**Vibrio vulnificus** infection: clinical manifestations, pathogenesis, and antimicrobial therapy

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There has been a dramatic increase in the number of reported cases of infection due to *Vibrio vulnificus* in Taiwan. Although the organism has been etiologically implicated in a variety of clinical syndromes, most cases of *V. vulnificus* infection are categorized as primary bacteremia, skin and soft tissue infection. The mortality was up to 50% in septic patients, most of them dying within 48 h of admission. In most of the cases involving *V. vulnificus* infection have underlying disease, particularly liver cirrhosis. The pathogenesis may attribute to several virulent factors, such as lipopolysaccharide, capsular lipopolysaccharide, cytolysin, metalloprotease and siderophore. Tetracycline was suggested as the drug of choice based on an animal study. Our previous *in vitro* data showed that cefotaxime and minocycline acted synergistically in inhibiting *V. vlunificus*. Furthermore, another *in vivo* animal study indicated that therapy using combined with cefotaxime and minocycline was distinctly more advantageous than therapy with the single antibiotic regimen for the treatment of severe experimental murine *V. vulnificus* infection. Recently, we also demonstrated that the newer fluoroquinolones, as single agents were as effective as the combination therapy both *in vitro* and *in vivo*.

**Key words:** Pathogenesis, primary bacteremia, necrotizing fasciitis, *Vibrio vulnificus* infection

*Vibrio vulnificus*, an opportunistic human pathogen, is a gram-negative halophilic marine bacterium that is endemic in the warm coastal waters. It is readily isolated from these waters, and in sediment, fish, and shellfish worldwide during summer months [1,2]. *V. vulnificus* infections in human hosts first illustrated in 1976 characteristically displayed three discernible syndromes: primary bacteremia, wound infection and gastrointestinal illness. The mortality rate was up to 55% in septic patients, most of them dying within 48 h with fulminate course after admission. The mortality rate was 25% among those who had wound infections [1]. Most patients admitted with *V. vulnificus* infection had an underlying disease, particularly liver cirrhosis, hemochromatosis, chronic renal failure or disease from immune compromised patients. Several virulent factors have been implicated to be the possible fulminate pathogenesis of infection, such as lipopolysaccharide, capsular lipopolysaccharide, cytolysin, metalloprotease and siderophore [3-7]. After the first reported case of *V. vulnificus* infection in Taiwan in 1985, there was an increased number of cases over the past decade, which may also be attributed to the high prevalence of hepatitis B and C infections, seagirt environment of Taiwan, and the habit of eating raw seafood [1,8]. These factors have drawn our considerable interest in investigating the clinical manifestations, pathogenesis, and antimicrobial therapy of *V. vulnificus* infection.

**History**

Infections of *V. vulnificus*, as the etiology suggests, can be traced back to the 5th century BC when Hippocrates [9] mentioned a man with ‘‘violent pain in his foot’’ who felt other symptoms like shivering, nausea, fever which were followed by a disturbance in consciousness. On the second day, the patient died after the whole foot became erythematous, swollen with small black blisters. Medical historians hypothesized this fatal infection due to *V. vulnificus*. In 1970, Roland proclaimed he had a 40-year-old patient with a generalized papular hemorrhagic rash, the manifestations of which included vomiting, diarrhea and fever that occurred after bathing and “clamming” in the New England coastal waters [10], the patient subsequently developed gangrene in the left leg and endotoxin shock. Though vesicle exudate grew *Vibrio parahemolyticus*, it was likely that the disease resulted from *V. vulnificus* [11], which was initially identified as a simple “halophilic lactose-positive marine *Vibrio*” which received its present name in 1979 [12]. Yuan *et al* [8] reported the first case of *V. vulnificus* infection at Kaohsiung in Taiwan in 1985. Thereafter, more cases were diagnosed, culminating in
3 cases in Taiwan in 1988, certified by Chuang et al [13]. Roughly, 200 sporadic cases have been encountered in Taiwan until 2001.

Microbiology
*V. vulnificus* is a gram-negative, pleomorphic, motile, curved, and rod-shaped bacteria. Most, if not all, *Vibrio* species are located in the marine or brackish water; therefore, *Vibrio*-linked illness tends to occur in the coastal areas in summer and fall when the waters are warmer and *Vibrio* counts are higher. The disease is always associated with seafood or seawater [1,2]. Classically, the strains are grouped into 2 biotypes [14] based on their physiological, biochemical (mainly indole test), serological and in host ranges differences. Biotype 1 is believed to compromise environmental and clinical isolates, and biotype 2 is thought to be pathogenic character for eels, but can nevertheless also be opportunistic pathogen for humans [15]. The genus *Vibrio* is oxidase positive and ferments glucose. *V. vulnificus* has the following characteristics: (1) is arginine dehydrogenase (ADH) negative and lysine decarboxylase (LDC) and ornithine decarboxylase (ODC) positive, can be differentiated from most *Aeromonas* spp. and *Plesiomonas* spp., which are ADH positive, and is sensitive to O129 (2,4-diamino-6, 7-diisopropylpteridine, vibriostatic compound), but the *Aeromonas* spp. are resistant to an antimicrobial susceptibility test [16]. (2) grows on *V. vulnificus* MacConkey agar, heart infusion medium, and thiosulfate citric bile salt sucrose (TCBS) agar [17], and the absence of sodium from media will inhibit the growth. (3) The colony of most *V. vulnificus* will be green on TCBS agar plate after overnight incubation, and can be distinguished from other *Vibrio* by the Voges-Proskauer test, sodium chloride tolerance, and lactose fermentation [18].

Epidemiology
The universal bacteria found, an halophilic organism, part of the natural bacterial flora, and is found in many varieties of shellfish (oysters and clams) favors the growth in the waters with a salinity of 0.7% to 1.6% and warm temperatures (higher than 20°C) [19]. As the water temperature increases during the summer, the number of shellfish harboring *V. vulnificus* similarly increases. According to various reports, up to 50% of oysters [20] and up to 11% of crabs [21] are cultured positive for the organism during the peak summer when the incidence of *V. vulnificus* wound infections and primary septicemia show a similar rise [1]. Infections have been documented worldwide [1,2,22-24]. As for Taiwan; 91% (72/77) strains were biotype 1 with the remaining five strains possibly also being biotype 1 too [25]. In a survey on the distribution of human-pathogenic *Vibrionaceae* of the seawater, there were 67 out of 1,167 *Vibrionaceae* isolate strains of *V. vulnificus* whose family, *Vibrionaceae*, exists autochthonously around the coastal waters [26].

Clinical Manifestations
*V. vulnificus* characteristically produces 3 perceivable syndromes [1,13,18]: (1) primary sepsis with high fever and chills without an apparent focus of infection when *V. vulnificus* is usually acquired through the gastrointestinal route. Primary septicemia is classically pertinent to consumption of raw oysters; (2) wound infection, resulting from cellulitis, caused by direct inoculation of the microorganism, which may result in tissue necrosis and secondary bacteremia (usually regarding exposure of chafed skin to salty water containing the microorganism or injuries applicable to the cultivation and/or preparation of seafood); and (3) gastrointestinal illness, characterized by vomiting, diarrhea, or abdominal pain. The microorganism is cultured from the stool but not from the blood, there is no evidence of wound infection in the patients. Other relatable types, such as pneumonia and endometritis have also been announced [27,28]. Thanks to the widespread obliterator vasculitis and vascular necrosis which are both the major features of skin lesion, penetration of antibiotics to the affected area may be severely hindered. Hence, early surgical debridement should be added as part of the whole treatment [29], this facilitates delivery of the medicine to the part where it is most needed. In addition, since most patients die within 48 h after hospitalization, an urgent decision is required within the first 24 h on whether the infected areas should be removed or not. Sometimes even amputation is considered, especially in cases appearing to be refractory to antimicrobial therapy.

Pathogenesis
*V. vulnificus* can secrete a variety of toxins that have been implicated in bacterial virulence and pathogenesis, including lipopolysaccharide (LPS), capsular polysaccharide (CPS), iron, metalloprotease, cytolysin, and other related toxins, may be considered pathogenesis of this infection, we discuss some important virulent factors here.

Iron
Iron plays a pivotal role in the pathogenesis of *V. vulnificus* infection. Patients with hemochromatosis and
other syndromes involving chronic iron overload are unusually susceptible to septicemia caused by *V. vulnificus* [30,31]. Raising the serum iron level by damaging the liver leads to a dramatic lowering of 50% of lethal dose (LD$_{50}$) and *V. vulnificus* is usually killed in blood from the healthy, but grows rapidly in blood from the patients with hemochromatosis [32] or when the iron saturation level of transferrin is raised to more than 50% [33]. This bacterium also can: (1) create vulnibactin, a siderophores, which can acquire iron from transferrin and lactoferrin and deliver iron to the bacterial cell by this high-affinity uptake system [7,34]. (2) Obtain iron from hemoglobin [35]. The avirulent isolates differed from those virulent bacteria, which can’t manufacture significant amounts of phenolate siderophore or utilize transferrin-bound iron [36]. (3) So, successfully utilizing iron to accelerate growth of bacteria can quickly release the lethal level of the tumor necrosis factor-alpha (TNF-α) and the lower activity of immune cells as a result of iron-overloading stimulated [33]. The structural gene of HupA and fur gene of *V. vulnificus* may be helpful for gaining iron from the host [37,38].

**Capsular Polysaccharide**

Capsular polysaccharide is vital to the virulence of *V. vulnificus* and has the complete carbohydrate structures from the bacilli declared [39]. The opaque (encapsulated) strains observed early, is a useful marker for potential virulence [40], whose determinant, CPS, was confirmed by the loss of virulence phenotype in acapsular transposon mutants [41]. The opaque capsule has been tested to protect the organism from complement-mediated lysis and phagocytosis by macrophages [42]. The antibodies from the CPS were protective against challenges with bacterial stains in both active and passive mouse models [43,44]. The amount of CPS expressed may vary with genetically determined phase variation or with environmental condition [45]. The CPS of *V. vulnificus* can stimulate the expression and secretion of proinflammatory cytokines in vivo, and in vitro and can activate the human peripheral blood mononuclear cells (PBMCs) [4]. The epimerase gene has been cloned, which was crucial for capsule expression in *V. vulnificus* [46].

**Metalloprotease**

*V. vulnificus* can secrete a 45-kDa zinc metalloprotease, an N-terminal 35-kDa polypeptide mediating proteolysis and a C-terminal 10-kDa polypeptide for efficient attachment to protein substrates and erythrocyte membranes, is an important virulence determinant [6], and can generate bradykinin through the activation of Hageman factor-plasma kallikrein-kinin system [47]. The bradykinin generation in infectious foci is critically involved in facilitation of intravascular dissemination of the bacteria and can prevent this invasion by a bradykinin antagonist [48]. When this protease was injected intradermally into the dorsal skin in vivo could cause hemorrhagic damage [49] through specific degradation of type IV collagen in the vascular basement membrane [50]. The C-terminal polypeptide may augment the hemorrhagic reaction of protease [51] and N-terminal 35-kD may mediate proteolytic effect. The gene (empV) encoding the extracellular metalloprotease of *V. vulnificus* CKM-1 has been cloned [52]. However, this virulent factor may not be an essential virulence in mice declared by Shao et al [53] and appears to be less important in the pathogenesis of *V. vulnificus* [54].

**Lipopolysaccharide**

Like gram-negative microorganisms, *V. vulnificus* can construct LPS, the LPS of this bacilli may cause mortality of intravenously infected rats [3], whose proinflammatory cytokine production [55] and stimulation of the activity of nitric oxide synthase were highly lethal, this could be rescued by inhibiting the nitric oxide synthase [56] or by decreasing proinflammatory cytokine levels [55,57]. The biotype 2 of *V. vulnificus* constitutes an LPS-based O serogroup which is phenotypically homogeneous and pathogenic for eels [58].

**Cytolysin**

*V. vulnificus* cytolsin, whose submicrogram amount is fatal to intravenously injected mice, is a water-soluble polypeptide with extreme toxicity. This virulence can induce apoptosis by elevation of cytosolic free Ca$^{2+}$ level [59] and the generation of superoxide anion, the release of cytochrome C from mitochondria, the activation of caspase-3, the degradation of poly (ADP-ribose) polymerase and DNA fragmentation [60,61]. The cytolsin has hemolytic effect that can lyse erythrocytes due to the formation of small pores on cell membrane by cholesterol mediated oligomerization of cytolsin [62]. Induction of nitric oxide synthase and nitrate oxide in vivo by cytolsin was promulgated by Kang et al [5]. It could increase vascular permeability, neutrophil sequestration in the lungs [63]. This mechanism for pulmonary damage may be attributed to hyperadhesiveness of pulmonary endothelial cells for neutrophils through mobilization of endothelial P-selectin to the cell surface [64]. It also could cause
mouse skin damage after intradermal injection [65]. The gene (vllY) encoding a novel hemolysin of *V. vulnificus* CKM-1 has been cloned and sequenced [66].

*V. vulnificus* can induce a septic shock-like syndrome, and that in *vivo* serum TNF-α parallels the degree of bacteremia. The increased mortality to *V. vulnificus* infection for cirrhotic mice depends on that factor [67]. Those virulent factors have been noted in relation to the production of inflammatory mediators in humans. The overproduction of proinflammatory cytokines, such as TNF-α, interleukin (IL)-1β, and IL-6, which were possibly connected with the clinical manifestations and mortality of septic shock, was noted in the sera of septicemic patients who could be rescued after decreasing the proinflammatory cytokine levels [55,57].

**Antimicrobial therapy**

**General drug of choice**

Because of the sporadic occurrence of *V. vulnificus* infection, it is very difficult to conduct a randomized, comparative study of different antimicrobials treatment. In *vivo* antibacterial test showed that the minimum inhibitory concentrations to inhibit 90% isolates (MIC$_{90}$s) for cefotaxime and ceftriaxone were found to be superior, and also, our published data showed fluoroquinolones were very active against *V. vulnificus*, MIC$_{90}$s for levofloxacin and ciprofloxacin were found to be both below 0.03 µg/mL (Table 1). On the other hand, tetracycline has been thought to be the drug of choice based on an *in vivo* animal study [68]. Several authors recommended the addition of aminoglycoside, whereas some authors advocated cephalosporins based on their clinical experience [69], and we had demonstrated that cefotaxime and minocycline synergistically inhibited *V. vulnificus* in *vitro* [70]. The *in vivo* study also ascertained that combination therapy with cefotaxime and minocycline for severe cases was distinctly advantageous than the single antibiotic regimen treatment with *V. vulnificus* infections [71], while Sanford et al [72] recommend doxycycline and ceftazidime based on the previous mentioned study. Recently, we also concluded the efficacies of the newer fluoroquinolones, such as ciprofloxacin, moxifloxacin, levofloxacin, gatifloxacin, sparflaxacin, and lomefloxacin, as single agents were as effective as the combination, both *in vitro* and *in vivo* [73].

**Bacteremia**

*V. vulnificus* is estimated to account for 95% of all seafood-related deaths in the United States [74]. In the early phase, sepsis syndrome caused by *V. vulnificus*, is clinically indistinguishable from that caused by other gram-negative bacilli. Secondary hemorrhagic bulla, necrotizing fasciitis, and previous exposure to seawater, or prior consumption of raw or semi-cooked seafood may be a clue for diagnosis. Tetracycline, whose alternatives are cefotaxime and ciprofloxacin, is the first line agent. In severe cases, it is recommended to combine cefotaxime (2 g i.v. every 6 h) with minocycline (100 mg i.v. every 12 h, with 200 mg loading). Instituting antibiotic therapy before development of hypotension has been shown to decrease mortality.

**Gastrointestinal infection**

*V. vulnificus* gastroenteritis in immunocompetent persons is usually acute but self-limited, and the case-fatality rates were 1% for gastroenteritis [2]; therefore, antimicrobial therapy was not routinely recommended. Patients with severe symptoms and signs, or with a high risk of systemic infections, especially those who were immunocompromised, did not seemingly necessitate effective antibiotics. However, this was not supported by well-designed clinical studies due to its self-limited manifestation, possibly underreported *V. vulnificus* affiliated infectious diarrhea, and absence of the well-designed controlled studies.

**Soft tissue infections**

Skin and soft tissue infections proofed which are from infected wound, cellulitis, and soft tissue abscess, to
necrotizing fasciitis, tissue necrosis, gangrene change and myositis are common in lower-extremity lesions [75], and may be complicated with bacteremia and secondary bullous formation. In a review of 29 episodes of *V. vulnificus* infection in Taiwan, 23 cases had skin manifestations. Hemorrhagic bullae was the most frequently skin manifestation (15 of 23) (Fig. 1), the second most common was necrotizing fasciitis (7 of 23) (Table 2). Usually, the patient has underlying chronic liver dysfunction, and predominant skin lesions with edema and subcutaneous bleeding, such as ecchymosis and purpura (Fig. 2), while superficial necrosis was not recognized, and was different from necrotizing fasciitis caused by streptococcal infection [76]. The *V. vulnificus* involved soft tissue infections can be fulminate and subsequent septic shock initiated, and are deadly within two days if not treated early enough. The fatality of sepsis has been identifiably increasing due to delayed antibiotic treatment. Launching antibiotic therapy before development of hypotension has been demonstrated to decrease mortality. In severe cases, especially with underlying diseases, combined cefotaxime (2 g i.v. every 6 h)-minocycline (i.v. or p.o. 100 mg every 12 h, with 200 mg loading) is recommended. Aggressive supportive therapy is also very important, including incision and drainage, and debridement of nonviable or necrotic tissue. Earlier operative exploration and debridement can shorten hospitalization [77].

**Table 2. Skin manifestations of *V. vulnificus* infection in Taiwan**

<table>
<thead>
<tr>
<th>Skin manifestation</th>
<th>No. of patients</th>
<th>n = 23 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemorrhagic bullae</td>
<td>15 (65.2)</td>
<td></td>
</tr>
<tr>
<td>Necrotizing fasciitis</td>
<td>7 (30.4)</td>
<td></td>
</tr>
<tr>
<td>Cellulitis</td>
<td>5 (17.2)</td>
<td></td>
</tr>
<tr>
<td>Pyomyositis</td>
<td>1 (4.3)</td>
<td></td>
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<tr>
<td>Lymphangitis</td>
<td>1 (4.3)</td>
<td></td>
</tr>
<tr>
<td>Ecchymosis</td>
<td>1 (4.3)</td>
<td></td>
</tr>
<tr>
<td>Toxic epidermal necrolysis</td>
<td>1 (4.3)</td>
<td></td>
</tr>
</tbody>
</table>

Note: Some patients had more than 1 skin lesion, *Data from reference 1.

**Special Infections**

**Meningitis**

Meningitis caused by *V. vulnificus* is extremely rare. Katz [78] depicted a boy who had underlying...
thalamus, got *V. vulnificus* meningitis 3 days later for having raw oysters, and had a successful 3 weeks chloramphenicol treatment. The authors recommended a high dose cefotaxime (2 g i.v. every 4 h) with minocycline (100 mg i.v. every 12 h, with 200 mg loading).

**Ocular infections**

*V. vulnificus* ocular infections, for which tetracycline ointment works very well as does ciprofloxacin therapy [79], are very rare and usually relevant to exposure to seafood or seawater. Nonetheless, suppurative *V. vulnificus* keratitis was successfully treated with combined cefazolin and gentamicin [69].

**Endocarditis**

Truwi et al. [80] expressed a case of *V. vulnificus* bacteremia complicated with endocarditis. The patient, who had underlying liver cirrhosis and took ampicillin plus aminoglycoside, was effectively treated within 42 days.

**Prevention**

Wound infections may be less preventable as they may occur in healthy individuals. Otherwise, seafood-handling requires glove-wearing to decrease the risk of infection, slight modifications in diet and activities are also keys for prevention. Those who are with underlying disease: Firstly, should avoid raw oysters, clams, shrimp, and fish owing to the high incidence of *V. vulnificus* in these foods, eat well-cooked seafood, and be aware of the risks of eating undercooked seafood. Secondly, should avert exposure of open wounds to seawater, to ocean water or to ocean products, especially, in the summer or in warm weather. Thirdly, they should notify professionals for caring all underlying medical conditions and medications [81].

**Conclusion**

The clinical manifestations of *V. vulnificus* infection cases showed a fulminate course with a high mortality rate. The incubation periods of this infection were very short, and the most striking clinical manifestations were skin lesions. In a review of 95 cases of primary bacteremia and 72 cases of wound infection caused by *V. vulnificus* were included in this summary (Table 3). Most of them were related to seafood consumption, and for the patients with primary bacteremia, 94% were associated with underlying disease, especially chronic liver disease. The pathogenesis of this infection may be attributed to several virulence factors. We recommended combined cefotaxime and minocycline for the treatment of severe *V. vulnificus* infection. Aggressive supportive therapy with incision and debridement of nonviable or necrotic tissue is also very important.

**References**

12. Farmer JJ 3rd. *Vibrio* (“Beneckea”) *vulnificus*, the bacterium...


