

Research Article

A Review of Important Virulence Factors of *Vibrio vulnificus*

Mohammed M. Kurdi Al-Assafi, Sahilah Abd Mutalib, Ma'aruf Abd Ghani
and Mohammed Aldulaimi

Faculty of Science and Technology, School of Chemical Sciences and Food Technology,
Universiti Kebangsaan Malaysia (UKM), 43600 UKM Bangi, Selangor D.E., Malaysia

Abstract: This study aim 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 (*vvhA*) 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 (*vcgC*), 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, *vvhA*

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 phagocytosis, 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

Corresponding Author: Sahilah Abd. Mutalib, Faculty of Science and Technology, School of Chemical Sciences and Food Technology, Universiti Kebangsaan Malaysia (UKM), 43600 UKM Bangi, Selangor D.E., Malaysia, Tel.: +60176537181

This work is licensed under a Creative Commons Attribution 4.0 International License (URL: <http://creativecommons.org/licenses/by/4.0/>).

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

Gene	Product	Reference
epimerase and wbpP	CPS	Zuppardo and Siebeling (1998)
VvhA	Hemolysin	Kim <i>et al.</i> (2009)
vvuA & viuB	Siderophores	Yokochi <i>et al.</i> (2013)
rtxA1	MARTXAVv	Jeong and Satchell (2013)
vvpE	Metalloprotease	Jones and Oliver (2009)
vvE	Elastolytic protease	Liu <i>et al.</i> (2007)
sodA	Superoxide dismutase (MnSOD)	Kang <i>et al.</i> (2007a)
cadA & cadB	Lysine decarboxylase	Kovacikova <i>et al.</i> (2010)
pilA	Pilin	Paranjpye and Storm (2005)
pilD	Prepilin peptidase	Paranjpye and Storm (2005)
OmpU	Outer membrane protein	Goo <i>et al.</i> (2006)
flgC, flgE and flhF	Flagella components	Kim <i>et al.</i> (2012)

(Kwak *et al.*, 2011). Furthermore, bacterial pathogenicity linked with its ability to adherence on the surface of host cells. The present article discuss 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 genes 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) *vvhA* 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.*, 2010). Pathogenic *vibrio* cause three major clinical illness: 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 (Bunchorntavakul and Chavalitdhamrong, 2012), risk factor for *V. vulnificus* also increases with liver disease

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 there 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 their 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, *vhA* type 1 and *vcg* C-type, whereas profile 2 (non-clinical type) consisted of genotypes of 16S rRNA gene type A, *vhA* type 2 and *vcg* E-type whereas profile 3 combined of 16S rRNA gene type B, *vhA* type 2 and *vcg* C-type (Sanjuan *et al.*, 2009). random amplification of polymorphic DNA (RAPD_PCR) typing showed variation at the virulence-correlated gene (*vcg*) 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. sinoaloensis*, phenotypic resemblance between *V. vulnificus* and *V. sinoaloensis* 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, ell, 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 transferrin, the genes encoding for siderophore (vulnibactin) biosynthesis (*viuB*) 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 (*vcg*) is found in 90% of clinical isolates (Bogard and Oliver, 2007) *NanA* gene is definite for the growth and virulence of *V. vulnificus* 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

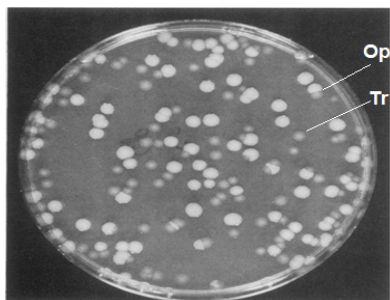


Fig. 1: Colony morphology of *V. vulnificus* on agar plate (Simpson *et al.*, 1987)

V. vulnificus (Goo *et al.*, 2006; Grau *et al.*, 2008) *wbpP* gene considered substantial factor involve in biosynthesis of CPS that important for virulence of *V. vulnificus* (Park *et al.*, 2006). Rugose Extracellular Polysaccharide (rEPS) gene cluster of *V. vulnificus* also contribute in expression of virulence factors in capsular and non-capsular strains (Grau *et al.*, 2008).

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 to 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 vibriosis 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.

Ironutilization: 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 cytolysin-hemolysin by increasing transcription of (*VvhA*) gene, cytolysin/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 LD₅₀ 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 toxin 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 MARTXAVv toxin in *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. vulnificus* 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 1 Secretion System (TISS) 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 TolC-like protein, TISS secret RTX toxin (Satchell, 2007), *Vibrio cholerae* MARTX toxin is secreted by an atypical TISS (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 ferric 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 LD₅₀ 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 be 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 siderophres (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 LD₅₀ 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 RtxA1 gene 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-cytolysin 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 LD₅₀ 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. parahaemolyticus* 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

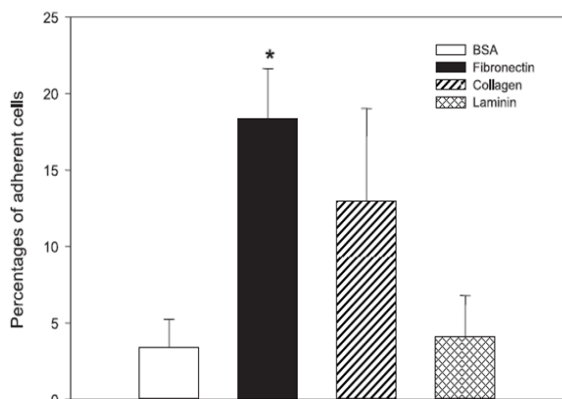


Fig. 2: Components of the extracellular matrix and adherence of *V. vulnificus* (Goo *et al.*, 2006)

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 Storm, 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 cytoadherence 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*, *vcgC*, 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*. Therefore 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.

ACKNOWLEDGMENT

This study was funded by Universiti Kebangsaan Malaysia-Faculty of Science and Technology.

REFERENCES

- Al-Mouqati, S., I.S. Azad, D. Al-Baijan and A. Benhaji, 2012. *Vibrio* detection in market Seafood samples of Kuwait by biochemical (API 20E) strips and its evaluation against 16s rDNA-based molecular methods. Res. J. Biotechnol., 7(3): 63-69.

- Arias, C.R., O. Oliveras-Fuster and J. Goris, 2010. High intragenomic heterogeneity of 16S rRNA genes in a subset of *Vibrio vulnificus* strains from the western Mediterranean coast. *Int. Microbiol.*, 13(4): 179-88.
- Biosca, E.G. and C. Amaro, 1996. Toxic and enzymatic activities of *Vibrio vulnificus* biotype 2 with respect to host specificity. *Appl. Environ. Microbiol.*, 62: 2331-2337.
- Biosca, E.G., J.D. Oliver and C. Amaro, 1996. Phenotypic characterization of *Vibrio vulnificus* biotype 2, a lipopolysaccharide-based homogeneous O serogroup within *Vibrio vulnificus*. *Appl. Environ. Microbiol.*, 62: 918-927.
- Biosca, E.G., H. Llorens, E. Garay and C. Amaro, 1993. Presence of a capsule in *Vibrio vulnificus* biotype 2 and its relationship to virulence for eels. *Infect. Immun.*, 61: 1611-1618.
- Biosca, E.G., C. Amaro, J.L. Larsen and K. Pedersen, 1997. Phenotypic and genotypic characterization of *Vibrio vulnificus*: Proposal for the substitution of the subspecific taxon biotype for serovar. *Appl. Environ. Microbiol.*, 63: 1460-1466.
- Biosca, E.G., R.M. Collado, J.D. Oliver and C. Amaro, 1999. Comparative study of biological properties and electrophoretic characteristics of lipopolysaccharide from eel-virulent and eel-avirulent *Vibrio vulnificus* strains. *Appl. Environ. Microbiol.*, 65: 856-858.
- Boardman, B.K. and K.J. Satchell, 2004. *Vibrio cholerae* strains with mutations in an atypical type I secretion system accumulate RTX toxin intracellularly. *J. Bacteriol.*, 186: 8137-8143.
- Boot, I.R., P. Cash and C. O'Byrne, 2002. Sensing and adapting to acid stress. *Antonie Van Leeuwenhoek*, 81: 33-42.
- Bross, M.H., K. Soch, R. Morales and R.B. Mitchell, 2007. *Vibrio vulnificus* infection: Diagnosis and treatment. *Am. Fam. Physician*, 76: 539-44.
- Brown, R.N. and P.A. Gulig, 2009. Roles of RseB, δ E and DegP in virulence and phase variation of colony morphotype of *Vibrio vulnificus*. *Infect. Immun.*, 77(9): 3768-378.
- Broza, Y.Y., N. Raz, L. Lerner, Y. Danin-Poleg and Y. Kashi, 2012. Genetic diversity of the human pathogen *Vibrio vulnificus*: A new phylogroup. *Int. J. Food Microbiol.*, 153: 436-443.
- Broza, Y.Y., Y. Danin-Poleg, L. Lerner, L. Valinsky, M. Broza and Y. Kashi, 2009. Epidemiologic study of *Vibrio vulnificus* infections by using variable number tandem repeats. *Emerg. Infect. Dis.*, 15: 1282-1285.
- Bunchorntavakul, C. and D. Chavalitdhamrong, 2012. Bacterial infections other than spontaneous bacterial peritonitis in cirrhosis. *World J. Hepatol.*, 4(5): 158-168.
- Canigral, I., Y. Moreno, J.L. Alonso, A. González and M.A. Ferrús, 2010. Detection of *Vibrio vulnificus* in seafood, seawater and wastewater samples from a Mediterranean coastal area. *Microbiol. Res.*, 165(8): 657-664.
- Choi, M.H., H.Y. Sun, R.Y. Park, Y.H. Bai, Y.Y. Chung, C.M. Kim and S.H. Shin, 2006. Human serum albumin enhances the hemolytic activity of *Vibrio vulnificus*. *Biol. Pharm. Bull.*, 29: 180-182.
- Duperthuy, M., J. Binesse, F. Le Roux, B. Romestand, A. Caro, P. Got, A. Givaudan, D. Mazel, G. Bachère and D. Destoumieux-Garzón, 2010. The major outer membrane protein OmpU of *Vibrio splendidus* contributes to host antimicrobial peptide resistance and is required for virulence in the oyster *Crassostrea gigas*. *Environ. Microbiol.*, 12: 951-963.
- Elmore, S.P., J.A. Watts, L.M. Simpson and J.D. Oliver, 1992. Reversal of hypotension by *Vibrio vulnificus* lipopolysaccharide in the rat by inhibition of nitric oxide synthetase. *Microb. Pathog.*, 13: 391-397.
- Fouz, B., F.J. Roig and C. Amaro, 2007. Phenotypic and genotypic characterization of a new fish-virulent *Vibrio vulnificus* serovar that lack potential to infect humans. *Microbiol.*, 153: 6-34.
- Giron, J.A., A.G. Torres, E. Freer and J.B. Kaper, 2002. The flagella of enteropathogenic *Escherichia coli* mediate adherence to epithelial cells. *Mol. Microbiol.*, 44: 361-379.
- Goo, S.Y., H.J. Lee, W.H. Kim, K.L. Han, D.K. Park, H.J. Lee, S.M. Kim, K.S. Kim, K.H. Lee and S.J. Park, 2006. Identification of OmpU of *Vibrio vulnificus* as a fibronectin-binding protein and its role in bacterial pathogenesis. *Infect. Immun.*, 74(10): 5586-5594.
- Goo, S.Y., Y.S. Ham, W.H. Kim, K.H. Lee and S.J. Park, 2007. *Vibrio vulnificus* IlpA-induced cytokine production is mediated by Toll-like receptor 2. *J. Biol. Chem.*, 282: 27647-27658.
- Grau, B.L., M.C. Henk, K.L. Garrison, B.J. Olivier, R.M. Schulz, K.L. O'Reilly and G.S. Pettis, 2008. Further characterization of *Vibrio vulnificus* rugose variants and identification of a capsular and rugose exopolysaccharide gene cluster. *Infect. Immun.*, 76(4): 1485-1497.
- Gray, L.D. and A.S. Kreger, 1987. Mouse skin damage caused by cytolysin from *Vibrio vulnificus* and by *V. vulnificus* infection. *J. Infect. Dis.*, 155: 236-24.
- Gulig, P.A., K.L. Bourdage and A.M. Starks, 2005. Molecular pathogenesis of *Vibrio vulnificus*. *J. Microbiol.*, 43: 118-131.
- Hilton, T., T. Rosche, B. Froelich, B. Smith and J. Oliver, 2006. Capsular polysaccharide phase variation in *Vibrio vulnificus*. *Appl. Environ. Microbiol.*, 72: 6986- 6993.

- Hor, L.I. and C.L. Chen, 2013. Cytotoxins of *Vibrio vulnificus*: Functions and roles in pathogenesis. *BioMedicine*, 3(1): 19-26.
- Hor, L.I., T.T. Chang and S.T. Wang, 1999. Survival of *Vibrio vulnificus* in whole blood from patients with chronic liver diseases: Association with phagocytosis by neutrophils and serum ferritin levels. *J. Infect. Dis.*, 179: 275-278.
- Hor, L.I., Y.K. Chang, C.C. Chang, H.Y. Lei and J.T. Ou, 2000. Mechanism of high susceptibility of iron-overloaded mouse to *Vibrio vulnificus* infection. *Microbiol. Immunol.*, 44(11): 871-878.
- Horseman, M.A. and S. Surani, 2011. A comprehensive review of *Vibrio vulnificus*: an important cause of severe sepsis and skin and soft-tissue infection. *Int. J. Infect. Dis.*, 15(3): 157-166.
- Iwamoto, M., T. Ayers, B.E. Mahon and D.L. Swerdlow, 2010. Epidemiology of seafood-associated infections in the United States. *Clin. Microbiol. Rev.*, 23: 399-411.
- Jeong, H.G. and K.J.F. Satchell, 2013. Additive function of *Vibrio vulnificus* MARTXVv and VvhA Cytolysins promotes rapid growth and epithelial tissue necrosis during intestinal infection. *PLoS Pathog.*, 8(3): e1002581.
- Jeong, H.G., M.H. Oh, B.S. Kim, M.Y. Lee, H.J. Han and S.H. Choi, 2009. The capability of catabolic utilization of N-acetylneuraminic acid, a sialic acid, is essential for *Vibrio vulnificus* pathogenesis. *Infect. Immun.*, 77(8): 3209-3217.
- Jeong, K.C., H.S. Jeong, J.H. Rhee, S.E. Lee, S.S. Chung, A.M. Starks, G.M. Escudero, P.A. Gulig and S.H. Choi, 2000. Construction and phenotypic evaluation of a *Vibrio vulnificus* vvpE mutant for elastolytic protease. *Infect. Immun.*, 68: 5096-5106.
- Jones, M.K. and J.D. Oliver, 2009. *Vibrio vulnificus*: Disease and pathogenesis. *Infect. Immun.*, 77: 1723-1733.
- Kang, I.H., E.J. Kim and J.K. Lee, 2009. Cadaverine is transported into *Vibrio vulnificus* through its CadB in alkaline environment. *J. Microbiol. Biotechnol.*, 19(10): 1122-6.
- Kang, I.H., J.S. Kim, E.J. Kim and J.K. Lee, 2007a. Cadaverine protects *Vibrio vulnificus* from superoxide stress. *J. Microbiol. Biotechnol.*, 17(1): 176-179.
- Kang, I.H., J.S. Kim and J. K. Lee, 2007b. The Virulence of *Vibrio vulnificus* is affected by the cellular level of superoxide dismutase activity. *J. Microbiol. Biotechnol.*, 17(8): 1399-1402.
- Kim, B.S. and J.S. Kim, 2002. Cholesterol induces oligomerization of *Vibrio vulnificus* cytolysin specifically. *Exp. Mol. Med.*, 34: 239-242.
- Kim, Y.R. and J.H. Rhee, 2003. Flagellar basal body flg operon as a virulence determinant of *Vibrio vulnificus*. *Biochem. Biophys. Res. Commun.*, 304: 405-410.
- Kim, C.M. and S.H. Shin, 2010. Regulation of the *Vibrio vulnificus* vvpE expression by cyclic AMP-receptor protein and quorum-sensing regulator SmcR. *Microb. Pathogen.*, 49(6): 348-353.
- Kim, S.M., D.H. Lee and S.H. Choi, 2012. Evidence that the *Vibrio vulnificus* flagellar regulator FlhF is regulated by a quorum sensing master regulator SmcR. *Microbiology*, 158(8): 2017-25.
- Kim, J.S., S.H. Choi and J.K. Lee, 2006a. Lysine decarboxylase expression by *Vibrio vulnificus* is induced by SoxR in response to superoxide stress. *J. Bacteriol.*, 188: 8586-8592.
- Kim, C.M., Y.Y. Chung and S.H. Shin, 2009. Iron differentially regulates gene expression and extracellular secretion of *Vibrio vulnificus* cytolysin-hemolysin. *J. Infect. Dis.*, 200: 582-589.
- Kim, J.S., M.H. Sung, D.H. Kho and J.K. Lee, 2005. Induction of manganese-containing superoxide dismutase is required for acid tolerance in *Vibrio vulnificus*. *J. Bacteriol.*, 187: 5984-5995.
- Kim, Y.R., B.U. Kim, S.Y. Kim, C.M. Kim, H.S. Na *et al.*, 2010. Outer membrane vesicles of *Vibrio vulnificus* deliver cytolysin-hemolysin VvhA into epithelial cells to induce cytotoxicity. *Biochem. Biophys. Res. Commun.*, 399: 607-612.
- Kim, H.Y., A.K. Chang, J.E. Park, I.S. Park, S.M. Yoon and J.S. Lee, 2007. Procaspase-3 activation by a metalloprotease secreted from *Vibrio vulnificus*. *Int. J. Mol. Med.*, 20: 591-595.
- Kim, Y.R., S.E. Lee, I.C. Kang, K. Nam, H.E. Choy and J.H. Rhee, 2013. A bacterial RTX toxin causes programmed necrotic cell death through calcium-mediated mitochondrial dysfunction. *J. Infect. Dis.*, 207(9): 1406-1415.
- Kim, I.H., J.I. Shim, K.E. Lee, W. Hwang, I.J. Kim, S.H. Choi and K.S. Kim, 2008a. Nonribosomal peptide synthase is responsible for the biosynthesis of siderophore in *Vibrio vulnificus* MO6-24/O. *J. Microbiol. Biotechnol.*, 18(1): 1835-1842.
- Kim, Y.R., S.E. Lee, C.M. Kim, S.Y. Kim, E.K. Shin, D.H. Shin, S.S. Chung *et al.*, 2003. Characterization and pathogenic significance of *Vibrio vulnificus* antigens preferentially expressed in septicemic patients. *Infect. Immun.*, 71: 5461-5471.
- Kim, H.R., H.W. Rho, M.H. Jeong, J.W. Park, J.S. Kim, B.H. Park, U.H. Kim and S.D. Park, 1993. Hemolytic mechanism of cytolysin produced from *Vibrio vulnificus*. *Life Sci.*, 53: 571-577.
- Kim, Y.R., S.E. Lee, H. Kook, J.A. Yeom, H.S. Na, S.Y. Kim, S.S. Chung, H.E. Choy and J.H. Rhee, 2008b. *Vibrio vulnificus* RTX toxin kills host cells only after contact of the bacteria with host cells. *Cell Microbiol.*, 10: 848-862.
- Kovacikova, G., W. Lin and K. Skorupski, 2010. The LysR-type virulence activator AphB regulates the expression of genes in *Vibrio cholerae* in response to low pH and anaerobiosis. *J. Bacteriol.*, 192: 4181-4191.

- Kreger, A. and D. Lockwood, 1981. Detection of extracellular toxin(s) produced by *Vibrio vulnificus*. Infect. Immun., 33: 583-590.
- Kwak, J.S., H. Jeong and K.J.F. Satchell, 2011. *Vibrio vulnificus* rtxA1 gene recombination generates toxin variants with altered potency during intestinal infection. P. Natl. Acad. Sci. USA, 108(4): 1645-1650.
- Kwon, K.B., J.Y. Yang, D.G. Ryu, H.W. Rho, J.S. Kim, J.W. Park, H.R. Kim and B.H. Park, 2001. *Vibrio vulnificus* cytolysin induces superoxide anion-initiated apoptotic signaling pathway in human ECV304 cells. J. Biol. Chem., 276: 47518-47523.
- Lee, B.C., S.H. Choi and T.S. Kim, 2008c. *Vibrio vulnificus* RTX toxin plays an important role in the apoptotic death of human intestinal epithelial cells exposed to *Vibrio vulnificus*. Microbes Infect., 10: 1504-1513.
- Lee, B.C., J.H. Lee, M.W. Kim, B.S. Kim, M.H. Oh, K.S. Kim *et al.*, 2008b. *Vibrio vulnificus* rtxE is important for virulence and its expression is induced by exposure to host cells. Infect. Immun., 76: 1509-1517.
- Lee, K.J., N.Y. Lee, Y.S. Han, J. Kim, K.H. Lee and S.J. Park, 2010. Functional characterization of the IIP protein of *Vibrio vulnificus* as an Adhesin and its role in bacterial pathogenesis. Infect. Immun., 78(6): 2408-2417.
- Lee, S.E., P.Y. Ryu, S.Y. Kim, Y.R. Kim, J.T. Koh, O.J. Kim, S.S. Chung, H.E. Choy and J.H. Rhee, 2004. Production of *Vibrio vulnificus* hemolysin *in vivo* and its pathogenic significance. Biochem. Biophys. Res. Commun., 324: 86-91.
- Linhartova, I., L. Bumba, J. Masin, M. Basler, R. Osicka, J. Kamanova *et al.*, 2010. RTX proteins: A highly diverse family secreted by a common mechanism. FEMS Microbiol. Rev., 34: 1076-1112.
- Linkous, D.A. and J.D. Oliver, 1999. Pathogenesis of *Vibrio vulnificus*. FEMS Microbiol. Lett., 174: 207-214.
- Litwin, C.M., T.W. Rayback and J. Skinner, 1996. Role of catechol siderophore synthesis in *Vibrio vulnificus* virulence. Infect. Immun., 64: 2834-2838.
- Liu, M. and J.H. Crosa, 2012. The regulator HlyU, the repeat-in-toxin gene rtxA1 and their roles in the pathogenesis of *Vibrio vulnificus* infections. Microbiol. Open, 1(4): 502-513.
- Liu, M., A.F. Alice, H. Naka and J.H. Crosa, 2007. The HlyU protein is a positive regulator of rtxA1, a gene responsible for cytotoxicity and virulence in the human pathogen *Vibrio vulnificus*. Infect. Immun., 75: 3282-3289.
- Liu, M., M. Rose and J.H. Crosa, 2011. Homodimerization and binding of specific domains to the target DNA are essential requirements for HlyU to regulate expression of the virulence gene rtxA1 encoding the repeat-in-toxin protein in the human pathogen *Vibrio vulnificus*. J. Bacteriol., 193: 6895-6901.
- Lo, H.R., J.H. Lin, Y.H. Chen, C.L. Chen, C.P. Shao, Y.C. Lai and L.I. Hor, 2011. RTX toxin enhances the survival of *Vibrio vulnificus* during infection by protecting the organism from phagocytosis. J. Infect. Dis., 203: 1866-1874.
- Maeda, H. and T. Yamamoto, 1996. Pathogenic mechanisms induced by microbial proteases in microbial infections. Biol. Chem. H-S., 377: 217-226.
- Maruo, K., T. Akaike, T. Ono and H. Maeda, 1998. Involvement of bradykinin generation in intravascular dissemination of *Vibrio vulnificus* and prevention of invasion by a bradykinin antagonist. Infect. Immun., 66: 866-869.
- McCarter, L.L., 1995. Genetic and molecular characterization of the polar flagellum of *Vibrio parahaemolyticus*. J. Bacteriol., 177: 1595-1609.
- McPherson, V.L., J.A. Watts, L.M. Simpson and J.D. Oliver, 1991. Physiological effects of the lipopolysaccharide of *Vibrio vulnificus* on mice and rats. Microbios, 67: 272-273.
- Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P.M. Griffin and R.V. Tauxe, 1999. Food-related illness and death in the United States. Emerg. Infect. Dis., 5(5): 607-625.
- Merkel, S.M., S. Alexander, E. Zufall, J.D. Oliver and Y.M. Huet-Hudson, 2001. Essential role for estrogen in protection against *Vibrio vulnificus*-induced endotoxic shock. Infect. Immun., 69: 6119-6122.
- Moreno, M.L.G. and M. Landgraf, 1998. Virulence factors and pathogenicity of *Vibrio vulnificus* strains isolated from seafood. J. Appl. Microbiol., 84: 747-751.
- Nilsson, W.B., R.N. Paranjypte, A. DePaola and M.S. Strom, 2003. Sequence polymorphism of the 16S rRNA gene of *Vibrio vulnificus* is a possible indicator of strain virulence. J. Clin. Microbiol., 41: 442-446.
- Okujo, N., T. Akiyama, S. Miyoshi, S. Shinoda and S. Yamamoto, 1996. Involvement of vulnibactin and exocellular protease in utilization of transferrin- and lactoferrin-bound iron by *Vibrio vulnificus*. Microbiol. Immunol., 40: 595-598.
- Oliver, J.D., 1989. *Vibrio vulnificus*. In: Doyle, M.P. (Ed.), Foodborne Bacterial Pathogens. Marcel Dekker, New York, pp: 569-599.

- Oliver, J.D., J.E. Wear, M.B. Thomas, M. Warner and K. Linder, 1986. Production of extracellular enzymes and cytotoxicity by *Vibrio vulnificus*. *Diagnost. Microbiol. Infect. Dis.*, 5(2): 99-111.
- Paranjpye, R.N. and M.S. Strom, 2005. A *Vibrio vulnificus* type IV pilin contributes to biofilm formation, adherence to epithelial cells and virulence. *Infect. Immun.*, 73(3): 1411-1422.
- Park, N.Y., J.H. Lee, M.W. Kim, H.G. Jeong, B.C. Lee, T.S. Kim and S.H. Choi, 2006. Identification of the *Vibrio vulnificus* wbpP gene and evaluation of its role in virulence. *Infect. Immun.*, 74(1): 721-728.
- Park, K.H., J.S. Kim, Y.R. Lee, Y.J. Moon, H. Hur, Y.H. Choi, C.H. Kim *et al.*, 2007. Low-density lipoprotein protects *Vibrio vulnificus*-induced lethality through blocking lipopolysaccharide action. *Exp. Mol. Med.*, 39: 673-678.
- Park, K.H., H.B. Yang, H.G. Kim, Y.R. Lee, H. Hur, J.S. Kim, B.S. Koo, M.K. Han, J.H. Kim, Y.J. Jeong and J.S. Kim, 2005. Low density lipoprotein inactivates *Vibrio vulnificus* cytolysin through the oligomerization of toxin monomer. *Med. Microbiol. Immunol.*, 194: 137-141.
- Rhee, J.E., J.H. Rhee, P.Y. Ryu and S.H. Choi, 2002. Identification of the cadBA operon from *Vibrio vulnificus* and its influence on survival to acid stress. *FEMS Microbiol. Lett.*, 208: 245-251.
- Rhee, J.E., K.S. Kim and S.H. Choi, 2005. CadC activates pH-dependent expression of the *Vibrio vulnificus* cadBA operon at a distance through direct binding to an upstream region. *J. Bacteriol.*, 187: 7870-7875.
- Rosche, T.M., Y. Yano and J.D. Oliver, 2005. A rapid and simple PCR analysis indicates there are two subgroups of *Vibrio vulnificus* which correlate with clinical or environmental isolation. *Microbiol. Immunol.*, 49: 381-389.
- Rosche, T.M., B. Smith and J.D. Oliver, 2006. Evidence for an intermediate colony morphology of *Vibrio vulnificus*. *Appl. Environ. Microbiol.*, 72: 4356-4359.
- Sanjuan, E., B. Fouz, J.D. Oliver and C. Amaro, 2009. Evaluation of genotypic and phenotypic methods to distinguish clinical from environmental *Vibrio vulnificus* strains. *Appl. Environ. Microbiol.*, 75: 1604-1613.
- Satchell, K.J., 2007. MARTX, multifunctional auto-processing repeats-in-toxin toxins. *Infect. Immun.*, 75: 5079-5084.
- Shao, C.P. and L.I. Hor, 2000. Metalloprotease is not essential for *Vibrio vulnificus* virulence in mice. *Infect. Immun.*, 68: 3569-3573.
- Shao, C.P., H.R. Lo, J.H. Lin and L.I. Hor, 2011. Regulation of cytotoxicity by quorum-sensing signaling in *Vibrio vulnificus* is mediated by SmcR, a repressor of hlyU. *J. Bacteriol.*, 193: 2557-2565.
- Shin, S.H., H.Y. Sun, R.Y. Park, C.M. Kim, S.Y. Kim and J.H. Rhee, 2005. *Vibrio vulnificus* metalloprotease VvpE has no direct effect on the iron-assimilation from human holotransferrin. *FEMS Microbiol. Lett.*, 247(2): 221-229.
- Simpson, L.M. and J.D. Oliver, 1983. Siderophore production by *Vibrio vulnificus*. *Infect. Immun.*, 41(2): 644-649.
- Simpson, L.M. and J.D. Oliver, 1987. Ability of *Vibrio vulnificus* to obtain iron from transferrin and other iron-binding proteins. *Curr. Microbiol.*, 15: 155-157.
- Simpson, L.M., V.K. White, S.F. Zane and J.D. Oliver, 1987. Correlation between virulence and colony morphology in *Vibrio vulnificus*. *Infect. Immun.*, 55: 269-272.
- Sonia, A.S. and A.P. Lipton, 2012. Pathogenicity and antibiotic susceptibility of *Vibrio* species isolated from the captive-reared tropical marine ornamental blue damselfish, *Pomacentrus caeruleus* (Quoy and Gaimard, 1825). *Indian J. Geo-Marine Sci.*, 41: 348-354.
- Staley, C., E. Chase and V.J. Harwood, 2013. Detection and differentiation of *Vibrio vulnificus* and *V. sinoaloensis* in water and oysters of a Gulf of Mexico estuary. *Environ. Microbiol.*, 15(2): 623-633.
- Strom, M.S. and R.N. Paranjpye, 2000. Epidemiology and pathogenesis of *Vibrio vulnificus*. *Microbes Infect.*, 2(2): 177-188.
- Sun, H.Y., S.I. Han, M.H. Choi, S.J. Kim, C.M. Kim and S.H. Shin, 2006. *Vibrio vulnificus* metalloprotease VvpE has no direct effect on iron-uptake from human hemoglobin. *J. Microbiol.*, 44: 537-547.
- Tabor, C.W. and H. Tabor, 1985. Polyamines in microorganisms. *Microbiol. Rev.*, 49: 81-99.
- Tanaka, Y., B. Kimura, H. Takahashi, T. Watanabe, H. Obata, A. Kai, S. Morozumi and T. Fujii, 2008. Lysine decarboxylase of *Vibrio parahaemolyticus*: kinetics of transcription and role in acid resistance. *J. Appl. Microbiol.*, 104: 1283-1293.
- Tison, D.L., M. Nishibuchi, J.D. Greenwood and R.J. Seidler, 1982. *Vibrio vulnificus* biogroup 2: new biogroup pathogenic for eels. *Appl. Environ. Microbiol.*, 44: 640-646.
- Vanaporn, M., M. Wand, S.L. Michell, M.S. Tyson, P. Ireland, S. Goldman *et al.*, 2011. Superoxide dismutase C is required for intracellular survival and virulence of *Burkholderia pseudomallei*. *Microbiology*, 157: 2392-2400.
- World Health Organization, 2008. Foodborne Disease Outbreaks: Guidelines for Investigation and Control. World Health Organization, Geneva.
- Wright, A.C. and J.G. Jr. Morris, 1991. The extracellular cytolysin of *Vibrio vulnificus*: inactivation and relationship to virulence in mice. *Infect. Immun.*, 59: 192-197.

- Wright, A.C., L.M. Simpson and J.D. Oliver, 1981. Role of iron in the pathogenesis of *Vibrio vulnificus* infections. *Infect. Immun.*, 34: 503-507.
- Wright, A.C., L.M. Simpson, J.D. Oliver and J.G. Jr. Morris, 1990. Phenotypic evaluation of acapsular transposon mutants of *Vibrio vulnificus*. *Infect. Immun.*, 58: 1769-1773.
- Wright, A.C., J.L. Powell, J.B. Kaper and G.J. Jr. Morris, 2001. Identification of a group 1-like capsular polysaccharide operon for *Vibrio vulnificus*. *Infect. Immun.*, 69: 6893-6901.
- Yokochi, N., S. Tanaka, K. Matsumoto, H. Oishi, Y. Tashiro *et al.*, 2013. Distribution of virulence markers among *Vibrio vulnificus* isolates of clinical and environmental origin and regional characteristics in Japan. *PLoS One*, 8(1): e55219.
- Yoshida, S., M. Ogawa and Y. Mizuguchi, 1985. Relation of capsular materials and colony opacity to virulence of *Vibrio vulnificus*. *Infect. Immun.*, 47: 446-451.
- Yu, H.N., Y.R. Lee, K.H. Park, S.Y. Rah, E.M. Noh, E.K. Song, Han *et al.*, 2007. Membrane cholesterol is required for activity of *Vibrio vulnificus* cytolyisin. *Arch. Microbiol.*, 187: 467-473.
- Zaidenstein, R., C. Sadik, L. Lerner, L. Valinsky, J. Kopelowitz and R. Yishai, 2008. Clinical characteristics and molecular subtyping of *Vibrio vulnificus* illnesses, Israel. *Emerg. Infect. Dis.*, 14: 1875-1882.
- Zuppardo, A.B. and R.J. Siebeling, 1998. An epimerase gene essential for capsule synthesis in *Vibrio vulnificus*. *Infect. Immun.*, 66(6): 2601-2606.