

## Antibiotic therapy for necrotizing fasciitis caused by *Vibrio vulnificus*: retrospective analysis of an 8 year period

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Received 14 March 2011; returned 18 June 2011; revised 17 October 2011; accepted 18 October 2011

**Objectives:** To compare the effectiveness of a third-generation cephalosporin alone, a third-generation cephalosporin plus minocycline, and a fluoroquinolone in patients with necrotizing fasciitis (NF) caused by *Vibrio vulnificus*.

**Methods:** A retrospective review of case notes was performed for 89 patients who presented with NF caused by *V. vulnificus* and underwent surgical intervention within 24 h of admission between 2003 and 2010. Data on comorbidities, clinical manifestations, laboratory studies, treatments and outcomes were extracted for analysis. These patients were grouped according to the antimicrobials prescribed: those who received only a third-generation cephalosporin (Group 1;  $n=18$ ); a third-generation cephalosporin plus minocycline (Group 2;  $n=49$ ); or a fluoroquinolone with/without minocycline (Group 3;  $n=22$ ).

**Results:** The mean age of the 89 patients included in the study was  $64.0 \pm 12.0$  years (range 33–89 years); 55% of the patients were male. There were no differences in age, sex or clinical characteristics among the three groups except that patients in Group 3 had a higher frequency of underlying chronic renal insufficiency than those in Groups 1 and 2 ( $P=0.009$ ). Groups 2 and 3 each had a significantly lower case fatality rate than Group 1 (61% in Group 1 versus 14% in Group 2,  $P=0.0003$ ; 61% in Group 1 versus 14% in Group 3,  $P=0.0027$ ), while no difference in case fatality rate was noted between Groups 2 and 3.

**Conclusions:** Our data suggested that, in addition to primary surgery, fluoroquinolones or third-generation cephalosporins plus minocycline are the best option for antibiotic treatment of NF caused by *V. vulnificus*.

**Keywords:** cephalosporins, fluoroquinolones, treatment effectiveness, mortality

### Introduction

*Vibrio vulnificus* is a motile, halophilic, rod-shaped, Gram-negative pathogen commonly found in warm estuarine environments. *V. vulnificus* infections in humans are infrequent and sporadic, but life threatening. *V. vulnificus* infections, mainly

manifesting as skin or soft tissue infections and/or septicemia,<sup>1,2</sup> can develop a fulminant course, which is associated with bacterial expression of toxins and enzymes, including capsular polysaccharides, metalloproteases, lipopolysaccharides and cytolysin.<sup>3–8</sup> If not promptly suppressed by eliminating the pathogen, the infection can rapidly exacerbate and progress to

the development of advanced skin or soft-tissue involvement. The severe form of *V. vulnificus* soft tissue infection, necrotizing fasciitis (NF), often leads to adverse consequences or even death within 24 h of admission,<sup>2,9–12</sup> particularly if associated with the development of sepsis or septic shock, with reported case fatality rates ranging from 26% to 71%.<sup>10–16</sup>

Antibiotic and surgical interventions (debridement, fasciotomy and/or amputation) are the main approach for treating these severe infections.<sup>11,15–17</sup> Early and aggressive surgery is one of the two key factors related to an optimal outcome of NF caused by this microbial pathogen because the necrotic tissue has an insufficient blood supply to achieve sufficient concentrations of any antimicrobial agent. The role of antibiotic therapy, the other key for the prognosis of NF, is to eradicate the viable pathogens in the inflamed but still well-perfused tissue, thus ensuring that NF does not spread further. A variety of antibiotic agents appear to be effective at killing *V. vulnificus*, including erythromycin, tetracycline, cephalosporins, minocycline and extended-spectrum penicillins, both *in vivo* and *in vitro*.<sup>18–20</sup> However, combined antibiotic therapy employing third-generation cephalosporins plus tetracycline or its analogues possesses a synergistic effectiveness against *V. vulnificus* infection and is more effective than any single-agent therapy with the aforementioned antibiotics for serious infections, as based on published clinical reports.<sup>7,8,11,15,16,21,22</sup> Recently, fluoroquinolones have been reported to be effective in animal studies.<sup>7,23</sup> However, these studies either did not take into account the surgical effect or lacked confirmation of their clinical effectiveness.

Over the past decade it has become common practice for medical centres in Taiwan to prescribe advanced classes of antibiotics, including third-generation cephalosporins (with or without tetracycline or its analogues) and fluoroquinolones to patients with severe *V. vulnificus* infections.<sup>7,8,11,15,16</sup> The optimal therapy for serious invasive infections caused by *V. vulnificus* is difficult to ascertain in a prospective trial, hence careful retrospective analysis of patient data is the best remaining option for the determination of optimal therapy. Therefore, we conducted this retrospective study to compare the clinical effectiveness of third-generation cephalosporins alone, third-generation cephalosporins plus tetracycline or its analogues, and fluoroquinolones for the treatment of NF caused by *V. vulnificus*.

## Patients and methods

### Study subjects and settings

Between January 2003 and December 2010, 192 consecutive patients aged >18 years who were diagnosed with *V. vulnificus* infections and hospitalized in the Chi Mei Medical Center (CMMC; a 2300 bed primary and tertiary teaching hospital) or the Chung Shan Medical University Hospital (CSMUH; a 1200 bed primary and tertiary teaching hospital) were included and a systematic review of each patient's records was performed. NF caused by *V. vulnificus* was diagnosed if the following conditions were met: (i) infected lesions were confirmed as NF by histopathological examination; and (ii) *V. vulnificus* was isolated from blood and/or wound cultures. During this study period, 93 patients (16 from CSMUH and 77 from CMMC) met these criteria. Among them, three patients had concomitant gastrointestinal symptoms on admission and did not have bacterial isolates from their stool cultures. Early surgical treatment (surgical intervention <24 h after admission) has been reported as an important prognostic

factor for NF caused by *V. vulnificus*.<sup>10–16,24</sup> Based on this rationale, we included 89 patients who had received surgical treatment within 24 h of admission from the 93 *V. vulnificus*-infected patients with NF who had their diagnosis confirmed by histopathological examination. Four patients, including one with a decision against surgical treatment and three with a subsequent surgical intervention at >24 h due to a delayed diagnosis, were excluded from our analysis. The remaining 89 patients were grouped according to antimicrobials prescribed: those who received only third-generation cephalosporin (Group 1), third-generation cephalosporin plus minocycline (Group 2) and fluoroquinolones with/without minocycline (Group 3). This study was approved by the Research Ethics Committee of each hospital.

### Data collection

We collected clinical and laboratory information, including demographic data, microbiological findings, clinical presentations and course, treatments administered and outcomes. The *V. vulnificus* isolates identified by conventional methods were further verified using the API-20E system (bioMérieux Vitex Inc., Hazelwood, MO, USA).<sup>25</sup> Antimicrobial susceptibility testing was performed using the Kirby-Bauer, broth dilution and Etest methods.<sup>25</sup> Initial empirical broad-spectrum antibiotics were given intravenously after the blood, wound or stool specimens were obtained. Antimicrobials were subsequently tailored, if necessary, based on the results of cultures or susceptibility tests and infection severity. Among these patients, those presenting with sepsis but having no other obvious source of infection were regarded as having primary septicæmia, while those with a recent history of wound exposure to seawater/marine creatures or a recent injury from handling seafood were considered to have primary wound infections.<sup>9,26,27</sup> The severity of illness on admission was evaluated with the first-day Acute Physiology and Chronic Health Evaluation (APACHE) II score.<sup>28</sup> Sepsis and septic shock were defined according to the International Sepsis Definitions Conference criteria.<sup>29</sup> Criteria for intensive care unit admission used at the hospitals were based on recommendations of the American College of Critical Care Medicine and the Society of Critical Care Medicine.<sup>30</sup> Case fatality was defined as death during hospitalization.

### Statistical analysis

Descriptive data are presented as means with standard deviations for continuous data and percentages for categorical data. Continuous variables were compared among groups with the use of one-way analysis of variance. When the *F* test was significant, *post hoc* comparison procedures with Scheffe's method were used. Categorical variables were compared by either the  $\chi^2$  test or Fisher's exact test if the expected value of at least one cell was <5. All statistical tests were two-tailed, with  $P < 0.05$  denoting statistical significance, and were performed using SAS version 8.2 (SAS Institute, Cary, NC, USA).

## Results

### Baseline characteristics among the three antibiotic treatment groups

The mean age of the 89 patients included in the study was  $64.0 \pm 12.0$  years (range 33–89 years); 55% of the patients were male. The mean APACHE II score on admission was  $13.1 \pm 5.0$ . The prescribed third-generation cephalosporins in Group 1 included ceftazidime ( $n=2$ ), cefotaxime ( $n=4$ ) and ceftriaxone ( $n=12$ ). The combination antibiotic therapy in Group 2 was ceftazidime plus minocycline ( $n=40$ ) or ceftriaxone plus minocycline ( $n=9$ ). Ciprofloxacin with minocycline ( $n=7$ ) or

without minocycline ( $n=15$ ) was prescribed for patients in Group 3. There were no differences in clinical characteristics among the three antibiotic treatment groups except chronic renal insufficiency ( $P=0.009$ ) and initial antibiotic treatment ( $P<0.0001$ ). Patients in Group 3 had a higher frequency of underlying chronic renal insufficiency than those in Groups 1 and 2. There was no difference in frequency of the initial treatment with either fluoroquinolones or third-generation cephalosporins plus minocycline among the three groups (44%, 51% and 59% in Groups 1, 2 and 3, respectively;  $P=0.647$ ) in spite of the difference in initial antibiotic class given among the groups. The baseline characteristics of the three groups are shown in Table 1.

### Patient outcomes among the three groups

Ten patients subsequently needed limb amputation(s); two of these patients eventually died. Seventy-two patients needed

intensive care; seventeen of these eventually died in the intensive care unit. There was no difference in the frequency of limb amputation or intensive care needed among the three groups. In Group 3 there was no difference in the case fatality rate between the patients treated with ciprofloxacin alone and those with ciprofloxacin plus minocycline (13% [2/15] versus 14% [1/7];  $P=1.000$ ). Both Groups 2 and 3 had a significantly longer hospital stay than Group 1, while no difference in length of hospitalization was noted between Groups 2 and 3. Twenty-one patients died during their hospitalization; the non-survival group had a significantly shorter hospital stay than the survival group ( $3.9\pm 3.8$  days versus  $28.4\pm 18.4$  days;  $P<0.0001$ ). Groups 2 and 3 each had a significantly lower case fatality rate compared with Group 1, while no difference in the case fatality rate was noted between Groups 2 and 3 (Figure 1). In each treatment group the case fatality rates did not significantly differ among the time periods (from years 2003–04 to years

**Table 1.** Baseline characteristics in the three antibiotic treatment groups ( $n=89$ )

Variable	Group 1 <sup>a</sup> ( $n=18$ )	Group 2 <sup>b</sup> ( $n=49$ )	Group 3 <sup>c</sup> ( $n=22$ )	<i>P</i>
Gender, male, no. (%)	8 (44)	25 (51)	16 (73)	0.175
Age (years, mean $\pm$ SD)	68.9 $\pm$ 9.2	62.5 $\pm$ 12.0	63.2 $\pm$ 13.4	0.148
APACHE II score (mean $\pm$ SD)	13.9 $\pm$ 4.9	12.7 $\pm$ 4.4	13.5 $\pm$ 6.3	0.601
Interval between symptom onset and treatment given (days, mean $\pm$ SD)	1.6 $\pm$ 0.9	1.4 $\pm$ 0.7	1.3 $\pm$ 0.8	0.229
Origin of infection, no. (%)				0.664
primary septicaemia	5 (28)	19 (39)	7 (32)	
wound infection	13 (72)	30 (61)	15 (68)	
Underlying disorders, <sup>d</sup> no. (%)				
diabetes mellitus	3 (17)	13 (27)	7 (32)	0.545
hepatic disorders <sup>e</sup>	5 (28)	14 (29)	5 (23)	0.873
chronic renal insufficiency	2 (11)	4 (8)	8 (36)	0.009
malignancy	0	5 (10)	1 (5)	0.301
Clinical presentations and laboratory findings, <sup>d</sup> no. (%)				
lesions involving $\geq 2$ extremities	1 (6)	5 (10)	0	0.277
blood pressure $<90/60$ mmHg	9 (50)	24 (49)	11 (50)	0.995
WBC count $>1.2\times 10^4$ or $<4000$ cells/mm <sup>3</sup>	13 (72)	28 (57)	17 (77)	0.201
haemoglobin $<14$ g/dL in males, or $<12$ g/dL in females	11 (61)	25 (51)	11 (50)	0.730
AST $>40$ IU/L	13 (72)	29 (59)	9 (41)	0.127
serum creatinine $>1.3$ mg/dL	11 (61)	26 (53)	14 (64)	0.661
serum albumin $<3.5$ mg/dL	7 (39)	12 (25)	9 (41)	0.290
Time of surgical intervention after admission (hours, mean $\pm$ SD)	11.1 $\pm$ 8.4	10.1 $\pm$ 7.5	10.0 $\pm$ 6.7	0.847
Initial antibiotic treatment, no. (%)				$<0.0001$
penicillin group or first/second-generation cephalosporin +/- aminoglycoside	1 (6)	21 (43)	9 (41)	
third-generation cephalosporin	9 (50)	3 (6)	0	
third-generation cephalosporin with minocycline (or analogue)	6 (33)	11 (22)	6 (27)	
fluoroquinolones	2 (11)	14 (29)	7 (32)	

APACHE: Acute Physiology and Chronic Health Evaluation; AST: aspartate aminotransferase; SD: standard deviation; WBC: white blood cell.

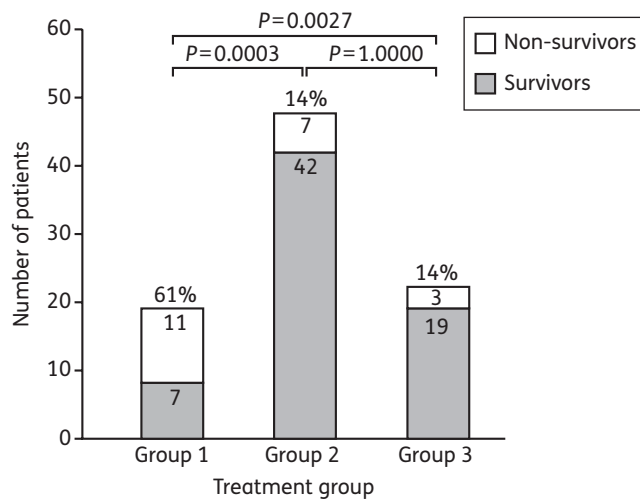
<sup>a</sup>Group 1: 18 patients were treated with only third-generation cephalosporin (ceftazidime, ceftriaxone or cefotaxime).

<sup>b</sup>Group 2: 49 patients were treated with third-generation cephalosporin (ceftazidime or ceftriaxone) plus minocycline.

<sup>c</sup>Group 3: 22 patients were treated with fluoroquinolone (ciprofloxacin) with or without minocycline.

<sup>d</sup>When patients fit into multiple categories, they were counted in each category.

<sup>e</sup>Included chronic hepatitis B, chronic hepatitis C, alcoholic hepatitis, liver cirrhosis and hepatocellular carcinoma.



**Figure 1.** Case fatality rates for the antibiotic treatment groups. Group 1 received a third-generation cephalosporin (ceftazidime, ceftriaxone or cefotaxime) alone. Group 2 received a third-generation cephalosporin (ceftazidime or ceftriaxone) plus minocycline. Group 3 received a fluoroquinolone (ciprofloxacin) with or without minocycline.

2009–10). The outcomes of these patients are summarized in Table 2.

## Discussion

This study demonstrated that the combination of third-generation cephalosporins plus minocycline was superior to single-agent therapy consisting of a third-generation cephalosporin for NF caused by *V. vulnificus*, a finding consistent with the report by Liu *et al.*<sup>11</sup> Furthermore, fluoroquinolones exhibited a similar mortality risk compared with third-generation cephalosporins plus minocycline and appeared to be better than third-generation cephalosporins alone for treating patients with NF caused by *V. vulnificus*, which was not described in the report of Liu *et al.*<sup>11</sup> and only found in previous animal experiments. To date, this is the first clinical report to assess the therapeutic effectiveness of antimicrobial therapy against NF caused by *V. vulnificus* infection.

While *V. vulnificus* has been reported to be susceptible to several antimicrobials based upon *in vitro* experiments,<sup>18–20</sup> antibiotics do not reach therapeutic levels at the site of infection due to necrosis and thrombosis of blood vessels supplying the affected areas, especially in the case of NF;<sup>17</sup> therefore, primary surgical intervention combined with optimal antibiotic therapy is essential. The choice of antibiotics with good tissue penetration seems to be important for treating patients with *V. vulnificus*-related NF. Recent animal experiments showed that fluoroquinolones (e.g. ciprofloxacin, moxifloxacin, levofloxacin and so on) have good tissue penetration abilities with very low MICs, which helps them to accumulate in phagocytes and inflammatory lesions and may increase their potency in clinical use.<sup>7,23</sup> Additionally, several *in vitro* studies have disclosed that exposure of many Gram-negative pathogens to antibacterial agents can result in both endotoxin and cytokine release, and this may worsen the outcomes in septic hosts,<sup>31–34</sup> though the

**Table 2.** Outcomes in the three antibiotic treatment groups (n=89)

Variable	Group 1 <sup>a</sup> (n=18)	Group 2 <sup>b</sup> (n=49)	Group 3 <sup>c</sup> (n=22)	P
Limb amputation needed, no. (%)	1 (6) <sup>d</sup>	6 (12) <sup>e</sup>	3 (21) <sup>f</sup>	0.684
ICU needed, <sup>g</sup> no. (%)	13 (72)	41 (84)	18 (82)	0.567
Hospital stay (days, mean ± SD)	10.5 ± 13.2	24.6 ± 21.1	28.1 ± 15.7	0.008 <sup>h</sup>
Fatality, no. (%)	11 (61) <sup>i</sup>	7 (14) <sup>j</sup>	3 (14) <sup>k</sup>	<0.0001

ICU: intensive care unit; SD: standard deviation.

<sup>a</sup>Group 1: 18 patients were treated with only third-generation cephalosporin (ceftazidime, ceftriaxone or cefotaxime).

<sup>b</sup>Group 2: 49 patients subsequently treated with third-generation cephalosporin (ceftazidime or ceftriaxone) plus minocycline.

<sup>c</sup>Group 3: 22 patients were treated with fluoroquinolone (ciprofloxacin) with or without minocycline. One of the seven patients receiving ciprofloxacin plus minocycline eventually died.

<sup>d</sup>The patient with below-knee amputation died of multiple organ failure.

<sup>e</sup>One patient with amputation of toes, three patients with amputation of fingers and two patients with foot amputation.

<sup>f</sup>One patient with amputation of toes and two patients with foot amputation. One of the two patients with foot amputation died of sepsis.

<sup>g</sup>Seventeen patients admitted to the ICU, including seven in Group 1, seven in Group 2 and three in Group 3, eventually died.

<sup>h</sup>Group 1 versus Group 2,  $P=0.026$ ; Group 1 versus Group 3,  $P=0.027$ ; Group 2 versus Group 3,  $P=0.930$ .

<sup>i</sup>Case fatality rate in years 2003–04, 2005–06, 2007–08 and 2009–10 were 60% (3/5), 50% (3/6), 75% (3/4) and 67% (2/3), respectively; the case fatality rates did not differ among these time periods ( $P=1.000$ ).

<sup>j</sup>Case fatality rate in years 2003–04, 2005–06, 2007–08 and 2009–10 were 17% (2/12), 10% (1/10), 13% (2/15) and 17% (2/12), respectively; the case fatality rates did not differ among these time periods ( $P=1.000$ ).

<sup>k</sup>Case fatality rate in years 2003–04, 2005–06, 2007–08 and 2009–10 were 14% (1/7), 17% (1/6), 0% (0/4) and 20% (1/5), respectively; the case fatality rates did not differ among these time periods ( $P=1.000$ ).

stress response of *V. vulnificus* when exposed to antibiotics remains unclear. Fluoroquinolones have been reported to have a low endotoxin-release potential and exhibit an immunomodulatory effect on septic hosts by attenuating the proinflammatory response *in vitro* and in animal models.<sup>35–37</sup> The use of fluoroquinolones could thus theoretically benefit patients infected with *V. vulnificus* in such situations. However, there is a lack of clinical data in humans to support this possibility. Our findings provide important clinical evidence that fluoroquinolones, which appear to be as efficacious as the combination of third-generation cephalosporin and minocycline, may qualify as a rational alternative choice for the antibiotic treatment of patients with *V. vulnificus*-related NF.

It should be acknowledged that our results are limited by the retrospective design of this study. The sporadic occurrence of *V. vulnificus* infections in humans makes conducting a clinical trial for determining the antimicrobial efficacy for *V. vulnificus* infection with NF during a finite study period extremely difficult. Hence some potential confounding factors should be taken into

account in our analysis. In our study, the non-survivors had a significantly shorter mean hospital stay (~4 days) than survivors; this is why Group 1 (with a higher case fatality rate) had a shorter hospitalization compared with Groups 2 and 3, which also indicates the fulminant course of this infection. In this potentially life-threatening infection by *V. vulnificus*, urgent surgical intervention within 24 h after admission is likely to enhance prognosis and play an essential role in reducing bacterial burden, improving blood supply and saving lives.<sup>10–16,24</sup> This is why our study subjects were restricted to patients having surgery within 24 h of admission, which was aimed at standardizing the surgical influence on the analysis of antibiotic efficacy, thus making the results more reliable. The *V. vulnificus*-infected patients with NF herein may not represent a clinically homogeneous population. The APACHE II score is well evaluated and widely accepted for assessing the disease severity in patients;<sup>28,29</sup> a greater APACHE II score has been reported to be associated with *V. vulnificus* mortality and is more representative and comprehensive than individual laboratory parameters for predicting mortality in patients with severe *V. vulnificus* infections.<sup>38</sup> The APACHE II score and other potential confounding factors, including history of liver disease, the presence of primary septicemia, hypoalbuminaemia, lesions involving two or more extremities, and the duration between illness onset and the treatment given,<sup>2,7–16</sup> which have been documented to be related to poor prognosis for patients infected with *V. vulnificus*, did not differ significantly among the three treatment groups. Some of the patients receiving ciprofloxacin were simultaneously treated with minocycline in our study. Although it is unclear whether a synergistic effect between fluoroquinolones and minocycline exists, the similar mortality risk herein between the patients treated with ciprofloxacin and those treated with ciprofloxacin plus minocycline, in conjunction with the lack of published evidence of an interaction between these antibiotics, makes it more likely that the effect is small. Further studies are required to validate this hypothesis. In addition, our study included a relatively large number of *V. vulnificus*-infected patients with NF,<sup>10–16,24</sup> which provided an important retrospective analysis for the evaluation of antibiotic effectiveness for this serious infection.

In conclusion, given the high case fatality rates associated with NF secondary to *V. vulnificus* infection, we recommend that clinicians initiate a prompt surgical intervention and simultaneously prescribe either fluoroquinolone or third-generation cephalosporins plus minocycline after admission when a presumptive diagnosis for this infection is made.

## Acknowledgements

We gratefully thank Professors H.-S. Lee, Ph.D. and R.-H. Wong, Ph.D., School of Public Health, Chung Shan Medical University, for help with the medical statistics.

## Funding

This study was carried out as part of our routine work.

## Transparency declarations

None to declare.

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