J Microbiol Immunol Infect
2003;36:81-88

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

Shyh-Ren Chiang[1], Yin-Ching Chuang[2]

Departments of [1]Internal Medicine and [2]Medical Research, Chi Mei Medical Center, Tainan, Taiwan, ROC

Received: March 11, 2003 Accepted: March 31, 2003

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.

Corresponding author: Dr. Yin-Ching Chuang, No. 901, Chunghwa Road, Yungkang City, Tainan Hsien, Taiwan 710, ROC. E-mail: chuangkenneth@hotmail.com

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^{o}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 gastro-intestinal 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 obliterative 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 proteolyic 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 oxidase synthase were highly lethal, this could be rescued by inhibiting the nitric oxidase 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. vulnific*us cytolysin, 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 cytolysin has hemolytic effect that can lyse erythocytes due to the formation of small pores on cell membrane by cholesterol mediated oligomerization of cytolysin [62]. Induction of nitric oxide synthase and nitrate oxide *in vivo* by cytolysin 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 vitro* 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 a*l [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, sparfloxacin, and lomefloxacin, as single agents were as effective as the combination, both *in vitro* and *in vivo* [73].

Table 1. Susceptibilities of clinical isolates of *V. vulnificus* to 16 antimicrobial agents

Antimicrobial agent	MIC (μg/mL)		
	50%	90%	Range
Ampicillin (n = 42)[a]	1.0	1.0	0.25-2.0
Cefotaxime (n = 42)[a]	≤0.03	0.06	≤0.03-1.0
Ceftriaxone (n = 42)[a]	≤0.03	≤0.03	0.03-0.12
Ceftazidime (n = 42)[a]	1.0	2.0	1.0-32.0
Imipenem (n = 42)[a]	0.12	0.12	0.06-0.12
Gentamicin (n = 42)[a]	2.0	4.0	1.0-8.0
Minocycline (n = 42)[a]	0.06	0.25	0.06-0.25
Moxalactam (n = 42)[a]	0.25	0.5	0.25-32.0
Cefoperazone (n = 42)[a]	0.06	0.12	≤0.03-2.0
Ofloxacin (n = 42)[a]	0.12	0.12	0.06-8.0
Ciprofloxacin (n = 46)[b]	0.03	0.03	0.015-0.03
Moxifloxacin (n = 46)[b]	0.06	0.06	0.03-0.06
Gatifloxacin (n = 46)[b]	0.03	0.06	0.015-0.06
Sparfloxacin (n = 46)[b]	0.06	0.06	0.015-0.06
Levofloxacin (n = 46)[b]	0.03	0.03	0.015-0.03
Lomefloxacin (n = 46)[b]	0.12	0.12	0.06-0.12

MIC = minimal inhibitory concentration
[a]Data from reference 70.
[b]Data from reference 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 one 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].

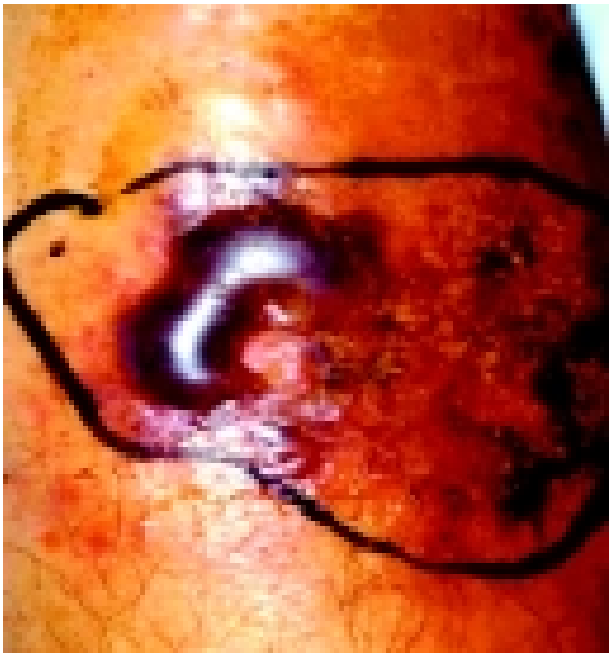

Fig. 1. The most frequently skin manifestation of *V. vulnificus* infection is hemorrhagic bullae.

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

Skin manifestation[a]	No. of patients n = 23 (%)
Hemorrhagic bullae	15 (65.2)
Necrotizing fasciitis	7 (30.4)
Cellulitis	5 (17.2)
Pyomyositis	1 (4.3)
Lymphangitis	1 (4.3)
Ecchymosis	1 (4.3)
Toxic epidermal necrolysis	1 (4.3)

Note: Some patients had more than 1 skin lesion, [a]Data from reference 1.

Special Infections

Meningitis

Meningitis caused by *V. vulnificus* is extremely rare. Katz [78] depicted a boy who had underlying

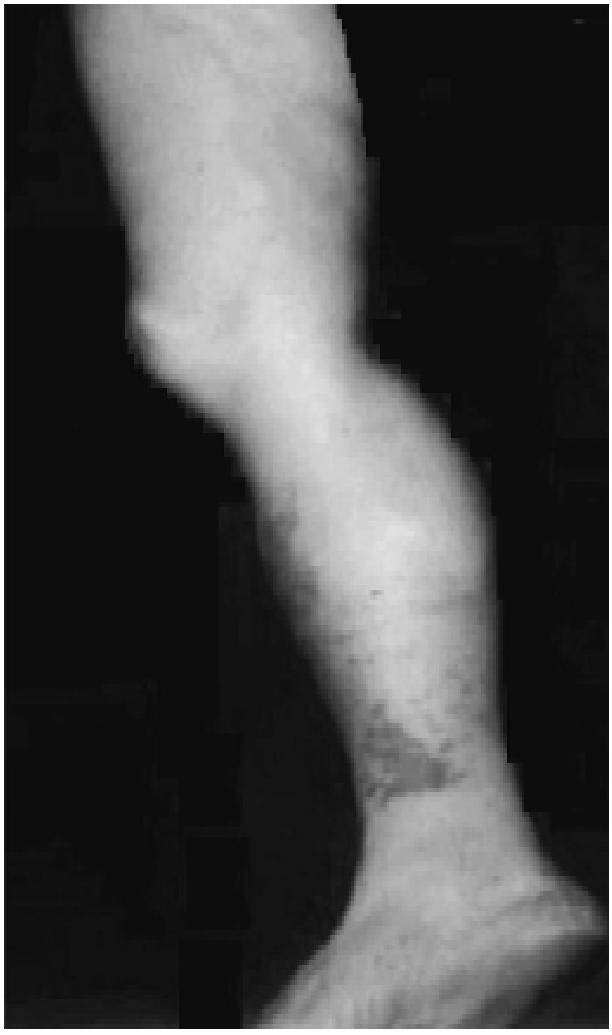

Fig. 2. A patient manifested as cellulitis after being bitten by a crab, the aspirated material from local wound grew *V. vulnificus*.

Table 3. Summary of *V. vulnificus* infections reported in the literature

	No. of cases (%)				
	Underlying disease	Seafood exposure	Skin manifestation	Surgical treatment	Mortality rate
Sepsis	89/94 (94)	41/52 (78)	69/106 (65)	13/35 (37)	51/94 (54)
Wound infection	44/80 (55)	71/78 (91)	30/69 (44)[a]	19/37 (51)	22/87 (25)

Note: Data from reference 1.
[a]Percentage of patients with secondary bacteremia.

thalassemia, 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

Truwit *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

1. Chuang YC, Yuan CY, Liu CY, Lan CK, Huang AH. *Vibrio vulnificus* infection in Taiwan: report of 28 cases and review of clinical manifestations and treatment. Clin Infect Dis1992; 15:271-6.
2. Hlady WG, Klontz KC. The epidemiology of *Vibrio* infections in Florida, 1981-1993. J Infect Dis 1996;173:1176-83.
3. McPherson VL, Watts JA, Simpson LM, Oliver JD. Physiological effects of the lipopolysaccharide of *Vibrio vulnificus* on mice and rats. Microbios 1991;67:141-9.
4. Powell JL, Wright AC, Wasserman SS, Hone DM, Morris JG Jr. Release of tumor necrosis factor alpha in response to *Vibrio vulnificus* capsular polysaccharide in *in vivo* and *in vitro* models. Infect Immun 1997;65:3713-8.
5. Kang MK, Jhee EC, Koo BS, Yang JY, Park BH, Kim JS, Rho HW, Kim HR, Park JW. Induction of nitric oxide synthase expression by *Vibrio vulnificus* cytolysin. Biochem Biophys Res Commun 2002;290:1090-5.
6. Miyoshi S, Wakae H, Tomochika K, Shinoda S. Functional domains of a zinc metalloprotease from *Vibrio vulnificus*. J Bacteriol 1997;179:7606-9.
7. Simpson LM, Oliver JD. Siderophore production by *Vibrio vulnificus*. Infect Immun 1983;41:644-9.
8. Yuan CY, Yuan CC, Wei DC, Lee AM. Septicemia and gangrenous change of the legs caused by marine *vibrio*. *Vibrio vulnificus*-report of a case. J Formos Med Assoc 1987;86:448-51.
9. Baethge BA, West BC. *Vibrio vulnificus*: did Hippocrates describe a fatal case? Rev Infect Dis 1988;10:614-5.
10. Roland FP. Leg gangrene and endotoxin shock due to *Vibrio parahaemolyticus*-an infection acquired in New England coastal waters. N Engl J Med 1970;282:1306.
11. Blake PA, Merson MH, Weaver RE, Hollis DG, Heublein PC. Disease caused by a marine *vibrio*: clinical characteristics and epidemiology. N Engl J Med 1979;300:1-5.
12. Farmer JJ 3rd. *Vibrio* ("Beneckea") *vulnificus*, the bacterium

associated with sepsis, septicemia, and the sea. Lancet 1979;2 (8148):903.
13. Chuang YC, Young CD, Chen CW. *Vibrio vulnificus* infection. Scand J Infect Dis 1989;21:721-6.
14. Amaro C, Hor LI, Marco-Noales E, Bosque T, Fouz B, Alcaide E. Isolation of *Vibrio vulnificus* serovar E from aquatic habitats in Taiwan. Appl Environ Microbiol 1999;65:1352-5.
15. Amaro C, Biosca EG. *Vibrio vulnificus* biotype 2, pathogenic for eels, is also an opportunistic pathogen for humans. Appl Environ Microbiol 1996;62:1454-7.
16. Khaitovich AB, Ved'mina EA. *Vibrio* and *Aeromonad* sensitivity to the vibriostatic O 129. Antibiot Med Biotekhnol 1987;32:446-9.
17. Desmond EP, Janda JM, Adams FI, Bottone EJ. Comparative studies and laboratory diagnosis of *Vibrio vulnificus*, an invasive *Vibrio* sp. J Clin Microbiol 1984;19:122-5.
18. Chuang YC, Huang KY. *Vibrio vulnificus.* In: Yu VL, Weber R, Raoult D, eds. Antimicrobial Therapy and Vaccines. Vol. I: Microbes. New York:Apple Trees Productions, LLC; 2002:773-7.
19. Koenig KL, Mueller J, Rose T. *Vibrio vulnificus*: hazard on half shell. West J Med 1991;155:400-3.
20. Tamplin M, Rodrick GE, Blake NJ, Cuba T. Isolation and characterization of *Vibrio vulnificus* from two Florida estuaries. Appl Environ Microbiol 1982;44:1466-70.
21. Davis JW, Sizemore RK. Incidence of *Vibrio* species associated with blue crabs (*Callinectes sapidus*) collected from Galeveston Bay, Texas. Appl Environ Microbiol 1982;43:1092-7.
22. Dalsgaard A, Frimodt-Moller N, Bruun B, Hoi L, Larsen JL. Clinical manifestations and molecular epidemiology of *Vibrio vulnificus* infections in Denmark. Eur J Clin Microbiol Infect Dis 1996;15:227-32.
23. Melhus A, Holmahl T, Tjernberg I. First documented case of bacteremia with *Vibrio vulnificus* in Sweden. Scand J Infect Dis 1995;27:81-2.
24. Park SD, Shon HS, Joh NJ. *Vibrio vulnificus* septicemia in Korea: clinical and epidemiologic findings in seventy patients. J Am Acad Dermatol 1991;24:397-403.
25. Hor LI, Gao CT, Wan L. Isolation and characterization of *Vibrio vulnificus* inhabiting the marine environment of the southwestern area of Taiwan. J Biomed Sci 1995;2:384-9.
26. Wu HS, Liu DP, Hwang CH, Chen MJ, Hwang JL, Liu Y, Shankuan LC, Lin CS, Wu TN. Survey on the distribution of *Vibrionaceae* at the seaport areas in Taiwan, 1991-1994. J Microbiol Immunol Infect 1996;29:197-209.
27. Kelly MT, Avery DM. Lactose-positive *Vibrio* in seawater: a cause of pneumonia and septicemia in a drowning victim. J Clin Microbiol 1980;11:278-80.
28. Tison DL, Kelly MT. *Vibrio vulnificus* endometritis. J Clin Microbiol 1984;20:185-86.
29. Zielinski CJ, Bora FW Jr. *Vibrio* hand infections: a case report and review of the literature. J Hand Surg (Am) 1984;9:754-7.
30. Brennaman B, Soucy D, Howard RJ. Effect of iron and liver injury on the pathogenesis of *Vibrio vulnificus*. J Surg Res 1987; 43:527-31.
31. Hor LI, Chang TT, Wang ST. 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 1999;179:275-8.
32. Bullen JJ, Spalding PB, Ward CG, Gutteridge JM. Hemochromatosis, iron and septicemia caused by *Vibrio vulnificus*. Arch Intern Med 1991;151:1606-9.
33. Hor LI, Chang YK, Chang CC, Lei HY, Ou JT. Mechanism of high susceptibility of iron-overloaded mouse to *Vibrio vulnificus* infection. Microbiol Immunol 2000;44:871-8.
34. Okujo N, Akiyama T, Miyoshi S, Shinoda S, Yamamoto S. Involvement of vulnibactin and exocellular protease in utilization of transferrin- and lactoferrin-bound iron by *Vibrio vulnificus*. Microbiol Immunol 1996;40:595-8.
35. Zakaria-Meehan Z, Massad G, Simpson LM, Travis JC, Oliver JD. Ability of *Vibrio vulnificus* to obtain iron from hemoglobin-haptoglobin complexes. Infect Immun 1988;56:275-7.
36. Stelma GN Jr, Reyes AL, Peeler JT, Johnson CH, Spaulding PL. Virulence characteristics of clinical and environmental isolates of *Vibrio vulnificus*. Appl Environ Microbiol 1992;58: 2776-82.
37. Litwin CM. Quackenbush J. Characterization of a *Vibrio vulnificus* LysR homologue, HupR, which regulates expression of the haem uptake outer membrane protein, HupA. Microb Pathog 2001;31:295-307.
38. Litwin CM, Calderwood SB. Cloning and genetic analysis of the *Vibrio vulnificus* fur gene and construction of a fur mutant by *in vivo* marker exchange. J Bacteriol 1993;175:706-15.
39. Reddy GP, Hayat U, Xu Q, Reddy KV, Wang Y, Chiu KW, Morris JG Jr, Bush CA. Structure determination of the capsular polysaccharide from *Vibrio vulnificus* strain 6353. Eur J Biochem 1998;255:279-88.
40. Moreno ML, Landgraf M. Virulence factors and pathogenicity of *Vibrio vulnificus* strains isolated from seafood. J Appl Microbiol 1998;84:747-51.
41. Wright AC, Simpson LM, Oliver JD, Morris JG Jr. Phenotypic evaluation of acapsular transposon mutants of *Vibrio vulnificus*. Infect Immun 1990;58:1769-73.
42. Kreger A, DeChatelet L, Shirley P. Interaction of *Vibrio vulnificus* with human polymorphonuclear leukocytes: association of virulence with resistance to phagocytosis. J Infect Dis 1981;144:244-8.
43. Devi SJ, Hayat U, Frasch CE, Kreger AS, Morris JG Jr. Capsular polysaccharide-protein conjugate vaccines of carbotype 1 *Vibrio vulnificus:* construction, immunogenicity, and protective efficacy in a murine model. Infect Immun 1995;63:2906-11.
44. Simonson JG, Siebeling RJ. Immunogenicity of *Vibrio vulnificus* capsular polysaccharides and polysaccharide-protein conjugates. Infect Immun 1993;61:2053-8.
45. Bush CA, Patel P, Gunawardena S, Powell J, Joseph A, Johnson JA. Morris JG. Classification of *Vibrio vulnificus* strains by the carbohydrate composition of their capsular polysaccharides. Anal Biochem 1997;250:186-95.
46. Zuppardo AB, Siebeling RJ. An epimerase gene essential for capsule synthesis in *Vibrio vulnificus*. Infect Immun 1998;66: 2601-6.
47. Miyoshi S, Shinoda S. Activation mechanism of human Hageman factor-plasma kallikrein-kinin system by *Vibrio vulnificus* metalloprotease. FEBS Lett 1992;308:315-9.
48. Maruo K, Akaike T, Ono T, Maeda H. Involvement of bradykinin generation in intravascular dissemination of *Vibrio vulnificus* and prevention of invasion by a bradykinin antagonist. Infect Immun 1998;66:866-9.
49. Chuang YC, Sheu HM, Ko WC, Chang TM, Chang MC, Huang KY. Mouse skin damage caused by a recombinant extracellular metalloprotease from *Vibrio vulnificus* and by *V. vulnificus* infection. J Formos Med Assoc 1997;96:677-84.
50. Miyoshi S, Nakazawa H, Kawata K, Tomochika KI, Tobe K, Shinoda S. Characterization of the hemorrhagic reaction caused

by *Vibrio vulnificus* metalloprotease, a member of the thermolysin family. Infect Immun 1998;66:4851-5.
51. Miyoshi S, Kawata K, Tomochika K, Shinoda S, Yamamoto S. The C-terminal domain promotes the hemorrhagic damage caused by *Vibrio vulnificus* metalloprotease. Toxicon 2001;39: 1883-6.
52. Chuang YC, Chang TM, Chang MC. Cloning and characterization of the gene (*empV*) encoding extracellular metalloprotease from *Vibrio vulnificus*. Gene 1997;189:163-8.
53. Shao CP, Hor LI. Metalloprotease is not essential for *Vibrio vulnificus* virulence in mice. Infect Immun 2000;68:3569-73.
54. Jeong KC, Jeong HS, Rhee JH, Lee SE, Chung SS, Starks AM, Escudero GM, Gulig PA, Choi SH. Construction and phenotypic evaluation of a *Vibrio vulnificus vvpE* mutant for elastolytic protease. Infect Immun 2000;68:5096-106.
55. Chae MR, Park BH, Kim JS, Rho HW, Park JW, Kim HR. Protective effect of C-reactive protein against the lethality induced by *Vibrio vulnificus* lipopolysaccharide. Microbiol Immunol 2000;44:335-40.
56. Elmore SP, Watts JA, Simpson LM, Oliver JD. Reversal of hypotension induced by *Vibrio vulnificus* lipopolysaccharide in the rat by inhibition of nitric oxide synthase. Microb Pathog 1992;13:391-7.
57. Shin SH, Shin DH, Ryu PY, Chung SS, Rhee JH. Proinflammatory cytokine profile in *Vibrio vulnificus* septicemic patients' sera. FEMS Immunol Med Microbiol 2002; 33:133-8.
58. Biosca EG, Oliver JD, Amaro C. Phenotypic characterization of *Vibrio vulnificus* biotype 2, a lipopolysaccharide-based homogeneous O serogroup within *Vibrio vulnificus*. Appl Environ Microbiol 1996;62:918-27.
59. Rho HW, Choi MJ, Lee JN, Park JW, Kim JS, Park BH, Sohn HS, Kim HR. Cytotoxic mechanism of *Vibrio vulnificus* cytolysin in CPAE cells. Life Sci 2002;70:1923-34.
60. Park JW, Jahng TA, Rho HW, Park BH, Kim NH, Kim HR. Inhibitory mechanism of Ca^{2+} on the hemolysis caused by *Vibrio vulnificus* cytolysin. Biochim Biophys Acta 1994;1194: 166-70.
61. Kwon KB, Yang JY, Ryu DG, Rho HW, Kim JS, Park JW, Kim HR, Park BH. *Vibrio vulnificus* cytolysin induces superoxide anion-initiated apoptotic signaling pathway in human ECV304 cells. J Biol Chem 2001;276:47518-23.
62. Kim HR, Rho HW, Jeong MH, Park JW, Kim JS, Park BH, Kim UH, Park SD. Hemolytic mechanism of cytolysin produced from *V. vulnificus*. Life Sci 1993;53:571-7.
63. Park JW, Ma SN, Song ES, Song CH, Chae MR, Park BH, Rho RW, Park SD, Kim HR. Pulmonary damage by *Vibrio vulnificus* cytolysin. Infect Immun 1996;64:2873-6.
64. Kim BS, Kim JS. *Vibrio vulnificus* cytolysin induces hyperadhesiveness of pulmonary endothelial cells for neutrophils through endothelial P-selectin: a mechanism for pulmonary damage by *Vibrio vulnificus* cytolysin. Exp Mol Med 2002;34:308-12.
65. Gray LD, Kreger AS. Mouse skin damage caused by cytolysin from *Vibrio vulnificus* and by *V. vulnificus* infection. J Infect Dis 1987;155:236-41.
66. Chang TM, Chuang YC, Su JH, Chang MC. Cloning and sequence analysis of a novel hemolysin gene (*vllY*) from *Vibrio vulnificus*. Appl Environ Microbiol 1997;63:3851-7.
67. Espat NJ, Auffenberg T, Abouhamze A, Baumhofer J, Moldawer LL, Howard RJ.A role for tumor necrosis factor-alpha in the increased mortality associated with *Vibrio vulnificus* infection in the presence of hepatic dysfunction. Ann Surg 1996;223: 428-33.
68. Bowdre JH, Hull JH. Cocchetto DM. Antibiotic efficacy against *Vibrio vulnificus* in the mouse: superiority of tetracycline. J Pharmacol Exp Ther 1983;225:595-8.
69. Penland RL, Boniuk M, Wilhelmus KR. *Vibrio* ocular infections on the U.S Gulf Coast. Cornea 2000;19:26-9.
70. Chuang YC, Liu JW, Ko WC, Lin KY, Wu JJ, Huang KY. *In vitro* synergism between cefotaxime and minocycline against *Vibrio vulnificus*. Antimicrob Agents Chemother 1997;41:2214-7.
71. Chuang YC, Ko WC, Wang ST, Liu JW, Kuo CF, Wu JJ, Huang KY. Minocycline and cefotaxime in the treatment of experimental murine *Vibrio vulnificus* infection. Antimicrob Agents Chemother 1998;42:1319-22.
72. Sanford JP, Gilbert DN, Moellering RC Jr, Sande MA. Selection of initial empirical antibacterial therapy on clinical grounds. In: Sanford JP, Gilbert DN, Moellering RC Jr, Sande MA,eds. The Sanford Guide to Antimicrobial Therapy. 27th ed. Dallas: Antimicrobial Therapy; 1997:2-44.
73. Tang HJ, Chang MC, Ko WC, Huang KY, Lee CL, Chuang YC. *In vitro* and *in vivo* activities of newer fluoroquinolones against *Vibrio vulnificus*. Antimicrob Agents Chemother 2002; 46:3580-4.
74. Oliver JD, Bockian R. *In vivo* resuscitation, and virulence towards mice, of viable but nonculturable cells of *Vibrio vulnificus*. Appl Environ Microbiol 1995;61:2620-3
75. Laughlin TJ, Lavery LA. Lower extremity manifestations of *Vibrio vulnificus* infection. J Foot Ankle Surg 1995;34:354-7.
76. Fujisawa N, Yamada H, Kohda H, Tadano J, Hayashi S. Necrotizing fasciitis caused by *Vibrio vulnificus* differs from that caused by streptococcal infection. J Infect 1998;36:313-6.
77. Halow KD, Harner RC, Fontenelle LJ. Primary skin infections secondary to *Vibrio vulnificus*: the role of operative intervention. J Am Coll Surg 1996;183:329-34.
78. Katz BZ. *Vibrio vulnificus* meningitis in a boy with thalassemia after eating raw oysters. Pediatrics 1988;82:784-6.
79. Massey EL, Weston BC. *Vibrio vulnificus* corneal ulcer: rapid resolution of a virulent pathogen. Cornea 2000;19:108-9.
80. Truwit JD, Badesch DB, Savage AM, Shelton M. *Vibrio vulnificus* bacteremia with endocarditis. South Med J 1987;80: 1457-9.
81. Martin G, Wright AM, Banakarim K. A case of fatal food-borne septicemia: can family physicians provide prevention? J Am Board Fam Pract 2000;13:197-200.