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The pathogenesis, detection, and prevention of *Vibrio parahaemolyticus*

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Vibrio parahaemolyticus, a Gram-negative motile bacterium that inhabits marine and estuarine environments throughout the world, is a major food-borne pathogen that causes life-threatening diseases in humans after the consumption of raw or undercooked seafood. The global occurrence of *V. parahaemolyticus* accentuates the importance of investigating its virulence factors and their effects on the human host. This review describes the virulence factors of *V. parahaemolyticus* reported to date, including hemolysin, urease, two type III secretion systems and two type VI secretion systems, which both cause both cytotoxicity in cultured cells and enterotoxicity in animal models. We describe various types of detection methods, based on virulence factors, that are used for quantitative detection of *V. parahaemolyticus* in seafood. We also discuss some useful preventive measures and therapeutic strategies for the diseases mediated by *V. parahaemolyticus*, which can reduce, to some extent, the damage to humans and aquatic animals attributable to *V. parahaemolyticus*. This review extends our understanding of the pathogenic mechanisms of *V. parahaemolyticus* mediated by virulence factors and the diseases it causes in its human host. It should provide new insights for the diagnosis, treatment, and prevention of *V. parahaemolyticus* infection.

Keywords: *Vibrio parahaemolyticus*, pathogenesis, virulence factor, detection, prevention

Vibrio parahaemolyticus, a kind of Gram-negative motile bacteria inhabiting marine and estuarine environments throughout the world (Wang et al., 2011a), is a major food-borne pathogen that causes diarrhea primarily after the consumption of raw or undercooked seafood (Bresee et al., 2002; Kawatsu et al., 2006). To ensure its survival in varying environments, *V. parahaemolyticus* has two different types of flagellar systems, allowing it to adapt to constantly changing environments. The polar flagellum is responsible for swimming (Broberg et al., 2011), whereas the lateral flagella are closely associated with the swarmer cell type transformation and biofilm formation (**Figure 1**). During infection, *V. parahaemolyticus* uses the adhesion factors to bind to the fibronectin and phosphatidic acid on the host cell, thus releasing different effectors and toxins into the cytoplasm, causing cytotoxicity and serious diseases (Gode-Potratz et al., 2011). *V. parahaemolyticus* is a multiserotype bacterium, containing at least 12 different O antigens and more than seventy different K antigens in its capsule. Consequently, serotyping has been widely used to detect *V. parahaemolyticus* and to study its pathogenesis (Xu et al., 2014). Among the serotypes, three serotypes (O3:K6, O4:K68, and O1:K untypeable) are extremely virulent and pathogenic to humans, and are regarded as the major agents of food-borne diseases (Jones et al., 2012). To date, the genomes of six strains from these different serotypes have been sequenced: strains RimD221063 and AQ3810 from O3:K6, the strains AN-5034, K5030, and Peru-466 from O4:K68, and the strain Vp10329 from O4:K12 (Makino et al., 2003; Broberg et al., 2011; Gonzalez-Escalona et al., 2011). The first fully sequenced and annotated genome of strain RimD221063 has been used as the reference sequence in cell biological and pathogenetic analysis of numerous clinical and environmental *V. parahaemolyticus* strains (Makino et al., 2003).

DISEASE CAUSED BY *V. parahaemolyticus*

In 1950, an first outbreak of disease caused by *V. parahaemolyticus* in the city of Osaka city of Japan was first reported, with acute gastroenteritis in 272 individuals, 20 of whom died (Fujino et al., 1953; Daniels et al., 2000). Since then, 802 outbreaks of food-borne diseases have been reported in 13 of the coastal provinces of eastern China, causing 17,462 individuals to become ill (Wang et al., 2011a). *V. parahaemolyticus* (40.1%) accounted for the greatest number of these outbreaks and cases (Liu et al., 2006; Chao et al., 2010). Similar diseases have also been frequently reported in many countries in Asia, Europe, Africa, and in the Americans (DePaola et al., 2000; Alam et al., 2002; Lozano-León et al., 2003; Martinez-Urtaza et al., 2005; Su and Liu, 2007; Chao et al., 2009). *V. parahaemolyticus* infection is also disseminated through open wounds, and often causes septicemia in severe cases (Wang et al., 2012). Recently, *V. parahaemolyticus* has been reported to be the major agent of acute hepatopancreatic necrosis syndrome afflicting penaeid shrimp, and has seriously damaged the shrimp aquaculture industry (Tran et al., 2013).

The food poisoning caused by *V. parahaemolyticus* usually occurs in summer (from June to October), and is predominantly associated with different kinds of seafood, including crab, shrimp,

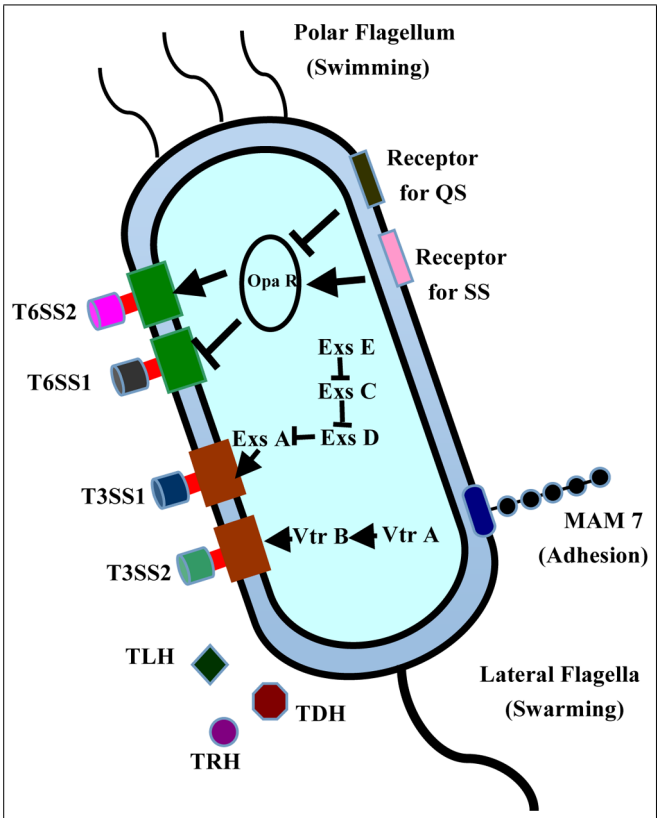

FIGURE 1 | Structures and virulence factors of *V. parahaemolyticus*. *V. parahaemolyticus* contains two T3SS systems, two T6SS systems, and expresses many toxins, including TLH, TRH, and TDH. MAM7 is responsible for its initial attachment to host cells. This bacterium has two different flagellar systems, allowing it to adapt to changing environments. The polar flagellum is responsible for swimming, whereas the lateral flagella are closely related to the swarmer cell transformation and biofilm formation.

shellfish, lobster, fish, and oysters (Lee et al., 2008; Nakaguchi, 2013; Jones et al., 2014; Cruz et al., 2015; Letchumanan et al., 2015). Among the whole range of seafood, shellfish is regarded as a high-risk food because it is infested with large populations of bacteria, including *V. parahaemolyticus*, to levels higher than those in the surrounding water (Peng et al., 2010; Anonymous, 2012). Once consumers eat undercooked, contaminated seafood, illness is inevitable (Rahimi et al., 2010). The typical clinical symptoms of *V. parahaemolyticus* poisoning are acute dysentery and abdominal pain, accompanied by diarrhea, nausea, vomiting, fever, chills, and water-like stools (Yeung and Boor, 2004; Shimohata and Takahashi, 2010). The feces of patients are mixed with mucus or blood, and their blood pressure decreases dreamily, leading to shock (Broberg et al., 2011). Some severely affected patients become unconsciousness, show recurrent convulsions, become pale or cyanotic, and even death (Nair et al., 2007). The distinct pathological changes in patients include the mild erosion of the jejunum and ileum, gastric inflammation, and internal organ damage (liver, spleen, lung congestion, etc.). To cure *V. parahaemolyticus* infection effectively, common treatment methods include antibiotics and oral rehydration. To avoid intense illness, it is recommended that some subpopulations, including patients suffering severe physical or immunodeficiency diseases, do not consume the seafood (Blake et al., 1979; Hlady and Klontz, 1996).

PATHOGENESIS OF *V. parahaemolyticus*

HEMOLYSIN, UREASE, AND PATHOGENESIS

Thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) are two major virulence factors of *V. parahaemolyticus*, and are closely related to its pathogenicity (**Table 1**). They have similar hemolytic activity *in vitro* and cause the lysis of human erythrocytes in excessively saline medium (Sakazaki et al., 1968; Miyamoto et al., 1969; Hondo et al., 1987; Honda et al., 1988). An epidemiological investigation indicated that TDH is one of the major pathogenic factors in *V. parahaemolyticus*, and is prevalent in almost all (95%) of clinical isolates. When secreted, it can lyse red blood cells and produces a special hemolysis ring on Wagatsuma blood agar plates (Nishibuchi et al., 1992; Honda and Iida, 1993; Liu, 2003). This is also known as "Kanagawa phenomenon" and is reported to be commonly associated with gastroenteritis (Miyamoto et al., 1969; Joseph et al., 1982). Previous reports have shown that two enzymatic activities of TDH are associated with bacterial pathogenesis. One is a hemolytic activity that is independent of lipid rafts. TDH binds to the membranes of red blood cells or host cells, and forms a pore on the membrane surface, ultimately leading to the permeation of the colloids of red blood cells (Matsuda et al., 2010). The other enzymatic activity is its cytotoxicity, TDH causes cells toxicity and forms a channel in the cell membrane, which induces an increase in the extracellular Ca^{2+} concentration and Cl^{-} secretion (Matsuda et al., 2010). When the osmotic pressure of the cell exceeds the upper limit for cell self-regulation, pathological and morphological changes were occur in the cell, resulting in cell expansion and even death. Like TDH, TRH causes similar levels of hemolysis *in vitro* (Takahashi et al., 2000; Ceccarelli et al., 2013).

Thermolabile hemolysin (TLH) is another hemolysin of *V. parahaemolyticus*, encoded by the *tlh* gene, and also causes the lysis of red blood cells (Shinoda et al., 1991; McCarthy et al., 1999; Wang et al., 2013b). TLH is expressed by all clinical and environmental strains of *V. parahaemolyticus* (Bej et al., 1999), and the gene is significantly upregulated under simulated intestinal infection conditions (Gotoh et al., 2010; **Table 1**). Besides, TLH shows typical lecithin-dependent phospholipase activity, and it also lyses human erythrocytes (Broberg et al., 2011). Therefore, it may play a key role in the process of human infection. Recent, studies have demonstrated that all three types of cells (Hela, Changliver, and RAW264.7 cells) display signs of severe cytotoxicity when treated with the purified TLH protein, and its effects are dose and time-dependent (Wang et al., 2012). Therefore, TLH may have similar biological functions similar to these of the TDH and TRH toxins, playing a key role in the *V. parahaemolyticus* infection. Early studies showed that urease induces the accumulation of intestinal fluid in the rabbit ileal loops test and causes gastrointestinal inflammatory lesions, confirming that urease is an important virulence factor in trh^{+} *V. parahaemolyticus* strains (Cal and Ni, 1996; Osawa et al., 1996). Urease is encoded by the *Uh* gene, and is generally involved in the formation of ammonia during the process of infection (Levin, 2006).

Table 1 | List of known virulence factors of *V. parahaemolyticus*.

Effectors	Gene	Biological activity	Effects on host cells
Toxins			
TDH	*tdh*	Forms pores on cells	Cytotoxicity and enterotoxicity
TRH	*trh*	Forms pores on cells	Cytotoxicity and enterotoxicity
TLH	*tlh*	Hemolysin activity or?	Cytotoxicity and ?
T3SS1 effectors			
Vop Q	*vp1680*	Forms pores and binds V-ATPase	Autophagy, cell lysis, MAPK activation, IL-8 secretion
Vop S	*vp1686*	Inhibition of Rho by AMPylation	Cells rounding, phagocytes invasion
VPA0450	*vpa0450*	Phosphatidylinositol phosphatase	Membrane blebbing, destabilization
Vop R	*vp1683*	Binds PIP2 in membrane	Promoting refolding of T3SS effectors
T3SS2 effectors			
Vop A/P	*vpa1346*	Inhibition of MAPK by acetylation of MKK	Blocking of phosphorylation and ATP binging
Vop T	*vpa1327*	Ras ADP-ribosylation	Cytotoxicity and yeast growth inhibition
Vop L	*vpa1370*	Actin nucleation	Stress fibers formation and cell shape changing
Vop C	*vpa1321*	Activation of Rac and CDC42 by deamidation	Invasion of non-phagocytic cells
Vop V	*vpa1357*	Actin binding and bundling	Enterotoxicity and blunting of villi
Vop Z	*vpa1336*	Inhibition of TAK1 and downstream MAPK and $NF\text{-}_kB$	Enterotoxicity and colonization
VPA1380	*vpa1380*	Cysteine catalysis dependent on IP6	Toxicity in yeast

T3SS1 INDUCES AUTOPHAGY AND CYTOTOXICITY

The type III secretion systems (T3SSs) are transmembrane apparatuses formed by the multicomponent protein complexes (Cornelis, 2006), that allow effectors or virulence proteins to be injected directly into the cytoplasm of the host cell (Dean, 2011; Chatterjee et al., 2013). There are two different T3SS systems in *V. parahaemolyticus*, designated T3SS1 and T3SS2 (Makino et al., 2003). T3SS1 is located on chromosome I, is encoded by the first pathogenicity island, and is present in almost every clinical and environmental *V. parahaemolyticus* strains (Paranjpye et al., 2012). T3SS1 gene expression is regulated by three exoenzyme S synthesis proteins (ExsC, ExsD, and ExsE) and heat-stable nucleoid structuring protein (H-NS; Kodama et al., 2010; Zhou et al., 2010). Several studies have shown that T3SS1 is cytotoxic, causing autophagy, cell rounding, and finally death (Burdette et al., 2008; Hiyoshi et al., 2010; Okada et al., 2010; Ritchie et al., 2012; Zhang and Orth, 2013). To date, four effectors have been determined in T3SS1 (**Table 1**): Vop Q, Vop S, VPA0450, and Vop R (VP1638), correspondingly (Broberg et al., 2010; Luong et al., 2010; Salomon et al., 2013a; Sreelatha et al., 2013; **Figure 2**).

The effector Vop Q (Park et al., 2004a) is necessary for the formation of autophagic vesicles in the process of *V. parahaemolyticus* infection (Matsuda et al., 2012). Many researchers have confirmed that the *V. parahaemolyticus* strain in which T3SS1 is deleted can be easily engulfed and degraded by macrophages, causing the apoptosis of the infected cells (Burdette et al., 2009; Sreelatha et al., 2013). These results indicate that T3SS1 effectively inhibits the ability of the host cells to phagocytose *V. parahaemolyticus* (Jegga et al., 2011). Vop Q is also reported to be an activator of the JNK, p38, and MAPK pathways in Caco-2 cells, and interacts with the C subunit of the vacuolar H^+-ATPase (Matlawska-Wasowska et al., 2010; Porta et al., 2011), leading to the secretion of the chemokine interleukin 8 (IL-8; Shimohata et al., 2011).

Vop S, another effector secreted by T3SS1, causes the death of macrophages by inhibiting NF-κB activity. Vop S contains a Fic domain at its C terminus, and also prevents actin aggregation and rapid re-aggregation by AMPylating the Rho family GTPases (Casselli et al., 2008; Yarbrough et al., 2009; Luong et al., 2010), resulting in the rounding of the infected cells (Kim and Jo, 2013). This change allows the pathogen to suppress the phagocytosis of infected cells by macrophages (Higa et al., 2013). Vop S also has exerts certain cytotoxicity effects on Hela cells.

VPA0450 is a typical phosphatidylinositide phosphatase, hydrolyzing phosphatidylinositide (4, 5)-bisphosphate (PI(4,5)P2) to D5 phosphate (PI4P; Krauss and Haucke, 2007). The resulting product, PI4P, disrupts the association between the membrane and the actin cytoskeleton, leading to membrane blebbing (Broberg et al., 2010). VPA0450 induces cell rounding and lysis by destroying the dynamics of the plasma membrane cytoskeleton, and it may play a complementary role with other effectors in the infection process (Broberg et al., 2010).

Vop R is encoded by the *vp1683* gene and is secreted by T3SS1. It also contains a similar phosphoinositide-binding domain (BPD) that is conserved in diverse type III effectors of both plant and animal pathogens (Salomon et al., 2013a). Vop R localizes to the host membrane by its N-terminal domain and specifically binds the phosphoinositide on the host cell. It may also play a key role in promoting the refolding of Type III effectors after their delivery into the host cells (Geissler, 2012; Hicks and Galan, 2013; Salomon et al., 2013a).

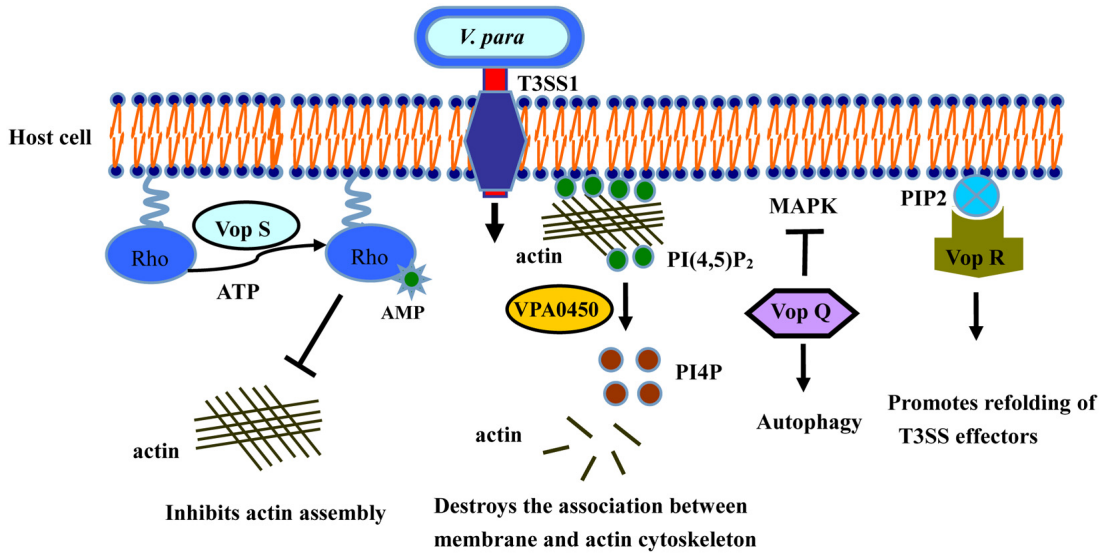

FIGURE 2 | Activities of T3SS1 effectors in cultured intestinal epithelial cells. Vop Q inhibits the MAPK pathway by acetylating MKK, and Vop S inhibits Rho by AMPylation, leading to cells rounding and phagocytes invasion. VPA0450 hydrolyzes phosphatidylinositide (4, 5)-bisphosphate (PI(4,5)P2) to D5 phosphate (PI4P) and disrupts the association between the membrane and the actin cytoskeleton, leading to membrane blebbing. Vop R binds to PIP2 in the membrane, thus promoting the refolding of the T3SS effectors.

T3SS2 MEDIATES ENTEROTOXICITY AND CYTOTOXICITY

T3SS2, a newly identified type of secretion system, is encoded by a pathogenicity island (*VP-PAL*) on chromosome II, and is found primarily in clinical isolates (Meador et al., 2007). T3SS2 has been associated with enterotoxicity in the rabbit ileal loop model, infant rabbits and piglets (Makino et al., 2003; Paranjpye et al., 2012), and has also been shown to cause cytotoxicity in intestinal cell lines such as Caco-2 cells and HCT cells (Park et al., 2004b; Hiyoshi et al., 2010; Ritchie et al., 2010; **Figure 3**). Seven known effectors have so far been identified and characterized in T3SS2 (**Table 1**): Vop C, Vop T, Vop Z, Vop A/P, Vop V, Vop L, and VPA1380 (Trosky et al., 2004; Kodama et al., 2007; Liverman et al., 2007; Yu et al., 2011; Zhang et al., 2012; Calder et al., 2014).

Vop A/P (VPA1346) is an acetyltransferase with 55% homology to Yop J of *Yersinia* spp. (Makino et al., 2003), which blocks the MAPKs signaling pathway by inhibiting the start and biological activity of mitogen-activated protein kinase (Trosky et al., 2004), thereby suppressing cell division *via* a new mechanism. Vop L (VPA1370) contains three Wiskott Aldrich homology 2 (WH2) domains and a C-terminal domain (VCD; Namgoong et al., 2011; Yu et al., 2011), which generally induces the formation of polarized actin fibers and accelerates the gathering of actin filaments by binding to actin monomers (Liverman et al., 2007). Notably, Vop L may provide a favorable microenvironment in which bacteria can replicate, thereby enhancing the uptake and invasion of *V. parahaemolyticus*.

Vop C disturbs the actin network and causes bacterial invasion by deamidating glutamine 61 in both Rac and CDC42, which occurs in their switch regions, resulting in the constitutive activation of the Rho family GTPases (Friebel et al., 2001; Zhang et al., 2012). The actin cytoskeletons of infected cells are further rearranged with the modification of these GTPases, thereby prompting the infected cells to engulf the bacteria (Zhang et al., 2012). Vop T modifies the small G protein with ADP-ribose using NAD^+ as the substrate *in vivo* and *in vitro* (Fraylick et al., 2002; Barbieri and Sun, 2004). Vop T inhibits yeast cell growth and is cytotoxic for Caco-2 and HCT-8 cells (Kodama et al., 2007).

Vop V has long repeat (LR) regions in its N- and C-terminal domains, composed of three types of repeated sequence units. It predominantly induces the enterotoxicity observed in the rabbit ileal loop model (Hiyoshi et al., 2011), and binds directly to F-actin, a polymeric form of actin, leading to the accumulation of F-actin filaments beneath the bacterial microcolonies in Caco-2 cells (Haglund and Welch, 2011). All the above results indicate that F-actin binding is required for the enterotoxicity caused by Vop V (Hiyoshi et al., 2011). However, the molecular mechanism of this enterotoxicity is still unclear, so further investigations are needed for underlying its crucial role during infection.

Vop Z, a novel effector secreted by the T3SS2 system, is responsible for fluid accumulation, cell detachment, and epithelial damage (Zhou et al., 2013). Intestinal colonization by *V. parahaemolyticus* and fluid accumulation are reduced when Vop Z is deleted (Zhou et al., 2013). Vop Z inhibits the activation of the MAPK and NF-κB signaling pathways by inhibiting the activation of the TAK1 kinase, resulting in a marked lesion, disrupting on the integrity of the tissue (Kajino-Sakamoto et al., 2008; Zhou et al., 2013). Therefore, it plays a critical role in the virulence of *V. parahaemolyticus*.

VPA1380 was recently identified as a critical effector of *V. parahaemolyticus* translocated by T3SS2 (Hiyoshi et al., 2011). It was detrimental to and exerted a toxic effect on yeast growth when it was expressed as an enhanced green fluorescent protein (eGFP) fusion protein, when yeast was used as a heterologous eukaryotic system (Calder et al., 2014). Bioinformatic analyses revealed

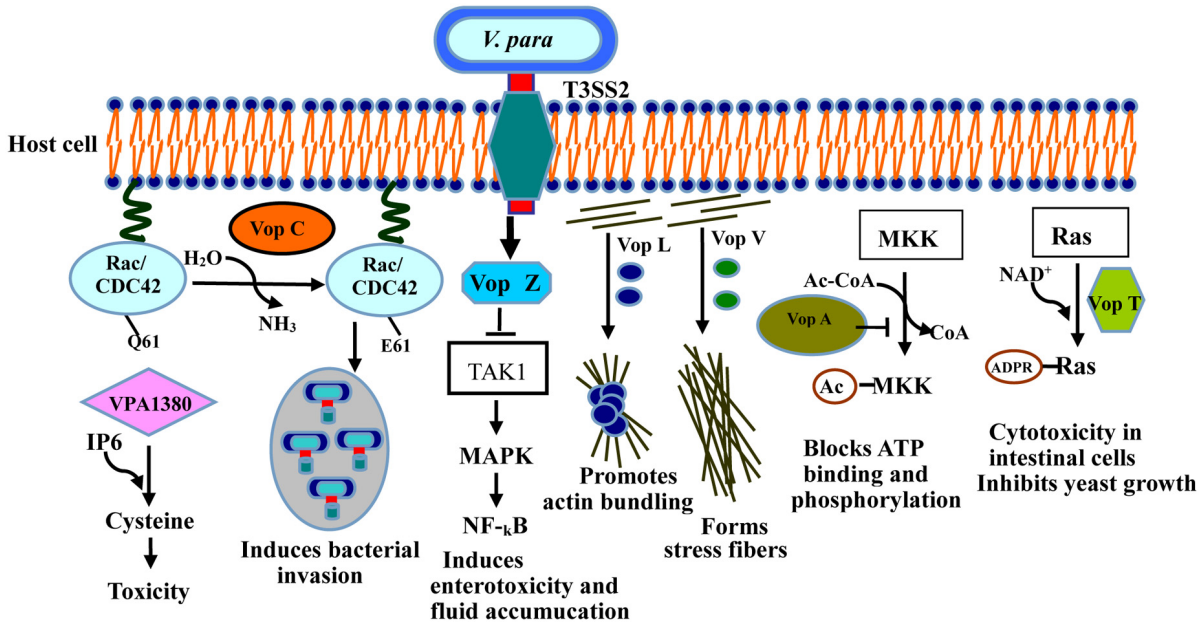

FIGURE 3 | Activities of the T3SS2 effectors in intestinal epithelial cells during infection. Vop C deamidates small GTPases such as Rac and CDC42, inducing bacterial invasion. Vop L dimerizes through its VCD domain, thus promoting actin bundling. Vop A inhibits MAPK by acetylating of MKK. Vop V binds directly to F-actin through its long repeat (LR) and C-terminal domain, forming the stress fibers. Vop T modifies Ras with ADP-ribose, and triggers yeast growth inhibition and cytotoxicity in intestinal cells. Vop Z inhibits the activation of the MAPK and NF-κB pathways by repressing of TAK1 kinase. VPA1380 catalyzes it targeted substrate.

that VPA1380 contains several inositol hexakisphosphate (IP6)-inducible cysteine protease domains, which are known to occur in large known toxins produced by other bacteria (Prochazkova and Satchell, 2008; Pruitt et al., 2009). VPA1380 is reported to be a typical cysteine protease, catalyzing its targeted substrate (Calder et al., 2014), so VPA1380 is possibly involved in the invasion of host cells by *V. parahaemolyticus*.

T6SS AND PATHOGENESIS

Recently, a type VI secretion system (T6SS) has been identified, and detected in many Gram-negative bacteria. It is a macromolecular machine consisting of a multicomponent protein complex (Ho et al., 2014). T6SS is responsible for delivering a series of toxic effector proteins into the cytoplasm of eukaryotic cells, allowing the effectors to disrupt the innate immune system and to kill the host cells (Coulthurst, 2013). The T6SS organelle is functionally analogous to T3SS, and may have a critical function in the process of bacterial infection (Salomon et al., 2013a). Interestingly, *V. parahaemolyticus* contains two different T6SS systems, designated T6SS1 and T6SS2 (Boyd et al., 2008; Yu et al., 2012). T6SS1 is encoded on chromosome I, is predominantly expressed in clinical isolates, and it is most active under warm conditions (O'Boyle and Boyd, 2014). T6SS2 has been found in both clinical and environmental isolates, is encoded on chromosome I, and is active under low-salt conditions (Salomon et al., 2013b). A homology analysis indicated that the T6SSs are present in most different *Vibrio* species, including *V. parahaemolyticus*, *V. cholerae*, *V. harveyi*, and *V. alginolyticus* (Pukatzki et al., 2006). Recently published medical research articles have reported that both T6SSs are necessary for the adhesion of *V. parahaemolyticus* to cells and are involved in intracellular trafficking and vesicular transport (Boyd et al., 2008; Yu et al., 2012; Salomon et al., 2013b). Only a few effectors of T6SS from *V. parahaemolyticus* have so far been reported. In recent research, two T6SS effectors that mediate its antibacterial activity were identified using proteomic, bioinformatic, and genetic analyses (Salomon et al., 2014). VP1388 is encoded within the T6SS1 gene cluster, whereas VPA1263 is encoded on chromosome II (Salomon et al., 2014). The two effectors contain the conserved MIX motif that is found in proteins with predicted cytotoxic domains, including VgrG and PAAR-repeat-containing protein (Pukatzki et al., 2007; Shneider et al., 2013).

DETECTION METHODS BASED ON VIRULENCE FACTORS

KANAGAWA TEST

Thermostable direct hemolysin is a virulence factor that contributes to the formation of a distinct hemolytic ring on blood cells agar plates in high concentrations of salt with D-mannitol as the carbon source, known as the "Kanagawa phenomenon" (KP; Honda and Iida, 1993; Nishibuchi and Kaper, 1995). In the past, the KP has been regarded as an important indicator in the identification of the pathogenic and non-pathogenic *V. parahaemolyticus* strains (Zhang and Austin, 2005; Ono et al., 2006). However, the detection of *V. parahaemolyticus* based on KP is time-consuming, labor intensive, and unreliable, and involves the evaluation of large numbers of samples (Park et al., 2004c; Wang et al., 2011b). Therefore, the development of specific, sensitive, and rapid methods to detect this bacterium is crucial for public health.

PCR DETECTION

Polymerase chain reaction (PCR) assays are being increasingly used to identify and distinguish specific pathogenic bacteria. Multiplex PCR protocols targeting the *toxR*, *tlh*, *tdh*, *trh*, and *fla* genes have been developed to detect the total and pathogenic *V. parahaemolyticus* from clinical and environmental samples (Rosec et al., 2009; Izumiya et al., 2011; Wang et al., 2011a; Hossain et al., 2013). Recently, a serogroup-*O*-specific PCR assay was used to detect and identify *V. parahaemolyticus* pathogens in clinical and environmental samples (Chen et al., 2012). Before 2012, multiplex real-time PCR with different fluorescent probes was used to detect total and pathogenic *V. parahaemolyticus* in different kinds of seafood (Ward and Bej, 2006; Nordstrom et al., 2007; Tyagi et al., 2009; Robert-Pillot et al., 2010). Garrido used multiplex real-time PCR to detect pathogenic *V. parahaemolyticus* in water and food samples. The limits of detection for this method were 0.24 CFU/g for *tdh*, and 0.44 CFU/g for *trh1*, and 0.52 CFU/g for *trh2* (Garrido et al., 2012). A quantitative PCR method combined with propidium monoazide has also been used to quantify the viable *V. parahaemolyticus* cells in raw seafood (Zhu et al., 2012). In general, detection methods based on PCR are quick, high accuracy and sensitivity, but the main disadvantages of that is badly controllability, and the PCR system often need to be optimized to gain the best detection results (Letchumanan et al., 2014).

Loop-mediated isothermal amplification (LAMP) is a specific and highly sensitive technique for DNA amplification under isothermal conditions with the specific primers, and has been widely used to detect pathogenic bacteria in food (Zhao et al., 2011; Qi et al., 2012). LAMP targeting the *tlh*, *tdh*, or *toxR* genes of *V. parahaemolyticus* is used for the sensitive and rapid detection of *V. parahaemolyticus* (Yamazaki et al., 2008; Nemoto et al., 2009; Chen and Ge, 2010). A novel LAMP *in situ* detection method was reported for the rapid detection of food-borne *V. parahaemolyticus* strains, which has greater specificity and is less time-consumption than regular LAMP and other PCR-based methods (Wang et al., 2013a). Recently, Zeng et al. (2014) developed a novel method that combines the LAMP assay with immunomagnetic separation to detect *V. parahaemolyticus* in raw oysters. The limit of detection was 0.19 CFU/g, thus providing a platform for the comprehensive detection of pathogenic strains using a virulence- gene-specific LAMP assay (Zeng et al., 2014). Although LAMP is an effective and economic method to rapidly detect the pathogenic bacteria at one temperature without the need of cycling, however, similar to PCR, the methods of targeted separation and enrichments severally affected the application of LAMP.

IMMUNOLOGICAL DETECTION

Immunological methods based on monoclonal antibodies are often used for the rapid detection and quantification of food-borne pathogens in seafood. Sandwich enzyme-linked immunosorbent assays based on monoclonal antibodies directed against TDH, TLH, and TRH have been used to identify these proteins in pathogenic clinical isolates of *V. parahaemolyticus* (Honda et al., 1989, 1990; Kumar et al., 2011; Sakata et al., 2012). However, these monoclonal antibodies do not detect all clinical and environmental *V. parahaemolyticus* strains because they cross-react with other bacteria (Prompamorn et al., 2013). An immunochromatographic assay was developed to detect the TDH hemolysin produced by *V. parahaemolyticus* in enrichment cultures from stool specimens (Kawatsu et al., 2006).

Today, recombinant antibody fragments, such as single-chain variable fragments (scFvs), have become an essential tool for research, diagnostic, and therapeutic purposes (Wang et al., 2014a). In 2012, our group has screened a high affinity scFv antibody successfully against a pathogenic factor TLH of *V. parahaemolyticus* by phage display. The screened scFv-LA3 antibody is specific to TLH antigen, and it is active against *Vibrio* cells possessing TLH (Wang et al., 2012). Our results indicated that scFv-LA3 recognizes specifically TLH produced by *V. parahaemolyticus* (Wang et al., 2014a), and it can be used as an antibody reagent to detect the TLH producing *V. parahaemolyticus* strains in seafood (Wang et al., 2012). Compared to the traditional full length Ig G antibody, the sensitivity of immunological method based on scFv is unsatisfactory, and the fact that current scFv antibodies have the poor stability, low solubility, and affinity seriously limits their diagnostic and clinic application. To improve the stability and solubility of scFv antibody, researchers have developed an Skp co-expressed system to express a functional scFv protein, and the Skp co-expressed scFv showed high solubility and binding activity to antigen TLH (Wang et al., 2013b).

OTHERS METHODS

In addition to the methods discussed above, many detection methods based on biochemistry and biophysics have been used to detect and identify *V. parahaemolyticus* strains. As early as Su et al. (2005), a chromogenic medium was used for the selective and specific detection of *V. parahaemolyticus* strains. Hayashi et al. (2006), developed a novel method for the early detection of viable and TDH- or TRH-producing *V. parahaemolyticus* in seafood using soft-agar-coated filter combined with multiplex PCR, which identifies seafood samples contaminated with *V. parahaemolyticus* within 2 days. A new enrichment broth containing the bile salt, sodium taurocholate (ST broth) was used for improving the isolation and detection of pathogenic *V. parahaemolyticus* from seafood (Raghunath et al., 2009). A novel light-scattering sensor based solid agar plate has also been used for the real-time detection and identification of *V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae* colonies (Huff et al., 2012). Dual-color flow cytometry was developed for the simultaneous detection of *V. parahaemolyticus* and *Salmonella typhimurium* in real samples. In this system, fluorescent quantum dots (QDs) labeled aptamers recognize the two bacterial species, and the sensitivity of detection was increased when QDs nanoparticles was used (Duan et al., 2013). Recently, Xiang et al. (2013) developed a real-time resistance measurement based on four different methods of detection *V. parahaemolyticus* by targeting the lecithin-dependent hemolysin gene: including LAMP, electrochemical ion bonding (crystal violet and Mg^{2+}), real-time monitoring, and derivative analysis. The limit of detection was 10 CFU/mL, and the results revealed that this method is more accurate, sensitive, and specific than culture methods.

PREVENTION AND CURES BASED ON VIRULENCE FACTORS

ANTIBODY NEUTRALIZATION AND INHIBITION

Given the widespread contamination by *V. parahaemolyticus* and because it is strongly pathogenic to humans, it is very important to prevent and treat the diseases caused by this bacterium. However, to date, no effective measures are available to treat the diseases caused by *V. parahaemolyticus*, and the prospect of developing such therapies is still not good. Excitingly, antibody molecules have become extremely potent candidates for therapeutic applications, and have been developed into an important class of drugs for the treatment of numerous infectious diseases (Hagemeyer et al., 2009). An scFv antibody directed against the pathogenic factor TLH of *V. parahaemolyticus* effectively neutralized the cytotoxicity of *V. parahaemolyticus* TLH, thus exerting a protective effect on various types of TLH-infected cells (Wang et al., 2012). In *V. parahaemolyticus*, the needle complex is formed by the needle subunit protein (VP1694), which contains only 88 amino acids, and its function only relies on a single polymerized protein (Tamano, 2000; Blocker et al., 2008). The needle subunit may be a useful target protein for screening an effective antibody or inhibitor that can prevent the formation of the needle complex (Davis and Mecsas, 2007). A specific and high-affinity scFv antibody directed against VP1694 (needle subunit) has been prepared, and may play an important role in inhibiting the assembly of T3SS (Wang et al., 2014b). Significantly, the above results showed that the specific and functional scFv antibodies against target antigens of *V. parahaemolyticus* have been prepared successfully, and it provides a solid foundation for the immunological diagnosis and prevention of the diseases caused by *V. parahaemolyticus*.

INHIBITOR-MEDIATED TARGETED THERAPIES

Type III secretion systems is conserved among different bacterial pathogens, and it may be an important potential therapeutic target (Gauthier et al., 2005; Mota et al., 2005). Like other Gram-negative pathogenic bacteria, *V. parahaemolyticus* contains a contact-dependent T3SS, which delivers several effectors into the cytosol of infected host cells (Makino et al., 2003; Park et al., 2004a; Sun et al., 2008; Worrall et al., 2011). The above description shows that T3SS might be a useful target for screening an effective inhibitor that can prevent the formation of the needle complex.

Many small chemical molecules have been shown to block assembly of T3SS, and some compounds broadly inhibit T3SS in many other bacterial pathogens (Izore et al., 2011). A high-throughput assay was developed to screen for a specific transcriptional inhibitor of the virulence factors in enteropathogenic *Escherichia coli*, to block the promoters of virulence associated factors and thus inhibit their transcription (Gauthier et al., 2005). Sulfonyl amino benzanilides and salicylidene anilides have been shown to inhibit the expression of T3SS- related genes, disrupting different pathways in enteropathogenic *E. coli* (Kauppi et al., 2003; Gauthier et al., 2005). Benzimidazoles also have also been shown to inhibit the transcription factors LcrF of *Yersinia pseudotuberculosis* and ExsA of *Pseudomonas aeruginosa* (Garrity-Ryan et al., 2010; Grier et al., 2010). Salicylidene acylhydrazide and thiazolidinone have been used to repress the formation and assembly of the needle complex, and to block the secretion of effectors in many bacterial pathogens, including *Shigella*, *Yersinia*, *Chlamydia*, and *Salmonella* spp. (Negrea et al., 2007; Veenendaal et al., 2009; Aiello et al., 2010). Other studies have suggested that thiazolidinones are multifaceted therapeutic agents for inhibiting bacterial infection (Dayam et al., 2006). In summary, the prevention and control of the diseases caused by *V. parahaemolyticus* mainly involve the use of antibiotics, or chemical molecules/drugs, but these inhibitors based on chemical molecules often lead to bacterial drug resistance or environmental residues of drug, resulting in enormous damage to the environment and human health (Pasqualinà et al., 2011; Silva et al., 2014b). Hence, it is very important to develop a feasible measure to improve this dilemma.

BACTERIOPHAGE-BASED THERAPIES

The increasing prevalence of bacterial antibiotic resistance has prompted a search for candidate agents to replace antibiotics in the effective treatment of bacterial diseases. In recent years, therapies based on bacteriophages have become a topical issue in this field. With the development of phage biology research and genome sequencing, theses methods have been applied to the diagnosis and treatment of bacterial diseases (Laanto et al., 2012; Silva et al., 2014a). A mycobacteriophage delivered by a non-virulent *Mycobacterium* was reported to effectively kill the *M. avium* and *M. tuberculosis*, and has become a model for phage therapies directed against intracellular bacterial pathogens (Broxmeyer et al., 2002; Peng et al., 2006). The therapeutic efficacy of phage therapies has been demonstrated in many infectious diseases caused by members of the genus *Vibrio*, including *V. vulnificus*, *V. harveyi*, *V. parahaemolyticus*, and *V. anguillarum* (Shivu et al., 2007; Crothers-Stomps et al., 2010; Mateus et al., 2014). Phage therapy can protect against experimentally induced vibriosis in the Atlantic salmon, and can effectively prevent mortality during vibriosis of in the brine shrimp and *V. anguillarum* infections during the production of fish larvae (Higuera et al., 2013; Martinez-Diaz and Hipolito-Morales, 2013; Silva et al., 2014b). Unlike antibiotics or chemical drugs, phage therapies are inexpensive, and more environmentally friendly, and do not induce microbial resistance, suggesting that phage therapy is a suitable alternative treatment for vibriosis in aquaculture industries (Silva et al., 2014b). One of the main challenges in using bacteriophages to control pathogens in seafood is to control the efficacy and safety of phage, and the market acceptance of use of phage. The detailed characterization of phage properties and understanding of phage–host interactions are essential requirements for the successful application of phage-based pathogen control (Tan et al., 2014).

CONTROLS AND PREVENTION

To reduce the risk of *V. parahaemolyticus* infections associated with seafood consumption, some strategies based on physical and chemical methods have been developed (Su and Liu, 2007). Thermal processing is a common approach to inactivating *V. parahaemolyticus* residues in seafood. Low-temperature freezing (at –18°C or –24°C) or high-temperature treatment (>55°C) for 10 min is reported to effectively inactivate or kill *V. parahaemolyticus* in oysters (Andrews et al., 2000). High-pressure processing (HPP) is another method that has also been used to destroy

pathogenic microorganisms in seafood, and has been used extensively to inactivate *V. parahaemolyticus* in oysters (Calik et al., 2002; Cook et al., 2002; He et al., 2002). Irradiation is another important method of eliminating *V. parahaemolyticus* from oysters. It does not kill the oyster or alter its sensory qualities at low doses, but the safety issues associated with radioactive materials limits its use (Andrews et al., 2003; Jakabi et al., 2003). Similar to the approaches discussed above, chemical reagents have been developed to reduce the bacterial contamination in seafood, including chlorine, electrolyzed oxidizing water and iodophors (Croci et al., 2002; Ren and Su, 2006). However, none of these effectively dislodge *V. parahaemolyticus* from oysters, and further research is required that focuses on the screening and development of new drugs.

CONCLUSION

Vibrio parahaemolyticus occurs naturally in marine, estuarine, and coastal environments throughout the world, and is the causative agent of food-borne gastroenteritis (Ceccarelli et al., 2013). The T3SSs are responsible for its cytotoxicity, and play a significant role in the induction of inflammatory chemokines in the host. T3SS1 is essential for systemic infection and the innate immune responses induced during intestinal infection, although the details of the mechanisms are still unclear, and the host targets remain to be determined (O'Boyle and Boyd, 2014). T3SS2 is associated with the enterotoxicity of *V. parahaemolyticus* in mammalian infection models *in vivo*, and has been reported to cause cytotoxicity in intestinal cell lines (Ham and Orth, 2012). The T6SSs, novel recently identified systems are necessary for the adhesion of *V. parahaemolyticus* to cells and are also involved in intracellular trafficking and vesicular transport. T6SS1 has antibacterial activity under warm conditions, enhancing the environmental fitness of *V. parahaemolyticus* (Salomon et al., 2013a), but our knowledge of the biological activity of T6SS2 is limited (Salomon et al., 2013a, 2014). Although a number of toxins and effectors associated with the pathogenesis of *V. parahaemolyticus* have been identified and characterized, but the detailed mechanisms of the total effectors of this bacterium, which have evolved to work together, and the distinct functions of individual effectors in causing pathogenicity are yet to be investigated. Further studies should focus on the correlation between T3SS and T6SS, and the non-invasive nature of *V. parahaemolyticus* warrants further investigation. Today, a large number of detection methods based on virulence factors are used for the detection and risk assessment of *V. parahaemolyticus.* However, to reduce the harm attributable to *V. parahaemolyticus,* specific, highly sensitive molecular methods are required to reliably identify and differentiate virulent and avirulent *V. parahaemolyticus* strains. The prevention and treatment of the diseases are still the key outcomes of future research, which should extend our understanding of the precise relationship between the disease in the human host and the pathogenicity of *V. Parahaemolyticus.* This review article provides insight into the control of the clinical risks posed by this potently virulent bacterium, which is extremely pathogenic to humans, by summarizing the molecular and therapeutic techniques available to future medical and immunological research. Effective control measures that combine novel drugs and targeted therapies must be developed to eradicate the risks posed to human health by this life-threatening disease exclusively.

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