Original Article

Seasonality, clinical types and prognostic factors of *Vibrio vulnificus* infection

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Abstract

Introduction: *Vibrio vulnificus* infection, an uncommon but life-threatening illness, manifests as two main types, primary septicemia and primary wound infections. Little information regarding the seasonality of *V. vulnificus* infections in tropical areas and prognostic factors of primary *V. vulnificus* wound infections is available.

Methodology: This retrospective study was conducted to include 159 *V. vulnificus*-infected admissions at our institution in southern Taiwan, 63 with primary septicemia (Group 1) and 96 with primary wound infections (Group 2), from 1999 to 2008, for analysis.

Results: The case-fatality rate was 24%. Eighty-eight percent of these cases occurred during April to November. During December to March, patients in Group 2 were less likely to have acquired the infection compared with those in Group 1. Group 1 was more likely to have comorbidities and a higher case-fatality rate compared to Group 2. In multivariate analysis, hemorrhagic bullous skin lesions/necrotizing fasciitis (P=0.024), lesions involving two or more limbs (P=0.043), and shock on admission (P=0.015) were related to an increased mortality risk, while surgery < 24 hours after admission (P=0.001) was related to a decreased mortality risk in Group 1; however, hemorrhagic bullous skin lesions/necrotizing fasciitis (P=0.045) was the only prognostic factor in Group 2.

Conclusion: The presence of hemorrhagic bullous lesion/necrotizing fasciitis is the main prognostic factor for primary septicemia or primary wound infections caused by *V. vulnificus*. Persons with an underlying immunocompromised status should avoid consuming raw/undercooked seafood or exposing wounds to seawater and should wear clothing during handling of seafood/fishing, especially in warmer months.

Key words: *Vibrio* species; seasonality; scoring system; risk factor; mortality

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Introduction

Vibrio vulnificus, which belongs to the family *Vibrionaceae*, is a virulent, Gram-negative, halophilic, rod-shaped, motile bacterium that often flourishes in warm estuarine seawaters or brackish environments with a salinity of 0.5% to 2.5% [1]. The bacteria are usually found in temperate or subtropical seacoast regions or countries, such as the coastal regions of the United States and East Asia [2-6]. Human infections due to *V. vulnificus*, initially described by Blake *et al*. [7], are uncommon but potentially life-threatening. The reported incidence and case-fatality rates of *V. vulnificus* infections are approximately 0.001 to 1.237 per million people and 10% to 54% over the past three decades, respectively [2-6,8-12]. *V. vulnificus* infections can be transmitted by eating contaminated or raw seafood or by exposure to seawater through a skin wound. The

infection exhibits two main clinical manifestations, including primary septicemia and primary wound infections, which can progress rapidly and become lethal even to an otherwise healthy person; therefore, promptly ascertaining this infection and instantly recognizing the risk factors present in these patients appears to be crucial. Most reports have focused on *V. vulnificus* infections with primary septicemia in high-risk persons who have underlying immunocompromised conditions and have consumed raw or undercooked seafood or shellfish, especially oysters [2-12]. With the increment in marine activities, the reported number of *V. vulnificus*-infected cases due to wound infection has been increasing over recent years [11,13]. However, the clinical characteristics and risk factors for primary wound infection caused by *V. vulnificus* are far less frequently investigated than infection from septicemia, and a comprehensive comparison between these two main types of *V. vulnificus* infections is lacking; information regarding the seasonality of *V. vulnificus* infections in tropical areas is also less reported compared to those from temperate or subtropical regions [5,6,9]. Therefore, we conducted a retrospective study to delineate the clinical features of patients with *V. vulnificus* infections with primary septicemia and those with primary wound infections to compare the clinical features and outcomes between the two types of patients and to identify the predictors of mortality in each group.

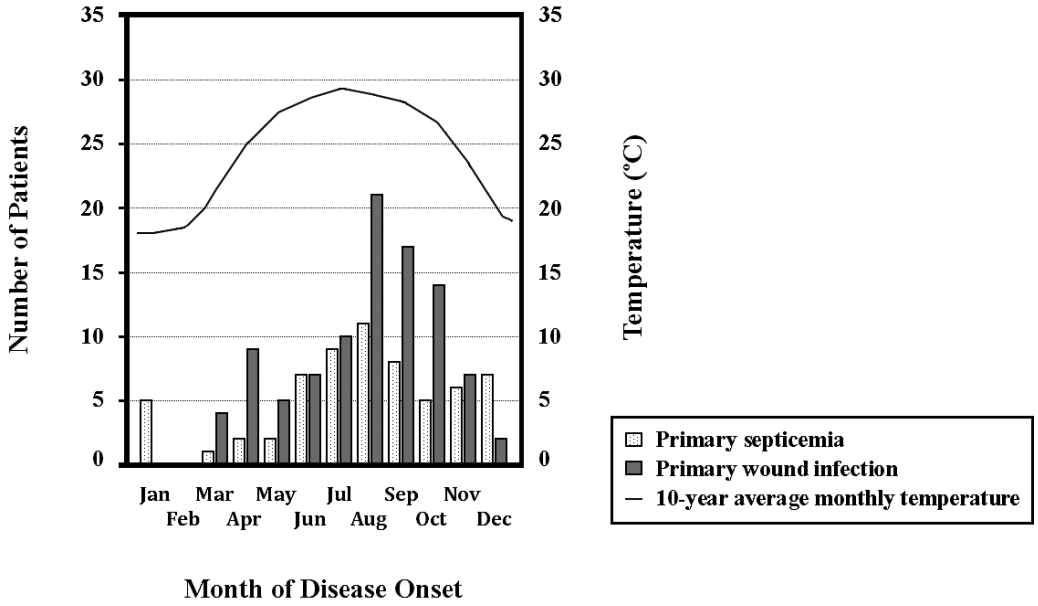
Methodology

We collected medical records of all adult patients with a final diagnosis of *V. vulnificus* infection who had been admitted to the Chi Mei Medical Center (a 2,300-bed primary and tertiary teaching hospital approximately 8.5 kilometers from the seacoast and located in southwest Taiwan) (120.2220ºE, 23.0209ºN) through a computer-aided systemic search from January 1999 to December 2008. *V. vulnificus* infections were diagnosed based on positive blood and/or wound culture results. *V. vulnificus*-infected patients having a history of wound exposure to marine environments or a recent injury from handling seafood in the past week before arrival were regarded as having primary wound infections, while those with sepsis but having no obvious source of infection were defined to have primary septicemia [2,4,8-10]. In total, 159 admissions identified as *V. vulnificus* infections, 63 having primary septicemia and 96 having primary wound infection, were included in the analysis. The average age of the 159 patients was 63.0 ± 12.3 years (range, 30-89 years), and 55% of those were men.This study was approved by the local research ethnics committee.

The sea surface temperature data for the coastal area of Tainan were obtained from the Central Weather Bureau of Taiwan. The 10-year average monthly seawater temperature was defined as the average of the mean surface seawater temperature for the month from 1999 to 2008.The clinical and laboratory information (demographic and microbiological data, clinical manifestations, treatments given, and outcomes) of all included patients were collected and analyzed. The *V. vulnificus* pathogens isolated by using conventional methods were further confirmed using the API-20E system (bioMe'rieuxVitek, Hazelwood, MO, USA), ID 32 GN System (bioMérieuxVitek, Hazelwood, MO, USA), and Vitek 2 ID-GNB identification card (bioMérieux, Durham, NC, USA) [14]. The broth dilution, Kirby-Bauer, and E-test methods were used for antibiotic susceptibility testing, which followed the recommendation of the Clinical and Laboratory Standards Institute [15]. Initial empirical broad-spectrum antibiotics were parenterally administered after collecting the wound and/or blood specimens. Sepsis and septic shock were definedaccording to the International Sepsis DefinitionsConference criteria [16]. Case fatality was defined as death during hospitalization.

Descriptive data are presented as numbers with percentages for categorical data and means with standard deviations for continuous data. Continuous variables were tested using either the Student's t test or Mann-Whitney U test, as appropriate. Categorical variables were compared by either the χ^2 test or Fisher's exact test (when the expected value was less than 5 in one cell), as appropriate. Variables regarding demographics, clinical features, laboratory results, and treatment modalities between survivors and non-survivors were compared. Significant variables obtained from the univariate analysis were further examined by Cox regression analysis using forward selection to identify significant risk factors for mortality. Hazard ratios (HRs) and 95% confidence intervals (CIs) were also calculated in the regression model. These statistical analyses were conducted using SAS version 8.2 (SAS Institute, Cary, NC, USA). Two-sided *P* values of < 0.05 were considered to be significant in all analyses.

Figure. Number of patients with *Vibrio vulnificus* infections by month of disease onset, the 10-year average monthly seawater temperature of the locality (1999-2008), and primary septicemia *versus* primary wound infections (n = 159)



Results

Seasonality, demographics, comorbidities, and clinical features

The 159 *V. vulnificus* infections occurred in Tainan (around 22° 59' 35" N/120° 12' 13" E) and all patients were Taiwanese. Among the 63 patients with primary septicemia, 47 had a recent history of consuming raw/undercooked seafood and the remaining 16 patients did not have the relevant information recorded. Ninety-six patients, including 24 with a recent history of existing wound exposure to seawater/marine creatures and 72 with a recent injury from handling seafood/fishing, had primary wound infections; of those, 85 were fishers/aquiculture workers, 5 were fish sellers, and 6 were working at other occupations. The 10-year average monthly seawater temperatures were over 20°C (68°F) in the locality from March to November. One hundred and forty cases (88%) occurred during April-November. The group with primary wound infections were less likely to have acquired the infection during the cooler (<20°C) months compared to those with primary septicemia (2/96 [2%] versus 12/63 [19%]; P = 0.0003). The greatest frequency of cases of primary septicemia and primary wound infection occurred in August. There were no cases of primary wound infection during the months of January and February and no cases of primary septicemia in February (Figure).

Patients with primary septicemia had a higher frequency of underlying liver diseases, malignancy, immunosuppressive agents or steroid use, chronic renal insufficiency, gastrointestinal symptoms, hypotension on admission, and lesions involving two or more limbs, as well as a lower percentage of non-comorbidity or skin/soft-tissue lesion involvements than patients with primary wound infections. The demographic and clinical characteristics of the two groups are shown in Table 1.

Laboratory findings, therapeutic modalities, and outcomes

On admission, patients with primary septicemia tended to have a lower percentage of white blood cell count abnormalities and a higher frequency of bacteremia, anemia, or hypoalbuminemia as well as the presence of elevated serum aspartate aminotransferase or creatinine values compared to those with primary wound infections. Treatment options for the 159 patients included surgical intervention (incision/drainage, debridement, fasciotomy, amputation, or combinations of these) combined with antibiotics (n = 140) or antibiotics alone (n = 19). The group with primary septicemia was significantly more likely to have had only antibiotic therapy, whereas patients with primary wound infections were more likely to have had surgical interventions within 24 hours of hospital admission. During their hospitalization, 38 patients died, yielding an overall case-fatality rate of 24%. The primary septicemia group had a significantly higher case-fatality rate compared to those with

Table 1. Demographic data, underlying diseases, and clinical features in *Vibrio vulnificus* patients with primary septicemia and those with wound infections.

Variable	All patients (n = 159)	Primary septicemia (n = 63)	Wound infection (n = 96)	*P* value
Age, mean ± standard deviation (years)	63.0 ± 12.3	65.1 ± 13.5	61.6 ± 11.3	0.078
Gender, male, No. (%)	88 (55)	32 (51)	56 (58)	0.350
Duration of symptoms before admission, mean ± standard deviation (days)	1.3 ± 0.7	1.3 ± 0.6	1.3 ± 0.7	0.719
Coexisting medical conditions [a]				
Liver disease [b]	47 (30)	27 (43)	20 (21)	0.003
Diabetes mellitus	46 (29)	22 (35)	24 (25)	0.177
Malignancy	22 (14)	18 (29)	4 (4)	<0.0001
Immunosuppressive agent/steroid used	37 (23)	22 (35)	15 (16)	0.005
Chronic renal insufficiency	15 (9)	13 (21)	2 (2)	<0.0001
Aplastic anemia	6 (4)	4 (6)	2 (2)	0.215
No comorbid diseases	47 (30)	6 (10)	41 (43)	<0.0001
Signs and symptoms [a]				
Fever/chills	122 (77)	51 (81)	71 (74)	0.307
Shock on admission	58 (37)	30 (48)	28 (29)	0.018
Gastrointestinal symptoms[c]	16 (10)	16 (27)	0	<0.0001
Skin/soft-tissue lesion involvement	140 (88)	44 (70)	96 (100)	<0.0001
Skin/soft-tissue lesions involving two or more limbs	18 (11)	17 (27)	1 (1)	<0.0001
Hemorrhagic bullous cutaneous lesions/necrotizing fasciitis	97 (61)	35 (56)	62 (65)	0.254

[a] When patients fit into multiple categories, they were counted in each category and expressed as number of patients (%)
[b] Hepatic disorders including chronic hepatitis B, chronic hepatitis C, alcoholic hepatitis, liver cirrhosis, or hepatocellular carcinoma.
[c] Gastrointestinal symptoms including nausea, vomiting, diarrhea, and/or abdominal pain.

primary wound infections (40% vs. 14%; $P < 0.0001$). Of the 38 deaths, 27 (71%) occurred within 72 hours after arrival. The laboratory findings, treatments administered, and outcomes of these patients are summarized in Table 2.

Multivariate analysis of risk factors for mortality

In patients with *V. vulnificus* infections with primary septicemia, further comparisons of the clinical variables between the survivors and non-survivors showed that non-survivors had higher proportions of hemorrhagic bullous skin lesions/necrotizing fasciitis, lesions involving two or more limbs, shock on admission, elevated creatinine levels, and a lower frequency of receiving surgical interventions within 24 hours of hospital admission than did the survivors. The above significant clinicalvariables were subjected to multivariate analysis, and four variables attained statistical significance related to mortality. The presence of hemorrhagic bullous skin lesions/necrotizing fasciitis ($P = 0.024$), lesions involving two or more limbs ($P = 0.043$) or shock on admission ($P = 0.015$) were associated with increased risk of mortality. Time to surgical treatment after arrival of less than 24 hours was associated with a decreased risk of mortality ($P = 0.001$).Among the 96 patients with *V. vulnificus* primary wound infections, when the significant factors for mortality obtained from the univariate analysis were subjected to multivariate analysis, only one variable–hemorrhagic bullous skin lesions/necrotizing fasciitis–was significantly associated with mortality ($P = 0.045$) (Table 3).

Discussion

Our study disclosed that patients with primary septicemia were prone to have underlying immunocompromised conditions with severe course and consequences compared to those with primary wound infections, implying that the host's immunocompetent status may influence the development of *V. vulnificus* infections. The majority of cases occurred during the months of April to November, especially for patients with primary wound infections. In the multivariate analysis, hemorrhagic bullous skin lesions or necrotizing fasciitis, lesions involving two or more limbs, and shock on admission were significant risk factors for mortality, while prompt surgical intervention within 24 hours after admission was a significant protective factor for mortality in patients with *V. vulnificus* infections with primary septicemia. On the other

Table 2. Laboratory findings on admission, treatment, and outcomes in patients with *Vibrio vulnificus* infections with primary septicemia and those with wound infections.

Variable [a]	All patients (n = 159)	Primary septicemia (n = 63)	Wound infection (n = 96)	*P* value
Laboratory findings				
WBC count > 1.2×10^4 cells/mm³ or < 4×10^3 cells/mm³	116 (73)	39 (62)	77 (80)	0.011
Hemoglobin <14 g/dl in males or <12 g/dl in females	76 (48)	40 (64)	36 (38)	0.001
Serum AST level > 40 IU/L	91 (57)	45 (71)	46 (48)	0.003
Serum creatinine level > 1.3 mg/dl	89 (56)	43 (68)	46 (48)	0.012
Serum albumin level < 3.5 mg/dl	43 (27)	24 (38)	19 (20)	0.011
Bacteremia	112 (70)	63 (100)	49 (51)	<0.0001
Treatment method				
Surgical intervention [b] plus antibiotics	116 (83)[c]	35 (80)[d]	81 (84)	0.482
Antibiotics alone	43 (27)	28 (44)	15 (16)	<0.0001
Antibiotic treatment				0.063
(1) Penicillin group or first-/second-generation cephalosporin with or without an aminoglycoside	27 (17)	15 (24)	12 (12)	
(2) Third-generation cephalosporin with minocycline (or analogue) or quinolone group	132 (83)	48 (76)	84 (88)	
Time to surgical treatment after admission < 24 hours	102 (73)[c]	26 (59)[d]	76 (79)	0.013
Limb amputation needed	9 (6)	4 (6)	5 (5)	0.761
Hospital stay, mean ± SD (days)	17.1 ± 16.5	19.5 ± 21.3	15.5 ± 12.3	0.142
Fatality	38 (24)	25 (40)	13 (14)	<0.0001

AST= aspartate aminotransferase; *SD*= standard deviation; *WBC*= white blood cell.
[a] Listed as number of patients (%), except as noted.
[b] Surgical intervention: incision and drainage, debridement, fasciotomy, and/or limb amputation.
[c] Total of 140 patients with skin/soft-tissue infection required surgery as denominator.
[d] Forty-four patients with skin/soft-tissue infection in the group required surgery as denominator.

hand, the presence of hemorrhagic bullous skin lesions/necrotizing fasciitis was the only predictor of mortality in patients with *V. vulnificus* primary wound infections.

The present study indicated that cases of *V. vulnificus* infection had a clear peak during the summer months, a result similar to the observations of previous reports from Japan and the United States [5,6,9]. Most infections occurred during the period from April to November, and the seasonality of *V. vulnificus* infections was apparently longer than that in Japan (June to November) and in the United States (May to October) [5,6,9]. The coast of the southeast Taiwan, where our institution is located, is the main fish and shellfish aquiculture area in the country, and the harvest period of the farmed fish and shellfish is usually from April to early November. The surface seawater alongside the coast generally exceeds 20°C (68°F) during this period, an ideal temperature for the growth of *V. vulnificus*, and the duration of warmer seawater in this tropical area is longer than that of the above temperate or subtropical regions/countries. It is not surprising that wound contact with seawater, injuries from handling seafood or fishing, or consuming raw/undercook seafood in the warmer/harvest months might account for the higher number of *V. vulnificus* infections, especially in those with primary wound infections. Although the average temperature of this locality is higher than 15°C (59°F) throughout the year, the strong northeasterly winds with accompanying huge waves usually arrive in winter in Taiwan, leading to a decline in marine activities, which may cause the apparent decrease in the occurrence of *V. vulnificus* primary wound infections during the period from December to February.

Animal models and human studies have shown that *V. vulnificus* can enter the host through skin wounds or gastrointestinal mucosal invasion and then multiply along with producing cytotoxins and enzymes, such as capsular polysaccharides, lipopolysaccharides, cytolysins, iron, hemolysins, and metalloproteases[17-22]. The toxic effects evoked by theses toxins/enzymes on skin or soft-tissue lesions

Table 3. Significant factors relevant to mortality in *Vibrio vulnificus*-infected patients with primary septicemia and those with wound infection separately, using univariate and multivariate analyses

Variable	Survival	Fatality	*P* value	HR (95% CI)	*P* value
(1) Patients with primary septicemia (n = 63)	**(n = 38)**	**(n = 25)**			
Immunosuppressive agent/steroid used	18 (47)	4 (16)	0.011		
Hemorrhagic bullous necrotic cutaneous lesions/necrotizing fasciitis	16 (42)	19 (76)	0.008	3.2 (1.2-8.8)	0.024
Lesions involving ≥ two limbs	5 (13)	12 (48)	0.002	2.6 (1.1-6.3)	0.043
Shock on admission	13 (34)	17 (68)	0.009	3.1 (1.3-8.0)	0.015
Serum creatine level > 1.3 mg/dL	21 (55)	22 (88)	0.006		
Time to surgical treatment < 24 hours after admission	23 (61)	4 (16)	<0.0001	0.16 (0.05-0.48)	0.001
(2) Patients with primary wound infection (n = 96)	**(n = 83)**	**(n = 13)**			
Hemorrhagic necrotic cutaneous lesions/necrotizing fasciitis	50 (60)	12 (92)	0.029	5.1 (1.1-21.5)	0.045
Bacteremia	38 (46)	11 (85)	0.015		
Shock on admission	19 (23)	9 (69)	0.002		
Serum creatine level > 1.3 mg/dL	33 (40)	13 (100)	<0.0001		
Serum albumin level < 3.5 mg/dL	13 (16)	6 (46)	0.020		
Serum AST level > 40 IU/L	35 (42)	11 (85)	0.004		
Surgical treatment + antibiotics	73 (88)	8 (62)	0.029		

AST= aspartate aminotransferase; *CI*= confidence interval; *HR*= hazard ratio.

clinically manifest in three stages: (i) inflammatory stage, (ii) bullous stage, and (iii) gangrenous stage [3,23]. These toxins/enzymes may trigger vigorous septic reactions and deteriorate the host's condition, thereby resulting in an increased mortality risk. Chuang *et al*.,based on animal model, reported thatthe severity of infection was proportionalto the quantity of *V. vulnificus* inoculated [24].The quantities of bacteria that can bedisseminated through the bloodstreammay have a greater chance to metastasizeto two or more extremities of the affected host when the infection is severe.These hypothetic mechanisms could explain howhemorrhagic bullous cutaneous lesions/necrotizing fasciitis (in patients who had either primary septicemia or primary wound infections), primary septicemia with secondary lesions involving two or more limbs, and primary septicemia with shock on admission are related to *V. vulnificus* mortality, and these results are compatible with those of otherpreviouslypublished reports [2,4,12, 25,26]. The only protective factor of *V. vulnificus* mortality for primary septicemia in the present study was time to surgical treatment within 24 hours after admission, a finding in concordance with the suggestion by Howard *et al*. and other groups [8,23,25,26] that early surgical intervention, including incision/drainage, debridement, fasciotomy, and/or even limb amputation (if necessary), plays an essential role in treating skin or soft tissue infections caused by *V. vulnificus*. Prompt surgical intervention appeared to be insignificant in treating patients with primary wound infections, which may be attributed to the fact that the majority of patients with primary wound infections were relatively healthy and the severity of their infections was usually not acute.

It should be acknowledged that our results are limited by its single-center study with a retrospective fashion. However, the uncommon occurrences of *V. vulnificus* infections in humans make conducting a large-scale prospective study quite difficult in a measurable period.Moreover, the number of patients in our report was comparable to the sample sizes of previously published studies involving *V. vulnificus* patients with study periods of one to 13 years [1-13,23,25,26].

Conclusion

We have learned that the presence of hemorrhagic bullous cutaneous lesions/necrotizing fasciitis is an important predictor of mortality for *V. vulnificus*-infected patients with either primary septicemia or primary wound infections. The presence of lesions involving two or more limbs and shock on admissionmay increase mortality risk and prompt surgical intervention with 24 hours after admission can benefit these patients with primary septicemia. Moreover, clinicians should advise persons with underlying immunocompromised status

to avoid consuming raw or undercooked seafood, exposing wounds to seawater (especially in warmer months), and to wear clothing (gloves) during handling of seafood/fishing. In addition tocomprehensive history-taking, the presentingsigns and symptoms (especially the presence of hemorrhagic bullous lesions/necrotizing fasciitis, lesions involving two or more limbs, or shock) could aidclinicians in determiningwhich patients clinically suspected with *V. vulnificus* infection should receive proper disposition and undergo specific therapeutic interventions.

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