole, trimethoprim and streptomycin (Waldor et al., 1996). The occurrence of this element and related ICEs (now termed SXT/R391 ICE family) is widespread in *Vibrio* spp. and elements of this family are found also in other Gram-negative bacteria (Bioteau et al., 2018; Partridge et al., 2018). In a German study on 63 seafood isolates of non-O1/non-O139 *V. cholerae*, the occurrence of class 1 integrons was observed in 60% of the investigated strains (Zhang et al., 2023). Conserved sequences of the SXT/R391 elements were found in 22% of the strains. These results disclose the potential to exchange ARGs and the frequent presence of these genomic features enhances the genetic plasticity of the species.

TABLE 7 Reported genetic localisation of AMR genes in *Vibrio* isolates from seafood.

Resistance to antimicrobial agents	AMR genes	Genetic localisation	Evidence for HGT	<i>Vibrio</i> species	Food source	Reference
Polymyxins: polymyxin B/colistin	Intrinsic <i>almG</i>	Chromosomal	No	Vv; Vc non-O1/non-O139	Seafood (most isolates)	Henderson et al. (2017), Massad and Oliver (1987)
β-Lactams	Intrinsic <i>bla</i> _{CARB-47/48}	Chromosomal	No	Vp	Seafood (all isolates tested, 83 total)	Janecko et al. (2021)
Quinolones: nalidixic acid, ciprofloxacin	<i>qnrVC6</i>	Flanked by IS-elements, conjugative plasmid	Yes	Vp	Shrimps (China, retail Hong Kong)	Liu et al. (2013)
Aminoglycosides Chloramphenicol Trimethoprim	<i>aacA3</i> , <i>aadA1</i> <i>catB2</i> <i>dfrA1</i>	Integron on conjugative plasmid	Yes	Vp	Shrimps (China, retail Hong Kong)	Liu et al. (2013)
β-Lactams: carbapenems Folate pathway inhibitors Trimethoprim Sulfonamides Phenicols Aminoglycosides Tetracyclines	<i>bla</i> _{NDM-1} <i>dfrA16</i> <i>sul1</i> , <i>sul2</i> <i>floR</i> <i>strA/strB</i> , <i>aad2</i> <i>tet(A)</i>	Conjugative plasmid	Yes	Vp	Shrimps (France, imported shrimps from Vietnam)	Briet et al. (2018), Ramírez-Castillo et al. (2023)
Ciprofloxacin	<i>gyrA</i> , <i>parC</i> alleles <i>qnrVC</i> variants	Chromosomal Plasmid	No Yes	Vp	Shrimps ^a (China)	Xu, Zheng, et al. (2023)
Streptomycin Tetracycline Folate pathway inhibitors	<i>strA/strB</i> <i>tet(A)</i> <i>dfrG</i> , <i>sul2</i>	Integrative conjugative element SXT/R391	Yes	Vp	Shrimps (China)	Bioteau et al. (2018)
Polymyxins: Colistin	<i>mcr-1</i>	Conjugative plasmid	Yes	Vp	Shrimp, China	Lei et al. (2019)
Tetracyclines	<i>tet(B)</i>	Plasmid	Yes	Vp	Shrimp, Mexico	Han et al. (2015)
β-Lactams/β-Lactamase inhibitors: ampicillin, amoxicillin, clavulanic acid, cefazolin, ceftiofur, meropenem	<i>bla</i> _{VCC-1} carbapenemase	Flanked by IS elements	Likely	Vc non-O1/non-O139	Shrimps (Canada, imported from India)	Mangat et al. (2016)
Meropenem wild-type phenotype ^b	<i>bla</i> _{NDM-1}	Conjugative plasmid	Yes	Vp	Shrimps (Germany, imported from Vietnam)	Personal information Hammerl (2024)

Abbreviations: AMR, antimicrobial resistance; HGT, horizontal gene transfer; Vc, *V. cholerae*; Vp, *V. parahaemolyticus*; Vv, *V. vulnificus*.

^aMeat products were also sampled, but shrimps represented the vast majority of samples.

^bMinimum inhibitory concentration (MIC) value for meropenem below the CLSI breakpoint (4 µg/mL), but slightly increased MIC value in comparison to other wild-type *V. parahaemolyticus* isolates (0.12 µg/mL).

Monitoring of AMR in vibrios from aquaculture production should aim to analyse ARGs conferring resistance to antimicrobials that are currently used or have been used in the past. As mentioned earlier, in 11 of the major producing countries about 73% of the applied antimicrobials were oxytetracycline, sulfadiazine and florfenicol, and 55% of the countries applied sulfadimethoxine, erythromycin, amoxicillin and enrofloxacin (Lulijwa et al., 2020). Thus, monitoring of AMR should include testing resistance against tetracyclines, sulfonamides, phenicols, macrolides, quinolones and β-lactams. Another source contributing to the prevalence of AMR in vibrios is the presence of antimicrobial compounds and ARGs in estuarine and coastal waters which originate from riverine runoff, sewage discharge and wastewater treatments plants (Zheng et al., 2021). The spread of ARGs through HGT is of special concern for compounds that are classified as CIA by the WHO. Carbapenem antibiotics are assigned to this group as they are used for treating multidrug-resistant Enterobacteriaceae for which only limited therapy options are available. Also, they are used for treatment of bacteria transmitted from non-human sources (Goh et al., 2022). Carbapenems constitutes the class of β-Lactam antibiotics with the broadest spectrum

against Gram-negative and Gram-positive bacteria. Isolates of non-O1/non-O139 *V. cholerae* that possessed carbapenemase activity have been detected in seafood from the Adriatic sea (Ottaviani et al., 2018) and isolates of *V. parahaemolyticus* and non-O1/non-O139 *V. cholerae* that possessed carbapenemase activity have been detected in imported seafood (Briet et al., 2018; Hammerl, 2024; Mangat et al., 2016; Zhang et al., 2023). In the latter cases all carbapenemase genes were associated with mobile genetic elements (plasmids and IS elements), so they were likely acquired by HGT. Other carbapenemase genes were located in a class 1 integron embedded on a plasmid (Aberkane et al., 2015). Among the different mobile genetic elements, plasmids have been identified as the most common way for propagation of carbapenemase genes (Goh et al., 2022). Research has shown that carbapenemase genes are widely distributed in the aquatic environment (e.g. VCC-1 encoding gene was identified in non-O1/non-O139 *V. cholerae* strains of German coastal waters, Hammerl et al., 2017) and in bacteria from aquatic food animals and are therefore likely to be transferred into the food chain. To date, 10 types of different carbapenemases have been found in *Vibrio* species, with NDM-1 as the most prevalent type (Goh et al., 2022). Some of the identified carbapenemases, e.g. VCC-1 (Mangat et al., 2016), are novel types, so far only found in *Vibrio* species (Goh et al., 2022).

Besides carbapenemase genes also extended-spectrum- β -lactamases (ESBL)-producing *Vibrio* spp. in seafood have been reported. ESBL are a major cause of resistance to 3rd and 4th generation of cephalosporins that belong to the class of CIAs (WHO). In a meta-analysis of studies on ESBL-producing Gram-negative bacteria from different kinds of seafood (Pearce et al., 2023) it was found that *Vibrio* spp. constituted the second most common group of ESBL producers after Enterobacterales in this type of food.

3.5.3 | Concluding remarks

- Few European studies on AMR of *V. parahaemolyticus*, *V. vulnificus* or non-O1/non-O139 *V. cholerae* isolated from seafood or from food-borne infections have been conducted. Despite this, a large spectrum of AMR is reported, some of which known to be intrinsic for some species (e.g. colistin, ampicillin).
- As regards *V. parahaemolyticus*, the prevalence of ampicillin resistance/intermediate resistance was between 70% and 100% in seven of nine studies, and resistance/intermediate resistance against streptomycin was higher than 30% (up to 70%) in six of eight studies.
- Non-O1/non-O139 *V. cholerae* strains showed colistin resistance (87% to 100%, including intermediate resistance) in four studies. Ampicillin resistance varied between 4% and 75% (five studies) and streptomycin resistance between 11% and 68% in four studies (clinical resistance and intermediate resistance).
- The low number of studies and investigated strains does not allow any conclusions to be drawn on AMR of *V. vulnificus*.
- *Vibrio* bacteria possess several MGEs that contribute to the horizontal transfer of ARGs. Mobile genetic elements of vibrios involved in the dissemination of ARGs are plasmids, integrons, ICEs, transposons and IS-elements. Almost for all antibiotic classes ARGs that are associated with mobile genetic elements have been detected in *Vibrio* spp.
- ARGs conferring resistance to antimicrobials used in aquaculture systems (e.g. tetracyclines, sulfonamides, phenicols) are of great concern, as they spread quickly within the microbiota of this environment and can enter the food chain. The dissemination of ARGs can be also promoted by the anthropic impact in estuarine and coastal waters.
- Resistance against carbapenems and 3rd/4th generation cephalosporins (MIAs categorised as authorised only for humans and highly important antimicrobials, respectively) associated with carbapenemases and ESBLs encoding genes in mobile elements are increasingly found in *V. parahaemolyticus*, *V. vulnificus* and non-O1/non-O139 *V. cholerae* and their detection in imported seafood has been reported.

3.6 | Persistence mechanisms of the relevant *Vibrio* spp. in different environments (SQ1.8)

Vibrio species are ubiquitous and widely distributed in aquatic environments worldwide. Their capability to survive is due to multiple strategies that allow the bacteria to deal with stressors as nutrient deprivation, changes in salinity and temperature and to resist predation by heterotrophic protists and bacteriophages. The mechanisms allowing a long-term survival are summarised below.

3.6.1 | Viable but non-culturable state

The bacteria must survive periods of harsh conditions, such as nutrient limitation or temperature extremes. To survive, the bacteria enter a specialised dormancy state, VBNC (Brumfield et al., 2021; Lutz et al., 2013). Changes in temperature, salinity, pH, oxygen concentration and starvation are all conditions that can induce the VBNC state (Akolkar & Matson, 2023). VBNC state cells have been shown to tolerate typically fatal stressors, including high-dose antibiotics and are able to resuscitate in vivo, e.g. VBNC *V. cholerae* O1 (attenuated vaccine strain CVD 101) was shown to convert to a culturable state during passage through human participants (Colwell et al., 1996) and *V. cholerae* non-O1/non-O139 and *V. parahaemolyticus* VBNC cells converted into the culturable form when co-cultured with eukaryotic cells such as HT-29 or Caco-2 cells (Senoh et al., 2012) indicating the potential for in vivo resuscitation.

3.6.2 | Biofilm formation

Bacterial attachment to environmental surfaces and aquatic organisms and the formation of multicellular structures (biofilms) have been shown to be critical for the survival of the genus *Vibrio*. Biofilms provide a protective environment and stability in a changing environment and seem to provide a substantial survival advantage to aquatic organisms. For instance, extraction of nutrients (e.g. chitin) can be facilitated by attachment to some biotic surfaces. Biofilm formation is a multistep process that begins with surface scanning and initial attachment, followed by the production of extracellular matrix components and the formation of microcolonies, and finally the development of highly organised, three-dimensional structures (Conner et al., 2016). The ability of vibrios to form biofilms depends upon specific structural genes (flagella, pili and exopolysaccharide biosynthesis) and regulatory processes (two-component regulators, QS and c-di-GMP signalling). The expression of different pili, for example, is related with the range of different surfaces colonised (Aagesen & Häse, 2012; Lutz et al., 2013). Besides, while vibrios share common regulatory proteins and signalling systems, there are several differences that establish the biofilm as something unique for each species, conditioned by the lifestyle to each *Vibrio* spp., e.g. differences in niche occupation, in the environmental parameters they respond to and even pathogenesis (Yildiz & Visick, 2009).

Of relevance, the human action in the marine environments is increasing the number of different particles that serve as surfaces for biofilm formation. It has been proposed that marine microplastics, that have been proposed as potential reservoirs and vectors for the transport of pathogenic *Vibrio* spp. (Noorian et al., 2023).

3.6.3 | Persister cells

Persister cells are described as slow- or non-growing subpopulations present within a growing culture, which are able to withstand multiple types of stressors, including exposure to antibiotics. In the case of antibiotic resistance, the tolerance of persister cells is non-genetic, not inheritable and results from a phenotypic switch from the normal, sensitive cell type to the tolerant, persister state (Ayrapetyan et al., 2015). While the relation between persister cells and VBNC state is still under debate (Liu et al., 2023), persister cells are described as a dormancy state in which cultivability is retained and growth on routine media is restored after the inducing stressors are removed. Although most studies of persister cells focus on antibiotic resistance, the persister state can also be induced by stressful environments such as starvation, oxidative stress, DNA damage, stressful pH and antibiotics (Ayrapetyan et al., 2015). At present, persister cells have been identified in almost every bacterial species examined including *V. cholerae* and *V. vulnificus* (Ayrapetyan et al., 2015; Van den Bergh et al., 2017).

3.6.4 | Anti-grazing strategies

Vibrio species interact with numerous organisms in their habitats, including heterotrophic protozoa that feed on them. Once in the interior of the protozoa, bacteria cells are processed in the phagosomes where they encounter a harsh environment that includes low pH, antimicrobial peptides, reactive oxygen/nitrogen species, proteolytic enzymes and low iron concentrations (Akolkar & Matson, 2023). To avoid protozoan predation, *Vibrio* spp. display various anti-grazing strategies, some of them include the formation of biofilms, production of proteases such as PrtV, secretion of ammonium and pyromelanin, expression of the type VI secretion system (T6SS) of *V. cholerae*, that kill the model amoeba *Dictyostelium discoideum*, and the MARTX type III of *V. vulnificus*, involved in the lysis of a wide range of eukaryotic cells, including amoebae (Espinoza-Vergara et al., 2020). In *V. parahaemolyticus* the type III secretion system 2 (T3SS2) mediate cytotoxicity and one of its effectors (VopC) allows the pathogen to escape from the vacuole (de Souza Santos & Orth, 2014). It has been suggested that the resistance of *Vibrio* spp. to the factors encountered inside of the phagosomes/food vacuoles in heterotrophic protozoa might serve as a pre-adaptation niche before entering a host, as the passage of bacteria within protozoa might activate specific factors used to resist the strategies that also contribute to the inactivation of bacteria within mammalian hosts (Espinoza-Vergara et al., 2020).

3.6.5 | Association with other aquatic organisms

Vibrios interact with many higher organisms and here we describe organisms that act as reservoirs. Different studies have demonstrated the positive chemotactic responses towards some components of the mucin (GlcNAc and Neu5Ac) present in algae and in the fish skin mucus (Reddi et al., 2018; Valiente et al., 2008). Besides, GlcNAc is also present in the chitin found in the exoskeleton of crustaceans, insects, eggs of arthropods, nematodes and in some diatom algae (Markov et al., 2015). The attraction and adherence to these components allows *Vibrio* association to zooplankton (primarily copepods), crustaceans, bivalves, fish, aquatic plants, phytoplankton, chironomids (Brumfield et al., 2021), all organisms that can act as potential reservoirs. Chironomid (non-biting midges) eggs are a natural reservoir for *V. cholerae*, and non-O1/non-O139 *V. cholerae* have been isolated from chironomid egg masses in several countries, highlighting their potential as an environmental reservoir (Noorian et al., 2023).

3.6.6 | Concluding remarks

- *Vibrio* species are ubiquitous and widely distributed in aquatic environments worldwide. The capability to survive and persist in such environmental niches is due to multiple strategies:
 - Changes in temperature, salinity, pH, oxygen concentration and starvation can induce the VBNC state in vibrios in which the bacteria are metabolically dormant;
 - *Vibrio* can form biofilms on several biotic and abiotic surfaces. In these communities, the bacteria survive several stressors;
 - A portion of the *Vibrio* population exist in a slow- or non-growing state (persister cells) that allows the bacterial survive several harsh conditions;
 - *Vibrio* spp. display various anti-grazing strategies to avoid protozoan predation; and
 - The preferred adhesion of *Vibrio* to chitin and mucin allows association to other aquatic organisms that can act as reservoirs, such as zooplankton (primarily copepods), crustaceans, bivalves, fishes, aquatic plants, phytoplankton, chironomids.

3.7 | Factors in the aquatic environments and in food that influence occurrence and growth of the relevant *Vibrio* spp., and affect transmission of their virulence and resistance determinants (AQ2)

3.7.1 | Factors in the aquatic environments and in food that influence occurrence and growth of the relevant *Vibrio* spp. (SQ2.1)

Vibrio species are autochthonous of the aquatic environments (primary marine and brackish waters) and may therefore occur in seafood deriving from such waters. In the aquatic environments as well as in seafood, *Vibrio* spp. are subjected to a multiplicity of factors that, individually and in combinations, may affect their ability to survive and grow. These factors are schematically presented in [Figure 3](#) and may be divided into:

- (i) **Extrinsic factors**, associated with the external conditions surrounding the microorganism in the aquatic environment or the conditions surrounding the food where *Vibrio* is present (e.g. temperature, (sea)water salinity, solar and UV radiation);
- (ii) **Intrinsic factors**, associated with the inherent properties of the seawater or the food hosting the microorganism and that, for foods, can be natural or related to food processing operations (e.g. pH, water activity of food, nutrient content);
- (iii) **Implicit factors**, depending on the interaction of the microorganism with the surrounding microbiota (e.g. microorganisms' interaction mechanisms such as predation, parasitism and commensalism phenomena, production of substances that are inhibitory/lethal to other organisms (antibiotics, bacteriocins, etc.) or that may support their survival or stimulate their growth).

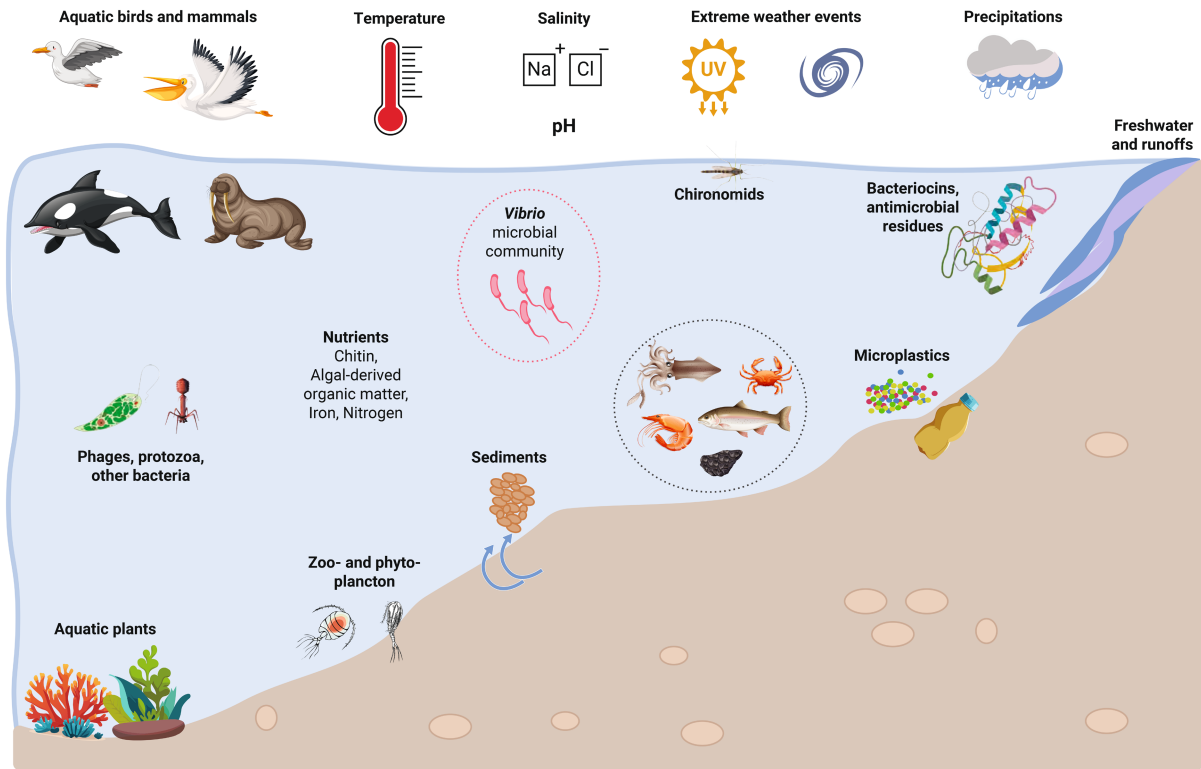


FIGURE 3 Factors that affect occurrence of *Vibrio* spp. of relevance in the aquatic environment (adapted from Brumfield et al., 2021).

A meta-analysis by Takemura et al. (2014) on the dynamics of Vibrionaceae in the environment pointed out that individual *Vibrio* species are susceptible to different degrees to environmental factors other than temperature. Given the potential short generation times of vibrios (≤ 20 min), rapid fluctuations of *Vibrio* populations in reaction to sudden and geographically restricted environmental variations should be considered with attention.

For example, factors relevant in one area, e.g. salinity and chlorophyll *a* of the Gulf Coast, were not found significant in Chesapeake bay on the Atlantic Ocean, where temperature, turbidity and dissolved oxygen were more predictive (Parveen et al., 2008; Zimmerman et al., 2007). Similar conclusions were drawn by Johnson (2015) based on multiple regions (Atlantic Ocean, Pacific Ocean, Gulf Coast). This reflects the complexity of interactions among environmental factors affecting the occurrence of *Vibrio* species, and the need for region-specific environmental models and validations.

3.7.1.1 | Temperature in the aquatic environment

Water temperature is a major driver for the occurrence and growth of *Vibrio* species, including those of PH relevance. Vibrios are mesophilic microorganisms and tend to be detected more frequently during warmer seasons in temperate areas, whereas their seasonality is less pronounced in tropical and subtropical waters (Urakawa & Rivera, 2006). In laboratory conditions, *Vibrio* species grow well $> 17^{\circ}\text{C}$ and up to an optimum at $30\text{--}40^{\circ}\text{C}$. *Vibrio* species are sensitive to low temperatures though, and around and below 10°C tend to shift to the VBNC status (see Section 3.6.1). The effect of water temperature on the occurrence of *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* has been addressed by several studies as reviewed by Johnson (2015) and Sheikh et al. (2022).

V. parahaemolyticus counts in seawater, bivalves (mainly oysters) or sediments have been reported to correlate with temperature in several studies in the USA (Blackwell & Oliver, 2008; DePaola et al., 1990, 2003; Duan & Su, 2005; Johnson et al., 2012; Parveen et al., 2008), in Asian countries (Fukushima & Seki, 2004; Ndraha & Hsiao, 2021; Rehnstam-Holm et al., 2014; Xu et al., 2020) and in Brazil (Sobrinho et al., 2010). Data from Northern American studies have been largely used to develop the quantitative models applied in the *V. parahaemolyticus* FAO/WHO risk assessments (FAO and WHO, 2011). In Europe, a French study in sites on the Atlantic coast showed that *V. parahaemolyticus* presence in seawater correlated with the mean temperature of the precedent week, while in mussels there was also a correlation with chlorophyll *a*. Also, the occurrence of *trh+* *V. parahaemolyticus* correlated with the mean temperature 2 days before sampling (Deter et al., 2010). In Germany, water temperature was the most important determinant for *Vibrio* abundance in the central Wadden Sea, although *V. parahaemolyticus* appeared less susceptible to lower temperatures in the sediments than other species (Böer et al., 2013). The role of temperature on *V. parahaemolyticus* occurrence and its combined effect with salinity was further confirmed for German North and Baltic Sea coasts (Fleischmann et al., 2022). In Italy, a statistically significant association of *V. parahaemolyticus* prevalence with the warmer seasons (April–October, average $T > 16.45^{\circ}\text{C}$) was found in a study on clams harvested in the estuary of the Po River (Northern Adriatic Sea). Interestingly, *tdh+* and/or *trh+* *V. parahaemolyticus* did not display significant correlations with any of the environmental parameters (Serratore et al., 2016). A second study performed in Italy, in Sardinian and Northern Adriatic Sea shellfish production areas, showed a correlation

between *V. parahaemolyticus* detection and water temperature, as well as correlation ($r=0.41$) between water temperature and *V. parahaemolyticus* counts (Suffredini et al., 2014). Finally, in studies performed in Galicia (Spain), the occurrence of *V. parahaemolyticus* in rias⁵⁰ was positively and significantly correlated with seawater temperature, but salinity was the dominant factor affecting its probability of detection, while temperature had an effect only in modulating the abundance in periods and areas of lower salinity (Martinez-Urtaza, Lozano-Leon, et al., 2008). On the contrary, in offshore areas, where seawater temperature followed a non-seasonal pattern, the occurrence of *V. parahaemolyticus* was mostly affected by zooplankton (Martinez-Urtaza et al., 2012). It should be noted that, while higher water temperatures are considered reliable predictors for greater *V. parahaemolyticus* abundance, it is not certain that pathogenic strains follow the same pattern, as there are indications that isolation rate of *tdh+* and/or *trh+* strains is higher in colder months, when the total *V. parahaemolyticus* counts are lower (DePaola et al., 2003; Williams et al., 2017).

V. vulnificus is a natural inhabitant of waters with a temperature between 10 and 33°C (optimum around 25–28°C). The lower temperature limit for the isolation of viable *V. vulnificus* from the environment is in the range 12–17°C depending on the studies and specific conditions of the areas (Froelich & Daines, 2020), while at temperatures < 10°C a shift towards the VBNC state (see Section 3.6.1) is reported (Oliver, 2015). Several *V. vulnificus* studies were undertaken, especially in the USA (Motes et al., 1998; Tamplin, 1994), to address the effect of seawater temperature on occurrence and concentrations. The cited studies (Motes et al., 1998; Tamplin, 1994) were used in the FAO/WHO risk assessment to develop the polynomial regression model describing temperature effects on levels of *V. vulnificus* in oysters (FAO and WHO, 2005b). Overall, while the importance of temperature on *V. vulnificus* occurrence and abundance is consistent among studies (Johnson, 2015), the combined effect of temperature and salinity is markedly relevant for this species (see Section 3.7.1.4). Among the few ecological studies performed in Europe dealing with the effect of temperature on *V. vulnificus* detection (Böer et al., 2013), water temperature was the relevant variable for *V. vulnificus* in bathing areas in Germany and small temperature variations⁵¹ significantly affected the probability of its detection (Cantet et al., 2013; Fleischmann et al., 2022). Two studies, performed in the Northern Adriatic Sea and in Mediterranean lagoons of the French coast, also showed that *V. vulnificus* detection was strongly associated with the warmest months of the year (Takemura et al., 2014).

V. cholerae is part of the autochthonous microbiota of temperate aquatic environments and it is often detected in brackish water in the temperature range 15–30°C, with observed greatest abundances around 20°C (Blackwell & Oliver, 2008; DePaola et al., 1983; Singleton et al., 1982). Temperature-response of *V. cholerae* has been extensively studied in cholera-endemic regions of the world, and cholera cases have been positively correlated to temperature globally (see Johnson, 2015). Several studies correlating *V. cholerae* concentrations (regardless of serogroups) to seawater temperatures have been developed also in other countries, including the USA (Chávez et al., 2005), Japan (Wang & Gu, 2005) and Mexico (Louis et al., 2003). The comprehension of the relation between temperature and *V. cholerae*, however, is hindered by the complexity of the interactions of this microorganism with other aquatic organisms (see Section 3.6.5), affecting its occurrence and levels (Fleischmann et al., 2022). In Europe, the detection rates of non-O1/non-O139 *V. cholerae* in the aquatic environments display a marked seasonality, with detection only between May and September and concentrations (real-time PCR) up to 1.8 log₁₀ gene copies/100 mL and 5.0 log₁₀ gene copies/100 mL in samples from the North and the Baltic Sea (Germany) (Cantet et al., 2013). A similar pattern was present in Mediterranean French lagoons, where *V. cholerae* was detected only during the warm season, with concentrations between 1 and 2 log₁₀ MPN/L of water (Kirschner et al., 2008). Interestingly, data from Austria support the correlation between water temperature and non-O1/non-O139 *V. cholerae* also for inland water bodies. A study from Kirschner et al. (2008) on lake Neusiedler See, a large Austrian alkaline lake, reports a highly significant correlation ($\rho=0.65$; $p<0.001$) between the detection rate of *V. cholerae* and temperature, as well as with zooplankton biomass and conductivity. Significantly, detection rate increased from non-detection in colder months, to 100% in summer (average $T=21.7^\circ\text{C}$). Thus, the effect of temperature on non-O1/non-O139 *V. cholerae* is evident in studies covering seasonal trends.

3.7.1.2 | Temperature in food

According to ICMSF (1996) (Table 8), the optimum growth temperature for the three major *Vibrio* species is 37°C with growth temperature ranges from 10 to 43°C for *V. cholerae*, 5–43°C for *V. parahaemolyticus* and 8–43°C for *V. vulnificus*. It should be noted, however, that data related to *V. cholerae* stem from testing of O1 (classic and El Tor) strains and may not fully represent the growth values for non-O1/non-O139 isolates.

Several studies provide predictive models on the effect of temperature on growth and inactivation of *Vibrio* in seafood (summarised in Annex F).

Growth/inactivation rates at temperatures ranging from freezing to the maximum growth temperature have been reported for *V. parahaemolyticus* in different oyster species (*C. gigas*, *C. virginica* and *Saccostrea glomerata*), in some crustaceans such as shrimps, prawns and marinated crabs, and in some finfish species such as salmon, flounder and tilapia. Some of these studies also addressed differences in the growth rates of specific pathogenic *V. parahaemolyticus* strains (*tdh+* and/or *trh+*, ST36) compared to non-pathogenic ones. In addition, secondary growth models – mostly based on the square root model – have been developed for oysters by Fernandez-Piquer et al. (2011), Parveen et al. (2013), Yoon et al. (2008) and

⁵⁰A coastal inlet formed by the partial submergence of an unglaciated river valley. It is a drowned river valley that remains open to the sea.

⁵¹e.g. in a temperature range of 15–20°C, an increment of 1°C in water temperature increased the probability of detecting *V. vulnificus* by 4%, while temperatures > 20°C, the same increment provided a tenfold increase of the detection probability.

Fletcher et al. (2024), for shrimps/prawn by Tang et al. (2015), Chen et al. (2019), Boonyawantang et al. (2012), Wu et al. (2023) and Ma et al. (2023), and for fish [salmon (*Oncorhynchus* spp.) by Yang et al., 2009; Kim et al., 2012; flounder (*Paralichthys* spp.) by Kim et al., 2012]. A few secondary models (Arrhenius, or other models), dealing with the survival/inactivation of *V. parahaemolyticus* in the temperature range between 0°C and the minimum growth temperature, are also available. Compared to *V. parahaemolyticus*, models on the effect of temperature on *V. vulnificus* concentration in seafood are limited, covering exclusively oysters (*C. gigas*, *C. virginica*), with secondary growth models provided by Kim et al. (2012) and DaSilva et al. (2012). No specific data on growth/inactivation rates of non-O1/non-O139 *V. cholerae* were retrieved, though some studies reported log increase/reduction of non-O1/non-O139 *V. cholerae* strains in prawns/shrimps (Wong et al., 1995).

TABLE 8 Information on the limits for growth of *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* (ICMSF, 1996).

Vibrio species	Temperature (°C)		pH		NaCl (%)		a_w		Atmosphere	
	Optimum	Range	Optimum	Range	Optimum	Range	Optimum	Range	Optimum	Range
<i>Vibrio cholerae</i> ^a	37	10–43	7.6	5.0–9.6	0.5	0.1–4	0.984	0.970–0.998	Aerobic	Aerobic/ anaerobic
<i>Vibrio parahaemolyticus</i>	37	5–43	7.8–8.6	4.8–11	3.0	0.5–10	0.981	0.940–0.996	Aerobic	Aerobic/ anaerobic
<i>Vibrio vulnificus</i>	37	8–43	7.8	5–10	2.5	0.5–5.0	0.98	0.96–0.997	Aerobic	Aerobic/ anaerobic

^aThese data are referred to *V. cholerae* O1 strains.

3.7.1.3 | Salinity

Salinity is considered the second most relevant environmental parameter to influence *Vibrio* abundance. *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* display significant differences in their halotolerance and salinity requirements. Consequently, salinity shifts in specific areas (e.g. gulfs, estuaries, rias) may affect the proportions of the different species and sustain temporary blooms of the species with the highest fitness for the established conditions.

V. parahaemolyticus, has strict salinity requirements, being unable to grow below 5 ppt NaCl while thriving in water environments at 15–25 ppt NaCl (DePaola et al., 2003). This species displays a noticeable halotolerance and adaptation to osmotic stress (Kalburge et al., 2014), e.g. in experimental settings simulating water systems subjected to salinity increase due to evaporation. Possibly because of the large salinity range supporting its growth, *V. parahaemolyticus* seems less affected by salinity than other pathogenic vibrios (Randa et al., 2004). This might also explain the observations on the different weight of salinity as a factor influencing *V. parahaemolyticus* prevalence and levels in temperate and tropical waters (FAO and WHO, 2011): in fact, in tropical areas, where limited oscillation of water temperature is present, salinity emerges as the major driver for *V. parahaemolyticus* abundance (Deepanjali et al., 2005), while in temperate areas both temperature and salinity are considered important (DePaola et al., 2003).

V. vulnificus is an obligate but moderate halophile, that inhabits marine and estuarine waters with salinities between 2 and 25 ppt (Oliver, 2015), with higher bacterial concentrations occurring in the range 5–20 ppt, and viability enhanced at 5–10 ppt (Parveen & Tamplin, 2013). *V. vulnificus* has a more complex relationship with the combination of temperature–salinity as, with the increase of salinity, the range of temperatures permissive for its growth tends to narrow. Therefore, at salinities > 30 ppt *V. vulnificus* occurrence is significantly reduced regardless of the environmental temperature (FAO and WHO, 2005b), at intermediate salinity (> 10 ppt) the permissive temperature is restricted to 22–30°C, and at low salinities (5–10 ppt) the growth range extends from 10 to 32°C (Randa et al., 2004).

Finally, non-O1/non-O139 *V. cholerae* usually colonises low-salinity and brackish waters (0.5–30 ppt) and its occurrence in freshwater (< 0.5 ppt) has been long recognised in association to pandemic cholera and its transmission through ingestion of contaminated water (Faruque Shah et al., 1998). *V. cholerae* occurrence and abundance is usually greater in the range 0.5–10 ppt, while higher salinities are less favourable (Takemura et al., 2014).

Few studies evaluated the significance of salinity on *Vibrio* occurrence in European marine areas. In Germany, for example, a survey dealing with bathing areas of the North and Baltic Sea, highlighted that salinity was a secondary factor to temperature for *Vibrio* spp. abundance, but it was the main modulator of the composition of the *Vibrio* community, enhancing occurrence of *V. parahaemolyticus* in areas with higher salinity, the North Sea, compared to areas as the Baltic Sea, where *V. vulnificus* was the predominant species (Fleischmann et al., 2022). Studies in France and Spain also underlined how a rapid shift of the salinity in confined areas such as lagoons and rias can lead to a rapid proliferation of a certain species, with *V. parahaemolyticus* occurrence in Galician rias increasing during periods of lower salinity in correspondence of freshwater discharge points (Martinez-Urtaza, Lozano-Leon, et al., 2008). Similarly, the rapid decrease of salinity (from 31–36 ppt to 2–16 ppt within 15 days) in French Mediterranean lagoons following freshwater flash floods, showed the increase of all three species of PH, with *V. parahaemolyticus* reaching the highest counts at a salinity of 10–20 ppt, *V. vulnificus* at 10–15 ppt and *V. cholerae* at 5–12 ppt (Esteves et al., 2015).

3.7.1.4 | Temperature and salinity interplay

In consideration of data and statistical analysis limitations, quite often, in environmental studies, either temperature or salinity are considered as sole driving factors for *Vibrio* occurrence. It has been long suggested, however, that *Vibrio* abundance in aquatic systems is dependent on both temperature and salinity simultaneously, although the interplay of these two factors may be disguised if studies or samples collection are conducted, for example, in a salinity range too narrow to appreciate correlations (Johnson, 2015; Zimmerman et al., 2007). Some studies specifically addressed the interdependency of temperature and salinity effects, especially for *V. vulnificus*. (Randa et al., 2004), using experimental and literature data, showed that – when considering the whole set of temperature and salinity values – *V. vulnificus* abundance is positively correlated to temperature and negatively correlated to salinity ($r_s = 0.310$ and $r_s = -0.451$, respectively). However, splitting the analysis according to salinity ranges, the relationship shows a mixed trend. In the range 5–10 ppt, in fact, *V. vulnificus* abundance does not correlate with temperature and large variations of concentrations are reported in this salinity range regardless of temperature. At higher salinities (range 20 to 25 ppt), instead, a positive correlation of *V. vulnificus* abundance to salinity can be established ($r_s = 0.354$).

3.7.1.5 | Solar and UV radiation

Direct exposure to solar radiation is a significant inactivation factor in the aquatic environment and higher sensitivity of *V. cholerae* to solar disinfection compared to other enteric pathogens has been reported (Berney et al., 2006). A prominent role of sunlight exposure on the induction of the lysogenic phage CTX Φ of toxigenic *V. cholerae* has been documented (Faruque et al., 2000). Furthermore, mechanisms of prophage induction, which enhance UV-sensitivity, and loss of culturability with induction of the VBNC state in water microcosms exposed to sunlight have been described for *V. parahaemolyticus* (Deepanjali et al., 2005; Zabala et al., 2009).

UV-C systems are routinely used for disinfection purposes, e.g. for water treatment in bivalve depuration settings. Based on experimental data (Joux et al., 1999; Mori et al., 2007; Nakahashi et al., 2014; Oguma et al., 2019), UV-A and UV-B (wavelength 315–400 nm and 280–315 nm, respectively), inducing DNA damage, cellular membrane damage and growth delay, may interfere with *Vibrio* spp. survival and replication. A \log_{10} inactivation rate constant k of 0.017 cm^2/mJ has been reported, for examples for *V. parahaemolyticus* subjected to UV-LED treatment at 300 nm (Oguma et al., 2019).

3.7.1.6 | pH

In general, *Vibrio* species are tolerant to alkaline pH, a feature that is exploited in culture media for their isolation, while they display extreme sensitivity to highly acidic environments (just a few minutes of survival at pH ~2; Koo et al., 2000; Mudoh et al., 2014). Some studies, however, highlighted that *V. parahaemolyticus* and *V. vulnificus* may retain viability after long-term storage (30 days) at pH ranging between 7.0 and 4.0 (Yoon et al., 2017) and that *V. vulnificus* clinical and environmental strains may produce biofilms in response to acidic environment (pH 5.5), a mechanism that may enhance survival and retention of infectivity in these conditions (Çam & Brinkmeyer, 2020). Overall, even though *Vibrio* species of PH relevance (and particularly *V. parahaemolyticus* and *V. vulnificus*) can adopt strategies to survive mild acidic environments, their optimal pH falls in the neutral/low alkaline area (pH 7.6–7.8) and efficient growth is supported up to highly alkaline conditions (pH ~10) (Mudoh et al., 2014). In general, the pH of aquatic environments is quite stable and, indeed, this factor does not emerge as a significant contributor to the variation of vibrios abundance (Takemura et al., 2014). Raw seafood pH is usually reported in the range between 6.0 and 7.0. Therefore, except for products that may have undergone acidification, this parameter is not relevant for modelling the growth of *Vibrio* spp. in seafood.

3.7.1.7 | Water activity

Vibrio species of PH relevance display optimal growth at a_w equal or above 0.98, with a minimum of 0.96 and 0.97 for *V. vulnificus* and *V. cholerae*, respectively, and a slightly higher tolerance for *V. parahaemolyticus* (0.94 according to ICMSF, 1996; 0.936 according to Miles et al., 1997). Considering the characteristics of raw seafood, a_w is generally a factor with limited relevance for the survival and growth of these *Vibrio* spp. while, due to susceptibility of vibrios to dehydration, it may affect processed (e.g. salted, dried) seafood products.

3.7.1.8 | Nutrient content

Vibrio spp. occur both as free-living organisms, using for their metabolism substances dissolved in the aquatic environment, and as attached to biological surfaces that can be used as a nutritive substrate. The most relevant of these substrates is chitin (see also Section 3.7.2.1), which is present in crustaceans, zooplankton (e.g. copepods) and phytoplankton (e.g. diatoms), and that can be used by almost all vibrios as a source of carbon and nitrogen through the production of an extracellular chitinase (Thompson et al., 2004). Further to this, *Vibrio* spp. can exploit algal-derived organic matter released following phytoplankton blooms. This was confirmed in experiments on Baltic Sea microcosms supplemented with cyanobacterial-derived organic matter (Eiler et al., 2007). More recently, the effect of microplastics on vibrios occurrence has been investigated. *Vibrio* spp., indeed, have been found in high abundances within the *plastisphere* microbiota

(summarised in Bowley et al., 2021); beside the relevance of abiotic surfaces for *Vibrio* attachment and spread, the ability of some *V. parahaemolyticus* and *V. alginolyticus* isolates to biodegrade a plastic polymer (polyvinyl alcohol-low linear density polyethylene-blended plastic films) was also reported (Raghul et al., 2014). Further to this, *Vibrio* occurrence is also modulated by essential micronutrients with limited external supply, such as iron (Fe). Fe concentration in the environment is relevant for proliferation of several *Vibrio* species, as evidenced in studies on Saharan dust pulses, an important source of iron to tropical marine waters, following which a 5- to 30-fold increase of *Vibrio* counts was registered (Westrich et al., 2016).

3.7.1.9 | Other biotic and abiotic factors

Vibrio proliferation in the aquatic environments is affected by several additional factors, e.g. water turbidity, phyto- and zooplankton content, chlorophyll *a*, dissolved oxygen and organic carbon, chitin, nitrogen, etc. (Brumfield et al., 2021). While all these factors play a role in *Vibrio* abundance, the meta-analysis conducted by Takemura et al. (2014) clearly showed that their correlation with *Vibrio* occurrence is inconsistent and not comparable to the predictive value of temperature and salinity. Further to this, *Vibrio* spp. occurrence is affected by their dual lifestyle, as either free-living microorganisms or as particle-associated bacteria (reviewed in Sampaio et al., 2022), the latter leading to specialised ecological niches with freshwater and estuarine plants, micro- and macroalgae, zooplankton and invertebrate animals (including bivalve shellfish and crustaceans) (Herrfurth et al., 2013; Parveen & Tamplin, 2013). Interaction with vertebrate animals may be relevant for *Vibrio* occurrence: aquatic birds, indeed, feed on plants and animals potentially colonised by *Vibrio* and their faeces could be a source of *Vibrio* dissemination to previously non-colonised water systems, especially in the case of migratory birds (Noorian et al., 2023). Specific interaction mechanisms enhancing survival of *Vibrio* spp. are addressed in Section 3.6.

3.7.2 | Factors in the aquatic environments and in food that affect transmission of their virulence and resistance determinants (SQ.2)

Vibrio spp. have been shown to evolve continuously by gaining fitness traits through HGT, contributing to the ability of *Vibrio* spp. to adapt to specific niches (Brumfield et al., 2021). HGT and recombination events may influence the emergence of pandemic clones, such as *V. parahaemolyticus* O3:K6, which appears to have acquired pathogenic characteristics from various bacterial species (Boyd et al., 2008). Broad distribution of virulence genes among environmental *Vibrio* spp. is well documented (Hasan et al., 2015; Klein et al., 2014; Nishibuchi et al., 1996; Sechi et al., 2000). Few factors have been described to affect the transmission of virulence or resistance determinants.

3.7.2.1 | Presence of chitin

Chitin is used by the different vibrios as a source of energy, carbon and nitrogen. Chitin is a long-chain polymer of *N*-acetylglucosamine and is the second most abundant polysaccharide in nature following the cellulose. Chitin was described to trigger the natural competence of *V. cholerae* in 2005 (Meibom et al., 2005). The *tfoX* gene is required for chitin-dependent natural transformation in *V. cholerae* and homologues of TfoX were identified in all sequenced Vibrionaceae members suggesting that chitin-induced natural transformation is a shared trait among the Vibrionaceae (Sun et al., 2013). Given the close association of vibrios to the zooplankton in marine environments, *V. cholerae* as well as *V. parahaemolyticus* and *V. vulnificus* are probably frequently in the state of natural competence enabling them to acquire novel traits providing an advantage in the respective environments (Escudero & Mazel, 2017).

3.7.2.2 | Presence of phages

The aquatic environments on earth are estimated to host $\sim 10^{52}$ phages, where they coexist in a dynamic equilibrium with their bacterial hosts. Phages can exist as free viruses (virulent phages) or integrated within bacterial genomes (temperate phages) as prophage (Molina-Quiroz & Silva-Valenzuela, 2023). Factors like salinity, high temperature, nutrient limitation and in vivo conditions such as the intestinal microenvironment induce the lytic stage by activating the SOS response (Nawel et al., 2022). In the aquatic environment, vibriophages can be found in the water column and associated with filter-feeding animals such as oysters (Richards et al., 2019). A recent study found 5674 prophage-like elements among 1874 sequenced *Vibrio* genomes at NCBI (Castillo et al., 2018). The authors reported that these vibrio phages carried virulence genes such as those encoding RTX toxin, collagenases or haemolysins, as well as ARGs, niche adaptation factors with high similarity to heavy metal resistance genes and even genes of the DNA uptake/recombination cascade, as the *dprA* gene. *Vibrio* prophages and temperate phages may constitute a major reservoir of virulence and niche adaptation traits in marine systems (Castillo et al., 2018).

⁵²ISO 21872-1:2017. Microbiology of the food chain – Horizontal method for the determination of *Vibrio* spp. Part 1: Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*. International Organization for Standardization, Geneva, Switzerland.

3.7.2.3 | Aquaculture systems

Aquaculture systems and farms have been designated as ‘genetic reactors’ and ‘hotspots for AMR genes’ where significant genetic exchange and recombination can occur, which can shape the evolution (Watts et al., 2017). The induction of the SOS response that is triggered under stressful conditions and during HGT (Escudero & Mazel, 2017) increases the transfer of ICEs of the SXT/R391 family (Burrus et al., 2006) and drives also conjugative transfer of plasmids. In aquaculture systems, in addition to antimicrobial compounds, biocides and metal-containing products (e.g. cages) are used. High concentrations of copper, cadmium, lead and mercury have been detected in fish feeds and residues of fertilisers have been found (Watts et al., 2017). The bacterial community is thus exposed to stressful conditions of combinations of heavy metals, antimicrobials and other co-selecting factors of natural or anthropic origin that are likely to increase the selection of AMR (Hiltunen et al., 2017).

3.7.3 | Concluding remarks

- The factors that affect survival and growth of the relevant *Vibrio* spp. in the aquatic environments and in food are:
 - factors associated with the external conditions in the aquatic environment or surrounding the food (extrinsic factors) as temperature, (sea)water salinity, solar and UV radiation;
 - factors associated with the inherent properties of the seawater or the food (intrinsic factors) as pH, water activity of food, nutrient content;
 - factors depending on the interaction of the microorganism with the surrounding microbiota (implicit factors) as predation, parasitism, commensalism, production of substances that are inhibitory/lethal to other organisms, etc.
- Among the factors affecting occurrence and growth of *Vibrio* spp., temperature is the most important driver for the abundance of the species of PH interest in both the aquatic environment and food (herein temperature limits for growth ranging from 5, 8 and 10 to 43°C for *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae*), followed by salinity in the environment (optimum range 15–25 ppt for *V. parahaemolyticus*, 2–20 ppt for *V. vulnificus* and 0.5–10 ppt for *V. cholerae*). Other factors as pH and a_w may be of relevance in processed seafood products.
- The interactions among environmental factors affecting the occurrence of *Vibrio* species display a high level of complexity and variability and require region-specific environmental models and validations.
- A limited number of ecological studies addressing the effect of environmental factors on the occurrence of *Vibrio* spp. in seafood production areas in the EU are available.
- Growth models have been developed for *V. parahaemolyticus* in oysters, shrimps/prawns and for some finfish (salmon and flounder) and for *V. vulnificus* in oysters.
- A broad distribution of virulence genes among environmental *Vibrio* spp. is well documented. The following factors affect the transmission of virulence or resistance determinants: presence of chitin (triggering natural competence), presence of phages (acting as reservoirs of these genetic determinants) and contaminants of the aquatic ecosystem such as antimicrobials and heavy metals.

3.8 | Impact of climate change on the occurrence and levels of the relevant *Vibrio* spp. in water environments and seafood (AQ3)

Human activities, principally through emissions of greenhouse gases, have unequivocally caused global warming, with global average surface temperature reaching, in 2011–2020, 1.1°C above 1850–1900 (IPCC, 2023). In 2023, the world saw the highest global temperatures in over 100,000 years, and heat records were broken in all continents through 2022 (Romanello et al., 2023). In Europe, the climate is changing fast and the extreme events that have taken place over the last years offer a foretaste of what is to come under future global warming. This entails a range of risks to human health, including those associated with changes in water quantity, quality and temperature (EEA, 2024). Climate change has the potential of causing, enhancing or modifying the occurrence and intensity of some food-borne diseases (EFSA, 2020a), and various physical manifestations of climate change are likely to have direct impacts on diseases, such as those mediated by pathogenic vibrios. These bacterial species grow in warm low-salinity waters, with their relative abundance in the natural environment tending to mirror environmental conditions (Baker-Austin et al., 2017). The most recent IPCC report indicates that climate change risks expected in the near term include an increase in food-borne, waterborne and vector-borne diseases (high confidence) (IPCC, 2023). With temperatures changing across the globe overall, areas suitable for vibrios will increase (Trinanes & Martinez-Urtaza, 2021). A recent study looking at projections for coastal warming showed that coastal areas suitable for *Vibrio* infections could expand significantly by 2100 under the most unfavourable climate warming scenarios (Trinanes & Martinez-Urtaza, 2021). As *V. parahaemolyticus* and other *Vibrio* species have replication times as rapid as 8–9 min (Aiyar et al., 2002; Joseph et al., 1982), this group of pathogens is one of the most responsive to favourable environmental stimuli (Aiyar et al., 2002; Joseph et al., 1982). Although the majority of human pathogenic diseases have been shown to be aggravated by climate change (Mora et al., 2022), the relative simplicity of the relationship between ambient

environmental temperature and *Vibrio* abundance is so clear that they have been deemed a key ‘barometer’ of climate change and climate-associated impacts (Baker-Austin et al., 2017).

3.8.1 | Data sources, tools and models to evaluate the impact of climate change on the relevant *Vibrio* spp. in water environments and seafood and their limitations (SQ3.1)

Warming of coastal areas is considered the most pervasive and obvious impact of climate change in aquatic ecosystems worldwide, particularly in light of recent observations demonstrating significant and rapid warming in most of the world's coastlines (Lima & Wetthey, 2012). From the late 1970s onwards, global mean SST started to rise rapidly (see Figure 4). Various data sources, tools and models are available to evaluate the impact of climate change on *Vibrio* spp. in water environments and seafoods. The most recent available data suggests that global SSTs are increasing at truly unprecedented rates, with the warming in 2023 alone record breaking compared to both the recent and long-term record. In addition to warming trends, various extreme weather events such as heatwaves and storms have increased in the last two decades. Unfortunately, there is currently a lack of high-quality climatic data indicating how specific climatic events have led to outbreaks and *Vibrio* infections in Europe.

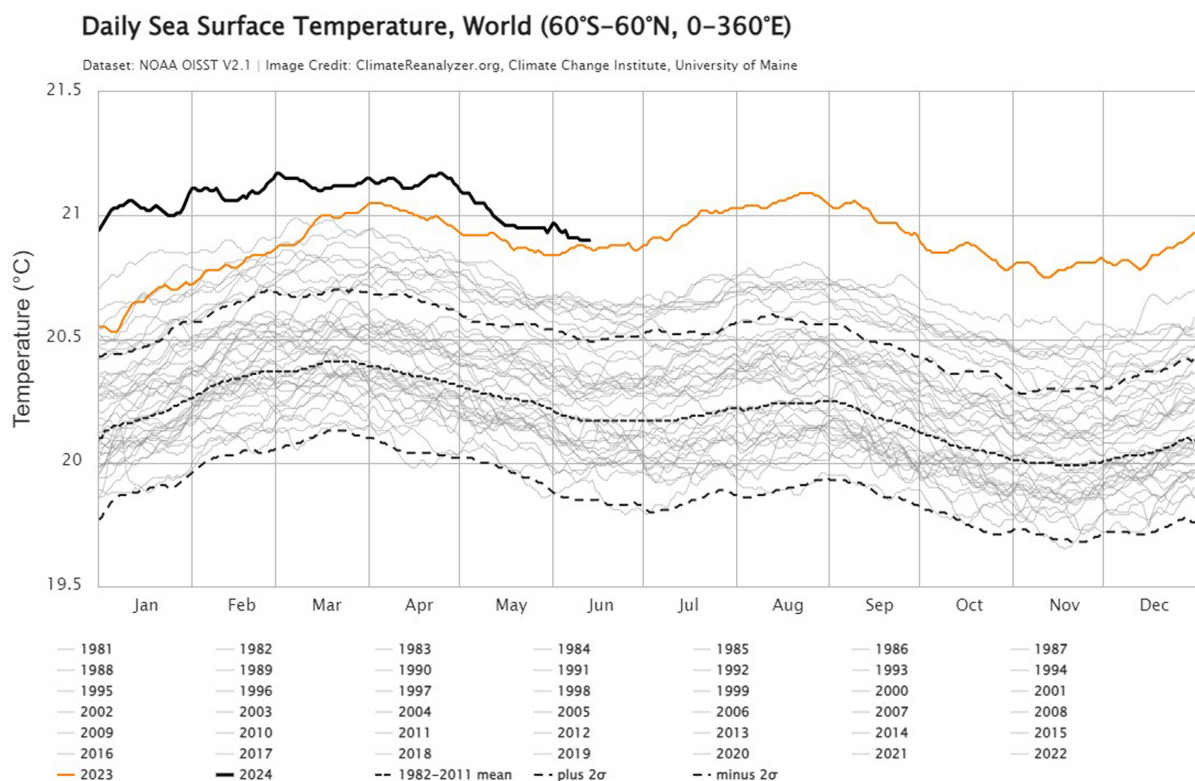


FIGURE 4 Climate warming of the global oceanic system. Daily sea surface temperatures for the world (60°S–60°N, 0–360°E) from 1981 to 12.6.2024. Climate Reanalyzer (n.d.) (Daily Sea Surface Temperature). Climate Change Institute, University of Maine. Retrieved (June 14, 2024), from <https://climatoreanalyzer.org/>.

Shared Socioeconomic Pathways (SSPs) are climate change scenarios of projected socioeconomic global changes up to 2100 as defined by the IPCC (2021). They are used to derive greenhouse gas emissions scenarios with different climate policies. The SSPs are part of a new framework that the climate change research community has adopted to facilitate the integrated analysis of future climate impacts, vulnerabilities, adaptation and mitigation (Riahi et al., 2017). Considering the medians under the very low greenhouse gases (GHG) emission scenario SSP1-2.6⁵³ and under the very high GHG emission scenario SSP5-8.5, the ocean temperatures at the surface (Figure 5) are expected to increase by 2100 (as compared to 1991–2010), respectively:

- 1.3°C (with a 90% spread from 0.2 to 2.5°C, henceforth referred to as a range of SSTs) and 3.5°C (2.2 to 5.8°C) for the Baltic Sea;
- 1.3°C (0.9 to 2.4°C) and 3.5°C (2.7 to 5.5°C) for the Black Sea;
- 1.1°C (0.6 to 2.1°C) and 3.4°C (2.6 to 5.0°C) for the Mediterranean Sea;
- 1.0°C (–0.7 to 1.8°C) and 2.4°C (1.2 to 4.6°C) for the North Sea.

⁵³The scenarios are referred to as SSPx-y, where ‘SSPx’ refers to the Shared Socio-economic Pathway or ‘SSP’ describing the socio-economic trends underlying the scenario, and ‘y’ refers to the approximate level of radiative forcing (in watts per square metre, or W/m²) resulting from the scenario in the year 2100.

Marine heatwaves are also projected to increase in frequency, duration, spatial extent and maximum intensity. Such changes could have widespread effects on marine species and cause the reconfiguration of marine ecosystems.

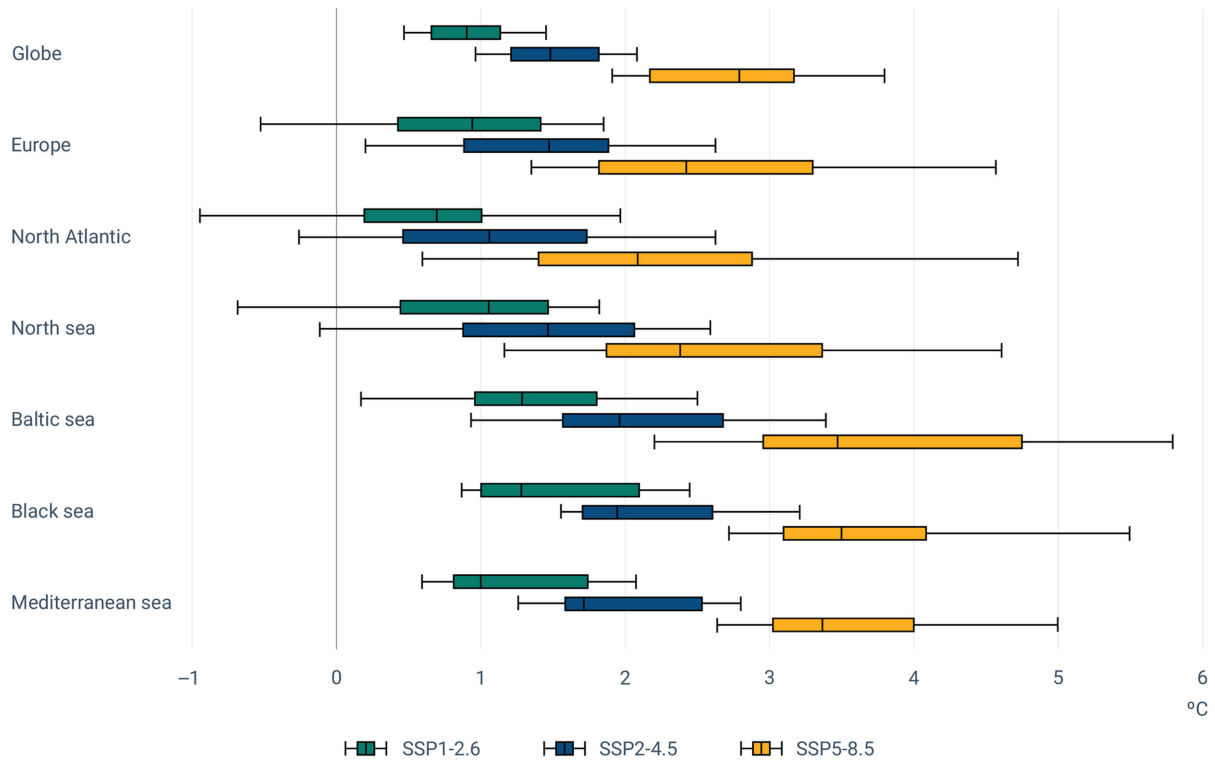


FIGURE 5 Projected sea surface temperature anomalies by 2100 under three different SSP scenarios (i.e. SSP1–2.6, SSP2–4.5 and SSP5–8.5) for European seas and global ocean as compared to 1991–2010. The boxplot shows the 5th, 25th, 50th, 75th and 95th percentiles (from <https://www.eea.europa.eu/en/analysis/indicators/european-sea-surface-temperature>).

Various data sets have become available over the last two decades that provide compelling evidence regarding the role of changing climate on infectious diseases mediated by pathogenic vibrios. Table 9 describes some key characteristics of the relevant data sources and approaches that have been undertaken to better understand the role of climate and vibriosis, with a focus where relevant in Europe. These include ecological and microbiological studies that show the impact of temperature and extreme weather events on vibrios, a small number of retrospective molecular studies (e.g. that use archived DNA/plankton samples alongside climatic data), epidemiological reports that link climate events to specific *Vibrio* infections and outbreaks, as well as risk mapping and *Vibrio* suitability approaches that can be used to infer how variables such as temperature and salinity impact the presence of these bacteria in the environment. There are various advantages as well as limitations to these different data sources that should be noted. However useful, there are few ecological and microbiological studies assessing the direct role of climate on *Vibrio* bacteria in Europe, although more published studies have emerged in the last decade. Molecular methods provide valuable information on the role of past climatic events on the abundance of *Vibrio* bacteria in the environment; however, most of the molecular studies that are focussed on analysing the relationship between climatic variables and *Vibrio* abundance are limited in geographical coverage, with only a small number of published studies using European derived samples (Vezzulli et al., 2012). In Europe, there is a lack of high-quality epidemiological studies, which has limited the study of climate and disease emergence. Especially, there are only a small number of reported shellfish-associated outbreaks linked to climatic anomalies from Europe (Baker-Austin et al., 2010; Martinez-Urtaza et al., 2013). Further to these studies, some specific *Vibrio* risk tools and calculators, addressing the risk of *V. parahaemolyticus* and *V. vulnificus* infection associated with seafood consumption considering environmental conditions at harvesting, are described under Section 3.10.

TABLE 9 Data sources of relevance that underpin the association between climate and disease emergence associated with vibrios.

Data source	Data used in studies	Outputs and geographical scope	Key studies	Applicability and limitations
Ecological studies of vibrios in Europe	Microbiological data from water and shellfish matrices	NW Europe, UK, the Netherlands, France	Ford et al. (2020), Schets et al. (2011), Sterk et al. (2015)	Studies are generally based on microbiological data from specific study sites and focusing on a small number of <i>Vibrio</i> species. Some but not all are linked to epidemiological risk and are generally descriptive in nature.
Retrospective molecular studies of vibrios using environmental samples	Temperature data coupled to long-term archived DNA samples such as continuous plankton recorder samples	North Atlantic, NW Europe	Vezzulli et al. (2012), Vezzulli et al. (2013), Vezzulli et al. (2016)	Some studies using molecular analysis of long-term stored environmental plankton samples provide compelling molecular evidence to suggest vibrios have emerged and proliferated in the last few decades in response to conducive environmental warming trends, such as following warming trends in certain areas (e.g. the North Sea from the 1980s onwards). Key limitation is limited geographical scope of studies and issues with comparing data to various microbiological data sets.
Epidemiological studies concerning vibrios and climate events	Various epidemiological data sets as coupled to climatic data	South America, USA, Canada, Europe and specific studies in NW Europe	Amato et al. (2022), Archer et al. (2023), Baker-Austin et al. (2013)	Studies mainly focus on the role of unusual climatic events such as heatwaves and oceanic anomalies in driving risk, as well as specific FBOs associated with such anomalies. European studies are limited by poor epidemiological metadata associated with human infections. Reporting capabilities may also have changed over time. Paucity of data linking climatic events and human health risk.
<i>Vibrio</i> suitability mapping approaches	Salinity, SST, population density and climatic change based on emission pathways ^a	Uses temperature and salinity to help predict risk of non-cholera <i>Vibrio</i> infections based on exposure using the model described by (Baker-Austin et al., 2013). Validated for Baltic Sea, possibly, has global applications. 7-day forecasting capability.	ECDC E3 Geoportal and NOAA Atlantic OceanWatch ERDDAP Server (Semenza et al., 2017; Trinanés & Martínez-Urtaza, 2021)	This tool predicts the suitability for <i>Vibrio</i> to be present in the environment using temperature and salinity data. It was developed to facilitate use in recreational (bathing) waters. The output does not provide information on the type of <i>Vibrio</i> spp. that may be involved. Considers only water exposure, though it is assumed that wound exposure and food exposure may result in similar infection rates. The underlying data behind this model is now over 10 years old. More recent studies (Trinanés & Martínez-Urtaza, 2021) provide useful information that consider projected warming trends and how these may impact future risks.
NCCOS <i>Vibrio</i> predictive model	Temperature, salinity and growth rate of Vp and occurrence of Vv in Chesapeake Bay.	Expected Vp levels at harvest and after postharvest handling in Chesapeake Bay, doubling time for Vp in specified regions, Vv occurrence in Chesapeake Bay.	Web-based product: https://coastalscience.noaa.gov/products/vibrio-predictive-models/	The model predicts Vp levels in certain specific geographical regions in the USA (e.g. Chesapeake Bay, Delaware, Pacific Northwest, etc.) and occurrence of Vv in Chesapeake Bay. Provides shellfish related guidance in specified regions. Needs to be adapted to other regions. The limitation is that relation between risk and abundance may vary in different regions based on strains, attack rates etc.

Abbreviations: FBO, food-borne outbreak; NCCOS, National Centers for Coastal Ocean Science; NOAA, National Oceanic and Atmospheric Administration; SST, sea surface temperature; Vp, *V. parahaemolyticus*; Vv, *V. vulnificus*.

^aVarious 'emission pathways' are used in climate change research. These are based on different emission trajectories for the globe (e.g. predicted reductions, stabilisations or increases in greenhouse gas emissions), generally to the end of this century. Two of the most commonly used are RCP (Representative Concentration Pathway) 4.5 and 8.5. Most of these studies/approaches use an exposure–response function between sea surface temperature and vibriosis cases and project the annual disease incidence to the end of the century under the 4.5 and 8.5 RCP scenarios (8.5 being the 'worst case scenario').

3.8.2 | Impact of climate change on the occurrence and levels of the relevant *Vibrio* spp. in the aquatic environment at a global level and in Europe (SQ3.2)

A key aspect related to vibrios of PH relevance such as *V. parahaemolyticus*, *V. vulnificus* and non-O1/non-O139 *V. cholerae* is that they tend to grow in warm, low-salinity waters, and their abundance in the natural environment mirrors ambient environmental temperatures (Baker-Austin et al., 2016). The close correlation between bacterial counts and environmental temperature makes vibrios an exceptional microbial group to study the interaction between microbiology, climate and infectious diseases (Figure 6). Numerous ecological studies have looked at the impact of temperature on *Vibrio* concentrations, which is a useful proxy to ascribe changing risks. Average levels of total *Vibrio* spp. abundance have been recorded in the Delaware and Chesapeake Bays (7.9×10^4 and 5.1×10^3 CFU/100 mL, respectively) (Parveen et al., 2020), where non-cholera *Vibrio* infections have recently emerged (King et al., 2019). Total *Vibrio* concentrations of 1.26×10^5 CFU/100 mL were recorded in the UK during a significant heatwave event (Ford et al., 2020).

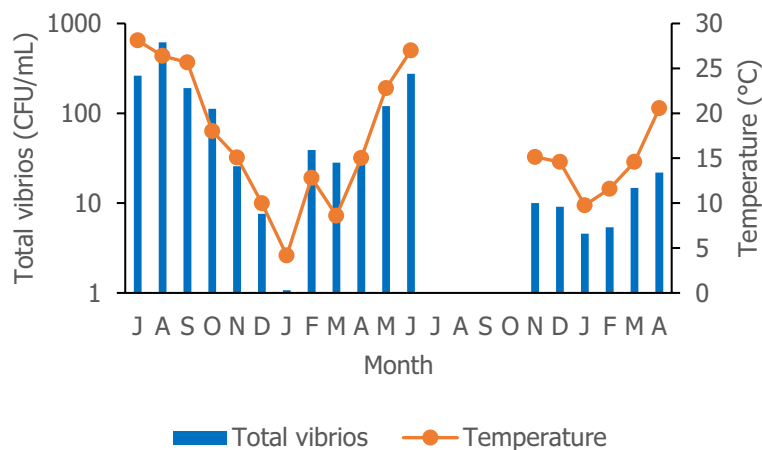


FIGURE 6 Relationship between total *Vibrio* abundance and ambient environmental temperatures (reproduced from Oliver, 2013).

Recently, non-cholera *Vibrio* species have undergone a global expansion, reaching new areas of the world that were previously considered adverse for these organisms (Trinanes & Martinez-Urtaza, 2021). Notable events in colder/temperate regions includes sporadic wound infections reported in the Baltic Sea (Baker-Austin et al., 2013), and outbreaks reported in Alaska (McLaughlin et al., 2005), Canada (Taylor, Cheng, et al., 2018), the East coast of the USA (Martinez-Urtaza et al., 2013) and Chile, the latter linked to the incursion of anomalously warm waters in the region (Martinez-Urtaza, Huapaya, et al., 2008). In all instances, the outbreaks corresponded with rapid warming of usually colder/temperate areas. There is also evidence of changes in the temporal distribution, e.g. extension in reported infections later into the year as observed in NW Europe into the autumn (Baker-Austin et al., 2013). There are various other studies, including an observed increase in *V. vulnificus* infections in the USA during El Niño years (Martinez-Urtaza et al., 2010), which shows how warming can increase infections during the winter. Although long-term data on the prevalence of these bacteria is largely lacking, there is compelling molecular evidence to suggest these bacteria have emerged and proliferated in the last few decades in response to conducive environmental warming trends (Vezzulli et al., 2016). Also, extreme weather events, such as heatwaves and storms, have been associated with *Vibrio* outbreaks and elevated reporting of *Vibrio* cases. Extreme event attribution aims to elucidate the link between global climate change, extreme weather events and the harms experienced on the ground by people, property and nature. To date, however, there is a lack of long-term evidence regarding specific extreme climatic events and *Vibrio* outbreaks. The science in this area, including extreme event attribution (Trinanes & Martinez-Urtaza, 2021),⁵⁴ is rapidly evolving.

Recent modelling studies using projections for global coastal warming indicate that coastal areas suitable for *Vibrio* infections (defined as areas showing SSTs above 18°C and sea surface salinities below 28 ppt) could cover 38,000 km of new coastal areas by 2100 under the most unfavourable climate warming scenarios. The population at risk in suitable regions almost doubled from 1980 to 2020 (from 610 million to 1100 million under the scenario of medium challenges to mitigation and adaptation, SSP2-4.5 – Trinanes & Martinez-Urtaza, 2021). Although this study does not distinguish between food and non-food-borne (e.g. recreational) routes of exposure, it is notable that areas conducive for these bacteria will not expand uniformly. Indeed, various temperate coastal areas and marine systems, and in particular regions representative of brackish/low-salinity systems are at particular risk. Such areas include large bodies of low-salinity water (e.g. Baltic Sea, transitional waters between the Baltic and the North Sea in Europe, the Black Sea), as well as coastal areas which have large riverine inputs or glacial melt inputs that may undergo rapid warming (Chesapeake Bay, Hudson Bay Canada, various coastal areas of NE USA, coastal areas of China, Alaska, etc.). A recent mapping study using a global-based coastal projection and different emission scenarios suggests such areas represent ‘hotspots’ of future *Vibrio* disease emergence (Trinanes & Martinez-Urtaza, 2021). Figure 7 displays the results of the analysis specifically undertaken for the European continent. It is notable that NE Europe represents a key

⁵⁴Extreme event attribution aims to elucidate the link between global climate change, extreme weather events, and the harms experienced on the ground by people, property and nature.

region undergoing increased risks associated with these bacteria. The combination of rapid warming, low salinity and high population densities (and associated exposures) in regions such as the Baltic Sea are important factors modulating risks.

Based on modelling, in 2022, 21 European countries identified *Vibrio* spp.-suitable areas, with a cumulative exposure risk of 2188 days, the third highest recorded. The affected coastline extended to 28,263 km, with an average annual increase of 136 km since 1982. Notably, Baltic Sea bordering nations, like Sweden and Finland, saw significant expansions in suitable coastline. Other countries, such as Belgium (207%) and the Netherlands (131%), also experienced expansions of *Vibrio* spp.-suitable areas (van Daalen et al., 2024).

It should also be considered that shifts in the environmental conditions may lead to changes in the *Vibrio* communities, with higher abundance of those variants better adapted to the new conditions. Warming in coastal areas, indeed, introduce a selective pressure on *Vibrio* populations, selecting for strains with a better fitness. Given the absence of present ecological studies in Europe to assess changes in the structure (i.e. composition) of *Vibrio* populations occurred in the last decades, anticipating how this selective pressure supplied by climate change will affect the *Vibrio* populations in Europe is extremely uncertain. It is unknown if these new conditions may promote the presence of virulent strains over the non-virulent ones or foster the evolution of new variants with virulence traits.

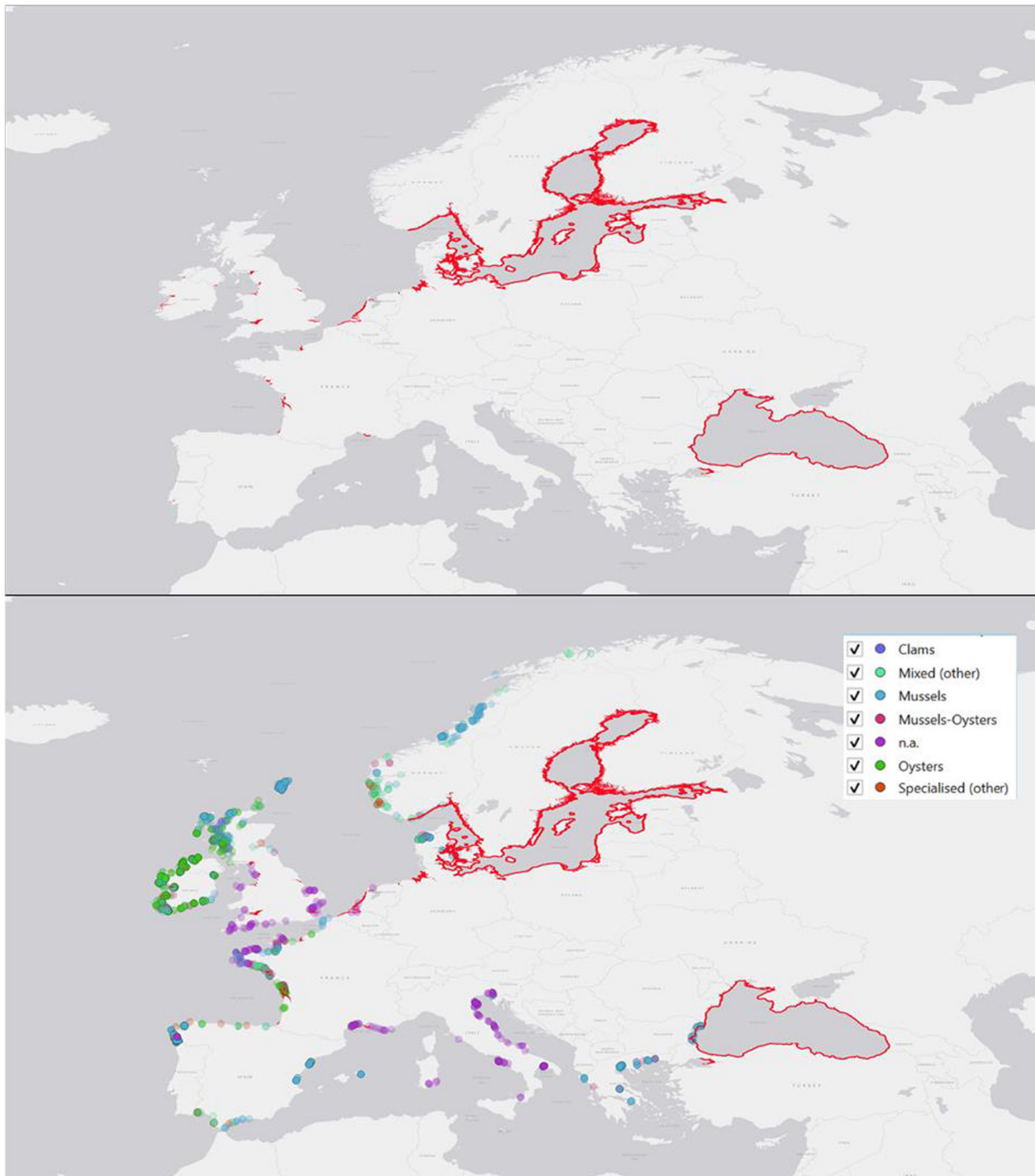


FIGURE 7 The map fields display the maximum monthly *Vibrio* suitability Index (red) and the EMODnet database on shellfish aquaculture in the EU (plus Norway and the UK). Data are missing for Germany, Portugal, Sweden and Croatia. The monthly suitability index is estimated from high-resolution sea surface temperature and sea surface salinity data within 10 km from the shoreline and it is a measure of the local suitability for *Vibrio* infections (when sea surface temperatures are above 18°C and sea surface salinities below 28 ppt). Reproduced from Trinanes and Martínez-Urtaza (2021).

3.8.3 | Impact of climate change on the occurrence and levels of the relevant *Vibrio* spp. in seafood at global level and in Europe (SQ3.3)

There are few data sets that systematically collect relevant information regarding the occurrence and levels of *Vibrio* species in seafood in Europe or globally, which makes the task of establishing the impact of climate change on seafoods particularly problematic. Numerous studies and modelling efforts (ANSES, 2012; Ndraha et al., 2022) have, however, indicated a clear link between elevated environmental temperatures and seafood-related *Vibrio* risks. A recent modelling study indicated that climate change could potentially increase the level of *V. parahaemolyticus* in oysters in Taiwan by 40%–67% in the near future (Ndraha et al., 2022). Elevated environmental temperature prior to and during the outbreak event tend to be a noticeable environmental signature leading to outbreaks. In 1997, a significant warm water incursion into the region of the Pacific Northwest was associated with an unprecedented *V. parahaemolyticus* outbreak (Annex G). In this event, 209 infections were reported, all associated with eating seafood harvested from California, Oregon and Washington in the USA and from British Columbia (BC) in Canada and one person died. This outbreak remains one of the largest ever reported in North America and clearly shows the interaction between anomalous seawater temperatures and disease transmission.

Table 10 summarises various *Vibrio*-associated seafood outbreaks, all caused by *V. parahaemolyticus*, that are likely to be linked to climate anomalies over the last 3 decades. Few studies have been published in Europe to date describing shellfish associated outbreaks (Baker-Austin et al., 2010; Martinez-Urtaza et al., 2016).

TABLE 10 Relevant food-borne *Vibrio*-associated outbreaks caused by *V. parahaemolyticus*.

Year	Country, region	Epidemiology	Climate-related evidence
1997	USA, Pacific Northwest	209 infections reported in Oregon and Washington in the USA and from British Columbia (BC). One fatality ^a	Mean Pacific coastal SSTs were significantly higher (typically 3–5°C) in areas where cases were subsequently reported. Unpublished data (Baker-Austin and Martinez-Urtaza). Clear expansion of anomalously warm water evident during outbreak, see Annex G
1999	Spain, Galicia	35 infections reported in Galicia, NW Spain	Significant sea surface anomaly reported from August–September 1999 (Baker-Austin et al., 2010)
2004	Chile	3400 cases reported in Puerto Montt, Chile. By the end of March 2005, the total number of cases in Chile was 10,783	SSTs were ~3°C higher than the average for this time of year (16°C) (Cabello et al., 2007; Fuenzalida et al., 2006)
2005	USA, Alaska	62 patients with GE. Vp serotype O6:K18 was isolated from most patients tested and from environmental samples of oysters	Notable anomaly. 2004 was the only year during which mean daily temperatures in July and August at the shellfish harvesting area did not drop below 15.0°C (McLaughlin et al., 2005)
2012	Spain, Galicia	100 reported infections in Galicia, NW Spain	Significant sea surface anomaly reported in shellfish harvesting area July and August 2012 (Martinez-Urtaza et al., 2016). Some evidence based on epidemiological data that post-harvest contamination of seafood may have played a role.
2012–2013	USA	During May–July 2012, Vp caused 28 illnesses in nine USA states. 104 persons from 13 states during May–September 2013	Significant SST anomaly along NE USA coastline from April–June 2012, preceding outbreaks. Unpublished data (Baker-Austin and Martinez-Urtaza)
2015	Canada	82 cases reported in British Columbia, vast majority during the summer	SSTs were above the historical levels in 2015 (Taylor, Cheng, et al., 2018)
2021	USA, British Columbia	52 clinically confirmed cases	Significant heat dome event in NW USA and Canada in July 2021. SSTs ~4°C higher than average in implicated area (Puget sound). Unpublished data (Baker-Austin and Martinez-Urtaza)

Abbreviations: GE, gastroenteritis; SST, sea surface temperature.

^aReference: MMWR Morb Mortal Wkly Rep. 1998 Jun 12;47(22):447–62; accessible at <https://www.cdc.gov/mmwr/preview/index98.html>.

3.8.4 | Concluding remarks

- Assessing the impact of climate change on *Vibrio* spp. in water environments and seafood involves utilising a variety of data sources, tools and models.
 - Data sources include environmental monitoring data, microbiological surveillance data, climate and oceanographic data as well as epidemiological case studies.
 - Tools include statistical analysis, risk mapping approaches and predictive modelling tools and retrospective molecular studies.

- Models include correlative and ecological models, process-based models and modelling and mapping approaches that use *Vibrio* suitability (areas showing SSTs above 18°C and sea surface salinities below 28 ppt) based on different future climate emission scenarios.
- The main limitations of these approaches are:
 - challenges with data availability and quality of epidemiological data, especially in Europe;
 - complexity of the interactions between environmental factors, which makes disentangling the effects of climate change on microorganisms difficult;
 - uncertainty inherent with current and future climate projections which impact the reliability of predictions;
 - lack of integration between different types of models, hindering comprehensive assessments.
- Climate change affects the occurrence and levels of the relevant *Vibrio* spp. in the aquatic environment by inducing a shift towards environmental conditions suitable and more conducive for *Vibrio* growth and persistence. It affects:
 - the geographic distribution of the coastal areas suitable for *Vibrio* spp. (expansion of the areas);
 - the temporal distribution of conditions suitable for *Vibrio* spp. (extension of the period);
 - the frequency, distribution and intensity of extreme weather events which may provide – within limited areas and/or time windows – conditions favourable to *Vibrio* spp. These include heatwaves and storms/flooding events.
- In Europe, warming coastal waters have led to an expansion of areas in which *Vibrio* spp. can proliferate, potentially resulting in increased risks of infections associated with seafood consumption and recreational activities. It is predicted that these regions will not increase in the future uniformly, and areas with brackish/low-salinity waters (e.g. Baltic Sea, transitional waters of the Baltic and the North Sea, the Black Sea), and coastal areas with large riverine inputs are at particular risk.
- Europe has also experienced an increase in extreme weather events such as heatwaves over the last two decades. Over this time Europe has witnessed a rise in *Vibrio* infections, including *V. vulnificus* and *V. parahaemolyticus*. There are, however, significant gaps in epidemiological data sets, with only a small number of published reports regarding the impact of climate change on seafood-related outbreaks in Europe.
- It is also possible that climate change may affect the structure of the *Vibrio* populations or speed up the evolution or selection for the emergence of new *Vibrio* variants, although limited data on this currently exists.
- The impact of climate change on the occurrence and levels of the relevant *Vibrio* spp. in seafood is not specifically addressed by current models. Epidemiological observations, however, placed a clear link between increased environmental temperatures and seafood-related *Vibrio* outbreaks.
- Based on knowledge on the effects on water environments, it is anticipated that the occurrence and levels of the relevant *Vibrio* spp. in seafood will increase both globally and in Europe, as they have done in aquatic settings, in response to manifestations of climate change such as coastal warming and extreme weather events as heatwaves, especially in low-salinity/brackish waters.

3.9 | Prevention and control measures along the food chain for the relevant *Vibrio* spp. (AQ4)

Prevention and control measures along the seafood chain for *Vibrio* spp. have been addressed in the risk assessments for France dealing with *V. parahaemolyticus* in seafood (ANSES, 2012) and for Germany dealing with pathogenic vibrios (non-cholera vibrios) in seafood (BfR, 2022). The AESAN Scientific Committee (Spain) considered measures for the prevention and control of *V. cholerae* applicable to imported frozen prawns and other fishery products (AESAN, 2024). Also both FAO/WHO risk assessments (FAO and WHO, 2020, 2021) cover post-harvest treatment technologies for reducing *V. parahaemolyticus* and *V. vulnificus* in seafood. In addition, several review papers deal with post-harvest interventions to reduce vibrios in seafood; some (Ndraha et al., 2020; Spaur et al., 2020) specifically consider *V. parahaemolyticus* in raw oysters, while others (Ekonomou & Boziaris, 2021; Kontominas et al., 2021; Ronholm et al., 2016) consider non-thermal processing of seafood. An overview of the measures contained in each of these documents can be found in Annex H. Of note is that some measures only apply to particular seafood groups and that several control measures were especially studied on oysters as RTE and high-value/price product.

Furthermore, it should be considered that measures to prevent food-borne vibriosis include consumer education. ANSES (2012) made following recommendation:

- to consider that the consumption of raw seafood in summer increases the risk of gastroenteritis (GE) caused by *Vibrio*.
- to place seafood in chilled storage as quickly as possible.
- to respect good hygiene practices when food handling and preparation: (i) consume within 2 h of taking out of the refrigerator and (ii) avoid contact between cooked foods and raw seafood to prevent cross-contamination.
- for patients with chronic liver disease, disease exposing to iron overload, or for immunocompromised persons, presenting increased susceptibility to *Vibrio* infections, to avoid eating raw or undercooked seafood (oysters, mussels, clams, shrimp in particular).

3.9.1 | Refrigeration storage/maintaining cold chain

An important measure to reduce or even prevent the proliferation of any vibrios that are potentially present is maintaining the cold chain from the catch or harvest to the retail outlet. While this is important for any seafood, it is particularly critical for foods intended to be consumed raw (BfR, 2022).

Temperature abuse that may occur during processing, transportation or storage can allow *V. parahaemolyticus* to grow to dangerously high concentrations in oysters (Ndraha et al., 2020). The utility of refrigerated storage was reported in many studies. For example, Gooch et al. (2002) showed that *V. parahaemolyticus* can multiply rapidly in live American oysters (*C. virginica*) oysters held at 26°C, showing an increase of 1.7 logs and 2.9 logs after 10 and 24 h, respectively. Mudoh et al. (2014) reported that *V. parahaemolyticus* concentrations in shellstock Eastern oysters (*C. virginica*) increased ~3 logs during a 10-day storage at 20°C, while the counts remained at the initial level or below at 5 and 10°C. Love et al. (2020) estimated that 75% of oysters in cold chains for US farmed oysters had a net decrease in *V. parahaemolyticus* from harvest to food retail/restaurant. The authors combined a predictive growth model (Parveen et al., 2013) with the tracked internal temperature of oysters. It averaged $4.4 \pm 2.7^\circ\text{C}$ but there were spikes in temperature in some shipments (18% of shipments experienced oyster temperatures $> 10^\circ\text{C}$ for 1 h or more).

3.9.2 | Icing

The icing method involves placing seafood in crushed ice or an ice slurry to chill the seafood as quickly as possible to lower their internal temperature, particularly after harvest, to prevent the growth of food-borne pathogens.

The use of ice slurries was shown to rapidly cool oysters (from 23.9 to 10°C within 12 min) with no significant changes on *Vibrio* spp. (Lydon et al., 2015). Also layered ice is an effective means of rapidly cooling oysters, even in warm summer conditions; resulting in 1.1–1.5 and 0.6–2.0 logs lower *V. vulnificus* and *V. parahaemolyticus* levels, respectively, in oysters, compared to those that were refrigerated post-harvest (Jones et al., 2017). Icing either immediately postharvest or just prior to truck loading did not reduce *V. vulnificus* or *V. parahaemolyticus* levels in oysters (Melody et al., 2008). This was also the case for *V. parahaemolyticus* in raw Pacific oysters (*C. gigas*) over 4 days (Phuvasate et al., 2012).

Of note is that on-board icing appeared to cause significantly higher oyster gaping, which could lead to economic losses unacceptable to the industry (Melody et al., 2008). Storing oysters in ice slurry for prolonged periods is not recommended as vibrios can multiply in the ice slurry water (Lydon et al., 2015).

3.9.3 | Mild heat treatment (with thermal shock)

In mild thermal treatment, oysters are submerged in water at 50°C for a specific processing time while thermal shock involves submerging oysters in water at a given temperature for a specific processing time followed by rapid cooling in ice water (Ndraha et al., 2020).

Submersion of live oysters in water at 50°C for 5 min reduced *V. parahaemolyticus* and *V. vulnificus* concentrations from $5 \log_{10}$ MPN/g to non-detectable levels (Andrews et al., 2000). In another study, 10 min in water at 50°C were needed to reduce *V. vulnificus* from $4.8 \log_{10}$ MPN/g to a non-detectable level; 5 min exposure led to a reduction to $2.3 \log_{10}$ MPN/g (Cook & Ruple, 1992). Ye et al. (2012) exposed inoculated live oysters to 40, 45 or 50°C for up to 20 min. A 15 min treatment at 50°C achieved $> 7 \log_{10}$ reduction.

A thermal shock reduced *V. parahaemolyticus* in oysters from 5 to $6 \log_{10}$ MPN/g to an undetectable level after being heated to 50°C for 8 to 10 min (Andrews et al., 2003). The authors noted that *V. parahaemolyticus* O3:K6 was more heat resistant than the non-pathogenic strains, and recommended a total processing time of at least 22 min at 52°C.

According to Ndraha et al. (2020), inaccurate control of time and temperature (e.g. exposure to $> 53^\circ\text{C}$) affected the sensory quality of oysters (Andrews et al., 2003), but even with strict temperature control, slight changes in colour and smell after treatment of oysters at 50°C for more than 5 min were reported (Ye et al., 2012).

3.9.4 | Freezing

Muntada-Garriga et al. (1995) determined the survival of *V. parahaemolyticus* in oyster meat homogenates at -18°C and -24°C and various bacterial levels ($2\text{--}7 \log_{10}$ CFU/mL). The numbers of *V. parahaemolyticus* showed a logarithmic decline over time with the time of total inactivation depending on the initial levels and temperature. Freezing of oyster meat homogenates inoculated with the higher bacterial levels ($5\text{--}7 \log_{10}$ CFU/g) at -18 and -24°C with storage for 15–28 weeks led to a complete inactivation.

Long-term storage of half-shell oysters at -20°C can reduce *V. parahaemolyticus* and *V. vulnificus* to non-detectable levels (Andrews, 2004). However, this does not occur in a reasonable storage time (< 6 months) when the initial levels were $> 3\text{--}4 \log_{10}$ CFU/g. Shen et al. (2009) showed that populations of *V. parahaemolyticus* decreased from $5.46 \log_{10}$ MPN/g to 1.66 and $0.38 \log_{10}$ MPN/g, after 75 days at -30°C of shucked and shell stock Zhe oyster (*Crassostrea plicatula*), respectively. No cells were detected ($< 3 \log_{10}$ MPN/g) in the shucked oysters after 60 days at -18°C . Detrimental effects of freezing were

greater at -18°C than at -30°C , consistent with greater bacterial damage at the higher temperatures due to the larger intracellular ice crystal formation.

Flash freezing followed by frozen storage can be used for reducing *V. parahaemolyticus* contamination in half-shell Pacific oysters (Liu et al., 2009). Although populations in the oysters declined only slightly (by $0.22 \log_{10}$) after the freezing process (-95.5°C for 12 min), subsequent storage of frozen oysters resulted in considerable reductions. The decline was 2.45, 1.71 and $1.45 \log_{10}$ and 4.55, 4.13 and $2.53 \log_{10}$ after 1 month and 6 months of storage at -10 , -20 and -30°C , respectively. Process validation confirmed that flash freezing, followed by storage at -21°C for 5 months, could achieve $> 3.52 \log_{10}$ reductions of *V. parahaemolyticus* in half-shell Pacific oysters.

A combination of vacuum packaging and freezing decreased *V. vulnificus* levels in oysters by $\sim 3 \log_{10}$ within 7 days post-freezing, and levels continued to drop throughout frozen storage achieving $\sim 5 \log_{10}$ reductions after 70 days. Vacuum-packed freezing was more effective compared to freezing under normal conditions (Parker et al., 1994).

3.9.5 | Treatment with sodium metabisulfite

Sodium metabisulfite (E 223) is authorised as a food additive in unprocessed cephalopods and crustaceans according to Regulation (EC) No 1333/2008. It is traditionally used to control a non-microbiological spoilage symptom of prawns known as melanosis or blackspot (Januário & Dykes, 2005).

According to Januário and Dykes (2005), the exposure of fresh prawns (*Penaeus monodon*) to seawater with sodium metabisulfite (1%) determined an additional decrease (up to $1.8 \log_{10}$ units) of the numbers of *V. cholerae* during chilled storage (1.5°C) for 14 days as compared to chilled untreated prawns ($\sim 4 \log_{10}$ units). Conversely, no significant additional reduction of the numbers of *V. cholerae* was observed in treated prawns stored at -25°C for 49 days as compared to the untreated ones.

3.9.6 | High-pressure processing

High-pressure processing (HPP) is a non-thermal treatment in which foods are subjected to isostatic pressures. Traditionally, this is an in-batch process that can be applied to solid and liquid prepacked foods. Pressure is transmitted instantaneously and uniformly in an isostatic manner so that all parts of the food are subjected to the same pressure simultaneously. An EFSA scientific opinion on the efficacy and safety of HPP of food has been recently published (EFSA BIOHAZ Panel, 2021). Within the industrial context, pressures of between 400 and 600 MPa are most often applied for microbial inactivation, with common holding times ranging from 1.5 to 6 min. The water used as pressure-transmitting fluid is often pre-chilled at 4 – 8°C .

HPP is used in the seafood industry, while it appears to be effective against the most common pathogenic and spoilage bacteria found on seafood products. Microbiological safety has been reported to be assured for many fish and seafood products with higher added value, such as shrimp, salmon, trout, cod and oysters (Ekonomou & Boziaris, 2021).

According to Roobab et al. (2022), 400–600 MPa inactivates common seafood vegetative pathogens, such as *Vibrio* and *Listeria monocytogenes*. HPP using 200–350 MPa is also useful to shuck oysters, lobsters, crabs, mussels, clams and scallops to increase recovery of the edible meat.

V. vulnificus proved to be more pressure sensitive compared to *V. parahaemolyticus* (Koo et al., 2006; Vu et al., 2018; Ye et al., 2012, 2013). Using inoculated oysters, 276 MPa applied for 5 min reduced *V. vulnificus* by > 5.4 logs, while less than 4 logs were achieved on *V. parahaemolyticus* (Koo et al., 2006). A 2-min treatment at 225 or 250 MPa reduced *V. vulnificus* in oyster meat by 4.8 and 7.1 logs and *V. parahaemolyticus* by 1.5 and 3.0 logs, respectively (Ye et al., 2012). In another study, using the same time–pressure conditions (at 20°C), figures were 5.1 and 6.7 logs and 2.6 and 4.1 logs, respectively (Ye et al., 2013). Vu et al. (2018) concluded that *V. vulnificus* was the most pressure sensitive among the four *Vibrio* species tested (i.e. *V. vulnificus*, *V. alginolyticus*, *V. cholerae* and *V. parahaemolyticus*); a > 5 log reduction in mussel homogenates is achieved using (i) 350–450 MPa for ≥ 1 min at 25°C for both *V. alginolyticus* and *V. cholerae*, (ii) 250 MPa for ≥ 3 min or 350–450 MPa for ≥ 1 min for *V. vulnificus* and (iii) 350 MPa for ≥ 3 min or 450 MPa for ≥ 1 min for *V. parahaemolyticus*. Hu et al. (2005) estimated the *D*-values at 276, 310 and 345 MPa as 3.3, 2.7 and 2.0 min using whole oysters inoculated with *V. parahaemolyticus* (O3:K6).

3.9.7 | Irradiation

Different technologies are available and used for the irradiation of food, having to a large extent the same effect on microorganisms. While gamma-rays are produced from a radioactive source, electron-beams and X-rays are produced by specific equipment converting other energy sources, without the involvement of any radioactive substance (EFSA BIOHAZ Panel, 2011). Irradiation methods using UV or LED are emerging technologies (Spaur et al., 2020). In Europe, the Scientific Committee on Food recommended that fish and shellfish could be irradiated at doses up to 3 kGy. A dose of 3 kGy was considered sufficient to effectively reduce non-sporeforming bacterial pathogens by 2 – $5 \log_{10}$ units for most of the fish and fishery products (SCF, 1998).

Irradiation was shown effective to lower the levels of *V. parahaemolyticus* and *V. vulnificus* in oysters. Jakabi et al. (2003) evaluated the effect of gamma radiation (0.5 to 3 kGy) on *V. parahaemolyticus* in oysters (*Crassostrea brasiliana*). A dose of 1 kGy yielded a 6 log₁₀ reduction in the level of *V. parahaemolyticus*. In another study, 1 kGy X-ray resulted in 4.9 and 2 log₁₀ reduction of *V. parahaemolyticus* in half and whole shell oysters, respectively, while >6.5 and 3.1 log₁₀ reduction were achieved using 2 kGy (Mahmoud & Burrage, 2009). Similarly, 1 kGy X-ray resulted in >6.5 and 3.2 log₁₀ reduction of *V. vulnificus* in half and whole shell oysters, respectively, while also >6.5 log₁₀ reductions were achieved using 3 kGy (Mahmoud, 2009). The D-value was estimated as 0.22 min by Hu et al. (2005) after exposing whole oysters inoculated with *V. parahaemolyticus* (O3:K6) to gamma irradiation at doses of less than 3 kGy. The radiation decimal reduction doses of *V. parahaemolyticus* and *V. vulnificus* in inoculated oyster homogenate using gamma irradiation were estimated as 0.159 and 0.140 kGy, respectively (Thupila et al., 2011). In the study by (Park & Ha, 2018), a 0.5, 1 and 2 kGy treatment using gamma irradiation reduced *V. parahaemolyticus* in shucked oysters (*Crassostrea gigas*) by 1.5, 3.2 and 4.2 log₁₀. A dose of 3 kGy did not kill the oysters or affect their sensory attributes (Jakabi et al., 2003).

Ready-to-eat (RTE) **shrimps** inoculated with *V. parahaemolyticus* were treated with 0.1, 0.2, 0.3, 0.5, 0.75, 1, 2, 3 and 4 kGy X-ray resulting in a 1.3; 2.4; 2.8; 3.1; 3.6; 4.9 and >6 log₁₀ reduction of *V. parahaemolyticus*, respectively. Park and Ha (2018) showed that a 0.5, 1 and 2 kGy gamma irradiation treatment reduced *V. parahaemolyticus* in **clams** (*Venerupis philippinarum*) by 1.5, 3.1 and 4.1 logs.

3.9.8 | Cooking

Live bivalve molluscs from B and C production areas (see Section 1.3) that have not been submitted for purification or relaying may be sent to a processing establishment, where they must undergo treatment to eliminate pathogenic microorganisms. A validated methodology must be used. Procedures based on the HACCP principles must be in place to verify the uniform distribution of heat. The permitted treatment methods, according to Regulation (EU) 853/2004, are:

- (a) sterilisation in hermetically sealed containers; and
- (b) heat treatments involving:

- (i) immersion in boiling water for the period required to raise the internal temperature of the mollusc flesh to not less than 90°C and maintenance of this minimum temperature for a period of not less than 90 s;
- (ii) cooking for 3–5 min in an enclosed space where the temperature is between 120 and 160°C and the pressure is between 2 and 5 kg/cm², followed by shelling and freezing of the flesh to a core temperature of –20°C; and
- (iii) steaming under pressure in an enclosed space satisfying the requirements relating to cooking time and the internal temperature of the mollusc flesh mentioned under (i).

According to BfR (2022), maintaining an interior temperature of 70°C in the food to be consumed for 2 min is a reliable method to ensure the inactivation of vibrios. CDC⁵⁵ recommends, for shellfish in the shell, to either boil until the shells open and continue boiling another 3–5 min or to add them to a steamer when the water is already steaming and cook them for another 4–9 min. Shellfish that do not open fully after cooking should not be consumed. Shucked oysters should either be boiled for at least 3 min, fried in oil for at least 3 min at 191°C, broiled 7.5 cm from the heat source for 3 min, or baked at 232°C for 10 min. ANSES (2012) provided the same heat treatments as examples. Also, the New Zealand Food Safety⁵⁶ recommends not to eat shellfish raw or undercooked and to cook them thoroughly until they are at least 65°C for 1 min (until they open and are firm to touch). Leftovers should be thoroughly reheated to a minimum core temperature of 75°C. Among other, Health Canada⁵⁷ also recommends cooking shellfish, especially oysters, thoroughly before eating and keeping raw and cooked shellfish separate.

3.9.9 | Interventions for bivalve molluscs

3.9.9.1 | Depuration

Depuration involves the storage of the live molluscs in tanks through which sufficiently clean, typically disinfected, seawater is circulated to facilitate depletion of mollusc microbial load through normal filtration activity of the molluscs (EFSA BIOHAZ Panel, 2015). Regulation (EC) No 853/2004 spells out the requirements for those purification centres.

Depuration thus maintains oyster viability while they filter clean salt water that either continuously flows through a holding tank or is recirculated and replenished periodically (Campbell et al., 2022). It is practised in several countries for a range of bivalve molluscan species (e.g. clams, oysters, mussels, scallops) and this process may be performed in static, flow through or recirculating systems using water treated with ultraviolet (UV) light, chlorine, iodine or ozone (Lee et al., 2008).

⁵⁵<https://www.cdc.gov/vibrio/prevention>.

⁵⁶<https://www.mpi.govt.nz/dmsdocument/1058-Food-safety-for-seafood-gatherers>.

⁵⁷<https://www.canada.ca/en/public-health/services/food-poisoning/vibrio/prevention-risks.html>.

While depuration has been reported to be effective in removal of human enteric bacteria endogenous bacteria, including coliforms, the reported efficacy for removal of *Vibrio* spp. has not been consistent (FAO and WHO, 2020) and has presented challenges (Campbell et al., 2022). Key depuration parameters like depuration processing time, water salinity, water temperature and water flow rate have been tested and the use of processing additives to enhance disinfection in oysters explored (Campbell et al., 2022). According to Spaur et al. (2020), also the feeding status, water to oyster ratio, and type and size of oysters varied in the studies. According to Ndraha et al. (2020), the efficacy of depuration varied between 1.2 and 3.9 log₁₀, depending on the time and temperature of processing, water salinity, the ratio of oyster to seawater, and the additional application of UV light, gamma irradiation or use of NaClO.

When considering temperature, in inoculated (at 10⁴⁻⁵ MPN/g) American oyster (*C. virginica*), 48 h depuration in artificial seawater at 15°C gave higher reductions of *V. parahaemolyticus* and *V. vulnificus* (2.1 and 2.9 logs, respectively) compared to 22°C. Extended depuration at 15°C for 96 h provided reductions of 2.6 and 3.3 logs, respectively (Chae et al., 2009). In inoculated (at 10⁴⁻⁶ MPN/g) Pacific oysters (*C. gigas*), depuration in recirculating seawater at 7–15°C reduced *V. parahaemolyticus* populations by > 3.0 log₁₀ after 5 days, while no significant reductions were obtained at 2 or 3°C (Phuvasate et al., 2012).

The impact of salinity was investigated in Pacific oysters. While *V. parahaemolyticus* was reduced by 2.1 log₁₀ in UV-irradiated water with a salinity of 10 ppt (at 12.5°C for 5 days), a reduction > 3.0 logs was achieved using 20–30 ppt (Phuvasate & Su, 2013). The study by (Larsen et al., 2015) using high-salinity depuration indicated a potential interaction between salinity and temperature. Oysters were held at a salinity of 35 ppt for 10 days at 20, 22.5 or 25°C. There was no significant effect of temperature on the reduction of *V. vulnificus* and *V. parahaemolyticus* throughout depuration but there was a tendency for lower temperatures to be less effective than the higher ones.

Ramos et al. (2012) compared depuration using UV light and chlorinated seawater from oysters inoculated with *V. parahaemolyticus* (at 10⁴⁻⁵ MPN/g) and *V. vulnificus* (at 10²⁻³ MPN/g). The UV light plus chlorine treatment was more efficient for controlling *V. parahaemolyticus*; after 48 h, the UV plus chlorine treatment reduced counts by 3.1 logs, the UV treatment by 2.4 log₁₀ and the control treatment by 2.0 logs. For *V. vulnificus*, the UV plus chlorine and the UV treatment were equally efficient (reductions of about 2.5 logs).

In conclusion, and according to Campbell et al. (2022), depuration from 4 to 6 days, low temperature, high salinity and flowing water effectively reduced *V. vulnificus* and *V. parahaemolyticus* in live oysters. However, further studies are needed to validate those experiments, in particular with naturally contaminated oysters and in conditions as close as possible to the diverse conditions applied in European shellfish purification centers.

3.9.9.2 | Relaying

Relaying involves moving live bivalve molluscs towards the end of the production phase, to designated seawater areas of relatively low microbial content with the explicit purpose of reducing the microbial hazards in the molluscs through the normal filtration processes. The relaying phase lasts at least 2 months (EFSA BIOHAZ Panel, 2015). According to Reg. (EC) No 853/2004, 'relaying' means the transfer of live bivalve molluscs to sea, lagoon or estuarine areas (that are subjected to classification and used exclusively for the natural purification of live bivalve molluscs) for the time necessary to reduce contamination to make them fit for human consumption. This does not include transferring bivalve molluscs to areas for further growth or fattening. Food business operators must immerse live bivalve molluscs in seawater at the relaying area for an appropriate period, fixed depending on the water temperature, which period must be of at least 2 months' duration unless the competent authority agrees to a shorter period based on the food business operator's risk analysis.

The potential for reducing levels of *V. parahaemolyticus* in live oysters by relaying them during warm weather to a site with elevated salinity and consistently low *V. parahaemolyticus* levels was shown by Taylor, Yu, et al. (2018), achieving a 4.5-log₁₀ decrease in *V. parahaemolyticus* levels after 14 days of relay in one occasion. Results suggested that both microbial community and environmental conditions at the relay sites can affect the effectiveness. Parveen et al. (2017) showed that high salinity relaying of oysters – in high-salinity field sites or recirculating aquaculture systems – was more effective in reducing *V. vulnificus* than *V. parahaemolyticus* in oysters (*Crassostrea virginica*).

3.9.9.3 | Other interventions for bivalve molluscs

Harvesting curfews and harvesting cessation are harvesting restriction approaches. Harvesting curfews aim at harvesting oysters under conditions corresponding to low contamination and growth of *V. parahaemolyticus*. Examples of harvesting curfews include early morning harvests (before the maximum daily temperature) or within specific tidal periods (FAO and WHO, 2021). Harvesting cessation can be used both as a reactive or proactive measure, i.e. as a response to reported illnesses or to prevent them. Proactive measures can be based on temperature or salinity levels in the environment (measured or based on remote sensing models) or can be based on harvesting restrictions during months associated with peaks of illness. The National Shellfish Sanitation Program (NSSP) also developed reactive measures including time to refrigeration and closures based on reported cases (FAO and WHO, 2021).

3.9.9.4 | Other technologies

Kontominas et al. (2021) conducted a review of recent studies on innovative seafood processing technologies applied for the preservation and shelf-life extension of seafood products including HPP, natural preservatives, ozonation, irradiation

(summarised in Section 3.9.6), pulse light technology and retort pouch processing. Ekonomou and Boziaris (2021) conducted a review of research studies for the past 20 years on the application of non-thermal methods for ensuring the microbiological safety and quality of fish and seafood, including apart from HPP (summarised in Section 3.9.5), also ultrasounds, non-thermal atmospheric plasma, pulsed electric fields and electrolysed water as alternative methods to conventional heat treatments. Phage treatment is covered in the review by Ronholm et al. (2016). Its use is discussed for adding a phage isolated from *V. parahaemolyticus* in the depuration water and to decontaminate *V. parahaemolyticus* from oyster meat. Spaur et al. (2020) covered acid treatment but its efficacy on vibrios was only assessed in culture media. These technologies are not covered in this assessment as there is currently only scarce information available on their efficacy on vibrios in seafood.

3.9.10 | Concluding remarks

- Maintaining the cold chain is required as temperature abuse that may occur during processing, transport or storage can allow vibrios to grow (to dangerously high concentrations) in seafood. This is particularly critical for seafoods intended to be consumed raw. Also icing can retard *Vibrio* growth in oysters.
- (Flash) freezing followed by long-term frozen storage reduces vibrios in oysters. A mild thermal treatment of oysters in water at 50°C with or without thermal shock reduces *V. parahaemolyticus* and *V. vulnificus* to a non-detectable level but needs to be well controlled to prevent sensorial changes of oysters.
- HPP is capable to reduce vibrios in seafood using industrially feasible conditions. Irradiation also proved to lower the levels of *V. parahaemolyticus* and *V. vulnificus* in oysters, and *V. parahaemolyticus* in shrimps and clams.
- Other innovative seafood processing technologies could be applied for the preservation and shelf-life extension of seafood products, such as ozonation, pulse light technology, ultrasounds and pulsed electric fields. There is currently only scarce information available on their efficacy on vibrios in seafood.
- Depuration under controlled conditions, although with variable reductions, is a post-harvest processing treatments for the segment of the market preferring consumption of live oysters.
- Furthermore, consumer education can help to prevent food-borne vibriosis. Food safety authorities have recommended to avoid eating raw or undercooked seafood by susceptible persons and to respect good hygiene practices with food handling and preparation.

3.10 | Risk assessment modelling options for the health impact of relevant *Vibrio* spp. in seafood in the EU (AQ5)

3.10.1 | Introduction

To identify the available modelling options, knowledge gaps and data needs to perform a risk assessment on the PH impact of the relevant *Vibrio* spp. in seafood (from Section 3.1), existing risk assessments were summarised (Section 3.10.2) and further characterised (Section 3.10.3). Several factors can inform the decision on which relevant *Vibrio* species and seafoods to select for a risk assessment addressing the PH impact in the EU. Data and knowledge gaps for such an assessment are spelled out in Section 3.10.4.

3.10.2 | Summary of available QMRAs (SQ5.1)

Table 11 summarises a selection of QMRAs or assessments presenting elements of a QMRA and considered of interest to include. Only two studies covered conditions in Europe, one QMRA (ANSES, 2012) and one qualitative risk assessment (BfR, 2022). *V. parahaemolyticus* and *V. vulnificus* but not non-O1/non-O139 *V. cholerae*, have been the subject of the risk assessments. Most studies addressed *V. parahaemolyticus*, and in seafood such as oysters, bloody clams, mussels, sea squirts, varieties of shrimp and different finfish. *V. vulnificus* specifically has only been assessed in raw oysters and a type of octopus.

Various tools and calculators to support risk assessment are also available as Excel files or on the web and have been described by FAO and WHO (2021). These range from tools to estimate abundance and doubling times of *V. parahaemolyticus* in oysters at different sites in the USA to the predicted risk of non-cholera *Vibrio* infections based on exposure estimated from salinity and SSTs in the Baltic Sea.

TABLE 11 A summary of published risk assessments (QMRA and other studies of interest) of *Vibrio* spp. in various types of seafood.

Hazard(s)	Food(s)	Title	Reference/source	Comment
Vp Vc ^a unspecified Vv	Cooked prawns Cooked prawns Oysters	A semi-quantitative seafood safety risk assessment	Sumner and Ross (2002)	Hazard/food combinations ranked using Risk Ranger
Pathogenic Vp	Raw oysters (<i>Crassostrea virginica</i>)	Quantitative risk assessment on the public health impact of pathogenic <i>Vibrio parahaemolyticus</i> in raw oysters	US FDA (2005)	The output is adapted and applied in the Vp calculator tool
Vv	Raw oysters (<i>C. virginica</i>)	Risk assessment of <i>V. vulnificus</i> in raw oysters	FAO and WHO (2005b)	The output is adapted and applied in the Vv calculator tool
Vc ^a O1 and O139	Warm water shrimp	Risk assessment of choleraenic <i>Vibrio cholerae</i> O1 and O139 in warm-water shrimp in international trade	FAO and WHO (2005a)	
Vp	Oysters (<i>C. virginica</i>)	An evaluation of the use of remotely sensed parameters for prediction of incidence and risk associated with <i>Vibrio parahaemolyticus</i> in Gulf Coast oysters (<i>Crassostrea virginica</i>)	Phillips et al. (2007)	Remote sensing data integrated into risk assessment
Vp	Bloody clams	Quantitative modeling for risk assessment of <i>Vibrio parahaemolyticus</i> in bloody clams in southern Thailand	Yamamoto et al. (2008)	
Vp	Raw horse mackerel	Quantitative risk assessment of <i>Vibrio parahaemolyticus</i> in finfish: a model of raw horse mackerel consumption in Japan	Iwahori et al. (2010)	
Vp	Raw oysters, bloody clams, raw horse mackerel	Risk assessment of <i>Vibrio parahaemolyticus</i> in seafood	FAO and WHO (2011)	
Vp	Mussels and oysters	Quantitative risk assessment of <i>Vibrio parahaemolyticus</i> in seafood (Évaluation du risque lié à <i>Vibrio parahaemolyticus</i> lors de la consommation de coquillages vivants)	ANSES (2012)	
Vp	Black tiger shrimps, cooked	The risk assessment of <i>Vibrio parahaemolyticus</i> in cooked black tiger shrimps (<i>Penaeus monodon</i>) in Malaysia	Sani et al. (2013)	
Vp	Raw oysters	A quantitative risk assessment model for <i>Vibrio parahaemolyticus</i> in raw oysters in Sao Paulo State, Brazil	Sobrinho et al. (2014)	
Vp	Bloody clams	Microbial risk assessment of <i>Vibrio parahaemolyticus</i> in bloody clams in Malaysia: A preliminary model from retail to consumption	Malcolm et al. (2016)	
Vp	Raw oysters	The risk of <i>Vibrio parahaemolyticus</i> infections associated with consumption of raw oysters as affected by processing and distribution conditions in Taiwan	Huang et al. (2018)	
Vp	Raw oysters	The risk assessment of <i>Vibrio parahaemolyticus</i> in raw oysters in Taiwan under the seasonal variations, time horizons, and climate scenarios	Ndraha and Hsiao (2019b)	
Vp	Chilled shrimps	Exposure Assessment and Sensitivity Analysis for Chilled Shrimp During Distribution: A Case Study of Home Delivery Services in Taiwan	Ndraha and Hsiao (2019a)	
Vp	Short mackerel	Preliminary quantitative microbial risk assessment of pathogenic <i>Vibrio parahaemolyticus</i> in short mackerel in Malaysia	Tan et al. (2019)	
Vp and Vv	Seafood	Risk assessment tools for <i>Vibrio parahaemolyticus</i> and <i>Vibrio vulnificus</i> associated with seafood	FAO and WHO (2020)	Evaluation of existing Vp and Vv risk calculators

(Continues)

TABLE 11 (Continued)

Hazard(s)	Food(s)	Title	Reference/source	Comment
Vp	Oysters	Influence of climatic factors on the temporal occurrence and distribution of total and pathogenic <i>Vibrio parahaemolyticus</i> in oyster culture environments in Taiwan	Ndraha and Hsiao (2021)	
Vp	Sea squirt (<i>Halocynthia roretzi</i>)	Quantitative microbial risk assessment of <i>Vibrio parahaemolyticus</i> food-borne illness of sea squirt (<i>Halocynthia roretzi</i>) in South Korea	Kang et al. (2021)	
Vc ^a and Vv	Whip-arm octopus	Risk assessment of vibriosis by <i>Vibrio cholerae</i> and <i>Vibrio vulnificus</i> in whip-arm octopus consumption in South Korea	Oh et al. (2021)	
Vp	Shellfish	Quantitative risk Assessment of <i>Vibrio parahaemolyticus</i> in shellfish from retail to consumption in coastal cities of Eastern China Advances in science and risk assessment tools for <i>Vibrio parahaemolyticus</i> and <i>V. vulnificus</i> associated with seafood	Ding et al. (2022) FAO and WHO (2021)	
Vp	Shellfish (oysters and sea urchins)	Prevalence, genomic characterisation, and risk assessment of human pathogenic <i>Vibrio</i> species in seafood	Neetoo et al. (2022)	Only risk associated with oyster consumption only was assessed, using Risk Ranger
Vp, Vv and non-cholera Vc	Seafood (oysters, mussels)	Bacterial food-borne <i>Vibrio</i> infections: health risk assessment of the occurrence of <i>Vibrio</i> spp. (non-cholera vibrios) in food	BfR (2022)	Qualitative assessment
Vp	Roasted shrimp	Quantitative risk assessment of <i>Vibrio parahaemolyticus</i> toxi infection associated with the consumption of roasted shrimp (<i>Penaeus monodon</i>)	Takoundjou et al. (2022)	
Vp	Oysters	Modeling the risk of <i>Vibrio parahaemolyticus</i> in oysters in Taiwan by considering seasonal variations, time periods, climate change scenarios, and post-harvest interventions	Ndraha et al. (2023)	
Vp	Shrimp (with vinegar)	Risk reduction assessment of <i>Vibrio parahaemolyticus</i> on shrimp by a Chinese eating habit	Xu, Liu, et al. (2023)	
Vp	Grey mullet (farmed finfish)	Quantitative risk evaluation of antimicrobial-resistant <i>Vibrio parahaemolyticus</i> isolated from farmed grey mullets in Singapore	Ong et al. (2023)	AMR risk assessment

Abbreviations: AMR, antimicrobial resistance; QMRA, quantitative microbiological risk assessment; Vc, *Vibrio cholerae*; Vp, *Vibrio parahaemolyticus*; Vv, *Vibrio vulnificus*.

^a*Vibrio cholerae* unspecified and O1/O139 are outside the scope of ToR 5 but included for completeness.

3.10.3 | Characterisation of available QMRAs (SQ5.2)

3.10.3.1 | Objectives/scope

The scope of the QMRAs range from harvest to consumption, but most studies include the harvest or the retail stage only as a starting point with the initial prevalence and concentration of *Vibrio* bacteria. Mitigations in the harvest or initial post-harvest processing include practices motivated by SST – considering the effects of seasons and climate change – (e.g. Ndraha & Hsiao, 2019b; US FDA, 2005), depuration (e.g. Ndraha & Hsiao, 2019b; Sobrinho et al., 2014) and washing of fish coming into port (e.g. FAO and WHO, 2011). Almost all studies evaluate the impact of time–temperature during storage at different stages and transport, and several evaluate the effect of cooking or other heating regimes (Annex I). In addition, the potential impact of different pathogen target levels on illnesses averted and percentage of harvest lost has been evaluated (FAO and WHO, 2005b, 2011; US FDA, 2005), as have more specific mitigations such as washing of fish cavities (Iwahori et al., 2010), mixing of food with different vinegars (Xu et al., 2022), different types of markets (Malcolm et al., 2016), different roasting of shrimp protocols (Takoundjou et al., 2022) and inactivation treatments such as thermal treatment, heat shock, irradiation (Ndraha & Hsiao, 2019b), ozone, UV (Sobrinho et al., 2014). The majority of QMRAs have a national or regional scope, also those by FAO and WHO (Annex I). The latter assessments opted for this approach due to data limitations and/or for illustration purposes.

3.10.3.2 | Hazard identification

The hazards covered in the QMRAs were pathogenic *V. parahaemolyticus*, antibiotic-resistant *V. parahaemolyticus*, *V. cholerae*, choleraogenic *V. cholerae* O1 and O139, *V. vulnificus* and *Vibrio* spp., but assessments of non-O1/O139 *V. cholerae* were lacking (Table 12 and Annex J). These hazards were evaluated in various combinations of food or food groups, i.e. bivalve molluscs (raw oysters, mussels, bloody clam, scallop) and cephalopods (raw whip-arm octopus), finfish (raw horse mackerel, short mackerel, wild and farmed finfish, farmed grey mullet), crustaceans (warm water shrimp, raw frozen black tiger shrimp, roasted shrimps, shrimps), sea urchins and sea squirts. The general population was considered in most QMRAs, especially for *V. parahaemolyticus*, although the US FDA (2005) assessment for this hazard also considered the susceptible population. The target populations for *V. vulnificus* were risk groups and susceptible persons (FAO and WHO, 2005b; Oh et al., 2021). Only a single QMRA addressed production and conditions in Europe, in this case regions in France (ANSES, 2012).

3.10.3.3 | Hazard characterisation

A range of health endpoints, from GE, severe illness to death have been included in the DR model for *V. parahaemolyticus*. Of the VFs summarised in Section 3.4, the existing DR models only relate pathogenic strains to the presence of *tdh* for *V. parahaemolyticus* while all *V. vulnificus* strains are considered pathogenic. The most frequently used *V. parahaemolyticus* DR model was developed by US FDA (2005) based on quite old human clinical feeding studies and a total of 20 subjects. Dose is expressed in pathogenic *V. parahaemolyticus*, with the pathogenicity characterised as 'Kanagawa phenomenon positive' (KP+) (see Section 3.4). This phenomenon is related to the presence of *tdh* genes, although several QMRAs apply this DR model while defining pathogenic strains as those possessing either *tdh* or *trh*, which implicitly assumes the same virulence associated with both VFs. Limitations of the feeding studies were pointed out: the immune status of volunteers prior to the experiment, their gender, age and health status were often not reported. When reported, study subjects were males between 25 and 40 years. Further, the dose was not administered with a food matrix, and antacids were administered in most cases (US FDA, 2005). The absence of a food matrix is expected to lead to an underestimation of the probability of illness, since no protection from the low gastric pH is provided to the administered microorganisms. In contrast, the use of antacids would contribute to overestimating the probability, given their protective effect on bacteria from low pH. The data were fitted with a beta-Poisson relationship, as a simplification of the hypergeometric DR model. Parameters α and β were estimated by maximum likelihood (ML), with $\alpha = 0.6$ and $\beta = 1.31 \times 10^6$ giving an ID_{50} (dose for 50% probability of GE) around 2.8×10^6 (Equation 1). Iwahori et al. (2010) estimated model parameters by extending the data using previously excluded feeding trials which resulted in different parameter values but concluded that the choice of DR parameters was not critical for the evaluation of control measures.

$$P_{\text{inf}} = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha}, \quad (1)$$

$$ID_{50} = \beta (2^{1/\alpha} - 1).$$

Epidemiological data were used to shift the location of the curve (on the x-axis), keeping the shape of the previous *V. parahaemolyticus* beta-Poisson DR (multiplying β by 27) leading to a new ID_{50} of 80×10^6 (US FDA, 2005). This was justified because the predictions of the original QMRA model exceeded US epidemiological data. This DR was used for estimating GE cases, both in the general and immunocompromised population. Using other epidemiological data, on culture-confirmed cases, the probability of septicaemia from *V. parahaemolyticus* was estimated separately for healthy or immunocompromised individuals and the probability of death following septicaemia was estimated. The occurrence of septicaemia was modelled as an event conditional on the occurrence of illness, but independent of the dose. The proportion of immunocompromised individuals in the US population was estimated from epidemiological data. The probability of septicaemia for the immunocompromised population was estimated at 0.12, and for the healthy population at 0.0165. Probability of death after septicaemia was estimated at 0.2. The uncertainty about α and β parameters was estimated by non-parametric bootstrap (US FDA, 2005).

Although *V. vulnificus* may cause mild to severe GE that may progress to septicaemia with a high mortality, the only health endpoint considered in the available DR model is septicaemia in the susceptible population (FAO and WHO, 2005b). This model was developed using US data on the estimated exposure and reported number of septicaemia cases between 1995 and 2001. The monthly mean *V. vulnificus* concentration in oysters in these years was estimated from sea water temperature. The number of raw oyster servings was estimated from oyster landings data, with an estimate that 50% of oysters were eaten raw and was calculated based on average oyster weight and typical number of oysters per serving. The mean meal size was assumed to be 196 g oyster flesh per serving. Around 7% of the meals were estimated to be eaten by the susceptible population. This percentage was the estimated prevalence of predisposing conditions among the adult population in the US in 1997. The monthly exposure estimate was then attributed to the monthly observed epidemiological data for the same time period to take seasonal variability into account. The level of reporting was not considered. A beta-Poisson DR model was fitted by ML. The DR assessment assumed that all strains are equally virulent and there are no

seasonal or regional changes in virulence. The best parameters in the DR model to estimate the probability of septicaemia for the immunocompromised population were $\alpha = 9.3 \times 10^{-6}$ and $\beta = 110 \times 10^3$ (FAO and WHO, 2005b). The ID_{50} estimate with these parameters is close to infinite values, which suggests more than 50% of the susceptible population is immune, which is unrealistic since the relationship is for the susceptible population and implies that the model cannot fully describe the DR relationship.

There are limitations associated with the available *V. parahaemolyticus* and *V. vulnificus* DR models (Figure 8). Both models are to a large extent based on quantification and epidemiological data specific to the USA, and doses estimated based on consumption in the US, and strains, oyster species and conditions around the coast of the US. Neither DR assessment has been validated against independent epidemiological data (FAO and WHO, 2020). Further, some assumptions related to pathogenic strains may not be valid (virulence, proportion of pathogenic strains to the total *V. parahaemolyticus* count) and strain variability and food matrix effects were not addressed. The assumptions behind the hypergeometric model are in agreement with the classical microbial mechanistic approach for DR (no threshold, single-hit model) (Haas et al., 1999), but the exact solution may be more appropriate than the beta-Poisson relationship (Teunis & Havelaar, 2000), in particular at low doses. Since the models are based on US epidemiological data, they reflect the distribution of human hosts, consumption, exposure and VFs in the US which may be similar to other parts of the world or not. Thus, validation on other epidemiological data, is needed. However, FAO and WHO (2020) concluded that if the ranges of expected exposures occur where DR models are linear, most often several logs lower than the ID_{50} , then the risk prediction model is also linear, and the DR models could be applied to estimate the relative effectiveness of control measures.

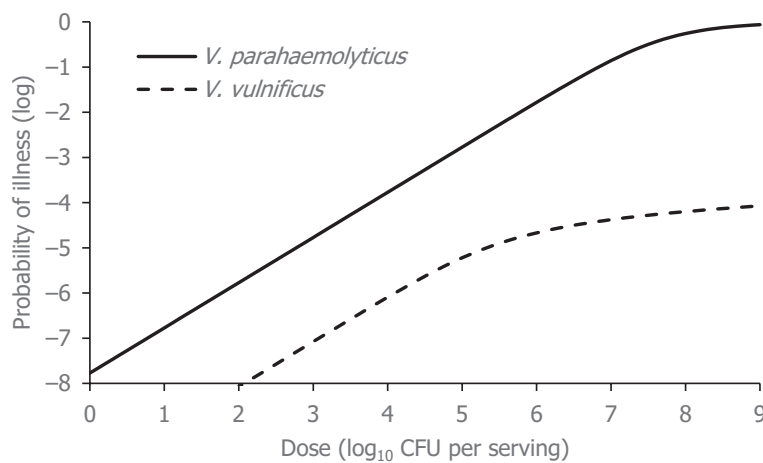


FIGURE 8 Dose–response models for *Vibrio parahaemolyticus* for estimating gastroenteritis cases, both in the general and immunocompromised population (US FDA, 2005) and for *Vibrio vulnificus* septicaemia for the susceptible population (FAO and WHO, 2005b).

3.10.3.4 | Exposure assessment

Initial concentration of pathogenic vibrios in seafood

The initial levels of *V. parahaemolyticus* have been estimated using models either relating environmental parameters, in most cases SST, to the concentration (\log_{10} CFU/g) in shellfish/oysters (ANSES, 2012; Ndraha et al., 2023; Ndraha & Hsiao, 2019b; Sobrinho et al., 2014), or using distributions based on the results of surveys or literature studies of concentration data in bloody clams (FAO and WHO, 2011; Huang et al., 2018; Malcolm et al., 2016; Yamamoto et al., 2008), mackerel (FAO and WHO, 2011; Iwahori et al., 2010; Tan et al., 2019), various sorts of shrimps (Sani et al., 2013; Takoundjou et al., 2022; Xu et al., 2022), sea squirt (Kang et al., 2021), shellfish (Ding et al., 2022) and grey mullets (Ong et al., 2023). For *V. parahaemolyticus* in oysters the most commonly used model was developed by linear regression (US FDA, 2005):

$$\text{mean } \log_{10} V. \text{ parahaemolyticus} / g = \alpha_{\text{reg}} + \beta_{\text{reg}} \times \text{SST} + \epsilon, \quad (2)$$

where α_{reg} and β_{reg} are parameters of the regression and ϵ is a normally distributed error term, $\text{Normal}(0, \sigma_{\text{reg}})$.

In most cases, Equation (2) was used directly, but with data on SST from the geographic area of interest. Sobrinho et al. (2014) and Ndraha et al. (2023) developed their own relationships for oysters based on national data. The latter used a model developed using a machine learning approach (Ndraha & Hsiao, 2021), involving many climate factors with the objective to predict SST due to climate change. In a prior study they used the US FDA model with Taiwan SST data and found that the predicted risk was different compared to when using the new model, indicating that it may not be valid to apply a model developed in one geographic area to another even when using local SSTs. An early illustration of an interesting approach to generate current and large-scale data is the study by Phillips et al. (2007), who used remote sensing to collect environmental data such as SST, turbidity, chlorophyll, later also applied in other studies (e.g. Ndraha and Hsiao (2019b)).

To estimate the dose of pathogenic *Vibrio* to apply in the DR models, the proportion of pathogenic *V. parahaemolyticus* was quantified in the US FDA (2005) QMRA based on the studies by DePaola et al. (2003) and Kaufman et al. (2003). These studies utilised a gene probe technique for enumeration of total and pathogenic *V. parahaemolyticus* considering only *tdh* but not *trh* genes which is a limitation. The study by DePaola et al. (2003) represented the Pacific Northwest region, while the study by Kaufman et al. (2003) represented the Mid-Atlantic and Northeast Atlantic regions. These assumptions were based on the data by Cook et al. (2002) showing that there was no apparent difference in the percentage of *tdh+* *V. parahaemolyticus* in oyster samples among the Gulf Coast, Mid-Atlantic and Northeast Atlantic regions (US FDA, 2005). The percentage of pathogenic *V. parahaemolyticus* strains defined as a Kanagawa-positive (KP+) or thermostable direct haemolysin-positive (TDH+) was approximated with a Beta distribution with an average of 2.33% for the Pacific Northwest and 0.18% for Atlantic regions (US FDA, 2005). The percentage of *V. parahaemolyticus*-positive samples containing pathogenic strains (*tdh+* and/or *trh+*) is highly variable between European studies. For example, in oysters in Catalonia (Lopez-Joven et al., 2015), between 2008 and 2010, the percentage of pathogenic strains in *V. parahaemolyticus*-positive samples, was 29.5%, while in Languedoc shore in oysters, in 2008, it was 5% (Rosec et al., 2012).

An approach applying proportions of pathogenic strains, defined as either KP+/*tdh+* or *tdh+*/*trh+*, to the estimated total number of *V. parahaemolyticus* at harvest, was used in all studies addressing pathogenic *V. parahaemolyticus*. The proportions were based on either the US-FDA data e.g. in oysters (ANSES, 2012), or data from surveys, e.g. in bloody clams (Yamamoto et al., 2008), in black tiger shrimps (Sani et al., 2013), in raw oysters (Sobrinho et al., 2014).

In the two QMRAs addressing *V. vulnificus*, Oh et al. (2021) estimated concentrations based on the analyses of whip-arm octopus samples from a market survey, whereas FAO and WHO (2005b) used a polynomial model, similarly to the *V. parahaemolyticus* model to estimate the concentration of the pathogen in raw oysters (Equation 3). All *V. vulnificus* detected were considered pathogenic.

$$\text{mean log}_{10} V. vulnificus / g = \alpha + \beta_1 \times \text{temp} + \beta_2 \times \text{temp}^2 + \epsilon, \quad (3)$$

with $\epsilon \sim \text{Normal}(0, \sigma)$.

Data – time and temperature

The change of *Vibrio* populations along the food chain being modelled is to a large extent affected by the time and temperature during harvesting, storage, transportation and processing. The distribution of time and temperatures in the stages covered in the assessments were based on literature data, surveys, observations or targeted studies, sometimes combined with expert opinion. For instance, FAO and WHO (2011), based their estimate on the time period from retail to cooking of bloody clams based on local individual practices and expert opinion. Time and temperatures during harvesting, storage, transportation and processing were data gaps commonly reported as limitations of the assessments.

Estimation of growth

Growth, depending on the rate of cooling at harvest, or the times above growth limiting temperatures during storage further up the chain, has been estimated with predictive growth models or by estimating growth rates from occurrence data along the distribution chain.

Predictive models. The US FDA *V. parahaemolyticus* QMRA (US FDA, 2005) employed a simple three-phase log linear model with no lag phase fitted to broth data reported in Miles et al. (1997) and calibrated to growth rates in oysters (Gooch et al., 2002). The calibration data were only representing 26°C and growth rates were four times slower than predicted by the Miles broth model (FAO and WHO, 2020). ANSES (2012) used a model from a more recent publication (Fernandez-Piquer et al., 2011) covering *V. parahaemolyticus* growth in live oysters over a greater temperature range. The Miles model showed a strong effect of salinity on the growth rate in broths and the US FDA model assumed a growth rate corresponding to a salinity of 2.7% NaCl, a condition that may vary across different regions. Other limitations or factors that have not been extensively investigated are the effect of *V. parahaemolyticus* strain and oyster species on growth rates though the limited data suggest systematic differences for both (FAO and WHO, 2020). Iwahori et al. (2010) used the Miles model but with adjustment factors based on *V. parahaemolyticus* growth in horse mackerel, which were also applied in farmed grey mullets (Ong et al., 2023). Kang et al. (2021) inoculated sea squirt with pathogenic strains and developed primary (Baranyi) and secondary models (square root model) for their QMRA. Growth increase during transport based on mean generation times estimated from experiments simulating transport of shrimps were applied in Takoundjou et al. (2022).

For *V. vulnificus*, a linear model developed based on growth rate data in Gulf Coast oysters (Cook, 1994, 1997) and minimum growth temperature from Kaspar and Tamplin (1993), was used in FAO and WHO (2005b). This model was evaluated by refitting data to a square root growth model and comparing predictions with observed rates in American and Asian oysters (FAO and WHO, 2020). The evaluation concluded that the original model is appropriate, although tending towards fail-safe predictions between 15 and 25°C, for predicting growth in at least American oysters (*C. virginica*) and possibly, only one data set available, also in Asian oysters (*C. ariakensis*). The applicability of the model to other seafood species is not evaluated. Oh et al. (2021) inoculated a mixture of *V. vulnificus* and *V. cholerae* strains, justified by similar growth rates,

in whip-arm octopus and developed primary (Baranyi) and secondary (polynomial) models for growth rate and lag phase duration.

Estimation from count data. Data of levels of *V. parahaemolyticus* in bloody clams at harvest and retail indicated that growth occurred in most samples. Based on the assumption that the time from harvest to retail was around 5 h the exponential growth rate was assumed to follow a Normal distribution with mean of 0.236 h^{-1} and standard deviation of 0.390 h^{-1} (FAO and WHO, 2011; Yamamoto et al., 2008), which was used in a simple exponential growth model. This distribution implies around a 25% probability of negative growth, i.e. inactivation, which was not commented on or explained in these studies.

Estimation of inactivation

The reported demarcation between inactivation and growth during storage covers a range of storage temperatures and is likely affected by several factors since different cut-off temperatures have been used (Huang et al., 2018). Inactivation is slow between 5 and 10°C and the definition is further complicated by problems related to detecting VBNC cells. Inactivation of *V. parahaemolyticus* in raw oysters was considered below a no-growth temperature of 8–10°C in the US FDA (US FDA, 2005) risk assessment, using a single rate recorded at 3°C based on Gooch et al. (2002), which may have overestimated inactivation. Huang et al. (2018) assumed inactivation to occur below 12°C, and developed secondary models (Arrhenius, square root models) between 4 and 12°C, based on experiments in inoculated raw oysters, while Spaur et al. (2020) considered < 6°C in their systematic review of post-harvest interventions when estimating log reductions during cold storage. Inactivation of *V. vulnificus* during storage was assumed at $0.041 \log_{10}$ per day at normal storage conditions, 3°C (Cook et al., 2002) and was assumed to be the same within the temperatures range 0 to 13°C using the same storage times as in the *V. parahaemolyticus* in oysters risk assessment (FAO and WHO, 2005b).

In Sobrinho et al. (2014), the effect of depuration combined with UV or ozone treatment was estimated based on few experimental samples using a non-parametric bootstrap approach, and log reductions described by normal distributions. Cooking or heat inactivation was considered in the consumer stage, and most studies used data on log reduction from the literature. For instance, in shrimps (Sani et al., 2013), grey mullets (Ong et al., 2023), and oysters (Ndraha et al., 2023). Malcolm et al. (2016) evaluated two heating regimes, minimally or moderately cooked bloody clams, with log reductions from the literature, and assuming the proportions of consumers applying them. Yamamoto et al. (2008) used a small survey to develop a beta distribution to describe the proportion of consumers cooking or undercooking bloody clams under the assumption that in the first case none and the second case all *V. parahaemolyticus* survives in the bloody clams. Tan et al. (2019) combined data from a kitchen study to estimate the maximum log reduction during cooking of short mackerel and took the minimum log reduction from the literature.

Relationships describing the log reduction of *V. parahaemolyticus* in shrimps treated with three different types of vinegars were developed by fitting distributions to the experimental data (Xu et al., 2022). Further, when evaluating different interventions, Ndraha et al. (2023) used distributions representing the variable log reductions of HPP, irradiation and thermal shock extracted from Spaur et al. (2020).

Estimation of removal and cross-contamination

Tan et al. (2019) addressed the effect of gilling, gutting and washing the eviscerated cavity on reducing the number of *V. parahaemolyticus* during consumer preparation and used a lognormal distribution for the reduction by fitting to data from published literature (Watanabe et al., 1994). Ong et al. (2023) used the same data and developed an additional log normal distribution to describe the log reduction due to only gilling and gutting but no washing of farmed grey mullets. The reduction of *V. parahaemolyticus* from the gills or the surface of fish due to washing of whole fish at landing was modelled in Iwahori et al. (2010) using data in Watanabe et al. (1994). Cross-contamination during handling was raised as a knowledge gap in several of the QMRAs but this process was not modelled in any of the QMRAs.

Estimation of consumption

Data on consumption, dietary habits, food origin and food types are very specific to the country or region of interest. In the QMRAs consumption data, i.e. serving size and frequency of consumption, were based on general national consumption surveys (e.g. raw horse mackerel – Iwahori et al., 2010, black tiger shrimps – Sani et al., 2013, bloody clams – Malcolm et al., 2016, whip-arm octopus – Oh et al., 2021, fish and seafood products – BfR, 2022), on literature data combined with general national consumption surveys (e.g. raw oysters – Huang et al., 2018), or small surveys carried out to complement the available information (e.g. bloody clams – Yamamoto et al., 2008, roasted shrimps – Takoundjou et al., 2022, oysters – Ndraha et al., 2023).

To illustrate the approaches and types of data needed in QMRAs related to European conditions we focus on the two European risk assessments in Table 13 to examine the feasibility in European countries. In ANSES (2012), the consumption was established from a combination of data sets. The overall consumption of oysters/mussels was established from landings data, in tons of shellfish (with shell), converted into tons of edible shellfish. The relative contribution, by area of origin (7 areas of production along the coast) to the overall market was estimated from open data available on the business's websites. Imports were neglected. The seasonality by month of consumption was estimated from sales data, and further

grouped by season. All oysters were assumed to be consumed raw. The average portion size was obtained from the national consumption survey (INCA1), with a mean portion size around 100 g for oysters and 56 g for mussels.

BfR (2022) established short-term consumption (relevant for acute risk) at 3.9 g/kg BW per day for marine/freshwater fish, 4.7 g/kg BW per day for shellfish and 2.6 g/kg BW per day for crustacean based on a national nutrition survey II (NVSII) (2005–2006). The frequency of consumers and frequency of consumption was also retrieved from this survey and showed that the majority (75%) of respondents never consume raw fish/seafood products. The frequency of consumption of such products indicated differences by age group and gender. Limitations highlighted were that the consumption data were based on studies conducted 15 years ago, and important changes in population, habits and products can have occurred, and the breakdown by age groups is inconclusive due to too small groups. Surveys in other European countries can be accessed in the EFSA Comprehensive European Food Consumption Database.⁵⁸ Dietary surveys results are very dependent on the methodology, e.g. 24-h dietary recalls underestimate foods that are not regularly consumed, as in the case of raw oysters (Diogène et al., 2023).

3.10.3.5 | Risk characterisation

Risk characterisation endpoints were the probability of illness (per serving, per person and year, or per a certain number of servings), or number of cases (per year or per 100,000 population, Annex I). The effects of the evaluated factors and control measures were in some studies reported as the relative change in risk due to the mitigation (e.g. Iwahori et al., 2010; Ndraha et al., 2023). Uncertainties were in most studies not addressed explicitly in quantitative terms but as limitations or data gaps. Since production, preparation and consumption practices vary with the type of seafood, results are presented per seafood group.

Bivalve molluscs (oysters, mussels, bloody clams)

Three risk assessments addressing *V. parahaemolyticus* in oysters highlight the importance of the initial total levels at harvest and the proportion of pathogenic strains but other variables that reduce or increase growth also have an impact on the predicted number of illnesses. These variables include shortening the time to refrigeration of oyster after harvest (US FDA, 2005), as well as temperatures during storage and transport (Sobrinho et al., 2014). A common result is the predicted effect of season on the occurrence of *V. parahaemolyticus*, with higher levels during summer in oysters (ANSES, 2012; Ding et al., 2022; Ndraha et al., 2023; US FDA, 2005) and in mussels (ANSES, 2012). For instance, in France an increased risk to consumers due to a higher temperature in the sea water was predicted for oysters (ANSES, 2012), and a 2°C higher than average temperature during summer increased the risk by a factor of 10. Further, 12 h storage at room temperature increased the risk by another factor of 6. Huang et al. (2018) suggested that maintaining temperatures < 12°C during oyster processing and transport would significantly reduce the annual number of *V. parahaemolyticus* infections. Thus, the potential effects of higher temperatures due to climate change are relevant and have been investigated in several studies. Ndraha et al. (2023) investigated different climate scenarios defined as different SSPs (see Section 3.8.1) and mitigations. Ndraha et al. (2023) showed that the SSP selected, the future time span considered, as well as different mitigations influenced the predicted risk of illness due to consumption of oysters in South Korea. In the time period 2041–2060 the risk per serving is predicted to increase by 18% to 145% depending on the season and SSP. Thermal processing or HPP were the most efficient mitigations also under the scenario of increasing global temperatures.

V. vulnificus numbers in oysters at harvest depend on SSTs and salinity, and numbers at consumption are influenced by temperature. Different process target levels achieved by post-harvest mitigation strategies, e.g. mild heat treatment, freezing with extended frozen storage, HPP, were assessed and found to have the capability to reduce risk substantially, from 32 reported cases to one case every 6 year or 8 cases per year depending on the target level, and with a 10-fold uncertainty of predictions (FAO and WHO, 2005b). The predicted effect of reducing the time oysters were unrefrigerated after harvest had less effect but the magnitudes were dependent on the assumptions made.

In Malaysia, the mean *V. parahaemolyticus* risk per undercooked meal of bloody clams was estimated as 6.1×10^{-4} (Malcolm et al., 2016). A sensitivity analysis indicated that the initial pathogenic *V. parahaemolyticus* counts and holding time (retail and home) were the major variable parameters that contributed to the variability in risk of illness per serving. A study in Thailand estimated the risk of illness due to *V. parahaemolyticus* following consumption of bloody clams to 5.6×10^{-4} per person and year, and the fraction of people that do not boil the clams sufficiently was the primary factor increasing risk (Yamamoto et al., 2008).

Cephalopods and tunicates

Two studies from South Korea addressed this seafood group. The mean probability of illness per person and day due to *V. parahaemolyticus* following consumption of sea squirt was 4.0×10^{-9} , and the impact of time and temperature after

⁵⁸<https://www.efsa.europa.eu/en/data-report/food-consumption-data>.

harvest was highlighted as important for the magnitude of risk (Kang et al., 2021). The only *V. vulnificus* assessment in this group considered whip-arm octopus and concluded that the risk per serving in South Korea was low (9×10^{-15} , Oh et al., 2021).

Shrimps

Risk characterisations with *V. parahaemolyticus* in this group covered cooked or roasted black tiger shrimps or unspecified shrimps from Malaysia, Cameroon and China, respectively. The risk was generally considered as low, with yearly 1.3 cases per 100,000 population (Sani et al., 2013), and <2 cases per million servings (Takoundjou et al., 2022), not least due to the heat treatments. Treatment of inoculated shrimps for up to 2 min with different recipes of vinegar were found to reduce risk up to a factor of 2.6 (Xu et al., 2022).

Finfish

Different scenarios were evaluated for raw horse mackerel and in the best-case scenario a contaminated meal contained a mean number of 9 pathogenic *V. parahaemolyticus* and was associated with a mean risk of 5.6×10^{-6} (Iwahori et al., 2010). This scenario was characterised by washing of whole fish at landing, storage and transportation in clean water, no increase in temperature before preparation, and washing of fish visceral cavities during preparation. The presence of contaminated water had a negligible effect, no wash at landing increased risk by 7%, exposure to higher temperatures before preparation increased risk by 50%, and no wash during preparation by 1400%. For short mackerel, besides cooking, washing was considered the main factor impacting on the magnitude of *V. parahaemolyticus* risk (Tan et al., 2019).

The main variables influencing the risk outputs following consumption of farmed grey mullets in Singapore was initial concentration of *V. parahaemolyticus*, and the cooking and washing of the fish cavity both when considering AMR and non-AMR *V. parahaemolyticus* (Ong et al., 2023).

3.10.4 | Evaluation of data and knowledge gaps (SQ5.3)

The selection of the scope of a risk assessment is a risk management decision and involves considerations of the magnitude of the problem (e.g. the number of food-borne cases, consumption patterns, production volumes), various management considerations (e.g. evaluation of control measures, preparedness for climate change, need to rank hazards, foods or management options), the feasibility to perform an assessment (i.e. availability of relevant data, unless the purpose for performing the assessment is to define the needed data). Knowledge gaps and data needs for a *Vibrio* QMRA will in part be general but will also be very specific for the risk management question to be addressed.

QMRA have been developed for both *V. parahaemolyticus* and *V. vulnificus* suggesting that these are possible subjects for an assessment also at the EU level (Annex I). Both species are of interest from food safety and climate change perspectives and the question arises how much new data and knowledge are needed to develop QMRAs relating to the EU. The majority of QMRAs addressed *V. parahaemolyticus* in oysters, and to a lesser extent in other seafoods. The latter reflecting that the main food source of infection can vary between countries putting different emphasis on different seafoods, e.g. the US (oysters) and Japan (sashimi and sushi). FAO and WHO (2021) concluded that the use of the framework and parameters from the *V. parahaemolyticus* model was generally successful to achieve a QMRA for *V. vulnificus* in terms of generating useful insights, e.g. the effect of interventions, and that when additional data were available some aspects of the risk assessment model could be validated (FAO and WHO, 2005b). However, considering the scope and limitations of the original QMRA publications for both *V. parahaemolyticus* and *V. vulnificus*, it is necessary to refine the approaches and to use country- or region-specific data to draw conclusions outside the USA.

The general knowledge and data gaps, related to both *Vibrio* species, include the need to validate DR models using epidemiological data from countries other than the USA, and to update the DR model with new information on virulence, pathogenicity, and host susceptibility. However, the existing models have been deemed sufficient to evaluate the relative effectiveness of mitigations. Further requirements are data on the production of the seafood of interest, i.e. processes, storage conditions, transportation conditions and its relative contribution to total consumption of this seafood in the relevant EU population. In addition, in relation to consumers in the EU, data on consumption frequencies and serving sizes of the seafood of interest, and preparation practices, consumer preferences and storage conditions are needed.

The specific data gaps, i.e. related to assessments of a specific *Vibrio* species and seafood combination, are data on the occurrence of the relevant pathogenic *Vibrio* in the water and the seafood of interest and the relation to factors in the environment. Predictive growth and inactivation models that have been validated in the seafoods of interest, as well as the proportion of pathogenic to total *V. parahaemolyticus* strains in the seafood are also needed.

These are the most important knowledge and data gaps but to what extent they affect the usefulness of a risk assessment is a matter of the intended scope. However, except perhaps for exploratory QMRAs, i.e. screening level risk assessments to identify specific knowledge and data gaps, or to develop estimates of relative risk levels relative to baselines, it is considered that the primary target species for an EU QMRA need realistically be *V. parahaemolyticus*, the reasons being both the known PH impact of this species and the availability of data. However, even for *V. parahaemolyticus*, data on the occurrence (prevalence and levels) in seafoods are limited in the EU context. For oysters the relationship describing the

levels based on SST can be applied but the validity for areas in Europe are not known. [Table 13](#) summarises the knowledge and data gaps that can be foreseen for a range of assessment questions related to a QMRA topic considered relevant at EU level; *V. parahaemolyticus* in bivalve molluscs or oysters. The scope of the assessment could be to evaluate the baseline risk and the effect of mitigations, especially in a climate change scenario. In view of the many gaps, it may be worthwhile to attempt an initial, exploratory, QMRA, with the objective to identify the most important gaps where studies are needed and those where proxy data can be used.

TABLE 12 Examples of variables and parameters expected to be relevant for a range of potential QMRAs addressing *V. parahaemolyticus* in oysters. The availability of this information, limitations and comments on the data gaps are also shown. The final variables will depend on the actual processes and mitigations being evaluated.

Module	Variable	Description	Limitation	Comment
QMRA step: Hazard characterisation				
Dose–response	Dose–response	Beta-Poisson model	The applicability of the model to EU conditions is not known. It is based on old and limited data, and calibrated to US epidemiology	Existing model can be used to estimate relative effectiveness of mitigations in linear dose-range
QMRA step: Exposure assessment				
Harvest	Water temperature	Distribution over seasons and climate change scenarios	Data may exist but uncertain if sufficient resolution	Depending on question, the geographic/spatial resolution may be important in relation to the objectives
	Salinity	Distribution over seasons and climate change scenarios	Data may exist but uncertain if sufficient resolution	The importance of salinity may differ with region and seafood
	Levels of Vp at harvest	Relationship with environmental parameters to model this or survey data needed	Quantitative relationship exists for oysters in some regions but not for other bivalves. Survey data largely missing	Relationship with environmental parameters and potential lag between change of environmental parameter and Vp levels, need to be established in European and/or other areas of production
	Levels of pathogenic Vp at harvest	Data on the proportion of pathogenic Vp	Limited data available for EU	Make assumptions based on literature data and evaluate uncertainty or perform survey
Post-harvest	Collection time	Distribution	Need to be collected or based on other QMRAs	Probably available among producers
	Time out of water	Distribution	Need to be collected or based on other QMRAs	Probably available among producers
	Air temperature	Distribution, for seasons and future climate	Need to be collected	Meteorological data and climate models may not have sufficient resolution
	Growth model	Predictive model	Broth models available	Need to validate in the seafood
	Effect of depuration	Predictive model or log reduction data	Variable effect in the literature indicates impact of local factors	See Section 3.9.9. The effect of depuration needs to be confirmed experimentally on different strains, oysters and environmental conditions
	Effect of HPP, irradiation, cooking, etc	Predictive model or log reduction data	Literature data	See Section 3.9. Much data in (Spaur et al., 2020)
	Temperature and time transport to retail	Distribution	Need to be collected	Surveys need to cover different types of distribution chains from producer to consumer
	Inactivation at retail	Predictive model or log reduction data	Point estimate of die-off rate or secondary model available	
	Time at retail	Distribution	Need to be collected	Literature data or collect from food business operators
	Temperature at retail	Distribution	Literature data	Not specifically for oysters

(Continues)

TABLE 12 (Continued)

Module	Variable	Description	Limitation	Comment
Home preparation	Consumption preference	Proportion eating oysters raw	Probably missing	May be possible to assume based on consumption surveys/literature
	Temperature of heat treatment	Distribution	Probably missing	May be possible to assume based on consumption surveys/literature
	Time of heat treatment	Distribution	Probably missing	May be possible to assume based on consumption surveys/literature
	Inactivation during heat treatment	Predictive inactivation model	No model used in QMRAs, but log reduction	
	Reduction during heat treatment	Distributions of log reductions	Alternative to inactivation model	Use the same as previous QMRAs
Consumption	Serving size	Distribution	Data in some EU/national databases	Possible to use EU data
	Frequency of consumption ^a	Proportion	Depending on survey type may be underestimation	Possible to use EU data
	Proportion of consumers ^a	Proportion	Depending on survey type may be underestimation	Possible to use EU data
	Production landing/volume ^b	Data depending on geographic scope	May or may not involve also imports and reflect actual consumption	
	Number of oysters per meal ^b	Distribution or constant	May not be in EU data bases	Possible to use EU or other data as an assumption
	Weight of an oyster ^b	Distribution or constant	Literature/available among producers	Should be possible to extract from literature or small survey depending on species
	The edible fraction ^b	Distribution or constant	Literature/available among producers	Should be possible to extract from literature or small survey depending on species

Abbreviations: QMRA, quantitative microbiological risk assessment; Vp, *Vibrio parahaemolyticus*.

^aNot required when only risk per serving is estimated but required when total cases per year or cases per some population is estimated.

^bRequired when no consumption data are available.

3.10.5 | Concluding remarks

- In total, 28 risk assessments⁵⁹ have been identified for *V. parahaemolyticus* in seafood (e.g. oysters, bloody clams, mussels, sea squirts, varieties of shrimp and different finfish) and *V. vulnificus* in raw oysters and a type of octopus; no risk assessment was identified for non-O1/non-O139 *V. cholerae* in seafood.
- The geographical scope of the majority of QMRAs is national or regional. Two studies covered conditions in Europe. Most QMRAs rely heavily on the first US FDA assessment of *V. parahaemolyticus* in raw oysters, which early on was applied also to *V. vulnificus*. These QMRAs apply a stochastic Monte Carlo simulation approach where distributions are used to address mainly variability. Uncertainties were mainly addressed as limitations or data gaps and not explicitly in quantitative terms.
- The scope of the QMRAs ranged from harvest – mostly included as a starting point for *Vibrio* prevalence and concentration – to consumption. For *Vibrio* in oysters, this initial occurrence is commonly estimated through regression relationship between mean numbers and SST. Changes in numbers of *Vibrio* spp. in seafood are estimated from time and temperature at different stages in the food chain considering growth (using predictive models or experimental data) and sometimes inactivation and removal/washing (log reductions).
- The beta-Poisson DR models for *V. parahaemolyticus* and *V. vulnificus* are developed based on old experimental data and/or epidemiological data from the USA and the applicability of the models to EU conditions are not known. In addition, the *V. parahaemolyticus* model is only based on *tdh+* strains. The health endpoints are for the general or susceptible populations and range from GE to severe illness for *V. parahaemolyticus* and septicaemia for *V. vulnificus*. Other endpoints or populations are evaluated by multiplying with the proportions of illness cases affected by these outcomes or the proportion of the population of interest to the whole population based on epidemiological and census data.
- Consumption data, dietary habits, food origin and food types are very specific to the country or region of interest and were based on several types of consumption surveys and/or additional consumer related data to estimate exposure.

⁵⁹QMRA's and assessments presenting elements of a QMRA.

- Risk assessment endpoints were the probability of illness (per serving, per person and year, or per a certain number of servings), or number of cases (per year or per 100,000 population). The effects of mitigations were commonly reported as factors representing the change in risk due to the mitigation (i.e. relative risks).
- Mitigations during harvest or directly after included practices motivated by SST – considering the effects of seasons and climate change – and by pathogen occurrence, such as time to refrigeration and depuration. In later stages, practices evaluated were time–temperature during storage and cooking/heating, the effect of setting different pathogen target levels on the number of illnesses averted and the subsequent percentage of non-compliant harvest lost (harvest above the target levels), as well as more specific mitigations (e.g. adding vinegars, washing of fish cavities).
- The impact of climate change scenarios on risk and the effect of different mitigations was only evaluated for *V. parahaemolyticus* in bivalve molluscs, most notably oysters.
- QMRAs have been developed for *V. parahaemolyticus* and *V. vulnificus*, and these are possible hazards for an assessment at the EU level, although it is necessary to refine the approaches and to use country- or region-specific data to draw conclusions outside the US. The selection of the scope of a risk assessment in terms of which *Vibrio* species, seafoods and questions to address, is subject to several considerations and is ultimately a risk management decision.
- A QMRA relevant in an EU context would be *V. parahaemolyticus* in bivalve molluscs (oysters), evaluating the baseline risk and the effect of mitigations, especially in a climate change scenario. The foreseeable specific knowledge and data gaps for a range of risk assessment questions on this topic are presented. An initial, exploratory, QMRA, to identify the most relevant gaps where studies are needed and those where proxy data can be used is suggested.

3.11 | Areas for future research on *Vibrio* spp. in seafood and aquatic environments (AQ6)

The knowledge gaps and the recommendations/priorities for future research related to *Vibrio* spp. in seafood and aquatic environments are shown in Table 13.

TABLE 13 List of knowledge gaps and priorities for future research related to *Vibrio* spp. in seafood and aquatic environments.

Knowledge gaps	Priorities for future research
Absence of epidemiological data on human non-cholera vibriosis in the EU	See recommendations under Section 5
Absence of representative and harmonised data on the occurrence (prevalence and level of contamination) of <i>Vibrio</i> spp. of PH in seafood marketed in the EU	To establish a EU-wide baseline survey on <i>Vibrio</i> spp. of PH relevance ^a in the most significant seafood (to be further defined in the technical specifications of this survey). Surveys should adopt harmonised protocols for sampling, analysis and characterisation of <i>Vibrio</i> isolates, and should be undertaken at:
Absence of baseline data to investigate trends linked to climate change	(i) retail stage, gathering data on the conditions of transport/storage, and origin (country of imports, FAO fishing area)
Absence of robust data on the proportion of <i>Vibrio</i> isolates from seafood marketed in the EU displaying specific genetic traits associated with pathogenicity	(ii) primary production level, gathering data on environmental conditions at sampling sites. This survey could also support identification of sentinel sites for long-term studies investigating <i>Vibrio</i> spp. trends To characterise the isolates collected in the baseline survey with as a prioritisation the determination of the proportion of seafood samples containing pathogenic Vp See also recommendations under Section 5
Absence of standardised, easy methods for quantitative analysis of the <i>Vibrio</i> spp. of PH relevance	To undertake standardisation of quantitative methods , e.g. based on MPN-PCR, for the enumeration of <i>Vibrio</i> spp. of PH relevance and of the <i>tdh+</i> and/or <i>trh+</i> strains of Vp
Lack of full characterisation of the performance of available standardised methods	To produce intercomparison data for ISO and FDA methods and allow the characterisation of methods' performance on VBNC cells
Differentiation of pathogenic strains within a <i>Vibrio</i> species is uncertain and characterisation of VFs of <i>Vibrio</i> spp. of PH relevance is incomplete	To perform characterisation studies of <i>Vibrio</i> isolates of clinical, food and environmental origin, preferably using WGS . See recommendations under Section 5
Absence of systematic WGS typing of <i>Vibrio</i> spp. isolates of food and clinical origin	
Identification of genetic traits associated with epidemic/pandemic potential of some <i>V. parahaemolyticus</i> strains is incomplete	To make use of machine learning approaches to identify eco-evolutionary drivers for pandemic expansion of specific Vp strains
Data on AMR of <i>Vibrio</i> spp. in seafood in the EU (EU production and imported seafood) are incomplete	To establish a EU-wide baseline survey on the prevalence of AMR in <i>Vibrio</i> spp. in seafood. Surveys should include <i>Vibrio</i> isolated from EU/EFTA produced aquaculture animals and imported seafood. Isolates should be phenotypically tested and subjected to WGS

(Continues)

TABLE 13 (Continued)

Knowledge gaps	Priorities for future research
ECOFF breakpoints for antimicrobial susceptibility testing of <i>Vibrio</i> spp. are currently missing	To engage in further research efforts and interlaboratory studies to determine, where missing, epidemiological cut-offs and/or clinical breakpoints for <i>Vibrio</i> spp. of PH relevance, considering also other species of potential PH interest such as <i>V. alginolyticus</i> , <i>V. fluvialis</i> , <i>V. mimicus</i>
The role and weight of mobile genetic elements of vibrios in ARGs dissemination is poorly defined	To engage in basic research to define the contribution of ICEs and superintegrons of <i>Vibrio</i> spp. to dissemination of ARGs and to clarify relevance of HGT in aquatic environments (e.g. aquaculture) and in microbiota of the human gut
The role of antimicrobials residues/degradation products on AMR development/dissemination in the aquatic environments is unclear	Basic research to investigate how the persistence of low concentrations of antimicrobials and their degradation products in aquaculture contribute to AMR of <i>Vibrio</i> spp.
The understanding of persistence mechanisms of <i>Vibrio</i> spp. in the environment is fragmented	Basic research on <i>Vibrio</i> spp. attachment to surfaces and biofilm formation. Ecological studies on the interactions between <i>Vibrio</i> spp. and zoo-/phyto- plankton
Models correlating environmental factors and growth/survival of the <i>Vibrio</i> spp. of PH relevance lack validation in the EU context	To develop and validate models correlating environmental factors, especially SST and salinity, and the occurrence of <i>Vibrio</i> spp. of PH relevance, with priority to Vp, in EU seafood production areas
Lack of specific attribution studies linking role of climate and climate change with human health risk	To gather baseline data on the abundance of <i>Vibrio</i> of PH relevance in the environment over different seasons through a baseline study (see above) and follow-up studies in sentinel areas to study trends associated with climate change. To gather data on the abundance of <i>Vibrio</i> of PH relevance in the environment during specific climatic events (e.g. heatwaves, floods) through targeted studies
Lack of an EU-wide, real-time system for prediction of conditions favourable to <i>Vibrio</i> spp. occurrence in seawater/seafood	To set-up maps of environmental suitability/risk for <i>Vibrio</i> spp. of PH interest in Europe using high-resolution sea surface salinity and sea surface temperature data, with a priority to coastal regions with shellfish harvesting areas in the EU. Following validation, this predictive tool would allow to direct sampling efforts to periods/areas of highest risk
Studies testing effectiveness of interventions on seafood, in particular seafood other than oysters, are scarce	To engage in studies on control measures to better characterise the relationship between the intervention and the reduction in levels of <i>Vibrio</i> spp. of PH relevance in various types of seafood. Such interventions include HPP, relaying and icing
Predictive microbiology models (and limitations thereof)	To develop and validate predictive microbiology models for <i>Vibrio</i> spp. of PH interest with priority to Vp growth and inactivation under relevant conditions in the seafood products considered for RA
Dose–response models (and limitations thereof)	To assess the applicability to the EU conditions and, if needed, update the current dose–response models with priority to Vp, considering information from outbreak investigations (consumption and abundance), strain virulence, pathogenicity and host susceptibility
Prioritisation of data/knowledge gaps	Development of an exploratory QMRA , with the objective to identify the most important gaps affecting risk to guide and allocate research

Abbreviations: AMR, antimicrobial resistance; ARG, antimicrobial resistance genes; FAO, Food and Agriculture Organization of the United Nations; FDA, Food and Drug Administration (United States of America); HGT, horizontal gene transfer; HPP, high-pressure processing; ICE, integrating conjugative elements; ISO, International Organization for Standardization; MPN, most probable number; PCR, polymerase chain reaction; PH, public health; QMRA, quantitative microbiological risk assessment; RA, risk assessment; SST, sea surface temperature; VBNC, viable but non-culturable; VF, virulence factor; Vp, *V. parahaemolyticus*; WGS, whole genome sequencing.

^a*V. parahaemolyticus*, *V. vulnificus* and non-O1/non-O139 *V. cholerae*.

4 | CONCLUSIONS

AQ1/For the relevant *Vibrio* spp., what is their occurrence and concentration in seafood, available analytical methods, pathogenicity to humans and virulence factors, as well as AMR and persistence mechanisms in different environments?

- The *Vibrio* spp. currently of highest public health relevance in the EU through consumption of seafood are *Vibrio parahaemolyticus*, *Vibrio vulnificus* and non-O1/non-O139 *Vibrio cholerae* (referred to as the relevant *Vibrio* spp.). Other species as *Vibrio alginolyticus*, *Vibrio fluvialis* and *Vibrio mimicus* may occasionally lead, particularly in individuals with underlying health conditions, to seafood-associated infections.
- Based on the available data, prevalence and concentrations of the relevant *Vibrio* spp. in seafood placed or intended to be placed on the EU market are estimated as follows:
 - Across seafood categories, the *V. parahaemolyticus* pooled prevalence estimate is 19.6% (95% CI 13.7–27.4), with the highest estimates for bivalve molluscs (27.8%; 95% CI 18.6–39.5) and gastropods (28.8%; 95% CI 10.5–58.3).

- About one out of five (18.4%; 95% CI 11.1–29.1) of *V. parahaemolyticus*-positive samples contained pathogenic (i.e. *tdh+* and/or *trh+*) strains. *V. parahaemolyticus* pooled mean concentration in bivalve molluscs is 1.91 log₁₀ CFU/g or MPN/g (95% CI 0.68–3.14), while in crustaceans is 3.33 log₁₀ CFU/g (single study).
- The *V. vulnificus* pooled prevalence estimate is 6.1% (95% CI 3.0–11.8) and is highest for bivalve molluscs with 9.9% (95% CI 4.0–22.6); the pooled mean concentration in bivalve molluscs is 1.84 log₁₀ CFU/g or MPN/g (95% CI 2.33–6.01).
 - Non-cholerae *V. cholerae* pooled prevalence estimate is 4.1% (95% CI 2.4–6.9) and the mean concentration in cockles is 1.34 MPN/g (single study).
 - No data were retrieved in either tunicates or echinoderms.
- Standardised microbiological methods are available for the detection of the relevant *Vibrio* spp. in seafood and for the enumeration of *V. parahaemolyticus* and *V. vulnificus*. These methods rely on culturing and include molecular tests for species identification and/or for the detection of genes associated with pathogenicity. Alternative approaches are available for the detection, enumeration and identification (e.g. LAMP-PCR, quantitative, digital and viability PCR, MALDI-TOF MS). For characterisation, serotyping and PFGE have been replaced by sequencing technologies [e.g. (cg)MLST schemes]. WGS is progressively more applied for full characterisation of clinical *Vibrio* isolates and in outbreak investigation but currently has limited use in official food control activities in the EU.
 - Pathogenic *Vibrio* species possess a wide array of virulence factors (VFs) that allow the colonisation and spread in the hosts. Their virulence is multifactorial; although with different impact, some VFs may be present in all species (i.e. capsule, flagellar mobility and pili), while other VFs are specific to certain species or strains within one species. The outcome of an infection with *Vibrio* species is determined both by bacterial and host factors.
 - *V. parahaemolyticus* mainly leads to acute GE, also in healthy individuals. Its pathogenicity is significantly associated with the haemolysins TDH and TRH, which are well-established pathogenicity markers. The type 3 secretion system 2 (T3SS2) has been also associated with pathogenic strains.
 - *V. vulnificus* infection are relatively rare and affect mainly individuals with underlying health conditions, possibly leading to sepsis and death. This species possesses several VFs but, as reliable discrimination of pathogenic from non-pathogenic strains is lacking, to date all *V. vulnificus* strains are considered potentially pathogenic.
 - *V. cholerae* non-O1/non-O139 is generally associated with self-limited GE or mild extraintestinal symptoms. However, in susceptible individuals, strains can cause more severe infections, sepsis and death. No single pathogenicity marker has been identified so far.
 - A large spectrum of AMRs, of which some are known as intrinsic resistances, has been reported in the few studies on *Vibrio* spp. isolates from seafood or food-borne infections in Europe. The AMRs most frequently detected were ampicillin (70%–100%; seven studies) and streptomycin (30%–70%; six studies) for *V. parahaemolyticus* and colistin (87%–100%; four studies), ampicillin (4%–75%; five studies) and streptomycin (11%–68%; four studies) for non-O1/non-O139 *V. cholerae*.
 - Antimicrobial resistance genes (ARGs) associated with mobile genetic elements, conferring resistance to various β -lactam types, quinolones, sulfonamides, aminoglycosides, tetracyclines, folate pathway inhibitors and phenicols, have been detected in *Vibrio* spp. Resistances against last-resort antimicrobials as carbapenems and 3rd/4th generation cephalosporins associated with mobile elements are increasingly found in the relevant *Vibrio* spp., and detection in imported seafood isolates has been reported.
 - The main persistence mechanisms of the relevant *Vibrio* species in the aquatic environment include the VBNC state, bio-film formation on biotic and abiotic surfaces, persister cells, anti-grazing strategies to avoid protozoan predation, and association with other aquatic organisms acting as reservoirs.

AQ2/What are the factors in the aquatic environments and in seafood that influence occurrence and growth of the relevant *Vibrio* spp., and affect transmission of their virulence and resistance determinants?

- Survival and growth of the relevant *Vibrio* spp. in the aquatic environments and in food are affected by temperature, (sea)water salinity, solar and UV radiation (extrinsic factors); pH, water activity of the food, nutrient content (intrinsic factors) and predation, parasitism and commensalism (implicit factors). Temperature is the most relevant driver for *Vibrio* abundance in the aquatic environment and in food, followed, in the environment, by salinity. Complex interactions among environmental factors make region-specific environmental models and validations required.
- Transmission of virulence or resistance determinants is affected by the presence of chitin and phages, and by factors of the aquatic ecosystem such as antimicrobials and heavy metals.

AQ3/What is the impact of climate change on the occurrence and levels of the relevant *Vibrio* spp. in water environments and seafood?

- Climate change induces a shift towards conditions in the aquatic environment suitable and more conducive for *Vibrio* growth and persistence. It affects: (i) the geographic distribution of the coastal areas suitable for *Vibrio* spp., with areas characterised by brackish/low-salinity waters (e.g. Baltic Sea, transitional waters of the Baltic and the North Sea, the

Black Sea) and coastal areas with large riverine inputs at higher risk; (ii) the temporal distribution of conditions suitable for *Vibrio* spp.; (iii) the frequency, distribution and potentially intensity of extreme weather events which may provide conditions favourable to *Vibrio* spp. Climate change may also affect the structure of the *Vibrio* populations in the aquatic environments or speed up the evolution or selection of new *Vibrio* variants.

- Although not specifically addressed by current models, it is anticipated that the occurrence and levels of the relevant *Vibrio* spp. in seafood will increase both globally and in Europe in response to coastal warming and extreme weather events as heatwaves, especially in low-salinity/brackish waters.

AQ4/What are the prevention and control measures along the seafood chain for the relevant *Vibrio* spp.?

- Maintaining the cold chain is important to prevent vibrios' growth in seafood. This is particularly critical for seafoods intended to be consumed raw.
- A mild thermal treatment of oysters in water at 50°C with or without thermal shock, (flash) freezing followed by long-term frozen storage, HPP using industrially feasible conditions or irradiation reduce vibrios in seafood.
- Depuration under controlled conditions, although with variable reductions, is a post-harvest processing treatments for the segment of the market preferring consumption of live oysters.

AQ5/What are the risk assessment modelling options for *Vibrio* spp. in seafood and which are the knowledge gaps and data needed to perform a risk assessment on the PH impact of the relevant *Vibrio* spp. in seafood at the EU level?

- The identified QMRA addressed *V. parahaemolyticus* in seafood such as oysters, bloody clams, mussels, sea squirts, shrimp and finfish, and *V. vulnificus* in raw oysters and a type of octopus. No risk assessment addressed non-O1/non-O139 *V. cholerae* in seafood. The majority of QMRAs had a national or regional scope and two covered conditions in Europe. Most rely heavily on the first US FDA assessment of *V. parahaemolyticus* in raw oysters, which was also applied to *V. vulnificus*. Their scope ranged from harvest to consumption. Changes in numbers of *Vibrio* spp. in seafood are estimated from time–temperature at different stages in the food chain considering growth and sometimes inactivation and removal/washing.
- The beta-Poisson DR models for *V. parahaemolyticus* (based on *tdh+* strains) and *V. vulnificus* have limitations and the applicability to conditions in EU is unknown. The endpoints are the probability of illness for the general or susceptible populations and range from GE to severe illness for *V. parahaemolyticus* and septicaemia for *V. vulnificus*. Other endpoints or populations are evaluated by applying proportions to these outcomes based on epidemiological data.
- Consumption data, dietary habits, food origin and food types are very specific to the country or region of interest and were based on several types of consumption surveys and/or additional consumer related data to estimate exposure.
- Mitigations during harvest or directly after, included practices motivated by sea surface temperature (SST) – considering the effects of seasons and climate change – and by pathogen occurrence, such as time to refrigeration and depuration. In later stages, practices evaluated were time–temperature during storage and cooking/heating, the effect of setting different pathogen target levels on the number of illnesses averted and the subsequent percentage of non-compliant harvest lost (harvest above the target levels), as well as more specific mitigations (e.g. adding vinegars, washing of fish cavities). The impact of climate change and different mitigations was only evaluated for *V. parahaemolyticus* in bivalve molluscs, most notably oysters.
- A QMRA relevant in the EU context would be *V. parahaemolyticus* in bivalve molluscs (oysters), evaluating the baseline risk and the effect of mitigations, especially in a climate change scenario.

AQ6/What are areas for future research on *Vibrio* spp. in seafood and aquatic environments?

- A key research priority is to establish an EU-wide baseline survey for the relevant *Vibrio* spp. in relevant seafood products, including at the primary production and retail stages. This survey will support: (i) gathering of representative and harmonised data on the relevant *Vibrio* spp.; (ii) definition of sentinel sites at primary production level to investigate temporal trends in *Vibrio* spp. occurrence; (iii) obtaining a representative collection of food isolates for characterisation (detection of VFs and AMR); (iv) establishing a baseline for future reference in relation to the effect of climate change.
- Other research needs include improving analytical methods (for detection, quantification and AMR testing) and their comparability, improve understanding of *Vibrio* VFs and discrimination of pathogenic strains, gathering data on *Vibrio* abundance during specific climatic events, reassess DR models, develop/validate models on the occurrence of *Vibrio* spp. (particularly *V. parahaemolyticus*) and environmental factors in the EU context, and develop an exploratory QMRA to identify the most important gaps and guide research.

5 | RECOMMENDATIONS

- To develop a case definition for human 'vibriosis' at EU level and to consider vibriosis for compulsory reporting.
- To systematically report data on *Vibrio* spp. occurrence in seafood collected in national monitoring programmes to the EU system for the monitoring and collection of information on zoonoses (Zoonoses Directive 2003/99/EC).

- To systematically characterise *V. parahaemolyticus* isolates of clinical and food origin for pathogenicity (*tdh* and *trh* genes) and *V. cholerae* isolates of clinical and food origin for serotype and/or presence of cholera toxin genetic determinants.
- To characterise by WGS a selection of isolates of *Vibrio* spp. of PH relevance of clinical, food and environmental origin to allow the implementation of genomic surveillance.
- To arrange computational resources to sustain the development and long-term operability of EU-wide *Vibrio* suitability maps operating with high-resolution data of sea surface temperature and sea surface salinity.
- To survey resistances against MIAs associated with mobile elements in *Vibrio* spp. isolates from seafood.

ABBREVIATIONS

AMR	antimicrobial resistance
AQ	assessment question(s)
ARG	antimicrobial resistance genes
BIOHAZ	EFSA Panel on Biological Hazards
CC	clonal complex
CDC	Centers for Disease Control and Prevention (United States of America)
CFU	colony forming unit(s)
cgMLST	core genome multi-locus sequence type
CI	confidence interval
CIA	critically important antimicrobial
CLSI	Clinical and Laboratory Standards Institute
CTX	cholera toxin
DR	dose–response
ECDC	European Centre for Disease Prevention and Control
ECOFF	epidemiological cut-off
ELS	extensive literature search
ENA	the European Nucleotide Archive
ESBL	extended-spectrum- β -lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization of the United Nations
FBO	food-borne outbreak(s)
FDA	Food and Drug Administration (United States of America)
FSANZ	Food Standards Australia New Zealand
Fur	ferric uptake regulator
GE	gastroenteritis
GHG	greenhouse gases
[HA]/protease	haemagglutinin protease
HACCP	hazard analysis and critical control points
HGT	horizontal gene transfer
HlyA	haemolysin A
HPP	high-pressure processing
I^2	intraclass correlation
ICE	integrating conjugative elements
ID ₅₀	dose for 50% probability of gastroenteritis
IPCC	Intergovernmental Panel on Climate Change
ISO	International Organization for Standardization
KP	Kanagawa phenomenon
LAMP	loop-mediated isothermal amplification
LOD	limit of detection
LOQ	limit of quantification
MALDI–TOF	matrix-assisted laser desorption ionisation–time of flight
MARTX	multifunctional autoprocessing repeats-in-toxin
MGE	mobile genetic elements
MIA	medically important antimicrobial
MIC	minimum inhibitory concentration
ML	maximum likelihood
MLST	multi-locus sequence typing
MLVA	multi-locus variable number tandem repeat analysis
MLVST	multi-virulence-locus sequence typing
MPN	most probable number
MS	mass spectrometry
NA	not applicable
NAG-ST	heat-stable enterotoxin

NCBI	the National Center for Biotechnology Information
NCCOS	National Centers for Coastal Ocean Science
NGS	next generation sequencing
NOAA	National Oceanic and Atmospheric Administration
NRL	National Reference Laboratory
PAI	pathogenicity island
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
pFv	fish virulence plasmid
PH	public health
QMRA	Quantitative Microbiological Risk Assessment
qPCR	quantitative PCR
QS	quorum sensing
RA	risk assessment
RASFF	Rapid Alert System for Food and Feed
RID	rho-inactivation domain
RMLE	restricted maximum likelihood estimator
RTE	ready-to-eat
SQ	sub-question(s)
SSP	shared socioeconomic pathway
SST	sea surface temperature
ST	sequence type
T3SS	type III Secretion System
T6SS	type VI Secretion System
TCP	toxin-co-regulated pilus
TDH	thermostable direct haemolysin
ToR	Terms of Reference
TRH	TDH-related haemolysin
US FDA	United States Food and Drug Administration
UV	ultraviolet
VBNC	viable but non-culturable
VF	virulence factor
VFDB	virulence factor database
WG	Working Group
wgMLST	whole genome multi-locus sequence type
WGS	whole genome sequencing
WHO	World Health Organization

ACKNOWLEDGEMENTS

The Panel wishes to thank the following for the support provided to this scientific output: Jens-Andre Hammerl (hearing expert), Jan Baele (EC DG-SANTE RASFF), the Pathogens in Food Database consortium (EFSA grant agreement GP/EFSA/BIOHAW/2023/05) and Kenneth Mulligan and Gianluca Rossi (EFSA staff). The Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

CONFLICT OF INTEREST

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WAIVER

In accordance with Article 21 of the Decision of the Executive Director on Competing Interest Management a waiver was granted to an expert of the Working Group. Pursuant to Article 21(6) of the aforementioned Decision, the concerned expert was allowed to take part in the discussions and in the drafting of the scientific output but was not allowed to take up the role of chair, vice-chair or rapporteur within that time frame. Any competing interests are recorded in the respective minutes of the meetings of the working group of the BIOHAZ Panel.

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How to cite this article: EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Nonno, R., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Baker-Austin, C., Hervio-Heath, D., ... Suffredini, E. (2024). Public health aspects of *Vibrio* spp. related to the consumption of seafood in the EU. *EFSA Journal*, 22(7), e8896. <https://doi.org/10.2903/j.efsa.2024.8896>

APPENDIX A

Uncertainty analysis

TABLE A.1 Potential sources of uncertainty linked to specific AQ or SQ in the assessment of the public health aspects of *Vibrio* spp. related to the consumption of seafood in the EU.

AQ or SQ	Source or location of the uncertainty	Nature or cause of the uncertainty
SQ1.1	Incomplete identification of <i>Vibrio</i> species of public health relevance (beyond Vp, Vv and non-O1/non-O139 Vc)	<p>It is possible that not all available information on human vibriosis cases due to seafood consumption has been retrieved. Multiple data sources (databases, literature, EU member state reports) were used to collect available data and the knowledge of the WG members was consulted.</p> <p>Intrinsic to epidemiological observations, the available information may not contain the complete data due to a systematic underestimation, as human cases may not be further investigated nor reported. Significant underestimation of vibriosis cases in the EU is also expected due to the lack of a specific surveillance system in the EU. Furthermore, the available information may not contain accurate data due to misattribution of cases to different/major species or misattribution of the exposure route (case being attributed to water exposure while being food-borne).</p> <p>Detection and hence reporting of certain species may be biased by the availability of recognised methods or by their established association with human disease.</p> <p>Finally, climate change may affect the occurrence of <i>Vibrio</i> species in seafood reported as occasional and sporadic cause of disease in individual with predisposing conditions, hence affecting their PH relevance.</p>
SQ1.2	<p>Incompleteness of retrieved information</p> <p>Inaccuracy of retrieved information due to the literature screening and data extraction</p> <p>Possible bias of the results</p> <p>Quality of reporting in the studies</p>	<p>It is possible that not all available relevant information on <i>Vibrio</i> occurrence in seafood has been retrieved through the literature review. To overcome this, a systematic review was used with sensitive search strings and the retrieved studies were cross-checked with published review papers.</p> <p>Inaccuracy intrinsic to the study methodology and the data sources (i.e. the retrieved publications) may affect several aspects of the data, including key outcomes, as reported prevalence and counts, or descriptors, as sampling period, area, food origin, analytical methods. To limit this source of uncertainty, only studies addressing surveys data were selected (e.g. studies dealing with methods' validation using naturally contaminated samples were excluded) and individual data on tested and positive/enumerated samples were retrieved for each seafood group and type. Bias assessment was performed at data extraction level.</p> <p>Representative sampling in terms of production areas and production period at primary production level is not used in the retrieved studies. Due to the possible bias of sampling in high-risk areas and during the warmer seasons in the studies, it is expected that the prevalence and counts are overestimated.</p> <p>Data at harvest and quantitative data are limited and not all seafood are well covered (in imports, origin, etc).</p> <p>The quality of reporting was sometimes limited as stated in Section 3.2 and this impacts the quality and precision of the results.</p>
SQ1.3–1.4	Incompleteness of information	<p>It is possible that not all available information on methods used for <i>Vibrio</i> spp. has been retrieved. Multiple data sources (databases, literature, EU member state reports) were used to collect available data and the knowledge of the WG members was consulted.</p> <p>It is possible that not all strengths, weaknesses, opportunities and threats associated with the contribution of analytical methods to RA have been captured.</p>
SQ1.5	<p>Incompleteness of retrieved information</p> <p>Scarcity of data, uncertainty to what extent representative</p>	<p>It is possible that not all available information on pathogenicity and VFs has been retrieved. Multiple data sources (databases, literature, EU member state reports) were used to collect available data and the knowledge of the WG members was consulted.</p> <p>Uncertainty intrinsic to the studies are present with regard to the significance of data considering that some VFs and corresponding genetic markers have been evaluated only in few instances on isolates from a restricted number/type of sources (limited variability and data not reproduced independently).</p> <p>Limited amount of data on the specific role of TRH in Vp pathogenicity (independently from TDH) and scarcity of data on genetic markers associated with pathogenicity of non-O1/non-O139 Vc</p>

TABLE A.1 (Continued)

AQ or SQ	Source or location of the uncertainty	Nature or cause of the uncertainty
SQ1.6–1.7	Incompleteness of information	It is possible that not all of the available information was retrieved concerning European data on AMR of Vp, Vv, and Vc from retail and primary production. Multiple data sources (databases, literature, EU member state reports) were used to collect data and, additionally, the knowledge of the WG members and of hearing experts was included.
	Inaccuracy of retrieved information due to data reporting	The AMR profiles of clinical strains of Vp and Vc may only partially reflect the AMR of strains causing food-borne infections as specific information on source of infection are lacking (travel associated cases, seawater contact, and other unknown origin represent confounding factors).
	Scarcity of data and limitations of studies	The number of European studies on AMR of vibrios is low, particularly for Vv; relevant phenotypes may not have been identified. Significant differences of reported AMR prevalence between studies may depend on variations of the investigated matrices, but can also be attributed to methodological factors (choice of different antibacterial compounds, application of different antimicrobial susceptibility tests, i.e. disc diffusion/MIC). Furthermore, the interpretative criteria (e.g. CLSI, EUCAST) for resistance or susceptibility may have changed over time.
SQ1.8	Incompleteness of information	It is possible that not all available information on <i>Vibrio</i> persistence has been retrieved. Multiple data sources (databases, literature, EU member state reports) were used to collect available data and the knowledge of the WG members was consulted.
SQ2.1	Incompleteness of information	It is possible that not all relevant information has been retrieved, which could leave out factors affecting growth and survival of <i>Vibrio</i> in the environment and in food or could underestimate/overestimate the role of certain factors. To reduce this uncertainty, results from the ELS for reviews were complemented by snowballing, non-systematic search, and expert knowledge.
SQ2.2	Incompleteness of information	It is possible that not all available information on factors affecting transmission of VFs and AMR has been retrieved. Multiple data sources (databases, literature, EU member state reports) were used to collect available data and the knowledge of the WG members was consulted.
AQ3	Incompleteness of data and information regarding climate change	With regard to data sets and sources of information used to infer the relationship between climate and vibrio risk, in Europe there is a limited availability of high-quality of data, particularly for epidemiology data sets. The complexity of interactions between environmental factors can make it difficult to isolate the effects of climate change e.g. on growth of these bacteria from other factors. Few attribution studies, which tie particular outcomes (e.g. outbreaks) to specific climate change induced events, are available. Model uncertainty is inherent to the tools adopted for climate change study, and uncertainties associated with future climate projections can impact the reliability of predictions. Additionally, there may be a lack of integration between different types of models, hindering comprehensive assessments.
AQ4	Scarcity of studies testing interventions on seafood, in particular seafood other than oysters	Most studies assessed the impact of interventions on reduction of vibrios in oysters while few studies are available in other seafoods, such as shrimps. Even for oysters, many studies tested the interventions in oyster meat or oyster homogenate.
AQ5	Incompleteness of information	It is possible that not all available relevant information on <i>Vibrio</i> QMRA and seafood has been retrieved which could limit the analysis in terms of missing some useful approaches, models, or data.
	Scarcity of data, uncertainty to what extent representative for conditions in Europe	The data and knowledge gaps have been listed in Section 3.11

Abbreviations: AMR, antimicrobial resistance; AQ, assessment question; CLSI, Clinical and Laboratory Standards Institute; ELS, extensive literature search; EUCAST, the European Committee on Antimicrobial Susceptibility Testing; MIC, minimum inhibitory concentration; PH, public health; QMRA, quantitative microbiological risk assessment; RA, risk assessment; SQ, sub-question; TDH, thermostable direct haemolysin; TRH, TDH-related haemolysin; Vc, *Vibrio cholerae*; VF, virulence factor; Vp, *Vibrio parahaemolyticus*; Vv, *Vibrio vulnificus*; WG, Working Group; WGS, whole genome sequencing.

ANNEX A

Protocol for the assessment of the public health aspects of *Vibrio* spp. related to the consumption of seafood in the EU

Annex A is available under the Supporting Information section on the online version of the scientific output.

ANNEX B

Food Standards Australia New Zealand information request

Annex B is available under the Supporting Information section on the online version of the scientific output.

ANNEX C

***Vibrio parahaemolyticus*, *Vibrio vulnificus* and non-O1/non-O139 *Vibrio cholerae* human cases associated with seafood consumption in Europe**

Annex C is available under the Supporting Information section on the online version of the scientific output.

ANNEX D

Analytical methods for the detection and enumeration of potentially enteropathogenic *Vibrio* spp. in seafood.

Annex D is available under the Supporting Information section on the online version of the scientific output.

ANNEX E

Antimicrobial resistances of the relevant *Vibrio* spp. from clinical and seafood associated isolates from Europe as reported in official reports, reviews and primary research papers

Annex E is available under the Supporting Information section on the online version of the scientific output.

ANNEX F

Effect of temperature on *Vibrio* spp. in seafood

Annex F is available under the Supporting Information section on the online version of the scientific output.

ANNEX G

***Vibrio parahaemolyticus* outbreak associated with an increase of seawater temperature (Pacific Northwest, 1997)**

Annex G is available under the Supporting Information section on the online version of the scientific output.

ANNEX H

Overview of the prevention and control measures along the seafood chain for *Vibrio* spp.

Annex H is available under the Supporting Information section on the online version of the scientific output.

ANNEX I

Characterisation of relevant (quantitative) microbial risk assessment of *Vibrio* spp. in various types of seafood

Annex I is available under the Supporting Information section on the online version of the scientific output.