

Clinical Usefulness of Real-Time Polymerase Chain Reaction for the Diagnosis of *Vibrio vulnificus* Infection Using Skin and Soft Tissues

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Abstract. *Vibrio vulnificus* is a halophilic gram-negative bacillus isolated in seawater, fish, and shellfish. Infection by *V. vulnificus* is the most severe food-borne infection reported in the United States of America. Here, we aimed to examine the clinical usefulness of polymerase chain reaction (PCR) using tissue specimens other than blood samples as a diagnostic tool for *V. vulnificus* infection. A retrospective study was conducted with patients who underwent real-time PCR of *toxR* in both blood and skin tissues, including serum, bullae, swab, and operation room specimens, between 2006 and 2009. The median *V. vulnificus* DNA load of 14 patients in real-time PCR analysis of serum at the time of admission was 638.5 copies/mL blood, which was within the interquartile range (IQR: 37–3,225). In contrast, the median value by real-time PCR using the first tissue specimen at the time of admission was 16,650 copies/mL tissue fluid (IQR: 4,419–832,500). This difference was statistically significant ($P = 0.022$). DNA copy numbers in tissues were less affected by short-term antibiotic administration than that in blood samples, and antibiotic administration increased the DNA copy number in some patients. We found, for the first time, that DNA copy numbers in tissues of patients infected by *V. vulnificus* were higher than those in blood samples. Additionally, skin lesions were more useful than blood samples as specimens for PCR analysis in patients administered antibiotics for *V. vulnificus* infection before admission.

INTRODUCTION

Vibrio vulnificus is a halophilic gram-negative bacillus that can be isolated from seawater, fish, and shellfish. Consumption of raw fish and shells that are contaminated or exposure to contaminated seawater results in *V. vulnificus* infection, which can cause primary septicemia or wound infection and gastroenteritis.¹ *Vibrio vulnificus* infection is the most severe disease among food-borne infections reported in the United States of America.²

Rapid and appropriate initial antimicrobial treatment can reduce mortality caused by *V. vulnificus* infection; therefore, rapid diagnosis is critical for achieving good clinical outcomes.³ Because *V. vulnificus* grows well in standard blood culture medium or nonselective medium (such as blood agar) routinely used for wound cultures, *V. vulnificus* is easily diagnosed after it has been cultured. However, *V. vulnificus* is also susceptible to most antibiotics and can be highly affected by antibiotic administration,⁴ which may complicate diagnosis. Polymerase chain reaction (PCR) has been used for the rapid diagnosis of *V. vulnificus* by targeting *V. vulnificus*-specific genes. In this respect, real-time PCR has been reported as a highly sensitive and specific diagnostic method for *V. vulnificus* infection in clinical practice.^{5,6}

Although quantification using real-time PCR has been shown to be correlated with disease severity or mortality in patients with *V. vulnificus* infection,⁷ no studies have examined the clinical utility of PCR using tissue specimens other than blood samples. Accordingly, in this study, we aimed to investigate the clinical usefulness of real-time PCR using

bullae, swab, and surgical specimens for patients suspected to have *V. vulnificus* infection.

MATERIALS AND METHODS

Of patients admitted to Chosun University Hospital between 2006 and 2009 who had real-time PCR testing for targeting of the *V. vulnificus*-specific *toxR* as previously described,⁶ including prospective research data on adults 18 years or older who had skin and soft tissue infections, such as cellulitis or necrotizing fasciitis, and who had blood and skin tissue culture tests,⁸ patients with skin and soft tissue infection who had real-time PCR analysis of both serum and skin tissues, including bullae, swab, and surgical specimens, at the time of admission were subjected to retrospective chart analysis of the presence or absence of antibiotic administration before admission.^{5,6} Bacterial species were identified using a VITEK 2 automated system (BioMérieux, Marcy-L'Etoile, France) in our hospital.

The study was approved by the Ethics in Human Research Committee of Chosun and Chonnam University Hospital.

Statistical analyses of all test items were carried out using Statistical Package for the Social Sciences (SPSS) for Windows, version 17.0 (SPSS Korea, Seoul, Korea). *Vibrio vulnificus* DNA copy numbers in serum and tissues, such as bullae and surgical specimens, were compared using the Wilcoxon signed rank test, a nonparametric paired *t* test. Differences with *P* values of below 0.05 were considered statistically significant.

RESULTS

A total of 14 patients were found to have at least one positive result from real-time PCR with both skin and blood specimens. Of these patients, the mean age was 58.9 years, and all but one patient was male (Table 1).

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TABLE 1
Clinical characteristics and diagnostic results in patients infected by *Vibrio vulnificus*

No. Age/Sex	Underlying disease	Lesion site	Interval A (day)	Antibiotic administration before admission	Positive culture	Antibiotics	Result	Interval B (day)	Tissue	qPCR (CP)	qPCR (Quantitation)
1	LC	Left leg	1	No	B (+)	Cefotaxime,	Death	0	Serum	30.55	1,380
62/M					W (+)	doxycycline		0	Bulla	18.55	1,950,000
2	LC	Right leg	3	Yes	B (-)	Cefotaxime,	Recovery	2	Serum	> 40	
50/M	DM				W (+)	doxycycline		3	Bulla	17.76	9,250,000
3	LC	Both legs	1	No	B (+)	Cefotaxime,	Recovery	2	Serum	34.8	16
81/M					B (+)	ciprofloxacin		4	Fascia	25.61	5,580
4	No	Both legs	0	No	B (+)	Cefotaxime,	Death	1	Serum	36.16	43.8
50/M					B (+)	ciprofloxacin		2	Bulla	28.34	937
5	MDS	Right arm	1	No	B (+)	Nafcillin,	Recovery	0	Serum	33.65	1,170
65/F					W (-)	ciprofloxacin		1	Bulla	23.41	460,000
6	Chronic alcohol drinker	Both ankles	2	No	B (+)	Vancomycin,	Recovery	0	Serum	34.31	500
57/M					W (-)*	meropenem		0	Swab	24.27	13,400
7	Chronic alcohol drinker	Both legs and buttock	0	No	B (-)	Cefotaxime,	Death	0	Serum	27.16	15,400
73/M					Lt. W (-)	ciprofloxacin		0	Left Bulla	30.33	253
8	HBV	Right leg	1	No	Rt. W (+)		Death	1	Right Bulla	17.84	913,000
42/M					B (-)	Cefotaxime,		0	Serum	31.11	2,300
9	Chronic alcohol drinker	Right leg	1	No	B (+)	ciprofloxacin	Death	0	Swab	23.82	18,000
48/M					W (+)	Cefotaxime,		0	Serum	30	6,000
10	LC	Both legs	2	Yes	B (-)	ciprofloxacin	Death	0	Swab	> 40	
60/M					B (-)	Cefotaxime,		2	Serum	32.75	63.6
11	LC, HTN	Left leg	2	No	W (-)	ciprofloxacin		2	Bulla	20.96	118,000
62/M		Left leg and wrist			B (-)	Cefotaxime,	Recovery	0	Serum	33.07	777
12	LC	Both legs	7	?†	W (-)	doxycycline		2	Bulla	23.06	330,000
74/M					B (-)	Cefotaxime,		0†	Serum	26.16	55,400
13	DM, chronic alcohol drinker	Leg	4	Yes	W (-)	doxycycline	Recovery	1†	Bulla	29.79	7,230
53/M					W (-)			2	Bulla	20.41	1,200,000
14	LC, HTN	Left leg	3	Yes	B (-)	Ceftriaxone,	Recovery	3	Serum	> 40	15,300
48/M					B (-)	doxycycline		3	Bulla	26.06	
					W (-)	Cefotaxime,	Recovery	1	Serum	32.47	76.5
					W (-)	doxycycline		2	Fascia	13.74	13,400,000

B = blood; COPD = chronic obstructive pulmonary disease; DM = diabetes mellitus; HB = hepatitis B; HC = hepatitis C; LC = liver cirrhosis; qPCR = quantitative polymerase chain reaction; W = wound; MDS = myelodysplastic syndrome; HBV = hepatitis B virus infection; HTN = Hypertension. Interval A, time from symptom onset to antibiotic administration; Interval B, time from antibiotic administration to sample collection.

* Performed wound culture test after 1 day.

† It was unclear whether antibiotics were administered before admission; PCR was performed using serum at the time of admission, a bulla sample on the day after admission, and a bulla sample at 2 days after admission.

Six of the 14 patients died (mortality rate: 42.9%). Of the 14 patients, bacteria were identified in blood or skin specimens from nine patients, and bacteria were cultured in both skin and blood samples from two of these nine patients and in either skin or blood samples from seven of these nine patients (four patients had blood cultures only and three patients had skin cultures only). Of the six patients whose blood culture tests identified *V. vulnificus* at the time of admission, three patients had follow-up blood culture tests around 24 hours, which did not yield cultured bacteria. In real-time PCR analysis of serum samples collected at the time of admission, the median *V. vulnificus* DNA load of 14 patients was 638.5 copies/mL blood (interquartile range [IQR]: 37–3,225). In contrast, the median real-time PCR value using the first tissue specimen at the time of admission was 16,650 copies/mL tissue fluid (IQR: 4,419–832,500); this difference was statistically significant ($P = 0.022$).

Of the 14 patients in this study, four had received antibiotics before admission, and nine were admitted without antibiotic administration; the status of the final patient was not clear. The mean serum DNA copy number of patients without antibiotic administration was $9,134 \pm 18,028$ copies/mL, whereas that of patients with antibiotic administration before admission was 35 ± 41 copies/mL. Additionally, the DNA copy number in tissues of patients without antibiotic administration was $272,822 \pm 646,606$ copies/mL, whereas that of patients with antibiotic administration was $5,695,825 \pm 3,358,658$ copies/mL.

Two patients (cases 2 and 13) were positive by real-time PCR using bullae samples but negative using blood samples. These patients had undergone antibiotic administration for 2 or 3 days before admission, respectively, and were then transferred to our hospital. In patient 2, blood culture tests did not identify any bacteria; however, culture tests with aspiration of bulla yielded cultured bacteria, and the DNA copy number was 9,250,000 copies/mL tissue fluid, showing a high copy number even after administration of antibiotics for 2 days. Patient 13 was treated for cellulitis at a local clinic for 3 days and was then transferred for bullae developed; in this patient, both skin and blood cultures were negative, and *V. vulnificus* DNA was not detected by PCR using blood samples but was detected (at 15,300 copies/mL) in bulla aspirates, despite the lack of detection of cultured bacteria from bulla aspirates.

Of the six patients whose blood samples were shown to have *V. vulnificus* in culture, no patients had received antibiotics for 24 hours or more prior to undergoing blood culture tests.

Of the five patients (10–14) for whom *V. vulnificus* was not identified in blood or skin samples, three patients had undergone antibiotic administration before admission, and one patient, for whom it was not clear whether antibiotics had been administered, was admitted after treatment at a local clinic 1 day before admission. Although *V. vulnificus* was not identified in blood and skin samples from these five patients, high DNA copy numbers were identified in the blood or bullae, particularly in bullae (Table 1).

For case 12, the DNA copy number from real-time PCR using bullae 1 day after admission was 7,230 copies/mL; this number increased to 1,200,000 copies/mL in bullae tests performed after an additional day of antibiotic

administration. For case 7, 253 DNA copies/mL were identified in bullae of the left leg in PCR analysis performed immediately after admission, whereas the copy number in the bullae of the right leg increased to 913,000 copies/mL after antibiotics were administered for an additional day.

DISCUSSION

Vibrio sepsis induced by *V. vulnificus* is a severe disease that causes rapidly progressing septic shock syndrome and is associated with mortality rates of 50% or more within a few days. Therefore, in cases of *V. vulnificus* infection, it is critical to administer antibiotics with a high antibacterial activity against *V. vulnificus* soon after rapid diagnosis.^{8,9} Because real-time PCR results can be obtained within 2 hours of testing, PCR can be used for rapid diagnosis. *toxR*, which is often used as a target gene, encodes a transmembrane DNA binding regulatory protein in *Vibrio* species and has been shown to have 100% specificity for *Vibrio* species in real-time PCR analyses.^{5,6} Moreover, in previous studies, PCR of *toxR* was shown to yield higher DNA copy numbers in patients who died of *V. vulnificus* sepsis than in patients who survived; thus, high DNA copy number by PCR is a clinically useful predictive factor for prognosis during the early stage of *V. vulnificus* infection.⁷ However, although skin or blood cultures should be performed during the early stages of infection for rapid diagnosis, many patients with *Vibrio* sepsis are admitted after antibiotic administration, and no studies have examined the usefulness of skin lesions as specimens for culture or PCR tests. Accordingly, in this study, we used blood and skin samples to determine the utility of skin samples as PCR specimens in the early diagnosis of *V. vulnificus* infection.

Of the six patients who were found to have *V. vulnificus* in blood cultures at the time of admission, follow-up blood culture tests were performed for three patients after 24 hours, yielding no bacteria. Moreover, according to a chart data analysis of 23 patients in whom *V. vulnificus* was identified in blood culture tests after admission to our hospital, eight patients had additional blood culture tests performed about 24 hours after antibiotic administration; these data showed that no patients remained positive for *V. vulnificus* after 24 hours (data not shown). Therefore, it is highly unlikely that the bacteria could be identified from blood culture tests in patients who have already received antibiotics, particularly tetracyclines or quinolones, before admission.

In the present study, a total of six patients were either admitted after antibiotic administration or had PCR tests performed using serum specimens after antibiotic administration. Of these patients, four were found to have *V. vulnificus* DNA in serum samples. Moreover, nine patients who underwent PCR using tissues and had received antibiotics before and after admission showed considerable numbers of DNA copies, even after antibiotic administration. Interestingly, cases 7 and 12 had higher DNA copy numbers in PCR tests performed after 1 and 2 days of antibiotic administration at our hospital than at initial admission. Therefore, these data showed that specimens from patients who were admitted after administration of antibiotics, particularly bullae or surgical specimens, were useful for diagnosis by PCR.

The present study showed that tissues had higher DNA copy numbers than blood samples. Therefore, we suggest that *V. vulnificus* may accumulate in lesions within tissues rather than in the blood. From our data, we indirectly speculated that tissues may have higher amounts of bacteria than blood. Additional studies comparing the amounts of bacteria present in blood and tissue samples are needed. Moreover, studies should clarify whether more DNA may be released immediately after antibiotic administration in tissue.

In conclusion, the present study showed, for the first time, that there were higher *V. vulnificus* DNA copy numbers in tissue samples than in blood samples from patients infected with *V. vulnificus*. Additionally, our findings suggested that skin lesions were more useful than blood samples for PCR-based diagnosis of *V. vulnificus* infection in patients admitted after administration of antibiotics active against *V. vulnificus*.

Received June 22, 2016. Accepted for publication February 13, 2017.
Published online May 8, 2017.

Financial support: This work was supported by a grant from the Clinical Medicine Research Institute of the Chosun University Hospital (2015).

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