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Case report

A case of *Vibrio vulnificus* related wound infection diagnosed by next-generation sequencing

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ABSTRACT

Vibrio vulnificus is a serious opportunistic human pathogen, which can cause primary septicemia, wound infection and gastroenteritis. In this case, wound and blood culture failed to grow the pathogen and a next-generation sequencing method was used to detect the pathogen as *V. vulnificus*.
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Introduction

Vibrio vulnificus infection can be an acute and rapidly fatal disease. The organism is a halophilic, Gram-negative bacillus found in warm estuarine water [1]. *V. vulnificus* usually infects people through two distinct mechanisms of entry: consumption of raw contaminated seafood (especially raw oysters) or exposure to seawater or seafood products [2,3]. People with immunocompromising conditions, especially chronic liver disease, alcoholism and hemochromatosis, have a higher risk of severe infection [4]. Interestingly, males, accounting for 86% *V. vulnificus* cases, are six times more likely to acquire *V. vulnificus* infection than females. Besides, *V. vulnificus* tends to infect older males (>40 years of age) with underlying risk conditions [5].

According to the clinical spectrum of disease, *V. vulnificus* infection can be divided into three types: primary septicemia, wound infection and gastroenteritis. The primary septicemia usually results from the consumption of contaminated seafood, which is the most lethal scenario with a mortality rate exceeding 50%. Characteristics of this disease include fever, chills, hypotensive septic shock, and typical skin lesions of cellulitis with ecchymosis and hemorrhagic bullae. Wound infection mainly results from handling contaminated seafood or exposing open wounds to water. Like primary septicemia, wound infection can progress rapidly to cellulitis, ecchymoses, and bullae, which can progress to necrotizing fasciitis and secondary septicemia. However, the mortality rate of wound infection (ca. 25%) is lower than that for primary septicemia [6–8]. Therefore, it is important to make an early diagnosis based on clinical symptoms, relevant examination, the likelihood of an infectious pathogen and start empiric therapy as soon as possible. However, prompt and proper diagnosis is not easy, especially when the pathogen is not identified through wound and blood culture. In this study, we used next-generation sequencing (NGS) method to detect the pathogen.

Presentation of case

A 55-year-old man presented to the emergency department with high fever, chills and excruciating pain in his left fifth finger at 8 p.m. He reported that he unexpectedly punctured his left fifth finger from a fin of Japanese sea perch when he selected fish for lunch at 6 a.m in the market. By 5 p.m, the patient felt pruritus of the finger and it began to swell. The patient went to the local hospital immediately. The doctor of the local hospital suggested further treatment at a referral hospital. By 7 p.m, the patient developed a high fever with chills and felt intense pain of his left fifth finger. His temperature was 103.3 °F when he arrived at the hospital. Cellulitis was not restricted to the primary wound site, extending from the finger to the upper arm. Blood testing revealed a white blood cell count of 12,690 cells/mm^3 and a ALT count of 103 U/L.

An emergency room physician thought that it was an skin and soft tissue infection possibly caused by a marine bacterium. He was

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treated with imipenem, but the temperature did not drop after treatment. The emergency room physician consulted immediately with an infectious disease specialist who was expert in *V. vulnificus* infection. Considering the history of exposing to seafood, infectious disease specialist gave priority to the waterborne pathogens, including Vibrio species and Aeromonas hydrophila, both of which can cause rapidly progressive infections similar to those seen in this case. The antibacterial drugs were transitioned to levofloxacin combined with piperacillin-tazobactam.

The following morning, the patient went into Burns and Wound Center for further treatment. Laboratory studies revealed a white blood cell count of 20,290 cells/mm^3 with 90% polymorphonuclear leukocytes, hemoglobin of 13.7 g/dL, platelets of 138,000 cells/mm^3, and C-reactive protein of 156 mg/L. The left fifth finger showed redness, swelling, tenderness and tense blisters (Fig. 1). Capillary filling test was negative and distal sensation was dysesthetic. The patient was treated with biapennem and levofloxacin. At the same time, the wound was coated with silver sulfadiazine cream and skin protectant. One day after admission, Gram stain of the blister fluid was negative. Three days after admission, wound culture of the blisters sample was negative for aerobic and anaerobic bacteria and fungi. One week after admission, three sets of blood cultures were still negative and laboratory studies basically returned to normal. The redness, swelling and pain of the finger were improved and the skin lesions did not progress to deep necrotic fasciitis (Fig. 2). His tissue sample was sent for pathogen detection by NGS at BGI-Shenzhen. Three days later, NGS detected the pathogen and confirmed *V. vulnificus* infection (Fig. 3). The result of polymerase chain reaction(PCR) was consistent with the findings of NGS (Fig. 4). On the two weeks of hospitalization, the left fifth finger of the patient had improved and the patient was discharged from hospital (Fig. 5). After two weeks, the left fifth finger was back to normal.

Discussion

V. vulnificus can be isolated from blood, exudates, blisters and cerebrospinal fluid, which is the gold standard of clinical diagnosis. After the institution of antibacterial agents, the positive rate of blood cultures is significantly reduced, while tissue or blister culture may still be positive. In this patient, blood culture and wound culture failed to identify the pathogen, which may be related to our early use of antibacterial agents, especially levofloxacin. As a result, we decided to try other ways to detect the pathogen.

Compared with the traditional culture method, polymerase chain reaction (PCR) for the diagnosis of *V. vulnificus* infection has made great progress due to its higher sensitivity and specificity. Blood and wound cultures should be sent during the early stages of infection for rapid diagnosis, however, many patients have already received antibacterials before admission, which may increase the negative rate of culture and complicate diagnosis. We found that skin lesions were more useful than blood samples as specimens for PCR analysis for *V. vulnificus* infection in patients with antibacterial administration before admission. The study showed that *V. vulnificus* DNA copy numbers in skin lesions were higher and less affected by antibacterial administration than those in blood samples [9]. In recent years, the next-generation sequencing, a new innovative technology, has gradually been applied into clinical laboratory testing for cancer, hereditary disorders and detecting microbial organisms. NGS is able to detect thousands of genetic variants in a single test run, while PCR employs a “one-gene one-test” approach by using specific sets of primers to detect one specific mutation. It is an emerging and valuable diagnostic method when other approaches fail to identify an organism or polymicrobial infecition, which creates precision and personalized medicine for infectious diseases [10]. In the present case, the patient had accepted antimicrobial treatment before administration and *V. vulnificus* was not identified in wound and blood cultures. His tissue sample was sent for pathogen detection and the result of NGS had confirmed the infection of *V. vulnificus*.

Prompt treatment with appropriate antimicrobial is essential for the best prognosis. It has already been demonstrated that the use of quinolone alone or the combination of tetracycline and third generation cephalosporin had the lowest mortality and should be the priority selection for *V. vulnificus* infection [11,12]. However, *V. vulnificus* infection may be increasingly difficult to treat as the inappropriate use of antimicrobials has increased resistance.

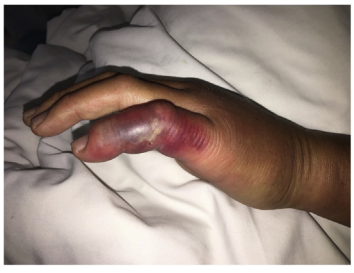

Fig. 1. On day 2. The left fifth finger showed redness, swelling, ecchymosis and tense blisters.

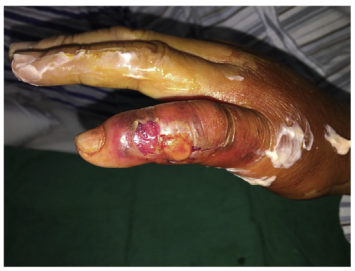

Fig. 2. On day 7. Redness and swelling gradually dissipated in about one week and the fundus was scarlet when blisters were removed.

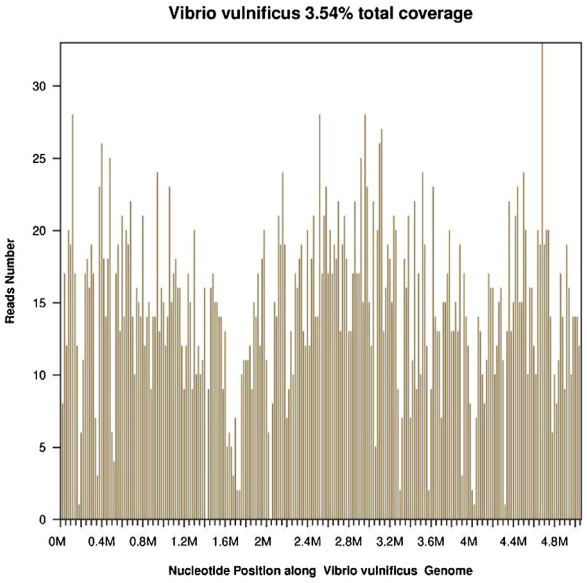

Fig. 3. Reads mapped to *V. vulnificus* derived from NGS data. A total of 3114 reads mapped to *V. vulnificus* in the reference database which contains 2473 bacterial genomes, and got a total coverage of 3.54%.

Fig. 4. PCR detection of *V. vulnificus* from tissue sample. Lane M: TAKARA DL2000. Lane 1: PCR product from tissue sample. Lane 2: negative control.

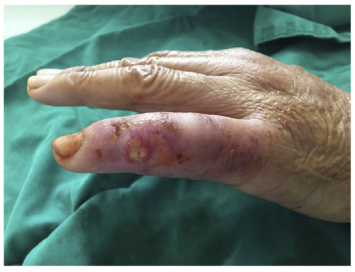

Fig. 5. On day 14. The left fifth finger was ultimately back to normal.

Therefore, antimicrobial should be customized in different countries as the first-line antimicrobial agent according to the recommended treatment and the reported resistance profile [13]. In our case, the patient was treated with biapennem and levofloxacin during hospitalization, which prevented the disease from getting worse. The antimicrobial resistance profile of *V. vulnificus* in China showed that the isolates were sensitive to piperacillin-tazobactam, imipenem, and levofloxacin, which indirectly proved the effectiveness of our treatment. Because of our early effective treatment, aggressive surgical debridement or amputation was not used in this patient.

Conclusion

Next-generation sequencing technology has its own unique advantages for infectious diseases, especially when routine examinations fail to find pathogens. This new technology will be gradually applied into the clinic to benefit more patients.

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Declarations of interest

None.

Author's statement

Linhui Li: Writing - original draft; Lingyan Wang: Writing - review & editing; Chunhong Zhang: Supervision; Peng Chen: Resources ; Xu Luo: Conceptualization.

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