Patients with Monomicrobial *Aeromonas* Necrotizing Fasciitis Had Higher Mortality Rates Than Those of *Vibrio vulnificus* Infections

Yao Hung Tsai (orma2244@adm.cgmh.org.tw)
Chiayi Chang Gung Memorial Hospital

Tsung-Yu Huang
Chiayi Chang Gung Memorial Hospital

Pei-An Yu
Chiayi Chang Gung Memorial Hospital

Liang Tseng Kuo
Chiayi Chang Gung Memorial Hospital

Chi-Lung Chen
Chiayi Chang Gung Memorial Hospital

Po-Yao Chuang
Chiayi Chang Gung Memorial Hospital

Cheng Ting Hsiao
Chiayi Chang Gung Memorial Hospital

Kuo Chin Huang
Chiayi Chang Gung Memorial Hospital

Research article

Keywords: Necrotizing fasciitis, Aeromonas hydrophila, Aeromonas sobria, Vibrio vulnificus, bacteremia

Posted Date: April 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-21986/v1

Abstract

Backgroud: Monomicrobial necrotizing fasciitis (NF) caused by *Vibrio vulnificus*, *Aeromonas hydrophila* and *Aeromonas sobria* are often associated with high mortality rates. The purpose of this study was to compare the independent predictors related to outcomes between of *Vibrio vulnificus* and *Aeromonas spp.* necrotizing fasciitis. We also investigated the risk factors association with mortality in patients with monomicrobial *Aeromonas* necrotizing fasciitis.

Methods: Monomicrobial necrotizing fasciitis caused by *Vibrio vulnificus* (60 patients) and *Aeromonas* species (31 patients) over an 11-year period were retrospectively reviewed. Differences in mortality, patient characteristics, clinical presentations, and laboratory data were compared between the *Vibrio vulnificus* and *Aeromonas species* groups, and between the death and the survival subgroups *Aeromonas* patients.

Results: Six patients in the *Vibrio vulnificus* group (10%) and eleven in the *Aeromonas* group (32.3%) died. *Vibrio vulnificus* patients had a significant higher incidence of bacteremia; however, the proportion of *Aeromonas* patients presenting bacteremia associated with death was significantly higher than that of *Vibrio vulnificus* group ($p = 0.0002$). *Aeromonas* isolates had a significant higher incidience of antibiotics resistance ($p = 0.0001$). The death subgroup of *Aeromonas* NF patients had a significantly higher incidence of bacteremia ($p = 0.001$), higher counts of banded leukocytes ($p = 0.026$), lower platelet counts ($p = 0.043$), lower total lymphocyte counts ($p = 0.013$), and lower serum albumin level ($p = 0.019$) than the survival subgroup.

Conclusion: Monomicrobial necrotizing fasciitis caused by *Aeromonas* spp. was characterized more fulminating and higher mortality than that of the *Vibrio vulnificus* infection even after early fasciotomy and third-generation cephalosporin antibiotic therapy. The death patients of *Aeromonas* group have a significantly higher proportion of antibiotics resistance and bacteremia, higher incidence of shock, lower lymphocyte counts, and lower albumin levels than the *Vibrio* patients who died.

Level of Evidence: Therapeutic level III.

Introduction

Necrotizing fasciitis which is a rapidly progressive, life-threatening soft-tissue infection with an average of mortality rate of 22.6 to 33% has been documented a medical and orthopaedic emergency in the past decade (1–6). Necrotizing fasciitis needs early diagnosis, emergent surgical debridement, and broad-spectrum antibiotic therapy when patients appear in the emergency department (1–5). Although type I necrotizing fasciitis which is a polymicrobial infection involving aerobic and anaerobic organisms was often reported, the incidence of monomicrobial necrotizing fasciitis has recently been reported to be as high as 55 to 87% (3–6). Recent literatures revealed that monomicrobial necrotizing fasciitis caused by gram-negative pathogens, such as *Vibrio vulnificus*, *Klebsiella pneumoniae*, *Aeromonas hydrophila*,

Pseudomonas spp. and *Escherichia coli*, could cause fulminant clinical courses and fatal syndrome (4–12).

Vibrio vulnificus and *Aeromonas* species, members of the Vibrionaceae family, are gram-negative pathogens and thrive in aquatic environments to cause acute gastrointestinal illness, soft-tissue infections and primary septicemia in humen (12–15). *Vibrio* spp. and *Aeromonas* spp. can produce similar necrotic skin lesions, such as pain, swelling, hemorrhagic bullae, subcutaneous bleeding, purpura, and necrosis, and are often associated with high mortality rates in patients with necrotizing fasciitis.

Nearly fifty patients with necrotizing fasciitis are admitted to our institution annually, and *Vibrio vulnificus* are the most frequent causative organism of monmicrobial necrotizing fasciitis (4, 10–14, 16). We have set up the team "*Vibrio* necrotizing soft tissue infection (NSSTI) Treatment and Research (VTR) Group", which consists of professional staff working in various departments, including emergency medicine, orthopedic surgery, infectious diseases, intensive care unit (ICU), plastic surgery, and hyperbaric oxygen treatment center. Our team has successfully decreased the mortality rate of *Vibrio* NF from 38–13% (4, 10–14, 16–19). However, we observed that the patients with monmicrobial necrotizing fasciitis caused by *Aeromonas hydrophila* and *Aeromonas sobria* still had the highest mortality rates up to 50% even we performed early diagnosis and immediately surgical interventions (4, 12, 14, 16).

The purpose of this study was to evaluate the specific characteristics of *Vibrio vulnificus* and *Aeromonas spp*. necrotizing fasciitis, and to compare the independent predictors related to outcomes between the two groups. We also investigated the risk factors association with mortality in patients with monomicrobial *Aeromonas* necrotizing fasciitis.

Subject And Methods

Study design and patient selection

We retrospectively reviewed the medical records of ninty-one patients with surgically confirmed monmicrobial necrotizing fasciitis caused by *Vibrio vulnificus* and *Aeromonas species* who were admitted to our hospital from July 2009 to December 2019. The cultured specimens, obtained from the wounds or the blood, were confirmed by microbiologic evaluation, and those enrolled patients were categorized into two groups: the *Vibrio vulnificus* group and the *Aeromonas species* group. Patients who had been confirmed polymicrobial necrotizing fasciitis were excluded.

There were 60 patients with *Vibrio vulnificus* (collected from March 2015 to December 2019) and 31 patients with *Aeromonas hydrophila* or *Aeromonas sobria* infection (collected from May 2008 to October 2019) were included. The most common complaints of patients with necrotizing fasciitis were pain and swelling of the involved limbs with edematous, patchy, erythematous, and hemorrhagic bullous skin lesions at the time of admission to the emergency room or at the time of consultation in the hospital ward. Ceftriaxone or ceftazidime combined with doxycycline or other antibiotics were administered in all patients, and the blood cultures were obtained before the antibiotic therapy. The emergent operations of

fasciotomy or limb amputation were performed, and the cultures of tissue specimens were obtained during the operation. Initial empiric antibiotics were continued after first surgery and adjusted based on the results of blood cultures and tissue tests few days later. Soft tissue reconstructions, such as skin grafts and flap reconstruction, were done until the infected necrotic tissue was controlled and stabilized (Figure 1).

Microbiology laboratory procedures

Identification of *Vibrio vulnificus*, *Aeromonas hydrophila* and *Aeromonas sobria* was based on standard phenotypic tests used in clinical microbiology laboratories. All strains were identified to the species level by conventional methods and were further verified by the API-20E and ID 32 GN Systems (bioMérieux Inc., Hazelwood, MO, USA), or the Vitek 2 ID-GNB identification card (bioMérieux Inc., Durham, NC, USA). Antimicrobial susceptibility of *Vibrio vulnificus* and *Aeromonas species* was performed by the hospital microbiology laboratory via the standard disk diffusion technique. These antimicrobial susceptibility tests were performed as recommended by the Clinical and Laboratory Standards Institute (CLSI), and the results were interpreted according to the CLSI criteria for these microorganisms. When the antimicrobial susceptibility test of the isolate revealed resistance in more than one antibiotics, the isolate of the patient was recorded as antibiotic resistance case.

Clinical assessment

The *Vibrio vulnificus* group composed of 41 men and 19 women, and the *Aeromonas* group was composed of 24 men and 7 women. The patients in the *Aeromonas* group were divided to the death subgroup and the survival subgroup. Age, gender, comorbidities, infection site, results of bacteriological tests, laboratory findings at the time of admission, interval between contact and admission, interval between diagnosis and first surgery, length of hospitalization, and clinical outcomes were reviewed for each patient.To assess clinical outcomes after treatment, mortality was defined as death because of progressive sepsis or medical complications within 6 months after first surgery.

Differences in mortality, patient characteristics, clinical presentation, underlying chronic diseases, infection site, first operative procedure, laboratory data and hospital course were compared between the *Vibrio vulnificus* and *Aeromonas species* groups, and between the death and the survival subgroups of *Aeromonas* patients.

Statistical analysis

Statistical analyses were performed with the use of SPSS Version 12.0 statistic software (SPSS, Chicago, Illinois). We used the Student's *t*-test for continuous variables and the Fisher exact test for categorical variables to examine significant relationships between these factors among the groups. A value of $p<0.05$ was considered significant.

Results

Patient outcomes

Culture findings confirmed that the cause of monomicrobial infection was *Vibrio vulnificus* in 60 patients, *Aeromonas hydrophila* in 26 patients, and *Aeromonas sobria* in 5 patients. Seventeen patients died, resulting in an all-cause in-hospital mortality rate of 18.7%: six in the *Vibrio vulnificus* group (10%), and eleven in the *Aeromonas* group (32.3%). The *Aeromonas* group had a significant higher mortality rate than the *Vibrio vulnificus* group (p = 0.005) (Table 1).

Patient characteristics in the *Vibrio vulnificus* group

The mean age of the *Vibrio vulnificus* group was 70.8 years (range, 34 to 95 years). There were 46 patients had reported contacting seawater or handling fish and raw seafood, four patients had injuried in the farm while working, two had acquired abrasion wounds, and 8 patients did not recall any injuries. Six patients died (a mean of 18.5 days after admission), resulting in an all-cause in-hospital mortality rate of 10%.

The estimated period from exposure or injury to presentation at the emergency room ranged from one to three days (mean, 1.72 days) prior to admission. The mean time-interval from treatment in the emergency room to the first operation was 5.03 hours. Thirty-one patients had upper limb skin lesions and 29 had lower limb skin lesions. All patients initially underwent fasciotomy and debridement initially. One patient underwent above-the-knee amputation after a few days due to progressive skin involvement following fasciotomy, and died on the 33th day. Thirty patients received skin grafts, and two patients received flap reconstruction. Twenty-three patients underwent repeated debridement with wound care after initial fasciotomy, and three of them died due to uncontrolled sepsis. Four patients did not undergo any surgery following fasciotomy, and two of them died.

Eleven patients had a history of hepatic dysfunction alone, such as liver cirrhosis, hepatitis B or C, or alcoholic liver disease, and two patients died. Twenty-one patients had hepatic dysfunction with other medical comorbidity, such as diabetes mellitus, chronic kidney disease, cancer, steroid usage, or gout, and three patients died. Four patients with a history of heart disease, such as heart valve insufficiency, hypertension, coronary heart disease, and one patient died. Twenty-two patients had a body temperature of >38.5^{o}C. Twenty-two patients (36.7%) were hypotensive with a systolic blood pressure of $\leq$ 90 mmHg. The mean hospital stay for patients with *Vibrio vulnificus* infection was 38.2 days (range, 2 - 67 days).

All *Vibrio vulnificus* isolates were susceptible to ceftazidime, ceftriaxone, levofloxacin, and tetracycline. *Vibrio vulnificus* specimens were obtained from wounds in 16 cases, from the blood in 18 patients, and from both blood and wounds in 26 patients.

Patient characteristics in the *Aeromonas* group

.The *Aeromonas* group had a mean age of 61.5 years (range, 15 - 85 years). Nine of the *Aeromonas hydrophila* patients, and two of the *Aeromonas sobria* patients died. Nine patients had contact with

seawater or seafood, six patients acquired abrasion wounds while working, and two had previous chronic ulcers of toes. One patient had contact with dirty water in a drain, one had received an injury while working with bamboo on a farm, and twelve did not recall any injuries. Eleven patients died a mean of 11.2 days after admission, and the all-cause in-hospital mortality rate was 32.3%.

The interval from symptoms to presentation at the ER ranged from 1 to 4 days (mean, 1.77 days). The mean time interval between treatment in the emergency room and the first operation was 4.13 hours. One patient had hepatitis C, diabetes mellitus, chronic kidney insufficiency, and heart failure. Ten patients had both hepatic dysfunction and diabetes mellitus Thirteen patients had the history of hepatic dysfunction with or without other comobidity. Three patients had diabetes mellitus with other medical conditions. Four patients had lesions in upper extremity and 26 patients had lesions in lower extremity. One patient had skin lesions on both upper and lower extremities. Thirty patients initially underwent fasciotomy with debridement, and one patient underwent an immediate above-the-knee amputation due to progressive uncontrolled initial sepsis. Eleven patients received skin grafts, and two patients received flap reconstruction. Seven patients underwent repeated debridement with wound care after initial fasciotomy, and two of them died. Nine patients did not perform any secondy surgery, and eight of them died due to uncontrolled sepsis. Ninteen patients (61.3%) had systolic blood pressure of ≤90 mm Hg at presentation to the emergency room, and ten patients died. The mean duration of hospital stay for the *Aeromonas* patients was 30.6 days (range, 2 to 90 days).

Aeromonas specimens were obtained from wounds in 16 cases, from the blood in 2 patients, and from both blood and wounds in 13 patients. The isolates of eleven *Aeromonas* patients were susceptible to amikacin, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, ertapenum, gentamicin, and tetracycline. Twenty *Aeromonas* isolates, including 16 of *Aeromonas hydrophila* and 4 of *Aeromonas sobria*, were resistant to either ertapenum, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, imipenem or ampicillin. Six patients of *Aeromonas hydrophila* and two of *Aeromonas sobria* who revealed antibiotics resistance died.

Comparison of *Vibrio vulnificus* and *Aeromonas* groups

Age, sex, fever, length of hospital stay, interval between contact and admission, interval between diagnosis and the first surgery, total white blood cell counts, banded leukocyte cells, and segmented forms of leukocytes did not differ significantly between the two groups. However, we found that patients with *Aeromonas* infection had a significantly higher incidence of ICU admission, shock status, lower platelet counts, and lower albumin level than patients with *Vibrio vulnificus* infection in the emergency room (Table 1 & 2).

Vibrio vulnificus patients had a significant higher incidence of bacteremia ($p = 0.02$). However, the proportion of *Aeromonas* patients presenting bacteremia associated with death was significantly higher than that of *Vibrio vulnificus* group ($p = 0.0002$). *Aeromonas* isolates had a significant higher incidience

of antibiotics resistance (p = 0.0001). Meanwhile, the *Aeromonas* patients who died were observed to have a significantly higher proportion of antibiotics resistance (p = 0.009), lower lymphocyte counts (p = 0.013), and lower levels of serum albumin (p = 0.008) compared to the *Vibrio* patients who died.

Comparison of Death subgroup and Survival subgroup of *Aeromonas* patients

Age, gender, interval between symptom and admission, interval between diagnosis of necrotizing fasciitis and first surgery, nature of first surgery, fever, antibiotics resistance, white blood cell counts, and segmend forms of leukocyted did not differ significantly between the death and survival subgroups (Table 3 & 4). The death subgroup had a significantly higher incidence of bacteremia (p = 0.001), higher counts of banded leukocytes (p = 0.026), lower platelet counts (p = 0.043), lower lymphocyte count of leukocytes (p = 0.007) and lower levels of serum albumin (p = 0.019) than the survival subgroup of *Aeromonas* NF patients.

Discussion

Monomicrobial gram-negative necrotizing fasciitis had been reported to have more fulminant clinical cources and higher motality than gram-positive necrotizing fasciitis in the past decade (4–11). *Vibrio vulnificus*, *Aeromonas hydrophila*, and *Klebsiella pneumoniae* were the most frequently reported gram-negative aerobic pathogens of necrotizing fasciitis in southwest Taiwan (4, 8, 10–21).

Our previous study revealed the patients with *Vibrio vulnificus* necrotizing fasciitis had higher mortality rate than those with *Aeromonas* necrotizing fasciitis (13). The patients with *Vibrio vulnificus* necrotizing fasciitis can be early identified due to the contact history with seawater or raw seafood when arriving emergency department. However, *Aeromonas* spp. often thrive in fresh or brackish water, sewage, soil, tap water and non-fecal organic materials, and the clinical signs and symptoms of necrotizing fasciitis are characteristically indistinguishable from *Vibrio vulnificus* infection at the time of presentation (13–19, 22, 23). Although we have set up a treatment algorithm for necrotizing fasciitis including early recognition, time interval of less than 6 hours from treatment in the emergency room to the first emergency fasciotomy or amputation, a third-generation cephalosporin plus tetracycline or gentamicin antibiotic therapy, and intensive unit care, we found monomicrobial *Aeromonas* necrotizing fasciitis had higher mortality than *Vibrio vulnificus* infection (32.3% vs. 10%; p = 0.005) in this study. As compared with those risk factors of mortality between two groups, the death patients of *Aeromonas* group have a significantly higher proportion of antibiotics resistance and bacteremia, higher incidence of shock, lower lymphocyte counts, and lower albumin levels than the *Vibrio* patients who died.

Monomicrobial necrotizing fasciitis and bacteremia caused by *Vibrio vulnificus*, *Aeromonas hydrophila* and *Aeromonas sobria* often occurs in patients with liver cirrhosis, hepatitis, alcoholic liver disease and diabetic mellitus (4, 7–19, 22, 23). *Vibrio vulnificus* can produce *V. vulnificus* hemolysin/cytolysin (VVH), *V. vulnificus* serine protease (VvsA) and *V. vulnificus* protease (VVP). VVH contributes to bacterial

invasion from the intestine to the blood stream and plays a significant role in development of hypotensive septic shock. VVP and VvsA are siginificantly produced in the interstitial tissues of limbs, and cause serious collagenolytic, hemorrhagic or edematous skin damage in the extremities through digestion of the vascular basement membrane and vasodilatation (24, 25). *Aeromonas hydrophila* and *Aeromonas sobria* can produce hemolysin, cytolytic enterotoxin, endotoxin, protease, lipases, and four types of secretory systems (types II, III, IV and VI), which are associated with extensive muscular necrosis, gastrointestinal tract infection and septicemia (12, 22, 23, 25, 26). In particular, those virulance factors of *Vibrio vulnificus* and *Aeromonas* species were commonly reported to impair the phagocytic activity of the reticuloendothelial system, and to result in bacterial translocation and bacteremia in patients with hepatic decompensation (22–28). We observed that 56 patients (56/91, 61.5%) were associated with hepatic dysfunction, and 14 patients (14/56, 25%) died. Hence we should pay more attention and treat those *Vibrio* or *Aeromonas* NF patients with hepatitis and liver cirrhosis aggressively.

Although *Vibrio vulnificus* isolates were recorded susceptible to ampicillin, amikacin, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, gentamicin, imipenem, piperacillin, and sufamethoxazole-trimethoprim by clinical microbiology laboratory, many countries demonstrated a small porportion of clinical isolates resistance to penicillin, ampicillin, amoxicillin, streptomycin, cephalexin and erythromycin (29, 30). In a study by Trinh et al., the survival rate was significantly higher with ceftriaxone-doxycycline (91%), ceftriaxone-ciprofloxacin (100%), cefepime-doxycycline (96%), and cefepime-ciprofloxacin (90%) combination therapy for *Vibrio vulnificus* septicemia (31). But otherwise recent literatures indicated that *Aeromonas* spp. increased the resistant abilities to aminoglycosides, carbapenems, and third–generation cephalosporins (23, 32–35). Rhee et al. reported that clinical *Aeromonas* isolates presented high antibiotics resistance rates of 15.5% for ceftriaxone and 15.5% for piperacillin/tazobactam (36). Our study demonstrated that *Vibrio vulnificus* NF patients (44/60, 73.3%) presented a significant higher proportion of bacteremia than the *Aeromonas* NF patients (15/31, 48.4%). However, the mortality rates of *Aeromonas* patients with bacteremia (10/15, 66.7%) was significantly higher than those of *Vibrio vulnificus* group (6/44, 13.6%). In the meanwhile, we found that the blood or wound samples of 20 *Aeromonas* NF patients showed antibiotics resistance to either ertapenum, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, imipenem or ampicillin, and eight of them died. We concluded that our empirical antimicrobial administration of ceftriaxone combined with doxycycline or other antibiotics were adequate and effective for *Vibrio vulnificus* infection, but were ineffective against *Aeromonas* isolates due to their higher resistance rates. Thus, application of the effective antimicrobial agents against *Aeromonas* spp. under the supervision of infectious doctors may improve the outcomes of *Aeromonas* NF patients.

Admission serum albumin levels and total lymphocyte counts (TLC) were used as the markers of nutritional status (37, 38). A serum albumin level < 3.5 g/dL and the TLC < 1500 cells/mm3 are definited as protein energy malnutrition (PEM), and the TLC < 900 cell/mm3 is related to severe malnutrition (37–39). Hypoalbuminemia and low TLC were commonly associated with increased rates of wound healing problems and surgical site infections in total joint arthroplasty, and presented a significantly higher in-hospital mortality in NF patients and cirrhotic patients (4, 13, 19, 38–43). Our previous studies had

demostrated the serum albumin level of ≤ 2.5 g/dL was significantly associated with mortality of NF patients (4, 11, 13, 19). In the present study, both *Vibrio* and *Aeromonas* NF patients showed the mean total lymphocyte counts < 1000 cell/mm3 and correlated with malnutrition, but the *Aeromonas* patients with the albumin level of 2.59 g/dL and TLC of 754.91 cells/mm3 revealed much severer malnutrition status than *Vibrio* NF patients. We also observed that *Aeromonas* patients who died had significantly lower levels of serum albumin and lower lymphocyte counts compared to the *Vibrio* patients who died. On the other hand, those *Aeromonas* necrotizing fasciitis patients had a significantly higher incidence of ICU admission, systolic blood pressure < 90 mmHg at ER, and lower platelet counts than *Vibrio vulnificus* patients, even though the initial clinical courses revealed similarly fulminant in the two groups. Therefore, the above results indicate that monomicrobial *Aeromonas* NF patients experienced more fulminant clinical course and higher mortality rate than those *Vibrio* NF patients.

As compared with the survival subgroup of *Aeromonas* NF patients, the death subgroup had a significantly higher incidence of bacteremia (10/11), higher banded leukocytes forms, lower platelet counts, lower lymphocyte count of leukocytes, and lower levels of serum albumin. Many literatures had confirmed monomicrobial *Aeromonas* bacteremia were associated with high mortality rates (22, 23, 32, 34, 36). The reasons contributing to mortality are the virulance factors of *Aeromonas* spp. not only can cause severe skin necrosis, but also impair phagocytosis and suppress host immune system through bloodstream invasion, especially in patients with severe malnutrition.

There are several limitations to our study. First, the number of monomicrobial *Aeromonas* cases is smaller than *Vibrio* cases because of its rarity in the long study period. We had excluded 25 polymicrobial patients who survived for precise identification of risk factors in monomicrobial *Aeromonas* NF patients. The second limitation of our study was that we did not measure whether these patients had the history of malnutrition before necrotizing fasciitis occurred. PEM is a common cause of secondary immune deficiency and susceptibility to major human infection which were often assiciated with underlying chronic illness (43, 44). There were 69 patients (75.8%) with hepatic dysfunction and/or diabetes mellitus revealed PEM in our study, and nutrition support should be aggressive administration in NF patients initially.

Conclusions

Monomicrobial necrotizing fasciitis caused by *Aeromonas hydrophila* and *Aeromonas sobria* was characterized more fulminating and higher mortality than that of the *Vibrio vulnificus* infection even after early fasciotomy and empiric third-generation cephalosporin antibiotic therapy. High mortality rates were significantly related to those NF patients with hepatic decompensation and malnutrition. The death patients of *Aeromonas* group have a significantly higher proportion of antibiotics resistance and bacteremia, higher incidence of shock, lower lymphocyte counts, and lower albumin levels than the *Vibrio* patients who died. Our study strengthens that early identification those risk factors and surgical intervention for those patients with *Aeromonas* and *Vibrio* NF may improve the survival rates.

Declarations

Abbreviations

NF, Necrotizing fasciitis; ICU, intensive care unit; CLSI, Clinical and Laboratory Standards Institute*; V. vulnificus*, *Vibrio vulnificus*; VVH. *vulnificus* hemolysin/cytolysin; VVP, *Vibrio vulnificus* protease; TLC, total lymphocyte counts; PEM, protein energy malnutrition.

Acknowledgements

The authors thank all the participants who participated in this study.

Availability of data and materials

Please contact author for data requests.

Authors' contributions

YHT: contributed the conception and design of the study and drafting the article. PAY: contributed acquisition of data. TYH: contributed analysis and interpretation of data. CLC and PYC: contributed analysis and interpretation of data. LTK: contributed final approval of the version to be submitted. CTH: participated in its design and coordination. KCH: contributed revising it critically for important intellectual content.

Funding

This work was supported by the Chang Gung Medical Research Program Foundation [CORPG6E0033 to YHT].

Ethics approval and consent to participate

This study protocol was approved by the Institutional Review Board of Chang Gung Medical Foundation((IRB:103-2081B). Consent to participate was not applicable.

Consent for publication

Not applicable

Conflicts of Interest:

All contributing authors declare no conflicts of interest.

References

1. Bellapianta JM, Ljungquist K, Tobin E, et al: Necrotizing fasciitis. J Am Acad Orthop Surg 2009; 17:174-182.
2. Morgan MS: Diagnosis and management of necrotizing fasciitis: a multiparametric approach. J Hosp Infect 2010;75:249-257.
3. Stevens DL, Bryant AE. Necrotizing soft-tissue infections. N Engl J Med 2017;377:2253–65.
4. Tsai YH, Huang KC, Shen SH, et al: Microbiology and surgical indicators of necrotizing fasciitis in a tertiary hospital of southwest Taiwan. Int J Infect Dis 2012; 16:e159-165.
5. Lee A, May A, Obremskey WT. Necrotizing soft-tissue infections: an orthopaedic emergency. J Am Acad Orthop Surg. 2019;27(5):e199-206.
6. Jabbour G, El-Menyar A, Peralta R, et al. Pattern and predictors of mortality in necrotizing fasciitis patients in a single tertiary hospital. World J Emerg Surg 2016; 11: 40.
7. Yahav D, Duskin-Bitan H, Eliakim-Raz N, Ben-Zvi H, Shaked H, Goldberg E, et al. Monomicrobial necrotizing fasciitis in a single center: the emergence of Gram-negative bacteria as a common pathogen. Int J Infect Dis 2014; 28: 13–6.
8. Rahim GR, Gupta N, Maheshwari P, Singh MP. Monomicrobial Klebsiella pneumoniae necrotizing fasciitis: an emerging life-threatening entity. Clin Microbiol Infect. 2019; 25:316–323
9. Park SY, Yu SN, Lee EJ, Kim T, Jeon MH, Choo EJ, Park S, et al. Monomicrobial gram-negative necrotizing fasciitis: An uncommon but fatal syndrome. Diagn Microbiol Infect Dis 2019;94:183–187.
10. Huang TU, Peng KT, Hsiao CT, FannWC, Tsai YH, Li YY, et al. Predictors for gram-negative monomicrobial necrotizing fasciitis in southern Taiwan. BMC Infect Dis 2020; 20:60.
11. Lee CY, Kuo LT, Peng KT, Hsu WH, Huang TW, Chou YC. Prognostic factors and monomicrobial necrotizing fasciitis: gram-positive versus gram-negative pathogens. BMC Infect Dis 2011;11:5.

12. Tsai YH, Shen SH, Yang TY, Chen PH, Huang KC, Lee MS. Monomicrobial Necrotizing Fasciitis Caused by *Aeromonas hydrophila* and *Klebsiella pneumoniae*. Med Princ Pract 2015;24(5):416–23.
13. Tsai YH, Hsu RWW, Huang TJ, et al: Necrotizing soft tissue infections and sepsis caused by Vibrio vulnificus compared with those caused by Aeromonas J Bone Joint Surg Am 2007; 89: 631-636.
14. Tsai YH, Huang KC, Huang TJ, et al: Fatal necrotizing fasciitis caused by Aeromonas sobria in two diabetic patients. Clin Orthop Relat Res 2009; 467; 846-849.
15. Chao CM, Lai CC, Huang SH, Lin SH. Comparison of skin and soft tissue infections caused by Vibrio and Aeromonas species. Surg Infections 2014;15:576-580.
16. Chang CP, Hsiao CT, Lin CN, Fann WC. Risk factors for mortality in the late amputation of necrotizing fasciitis: a retrospective study. World J Emerg Surg. 2018;13:45
17. Huang TY, Hung CH, Lai LJ, Chuang HJ, Wang CC, Lin PT, et al. Implementation and outcomes of hospital-wide computerized antimicrobial approval system and on-the-spot education in a traumatic intensive care unit in Taiwan. J Microbiol Immunol Infect 2018;51(5):672–80.
18. Tsai YH, Hsu RW, Huang KC, Chen CH, Cheng CC, Peng KT, et al. Systemic *Vibrio* infection presenting as necrotizing fasciitis and sepsis. A series of thirteen cases. J Bone Joint Surg Am 2004;86(11):2497–502.
19. Tsai YH, Hsu RW, Huang KC, Huang TJ. Laboratory indicators for early detection and surgical treatment of *Vibrio* necrotizing fasciitis. Clin Orthop Relat Res 2010;468(8):2230–7.
20. Cheng NC, Yu YC, Tai HC, et al : Recent trend of necrotizing fasciitis in Taiwan: focus on monomicrobial Klebsiella pneumoniae necrotizing fasciitis. Clin Infect Dis 2012;55:930-939.
21. Lee SSJ: Klebsiella pneumoniae is an emerging major pathogen in necrotizing fasciitis. Clin Infect Dis 2012;55:940-942.
22. Wu CJ, Chen PL, Tang HJ, et al: Incidence of Aeromonas bacteremia in southern Taiwan: Vibrio and Salmonella bacteremia as comparators. J Microbiol Immunol Infect 2014;47:145-148.
23. Syue LS, Chen PL, Wu CJ, Lee NY, Lee CC, Li CW, et al.Monomicrobial *Aeromonas* and *Vibrio* bacteremia in cirrhotic adults in southern Taiwan-similarities and differences. *J Microbiol Immunol Infect* 2016; 49 (4): 509-515.
24. Kashimoto T, Akita T, Kado T, Yamazaki K, Ueno S. Both polarity and aromatic ring in the side chain of tryptophan 246 are involved in binding activity of *Vibrio vulnificus* hemolysin to target cells. Microb Pathog 2017;109:71–77.
25. Elgaml A, Miyoshi SI. Regulation systems of protease and hemolysin production in *Vibrio vulnificus*. Microbiol Immunol 2017; 61:1–11.
26. Bhowmick UD, Bhattacharjee S. Bacteriological, clinical and virulence aspects of *Aeromonas*-associated diseases in humans. Pol J Microbiol 2018; 67: 137-149.
27. Pessoa RBG , de Oliveira WF , Marques DSC, Correia MTS , de Carvalho EVMM , CoelhoLCBB. The genus *Aeromonas*: a general approach. Microb Pathog 2019; 130: 81-94.

28. Chung PY, Yang TY, Huang TW, Tsai YH, Huang KC, Weng HH. Hepatic disease and the risk of mortality of *ibrio vulnificus* necrotizing skin and soft tissue infections: A systematic review and metaanalysis. Plos One 2019;14:e0223513.
29. Elmahdi S, DaSilva LV, Parveen S. Antibiotic resistance of Vibrio parahaemolyticus and Vibrio vulnificus in various countries: a review. Food Microbiol 2016; 57:128–134.
30. Bier N, Schwarts K, Guerra B, Strauch E. Survey on antimicrobial resistance patterns in Vibrio vulnificus and Vibrio cholera non-O1/non-O139 in Germany reveals carbapenemase-producing Vibrio cholera in coastal waters. Front. Microbiol. 2015; 6:1179.
31. Trinh SA, Gavin HE, Satchell KJF. Efficacy of ceftriaxone, cefepime, doxycycline, ciprofloxacin, and combination therapy for Vibrio vulnificus foodborne septicemia. Antimicrob Agents Chemother. 2017;61:e01106.
32. Nolla-Salas J, Codina-Calero J, Valles-Angulo S, Sttges-Serra A, Zapatero-Ferrandiz A, Climent MC, Gomez J, Masclans JR. Clinical significance and outcome of *Aeromonas* infections among 204 adult patients. *Eur J Clin Microbiol Infect Dis.* 2017;36(8):1393–1403.
33. Aravena-Román M, Inglis TJJ, Henderson B, Riley TV, Chang BJ. Antimicrobial susceptibilities of Aeromonas strains isolated from clinical and environmental sources to 26 antimicrobial agents. Antimicrob Agents Chemother 2011; 56:1110–1112.
34. Tang HJ, Lai CC, Lin HL, Chao CM. Clinical manifestations of bacteremia caused by Aeromonas species in Southern Taiwan. PLoS One 2014;9:e91642
35. Chen PL, Ko WC, Wu CJ. Complexity of β-lactamases among clinical *Aeromonas* isolates and its clinical implications. J Microbiol Immunol Infect 2012: 45: 398-403.
36. Rhee JY, Jung DS, Peck KR. Clinical and therapeutic implications of Aeromonas Bacteremia: 14 years nation-wide experiences in Korea. *Infect Chemother* 2016;48:274–284.
37. Gunarsa RG, Simadibrata M, Syam AF. Total Lymphocyte Count as a Nutritional Parameter in Hospitalized Patients. Indonesian J Gastroenter Hepatol Dig Endosc 2011;12:89-94.
38. Yuwen P, Chen W, Lv H, Feng C, Li Y, Zhang T, et al. Albumin and surgical site infection risk in orthopaedics: a meta-analysis. BMC Surg2017;17:7.
39. Gu A, Malahias MA, Strigelli V, Nocon A., Sculco TP, Sculco PK. Preoperative malnutrition negatively correlates with postoperative wound complications and infection after total joint arthroplasty: a systematic review and meta-analysis. J Arthroplasty2019;34:1013–24.
40. Yi PH, Frank RM, Vann E, Sonn KA, Moric M, Della Valle CJ. Is potential malnutrition associated with septic failure and acute infection after revision total joint arthroplasty? Clin Orthop Relat Res. 2015;473:175–182.
41. Pruzansky JS, Bronson MJ, Grelsamer RP, Strauss E, Moucha CS. Prevalence of modifiable surgical site infection risk factors in hip and knee joint arthroplasty patients at an urban academic hospital. J Arthroplast. 2014;29:272–6.
42. Chang CP, Fann WC, Wu SR, Lin CN, Chen IC, Hsiao CT. Diagnostic performance of initial serum albumin level for predicting in-hospital mortality among necrotizing fasciitis patients. J Clin Med

2018;7:435.

43. Maharshi S, Sharma BC, Srivastava S. Malnutrition in cirrhosis increases morbidity and mortality. J Gastroenterol Hepatol. 2015;30:1507–1513.
44. Schaible UE, Kaufmann SHE. Malnutrition and infection: Complex mechanisms and global impacts. PLoS Med 2007; 4: e115.

Tables

Table 1. Comparison Between *Vibrio vulnificus* Group and *Aeromonas* Group for Charcteristics at First Consultation and Treatment

Variable		*Vibrio vulnificus* Group (N=60)	*Aeromonas* Group (N=31)	P value
Age (years)	Mean	70.8	61.5	0.2
Sex				0.26
Male		41	24	
Female		19	7	
Mortality rate (%)		10	32.3	0.005*
Death		6	11	
Survivals		54	20	
Timing From Contact to Presentation at ER (days)	Mean	1.72 ±0.38	1.77 ±0.51	0.34
Timing From First Consultation to First Operation (hours)	Mean	5.03 ±3.28	4.13 ±3.55	0.23
First Operation				
Fasciotomy (death)		60 (6)	30 (10)	
Amputation (death)		0 (0)	1 (1)	
Final Operation				
Amputation (death)		1 (1)	2 (1)	
Split-thickness skin graft (death)		30 (0)	11 (0)	
Flap (death)		2 (0)	2 (0)	
Debridement (death)		23 (3)	7 (2)	
Without secondary operation (death)		4 (2)	9 (8)	
Underlying Chronic disease (death)				
Hepatic dysfunction and DM		6 (0)	10 (2)	
Hepatic dysfunction and DM with others		5 (1)	1 (0)	
Hepatic dysfunction alone		11 (2)	7 (3)	
Hepatic dysfunction with others		10 (2)	6 (4)	
Diabetes Mellitus alone		7 (0)	0 (0)	
Diabetes mellitus with others		3 (0)	3 (1)	
Steroid intake		3 (0)	1 (1)	
Gout with/without others		6 (0)	1 (0)	
Heart disease		4 (1)	1 (0)	
None		5 (0)	1 (0)	
Wound location				
Upper extremity (death)		31 (2)	4 (0)	
Lower extremity (death)		29 (4)	26 (11)	
Upper & lower extremity (death)		0 (0)	1 (0)	
ICU admission		36	26	0.03*
Hospital days	Mean	38.02 ±18.12	30.61±24.35	0.1

* mean $p < 0.05$ and the difference was significant

Table 2. Comparison Between the *Vibrio vulnificus* Group and *Aeromona* Group for Clinical Findings and Laboratory data at First Consultation in the ER

		Vibrio vulnificus Group (N=60)	*Aeromona* Group (N=31)	P value
Shock at first consultation		22	19	0.03*
Deaths		3	10	0.02*
Survivals		19	9	
Fever at first consultation		22	10	0.81
Deaths		3	2	
Survivals		19	8	
Positive culture				
Wound		16	16	
Blood		18	2	
Wound and Blood		26	13	
Presentation of bacteremia		44	15	0.02*
Deaths with bacteremia		6	10	0.0002*
Antibiotic resistance cases		0	20	0.0001*
Deaths with antibiotics resistant		0	8	0.009*
Deaths without antibiotics resistant		6	3	
White Blood Cell Count (cells/mm3)	Mean	14495.0 ± 9502.3	12309.7± 9478.9	0.3
Deaths		7616.7 ± 4952.7	9454.6 ± 7165.5	0.58
Survivals		15259.3 ± 9604.7	13880.0 ± 10369.6	0.59
Band Forms(%)	Mean	7.41 ± 7.99	11.15 ± 9.65	0.052
Deaths		11.5 ± 9.35	16.27 ± 9.97	0.35
Survivals		6.92 ± 7.82	8.33 ± 8.43	0.5
Segmented Forms (%)	Mean	78.8 ± 13.1	74.6 ± 11.3	0.13
Deaths		66.8 ± 11.7	69.8 ± 13.7	0.66
Survivals		80.1 ± 12.6	77.3 ± 9.0	0.35
Lymphocyte count (cells/mm3)	Mean	984.01 ± 630.18	754.91 ± 720.81	0.12
Deaths		780.33 ± 510.50	302.86 ± 194.38	0.013*
Survivals		1006.65 ± 642.19	1003.54 ± 785.56	0.98
Platelet Count (per mm3)	Mean	146550 ± 60446	104000 ± 69037	0.003*
Deaths		91333 ± 30878	70454 ± 49092	0.36
Survivals		152685 ± 59949	122450 ± 72437	0.07
Albumin (g/dL)	Mean	3.52 ± 0.52	2.59 ± 0.76	0.001*
Deaths		3.03 ± 0.53	2.16 ± 0.58	0.008*
Survivals		3.57 ± 0.50	2.82 ± 0.76	0.001*

Shock at first consultation: Systolic blood pressure ≦90mm Hg; Fever at first consultation: Body temperature ≥38.5°C.

* mean two-tailed $P<0.05$ and the difference was significant

Table 3. Comparison Between the Deaths and Survivals of Aeromonas patients for Basic Characteristics and Surgical Outcome

Variable	Deaths (n = 11)	Survivals (n = 20)	p Value
Age (mean years)	62.4	62.3	0.99
Gender			0.52
Male	9	15	
Female	2	5	
Timing from symtom to presentation in ER (mean days)	1.91	1.7	0.45
Timing from diagnosis at ER to first operation (mean hours)	4.36	4	0.79
Underlying chronic disease			
Hepatic dysfunction & DM	2	8	
Hepatic dysfunction & DM with CKD	0	1	
Hepatic dysfunction alone	3	4	
Hepatic dysfunction with others	4	2	
Diabetes mellitus with others	1	2	
Steroid intake	1	0	
Gout	0	1	
Heart disease	0	1	
None	0	1	
Wound location			
Upper extremity	0	4	
Lower extremity	11	15	
Upper and lower extremities	0	1	
First operation			0.355
Fasciotomy	10	20	
Amputation	1	0	
Final operation			
Amputation	1	1	
Split-thickness skin graft	0	11	
Flap	0	2	
Debridement	2	5	
Without secondary operation	8	1	
Systolic blood pressure (mm Hg)			0.014*
≦ 90	10	9	
> 90	1	11	
Body temperature (℃)			0.202
>38.5	2	8	
<38.5	9	12	
Hospital days (mean)	11.2	41.3	0.0003*

* mean $p < 0.05$ and the difference was significant

Table 4. Comparison Between the Deaths and Survivals of *Aeromonas* NF patients for Bacteriological Findings and Laboratory data at First Consultation in the ER

		Deaths (N=11)	Survivals (N=20)	P value
Pathogen				
Aeromonas hydrophilia		9	17	
Aeromonas sobria		2	3	
Positive culture				
Wound		1	15	
Blood		0	2	
Wound and Blood		10	3	
Presentation of bacteremia		10	5	0.001*
Antibiotic resistance cases				
With antibiotics resistant		8	12	0.69
Without antibiotics resistant		3	8	
White Blood Cell Count (cells/mm3)	Mean	9454.6 ± 7165.5	13880.0 ± 10369.6	0.22
Band Forms(%)	Mean	16.27 ± 9.97	8.33 ± 8.43	0.026*
Segmented Forms (%)	Mean	69.8 ± 13.7	77.3 ± 9	0.078
Lymphocyte count (cells/mm3)	Mean	302.86 ± 194.38	1003.54 ± 785.56	0.007*
Platelet Counts (per mm3)	Mean	70454 ± 49092	122450 ± 72437	0.043*
Albumin (g/dL)	Mean	2.16 ± 0.58	2.82 ± 0.76	0.019*
≦2		5	2	0.037*
>2		6	18	

* mean $P<0.05$ and the difference was significant

Figures

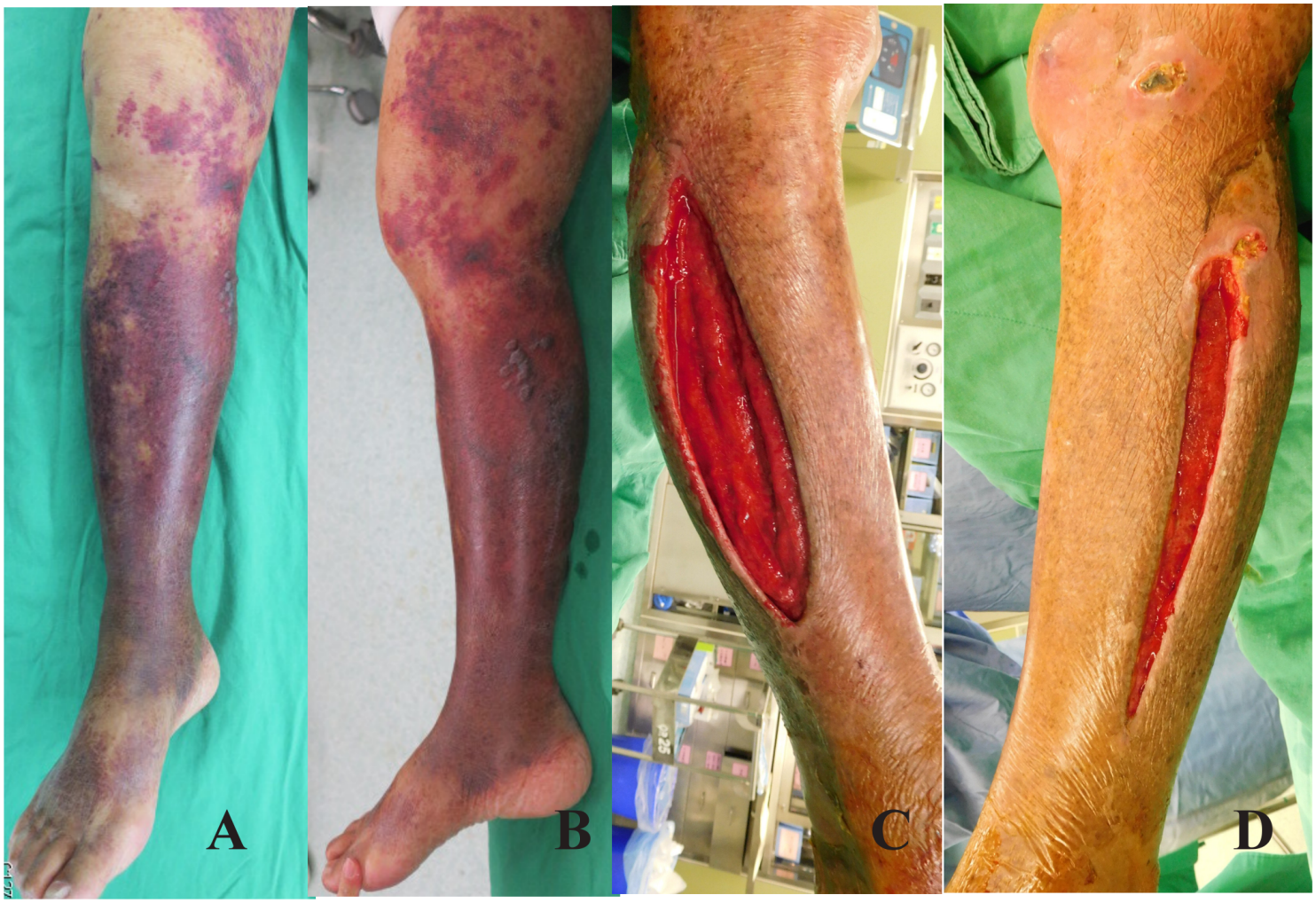

Figure 2

A 78-year-old male with a history of hepatitis C and liver cirrhosis had left leg pain and swelling for 2 days with unknown causes. (A)(B) Preoperative photographs of left lower leg revealed severe patchy purpura and edema in the emergency room. (C)(D) After emergency fasciotomy, the blood and wound cultures confirmed the presence of Aeromonas sobria. He had received skin graft on the 59th day after fasciotomy and discharged on 70th day.