Vibrio spp. infections

Craig Baker-Austin[1], James D. Oliver[2,3], Munirul Alam[4], Afsar Ali[5], Matthew K. Waldor[6], Firdausi Qadri[4] and Jaime Martinez-Urtaza[1]*

Abstract | *Vibrio* is a genus of ubiquitous bacteria found in a wide variety of aquatic and marine habitats; of the >100 described *Vibrio* spp., ~12 cause infections in humans. *Vibrio cholerae* can cause cholera, a severe diarrhoeal disease that can be quickly fatal if untreated and is typically transmitted via contaminated water and person-to-person contact. Non-cholera *Vibrio* spp. (for example, *Vibrio parahaemolyticus*, *Vibrio alginolyticus* and *Vibrio vulnificus*) cause vibriosis — infections normally acquired through exposure to sea water or through consumption of raw or undercooked contaminated seafood. Non-cholera bacteria can lead to several clinical manifestations, most commonly mild, self-limiting gastroenteritis, with the exception of *V. vulnificus*, an opportunistic pathogen with a high mortality that causes wound infections that can rapidly lead to septicaemia. Treatment for *Vibrio* spp. infection largely depends on the causative pathogen: for example, rehydration therapy for *V. cholerae* infection and debridement of infected tissues for *V. vulnificus*-associated wound infections, with antibiotic therapy for severe cholera and systemic infections. Although cholera is preventable and effective oral cholera vaccines are available, outbreaks can be triggered by natural or man-made events that contaminate drinking water or compromise access to safe water and sanitation. The incidence of vibriosis is rising, perhaps owing in part to the spread of *Vibrio* spp. favoured by climate change and rising sea water temperature.

[1]*Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth, UK.*

[2]*Department of Biological Sciences, University of North Carolina, Charlotte, NC, USA.*

[3]*Duke University Marine Laboratory, Beaufort, NC, USA.*

[4]*icddr,b, formerly known as the International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh.*

[5]*Department of Environmental and Global Health, College of Public Health & Health Professions and Emerging Pathogens Institute (EPI), University of Florida, Gainesville, FL, USA.*

[6]*Department of Microbiology, Harvard Medical School, Boston, MA, USA.*

**e-mail: craig.baker-austin@cefas.co.uk*

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Vibrio spp. are a group of common, Gram-negative, rod-shaped bacteria that are natural constituents of freshwater, estuarine and marine environments[1]. *Vibrio* spp. share several biological and genomic features. Their genomes are divided between two chromosomes, which have been shaped by recombination and horizontal gene transfer (HGT; that is, the acquisition of genetic material by transfer from other organisms). Although these pathogens may be genomically diverse, they all originate from aquatic and marine environments: they prefer warm, brackish (slightly salty) water, and their abundance in the natural environment tends to mirror environmental temperatures. Numerous studies show that sea water is the ecological niche of many microorganisms. Indeed, many bacterial pathogens are frequently encountered in sea water, including *Escherichia coli*, *Salmonella* spp., *Proteus* spp., *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and *Staphylococcus aureus*. These bacteria have been shown to cause an array of clinical manifestations, including otorhinolaryngological, ophthalmological, digestive and dermatological infections[2]. *Vibrio* spp. are responsible for the majority of human diseases attributed to the natural microbiota of aquatic environments and seafood[3]; the most common pathogenic species are *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio alginolyticus*. Cases of *Vibrio* spp. infections have a marked seasonal distribution, with most cases occurring during warmer months. *Vibrio* spp. infections are usually initiated from exposure to contaminated water or consumption of raw or undercooked contaminated seafood and cause a variety of symptoms in humans[4] (TABLE 1).

Human diseases caused by pathogenic bacteria of the *Vibrio* genus can be divided in two major groups: cholera and non-cholera infections. *V. cholerae* is the aetiological agent of cholera[5], a severe diarrhoeal illness usually caused by ingestion of contaminated food or water, although person-to-person transmission is also possible. Of note, *V. cholerae* can also be found in fresh water. Non-cholera *Vibrio* spp., such as *V. parahaemolyticus* and *V. vulnificus*, cause vibriosis, a group of infections with different clinical manifestations depending on the pathogen species, route of infection and host susceptibility. For example, ingestion of non-cholera bacteria can cause mild gastroenteritis or primary septicaemia (that is, septicaemia following ingestion of raw or undercooked contaminated food), whereas exposure of skin wounds to contaminated water can cause wound infection that can result in secondary septicaemia. Non-cholera *Vibrio* spp. occupy habitats of moderate or high salinity and can be found in sea water and seafood. These bacteria are the most important environmental human pathogens that originate from aquatic and marine habitats (FIG. 1).

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Table 1 | **Most important *Vibrio* spp. that can cause infections in humans**

Species	Source of infection			Route of infection		Clinical manifestations
	Seafood	Sea water	Fresh water	Oral	Wound exposure	
Vibrio cholerae (O1 or O139 strains)	Rarely	Rarely	Yes	Yes	Rarely	Cholera and gastroenteritis; rarely wound infections
Vibrio cholerae (other strains)	Yes	Yes	No	Yes	Yes	Gastroenteritis and wound and ear infections; rarely primary septicaemia
Vibrio parahaemolyticus	Yes	Rarely	No	Yes	Yes	Gastroenteritis and wound infections; rarely sepsis
Vibrio vulnificus	Yes	Yes	No	Yes	Yes	Gastroenteritis, wound infections and sepsis
Vibrio alginolyticus	No	Yes	No	No	Yes	Most commonly ear and wound infections; rarely sepsis
Vibrio fluvialis	No	Yes	No	Yes	Yes	Gastroenteritis; more rarely wound, eye and ear infections
Vibrio hollisae[a]	Yes	Yes	No	Yes	No	Gastroenteritis and wound infections; rarely sepsis
Vibrio mimicus	Rarely	Yes	No	Yes	Yes	Gastroenteritis; more rarely wound, eye and ear infections
Vibrio metschnikovii	No	Yes	No	Probably	No	Gastroenteritis and sepsis

[a]Also known as *Grimontia hollisae*.

The exact number of *Vibrio* spp. infections worldwide is uncertain because of limitations in existing surveillance systems, under-reporting or failure to report infections, differences in reporting procedures and the lack of international systems of epidemiology. *V. cholerae* has played a substantial part in shaping human history. Although cholera is rare in the developed world, it continues to represent a major cause of morbidity and mortality worldwide, especially in developing countries in Asia, Africa and Latin America, where *V. cholerae* is endemic, population density is high, sanitation is poor and access to safe drinking water is scarce. By contrast, vibriosis outbreaks are becoming increasingly frequent in developed countries, owing in part to a raised ocean temperature that favours the spread of non-cholera *Vibrio* spp.[6]. In countries that gather epidemiological data on these pathogens, such as the United States, large numbers of both waterborne[7] and foodborne[8,9] vibriosis predominate.

This Primer provides an overview of the ecology, epidemiology and public health relevance of *Vibrio* spp. as well as the mechanisms of disease and provides an outline of the diagnosis, treatment and outlook associated with *Vibrio* spp. infections. We focus on *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* as these are the most studied pathogens of greatest epidemiological importance; we highlight recent research work that has redefined our understanding of these pathogens, outline current knowledge gaps and speculate on future research work in this important set of pathogens.

Epidemiology

Vibrio spp. represent a diverse group of human pathogens, and the epidemiology associated with these bacteria is similarly complex. Although national and international agencies such as the Centers for Disease Control and Prevention (CDC) and the WHO gather global epidemiological data on these pathogens, currently no global systematic surveillance framework exists, and few individual countries have dedicated surveillance systems for *Vibrio* spp. One exception is in the United States, where epidemiological data have been gathered systematically since 1988 through the Cholera and Other *Vibrio* Information Service (COVIS) CDC programme[7]. Vibriosis has been notifiable in all 50 states of the United States since 2007. This type of national surveillance system is extremely useful because it provides key insights into routes of exposure, changes in incidence and geographical and epidemiological characteristics associated with *Vibrio* spp. infections. A 2012 estimate suggests a substantial increase in foodborne-associated *Vibrio* spp. infections in the United States[7]. The annual incidence of reported vibriosis increased over threefold from 0.09 cases per 100,000 population in 1996 to 0.29 cases per 100,000 population in 2010 (REF.[7]).

Vibrio cholerae

An estimated 3–5 million people contract cholera worldwide annually[10,11], with ~100,000 deaths[12] (BOX 1); in endemic countries, about half of the deaths occur in children of <5 years of age[13]. Cholera has its highest incidence in children of <5 years of age; about half of all cholera cases occur in this age group, although the incidence varies annually[14], presumably related to climate and hitherto unknown factors. For centuries, cholera has been endemic in Asia, mainly in the Ganges delta of the Bay of Bengal[15], Bangladesh and India[16]. Asiatic cholera has exploded at several different times, spread rapidly and resulted in waves of global pandemics (FIG. 2). The spread of cholera outside of Asia is largely mediated by human activities[17,18]. Cholera was likely introduced through infected humans to Africa, Haiti and Latin America, and probably also historically to Europe and

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the United States[19,20]. The seventh pandemic continues to be a major public health threat for 175 countries in Asia, Africa and the Americas. Cholera has long been established as a climate-driven disease[21,22], and in major endemic settings of Asia, it shows seasonal peak patterns at defined times of the year[23].

The infection pattern is not well defined for many affected regions, although major factors associated with cholera outbreaks worldwide include sea surface temperature[24,25], sea surface height[24] (that is, alterations in average sea surface topology), the temperature of fresh water[26], plankton blooms[27,28], precipitation[29] and flooding[30]. The epidemiological patterns of cholera suggest a strong estuarine link for the disease, as outbreaks tend to start in the coastal regions before cases occur in inland areas[31,32]. Epidemiological studies have shown that *V. cholerae* is transmitted mainly in a faecal–oral mode via contaminated drinking water, although the bacterium can also transmit through person-to-person close contact[33,34] and through food[35]. *V. cholerae* has >200 serogroups that are distinguished on the basis of the chemical composition of the O-antigen of lipopolysaccharide (LPS). O-Antigens are immunodominant and can be used for serological classification of *V. cholerae* on the basis of the characteristics of the sera generated by immunization with different serogroups. The vast majority of cholera cases are caused by serogroup O1. The O1 serogroup is divided into two main serotypes: Ogawa and Inaba. The serogroup O1 is also divided into biological variants known as biotypes — classical and El Tor — and both of these biotypes can be either Ogawa or Inaba serotypes. The classical and El Tor biotypes differ in a variety of phenotypic traits; the classical biotype was responsible for the first six cholera pandemics (which are considered concluded), whereas the ongoing seventh pandemic is caused by the El Tor biotype, which in 1961 replaced the classical biotype[16] (FIG. 2). El Tor was replaced temporarily in 1992 by a

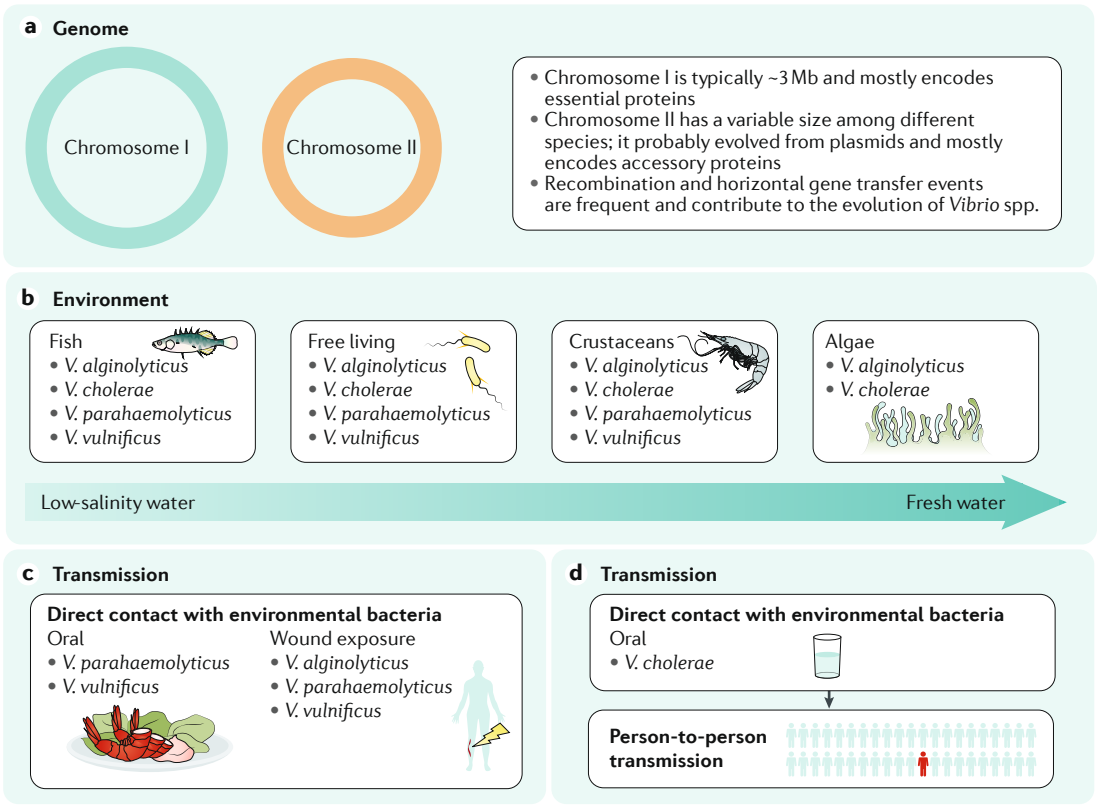

Fig. 1 | **A unifying theme of *Vibrio* spp.** Pathogenic *Vibrio* spp. share several biological, clinical and environmental characteristics that set them apart as important human pathogens. **a** | These bacteria share interesting genomic structures, including two chromosomes, which are frequently shaped by recombination and intense horizontal gene transfer events. **b** | All *Vibrio* spp. grow in warm (typically ≥15 C°) sea water and brackish water, and *Vibrio cholerae* can also be found in fresh water. *Vibrio* spp. can persist in a free living state, colonize fish and marine invertebrates or be associated with plankton, algae and abiotic detritus[74]. *Vibrio* spp. can also form biofilms on biotic and abiotic surfaces, an ability that has an essential role in their environmental persistence[215]. **c** | However, *Vibrio* spp. differ in the routes of transmission to humans; non-cholera *Vibrio* spp., such as *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio alginolyticus*, represent an important and growing source of infections through contaminated seafood and direct exposure to water. **d** | The causative agent of cholera — *V. cholerae* — is the only *Vibrio* sp. that has both human and environmental stages in its life cycle[74]. *V. cholerae* can be found in fresh water as free living, in clusters of many cells forming biofilms or in association with plankton (a widely recognized environmental reservoir). From contaminated water, *V. cholerae* can be transmitted directly to the human population[216,217]. In contrast with other pathogenic non-cholera *Vibrio* spp., *V. cholerae* is also transmitted person to person and through the faecal–oral route.

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Box 1 | **Countries reporting cholera deaths and imported cases in 2016**[a]

≤10 deaths
Afghanistan, Angola, Bhutan, Burundi, India, Mozambique, Niger, Republic of the Congo, Thailand and Zimbabwe

10–99 deaths
Benin, Dominican Republic, Kenya, Malawi, Myanmar, Nigeria, South Sudan and Uganda

≥100 deaths
Democratic Republic of the Congo, Haiti, Somalia, Tanzania and Yemen

Imported cases
Australia, Canada, Denmark, Dominican Republic, Japan, Netherlands, South Korea, United Kingdom and United States

[a]Data from WHO Global Map Gallery.

non-O1 serogroup strain, which initiated a massive outbreak of cholera-like disease in the coastal villages of India[31] and Bangladesh[36,37]. The rapid spread of this non-O1 serogroup strain, designated O139 Bengal, in most of Asia was thought to herald the beginning of the eighth pandemic, but this organism did not spread out of Asia and accordingly was not classified as a pandemic[38]. The O139 Bengal strain-associated cholera waned by 1996 but with an upsurge in 2002 (REF.[39]) and again in 2005 (REF.[36]); since then, the proposed eighth pandemic pathogen O139 Bengal is not associated with any major outbreak and is only sporadically found. The El Tor biotype continues as the major causative agent of cholera worldwide[36].

Non-O1 and non-O139 *V. cholerae* strains are the causative agents of sporadic, yet severe, gastrointestinal and extraintestinal infections[40], and compared with *V. cholerae* O1 and O139 they are relatively understudied human pathogens. During the Northern Hemisphere summer of 2014, there was a noticeable spike in reported non–O1 and non–O139 *V. cholerae* infections in the Baltic Sea area, corresponding both temporally and spatially with a substantial heatwave event[1,41]. Most cases involved self-limiting ear and soft tissue infections associated with swimming or water exposure.

Vibrio parahaemolyticus

V. parahaemolyticus is a ubiquitous inhabitant of temperate and tropical coastal areas around the world. The epidemiology of *V. parahaemolyticus* is characterized by sporadic cases of infection along coastal areas, mostly associated with the consumption of raw or undercooked contaminated seafood over the warmer months[1], although wound exposure to contaminated water can also cause infection. *V. parahaemolyticus* does not spread via person-to-person transmission or the faecal–oral route[42]. Until the late 1960s, cases of *V. parahaemolyticus* infections were geographically restricted to Japan, but starting in 1969 *V. parahaemolyticus* infections were reported in geographically diverse locations, including the Atlantic, Pacific and Gulf States and Hawaii in the United States[43], where according to 2011 estimates there are ~30,000 infections each year[8]. The first well-documented outbreak of *V. parahaemolyticus* in the United States was detected in Maryland in 1971, associated with crab ingestion[44]. Strains isolated from patients and from the suspected foods in the United States showed different serotypes from those isolated in Japan[45]. A comprehensive analysis of the sources of outbreaks in the United States led to the conclusion that the ingestion of raw or improperly cooked contaminated food was the most probable source of the *V. parahaemolyticus* infections, although cross contamination of cooked food (for example, with contaminated water) might have been a secondary vehicle[43]. Following the detection of *V. parahaemolyticus* in clinical cases in the United States, reported infections caused by this organism rapidly increased throughout the 1970s, with sporadic cases and outbreaks reported in Europe, Africa, New Zealand and most of the Asian countries[46], thereby turning *V. parahaemolyticus* into a major seafood-borne pathogen and a global public health concern. Infections were associated with heterogeneous groups of diverse strains, although some groups prevailed in each geographical area. The epidemiology of *V. parahaemolyticus* experienced a radical change in 1996, when a sudden increment of gastroenteritis cases was detected in Calcutta, India. In contrast to most of the previous outbreaks, all the isolates from these cases clustered in a single homogeneous group, a variant of the serotype O3:K6 containing the same virulence traits: the thermostable direct haemolysin (Tdh), encoded by *tdh*, but not the Tdh-related haemolysin (Trh), encoded by *trh*[47]. Using molecular typing techniques, these isolates were clearly differentiated from other O3:K6 strains recovered prior to the epidemic onset in Calcutta[48]. The emergence of this new O3:K6 strain began a prolific expansion of *V. parahaemolyticus* illnesses throughout most Southeast Asian countries in only 1 year[47,48]. This strain has continued its pandemic expansion over recent years: infections associated with this strain have been detected in almost all continents, and the O3:K6 strain has become endemic in many areas where it was introduced (FIG. 2). Although there are no definitive data, possible factors contributing to *V. parahaemolyticus* spread include ballast water discharge and shellfish transport. Climate warming, such as spells of anomalously warm weather, can have a role in initiating outbreaks[49].

V. parahaemolyticus was the leading cause of bacterial infectious diarrhoea in the southern China region during 2007–2012, with serotype O3:K6 strains being the most prevalent isolates[50]. The pandemic strain (that is, the Calcutta O3:K6 strain) was the first example of cross-continental spreading of this pathogen until 2012, when strains belonging to the highly pathogenic ST36 (multilocus sequence type 36) clonal type were detected outside of their normal endemic region in the US Pacific Northwest, causing infections along the northeast coast of the United States and northwest Spain[51,52] (FIG. 2). The ST36 clonal type is of some concern because of its increased virulence potential compared with other pathogenic *V. parahaemolyticus* strains[49,53]; this increased virulence could mean that smaller bacterial loads of this strain can be sufficient to cause infection, although the precise molecular mechanisms underlying the increased virulence remain to be elucidated. *V. parahaemolyticus* infections are

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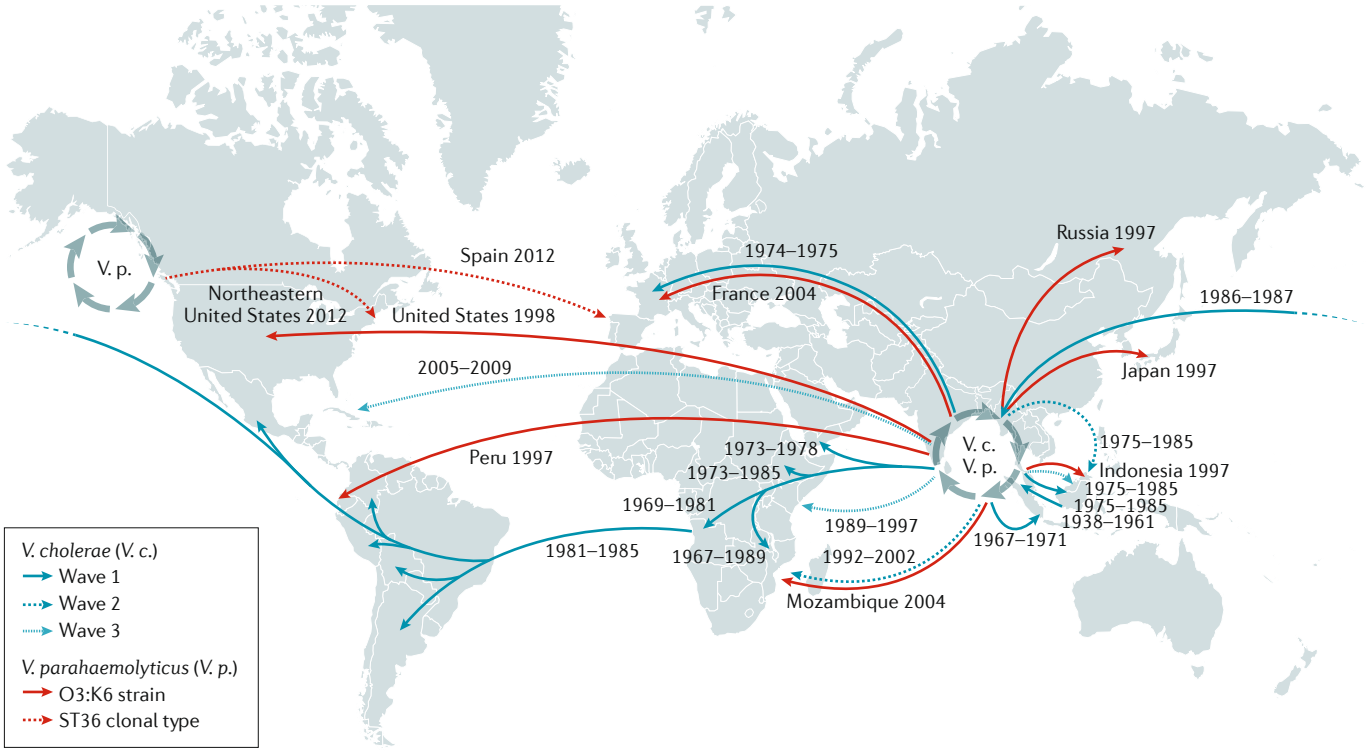

Fig. 2 | ***Vibrio cholerae* and *Vibrio parahaemolyticus* pandemic spread.** A key aspect of *Vibrio cholerae* and *Vibrio parahaemolyticus* (which sets them apart in the *Vibrio* spp.) is their ability for pandemic expansion. *V. cholerae* and *V. parahaemolyticus* have very similar genomic structures. Although much is known regarding the mechanism of virulence associated with these bacteria, it is not clearly understood why particular strains can gain a foothold in a particular region and cause outbreaks. Certainly, particular strains such as *V. cholerae* El Tor and *V. parahaemolyticus* O3:K6 seem to have evolved a competitive advantage, which could explain why they can initiate and maintain substantial outbreaks, leading to pandemic expansion. The current seventh pandemic expansion of *V. cholerae* (El Tor biotype) started from the Bay of Bengal in the 1960s and spread in at least three independent but overlapping waves. The date ranges on the transcontinental transmission events of the different waves indicate the introduction of the pathogen in the different geographical areas, as inferred by phylogenetic analysis. The cross-continental spread of the first pandemic *V. parahaemolyticus* serotype, O3:K6, started from the original place of emergence in Southeast Asia in the 1990s, whereas the dispersal of the Pacific Northwest clonal type ST36 is a more-recent phenomenon. The spread of the O3:K6 serotype was restricted to Asia until 1997, when O3:K6 isolates were detected in Peru in June 1997[218] and subsequently in Chile at the end of the same year[199], initiating the pandemic expansion. From the emergence of infections along the coasts of South America in 1997, this pandemic serogroup disseminated globally, with outbreaks and infections detected in the United States in 1998[180], Russia in 1997[47], France and Spain in 2004 (REFS[219,220]), Mozambique in 2004 (REF.[221]) and Italy in 2007 (REF.[222]). Adapted from REF.[191], Macmillan Publishers Limited; Data for *V. parahaemolyticus* from REF.[40].

estimated to cause a substantial percentage of the total cases of foodborne infections in the United States[9], where foodborne *Vibrio* spp. infections have been increasing[7] (FIG. 3). The WHO–Food and Agricultural Organization (FAO) risk assessments regarding this pathogen in shellfish were published in 2011 and form the basis of international control measures frequently adopted to reduce risk from seafood[54].

Vibrio vulnificus. This pathogen is common in estuarine waters and has been isolated from a range of different environmental sources, including sea water, sediment and seafood produce[42,55]. Unlike *V. cholerae* and *V. parahaemolyticus*, *V. vulnificus* is an opportunistic pathogen, with virtually all cases occurring in those with an underlying disease. The most common risk factors are liver diseases (such as cirrhosis or hepatitis), diabetes mellitus and malignancies. Previous studies have indicated that individuals with chronic liver disease such as cirrhosis are up to 80-fold more likely than healthy individuals to develop *V. vulnificus*-associated primary septicaemia[56]. *V. vulnificus* infection is typically most prevalent in individuals of 45–60 years of age and in men, who make up 85–90% of patients; 85.6% of *V. vulnificus* cases reported to the US FDA between 1992 and 2007 were in men[55]. This age and sex specificity is probably explained by the fact that susceptible patients with cirrhosis are predominantly of middle age[57] and men make up the bulk of persons with cirrhosis[57]. Similar to *V. parahaemolyticus*, infections associated with *V. vulnificus* originate from two distinct sources: consumption of contaminated seafood, in particular molluscan shellfish, resulting in gastroenteritis or primary septicaemia, which is often associated with consumption of oysters, where this bacterium can occur in large numbers ($\geq 10^5$ per gram); or exposure of wounds to sea water or seafood products, resulting in wound infections and secondary septicaemia. However, unlike *V. parahaemolyticus*, *V. vulnificus* is a highly fatal human pathogen: it is responsible for >95% of seafood-related deaths in the United States[55]

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and has the highest case fatality rate (~50%) of any foodborne pathogen[58]. Wound infections associated with *V. vulnificus* are usually contracted during recreational activities such as swimming, fishing and handling seafood[2] and have a substantial mortality (~25%)[2,55]. Three biotypes (a classification approach based on biochemical characteristics) of *V. vulnificus* have been identified[55] Biotype 1 is responsible for both the majority of ingestion cases (primary septicaemias) and most wound infections. Biotype 2 is the causative agent of a rapidly fatal septicaemia in farmed eels and rarely in humans[59]. Biotype 3 causes human wound infections that, to date, have mostly been reported among tilapia aquaculture workers in Israel[60], although an infection was recently reported in Japan[61].

Globally, surveillance data regarding *V. vulnificus* infections are not gathered systematically, making wider geographical and epidemiological comparisons problematical. Where only fragmentary surveillance data exist (for example, published peer reviewed reports of infections), such as studies in Europe[42,62], China[63], Taiwan[64], South America[65], Japan[66] and Korea[67], infections tend to affect middle-aged men with underlying disease. *V. vulnificus* is a rare cause of infection (~100 cases per year in the United States), but published studies demonstrate an increase in disease in the United States and potentially also in Europe[7,62,68]. Incidence of infection is related to environmental distribution, and *V. vulnificus* exhibits quite distinct temperature and salinity tolerances, as it can be found in warm (13–30 °C) and brackish (2–25 parts per thousand NaCl) waters. The WHO risk assessment on *V. vulnificus* was published in 2004 (REF.[69]). Other *Vibrio* spp. that can infect humans are described in BOX 2.

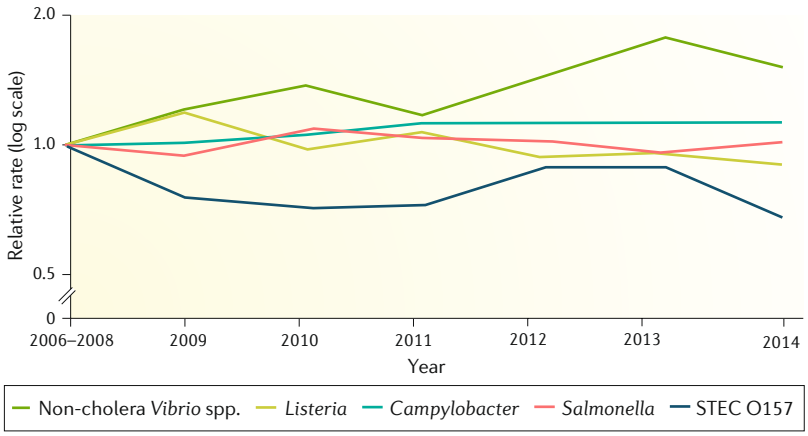

Fig. 3 | **Foodborne infections reported in the United States.** Relative rates (compared with 2006–2008 rates) of culture-confirmed foodborne infections caused by different pathogens. Of all the major foodborne pathogens monitored in the United States, non-cholera *Vibrio* spp. represent the only group showing a clear increase in incidence. Although the absolute number of cases of vibriosis is low (for example, 216 in 2014, compared with ≥7,400 of salmonella and ≥400 of Shiga-toxin producing *Escherichia coli* (STEC) O157 infection), under-reporting of these infections is substantial, owing to misdiagnosis and lack of systematic screening, among other factors. For example, the number of *Vibrio parahaemolyticus* infections is estimated to be ~140-fold higher — thus, for each laboratory-confirmed infection, a hundred more are estimated to elude identification[8]. Adapted with permission from REF.[223], Centers for Disease Control and Prevention.

Mechanisms/pathophysiology

Vibrio cholerae

Of all the studied pathogenic *Vibrio* spp., *V. cholerae* is the most well understood. *V. cholerae* is the paradigmatic non-invasive mucosal pathogen: following ingestion of contaminated water or food by the host, the pathogen proliferates to high density along the mucosal surface of the small intestine but does not disrupt the integrity of the epithelial barrier or cause substantial damage to epithelial cells[70] (FIG. 4). Instead, the bacteria prompt an intense secretory response, resulting in profuse watery diarrhoea that, if untreated, frequently results in death due to dehydration within 1–2 days. Studies in a variety of animal models and in human volunteers have demonstrated that choleric diarrhoea is primarily a response to a pathogen-secreted factor, cholera toxin (CT)[71]. Deletion of *ctxA* and *ctxB* (encoding cholera enterotoxin subunit A and B, respectively) from pathogenic *V. cholerae* abrogates the bacterial capacity to induce diarrhoea in animal models, and administration of purified CT is sufficient to induce diarrhoea in human volunteers[72].

Additional bacterial factors that contribute to cholera pathogenesis have also been identified through studies in animal models of disease, and some of these have been confirmed in studies in human volunteers[73]. Several studies demonstrate that clinically apparent *V. cholerae* infection induces protective immunity against subsequent infection in humans[74]. A key virulence factor in infant mice is the toxin-co-regulated pilus (Tcp), a type IV pilus (a filamentous surface appendage). Tcp produced by adjacent *V. cholerae* bacteria can bind to each other, tethering bacteria together and facilitating microcolony formation within the intestines of infected animals, and can also facilitate adhesion to enterocytes[75]. Human volunteer studies have demonstrated that Tcp is also essential for *V. cholerae* colonization of the human intestine[76], suggesting that findings from infant mouse model hosts are relevant for understanding *V. cholerae* colonization of the human intestine. In addition to Tcp, many other cell structures and processes, including the LPS O-antigen, cell curvature[77], motility and metabolic processes, have been implicated in *V. cholerae* intestinal colonization[73].

Regulation of gene expression. Once the bacteria reach the host intestine, a complex network of regulatory inputs governs expression of *V. cholerae* virulence factors, many of which are not typically expressed in vitro. Transposon insertion sequencing (a high-throughput technique that combines transposon insertional mutagenesis with next-generation sequencing to identify the genes involved in a specific function) has aided in comprehensive, genome-wide identification of genes that facilitate intestinal colonization[78]. Environmental factors present in the intestine, including bile, bicarbonate, reduced oxygen tension and unsaturated fatty acids, contribute to the co-expression of genes for the biogenesis of Tcp and CT and other genes associated with colonization[79]. Transduction of these environmental stimuli is mediated by membrane-localized transcription factors, including CT transcriptional activator (encoded by *toxR*) and Tcp biosynthesis protein P and H (encoded by *tcpP* and *tcpH*, respectively), which induce

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Box 2 | **Other *Vibrio* spp. that can cause human disease**

Around a dozen species of *Vibrio* cause infections in humans (TABLE 1). *Vibrio alginolyticus* is ubiquitous in sea water and tends to cause superficial wound and ear infections (otitis media and otitis externa), which can be resolved using appropriate antibiotics, although rarely these infections can progress to septicaemia and necrotizing fasciitis, particularly in individuals with a compromised immune system. The incidence of these infections considerably increases during warmer months[41], and a study of vibriosis in Florida, United States (1998–2007), identified *V. alginolyticus* as a relevant cause of infection, with 131 cases (almost 20% of all vibriosis infections) reported during this time period[68]. In most reports, *V. alginolyticus* wound infections resulted from exposure of cuts or abrasions to contaminated sea water[1]. Numerous sporadic reports of *V. alginolyticus* have also been documented in Europe[62,177,206,207].

A number of other *Vibrio* spp. are associated with human infections, including *Vibrio mimicus*, *Vibrio cincinnatiensis*, *Vibrio hollisae*, *Vibrio furnissii*, *Vibrio fluvialis* and *Vibrio metschnikovii*. Although these clinically important bacteria are capable of causing different types of vibriosis, these infections are relatively rare. However, *V. fluvialis* is increasingly being considered an emerging foodborne pathogen[208] of public health concern[209].

expression of Tcp virulence regulatory protein (encoded by *toxT*, also known as *tcpN*), an activator of virulence gene expression[79]. Expression of the *toxT* regulon (a group of genes controlled by a common regulator) is also modulated by metabolic signals and quorum sensing. In contrast to several other pathogens, the expression of *V. cholerae* virulence genes is typically repressed at high cell densities[80]. In vivo gene expression studies have suggested that there is a temporal pattern of *V. cholerae* gene expression within the intestine. Genes expressed early in infection enable colonization, whereas genes expressed late in infection are thought to prepare the pathogen for growth in the environment outside of the host intestine as well as to promote the transmission of the pathogen to new hosts; in fact, for several hours after the pathogen has been excreted, it is thought to be in a hyperinfectious state that facilitates its transmission between hosts[73,74,81,82]. Genes encoding CT and Tcp are not present in all *V. cholerae* O1 isolates. *ctxA* and *ctxB* are embedded in the genome of CTXϕ, a lysogenic (that is, integrating its viral genome into the host's) filamentous bacteriophage that has been independently acquired by a subset of *V. cholerae* lineages[83]. Similarly, genes encoding Tcp are present in only a subset of *V. cholerae* isolates — generally those of the O1 serogroup[73], which are thought to account for all known cholera pandemics. Notably, only strains that produce Tcp can be infected by CTXϕ, as Tcp serves as the primary bacteriophage receptor. Consequently, the evolution of pathogenic *V. cholerae* is presumed to have involved sequential acquisition of the Tcp-encoding pathogenicity island (a pathogenicity island is a set of contiguous genes involved in virulence that are acquired by HGT) and then CTXϕ. The O139 pathogenic isolates that temporarily became prevalent in 1992 are thought to have arisen as a result of serogroup conversion of a toxigenic O1 progenitor[84].

Vibrio parahaemolyticus

Despite the prevalence of *V. parahaemolyticus*-induced gastroenteritis, there is limited understanding of how this pathogen causes disease in the intestine. Almost all clinical isolates of *V. parahaemolyticus* show β-haemolysis activity when cultured on specialized blood agar (Wagatsuma agar), whereas almost all isolates from other sources are non-haemolytic; this difference is known as the Kanagawa phenomenon. Virulent *V. parahaemolyticus* strains produce a variety of recognized virulence factors during pathogenesis. Of these, Tdh[85,86], which is responsible for the Kanagawa phenomenon, and Trh[87] are currently the most predictive overall indicators of potential virulence. Most infections are associated with strains that possess *tdh* and *trh*, although there are notable published exceptions[88]. Both Tdh and Trh share several biological properties, including haemolytic activity, enterotoxicity and cytotoxicity[89]. Whole-genome sequencing efforts have identified that pathogenic isolates of *V. parahaemolyticus* also encode two type III secretion systems (T3SS): T3SS1 and T3SS2 (REFS[90,91]). Secretion systems are multiprotein structures that mediate the translocation of bacterial effector proteins directly into eukaryotic cells[90,92]. T3SS1 and T3SS2 also ensure *V. parahaemolyticus* survival in the environment[20]. *toxR*, an ancestral locus in *Vibrio* spp., is required for *V. parahaemolyticus* fitness in vivo and for induction of expression of the T3SS2-related genes associated with gastroenteritis[93]. During infection, *V. parahaemolyticus* uses adhesion factors to bind to fibronectin and phosphatidic acid on the host cell, and it uses T3SS1 and T3SS2 to transport different effectors and toxins into the cytoplasm, causing cytotoxicity and serious diseases[94].

In part, the paucity of knowledge of pathogenicity and virulence in *V. parahaemolyticus* results from the absence of an oral-infection-based animal model to replicate human disease; however, progress in this regard has enabled us to infer the crucial steps in how these infections develop[91]. Strain-to-strain variability in the infectious doses required to initiate *V. parahaemolyticus* gastroenteritis have been noted, with Pacific Northwest strains (for example, ST36 clonal type) demonstrating a substantially increased attack rate (a biostatistical measure of frequency of morbidity (or speed of spread of a specific pathogen) in an at-risk population) of 30%, corresponding to an infectious dose of 10^3–10^4 cells[49]. This infectious dose is significantly lower than the previous risk assessment analysis on pathogenic strains of *V. parahaemolyticus*[95].

Vibrio vulnificus

Unlike *V. cholerae* and *V. parahaemolyticus*, *V. vulnificus* is an opportunistic pathogen. Most patients with *V. vulnificus* infection (≥80%) also have liver disease[55], which leads to elevated serum iron, which the bacterium requires for successful tissue growth and invasion. Indeed, most cases of *V. vulnificus* infection occur in men with underlying conditions resulting in elevated serum iron levels, primarily alcohol-associated liver cirrhosis. *V. vulnificus* produces both catechol and hydroxamate siderophores[96] (high-affinity iron-chelating systems), but the fact that the organism requires high serum iron levels to produce a successful infection suggests that these siderophores are not able to scavenge iron from transferrin or other iron-binding proteins in human serum[97] and, therefore, require an iron-saturated transferrin to obtain this key element. Because the liver is the largest source of iron in the human body, conditions associated

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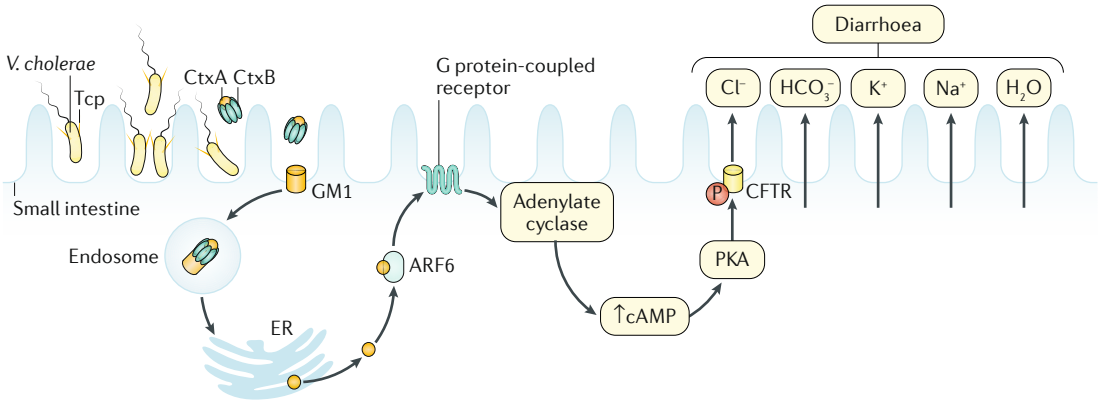

Fig. 4 | **Pathogenesis of cholera in humans.** Once in the human host, after reaching the small intestine, *Vibrio cholerae* begins expressing genes encoding virulence factors, such as toxin-co-regulated pilus (Tcp) and cholera toxin. Cholera toxin is composed of two subunits, CtxA and CtxB, and binds to the ganglioside (a sialylated glycosphingolipid) GM1 on the plasma membrane of enterocytes via the pentameric CtxB subunit. Bound cholera toxin is endocytosed, then undergoes retrograde transport to the endoplasmic reticulum (ER), where the subunits dissociate. Release of the enzymatic CtxA subunit from the ER into the cytosol enables its allosteric activation by ADP ribosylation factor 6 (ARF6). The ARF6-bound, activated CtxA subunit in turn activates adenylyl cyclase by catalysing ADP ribosylation of a G protein-coupled receptor. Increased cellular levels of cAMP lead to protein kinase A (PKA)-dependent phosphorylation (P) of the cystic fibrosis transmembrane receptor (CFTR), which induces the efflux of ions and water into the lumen of the small intestine that causes diarrhoea.

with liver dysfunction and release of iron into the serum clearly increase the risk of *V. vulnificus* infection[55]. The crucial roles that iron has in all aspects of *Vibrio* spp. life, including pathogenesis, have been superbly summarized[98], and, in *V. vulnificus*, the role of iron in the viable but non-culturable state (a state of suppressed metabolic activity in which the bacteria do not divide; bacteria in this state cannot be grown in culture but remain viable and potentially able to regrow under the appropriate environmental cues), chemotaxis, motility and survival in a host was also described[99].

Virulence factors. Numerous studies have been published on the putative virulence factors of *V. vulnificus*; however, unlike in *V. cholerae*, there is much yet to be learned as to which gene products are crucial to the ability of this pathogen to cause such rapidly fatal infections. Thus far, the only virulence factor that has been demonstrated to be essential for successful infection is the bacterial capsule, as non-encapsulated bacteria are readily phagocytosed by macrophages[55]. The capsular LPS of the bacterium is an endotoxin; bacterial endotoxins stimulate inflammation and cytokine production by activated macrophages and B cells, leading to vasodilation, increased capillary permeability and activation of the complement and coagulation pathways. *V. vulnificus* endotoxin is probably responsible for causing substantial hypotension and generalized organ failure that can be fatal[100]. Of note, oestrogen has been reported to protect women against the endotoxin produced by this pathogen[101]. Also important for successful infections is a multifunctional-autoprocessing repeats-in-toxin (MARTX)[102], essential for bacterial dissemination from the intestine[103]; the massive tissue destruction that characterizes both gastrointestinal and wound infections probably results from powerful collagenases, metalloproteinases and lipases and/or phospholipases that the bacterium produces[55,104]. Other virulence factors include the *tonB* systems, which are involved in tissue invasiveness and flagellum expression[105], *vvpE*, which mediates intestinal colonization, a mucin-binding protein encoded by *gbpA*, which is essential for pathogenesis[106,107], and the two-component signal transduction system *gacS–gacA*, which regulates biofilm formation and virulence[108].

Biotypes. Complicating our understanding of pathogenesis is the fact that there are three biotypes of *V. vulnificus*[55]. In addition, cells of biotype 1 strains are composed of two genotypes: a clinical (C) genotype, which is responsible for virtually all cases of primary septicaemia, and an environmental (E) genotype, which is associated with almost all of the wound infections[109]. These genotypes have substantial differences in DNA sequences and correlate well not only with human virulence but also with different isolation sources. Whereas 90% of human clinical isolates are the C-genotype, 85–90% of bacteria from environmental sources (shellfish, water, etc.) are the E-genotype[55]. Although whole-genome sequencing has been carried out on strains of the two genotypes[110,111], to date such studies have not definitively elucidated which genes are essential for human virulence or why E-genotype cells enjoy a substantial environmental survival advantage over cells of the C-genotype in estuarine environments. Why wound infections are of the E-genotype, which typically does not cause septicaemias, is not yet known, although whole-genome sequencing of additional strains will probably identify gene variations that will help to answer these questions. The virulence factors required to cause these life-threatening wound infections probably include very efficient tissue-degrading enzymes, which lead to the characteristic necrotizing fasciitis (rapid and widespread necrosis of the subcutaneous tissue and the fascia) of these infections. As patients with

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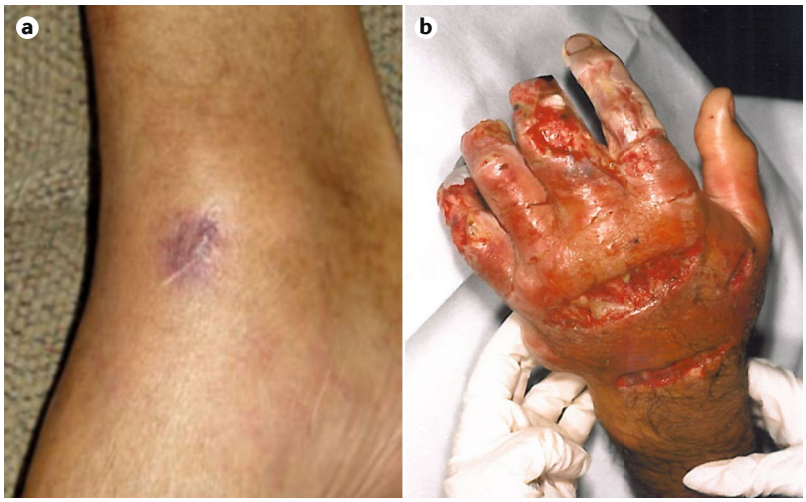

Fig. 5 | ***Vibrio vulnificus* wound infection. a** | Frequently, fatal *Vibrio vulnificus* infections can be initiated via minute entry sites. In this patient, the infection progressed to secondary septicaemia and led to a fatal outcome in ≤48 hours after exposure. **b** | *V. vulnificus* wound infections are also life altering, with tissue necrosis that necessitates tissue debridement and frequently finger and limb amputation. Images courtesy of J. D. Oliver, University of North Carolina, NC, USA.

wound infection exhibit a sex difference similar to that seen in patients with septicaemia, the E-genotype bacteria causing wound infections likely have virulence factors similar to those produced by C-genotype bacteria.

Importance of gene transfer

A key characteristic shared between all pathogenic *Vibrio* spp. is the ubiquity of pathogenicity markers acquired via HGT. Certainly, the best-known example of HGT contributing to the emergence of virulent *Vibrio* spp. is the presence of CTXϕ in toxigenic strains of *V. cholerae*[112]. The major virulence genes in *V. cholerae*, which are clustered in several chromosomal regions, seem to have been recently acquired from bacteriophages or through undefined HGT events[113], outlining the key role that this process has had on influencing virulence in this pathogen. Of note, environmental factors can influence the efficiency of HGT. In *V. cholerae*, high concentrations of chitin (a fibrous polysaccharide that is a major component of the exoskeleton of arthropods, such as crustaceans, and the scales of fish) — found naturally in the marine environment — induce an increase in competence and the ability to uptake DNA[114]. Similarly, in *V. vulnificus*, chitin has been shown to provide a substratum for *Vibrio* spp. to attach and exchange genetic information, leading to considerable HGT[115]. Finally, molecular analysis and comparative genomic analysis of pre-pandemic and pandemic strains revealed that the emergence of *V. parahaemolyticus* pandemic strains could be associated with recombination and HGT[116,117].

Diagnosis, screening and prevention

Diagnosis of cholera

Cholera is a severe form of diarrhoea characterized by rice-water-like stool, which comes out forcibly (purging) at about 1 litre per hour, causing rapid fluid loss (dehydration). This severe diarrhoea is often associated with nausea and vomiting. About 75% of *V. cholerae* infections are asymptomatic[118]; among symptomatic infections, ~5% of cases are mild, 35% are considered moderate and ~60% of infections are considered severe[119,120]. The incubation period of *V. cholerae* is typically 5 days, although it can range from a few hours to several days[121]. An individual may be contagious (that is, expels viable bacteria in the faeces) for up to 14 days[122]. Upon presentation in clinical settings, clinical stool samples and blood samples are typically taken for standard microbiological identification (see below).

From a public health perspective, because of the potential of *V. cholerae* for spreading and the devastating consequences of epidemics, the management of cholera outbreaks requires immediate identification of cases[123]. Recent advances in rapid diagnostic tests (RDTs) have shown promise, particularly in settings where availability of microbiological and diagnostic facilities (for example, for PCR or serotyping analysis) are limited. Most RDT methods are based on the detection of *V. cholerae* O1 and O139 antigens in human stool samples[124–126]. These RDTs could be particularly useful in settings where they can be used alongside established and accredited testing methods and where rapid surveillance of cholera in a population can be used for management and preventive action. A WHO overview and guidance note of RDTs has been recently published[123].

Diagnosis of vibriosis

A clinician could suspect vibriosis if a patient has watery diarrhoea and has recently eaten raw or undercooked seafood, especially oysters, or when a wound infection occurs after exposure to sea water[127]. For *V. parahaemolyticus*, the vast majority of infections are mild and self-limiting[1]. Following ingestion, the incubation period usually lasts 12–24 hours; the typical clinical characteristics of *V. parahaemolyticus* infections include abdominal cramps, diarrhoea, nausea, headaches, fever and chills[42]. Certainly, for more-serious cases of gastrointestinal vibriosis (for example *V. vulnificus* infection, with ~90% of cases requiring hospitalization) it is vital to obtain exposure history if the patient has underlying conditions, such as diabetes mellitus or liver diseases. *V. vulnificus* infections are characterized by an average 48-hour incubation period between the ingestion and onset of symptoms and an average 16-hour incubation period in the case of wound exposure[55], highlighting the need for rapid diagnosis. *V. vulnificus* wound infections can be severe and result in necrotizing fasciitis (FIG. 5). The appropriate clinical samples (stool, blood or wound or ear secretions) are collected for microbiological confirmation of diagnosis.

Microbiological diagnosis

Vibrio spp. are typically easy to culture from clinical samples. Thiosulfate citrate bile-salts sucrose (TCBS) agar is the standard medium used commonly for the selective isolation and further subculturing of *Vibrio* spp. Strains that are able to metabolize sucrose, such as *V. cholerae* and *V. alginolyticus*, will form yellow colonies on TCBS agar media, whereas other pathogenic species such as *V. parahaemolyticus*, *V. mimicus* and *V. vulnificus* produce green colonies. Other media types, such as blood agar and CHROMagar to isolate *V. parahaemolyticus*

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and cellobiose-polymyxin B-colistin (CPC) media for isolation of *V. vulnificus*[128], can be used to culture colonies that appear green on TCBS agar. In the United States, blood agar is frequently used for initial isolation of *Vibrio* spp. from clinical samples. Samples that yield positive culture results can be sent to specialized laboratories (national reference laboratories in Europe and state clinical laboratories in the United States) for downstream confirmatory testing typically using species-specific PCR methods. Well-established and robust tests are available for all major *Vibrio* spp. pathogens, with associated conventional PCR[129–131] and real-time PCR assays[132–135] for species-level confirmation. More-in-depth molecular characterization, such as PCR of the virulence genes (for example, *ctx* (including *ctxA* and *ctxB*) PCR testing of *V. cholerae*[136] (to identify toxigenic strains), *tdh* and *trh* analysis of *V. parahaemolyticus*[130] and a variety of virulence-associated PCR tests for *V. vulnificus*[137,138]), is frequently carried out during further clinical investigations. *V. parahaemolyticus* strains of major clinical significance can be further subtyped using specific PCR methods to identify pandemic strains such as those originating from pandemic groups[139] and ST36 clonal types[140]. Biochemical tests such as appropriate serological testing to further subtype strains, particularly those originating from groups commonly associated with human infections, are frequently used for *Vibrio* spp. identification purposes; however, these methods have some limitations. For example, these methods are slow, tedious, require staff with a good knowledge for interpretation of results, have subjective interpretation and are expensive[141,142].

Cholera prevention

Improvements in water supply, sanitation and food safety, coupled with community awareness, represent the most effective means of preventing cholera and other diarrhoeal diseases. Water, sanitation and hygiene (WASH) improvements in cholera-endemic countries have contributed to mitigate cholera outbreaks. Studies in Bangladesh have demonstrated that household members of patients with cholera are at a much higher risk of developing cholera than the general population; a hospital-based hygiene and water treatment intervention (Cholera-Hospital-Based-Intervention-for-7-Days (CHoBI7)) has shown great promises in reducing cholera infection among the household members of patients with cholera[143].

Cholera vaccines have been very effective in controlling cholera; nevertheless, mass vaccination at the country level is not always easy for several reasons, including political or cultural hesitancy in vaccine acceptance, costs (as a booster dose of vaccine is also recommended) and lack of adequate supportive infrastructure for the storage and delivery of vaccines.

Cholera vaccines. The first cholera vaccines developed required parenteral administration; however, this administration route had limited efficacy and short duration of protection. As the oral route elicited increased mucosal antibody responses against cholera, a shift from parenteral vaccines to oral vaccine development occurred in the 1980s. Both live-attenuated and inactivated oral cholera vaccines (OCVs) have been developed and tested[144–146]. At present, three killed whole-cell OCVs have been WHO prequalified (TABLE 2). Dukoral has a protective efficacy of >60% and has indirect herd protection, bringing the overall level of protection to >90%[147]. Dukoral is licensed in >80 countries and has been used for vaccine campaigns and as a travellers' vaccine to protect against cholera as well as diarrhoea caused by enterotoxigenic *E. coli*. However, this vaccine has not been routinely adopted for public health use, owing to its high cost and logistic issues with vaccine administration, as it needs bicarbonate buffer for reconstitution before intake. Affordable OCVs that do not require buffer for administration have been developed, including ORC-Vax and modified ORC-Vax (mORC-Vax), which include killed whole-cell *V. cholerae* O139. mORC-Vax has been used in Vietnam since 1997 in the national immunization programme[148]. The International Vaccine Institute (IVI) in South Korea has supported the technology transfer of the WHO prequalified OCV Shanchol to Incepta Pharmaceuticals in Bangladesh to produce a vaccine, Cholvax, that is undergoing clinical trials in Bangladesh (NCT02742558) before going through licensure. In addition, another OCV, OraVacs, is similar to Dukoral and is licensed for use in China and the Philippines[149]. The single-dose, live OCV CVD 103-HgR (Vaxchora) is at present approved by the FDA for use in travellers in some countries[150]. New-generation cholera vaccines are in phase I/II clinical trials (NCT02823899) as well as in preclinical studies[151]. All these efforts are expected to increase the current global OCV production and meet the needs of cholera-endemic and epidemic settings[152].

The WHO recommends the use of OCVs for control of both endemic and epidemic cholera by pre-emptive vaccination and reactive vaccination (that is, vaccination in response to an existing epidemic), respectively[122]. With affordable OCVs commercially available in many countries and through the WHO OCV stockpile (established in 2013) for countries that request it, >17 million doses of OCVs have been used in 40 mass administration campaigns for control of outbreaks, endemic cholera and humanitarian crisis situations globally[153]. The WHO prequalified vaccines are recommended for use in anyone of ≥1 year of age (Euvichol and Shanchol) or ≥2 years of age (Dukoral). No vaccine is registered for use in pregnant women; however, the WHO recommends that pregnant women are included in OCV campaigns. Analysis of large OCV trials has shown that Shanchol is safe in pregnancy with no adverse events in newborn babies[154,155]. The two-dose Shanchol regimen has around 60% protective efficacy, lasting ≥5 years[156]. The longevity of protection is better in those of ≥5 years of age, and vaccines provide indirect herd protection, which can lead to ≥90% protective efficacy. A single-dose Shanchol regimen gave ≥6 months of protection from severe cholera requiring hospitalization in those of ≥5 years of age in Bangladesh[157]. Although vaccines can be used to combat cholera in disadvantaged areas, the efficacy of each cholera vaccine with regard to long-term prevention of cholera remains hotly debated. Amid these debates, different research groups are currently

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pursuing a new generation of cholera vaccines that would potentially have long shelf life, be easy to transport and store at room temperature and be administered as appropriate with long protective immunity. However, at present, the affordable OCVs are being used to control epidemic and endemic cholera globally. There remains a shortage of OCVs since demand has increased after the establishment of the stockpile.

Prevention of vibriosis

A range of preventive approaches have been successfully applied to prevent non-cholera *Vibrio* spp. infections, particularly from seafood sources. Common regulatory control measures include monitoring harvest waters and high-risk food items (such as oysters) by microbiological analysis[9]. Control measures developed in the United States, where vibriosis originating from seafood produce is increasing, include state-level risk assessments and appropriate cold-chain processing of shellfish based on tightly controlled risk appraisals. Postharvest processing methods, such as high-pressure treatment, irradiation, quick-freezing and pasteurization, are available to make seafood produce safe[9]. These approaches are effective[158]. In April 2003, California implemented a regulation restricting the sale of raw oysters harvested from the Gulf of Mexico from 1 April to 31 October unless they were processed to reduce *V. vulnificus* to nondetectable levels using appropriate postharvesting processing methods. This regulation led to a considerable drop in the number of reported *V. vulnificus* infections and deaths in California associated with raw oysters[158]. However, in other US states, *V. vulnificus* infections remain a public health issue. In addition, educational efforts to reduce the risk of infection have been initiated to raise awareness in consumers, restaurant proprietors and physicians about the hazards of eating raw shellfish[159]. However, there remains little evidence to suggest that these efforts have been successful, as seafood-associated infections have been increasing[7].

There have been advances in the use of remote sensing methods, such as those that can directly measure sea surface temperature, as an approach to reduce risks associated with *Vibrio* spp. and as a forecasting method to identify at-risk areas. The European Centre for Disease Prevention and Control has established a global *Vibrio* spp. suitability mapping tool that enables 24-hour updated data[160]. These remote-sensing-enabled methods are increasingly being applied to study outbreaks retrospectively and determine risk factors for outbreaks, and they could offer advance warnings, such as rapid warming in temperate areas, that indicate an increased risk of the presence of *Vibrio* spp.[41]. Decreasing the incidence of wound infections is challenging as these mostly occur in individuals engaged in recreational activities in coastal waters. However, the CDC recommends limiting exposure to water

Table 2 | **Characteristics of oral cholera vaccines**

Vaccine	Manufacturer	Composition	Additional notes	WHO pre-qualification
Dukoral	SBL Vaccine (Solna, Sweden); now Valneva (Montreal, Quebec, Canada)	Killed whole cells of *Vibrio cholerae* O1 (classical and El Tor biotypes) and recombinant native subunit B of cholera toxin	• Requires buffer for administration • Two-dose (≥6 years of age) and three-dose (2–5 years of age) vaccine. Each dose of vaccine comes with a sachet containing sodium hydrogen carbonate. The granules are dissolved in water and mixed with the vaccine before intake	2001
ORC-Vax and mORC-Vax	VabioTech (Hanoi, Vietnam)	Killed whole cells of *V. cholerae* O1 (classical and El Tor biotypes) and *V. cholerae* O139	• No buffer is needed • Available only in Vietnam • Two-dose vaccine recommended for people ≥1 year of age. Single-dose 1.5-ml vial and multiple-dose vial (five doses per vial) formulations	No
Shanchol	Shantha Biotechnics (Hyderabad, India); now Sanofi Pasteur (Mumbai, India)	Killed whole cells of *V. cholerae* O1 (classical and El Tor biotypes) and *V. cholerae* O139	• No buffer is needed • Two-dose vaccine recommended for people ≥1 year of age. The formulation is in single-dose vials of 1.5 ml volume	2011
Euvichol	Eubiologics (Seoul, Republic of Korea)	Killed whole cells of *V. cholerae* O1 (classical and El Tor biotypes) and *V. cholerae* O139	• No buffer is needed • Two-dose vaccine recommended for people ≥1 year of age. The formulation is in single-dose vials of 1.5 ml volume	2015
Cholvax	Incepta Pharmaceuticals (Dhaka, Bangladesh)	Killed whole cells of *V. cholerae* O1 (classical and El Tor biotypes) and *V. cholerae* O139	• No buffer is needed • Available only for Bangladesh market • Two-dose vaccine. Recommended for people ≥1 year of age. The formulation is in single-dose vials of 1.5 ml volume	No
OraVacs	Shanghai United Cell Biotechnology (Shanghai, China)	Killed whole cells of *V. cholerae* O1 and recombinant subunit B of cholera toxin	• Enteric coated capsule • Three-dose regimen for people of ≥11 years of age	No
CVD 103-HgR (Vaxchora)	PaxVax (Redwood City, California, United States)	Live-attenuated oral cholera vaccine composed of *V. cholerae* O1 (Inaba)	• Requires buffer for administration • Currently indicated as a single-dose regimen for people of 18–64 years of age	No[a]

[a]Approved by the FDA and licensed in Australia, Canada, New Zealand, Switzerland and the United States.

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Table 3 | **Treatment options for the main human *Vibrio* spp. infections**

Species	Clinical manifestation	Treatment regime
Vibrio cholerae (O1 or O139 strains)	Cholera	• Oral rehydration solution and intravenous fluid replacement • For children with severe malnutrition and diarrhoea, zinc treatment is recommended • Antibiotic treatment (for example, azithromycin and ciprofloxacin) can speed up recovery
Vibrio cholerae (other strains)	Gastroenteritis	A single dose of ciprofloxacin or doxycycline
	Wound infections and sepsis	• Antibiotic treatment with doxycycline (usually topical) • For severe infections and sepsis, add a third-generation cephalosporin
	Ear or eye infections	Topical antibiotics
Vibrio parahaemolyticus	Gastroenteritis	• Treatment is not necessary in mild cases, but patients should drink plenty of liquids to replace fluids lost through diarrhoea • Patients ill and with a high fever or an underlying medical condition should receive oral antibiotic therapy with doxycycline or quinolone
	Wound infections and sepsis	• Treatment is not often necessary because the infection is self-limiting • Antibiotics typically used are doxycycline, with or without a third-generation cephalosporin for severe wound infection or sepsis; ciprofloxacin is an acceptable alternative • Rarely, patients with necrotizing fasciitis require surgical debridement
Vibrio vulnificus	Gastroenteritis	Antibiotic treatment with doxycycline and usually a third-generation cephalosporin
	Wound infections and sepsis	• Antibiotic treatment with doxycycline and usually a third-generation cephalosporin • Patients with necrotizing fasciitis require surgical debridement
Vibrio alginolyticus	Wound infections (rarely sepsis)	Antibiotic treatment with doxycycline
	Ear or eye infections	Topical antibiotics
Vibrio fluvialis, *Vibrio hollisae* and *Vibrio mimicus*	Gastroenteritis	A single dose of ciprofloxacin or doxycycline
	Wound infections and sepsis	• Antibiotic treatment with doxycycline • For severe infection and sepsis, add a third-generation cephalosporin
	Eye and ear infections	Topical antibiotics
Vibrio metschnikovii	Gastroenteritis and sepsis	• Enteric infections can be treated with a single dose of ciprofloxacin or doxycycline • For severe infections and sepsis, add a third-generation cephalosporin

and shellfish in individuals who have underlying risk conditions that could increase the probability or the severity of *Vibrio* spp. infections[127]. This recommendation is particularly relevant for *V. vulnificus* and where exposure-associated infections are easily preventable in at-risk groups.

Management

Treatment of *Vibrio* spp. infections varies depending on the pathogen responsible, route of transmission and observed clinical manifestations (TABLE 3). Many *Vibrio* spp. infections are self-limiting and do not require medical intervention beyond supportive care (such as drinking plenty of liquids to replace fluids lost through diarrhoea)[127]. However, important medical interventions are required where more-serious infections or clinical presentations are evident.

Cholera

Only about one in five individuals that aquire toxigenic *V. cholerae* exhibits symptomatic cholera, and the severity of the disease depends on both the pathogenic factors of *V. cholerae* and the host factors, such as age and nutritional and immune system status. Cholera can still be a fatal disease, and mortality in persons with severe cholera can exceed 70% if prompt clinical diagnosis and appropriate management are not available[162]. Therapy includes rehydration (orally or intravenously), antibiotics and nutritional supplements in malnourished individuals.

Rehydration therapy and maintenance of hydration. Patients with cholera should be treated with oral rehydration solution (ORS), promoting replacement of water and electrolytes lost by patients through frequent passage of voluminous rice-watery stool (TABLE 3). The WHO guidelines should be followed to evaluate the dehydration status of the patients[163]; the assessment is based on the clinical evaluation and examination of the patient's level of consciousness, eyes, tongue, thirst, skin turgor and rate of radial pulse. For mild-to-moderate cases, ORS is used both for rehydration (the initial therapy to restore fluid and electrolytes until the deficit has been replaced) and maintenance of hydration while diarrhoea persists. In addition to glucose-based ORS, rice ORS (ORS in which rice powder replaces glucose) is useful in reducing stool output during both rehydration and maintenance therapy[163,164]. The patient should be kept under observation and dehydration status should be assessed periodically. Patients with severe dehydration, and many with moderate dehydration (frequent vomiting ≥3 times in an hour), require emergency administration of intravenous fluid[164]. Ringer's lactate is a commonly used rehydration fluid; normal saline or cholera saline can also be used. For both adults and children, initial bolus therapy (the first part of the infusion) should be repeated if danger signs (that is, hypovolemic shock) are present after the initial bolus or if the radial pulse remains weak or undetectable. Intravenous fluid should be delivered in continuous infusion until the

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fluid deficit has been replaced; ORS can then be initiated to keep up with ongoing fluid losses from persistent purging of watery diarrhoea. Each stool that occurs during maintenance therapy should be replaced with a volume of ORS similar to that given during maintenance therapy. Ideally, maintenance therapy should be with ORS but can be performed with intravenous fluid in addition to that prescribed initially. Children with severe acute malnutrition should be managed carefully to prevent acute fluid overload and heart failure; in these patients, the intavenous rehydration process is spaced over 10–12 hours[165].

Antibiotic therapy. Antibiotic treatment is initiated following resolution of the initial fluid deficit and cessation of vomiting. This therapy reduces the total volume of stool passed and shortens the period of faecal excretion of *V. cholerae*[166]. The choice of antibiotic can be dictated by local antimicrobial susceptibility profiles. At present, azithromycin and ciprofloxacin are commonly used[122,167]. However, the pattern of resistance of pathogens can fluctuate in accordance with withdrawal of use of a specific antibiotic in the community. Because of widespread resistance to antibiotics, surveillance is needed to detect changing sensitivity patterns for determining the drug of choice for treating cholera. Molecular analyses are also conducted to determine the mechanism of resistance and its global spread[168]. When the local *V. cholerae* isolates are sensitive to azithromycin, children are given 20 mg per kg and adults are given 1 g, each as a single-dose regimen[169]. Ciprofloxacin is given to children in a dose of 15 mg per kg twice daily for 3 days. On the basis of the antibiotic resistance pattern, a single dose of 300 mg doxycycline in adults may be prescribed. Prophylactic administration of antibiotics during outbreaks or for travellers is not advised.

Zinc supplementation. Zinc therapy in children reduces the morbidity and mortality associated with cholera and other diarrhoeal diseases, as zinc can decrease the duration and severity of diarrhoea[170]. Zinc is given daily to children (6 months to 5 years of age) with diarrhoea, including those with cholera, as an adjunct therapy, starting as soon as the vomiting subsides, for 10 days[171]. For children with severe malnutrition and diarrhoea, zinc treatment is recommended for 14 days[172]. The dehydration status is determined on the basis of clinical signs and symptoms[164,173]. If there are no signs of dehydration, the preferred treatment is with ORS to prevent dehydration and continued oral feeding is advised for quick recovery.

Complex emergencies. Cholera is increasingly being reported among populations that have never previously witnessed this disease (as in Haiti[174] and Yemen[175]) and in cholera-endemic settings that experience natural or man-made disasters (as in, for example, some African countries, Iraq and Pakistan). In addition to traditional interventions (such as improvements and access to safe water, sanitation and hygiene), prevention and mitigation of cholera in these complex settings also require a portfolio of unconventional strategies, including social, political and educational awareness with implementation of existing treatment and prevention strategies and new scientific-based tools. In these complex scenarios, first responders (such as Doctors without Borders, the United Nations, the WHO and different non-governmental organizations) need to engage local partners (including Ministry of Health and community leaders) and potentially volunteer groups with empathy and emphasize the disastrous consequences of the outbreak if it is not counteracted fast and effectively. The involvement of local organizations in the affected areas could help first responders to mitigate disease transmission through practising optimal sanitation, hygiene, clean food and water intake and avoiding contact with the belongings of patients with cholera. Moreover, locals can ensure that any person showing symptoms of cholera must seek immediate attention at their nearby hospitals and/or clinics to receive adequate treatment. Finally, scientists are also investigating whether a cocktail of vibriophages (bacteriophages that specifically infect V. cholerae) can be used as an alternative therapy for cholera treatment[176].

Vibriosis

V. alginolyticus is often associated with ear and soft tissue infections, which can be readily treated using appropriate antibiotics such as doxycycline[177]. Gastroenteritis associated with *V. parahaemolyticus* infection is typically self-limiting and resolves within 72 hours[42]. Very rarely, medical intervention is required, although antibiotic therapy is sometimes used if infections do not resolve or progress to systemic infections. However, given the extremely rapid development of *V. vulnificus* infections, rapid diagnosis of both septicaemia and localized wound infections is absolutely essential. Often crucial to this end is obtaining good patient histories that indicate raw seafood (particularly oyster) consumption and/or water exposure. Along with rapid diagnosis, prompt and appropriate antibiotic treatment is also needed, as a treatment delay of ≥72 hours after symptom development can raise the case fatality rate to 100%[178]. Hospitalized patients with *V. vulnificus* infection frequently require tissue debridement (removal of necrotic tissue) and/or amputation of the affected limbs to limit spread of the infection. Antibiotic treatment recommendations for *Vibrio* spp. infections include tetracyclines (for example, doxycycline and tetracycline), fluoroquinolones (ciprofloxacin and levofloxacin), third-generation cephalosporins (cefotaxime, ceftazidime and ceftriaxone), aminoglycosides (amikacin, apramycin, gentamicin and streptomycin) and folate pathway inhibitors (trimethoprim–sulfamethoxazole)[179,180]. The CDC recommends a treatment course of doxycycline (oral or intravenous twice a day for 7–14 days) and a third-generation cephalosporin (intravenous or intramuscular every 8 hours for 7–14 days). However, a review of antibiotic usage data suggests that for *V. vulnificus*, treatment that includes either quinolone or tetracycline is associated with lower mortality than treatment with cephalosporin alone[181]. Some resistance to antibiotics has been observed in non-cholera *Vibrio* spp. such as *V. parahaemolyticus* and *V. vulnificus* from environmental sources[179,182,183]. There is increasing interest

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Box 3 | **Key approaches to tackle *Vibrio* spp. infections**

Translational approaches focusing on the following key areas could improve the management and understanding of *Vibrio* spp. infections and help to reduce the incidence of disease.

Improving access to molecular methods
Advances in molecular methods for strain typing, such as whole-genome sequencing, can help to answer key research questions, such as the source, transmission and dynamics of *Vibrio* spp. outbreaks. These methods are being widely applied in high-income countries, yet using these techniques in areas where *Vibrio* spp. infections and outbreaks are often endemic remains challenging. Overcoming this hurdle requires international cooperation and the sharing of data and resources as well as standardized methods, techniques and protocols.

Defining environmental pro-epidemic conditions
The ability to retrospectively scrutinize the environmental cues before and during outbreaks offers tantalizing insights into the specific conditions that drive the onset and spread of infectious diseases, including *Vibrio* spp. infections. The synthesis of a wealth of available environmental data — including sea surface temperature, salinity, turbidity and chlorophyll level, among others — to build up a clear picture of the conditions that can trigger outbreaks will undoubtedly help to refine risk assessment and management approaches. These approaches will become even more-important in a setting of global climate warming, and the sharing of data and resources is essential to develop this emerging area of science.

Improving epidemiology
The effect of these diverse pathogens highlights the need for continued and improved global epidemiology and surveillance, as the analysis of epidemiology data is crucial to determine key aspects related to infections, such as the source, route of transmission, host susceptibility and changes in incidence, among others. Countries with well-established systems such as the United States can represent a useful blueprint.

in the generation of new therapeutic approaches for specific *Vibrio* spp. pathogens. New monoclonal antibodies against the carboxyl terminus of *V. vulnificus* putative MARTX, RtxA1, effectively provided protective immunity in a mouse model of *V. vulnificus* infection[161]. Such approaches could be useful in particular for at-risk groups (for example, people handling shellfish produce).

Quality of life

In areas of the world where sanitation and access to clean drinking water are lacking, cholera continues to represent an important cause of morbidity and mortality. Advances in rehydration therapy have made cholera a treatable disease with a low case fatality rate[147]. However, individuals who survive cholera can have both short-term and long-term health consequences. The immediate consequences include loss of body weight and physical vigour, resulting in restrictions on an individual's day-to-day quality of life. The purging of watery stool results in expulsion of healthy gut commensal microbiota, and the 3-day course of antibiotic therapy could enable drug-resistant pathogenic Enterobacteriaceae bacteria to be selectively enriched in the gut[184]. Colonization of the gut by drug-resistant pathogenic genera is causally related to frequent diarrhoea in children of ≤5 years of age[185], with long-term consequences such as persistent microbiota immaturity (as the prevalence of drug-resistant species affects the normal commensal gut microbiota)[186], which could contribute to malnutrition and lead to irreparable growth stunting and impaired cognitive function. Although the WHO prequalified OCVs have been successfully used in vaccination programmes to prevent cholera in many countries, for a vast number of people the access to vaccines when there is an outbreak still remains challenging owing to the underlying financial and logistic issues globally. Many studies have estimated the cost-effectiveness of vaccine use following WHO criteria and found it both considerable and incremental. The incremental cost-effectiveness ratios for cholera in Zanzibar, Africa, were estimated to be US$750,000 per death averted, US$6,000 per case averted and US$30,000 per disability-adjusted life-year averted, regardless of the differences in the health-care providers and the societal perspectives on the effect of cholera on individuals and on the use of vaccines[187].

Cases of vibriosis are typically self-limiting (for example, *V. parahaemolyticus*-associated gastroenteritis), with few notable long-term quality-of-life issues associated with these infections. However, *V. vulnificus*-associated wound infections can frequently result in life-altering clinical interventions such as limb or finger amputation and substantial tissue debridement (FIG. 5). *V. vulnificus* infections are also the most expensive marine-acquired infections[188], underlying the substantial and long-term[189] medical interventions often required. A key feature of vibriosis (in particular wound infections) is the need for stringent education programmes for at-risk groups, such as individuals with underlying risk conditions (for example, liver disease, a compromised immune system and diabetes mellitus).

Outlook

Vibrio spp. infections have remained a scourge to humankind for over a millennium[190], and *V. cholerae* and *V. parahaemolyticus* have caused numerous pandemic outbreaks[47,191]. Many advances have been made in our knowledge of the mechanism of persistence of pathogenic *Vibrio* spp. in aquatic reservoirs, the transmission of bacteria from reservoirs to humans causing infections and the pathobiology of disease, and progress has been made in rapid diagnosis and effective intervention strategies. Nevertheless, *Vibrio* spp. infections are expected to rise in the future. Such a rise could be attributed to global warming, continued population growth (particularly in developing countries where poverty is common), protracted wars and dwindling access to safe drinking water and optimal sanitation. Given that *Vibrio* spp. infections — particularly cholera — disproportionately affect individuals living in resource-poor countries, the mobilization of resources and timely distribution and storage of supplies, including ORS, vaccines, antibiotics and water purification tablets, are central to mitigate these threats. In addition, risk awareness campaigns and information on management of *Vibrio* spp. infections through mass media, including social media, would be beneficial. Of note, the majority of *Vibrio* spp. infections are seasonal and primarily driven by increased temperature, rainfall events and contamination of aquatic reservoirs with *Vibrio* spp. pathogens. The transient bloom of *Vibrio* spp. in aquatic reservoirs during warm months is thought to 'spill over' to susceptible human populations. Basic and translational research in different areas will contribute to reduce the burden of *Vibrio* spp. infections (BOX 3).

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Cholera

Cholera is a preventable disease, yet it remains a persistent source of morbidity and mortality, particularly in resource-limited countries with insufficient access to clean water and appropriate sanitation, and can emerge rapidly in areas where basic environmental infrastructures are disrupted (BOX 4). The most effective means to tackle cholera outbreaks are coordinated and multidisciplinary approaches and the availability of appropriate vaccines. Studies have shown that knowledge of the local understanding of cholera can be used to help to manage outbreaks[192], and attention to local social and cultural features of cholera can help to increase vaccine coverage[193]. Thus, sociocultural sciences can contribute to understanding and mitigating cholera outbreaks. Recent investigations have demonstrated the effective killing of *V. cholerae* by vibriophages[194]. Manipulating the environmental vibriophage populations could be a future approach to ameliorate cholera outbreaks, promptly shorten cholera epidemics at the community level and limit *V. cholerae* spread from environmental blooms.

However, many questions remain unanswered. For example, how can toxigenic *V. cholerae* persist in aquatic reservoirs during interepidemic periods and erupt sporadically in some regions, whereas it completely disappears in other regions? Why do only 20–25% of individuals with *V. cholerae* infection exhibit symptomatic cholera, whereas most individuals are asymptomatic carriers? With the advent of modern molecular techniques, including metagenomics analysis, real-time PCR and single-cell sorting with automated genetic characterization, *V. cholerae* from both aquatic reservoirs (including biofilm bacteria) and stool and/or rectal swab samples of household members of patients with cholera should be examined to address these questions. Knowledge gained from these suggested investigations can potentially help to identify risk factors for *Vibrio* spp. infections and design appropriate and effective intervention strategies to mitigate *Vibrio* spp. infections.

Box 4 | **Emergence of cholera in Haiti and Yemen**

In October 2010, a massive cholera epidemic broke out in Haiti after ≥100 years[210], following a severe earthquake earlier that year, resulting in a complete breakdown of health infrastructures, including safe drinking water supply, sanitation and hygiene. Available scientific and circumstantial evidence[211] supports the hypothesis that cholera was introduced in Haiti by a Nepalese UN peace-keeping trooper who had *Vibrio cholerae* infection but was asymptomatic[212]. Subsequently, infections were also reported in the Dominican Republic, Cuba and Mexico, highlighting the recent expansion of cases[17]. The initial explosive wave of cholera cases in Haiti between 2010 and 2012, followed by periodic occurrence of the disease, confirms that cholera is now endemic in Haiti, as in Asia and Africa[20,174], and undergoes regular annual seasonal epidemic peaks[213,214].

In October 2016, a major cholera outbreak was reported by the WHO in Sana'a, the capital city of Yemen, driven primarily by war that has disrupted drinking water supply, sanitation and hygiene and promoted the internal displacement of the Yemeni population, which further exacerbated the disease burden. Until November 2017, >900,000 cases had been reported, with 2,196 fatalities[175]. According to the WHO, this outbreak is unusual because of its rapid spread; however, cholera can spread rapidly in a naive population (a population lacking prior immunity).

Both Haiti and Yemen cholera events highlighted the fact that cholera can break out in any country or area that has experienced sustained natural and/or man-made disasters (such as conflicts and war). *V. cholerae* can be of indigenous or exogenous (imported via asymptomatic carrier or through an environmental source) origin. In conclusion, international communities and aid organizations should be ready to act swiftly, as cholera always strikes resource-poor countries lacking experience in dealing with the disease.

Changing epidemiology

A notable data gap in the field of *Vibrio* spp. research is the availability of surveillance data regarding *Vibrio* spp. infections globally. For instance, in Europe, vibriosis is not a notifiable infection[42,62]; as a result, there is a paucity of epidemiological and surveillance data regarding these pathogens. Good-quality national surveillance systems do exist, such as COVIS and FoodNet in the United States[7], and the data gathered from such systems are invaluable in building up a clear picture of the routes of exposure and changes in incidence and regional effects that these pathogens can have (FIG. 3). We advocate developing a centralized depository of epidemiological data that can be used by researchers in the field of *Vibrio* spp. microbiology.

Climate warming, in particular rapid warming of coastal regions caused by climate change[195], is likely to greatly expand the geographical extent and effects of pathogenic *Vibrio* spp.[1,6,21,49,196] (FIG. 6). Striking evidence of this process has emerged since 2000, including key studies assessing the link between the changing abundance of these bacteria in the environment and environmental warming[196,197], the increasing number of reported wound infections observed in temperate areas[161] and shellfish-associated outbreaks at high latitudes[198,199]. These studies, coupled with the preference of these pathogens for growing in warm, brackish water, suggest that these bacteria are likely to continue causing infections under a warming climate scenario (FIG. 6).

Methods such as whole-genome sequencing have provided incredible insights into the genomic structure, evolution[19,20] and virulence capabilities of key members of the *Vibrio* genus. Whole-genome sequencing has enabled the determination of the source of outbreaks[18] and the establishment of the evolutionary history of causative strains[191]. However, there remain many questions that advances in strain genotyping methods will help to resolve, such as why the Bay of Bengal region has been the source of many pandemic waves of *V. cholerae*[191] and pandemic O3:K6 *V. parahaemolyticus*[47] (FIG. 2), or how the rapid geographical expansion of infections following the establishment of strains with pandemic potential was possible. Certainly, with cholera, human travel has been an effective long-distance mechanism of transmission[200]. However, *V. parahaemolyticus* has a very different lifestyle and disease transmission route. The rapid expansion of *V. parahaemolyticus* during pandemic spreading, such as the O3:K6 strain in the 1990s and early 2000s[47] and the 2012 long-distance expansion of the Pacific Northwest ST36 clonal type[51,201], suggests that mechanisms such as the transfer of shellfish or ballast water movement[202–204] are involved. Novel sequencing and bioinformatic approaches to infer the evolutionary timelines coupled to these physical transmission routes may help to shed light on epidemic spreading.

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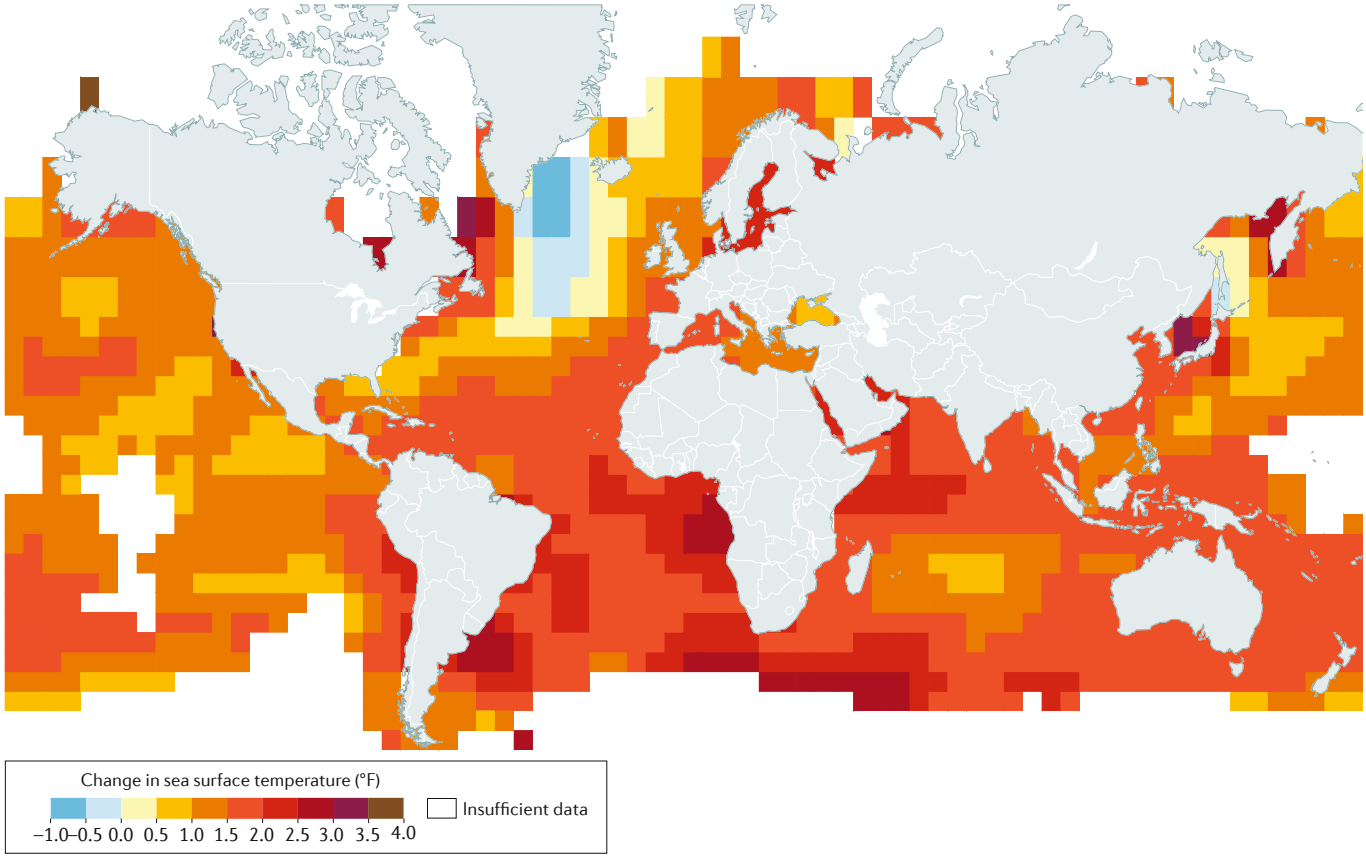

Fig. 6 | **Effect of climate change on *Vibrio* spp. infections.** Average change in the sea surface temperature around the world between 1901 and 2015. Measurements are based on a combination of direct measurements and satellite measurements. Data show that in most parts of the world the oceans have become warmer, although regionally a decrease in the temperature has occurred (for example, in the North Atlantic). Overall, rising sea surface temperatures favour the spread of *Vibrio* spp. and increase the population at risk of infection. Based on Figure 2.21 from Hartmann, D.L., et al, in Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (eds Stocker, T.F. et al). (Cambridge University Press, Cambridge and New York, 2013).

A key area of current work is determining the environmental features that drive *Vibrio* spp. outbreaks. Retrospective studies that have analysed key environmental features before, during and after *Vibrio* spp. outbreaks are identifying key factors involved in pathogen proliferation, such as anomalous temperature-driven events in non-cholera *Vibrio* spp.[41,49,62], and the role of temperature, sea surface height, plankton blooms, precipitation and flooding in *V. cholerae* emergence[24,26,27,29,30]. The methods that frequently use remote-sensing-based approaches are now providing key insights into what specific environmental conditions help to drive outbreaks[22]. Linking these approaches alongside epidemiological and climatic models such as regional climate change models (for example, with different emission scenarios) may help to identify where outbreaks are likely to emerge in the future[205]. This information is particularly relevant given predictions regarding warming in many coastal regions driven by climate change.

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Author contributions

Introduction (C.B.-A.); Epidemiology (A.A., M.A. and J.M.-U.); Mechanisms/pathophysiology (J.D.O. and M.K.W.); Diagnosis, screening and prevention (J.D.O. and F.Q.); Management (J.D.O. and F.Q.); Quality of life (M.A. and C.B.-A.); Outlook (A.A., C.B.-A. and J.M.-U.); Overview of Primer (C.B.-A.).

Competing interests

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