ENVIRONMENTAL RESERVOIRS AND MECHANISMS OF PERSISTENCEOF VIBRIO CHOLERAE

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ABSTRACT

Vibrio cholerae, which causes cholera, is now known to be acquired from ambient sources between epidemics. This bacteria can now be found outside of endemic locations because to developments in molecular technology. V. cholerae persists in aquatic environments by intra- and interspecific mechanisms such responsive gene regulation, biofilm formation on biotic and abiotic surfaces, and interactions with many other species. This review discusses how this bacteria survives in the environment. We will describe how V. cholerae survives hunger, temperature, and salinity changes and heterotrophic protist predation.

Keywords: *protozoa, biofilms, chitin, zooplankton, predation, stress, starvation adaptation, viable but non- culturable*

INTRODUCTION

Since 1817, cholera epidemics have been scientifically recorded (Pollitzer, 1954). Sir John Snow was the first to relate dirty water to cholera outbreaks and minimize infection in 1849. (Snow, 1855). His discovery may have started Vib-rio cholerae ecological studies. Epidemiology was born. V. cholerae was discovered as an autochthonous aquatic bacteria 120 years later (Colwell et al., 1977). O1 and O139's CT and toxin co-regulated pilus genes cause cholera in V. cholerae's 200 serogroups (TCP; Chatterjee et al., 2007). Most V. cholerae strains in non-endemic areas are non-toxigenic (Faruque et al., 2004; Haley et al., 2012; Islam et al., 2013), indicating that human host connections constitute a minor element of the life cycle and not necessary for environmental persistence.

Vibrio cholerae dwells in tropical and temperate seas worldwide, including the Bay of Bengal, where pandemics continue (e.g., USA, South America, Aus- tralia, Sweden, and Italy, e.g., Vezzulli et al., 2009; Collin and Rehnstam-Holm, 2011; Schuster et al., 2011; Islam et al., 2013; Tall et al., 2013; Figure 1). Breakthroughs in genetic detection and ecology demonstrate that V. cholerae is a widespread aquatic species that may infect humans (Sack et al., 2004).

V. cholerae may adapt to nutrient limitation, salinity, temperature, heterotrophic protist, and bacteriophage predation. A viable but non-culturable (VBNC) state may result from unfavourable conditions (Colwell, 2000; Thomas et al., 2006). V. cholerae biofilms on chitinous and gelatinous zoo- and phytoplankton (Huq et al., 1996; Akselman et al., 2010; Shikuma and Hadfield, 2010). Mobile host biofilms improve stress tolerance, nutrition, and dispersion (Costerton et al., 1995; Hall-Stoodley et al., 2004). Summary of V. cholerae's water adaption and persistence.

SURFACE COLONIZATION AND BIOFILM FORMATION ENHANCE V. cholerae PERSISTENCE

Aquatic bacteria benefit from liquid–

surface nutrient access (Dawson et al., 1981). Hence, surface adhesion may enable nutrient-poor bacteria thrive (Dawson et al., 1981; Figure 2). Certain surfaces nourish germs (e.g., chitin; Nalin et al., 1979). Hence, V. cholerae inhabits many abiotic surfaces.

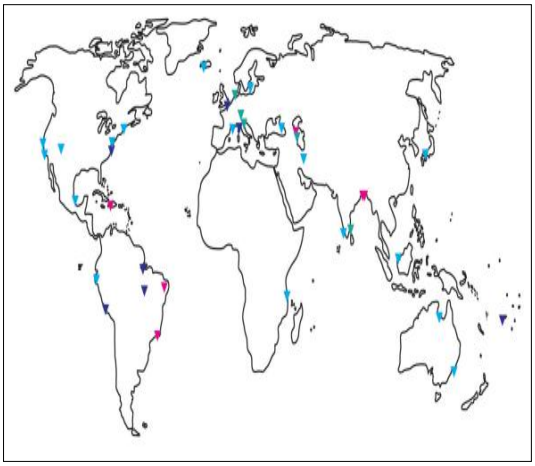

FIGURE 1 | Global distribution of Vibrio cholerae.

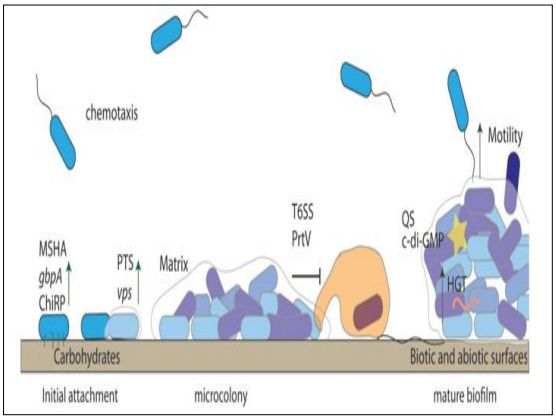

FIGURE 2 Biofilm formation enhances Vibrio cholerae persistence.

ships, zooplankton, macroalgae, and floating aggregates (Hood and Winter, 1997). (2006).

Pilin subunit-expressed pili form biofilms on Vibrio cholerae. Pilin subunits and pili help V. cholerae stick to surfaces (Boyd and Waldor, 2002; Aagesen and Häse, 2012). V. cholerae, like other Vibrionaceae, is chitinolytic and has numerous genes to adhere and degrade this ecologically important substrate (Meibom et al., 2004; Hunt et al., 2008). GlcNAc/NAG, the second most prevalent organic polymer, feeds bacteria carbon (Rinaudo, 2006; Martínez et al., 2009). GbpA and MSHA bind V. cholerae chitin (Chiavelli et al., 2001; Figure 2). Chitin and TCP interact. Undifferentiated biofilms without TCP degrade chitin less efficiently, decreasing ecological fitness (Reguera and Kolter, 2005).

V. cholerae develops biofilms after adhesion (Yildiz and Visick, 2009). Type IV pili, flagella, and Vibrio polysaccharide promote V. cholerae biofilm growth (VPS; Watnick and Kolter, 1999). VPS cells, microcolonies, biofilms (Watnick and Kolter, 1999; Watnick et al., 2001). The rugose V. cholerae colony type is more resistant to chlorine, low pH, osmotic and oxidative stress, anti-bacterial serum, SDS, and phage (N. (Sun et al., 2013). Little environmental rugose V. cholerae reports exist, making VPS's environmental protection value unclear (Ali et al., 2002; Jubair et al., 2012).

Two big chromosomal carbohydrate biosynthesis operons include VPS structural genes (Yildiz and Schoolnik, 1999; Fong et al., 2010). vpsI has A–K, whereas vpsII has L–Q. Biofilm is produced by six vps operon genes (rbmA–F) (Fong and Yildiz, 2007; Absalon et al., 2011; Berk et al., 2012). VPS development requires carbs in biofilm management. Sugars and regulators alter VPS expression. VpsR and VpsT produce VPS c-di-GMP-dependently (Yildiz et al., 2001; Casper-Lindley and Yildiz, 2004). Srivastava (2011). Environmental circumstances influence bacterial surface association through C-di-GMP (Yildiz, 2008).

Bacteria require quorum sensing (QS) to form biofilms (Liu et al., 2007; Muller et

al., 2007). At high V. cholerae cell densities, the QS response regulator, HapR, positively modulates the transcription of hapA, which encodes the hemagglutinin protease (HAP; Jobling and Holmes, 1997; Zhu et al., 2002), cytR, a biofilm repressor, and flagellum biosynthesis genes (Yildiz et (Jobling and Holmes, 1997; Zhu et al., 2002; Hammer and Bassler, 2003; Zhu and Mekalanos, 2003; Yildiz et al., 2004). QS and c-di-GMP characteristics accelerate environmental adaption and survival. Moving from free-swimming to linked lives (Yildiz and Visick, 2009; Srivastava and Waters, 2012) improves predation resistance, natural cooperation, and horizontal gene transfer (HGT; Lo Scrudato and Blokesch, 2012). (Matz et al., 2005). Biofilms support V. cholerae without nutrients. Temperature, salinity, and pH affect adaptation.

"VIABLE BUT NON-CULTURABLE" V. cholerae IN PLANKTON

Smaller than starved cells, VBNC cells are metabolically active (Nilsson et al., 1991; McDougald et al., 1998; Oliver, 2010). Since V. cholerae became VBNC (Xu et al., 1982), numerous bacteria—pathogens and non-pathogens—have entered under unfavorable circumstances (McDougald et al., 1998, 1999; Oliver, 2005, 2010). V. cholerae may generate VBNCs under high temperatures, salinity, and nutrient deprivation (Colwell et al., 1985; Ravel, 1995; Carroll, 2001; González-Escalona, 2006; Thomas, 2006; Mishra, 2012). Crustaceans, algae, chironomid egg masses, and bacterioplankton contain V. cholerae VBNC cells (e.g., Louis et al., 2003; Binsztein et al., 2004; Alam et al., 2007; Halpern et al., 2007). VBNC cells and culturable cells of V. cholerae are detected in bacterioplankton and biofilm consortia on biotic and abiotic surfaces (Alam et al., 2006). Mishra et al. (2012) observed VBNC V. cholerae pathogenicity and colonization in freshwater microcosms, validating the state's cholera epidemiology.

As circumstances improve, VBNC resuscitates and divides cells (McDougald et al., 1998). Marine diffusion chambers may resuscitate VBNC-stage Vibrio vulnificus (Oliver et al., 1995). Temperature rise (Nilsson et al., 1991; Mishra et al., 2012) Although numerous variables may promote VBNC development in distinct species, dietary increase may cause resuscitation (Binsztein et al., 2004; Senoh et al., 2010).

Animal digestion makes VBNC V. cholerae cells culturable (Colwell et al., 1985; Alam et al., 2007; Asakura et al., 2007). Human volunteers colonized their intestines with VBNC V. cholerae cells for 7 weeks and ejected culturable cells (Colwell et al., 1996). VBNC cells contain V. cholerae. before dying (Weichart et al., 1997). Co-incubation with eukaryotic cells cannot resuscitate long-term VBNC cells (more than 91 days; Senoh et al., 2010).

QS runs VBNC now. QS generates biofilm from culturable V. cholerae for VBNC (Kamruzzaman et al., 2010). QS autoinducers revived Bangladeshi surface water VBNC cells. Two autoinducers increased CFU counts after 4–5 h. (Bari et al., 2013). Non-growing VBNC cells may create ROS after meal addition, rendering them unviable on agar plates. Diet may cause metabolic imbalance, ROS generation, and cell death (Bloomfield et al., 1998). Catalase or peroxide-degrading chemicals may revive

VBNC Escherichia coli (Mizunoe et al., 1999), and removing hydrogen peroxide from starved cells can prevent VBNC development (Arana et al., 1992). Low-temperature incubation reduces catalase activity, rendering V. vulnificus ROS susceptible (Kong et al., 2004).

Recent findings show VBNC cells resuscitate stochastically (Epstein, 2009). The authors say dormant cells randomly awaken and multiply under favorable circumstances. Revived cells are environmental "scouts" (Buerger et al., 2012a,b). Poor circumstances will kill Scouts. Favorable circumstances increase genetic diversity. Long-term incubation on single-cell microtiter plates randomly culturable marine and soil bacteria. 48–72 h subculture 3–4-week-old strains (Buerger et al., 2012b). Hence, the low-cost population-based VBNC state enables bacteria to sleep for long periods and maybe wake up when a trigger, such as an initiating signal, is available or randomly test their surroundings to develop when circumstances are suitable. Stochastic VBNC resuscitation signals may explain V. cholerae epidemics.

Vibrio cholerae RESPONSES TO ENVIRONMENTAL STRESSES – BOTTOM-UP CONTROL OF V. cholerae

Temperature, salinity, phytoplankton, and zooplankton affect Vibrio spp (Turner et al., 2009, 2013; Johnson et al., 2010; Asplund et al., 2011). Above 15°C, vibrio spp (Blackwell and Oliver, 2008; Lama et al., 2011; Johnson et al., 2012). Several studies show that Vibrio spp. abundance follows a temperature-dependent seasonal cycle (e.g., Louis et al., 2003; Binsztein et al., 2004). Temperature affects V. cholerae adhesion to chitinous zooplankton. MSHA and GbpA colonization factors bind to chitin above 15°C (Castro-Rosas and Escartìn, 2005; Turner et al., 2009; Stauder, 2010). V. cholerae was more commonly planktonic than attached to plankton at Chesapeake Bay water temperatures over 19°C (Louis et al., 2003). Hence, cholera pandemic causes need environmental assessments, data collecting, and analysis.

Temperature and freshwater input affect marine water salinity. 35 ppt. Nonetheless, freshwater influx from rivers or rain run-off may decrease salinity at coastal and estuary settings (Jutla et al., 2011), whereas solar evaporation, particularly in the tropics, may increase it. Vibrio prefers salinities <25 ppt (e.g., Jiang, 2001; Thomas et al., 2006; Baker-Austin et al., 2010). Melanin protects high-salinity V. cholerae (Coyne and al-Harthi, 1992). (2009). Numerous studies link salinity to V. cholerae incidence (Singleton et al., 1982; Johnson et al., 2010), although others do not (Johnson et al., 2012). Surface attachment—essential for environmental persistence—was unaffected by salt, according to Stauder et al. (2010). (see Section "Association with Other Organisms").

Spring/autumn coastal/estuarine discharge increases nutrients. This may encourage phytoplankton and zooplankton blooms, which provide V. cholerae with chitinous surfaces. Bacterivorous predators may not prevent population growth.

Waste and cell lysis harm micronutrient patches (Blackburn et al., 1998). Planktonic bacteria need nutrient patches (for a review of see, Stocker and Sey- mour, 2012). V. cholerae has one sodium-powered sheathed polar flagella (Hranitzky et al., 1980). (1999). V. cholerae has several duplicated chemotaxis-related genes, proving its environmental survivability (Heidelberg et al., 2000). Many V. cholera chemotaxis genes are auxiliary or have unknown

environmental roles (Gosink et al., 2002). V. cholerae is chemotactic for all amino acids, making proteins, peptides, and amino acids important aquatic nutrients (Freter and O'Brien, 1981). V. cholerae responds to oligosaccharides by upregulating chemotaxis genes to attach to chitinous species (Meibom et al., 2004).

V. cholerae survives and gets nutrition from biofilm and VPS. Sugars, phosphorus, and nitrogen affect V. cholerae biofilm formation. Glucose and mannose create biofilm VPS (Kierek and Watnick, 2003; Moorthy and Watnick, 2004). PTS activates V. cholerae biofilm formation and VPS gene transcription (Houot and Watnick, 2008; Houot et al., 2010). V. cholerae PTS that reacts to intracellular nitrogen concentrations may inhibit VPS synthesis. The receptor molecule and signaling mechanism are unclear (Houot et al., 2010).

Phosphorous affects surface colonization. PhoBR, a two-component system, enables V. cholerae survive planktonically in phosphorus-depleted environments. PhoR phosphorylates response regulator PhoB, limiting VPS synthesis (Pratt et al., 2009; Sultan et al., 2010).

eDNA settles planktonic V. cholerae cells in biofilms (Haugo and Watnick, 2002). CytR inhibits VPS and biofilm formation (Haugo and Watnick, 2002). DNase-rich V. cholerae may consume eDNA (Focareta and Manning, 1991). (2011).

"Feast-to-famine" bacteria store nutrients when aquatic food source varies. Starvation may provide carbon from bacterial glycogen granules (Preiss and Romeo, 1994). Nutrient-rich V. cholerae boosts glycogen precursors (Kan et al., 2004). Cholera patients' nutrient-poor rice water feces contain glycogen granules (Bourassa and Camilli, 2009), suggesting that glycogen storage may nourish V. cholerae in aquatic environments. RpoS protects against acidity, salt, and hydrogen peroxide destruction by activating glycogen and Pi storage (Jahid et al., 2006). V. cholerae contains 100 times more membrane-bound Pi granules than E. coli (Ogawa et al., 2000). Pi-deficient V. cholerae mutants had decreased motility, abiotic surface adhesion, and delayed adaptation to 200 mM calcium media (Ogawa et al., 2000).

Cofactors like iron impede cellular metabolism (Wackett et al., 1989). (1992). Water depth impacts iron content (Martin and Michael Gordon, 1988).

V. cholerae has many iron transport routes and sensors for low-iron conditions (Heidelberg et al., 2000; Wyckoff et al., 2006, 2007). Feo and vibriobactin bind ferrous iron (Griffiths et al., 1984). (2006). The ferric uptake regulator (Fur) represses iron-regulated gene promoters in iron-rich environments (Bagg and Neilands, 1987). V. cholerae employs Vibrio fluvialis siderophores like flu-vibactin (Yamamoto et al., 1993).

V. cholerae may withstand nutritional deficiency. Jubair et al. (2012) tested V. cholerae's long-term starvation survival in the lab (700 days). Persister phenotype separates starving from VBNC cells. Phosphate and chitin, V. cholerae nutrition, increased persister cell production. 40-day-fasted V. cholerae preserved chitin attachment ligands (Pruzzo et al., 2003). These studies show the relevance of interacting with chitinous creatures, as indicated in Section "Interactions. "Top-down predatory micrograzer control

Heterotrophic protists kill germs, whereas nutrients control V. cholerae "bottom-up" (Hahn and Höfle, 2001; Matz and Kjelleberg, 2005). Bacteria-eating protists and zooplankton devour V. cholerae. Stress management affects V. cholerae's

persistence and seasonal accumulation. Ciliates and heterotrophic nanoflagellates (HNFs) eliminate V. cholerae from ambient water in Gulf of Mexico microcosms (Martínez Pérez et al., 2004). V. cholerae ciliates and flagellates may graze 600–2,000 bacteria cell−1 h−1 (Macek et al., 1997). Heterotrophic protists enhance grazing mortality, limiting V. cholera levels, according to Worden et al. (2006). At severe phytoplankton blooms with four doublings per day, V. cholerae may overcome grazing pressure and achieve an infectious dosage.

Biofilm-encased Vibrio cholerae cells survive HNFs (Matz et al., 2005). VPS from predation causes biofilm and smooth-to-rugose morphology (Matz et al., 2005). VPS protected V. cholerae cells against predators, preventing biofilm grazing (Sun et al., 2013). Biofilms protect and express QS-regulated anti-protozoal factors that plankton cannot.

In field testing, QS-deficient V. cholerae was more grazeable than the wild variety. V. cholerae modulates anti-protozoal activity by QS and other mechanisms (Erken et al., 2011). QS was more predator-resistant than VPS mutants to surface grazing by the amoeba Acanthamoeba castellanii and the HNF Rhynchomonas nasuta (Sun et al., 2013). QS secretes PrtV protease and an uncharacterized anti-protozoal component (Matz et al., 2005) to guard against Tetrahymena pyriformis, Cafeteria roen-bergensis, and Caenorhabditis elegans (Vaitkevicius et al., 2006).

T6SS inhibits Dictyostelium discoideum, mammalian macrophage, and E. coli predation (Pukatzki et al., 2006; MacIntyre et al., 2010). Three T6SS proteins—VgrG-1, -2, and -3—form syringes that puncture the cell membrane and transport VasX, a virulence factor, into D. discoideum (Pukatzki et al., 2007; Miyata et al., 2011). All V. cholerae strains contain this mechanism, however expression varies (Ishikawa et al., 2009). (2012). Non-O1/non-O139 strains express T6SS constitutively, unlike pandemic El Tor strains (Miyata et al., 2010).

Digestion protects bacteria against protozoans. Clinical and environmental V. cholerae strains may survive intracellularly in amoeba (Abd et al., 2004, 2005; Jain et al., 2006). Free-living amoeba increase V. cholerae development (Thom et al., 1992; Sandström et al., 2010; Valeru, 2012), confirming their environmental reservoir function. V. cholerae cells in amoeba stress-resistant cysts may migrate throughout aquatic ecosystems (Thom et al., 1992; Abd, 2004). Brown and Barker, 1999. Amoeba cysts may spread cholera (Winiecka-Krusnell and Linder, 2001). V. cholerae and amoeba have been widely investigated, but little is known about their intracellular survival mechanisms (Thom et al., 1992; Abd, 2005, 2007; Sandström, 2010). Inducible acid tolerance helps digestive vacuoles survive (Merrell and Camilli, 1999). ToxR protects amoeba (Valeru et al., 2012). OmpU and OmpT, controlled by ToxR, may help survival. Experimentally, protozoan host cell attachment is unknown (Abd et al., 2009, 2011). MSHA/capsule/LPS O side chain adhesins are inactive (Lock et al., 1987; Abd et al., 2009).

Protozoa's survival methods are unknown (Rowbotham, 1980; Bozue and Johnson, 1996; Brandl et al., 2005). Environmental prevalence and VBNC resuscitation should study V. cholerae–protozoa interactions. Bacteria need to tolerate predatory protists and feces. Understanding how higher species boost V. cholerae fitness is critical

to its persistence and spread.

Phage, predatory bacteria, and phagotrophic protists alter V. cholerae abundance and serogroup prevalence. CTX phage (CT) renders non-toxigenic strains hazardous (Miller and Mekalanos, 1988; Pearson et al., 1993; Waldor and Mekalanos, 1996). During cholera outbreaks, V. cholerae cells and phages grow in Bangladesh's aquatic environment (Faruque et al., 2005). Phages decrease cholera. Myoviridae species dominated V. cholerae lytic bacteriophage in stool samples (Seed et al., 2011). Environmental investigations found Myoviri-dae in cholera-stricken Peru, Calcutta, and Kenya (Maina et al., 2013). A continuous culture experiment reveals that phage manage V. cholerae populations more than nutrition (Wei et al., 2011). Bdellovibrio may infect V. cholerae (Chen et al., 2012). Predatory bacteria-V. cholerae interactions are unknown.

ASSOCIATION WITH OTHER ORGANISMS

Vibrio cholerae interacts with aquatic creatures besides heterotrophic protists. Ducks, fish, mussels, diatoms, cyanobacteria, and dinoflagellates interact with it (Islam et al., 1999). Figure 3 (Binsztein et al., 2004; Akselman, 2010). Since 1980's copepod cell discovery, V. cholerae's connection with zooplankton has been studied (Huq et al., 1983; Tamplin et al., 1990). Zooplankton consume autotrophic and heterotrophic bacterio-, nano-, and micro-plankton and are devoured by bigger plankton like bug and crab larvae and fish. Well-studied interactions between V. cholerae and chitinous zooplankton such copepods and cladocerans (Nalin et al., 1979; Huq et al., 1983; Rawlings et al., 2007). Significant experiments link cholera transmission to zooplankton (Huq et al., 1996, 2005; Colwell et al., 2003). A famous experiment removed V. cholerae 99% using sari fabric filtration. (Huq, 1996). Villagers cleaned water using this approach because field testing showed it decreased cholera cases (Colwell et al., 2003; Huq, 2010). de Magny et al. (2011) found that the cladocerans Monia and Diphanosoma and the rotifer Brachionus angularis were strongly related with V. cholerae and cholera outbreaks, suggesting that various zooplankters may predict epidemics. Acartia tonsa possesses more V. cholerae than co-occurring copepods (Huq et al., 1983; Binsztein, 2004; Rawlings, 2007; Lizárraga-Partida, 2009, for more, see Pruzzo, 2008).

V. cholerae may eat numerous biotic surfaces due to its lifestyle. V. cholerae's QS-regulated HAP may disintegrate chironomid egg masses' gelatinous matrix (Broza and Halpern, 2001; Halpern et al., 2004). (2003). Acinetobacter, Aeromonas, Klebsiella, Shewanella, and Pseudomonas outnumbered V. cholerae on egg masses (3.9 104 per egg mass; Halpern et al., 2007). V. cholerae's egg mass destruction may feed them. 99.7% of V. cholerae on egg masses were VBNC, maybe because they express bacteriocins or compete for resources and space (Halpern, 2011). V. cholerae has been found in all four phases of chironomid development, from egg to adult (Broza and Halpern, 2001; Halpern et al., 2003; Broza, 2005), indicating they transmit cholera. Air-collected chironomids 3 kilometers from water

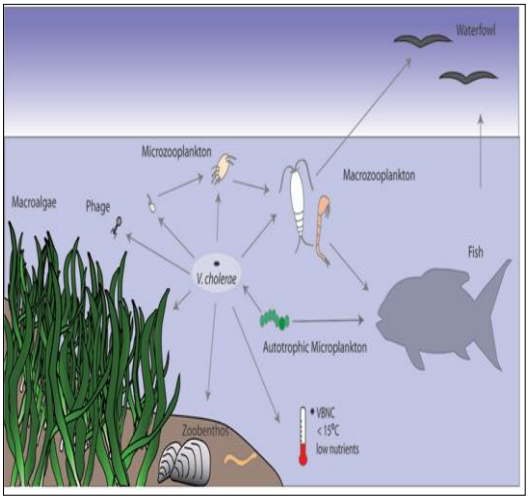

FIGURE.3 Vibrio cholerae interactions with other organisms and the environment.

V. cholerae midges may transfer illness between aquatic bodies (Broza et al., 2005). Toxigenic V. cholerae serogroups have not been connected to chironomids (Halpern, 2011).

V. cholerae and phytoplankton are well-studied (e.g., Tamplin et al., 1990; Lobitz et al., 2000; Turner et al., 2009). Diatoms, dinoflagellates, cyanobacteria, and macroalgae promote V. cholerae (e.g., Vezzulli et al., 2010). Microalgae hold V. cholerae. Seeligmann et al. (2008) discovered 1–10 VBNC V. cholerae per algal cell off Argentina. V. cholerae may grow in wet conditions because phytoplankton cells produce nutrients and salts (Islam et al., 1989; Tamplin et al., 1990; Binsztein et al., 2004). V. cholerae may grow during phytoplankton blooms (Mouriño-Pérez et al., 2003). Distant chlorophyll-a sensing may predict cholera (Lobitz et al., 2000).

Plant-derived polyamine norspermidine binds V. cholerae to macroalgae (Hamana and Mat- suzaki, 1982). NspS and MbaA enhance biofilm development (Karatan et al., 2005). Brown algal photosynthesis promotes V. cholerae colonization and VPS-dependent biofilm development through mtlA transcription (Ymele-Leki et al., 2013). Mannitol may provide V. cholerae cells carbon or osmoprotection (Ymele-Leki et al., 2013).

Phytoplankton and zooplankton-eating fish carry Vibrio cholerae (Senderovich et al., 2010). Israeli marine and freshwater fish contained 5 103 V. cholerae cells/gram intestinal contents (Senderovich et al., 2010). Sick or homogenized Japanese ayu from numerous rivers had non-O1 V. cholerae in their kidneys, livers, and spleens (Kiiyukia et al., 1992). Suckling mice collected culture supernatant lacking CT genes. Ducks and fish spread V. cholerae (Ogg et al., 1989). Seabirds consume phytoplankton. Airborne planktonic organisms on bird feathers may transmit V. cholerae (Halpern et al., 2008).

Planktonic organisms research environmental V. cholerae.

Benthic communities have V. cholerae (e.g., Covazzi Harriague et al., 2008; Vezzulli et al., 2009; Collin and Rehnstam-Holm, 2011). Bivalves connect benthos to plankton. Oysters, mussels, and Vibrio spp (e.g., Olafsen et al., 1993; Maugeri et al., 2001; Kirs et al., 2011). Raw fish poisons. V. cholerae, a deadly marine illness, may be transmitted via mussels (Wright and Harwood, 2013). (Murphree and Tamplin, 1995; Bauer et al., 2006; Haley, 2012).

Hemocytes and hemolymph compose the bivalve immune system (i.e., lysosomal enzymes and antimi- crobial peptides; Mitta et al., 2000; Pruzzo et al., 2005). Bivalve tissue bacteria must withstand hemolymph and hemocyte engulfment. Vibrios and Mytilus edulis hemocytes resist depuration (Murphree and Tamplin, 1995). (2010). Many Vibrios prevented adult M. edulis filtration without sticking to the mussels' gills (Birkbeck et al., 1987), suggesting another mechanism. Mussels carry V. cholerae (Collin et al., 2012). Clinical non-

O1/O139 strains absorbed slower. Clinical strains outlast environmental strains, therefore mussels prefer them. Depuration reduces environmental strains better than clinical strains. Collin et al. (2012) identified a virulent El Tor strain that bivalves refused. These studies demonstrate how V. cholerae develops and chooses pathogenic variants.

Filter feeders bring V. cholerae to the benthos, although sediments may isolate more than in planktonic phase (Covazzi Harriague et al., 2008; Vezzulli et al., 2009). Cholera-contaminated sediments may seed the water column as temperatures rise in winter (Vezzulli et al., 2009). 50% nematodes ate bacteria. Top-down grazing nematodes suppress Vibrio spp (Vezzulli et al., 2009). Vaitkevicius et al. (2006) showed that V. cholerae's extracellular protease PrtV kills C. elegans after ingestion. Killing required no CT or TCP. PrtV prevented C. roenbergensis and T. pyriformis grazing. HapR-resistant nematodes. QS response regulator hapR helps natural and lab grazing resistance (Matz et al., 2005). (2011). Hence, V. cholerae resists top-down control by predatory eukaryotes via several genetic mechanisms.

CONCLUSIONS

Vibrio cholerae changed history. It transmitted infections and promoted epidemiology over 150 years. Ecology and persistence are less investigated than infection and outbreaks. Non-toxigenic and genetically diverse environmental V. cholerae strains may survive. The bacteria can tolerate nutritional shortage, iron limitation, salinity, and temperature variations genetically. Biofilm growth adapts. Chitinous surfaces promote stress resistance and nutrient availability. Biofilms shelter microeukaryotes. Biofilms may create protective chemicals or shield against predators. Human infectious pathogenicity comprises several anti-predator gene systems. The co-incidental virulence theory posits that predator-prey conflict rather than human hosts causes virulence variables. V. cholerae lives in both marine and freshwater habitats. These processes will elucidate ecology, evolution, and biology over 150 years.

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