A Review of Important Virulence Factors of Vibrio vulnificus

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Abstract: This study aims to review important virulence factors of Vibrio vulnificus that implicated in pathogen city of this bacteria, Vibrio vulnificus is a gram negative marine bacterium causing food borne infections include septicemia, gastroenteritis and wound infection in humans and marine vertebrates; as well as possess variety of virulence factors; the virulence factors of V. vulnificus are not yet well explained. The extracellular Capsule Polysaccharide (CPS) of V. vulnificus is a primary virulence factor which allows bacteria survival in the human host. The ability of V. vulnificus to cause disease is associated with the production of “Multifunctional-Auto processing RTX” (MARTXVv) toxin that encodes by RtxA1 gene, HlyU plays an essential role in regulation of the virulence of the human pathogen, HlyU regulate the expression of the repeat-in-toxin (RtxA1) gene, hemolysin gene (vhA) play an additive role for pathogenesis of V. vulnificus. In this review we focus on the main important virulence factors, the extracellular Capsule Polysaccharide (CPS), RTX” (MARTXVv) toxin, Virulence-correlated gene (vgcC), adherence of bacteria to the host cells and resistance to acid stress. These virulence factors serve as a credible biomarker to detection of potentially virulent factors of V. vulnificus strains.

Keywords: Capsule polysaccharide, rtxA1, Vibrio vulnificus, virulence-correlated gene, virulence factors, vha

INTRODUCTION

Vibrio vulnificus is gram-negative halophilic motile bacterium that spreads worldwide in estuarine and warm coastal waters that already salt and brackish water frequently contaminates oysters and other seafood (Horseman and Surani, 2011). This bacterium classified in Gammaproteo bacteria group, family Vibrionaceae (Broza et al., 2009). V. vulnificus considered a highly pathogenic marine bacterium which implicated in a variety of infections in humans those are consuming a contaminated seafood or exposure of skin wounds for water contaminated with V. vulnificus that also can cause septicemia, primary sepsis for patients with chronic liver disease, immunodeficiency and iron storage disorders. Moreover, its increases mortality rates as a result of the bacterial infection rank among the highest foodborne pathogens, up to 50%, among individuals with immune compromised (Mead et al., 1999).

Pathogenesis of V. vulnificus related to a variety of factors have been implicated as possible virulence determinants for V. vulnificus, these factors including extracellular Capsule Polysaccharide (CPS), cytotoxins and many hydrolytic enzymes, hemolysin/cytolysin (Gray and Kreger, 1987; Hor and Chen, 2013) an elastolytic protease (Oliver et al., 1986) production of lecithinase, lipase, protease and collagenase (Moreno and Landgraf, 1998). The ability of virulent strains of V. vulnificus to overcome iron limitations by using iron bound to iron-binding proteins (Shin et al., 2005). The major virulence factor of V. vulnificus is (CPS) which protect the bacteria from bactericidal and phagocytosis and allows survival in the human host (Grau et al., 2008) encapsulated strains of V. vulnificus possessing the ability to resist destruction by stomach acid and serum phosphatase, presence of capsule correlated with bacterial virulence, presence of capsule enables V. vulnificus to avoid host immune cells and complement (Grau et al., 2008). Lipopolysaccharide, cytotoxins, pili and flagellum also implicated in the virulence of V. vulnificus (Horseman and Surani, 2011). The ability of V. vulnificus to production of cytotoxins increase the chance of this bacterium to cause disease, there are two important cytotoxins associated with invasiveness and damage of infected tissues called hemolysin/cytolysin (VvhA) and “Multifunctional-Autoprocessing RTX” (MARTXVv) toxins, VvhA have the ability to lysis erythrocytes (Kreger and Lockwood, 1981), MARTXVv toxin belong to RTX toxin family that cause death of human intestinal epithelial cells (Lee et al., 2008c) and intragastric route of infection in mice (Kwak et al., 2011). Furthermore, bacterial pathogenicity linked with its ability to adherence on the surface of host cells. The present article discus and reviews the previous studies on the role of important
virulent factors that implicated in pathogenesis of *V. vulnificus*.

Ability of *V. vulnificus* to cause diseases associated with releasing cytotoxins and enzymes that controlled by one or more genes (Table 1), these gens can be used as a virulence marker in genotyping. *NanA* gene responsible of adhesion of *V. vulnificus* that causes infection in mice (Jeong et al., 2009), *wbpP* Gene considered important virulence factor of *V. vulnificus*, it is essential for pathogenesis and capsular polysaccharide biosynthesis of *V. vulnificus* (Park et al., 2006), in capsular and non-capsular strains rugose Extracellular Polysaccharide (*rEPS*) gene cluster is important for the expression of virulence factors (Grau et al., 2008) *vwhA* gene, coding for hemolysin enzyme is important for red blood cells lysis and death of epithelial cells (Kim et al., 2010).

This review discusses important factors implicated in virulence of *V. vulnificus* to determine the virulence factors related with pathogenicity that can be used as a biomarkers in molecular detection of environmental and clinical isolates.

### RESULTS AND DISCUSSION

**Pathogenicity of *V. vulnificus***: Pathogens responsible for seafood-associated infections include broad spectrum types of bacteria such as *Vibrio* species, *Shigella*, *Staphylococcus aureus*, *Campylobacter*, *Salmonella*, *Yersinia*, *Listeria*, *Bacillus*, *Clostridium perfringens* and certain strains of *Escherichia coli*; pathogenic viruses such as polio, rotaviruses and other viruses, parasites and protozoa (Iwamoto et al., 1999). Pathogenic *vibrio* cause three major clinical illnesses: wound infections, gastroenteritis and septicemia. Epidemiologic studies refers to the majority of these infections are foodborne and associated with consumption of raw or undercooked shellfish and contamination of open wounds in marine environments (Bross et al., 2007).

*V. vulnificus* is a pathogenic marine bacterium it’s an agent of foodborne diseases that threatening life of many patients sever from septicemia, soft tissue infection and possibly gastroenteritis in individuals with predisposed conditions, including liver damage, high levels of iron and immune-compromised cases (Linkous and Oliver, 1999; Strom and Paranjpye, 2000). *V. vulnificus* is an opportunistic human pathogen that may cause a high lethality rate with persons sever from gastroenteritis, severe necrotizing soft-tissue infections and primary septicemia (Canigral et al., 2010), *V. vulnificus* also considered pathogen for fish where isolated from infected and died fish (Sonia and Lipton, 2012).

Ability of *V. vulnificus* to cause diseases attributed to complex process and virulence factors that involve products of many virulent genes Table 1, its remarkable for the invasive nature of the infection by severe tissue damage and a fulminating progression. Moreover, many potential factors contributed in virulence of *V. vulnificus* such as capsule polysaccharide, endotoxin, iron sequestering systems, cytolytic hemolysin, elastase, phospholipase, the ability to evade destruction by stomach acid, lipopolysaccharide, pili, flagellum and other exotoxins, only few of these factors confirmed as a cause of pathogenicity including capsule and iron acquisition systems be essential for virulence (Linkous and Oliver, 1999; Biosca et al., 1999; Strom and Paranjpye, 2000; Kim et al., 2003; Gulig et al., 2005; Park et al., 2006). Therefore, the identification and characterization of more virulence factors of *V. vulnificus* are necessary in the development of improved treatment and prevention as well as for discovering novel approaches in the control and prevention spread of this pathogen.

Most reports refer to contribution of *V. vulnificus* in infection of human with chronic liver disease such as Cirrhosis, immunodeficiency, cutaneous lesions or hematological disorders characterized by elevated iron levels (Zaidenstein et al., 2008; World Health Organization, 2008). Primary bacteremia and Gastroenteritis mostly result from the consumption of contaminated raw oysters, shellfish, shrimp and other seafood. Skin and soft-tissue infection usually result from exposure of open wounds to contaminated water and handling seafood contaminated with this organism. Chronic liver disease, especially cirrhosis considered important risk factor for *V. vulnificus* infection (Bunchrontavakul and Chavalitdhamrong, 2012), risk factor for *V. vulnificus* also increases with liver disease.

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**Table 1: List of genes involve in virulence of *V. vulnificus***

| Gene       | Product                      | Reference                                |
|------------|------------------------------|------------------------------------------|
| epimerase  | CPS                          | Zuppardo and Siebeling (1998)            |
| wvbA       | Hemolysin                    | Kim et al. (2009)                        |
| vvuA & viuB| Siderophores                 | Yokochi et al. (2013)                    |
| rtxA1      | MARTXAVv                     | Jeong and Satchell (2013)                |
| vvpE       | Metalloprotease              | Jones and Oliver (2009)                  |
| vvE        | Elastolytic protease         | Liu et al. (2007)                        |
| sodA       | Superoxide dismutase (MsSOD) | Kang et al. (2007a)                      |
| cadA & cadB| Lysine decarboxylase         | Kovačičkova et al. (2010)                |
| pilA       | Pilin                        | Paranjpye and Strom (2005)               |
| pilID      | Preplin peptidase            | Paranjpye and Strom (2005)               |
| OmpU       | Outer membrane protein       | Goo et al. (2006)                        |
| flgC, flgE and flhF | Flagella components | Kim et al. (2012) |
due to alcoholism and persons infected by chronic hepatitis such as hepatitis B or C (Jones and Oliver, 2009).

*V. vulnificus* considered one of the most rapidly fatal pathogens known that cause endotoxic shock with fatality rates over 60% for persons consuming a contaminated raw shellfish, individuals over the age of 50 years more age groups suffer from endotoxic shock with this organism. Hormone levels played a role in this gender-based response to infection, therefore females who extirpate there ovaries were more likely to develop endotoxic shock. Interestingly, estrogen replacement therapy significantly decreased mortality in male rats; increase level of estrogen is providing protection against *V. vulnificus* lipopolysaccharide-induced endotoxic shock (Merkel et al., 2001).

**Genotypic characterization of V. vulnificus:** Genotypic characteristics of virulence strain have been largely unsuccessful than phenotypic characterization, genotypic characteristics for rapid differentiation of strains with human virulence potential, genotyping based on Deoxyribonucleic Acid (DNA) polymorphisms and sequencing of 16S ribosomal Ribonucleic Acid (16S rRNA). The studies showed generally two major genotypes of the 16S ribosomal Ribonucleic Acid (16S rRNA) with variants polymorphism, majority of strains belonging to genotype A correlated with the non-clinical (environmental) and types B that significantly clinical isolates (Nilsson et al., 2003; Arias et al., 2010). *V. vulnificus* isolates from different sources classified according to virulence factor types to three profiles, the three main genotypic profiles, Profile 1 (clinical type) consisted of genotypes of 16S rRNA gene type B, vvhA type 1 and veg C-type, whereas profile 2 (non-clinical type) consisted of genotypes of 16S rRNA gene type A, vvhA type 2 and veg E-type whereas profile 3 combined of 16S rRNA gene type B, vwhA type 2 and veg C-type (Sanjuan et al., 2009). random amplification of polymorphic DNA (RAPD-PCR) typing showed variation at the virulence-correlated gene (vsg) locus between C-type and E-type sequence, correlating with the clinical and non-clinical isolates, respectively (Rosche et al., 2005). Sequence analysis of 16S rDNA also used for genotyping processes and differentiate between more related vibrio species like *V. vulnificus* and *V. sinaloensis*, phenotypic resemblance between *V. vulnificus* and *V. sinaloensis* the possibility that it could outcompete the pathogen in warm, estuarine waters need for a better understanding of this species (Staley et al., 2013).

**Virulence factors of V. vulnificus:** *V. vulnificus* is a pathogenic bacteria that is virulent for humans, eel, shrimps and fish (Al-Mouqati et al., 2012). In term of virulence, *V. vulnificus* classified to three biotypes based on phenotypical and serological characteristics (Biosca et al., 1997; Tison et al., 1982), Human isolates are classified into biotype1 and biotype 3 and isolates cause diseases in fish and eel that is belong to biotype 2 (Fouz et al., 2007; Broza et al., 2012). However, some reports worldwide shows a few human infections caused by biotype 2 isolates which belong to Serovar E (SerE). Some virulence factors proposed as a cause of human infection with biotype 2, the extracellular products of biotype 2 strains exhibit hydrolytic/toxic activities and lethality for the eel similar to those produced by the biotype 1 strain (Biosca and Amaro, 1996) the mechanism of virulence for biotype 2 *V. vulnificus* strains in eels still need more studies. Lipopolysaccharides (LPS) expression is implicated of high prevalence of serovar E of *V. vulnificus* isolates in infected eels (Biosca et al., 1997; Biosca et al., 1993).

A broad spectrum of factors was implicated as possible virulence determines for *V. vulnificus* in different disease cases; Virulence of the bacterium has been closely linked to the presence of a surface-exposed acidic capsular polysaccharide that plays an additional role in pathogenesis by modulating inflammatory-associated cytokine production, Hemolysin/cytolysin (Gray and Kreger, 1987) an elastolytic protease (Oliver et al., 1986). The ability of virulent strains of *V. vulnificus* to overcome iron limitations by using iron bound to iron-binding proteins (Shin et al., 2005). siderophores that are able to acquire iron from transferring, the genes encoding for siderophore (vulnibactin) biosynthesis (vibl) can used as a biomarker for virulence in *V. vulnificus* (Yokochi et al., 2013). CPS is a major virulence factor of *V. vulnificus* which help of the bacteria to survival in the human host (Grau et al., 2008). In term of virulence, *V. vulnificus* strains demonstrate two different forms, virulent and a virulent forms, presence of capsule in virulent strains increase the ability to resist serum antibodies and immune cells and evade destruction by stomach acid (Horseman and Surani, 2011). Production of cytotoxin by *V. vulnificus* increase ability to cause disease, production of “Multifunctional-Autoprocessing RTX” (MARTXVv) toxin and The RtxA1 toxins are an important factor for virulence with the intragastric route of infection in mice and overwhelmingly cytotoxic to host cells (Kwak et al., 2011; Liu and Crosa, 2012). Adherence of bacteria to surfaces of host cells is another important virulence factor for *V. vulnificus* and other pathogens, Possess of single polar flagellum important for motility that increase ability of *V. vulnificus* strains to cause diseases (Kim et al., 2012).

Virulence-correlated gene (vsg) is found in 90% of clinical isolates (Bogard and Oliver, 2007) NanA gene is definite for the growth and virulence of *V. ulnificus* after injection in mice (Jeong et al., 2009). fibronectin-binding protein as an outer membrane protein (OmpU) is important for the expression of virulence factors of
The extracellular Capsule Polysaccharide (CPS): CPS is important for virulence and many lethality reports in animal models are mostly related to CPS expression (Simpson et al., 1987). *V. vulnificus* virulence associated with (CPS) expression that can be distinguished tow colony morphology, encapsulated colonies has been described as Opaque (Op), Translucent (Tr) colony morphology with decrease expression of (CPS) and Intermediate colony morphology (Int) in which the colonies appear less opaque but are not fully translucent on solid media such as TCBS and Brain heart infusion (Fig. 1) (Simpson et al., 1987; Rosche et al., 2006). However, isolates with (Op) colony morphology have a thick capsule that increase the virulence, whereas, the translucent colonies are less thickness with absent or incomplete CPS (Brown and Gulig, 2009; Hilton et al., 2006; Wright et al., 2001; Biosca et al., 1993).

Previous studies shows clearly correlation between colony morphology and virulence, CPS is an essential virulence factor of *V. vulnificus* which allows of these bacteria to survival in the human host (Zuppardo and Siebeling, 1998) therefore, the isolates cannot express the CPS reduced the virulence (Simpson et al., 1987; Wright et al., 1990). Bacterial strains that have capsular materials develop resistance against host serum bactericidal action, antiphagocytosis, tissue damages and high lethality for mice (Yoshida et al., 1985), in the other hand, other studies proved presence of capsule not essential for vibrosis for eels when injected through intra-peritoneal rout, this study suggests the role of capsular materials in initial stage of infection to help of pathogen to adherence on the surface of host (Biosca et al., 1993). Generated mutants in epimerase gene which responsible of CPS that lost the ability to produce CPS (Zuppardo and Siebeling, 1998).

Extracellular enzymes: Environmental and clinical strains of *V. vulnificus* have ability to produce broad spectrum of exoenzyme that correlated with the virulence (Oliver et al., 1986) Most of extracellular enzymes are produced by *V. vulnificus* such as elastase, protease, hemolysin, collagenase, DNase, lipase, phospholipase, mucinase, chondroitin sulfatase, hyaluronidase and fibrinolysin (Oliver, 1989; Gray and Kreger, 1987; Oliver et al., 1986; Wright and Morris, 1991). An elastolytic protease showed toxic for mice after the injection in blood stream (Jeong et al., 2000). Other studies have also shows the proteases in *V. vulnificus* has a role in bradykinin production (Maruo et al., 1998) bradykinin generated by kinin system that is enzymatic cascade mediated by specific receptors (B1 and B2). Activation of kinin system is particularly important in regulation of blood pressure and inflammatory reactions because bradykinin able to increase permeability of blood vessels and to cause vasodilatation of veins and arteries. Increase permeability of blood vessels facilitates dissemination of this pathogen through vascular system that allowing developing septicemia. Based on these reports, protease produced by *V. vulnificus* may play important role in the pathogenesis of this organism.

Hemolysin is another important exoenzyme produced by *V. vulnificus* that help in lysis of host red blood cells; hemolysin is implicated in high lethality of mice that injected intravenous rout by low concentration of this exoenzyme purified from *V. vulnificus*. Furthermore, hemolysin can be produced in vivo in mice during disease development; this finding leads to hypothesis that suggests this exoenzyme may be has a role in develop the disease and increase pathogenesis of *V. vulnificus* (Gray and Kreger, 1987).

Endotoxins: Endotoxin is a part of outer membrane of cell wall in gram negative pathogens that released when the bacteria ruptured, the term endotoxin refer to cell associated toxin that bin in cell membrane such as, LPS, the toxic ability of LPS associated with type of lipids in the cell membrane called (Lipid A) and polysaccharides that activate immune system. LPS considered as a major virulence factor of *V. vulnificus* as well as in wound infections associated with this pathogen observe inflammatory symptoms of septicemia, LPS molecules of *V. vulnificus* caused decreases of atrial pressure in mice injected with LPS molecules that lead to decline and death in one hour, same response seems with injection similar amount of LPS from *Salmonella typhimurium* (McPherson et al., 1991).

LPS molecules in *V. vulnificus* also develop endotoxic shock, tissue necrosis and small cytokine because LPS is a pyrogen that causes release of Tumor Necrosis Factor (TNF) (McPherson et al., 1991; Rhee et al., 2005). The overproduction of TNF believed its
main factor contribute in high fatality in Gram-negative septicemia that lead to stimulation of nitric oxide synthase in response to LPS leads to develop symptoms of endotoxic shock. Injection of purified V. vulnificus LPS to mice leads to sudden drop of pressure in arteries that cause death of this animals during one hour (Elmore et al., 1992).

Furthermore, the acidic polysaccharide capsule of V. vulnificus contribute directly in stimulates this bacteria to expression and secretion of TNF in human and mice (McPherson et al., 1991; Park et al., 2007). Injection of N-monomethyl-L-arginine shows reduce the effects of LPS, N-monomethyl-L-arginine an inhibitor of nitric oxide synthase refer to contribution of nitric oxide synthase in stimulation of immune response against LPS (Elmore et al., 1992). Comparative studies of the LPS of eel-virulent and eel-avirulent V. vulnificus isolates, shows only serological differences between two biotypes of V. vulnificus (Biosca et al., 1999).

Interestingly, estrogen and Light Density Lipid (LDL) cholesterol have a role in minimize the effects of LPS, where it’s noted increase survival and low fatality among mice injected by LDL prior to LPS exposure (Park et al., 2007). A high level of estrogen in female associate in protection of female against the endotoxic activity of LPS in V. vulnificus and is elucidate the reason decrease the infection developed among females compared with males (Merkel et al., 2001). Whoever, on reports indicates relationship between level of estrogen and LDL in blood and reduce the effect of LPS but these results manifest the role of LPS limited in activation of host responses and host damage.

Iron utilization: Environmental and clinical isolates of V. vulnificus have several virulence factors, environmental isolates show low ability to human pathogenesis, ability of V. vulnificus to cause diseases increases with increased level of iron in host serum due to V. vulnificus is ferrophilic bacterium its growth demand high levels of iron available in serum (Bogard and Oliver 2007), therefore, patents suffering from liver dysfunction, hemochromatosis and cirrhosis acquisition of iron in high levels that used as essential nutrient, high level of iron in plasma can be facilitated through possess hemolytic factors that help to release the iron from hemoglobin (Hor et al., 1999). Iron stimulates the production of cytolsin-hemolysin by increasing transcription of (VvhA) gene, cytolsin/hemolysin is the most virulent exotoxin produced by V. vulnificus implicated in virulence of V. vulnificus (Kim et al., 2009). There is correlation between pathogenesis of V. vulnificus and possessing of several putative virulence factors:

- Phenolatesiderophore production
- Transferrin-bound iron utilization
- Production of hemolysin (Wright et al., 1981)

Virulence of clinical and environmental isolates of V. vulnificus directly correlated with host serum iron level (Yokochi et al., 2013), there are several iron uptake systems help strains of V. vulnificus to obtain of iron from human host, transferrin, siderophores and lactoferrin, are examples of these mechanisms, ability of make infection appears to related with increase in transferrin saturation (Wright et al., 1981), V. vulnificus strains be also able to produces tow kind of siderophores, phenolate and hydroxamate siderophores, possess of phenolate siderophore increase ability of virulent isolates to obtain iron from high saturated transferrin (Simpson et al., 1987; Litwin et al., 1996), lactoferrin and ferritin iron-binding proteins have been reported shows same results with iron acquisition (Simpson and Oliver, 1987). Acquisition of iron is required for V. vulnificus virulence, mutation in genes that regulate phenolate siderophore production for virulent strain which lost ability to produce siderophore also lost ability to use transferrin at 100% iron saturation and virulence reduced (Litwin et al., 1996).

High levels of iron in serum clearly related with the virulence of V. vulnificus in mice treated with iron dextran, the mice treated with iron shows susceptible to V. vulnificus infection (Hor et al., 2000), there for persons with high level of iron serum as a result of chronic liver and blood diseases are more susceptible to V. vulnificus infections (Gulig et al., 2005; Jones and Oliver, 2009). Intraperitoneal injection of V. vulnificus in mice treated with ferric ammonium resulted in a much lower LD_{50} compared to untreated mice (Wright et al., 1981) Ferric ammonium treatment was found to lead to liver damage and an increase in serum iron. The majority of iron in the serum is bound to transferrin. V. vulnificus possesses several iron-scavenging siderophores that are able to acquire iron from transferrin (Simpson and Oliver, 1983). Catechol siderophore (vulnibactin) is the major system that helps V. vulnificus to release iron from transferring, Mutations in the genes encoding for siderophore (vulnibactin) biosynthesis (vvuA, viuB, vvsA and vvsB) in V. vulnificus were found to diminish virulence in mice (Yokochi et al., 2013; Kim et al., 2008a).

**Multifunctional-Autoprocessing RTX” (MARTXVv) toxin:** MARTXVv is one of important large cytotoxin that involved in pathogenicity of V. vulnificus through lyse a variety of cells therefore its considered as an important factor contribute in virulence of V. vulnificus for mice by the intragastric route (Kwak et al., 2011; Lo et al., 2011; Shao et al., 2011). rtxA1 gene that coding for MARTXVv toxin in v. vulnificus Biotype 1 shows genetic variation, there are four distinct variants of rtxA1 that encode toxins in different structures and domain of effects (Liu et al., 2011; Kim et al., 2008b).
In addition, rtxA1 gene also related to production of MARTX toxin in *V. cholera* as one of virulence factors in this pathogen and in *V. vulnificus* (Jeong and Satchell, 2013). RtxA1 gene with large open reading frame (15,618 bp) of *V. cholera* genome and other Vibrio species, in *V. vulnificus* there are two homologs of the *rtxA1* gene identified (Liu et al., 2007; Kim et al., 2008b). Gene cluster include rtxE next to *rtxA1* operon encodes for MARTX in *V. vulnificus* and *V. cholera*, mutation in *rtxE* gene in this cluster leads to block secretion of RTX and reduce toxicity against host cells, therefore most reports suggested RtxE is essential for the virulence of this bacterium (Lee et al., 2008b). The disruption of the *rtxE* gene also blocked secretion of RtxA1 to the cell exterior and resulted in a significant reduction in cytotoxic activity against epithelial cells. The Type I Secretion System (T1SS) composed of four-component proteins: an ATP-Binding Cassette (ABC) transporter RtxB, a membrane fusion protein RtxD, an additional ATP-binding protein RtxE and a ToC-like protein, T1SS secret RTX toxin (Satchell, 2007), *Vibrio cholerae* MARTX toxin is secreted by an atypical T1SS (Boardman and Satchell, 2004; Linhartova et al., 2010).

The mechanism by which RtxA1 toxin mediate death of host cells elucidated by form small pore in cell membrane of host cells and increase in the intracellular concentration of Ca\(^{2+}\) and activate the calcium-dependent mitochondrial pathways that caused calcium sequestration in the mitochondria and irreversible mitochondrial membrane dysfunction which cause drop the level of Adenosine Triphosphate (ATP), finally, the distraction of the integrity of the plasma membrane (Kim et al., 2013), RTX also increase survival of pathogens by protect them against phagocytosis (Lo et al., 2011).

In mice infected orally with *V. vulnificus* shows the ability to secret MARTXVv that play an important role in growth and prevalence of this pathogen in the gut through made small pores in small intestine helps this pathogen to transport to other organs. Histopathology records refer to role of this cytotoxin in disruption of small intestinal villi, epithelial necrosis and small intestinal inflammation in the mice, therefore deletion mutation of *rtxA* gene leads to stop releasing MARTXVv cytotoxin and reduce ability of this pathogen to cause intestinal epithelial tissue damage and unable this bacterium to cause infection in humans intestinal (Jeong and Satchell, 2013).

**Cytolysin-hemolysin VvhA:** Virulence of *V. vulnificus* lies in its ability to produce cytotoxins that induce cytolysis and death of erythrocytes in different animal species through made small pores in the cell membrane (Kim et al., 2010, 2013), this cytotoxin called *V. vulnificus* hemolysin/cytolysin (vvhA) that is an important virulence factor contributes to release iron through its hemolytic activity (Wright and Morris, 1991; Yokochi et al., 2013). The first report about role of cytolytic activity of vvhA in hamster ovary cells and mammalian erythrocytes (Kreger and Lokwood, 1981). Furthermore vvhA gene regulate iron uptake by ferrocyanide uptake regulator (Fur)-binding box and mutation in fur region leads to disable in iron utilization (Kim et al., 2009; Jeong and Satchell, 2013).

Hemolysin cause variety of pathological effect includes necrosis of soft tissue when injected into mice, accumulation of fluid in intestinal tissues (Gray and Kreger, 1987), increased vascular permeability and apoptosis of endothelial cells when exposure to this cytotoxin induce production of nitric oxide (Kwon et al., 2001). High lethality of hemolysin attributed to its ability to form small pores in plasma membrane that this leads to increase permeability of vascular system and cause hypotension (Kim et al., 1993). Although of these ability to virulence but inactivation of the gene vvhA responsible of production of hemolysin indicate that did not major change in the LD\(_{50}\) among of mutant strains and wild strains after injection into iron-loaded mice (Lee et al., 2004) that means not only hemolysin implicated in the lethality and tissue damage resulting from infection with *V. vulnificus* (Wright and Morris, 1991). In the recent study shows there is collaboration between vvhA and MARTXAVv toxins to cause necrosis and tissue damages (Jeong and Satchell, 2013).

Other studies suggested the Outer Membrane Vesicles (OMVs) induced cell death through mediated in VvhA delivery to host cells (Kim et al., 2010), presence of membrane-bound cholesterol increased VvhA binding to cell membrane through interaction between VvhA-bearing OMVs and cholesterol on the surface of host cell and increase virulence activity of this cytotoxin (Yu et al., 2007). There for, cholesterol confine in the host cells decrease ability of OMV to deliver VvhA to host cells (Kim et al., 2010). Hemolysin receptors on cholesterol facilitate binding of VvhA and either increased or decreased this cytotoxin activity in case of bound or free cholesterol, in case bound cholesterol, hemolysin monomers can be inactivated by stabilized in human serum albumin and inhibited oligomerization can be enhanced with exogenous cholesterol (Choi et al., 2006; Kim and Kim, 2002). Effects of cholesterol on hemolysin embolden researchers to study the effects of Low-Density Lipoprotein cholesterol (LDL) on virulence of *V. vulnificus* (Park et al., 2005). There are no observed differences in death percentage between wild type and vvhA mutant the strain injected together by LDL cholesterol, this finds led to the hypothesis which supposed the role of vvhA in protect host cells against these toxins and no effect on hemolysin activity (Park et al., 2007).
Metalloprotease: Metalloprotease is a type of protease enzymes have variety of metals in active site involve in mechanism of action, this class of protease coded by vvpE gene in V. vulnificus and purified VvpE cause damage for cutaneous tissues, hemorrhage and increase permeability of vascular tissues leads to edema in mice (Jones and Oliver, 2009; Jeong et al., 2000), production of vasodilator called bradykinin can be increase vascular permeability caused by VvpE (Maeda and Yamamoto, 1996). Bradykinin was important for virulence of V. vulnificus through increase prevalence of V. vulnificus into the bloodstream (Maruo et al., 1998). VvpE contributes to destroy collagen type IV in basement membrane and cause local tissue damage, moreover degeneration of Immunoglobulin A (IgA) and lactoferrins, which are responsible for mucosal immunity (Kim et al., 2007; Kim and Shin, 2010). vvpE appears to regulate swarming ability of V. vulnificus, mutation of the VvpE gene encoding for metalloprotease resulted in reducing of swarming ability that was retrieved after vvpE gene complementation. Further investigations demonstrated Iron required for vvpE transcription elucidate its ability to destroy proteins containing iron to be available to induce transcription and used by siderophores (Okujo et al., 1996; Shin et al., 2005). Interestingly, other studies have shown metalloprotease is not required for iron acquisition (Sun et al., 2006) both virulent and avirulent strains of V. vulnificus possess the ability to produce metalloprotease and no change in LD$_{50}$ between vvpE mutant strains and wild strains (Jeong et al., 2000; Shao and Hor, 2000). These evidences lead to supposition that metalloprotease may not be contribute significantly in the virulence of V. vulnificus.

Role of HlyU in regulation of rtxA1 and vvhA: In V. vulnificus human pathogen HlyU plays an essential role in regulation of virulence of this pathogen through regulation of RtxA1 gene expression, role of HlyU in regulation of RtxA1gene expression considered as a positive control for virulence of important virulence factors accounting for the fulminating and damaging nature of V. vulnificus infections (Liu and Crosa, 2012). In addition to rtxA protein regulation, HlyU play an essential role in regulate production of hemolysin-cytoylisin and elastolytic protease in V. vulnificus and V. anguillarum (Kim et al., 2003; Liu et al., 2011; Shao et al., 2011; Liu and Crosa, 2012), expression of rtxA1 gene repressed by Histone-like Nucleoid Structuring protein (H-NS), hlyU protein depressing expression of rtxA1 by joined to a region upstream of the rtxA1 operon promoter, demonstrate the displacing of H-NS and allowing rtxA1 expression and retrieval invasion of bacteria (Kim et al., 2003). Although the mutation of vvhA and vve not affect virulence and LD$_{50}$ of V. vulnificus but mutation of hlyU led to rescission of cytotoxic activity of rtxA1, that refer to the role of hlyU protein in positive regulation and expression of cytolysin/hemolysin and elastolytic protease that encoded by vvhA and vve respectively (Wright and Morris, 1991; Liu et al., 2007).

Resistance to acid stress: Enterobacteriaceae is a large family include pathogenic bacteria causes infection in gastrointestinal tract in acidic environment, thus, Resistance to acid is an important factor for virulence of these bacteria include Escherichia coli, Vibrio cholerae and Vibrio vulnificus (Boot et al., 2002). V. vulnificus as a member of enterobacteriaceae family cause food-borne gastroenteritis that may travel and colonize in low pH environment of the stomach and intestinal lumen, acidic environment induce these bacteria to release amino acid decarboxylase which form amines as a response to high acidity (Tabor and Tabor, 1985), accumulation of amines surrounding the cells contribute in neutralize the external pH and protect cells from high acidity. Lysine decarboxylase one of amino acid decarboxylases contribute to the acid resistance of V. vulnificus by convert lysine to cadaverine (Rhee et al., 2002) and V. paraaemolyticus also possesses lysine decarboxylase pathway to avoid lethal acidity (Tanaka et al., 2008). In V. vulnificus, superoxide stress also involve in synthesis of Lysine decarboxylase in addition to acid stress (Kim et al., 2006a), level of cellular superoxide is elevated when exposure to low pH (Kim et al., 2005). Lysine decarboxylase encoded by cadA and cadB genes are activated in acid environments and neutral environments under anaerobic conditions (Kovacikova et al., 2010; Kang et al., 2009), mutation of cadA and cadB genes demonstrate low tolerance to acid stress (Tanaka et al., 2008). Mn-containing Superoxide Dismutase (MnSOD) encoded by sodA that is activated by SoxR in acid environments, increase of cadaverine level reduce MnSOD production under superoxide stress in V. vulnificus (Kim et al., 2006a; Kang et al., 2007a).

Cellular level of Superoxide Dismutase (SOD) is significantly effect on the virulence and an increase in SOD level through MnSOD induction by SoxR under superoxide stress is essential for virulence of V. vulnificus. Mutant of SOD reduce virulence in V. vulnificus when intraperitoneal injection of mutant strains into mice, sodC mutant, sodA mutant and sodB mutant loss CuZnSOD, MnSOD and FeSOD, respectively. The survival of SOD mutants under superoxide stress also decreases in the same order (Kang et al., 2007a, b; Vanaporn et al., 2011).

Adherence to the host cells: Attachment of pathogens on the surface of host cells is an important factor has been well documented, adherence of pathogenic bacteria on a variety of cells considered among various virulence-related phenotypes, bacterial adhesion is essential step for their survival and critical for the initial pathogenic interaction. A bacterial cell possesses pili as surface receptors are required for virulence since they are increase invasion of a host because pili are used for
adherence to host cells (Kim et al., 2008b). pilA and pilD are important genes which encoding for pilin structural protein and prepilin peptidase, respectively, were generated in V. vulnificus to forming pili contribute in adherence of this bacteria on epithelial cells in infected organs and biofilm formation, therefore, induce mutation in pilA and pilD genes led to loss of adhesion ability and biofilm formation on epithelial cells and a little increase in LD50 for pilA and pilD for mutant strains in compare with wild strains when injected in mice, in contrast, mutation in pilD gene also effect on overall cytotoxicity through loss of ability to secretion of protease, cytolysin and chitinase (Paranjpye and Strom, 2005). Membrane-bound lipoprotein (IlpA) is other important factors contributing in adherence of bacteria on host cell (Goo et al., 2006, 2007). (IlpA) proposed as an important virulence factors in V. vulnificus its stimulate immune response in human monocytes through production of proinflammatory cytokines and attachment of V. vulnificus to epithelial cells in human small intestines, mutation for ilpA gene demonstrate decrease in cytadherence of a V. vulnificus on human epithelial cells that refer to the potential role of the IlpA protein in adhesion of pathogenic microorganism (Lee et al., 2010; Goo et al., 2007). outer membrane protein is a major protein in outer membrane involved in the adherence to the host cells and encoded by OmpU gene in V. vulnificus (Goo et al., 2006) and V. splendidus (Duperthuy et al., 2010). High molecular weight glycoprotein called fibronectin is one of components of extracellular matrix in animal cells, V. vulnificus shows high ability to adhere to fibronectin in presence of OmpU protein Fig. 2), thus, mutation in OmpU reduce ability of this pathogen to adhere in mammalian cells in compare with wild strains (Goo et al., 2006).

Interestingly Decrease of adherence ability in OmpU and IlpA mutants accompanied with reduced cytotoxicity, these results propose the cell contact is required for V. vulnificus cytotoxicity and local damage (Goo et al., 2006, 2007).

Flagella: Motility in liquid and semi-liquid media is important for bacterial to pathogenesis and biofilm formation, possess of flagella required for bacterial virulence. A single polar flagellum push the bacterium in liquid (swimming), while multiple lateral flagella help the bacterium to move on the surfaces (swarming) (McCarter, 1995), in addition to the previous factors motility could be suggested to be another virulence determinant (Giron et al., 2002). V. vulnificus and other related vibrio are motile organisms by one polar flagellum, whereas, V. cholera. Interestingly in V. vulnificus, there are several flagellar genes encode for flagella component formation and associate with this bacterium pathogenesis, flgC, flgE and flhF (Kim and Rhee, 2003; Lee et al., 2004; Kim et al., 2012) deletion mutation for these genes led to loss of flagella components resulted in significant decreases in motility, cellular adhesion and cytotoxicity compared to those of the wild strains, therefore, injection of flgC, flgE and flhF mutants in mice resulted in increased LD50 indicating that the flagellum is necessary for adhesion and cytotoxicity that play role in virulence (Kim and Rhee, 2003; Lee et al., 2004; Kim et al., 2012).

CONCLUSION

In the present study outlined important factors implicated in virulence of V. vulnificus, these bacteria has classified as a medically important pathogen causing prevalence of septicemia, skin and wound infections. Ability of this pathogen to cause disease is associated with possess variety of virulence factors, MARTXVv, vvhA, vgcC, Lysine decarboxylase and other extracellular enzymes, these virulence factors can used as biomarkers to predict potential virulent strains of V. vulnificus. In the other side, some of suggested virulence factors such as VvpE and CPS turned out irrelevant to induce and regulate virulence of V. vulnificus. Therefor more researches are needed to prove role of other virulence factors in pathogenicity and characterise molecular and cellular interactions between host cells and V. vulnificus that this lead to intra-species genetic transfer and virulence factors.

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