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Review

A comprehensive review of *Vibrio vulnificus*: an important cause of severe sepsis and skin and soft-tissue infection

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SUMMARY

Vibrio vulnificus is a halophilic Gram-negative bacillus found worldwide in warm coastal waters. The pathogen has the ability to cause primary sepsis in certain high-risk populations, including patients with chronic liver disease, immunodeficiency, iron storage disorders, end-stage renal disease, and diabetes mellitus. Most reported cases of primary sepsis in the USA are associated with the ingestion of raw or undercooked oysters harvested from the Gulf Coast. The mortality rate for patients with severe sepsis is high, exceeding 50% in most reported series. Other clinical presentations include wound infection and gastroenteritis. Mild to moderate wound infection and gastroenteritis may occur in patients without obvious risk factors. Severe wound infection is often characterized by necrotizing skin and soft-tissue infection, including fasciitis and gangrene. *V. vulnificus* possesses several virulence factors, including the ability to evade destruction by stomach acid, capsular polysaccharide, lipopolysaccharide, cytotoxins, pili, and flagellum. The preferred antimicrobial therapy is doxycycline in combination with ceftazidime and surgery for necrotizing soft-tissue infection.

1. Introduction

Vibrio vulnificus is a halophilic, motile, Gram-negative bacillus capable of causing severe to life-threatening infection in susceptible individuals. The spectrum of illness can vary from gastroenteritis to 'primary sepsis' and necrotizing fasciitis. The case-fatality rate has been reported to exceed 50% in primary sepsis. Infection may result from consuming or handling contaminated seafood (usually shellfish such as oysters) or from exposing open wounds or broken skin to contaminated salt or brackish water.[1–5]

V. vulnificus is usually found worldwide in coastal or estuarine environments with water temperatures from 9 to 31 °C. The organisms preferred habitat, however, is considerably more selective and has been reported to be water temperatures in excess of 18 °C and salinities between 15 to 25 parts per thousand (ppt).[6–8] However, salinities at or greater than 30 ppt will substantially reduce the burden of *V. vulnificus* regardless of the water temperature.[8] As a result, most cases of infection can be traced to tropical or subtropical sources. Even so, small outbreaks related to local seawater or seafood exposure have occurred in temperate climates as far north as Denmark during the summer months.[6] A recent report from Israel described several cases of soft-tissue infection resulting from the handling and processing of fish (tilapia or carp) raised in freshwater aquaculture ponds.[9]

Three biotypes of *V. vulnificus* are known to cause severe human disease. Biotype 1 is found worldwide in salt or brackish water. Biotype 2 occupies a more specific niche and is found in saltwater used for eel (genus *Anguilla*) farming in the Far East and Western Europe.[6,7] Biotype 3 is the strain associated with freshwater fish farming in Israel. Genomic analyses of biotype 3 indicate it is a hybrid of biotypes 1 and 2.[9] Biotype 1 is the most common strain and is responsible for the entire spectrum of illness, including the primary sepsis associated with the often-quoted fatality rate in excess of 50%. Biotype 2 is usually a serious pathogen of eels, but on rare occasions may cause wound infections in humans.[3,6,7] Although biotype 3 can cause severe soft-tissue infections requiring amputation, the mortality rate appears to be less than 8%.[9]

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Most reported cases of infection with *V. vulnificus* have occurred in patients with chronic liver disease, immunodeficiencies, or hematological disorders characterized by elevated iron levels.[1–7,9–12] Contamination with *V. vulnificus* can be difficult to detect because the organism has no effect on the appearance, taste, or odor of seafood, particularly raw oysters.[13] Because *V. vulnificus* is ubiquitous in warm marine environments, water quality also has little impact on the risk of infection.[12]

In this report, we describe a severe case of soft-tissue infection and review much of the current literature concerning *V. vulnificus*. We also provide recommendations for treatment and prevention in populations at high risk of infection.

2. Illustrative case

A 53-year-old male was admitted to the emergency department (ED) with a 2-day history of pain in both arms, which he characterized as 10/10. The patient also complained of subjective chills and fever. Vital signs on admission included a temperature of 37.2 °C, heart rate of 73/min, respiratory rate of 20/min, blood pressure of 75/51 mmHg, and an oxygen saturation of 100% on room air. The patient was noted to be grimacing but alert, and oriented times 3 and cooperative. The left arm was erythematous with petechiae to the medial aspect of the lower arm. The right arm was erythematous to the anterior forearm with serosanginous bullae.

Pertinent laboratory data included a white blood cell count (WBC) of 8.6×10^9/l with 54% segmented neutrophils, 27% band neutrophils, 8% lymphocytes, 11% monocytes; hemoglobin of 11.5 g/dl (7.1 mmol/l), hematocrit of 34.1%, platelet count of 30×10^9/l; prothrombin time (PT) of 15.7 s; activated partial thrombin time (aPTT) of 35.1 s; international normalized ratio (INR) of 1.6; fibrinogen of 831 mg/dl (24.4 μmol/l), D-dimer of 6.3 mg/l FEU (fibrinogen equivalent units); serum creatinine of 5.8 mg/dl (512.7 μmol/l); blood urea nitrogen (BUN) of 52 mg/dl (18.6 mmol/l), sodium of 121 meq/l (121 mmol/l); potassium of 5.8 meq/l (5.8mmol/l); bicarbonate (HCO_3^-) of 11 mmol/l; glucose of 84 mg/dl (4.7 mmol/l); total bilirubin of 1.3 mg/dl (22.2 μmol/l); albumin of 2.6 g/dl; alkaline phosphatase of 203 U/l; acetaminophen of 47.6 μg/ml (315.1 μmol/l); pH of 7.25; pCO_2 of 21 mmHg; and a B-type natriuretic peptide (BNP) of 1007 pg/ml. Blood and blister fluid cultures were collected and sent to the laboratory for Gram stain, culture, and susceptibility testing.

Upon questioning, the patient provided no history of exposure to saltwater. He was placed on vancomycin 1 g every 12 h. Past medical history included hepatitis C (presumably from multiple transfusions), chronic renal insufficiency, cirrhosis, hypertension, congestive heart failure, and ethanol abuse (stopped 2.5 years prior to admission). The patient's toxicology screen was negative. While in the ED, he was given a bolus followed by a maintenance infusion of normal saline. The patient was also placed on a norepinephrine infusion and titrated to maintain a systolic blood pressure >90 mmHg.

He was transferred to the intensive care unit (ICU). Vancomycin was discontinued the next day following a nephrology consult and was replaced with clindamycin 900 mg every 8 h. Ertapenem 500 mg every 24 h was also added to the patient's antimicrobial regimen. Hemorrhagic bullae appeared within the first 24 h and the affected integument extended from both hands to the elbows, with the right hand (Figure 1) more involved than the left (Figure 2). Eventually much of the area encompassing both forearms became necrotic. The presumptive diagnosis was necrotizing fasciitis. Blood cultures were negative; however blister fluid cultures were positive at 48 h for *V. vulnificus*. The bacterium was susceptible at 72 h to ampicillin, ampicillin–sulbactam, aztreonam, cefazolin, ciprofloxacin, gentamicin, imipenem, piperacillin–tazobactam, and trimethoprim–sulfamethoxazole. When questioned about the laboratory finding, the patient admitted to shucking and eating at least 8 raw oysters a few days prior to admission. The antimicrobial regimen was changed to doxycycline 100 mg intravenous (IV) every 12 h and ceftazidime 1 g every 24 h (adjusted to renal function).

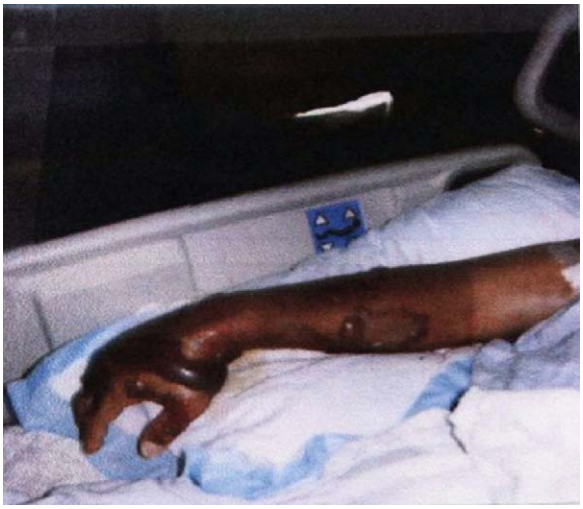

Figure 1. Right arm about 24 h after admission.

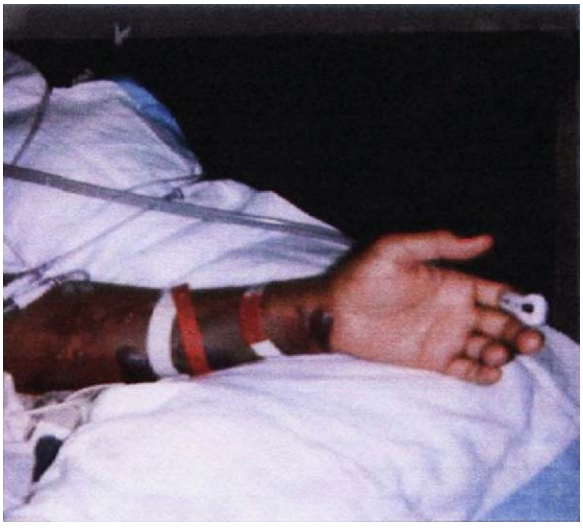

Figure 2. Left arm about 24 h after admission.

The patient's hypotension, metabolic acidosis, and thrombocytopenia responded to treatment and norepinephrine was discontinued by 48 h after admission. Hemodialysis was also initiated. A plastic surgery consult was requested when the patient stabilized, and debridement was performed on hospital days 6 and 15. The operative report indicated the infection was limited to the skin and subcutaneous fat and did not involve the fascia. The final diagnosis was therefore changed to necrotizing cellulitis. Skin grafts were performed on hospital days 36 and 39. The patient continued to improve, although he remained on hemodialysis post-discharge. He was discharged in stable condition on hospital day 40.

3. Epidemiology

V. vulnificus is found on all coastlines of the USA where water temperatures are sufficiently warm to support growth. It is one of

several species of the family *Vibrionaceae* found in US waters documented to cause human disease. Other important pathogenic species include *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio fluvialis*, *Photobacterium* (*Vibrio*) *damsela*, nontoxigenic *Vibrio cholerae*, and the rare toxigenic (cholera toxin-producing) serogroup (01) of *V. cholerae*.[2,14–16] In 2007, the last complete year of data, 95 cases of *V. vulnificus* infection were reported to the US Centers for Disease Control and Prevention (CDC). The outcome was known for 83 of these 95 cases; 30 patients (36%) died. This corresponds to 83% of all Vibrio-related deaths. The 95 cases accounted for 107 isolates. Most isolates were obtained from blood (64%) and wound (29%) cultures. *V. vulnificus* was the most common (34%) cause of Vibrio-related illness in the Gulf Coast states (Texas, Louisiana, Mississippi, Alabama, and Florida), but accounted for only 17% of cases nationwide. Most cases of *V. vulnificus*-related illness reported to the CDC since reporting became mandatory have either occurred along the Gulf Coast or can be traced to a Gulf Coast origin. The latter would include contaminated seafood sold in other regions of the USA. Most cases have occurred in the warm months from April to October.[15]

The spectrum of illness caused by *V. vulnificus* essentially manifests in three syndromes: gastroenteritis, 'severe sepsis', and skin and soft-tissue infection. As mentioned previously, 'sepsis or severe sepsis' appears to have the worst outcome (>50% mortality) and may be the most common presentation of infection. Gastroenteritis and primary septicemia usually result from the consumption of contaminated seafood, especially raw oysters. Skin and soft-tissue infection typically results from handling contaminated seafood or from exposure of open wounds to water in which the organism thrives. As mentioned previously, reports from Israel indicate freshwater fish may harbor the organism. However, in the USA, all reported cases have been traced to an organism acquired from a marine environment or brackish inland lakes. Biotype 1 is by far the predominant cause of human infection and presumably the likely etiology of all reported cases in the USA.[6,7,16]

The prominent risk factor for *V. vulnificus* infection is chronic liver disease, especially cirrhosis due to alcoholism or chronic hepatitides such as hepatitis B or C.[1–7,9–12,16,17] Alcohol consumption of as little as 30 ml/day was shown to increase the risk of infection in one study.[17] Other risk factors include immunodeficiencies involving macrophage and neutrophil dysfunction, such as those due to underlying cancers or immunosuppressive chemotherapy (e.g., for cancer and arthritis), acquired immunodeficiency syndrome, end-stage renal disease (especially patients receiving parenteral iron), gastrointestinal disorders (e.g., surgery, ulcers, achlorhydria), diabetes mellitus, and hematological disorders characterized by elevated iron levels such as hemochromatosis[1–7,10–12,16,18] (Table 1). Other potential contributing factors include chronic disease, especially heart disease, and advanced age (>60 years). As noted later in this discussion, the vast majority of *V. vulnificus*-related illness has occurred in males. The bacterium grows rapidly when transferrin saturation exceeds 70%.[16,19]

Table 1
Percentage (%) of patients with risk factors by clinical syndrome and epidemiologic study

Risk factors	Study, year [Ref.]					
	Blake et al., 1979 [11]	Tacket et al., 1984 [5]	Klontz et al., 1988 [1] [c]	Paik et al., 1995 [70]	Shapiro et al., 1998 [4]	Dechet et al., 2008 [2]
Gastrointestinal			*n* = 7		*n* = 23	
Liver disease					14	
Alcoholism					14	
Diabetes mellitus			14		5	
Gastrointestinal disease/surgery[a]			28		11	
Heart disease					10	
Hematological disorder					0	
Immunodeficiency[b]					5	
Malignancy					16	
Renal disease			14		5	
Any chronic disease			28		35	
Primary septicemia	*n* = 24	*n* = 18	*n* = 38	*n* = 92	*n* = 181	
Liver disease	75		66	79	80	
Alcoholism				73	65	
Diabetes mellitus				4	35	
Gastrointestinal disease/surgery[a]					18	
Heart disease					26	
Hematological disorder					18	
Immunodeficiency[b]					10	
Malignancy					17	
Renal disease					7	
Any chronic disease	96	89			97	
Wound infection	*n* = 15	*n* = 9	*n* = 17		*n* = 189	*n* = 428
Liver disease	0		12		22	20
Alcoholism					32	22
Diabetes mellitus					20	23
Gastrointestinal disease/surgery[a]					10	7
Heart disease					34	34
Hematological disorder					8	6
Immunodeficiency[b]					9	7
Malignancy					10	12
Renal disease					7	16
Any chronic disease	33	56			68	74

[a] Includes gastritis, pancreatitis, regional enteritis, peptic ulcer disease, ischemic bowel disease.
[b] Includes HIV; patients receiving chemotherapy or immunosuppressive drugs (including chronic corticosteroid use) for cancer, organ transplantation, rheumatoid arthritis, SLE, or other autoimmune disorders; and leukopenia or neutropenia.
[c] Includes ≤12 total patients listed in Tacket et al. data, Ref. 5.

Chronic liver disease is associated with impaired iron metabolism, which may partially explain the pathophysiologic mechanism associated with *V. vulnificus* and this disorder.[12] It has been estimated that susceptible individuals correspond to between 7% and 16% of the adult population in the USA.[12] 'Healthy patients', i.e., patients with no identifiable risk factors for *V. vulnificus* infection, are believed to account for < 5% of all reported US cases of primary sepsis.[12] Individuals with chronic liver disease or various immunodeficiencies have been reported to be up 80 times more likely to develop primary sepsis than healthy individuals.[20]

The various estimates of the population at risk assume all susceptible individuals are truly at risk. However, fairly recent data suggest that chronic liver disease or even cirrhosis is not an absolute risk factor for *V. vulnificus* infection. This observation is supported in part by the apparent low attack rate in individuals with liver disease, the largest high-risk group. It has been estimated that less than one case of *V. vulnificus*-related illness occurs in 10 000 meals of raw oysters served to individuals with liver disease.[12] It is also quite possible that only a subset of this group constitutes the true high-risk population. The other possibility is that not all environmental strains are equally virulent. Additional evidence supporting an unequal risk within the liver disease population comes from a pilot study designed to establish biomarkers for determining individuals at risk for *V. vulnificus* infection.[21,22] In the study, the investigators exposed peripheral blood mononuclear cells (PBMCs) collected from donors with liver disease to the bacterium. The authors reported that levels of proinflammatory cytokines elicited by the PBMCs in response to *V. vulnificus* had no association with serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), or the AST/ALT ratio obtained at baseline from the PBMC donors. However, PBMC glutathione levels, a measure of cellular oxidative stress, correlated directly and highly significantly with the release of proinflammatory cytokines. PBMC glutathione is inversely proportional to the oxidative stress. Oxidative stress is a common denominator for many of the high-risk populations. The authors concluded that individuals with biomarkers for oxidative stress have an increased susceptibility to *V. vulnificus* sepsis.[21]

4. Pathophysiology

V. vulnificus possesses multiple virulence determinants.[3,22] The organism has the ability to evade destruction in highly acidic environments such as the stomach. It is believed that this is accomplished by two different mechanisms.[3] In the presence of low pH and secondary oxidative stress, *V. vulnificus* can up-regulate production of lysine decarboxylase and manganese superoxide dismutase (MnSOD).[23–25] Lysine decarboxylase converts lysine to cadaverine, which functions as both an acid neutralizer (through deamination) and a superoxide radical scavenger.[3,24] The other inducible enzyme, MnSOD, also contributes to acid neutralization and reduction of oxidative stress, however, the exact mechanism involved in acid neutralization has not been elucidated.[24,25] Current evidence suggests superoxide generation rather than acid stress may be the initial triggering event for expression of both enzymes.[3] This observation is based on a study reporting poor survival of *V. vulnificus* when exposed to low pH under laboratory conditions.[26] However, another study reported dramatically improved survival if the organism was exposed to slightly acid pH prior to low pH.[27] This observation would support the argument that oxidative stress or possibly even other stress responses must be active to provide the bacterium protection from low pH.[3]

Once *V. vulnificus* evades the upper gastrointestinal tract defenses, it can penetrate the intestinal wall and enter the bloodstream. One of the first innate immune mechanisms the organism encounters is complement. Complement activation and opsonization are necessary for phagocytosis of *V. vulnificus* by polymorphonuclear leukocytes.[3,28] As described later in more detail, the exact role, or perhaps more precisely the relative contribution, of polymorphonuclear leukocytes or neutrophils in eliminating the bacterium is not known. However, current evidence indicates neutrophils like macrophages secrete cytokines that recruit additional leukocytes to the site of infection following complement-mediated phagocytosis.[29] Several of these cytokines are believed to mediate the systemic inflammatory response syndrome so often seen with *V. vulnificus*-associated disease. Among the proinflammatory cytokines specifically induced and expressed in patients infected with the bacterium are interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor alpha (TNF-α).[21,29,30] Although these mediators are likely promoters of the sepsis syndrome, deficiencies of proinflammatory cytokines such as those found in some patients with *V. vulnificus* infection and chronic liver disease are paradoxically associated with poor outcomes.[21] This would suggest that either extreme, i.e., inadequate cytokine expression or overactive cytokine induction, may be or is a major contributing factor to the high mortality associated with the organism. Cytokine release in response to the bacterium may be facilitated in part by the interaction of 'pathogen-associated molecular patterns' with toll-like receptors found on intestinal epithelial cells and macrophages.[31–33] A surface lipoprotein and flagellin, a subunit of the flagellum, have been identified as the 'pathogen-associated molecular patterns' associated with *V. vulnificus*.[32]

An in vitro study demonstrated that survival of *V. vulnificus* in human blood is inversely correlated with neutrophil phagocytosis.[34] The blood of patients with chronic liver disease appears to have decreased neutrophil activity in comparison to blood of normal volunteers. The response of neutrophils has been implicated as a disease determinant for *V. vulnificus*.[34] The organism has shown the ability to reduce the number of lymphocytes and macrophages through apoptotic activity.[31,35,36] However, it does not appear to have an impact on the quantity or the activity of neutrophils.[31,36]

Surface expression of capsular polysaccharide (CPS) is another virulence factor *V. vulnificus* uses to survive the immune response. This is accomplished in at least three different ways. CPS provides resistance to opsonization by complement and subsequent phagocytosis by macrophages, it confers resistance to the bactericidal effects of serum, and finally, encapsulation masks immunogenic structures that would normally activate nonspecific host responses.[3,22]

As mentioned previously, hematological disorders characterized by elevated iron levels are a risk factor for *V. vulnificus* infection. The exact relationship between serum iron and virulence for the bacterium remains unclear. However, serum iron levels have been shown to correlate directly with the infectious dose of *V. vulnificus* injected into laboratory mice.[37] Iron is believed to facilitate *V. vulnificus* infection by enhancing growth of the organism and possibly by reducing the activity of neutrophils.[38] *V. vulnificus* has been shown to produce at least two siderophores: vulnibactin (a catechol) and an unnamed hydroxamate siderophore. Vulnibactin is the primary siderophore for acquiring iron from media and for scavenging iron from transferrin and holotransferrin.[3,22] Deferoxamine, a hydroxamate siderophore derived from *Streptomyces pilosus*, has clinical utility as a chelating agent in acute iron intoxication and chronic iron overload disorders. It has been shown to promote iron uptake and growth of *V. vulnificus* and other ferrophilic organisms in vitro.[39] Deferoxamine has been reported to be a risk factor for *Yersinia enterocolitica* sepsis and mucormycosis (*Rhizopus sp*, Cunninghamella) when used as a therapeutic agent for chronic iron or aluminum overload disorders

(e.g., hemochromatosis, sideroblastic anemia, thalassemia, dialysis).[39–43] The significance of the threat posed by the drug in patients with excess iron is not known because *Y. enterocolitica* sepsis and mucormycosis may occur in this population with or without the use of deferoxamine. Nevertheless, it would seem reasonable to conclude the drug adds some degree of risk for *V. vulnificus* infection independent of the patients iron stores.[42,43] Other iron chelators such as deferiprone and deferasirox appear to inhibit growth of *V. vulnificus* in vitro and may even possess antibacterial properties.[42,44] In addition to siderophore-mediated uptake, the bacterium has the ability to trap unbound iron through a heme receptor (HupA).[3,22,45]

In addition to the mechanisms described above, *V. vulnificus* elaborates an exotoxin – hemolysin (VvhA) – a protein that facilitates the release of iron from hemoglobin. VvhA may contribute to the bacterium's virulence not only through hemolytic activity but also through other cytotoxic effects.[22] Intradermal injection of purified toxin into mice resulted in acute cellulitis with extensive extracellular edema, damaged or necrotic fat cells, capillary endothelial cells, and muscle cells, and mild inflammatory infiltration.[46] On a more microscopic level, other studies of affected tissues have demonstrated increased vascular permeability, apoptosis of endothelial cells, induction of inducible nitric oxide synthase activity, increased nitric oxide production, and possibly increased neutrophil recruitment.[3,22] The toxin causes cell death by pore formation in the cellular membrane.[47] Despite all the potentially deleterious effects, the relative contribution of hemolysin to *V. vulnificus*-associated disease is not known. There is also data suggesting the toxin may play a minor or even insignificant role in the infectious process. This observation is based on an in vivo study in mice using a wild strain and a mutant strain without the gene (*vvhA*) for hemolysin production. When both were injected intraperitoneally into iron-loaded mice, the mutant strain produced overall tissue damage and necrosis comparable to the wild strain.[48] There was also no difference in median lethal dose (LD_{50}). However, in another study using a hemolysin-deficient mutant, there was a small difference in mortality favoring the wild type when both strains were force-fed to mice. Histological examination of the stomach and intestinal mucosa revealed severe necrotizing enteritis in the mice fed the wild strain.[49] These results would suggest that VvhA may not be responsible for the lethality of *V. vulnificus* and may be only a relative contributor to the tissue damage seen with the organism. The most likely role of hemolysin in the disease process would be facilitating bloodstream invasion following oral ingestion of *V. vulnificus*.[49] Another possible explanation for the contradictory body of evidence may be that the toxin is not usually expressed in vivo; however other studies have not shown this to be the case.[22,46,50] Thus, hemolysin may play a greater role in environmental survival than human infection.[3]

Another toxin previously believed to contribute to the bacterium's virulence is VvpE, an extracellular metalloprotease. Purified VvpE has been shown to cause tissue necrosis, increased vascular permeability leading to edema, and bullous lesions, all characteristic of systemic disease caused by *V. vulnificus*.[3,22] The increased vascular permeability caused by the enzyme is associated with the generation of bradykinin.[22,51] Bradykinin, a vasodilator, appears to be essential for dissemination of the organism.[22,52] Other effects of VvpE include degradation of type IV collagen and activation of procaspase 3. The former is a structural component of basement membranes and the latter is involved in cellular apoptosis.[3,51] Despite all these deleterious effects, like hemolysin, there appears to be controversy concerning the enzyme's contribution to *V. vulnificus*-associated disease. Studies of experimental infection using mutant strains deficient in VvpE showed no difference in LD_{50} in comparison to the parent strain in local and systemic infections.[3,22,49]

As mentioned previously, mutant *V. vulnificus* strains unable to produce either hemolysin or VvpE were similar in cytotoxicity to the wild strains. To further help define the role of these toxins, a double mutant was created, one without VvpE (*vvpE*) and hemolysin (*vvhA*) genes.[3,49,53,54] In mice, HEp-2 cells, and HeLa cells, this strain was also found to be highly cytotoxic.[49] This would suggest another toxin is primarily responsible for the severe disease seen with *V. vulnificus*. Currently, the strongest evidence supports RtxA1 (VvRtxA) as that other toxin.[3,54,55] RtxA1 is a member of the RTX family of toxins and is 80% to 90% homologous with RtxA, an accessory toxin found in *V. cholerae*.[53,54] At this time, RtxA1 appears to be the key determinant responsible for the characteristic cytotoxicity seen with *V. vulnificus*.[3,54,55] The toxin is believed to trigger excessive production of reactive oxygen species (ROS) by the host, leading to necrotic cell death and apoptosis.[55] To further define the role of RtxA1, mutant strains deficient only in the toxin were found to be clearly less cytotoxic than the wild strain.[53]

Studies of various mutants exposed to HeLa cells have reinforced our knowledge of the contribution of the previously described toxins to *V. vulnificus* infection. Mutants without all three toxins (RtxA1, VvpE, hemolysin) and double mutants without RtxA1 and hemolysin were found to be totally devoid of cytotoxicity.[3,53]

Lipopolysaccharide (LPS), a known pyrogen, is believed to mediate the endotoxic shock associated with severe disease. Direct intraperitoneal (i.p.) injection of LPS endotoxin from *V. vulnificus* into laboratory rodents resulted in dramatic decreases in mean arterial pressure and rapid death of the animals.[3,30] The effect of lipopolysaccharide on blood pressure appears to be mediated through nitric oxide synthetase activity.[3] Interestingly, low-density lipoprotein (LDL) cholesterol and estrogen may be protective against LPS. In the animal model, mice pretreated with LDL cholesterol demonstrated lower or delayed mortality following exposure to LPS than controls.[3] The evidence for estrogen is observational. Males disproportionately outnumber females in terms of cases of *V. vulnificus*-related infection.[56]

Other virulence factors affect attachment and motility. Cell to cell contact with adherence and production of pili is essential for the cytotoxic effects of *V. vulnificus*.[3,22,53] Mutants with the inability to encode for either the pili structural protein (gene *pilA*) or prepilin peptidase (gene *pilD*) demonstrated a loss of attachment and slight increases in LD_{50} in comparison to the parent strain.[7,57] Furthermore loss of *pilD* resulted in an overall reduction of cytotoxicity.[3,22,57] Two other membrane proteins, OmpU and IlpA, are also believed to contribute to the virulence of *V. vulnificus*. OmpU is an outer membrane protein capable of binding fibronectin, while IlpA, a surface lipoprotein, appears to be an immunostimulant.[33,58] Studies of mutants devoid of either protein and injected i.p. into mice showed reduced cytotoxicity but only a sight increase in LD_{50} in comparison to the wild strain.[33,58] This would suggest both proteins contribute primarily to local tissue effects but are not essential for lethality.[3] In *V. vulnificus*, the flagellum is not only an organelle responsible for motility but also appears to enhance pathogenesis. Loss of motility may lead to decreased adhesion to the host cells and an inhibition of cytotoxin delivery.[3,59]

5. Clinical presentation

As mentioned previously, *V. vulnificus*-related infections usually manifest as one of three clinical syndromes: gastroenteritis, primary sepsis, and wound infection. Rare cases of spontaneous bacterial peritonitis,[60] pneumonia,[61] endometritis,[62] meningitis,[63] septic arthritis,[64] osteomyelitis,[65] endophthalmitis,[66,67] and keratitis[67] have also been reported and should be considered atypical presentations. Gastroenteritis is characterized by complaints (in

descending order of frequency) of abdominal pain or cramps, nausea, vomiting, diarrhea, fever, and chills.[1,4] The bullous skin lesions observed with other forms of *V. vulnificus*-related disease do not occur with gastroenteritis. Gastrointestinal symptoms are typically described as mild to moderate or even self-limited and may occur in individuals without the previously identified risk factors for the organism.[1,4] Death is rare, but has been reported in one series as high as 9%. It is likely that most if not all fatalities associated with *V. vulnificus* gastroenteritis resulted from undiagnosed sepsis.[4]

Severe sepsis in some series has been described as the most common presentation of *V. vulnificus*-associated disease or at the very least, a close second to wound infection.[1,4,5,11] Primary sepsis is usually characterized by bacteremia without an obvious focus of infection. Positive tissue culture (bullous lesion or exudate collected during debridement) without evidence of open wounds on presentation would also be included in this category. The portal of entry is believed to be the small intestine or the proximal colon (cecum) with the ileum as the most likely site.[68] Symptoms typically occur within 7 days, although they may be delayed up to 14 days in cases of the ingestion of raw or undercooked seafood.[69] The most common source of infection in the USA is consumption of raw oysters from the Gulf Coast.[4] All Gulf Coast oysters are reported to harbor *V. vulnificus* during the summer months. Primary sepsis typically presents with an abrupt onset of fever and chills. Metastatic infection characterized by cutaneous lesions such as bullae, cellulitis, ecchymosis, or even 'generalized macular or maculopapular eruptions' are common and usually occur on the lower extremities or the trunk. Gastrointestinal complaints such as nausea, vomiting, and abdominal pain often precede fever, chills, and cutaneous manifestations. Cutaneous lesions may progress to necrotic ulcers, necrotizing fasciitis, necrotizing vasculitis, or myonecrosis.[1,4,5,11,69,70] Septic shock (systolic blood pressure <90 mmHg) was reported in almost two-thirds of patients in one series.[4] Hypotension during the first 12 h or leukopenia is often associated with a very poor prognosis.[1] Another series reported mental status changes characterized by obtundation, lethargy, or disorientation in half the patients.[1,4] Thrombocytopenia is also very common and in an early study by the CDC, the frequency of disseminated intravascular coagulation (DIC) was only slightly less than the previously mentioned mortality rate of 50%.[11] At least one report has described a case complicated by purpura fulminans.[71]

Wound infection often differs from primary sepsis only in the presence of a cutaneous portal of entry. Infection may result from entry of *V. vulnificus* into pre-existing wounds or from inoculation of traumatic injuries. Pre-existing wounds may become infected by simply swimming or wading in environments containing the bacterium. The types of traumatic injuries most commonly associated with *V. vulnificus* infection include puncture wounds, lacerations, scratches, or abrasions sustained while handling contaminated shellfish, finfish, knives (or other devices used in the cleaning or processing of seafood), or fishhooks and those sustained while stepping on or handling sharp objects or marine organisms (e.g., crustaceans or possibly stingray spines) in contaminated water.[1,2,4,5,11] The severity of infection may vary from mild and self-limited to severe. Like gastroenteritis, mild to moderate wound infection may affect 'normal' individuals, i.e., those not characteristic of a previously mentioned high-risk group.[1,2,4,5,11] Similar to primary sepsis, symptoms usually occur within 7 days, although could be delayed as long as 12 days following exposure.[1,4,6,11] More serious infection is characterized by cutaneous lesions such as bullae, cellulitis, or ecchymosis surrounding the site of inoculation. Intense pain and swelling are also present. Skin manifestations may progress to necrotizing fasciitis, necrotizing vasculitis, or gangrene.[1,2,4,5,6,11] In contrast to primary sepsis, skins lesions are limited to the affected limb or area of inoculation; metastatic infection is not observed. Bacteremia and secondary sepsis is common with more severe wound infection. Systemic effects include fever, hypotension or shock, chills, and mental status changes.[1,2,4,5,6,11] Leukocytosis is common, however thrombocytopenia and DIC appear to be limited to the more severe cases.[11,72–74] Vomiting and diarrhea are also far less common than in other forms of *V. vulnificus* infection regardless of severity[1,2,5,11] (Table 2).

6. Treatment

V. vulnificus is usually susceptible in vitro to multiple antimicrobial agents. Among the classes of antimicrobial drugs reliably active against the organism are third-generation cephalosporins (cefotaxime, ceftriaxone, or ceftazidime), beta-lactam–beta-lactamase inhibitors (piperacillin–tazobactam), carbapenems (imipenem–cilastatin), tetracyclines (minocycline or doxycycline), aminoglycosides (gentamicin or amikacin), fluoroquinolones (ciprofloxacin, moxifloxacin, or levofloxacin), and miscellaneous agents such as trimethoprim–sulfamethoxazole or chloramphenicol.[75–81] The CDC recommends treatment of wound infections in adults with ceftazidime 1 to 2 g IV/intramuscular (IM) every 8 h in combination with doxycycline 100 mg IV/peroral (PO) twice daily (7 to 14 days). The literature consensus for primary sepsis is similar, with ceftazidime 2 g IV every 8 h in combination again with doxycycline 100 mg IV/PO every 12 h. Combination antimicrobial therapy appears to be the preferred regimen for serious infection, although some animal studies suggest monotherapy (e.g., fluoroquinolone) may be just as effective. An alternative regimen in adults includes cefotaxime 2 g every 8 h in combination with ciprofloxacin 400 mg every 12 h. Ciprofloxacin and cefotaxime can also replace doxycycline and ceftazidime, respectively, in the preferred or consensus regimen. Although rare in children, the CDC recommends trimethoprim–sulfamethoxazole and an aminoglycoside for pediatric patients with wound infections.[82]

Surgical intervention should be considered absolutely essential for patients with a severe soft-tissue infection such as necrotizing fasciitis.[83–85] Antimicrobial therapy alone is usually ineffective and may not achieve therapeutic levels at the site of infection due to thrombosis of the blood vessels supplying the affected area. Aggressive surgical debridement is necessary to remove necrotic tissue and reduce the bacterial burden. Survival with necrotizing fasciitis has been shown to improve dramatically when adequate debridement and fasciotomy are performed early.[73] Another nonpharmacologic intervention, hyperbaric oxygen, has been advocated as adjunctive therapy to aggressive antimicrobial therapy.[86] One anecdotal report suggested this combined approach may have eliminated the need for grafting in a patient with necrotic lesions of the right hand, arm, and shoulder.[80]

7. Prevention

The CDC has published recommendations for preventing *V. vulnificus* infections. These are especially applicable to individuals with liver disease or those who are immunocompromised. The recommendations are listed below and can be found on the CDC website (http://www.cdc.gov/nczved/divisions/dfbmd/diseases/vibriov/):

1. Do not eat raw oysters or other raw shellfish.
2. Cook shellfish (oysters, clams, mussels) thoroughly.
3. For shellfish in the shell, either (a) boil until the shells open and continue boiling for 5 more minutes, or (b) steam until the shells open and then continue cooking for 9 more minutes. Do not eat

Table 2
Percentage (%) of patients with various signs and symptoms by clinical syndrome and epidemiologic study

	Study, year [Ref.]					
	Blake et al., 1979 [11]	Tacket et al., 1984 [5]	Klontz et al., 1988 [1][b]	Paik et al., 1995 [70]	Shapiro et al., 1998 [4]	Dechet et al., 2008 [2]
Gastrointestinal			*n* = 7		*n* = 23	
Nausea					71	
Vomiting			29		68	
Diarrhea			100		84	
Fever			57		59	
Chills			43			
Mortality			0		9	
Primary septicemia	*n* = 24	*n* = 18	*n* = 38	*n* = 92	*n* = 181	
Nausea		58	59		58	
Vomiting	21	54	42	25	54	
Diarrhea	17	58	42	33	58	
Abdominal pain/cramps		53	34	26	53	
Fever	92	91	92	47	91	
Chills	82		53	43		
Mental status change		49	50		49	
Bullae			37	57		
Ecchymosis			32			
Cellulitis			50			
Fasciitis			18			
Myonecrosis			5			
Hypotension[a]	38	64	32	52	64	
Mortality	46	61	55	58	61	
Wound infection	*n* = 15	*n* = 9	*n* = 17		*n* = 189	*n* = 428
Nausea		38				38
Vomiting	20	50	6			26
Diarrhea	0	13	6			18
Abdominal pain/cramps		0	0			11
Fever	80	88	65		76	72
Chills	46	100	29			
Mental status change			18			
Bullae			41			48
Ecchymosis			18			
Cellulitis			88		91	85
Fasciitis						
Myonecrosis			12			
Hypotension[a]	14	22	12		30	25
Mortality	7	22	24		17	17

[a] Systolic blood pressure <90 mmHg for Klontz et al. (Ref. 1), Shapiro et al. (Ref. 4), and Dechet et al. (Ref. 2); <85 mmHg for Tacket et al. (Ref. 5); <80 mmHg for Blake et al. (Ref. 11); not defined for Paik et al. (Ref. 70).
[b] Includes ≤12 total patients listed in Tacket et al. data, Ref. 5.

those shellfish that do not open during cooking. Boil shucked oysters at least 3 minutes, or fry them in oil at least 10 minutes at 375 °F.
4. Avoid cross-contamination of cooked seafood and other foods with raw seafood and juices from raw seafood.
5. Eat shellfish promptly after cooking and refrigerate leftovers.
6. Avoid exposure of open wounds or broken skin to warm salt or brackish water, or to raw shellfish harvested from such waters.
7. Wear protective clothing (e.g., gloves) when handling raw shellfish.
8. Wear protective footwear (e.g., wading shoes) when wading in warm salt or brackish water.[87]

8. Final comments about the illustrative case

The case presented earlier describes a patient with a necrotizing soft-tissue infection and sepsis. The patient had shucked and eaten raw oysters a few days prior to admission. The hands were never examined closely for lacerations or other skin breaks. Although not mentioned in the case, the patient was admitted in mid-July when water temperatures and *V. vulnificus* colony counts are typically high.[4,8] The oysters were purchased from a vendor along the upper Gulf Coast, however the source or location of harvest was not known. The patient had chronic liver disease, a well-recognized risk factor for *V. vulnificus* infection. The lack of an obvious wound and a history of raw oyster ingestion would suggest a diagnosis of primary sepsis. However, this case may be more of a medical enigma than it appears. A more complete analysis of potential portals of entry suggests the site may not be limited to the gastrointestinal tract. It is also quite possible the organism entered through both hands, or even a combination of both hands and the gastrointestinal tract. The uniform and symmetric distribution as well as the ascending progression of the soft-tissue injury suggests a cutaneous portal of entry, presumably from both hands. Various contact points on oyster shells are typically razor sharp and easily capable of inflicting fine painless lacerations. The failure to use gloves or some type of protective device in the shucking process may have resulted in abrasions or lacerations that were not particularly conspicuous on admission. Oysters are usually shucked by holding the bivalve in a protected hand and inserting an oyster knife (or any similar dull, tapered, thin, and flat device) between the shells adjacent to or directly into the 'hinge'. The 'hinge' is the fibrous connection between the valves (shells) and is located at the narrow end of the oyster.[88,89] Cases of spontaneous cellulitis associated with *V. vulnificus* have been reported on limbs without an obvious portal of entry.[5,73,83,90–93] This would suggest that visible skin breaks or wounds are not absolutely necessary for cases of soft-tissue infection of limbs exposed to contaminated water or seafood. Therefore, it seems likely *V. vulnificus* may be capable of invading and producing disease through micro-injury of the skin.

Alternatively, the portal of entry could have been the gastrointestinal tract since, to the best of our knowledge, the

oysters were eaten raw. It would be easy to conclude this was a case of primary sepsis. On admission, the patient was in septic shock and had obvious skin involvement including bullae. Both hands were extremely painful. Cutaneous lesions, particularly bullae, are common and considered blood-borne or metastatic sequelae of primary sepsis. The usual sites of skin manifestations are one or both lower extremities and the trunk.[5,11,68,94] In contrast to wound infection, cutaneous lesions associated with primary septicemia occur less frequently on the arms and especially the hands. To the best of our knowledge, all case reports of upper extremity involvement have been characterized by either diffuse distribution involving bilateral upper and lower extremities or a single limb.[11,95–97] The distribution of metastatic skin manifestations with the same bilateral symmetry seen in our patient has not been described in other series. The presumed sources of the infection, i.e., raw oysters, were probably in close contact with the affected integument. The blood cultures were also negative. The patient did not receive any antibiotics prior to the collection of blood cultures. Although the presence of the organism in the blood is not an absolute requirement for the diagnosis of primary septicemia, many reported cases with cutaneous involvement have been characterized by bacteremia.[1,5,11] The patient also reported no gastrointestinal symptoms prior to admission.

The bacterium could have entered our patient through the hands and the gastrointestinal tract. It would be very difficult or even impossible to determine the likelihood of this happening. This would require the presence of manifestations unique to primary sepsis as well as those unique to severe wound infection. Unfortunately, there is considerable overlap with the signs and symptoms associated with both syndromes. Literature analyses of either primary septicemia or wound infection excluded patients with more than one potential access point for the organism.[1,4,11,17] As a result, there is very little information describing the spectrum of illness in patients with more than one possible portal of entry.

9. Conclusions

V. vulnificus is a motile, halophilic, Gram-negative bacillus found worldwide, primarily in warm coastal waters. The bacterium is capable of causing illness ranging from mild gastroenteritis to necrotizing soft-tissue infection and septic shock. Severe infection occurs primarily in patients with chronic liver diseases, immunodeficiency, and iron storage diseases. Despite the large number of individuals at risk for infection, the reported number of cases of *V. vulnificus*-related disease is relatively small. Most infections in the USA occur from April to October when bacterial counts are higher. Most cases result from ingestion or handling of raw contaminated seafood (usually raw oysters in the USA) or from exposure of open wounds to seawater. The organism possesses several virulence determinants including resistance to stomach acid, exotoxins, capsular polysaccharide, siderophores, destruction of macrophages and lymphocytes through apoptosis, lipopolysaccharide, pili, and flagellum. *V. vulnificus*-related infection is usually characterized by three syndromes: gastroenteritis, primary sepsis, and wound infection. Primary sepsis is caused by ingestion of raw or uncooked seafood and has the worst prognosis with a case-fatality rate exceeding 50%. Several classes of antimicrobial agents are active in vitro against *V. vulnificus*. Ceftazidime in combination with doxycycline is the preferred antimicrobial regimen for hospitalized patients. The CDC has published recommendations for preventing infection with *V. vulnificus*. These recommendations are particularly applicable to high-risk individuals with chronic liver diseases, immunodeficiency, or iron storage diseases.

Conflict of interest

All authors report no conflicts of interest and received no financial or other support in the preparation of this manuscript.

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