Infection and Drug Resistance

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CASE REPORT

A Case Report of Cutaneous Anthrax Diagnosed by Using a Metagenomic Next-Generation Sequencing (mNGS) Approach

Yushan Liu[1,2], Gezhi Zheng[1,3], Jing Li[1,3], Nan Yang[1–4], Juan Li[1,2], Zhengwen Liu[1–4], Qunying Han[1–4], Yingren Zhao[1–4], Fenjing Du[1,3], Yingli He[1,*], Taotao Yan[1,*]

[1]Department of Infectious Diseases, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, People's Republic of China; [2]Institution of Hepatology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, People's Republic of China; [3]Clinical Research Center for Infectious Diseases, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, People's Republic of China; [4]Shaanxi Clinical Research Center for Infectious Diseases, Xi'an, Shaanxi, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yingli He; Taotao Yan, Department of Infectious Diseases, The First Affiliated Hospital of Xi'an Jiaotong University, No. 277 Yanta Road(w), Xi'an, Shaanxi, People's Republic of China, Tel +86 189-9123-2863; +86 186-9148-5175, Email heyingli2000@xjtu.edu.cn; yantaotao_sweet@163.com

Abstract: Anthrax is caused by *Bacillus anthracis*. Humans are mainly infected through contact with the fur and meat of livestock. The cutaneous form is the most common form. The skin lesions of typical cutaneous anthrax are characterized by shallow ulcers with black crusts, surrounded by small blisters and nonpitting edema of nearby tissues. Metagenomic next-generation sequencing (mNGS) is a new pathogenic detection method which is rapid and unbiased. We reported the first case of cutaneous anthrax diagnosed by mNGS. Ultimately, the man received prompt antibiotic therapy and had a good prognosis. In conclusion, mNGS is proved to be a good method for etiological diagnosis, especially for rare infectious diseases.

Keywords: cutaneous anthrax, metagenomic next-generation sequencing, mNGS, case report, etiology diagnosis

Introduction

Anthrax is a zoonotic disease of herbivores caused by sporulating gram-positive *Bacillus anthracis*. This disease is common in people who have frequent contact with livestock such as herdsmen, veterinarians and slaughter workers.[1] *B. anthracis* has the potential to become a biological weapon that could lead to a public health security threat.[2] Anthrax is also a global disease and there are approximately 20,000 to 100,000 cases of anthrax reported each year.[3] In the past 10 years, the annual reported incidence of anthrax in China has been less than 400 cases. A total of 2907 cases of anthrax were reported from 2012 to 2021 in China. Cases were mainly distributed in western and northeastern China, and the five provinces with the highest incidence were Guizhou, Xinjiang, Gansu, Sichuan, and Qinghai provinces. People are infected with anthrax mainly through the following routes: cutaneous, inhalation, ingestion, or injection. The cutaneous form is the most common with a prevalence of 95%.[4]

According to the diagnostic criteria of CDC, an established case of anthrax is defined as clinically compatible with isolation of *B. anthracis* or with at least two positive supporting tests by serological or other confirmatory assays.[5,6] Culture results are positive in 60% to 65% of cases,[7] and traditional bacterial culture is time-consuming. Routine culture or real-time PCR is most commonly used to diagnose.[2] Metagenomic Next-Generation Sequencing (mNGS) is a new assay, providing accurate DNA/RNA detection through high-throughput and rapid detection. This allows detection of all potential pathogens (bacteria, viruses, fungi and parasites) in clinical samples. In the last few years, mNGS has been proved to be a new method for the diagnosis of some rare pathogens such as scrub typhus[8] and kala-azar.[9] As far as our knowledge by searching NCBI, there are no studies using mNGS to diagnose anthrax. Previously published anthrax case reports mostly relied on culture and smear for diagnosis.[1,10–12] Here, we reported the first case of cutaneous anthrax diagnosed by mNGS. On the basis of the clinical

Received: 30 March 2023
Accepted: 24 May 2023
Published: 7 June 2023

features of this patient's skin lesions, we considered the possibility of cutaneous anthrax. We did the blister fluid cultures and smears at the same time mNGS was done. 16 hours later, the positive results of mNGS confirmed our inference. However, a bacterial smear was negative. Bacteria culture of blister fluid presented same consequent after 5 days. As we did not have any other etiology other than mNGS, this patient was defined as a suspected case according to the criteria of the CDC.[5] Finally, the patient received timely treatment and had a good prognosis during our follow-up.

Case Report

A 37-year-old male sought medical attention with a complaint of rash on the right forearm for 5 days and fever for 3 days. At first, several erythematous discrete papules were observed on right forearm, and the papules became blisters and enlarged rapidly in 2 days. Afterwards, the blisters were bleeding and ruptured, accompanied by obvious swelling of the right upper limb, severe pain, chills and fever, and the highest temperature was 39°C. He felt marked fatigue and poor food intake but had no shortness of breath or cough. He was admitted to the emergency department of the first affiliated hospital of Xi'an Jiaotong University on August 14, 2022. The patient, born in Zhouzhi County, Xi'an, Shaanxi, a farmer by profession, had bought two cattle 2 months earlier and had personally fed them. There have been previous cases of anthrax reported in the county of Zhouzhi.[13] The cattle are in good health and there were no similar clinical signs in the surrounding people. He had two damaged skin in the forearm 10 days before the onset of the disease. The man had no history of any trauma or insect bites. The vital signs of the patient were not stable, with a pulse of 128 beats/min, a respiratory rate of 20 times per minute, and a body temperature of 38.1 °C. The right front chest and the back of the skin were congestive (Figure 1A). The forearm skin was redness with numerous fuchsia blisters and partially ruptured (Figure 1B). The right wrist joints, elbow joints were swollen. The right upper extremity had high muscle tone and limited movement. A 4cm×4cm×4cm lymph node was palpable in the right axilla, which was enlarged, firm, and poorly mobile.

Figure 1 (**A**) The congestive right chest. (**B**) Purple and faint yellow blisters in right forearm. (**C**) Swollen soft tissue of the chest wall and swollen lymph nodes (the area circled) on the right armpit in CT. (**D**) Swollen right forearm and elbow joint.

Table 1 mNGS Results of the Patient's Skin Blister

Genus			Species			
Type	**Pathogen**	**Reads**	**Pathogen**	**Confidence Level**	**Reads**	**Coverage**
G+ bacteria	*Bacillus*	985	*Bacillus anthracis*	High	952	33.42%

Laboratory tests showed for WBC count (25.01×10^9/L), neutrophils (93.2%), along with C-reactive protein (149.8mg/L) and procalcitonin (0.992μg/L) levels. There is no inflammation of the lungs on a CT scan of the chest, and enlarged axillary lymph nodes are seen. (Figure 1C). CT scan of the right upper extremity shows signs of infection in the soft tissues of the skin (Figure 1D).

An infectious disease specialist consulted the patient and diagnosed anthrax as the most likely disease, based on the patient's clinical features and historical epidemiology. The patient was immediately admitted to infectious department and got quarantined.

We extracted skin blister, which was used to do pathogen detection, included PACEseq metagenomic next-generation sequencing (mNGS) (Hugobiotech, Beijing, China). The skin blister of patient was collected to extract nucleic acid (QIAamp DNA Micro Kit), which was used for library construction (QIAseq™ Ultralow Input Library Kit) and high-throughput sequencing on an Illumina Nextseq platform. The filtered raw data removed human reads by mapping reads to human reference genome using SNAP software and compared to the microbial Genome Database (ftp://ftp.ncbi.nlm.nih.gov/genomes/) using Burrows-Wheeler Alignment to obtain the final microbial composition of the samples. mNGS was detected *Bacillus anthracis* (952 reads), accounting for 33.42% of nucleotide sequence coverage (Table 1). The bacterial culture and smear results were negative. Given the high sequence number and coverage, we considered the presence of *Bacillus anthracis* infection in this patient in the clinical context.[14] 1.6 million IU penicillin sodium 4 times a day and ciprofloxacin 200 mg 2 times a day was given intravenously. To reduce inflammation, 5 mg of dexamethasone was given for 5 days. We performed a dressing change for the local wound. In order to reduce the edema of the patient's forearm, we asked the patient to raise the limb. We also administered fluids for correction of electrolyte imbalance and adequate nutritional support. The next day, the patient's condition continued to progress, with a rise in temperature and the development of new blisters (Figure 2A).4 days later, the patient's temperature returned to normal, and the swelling and pain of the right upper reduced (Figure 2B). Axillary lymph nodes were significantly shrunk. 9 days later, there was a marked reduction in the number of blisters on the forearm, and some skin began to scab (Figure 2C). After 14 days, the

Figure 2 (**A**) New dark purple and faint yellow blisters developed, some of which were ulcerate at day 2. (**B**) The blister fluid ruptured and formed shallow ulcers at day 4. (**C**) The number of fluid blisters decreased and some began to scab at day 9. (**D**) Black crusts formed and began to fall off at day 14. (**E**) Some crusts were exfoliated at day 28.

swelling of the limb was significantly reduced and the activity was good (Figure 2D). The eschar began to fall off and gradually recovered (Figure 2E).

Discussion

A cattle farmer had a sudden onset of papules with rapid progression to pustules, accompanied by the presentation of febrile axillary lymphadenopathy. Cutaneous anthrax lesions often arise on exposed areas such as the face, neck, and hands. The characteristic lesion is a painless ulcer with serotonin secretion surrounded by extensive edema that is not proportional to the size of the lesion and later forms a black anthrax in the center. Systemic symptoms may manifest as malaise, hypothermia and localized lymph node enlargement, which is usually mild. Although the province of Shaanxi in China has been an area with a low prevalence of anthrax, there have been sporadic reports of cases in recent years. From 1970 to 2015, 97 cases of anthrax have been reported in the patient's home county of Zhouzhi.[13] In view of those, the possibility of cutaneous anthrax needs to be considered. The diagnosis of anthrax requires a combination of epidemiological history and clinical presentation, as well as pathogen support including culture, PCR and serological testing. There were no positive clues in the etiological smear, and the culture results generally took 3–5 days. PCR testing and ELISA of anthrax needed to be sent to the provincial CDC. By searching the literature, we learned that mNGS had been used to diagnose some pathogens.[8,9,15–17] To identify the pathogen and exclude the possibility of other infections rapidly, we gave priority to sending mNGS for detection with the consent of the patient and his family.

We repeatedly sent the pathogenic smear and culture, but did not find positive results. Why do cultures negative but mNGS positive results occur? We analyzed the reasons for this situation. Probably *B. anthracis* is very sensitive to antibiotics, but the patient was treated with piperacillin sodium and tazobactam antibiotics in the emergency room.

In addition to the use of antibiotics and dexamethasone, we also performed regular dressing changes for local skin lesions. The patient's local skin lesions were significantly improved, and the infectious inflammatory indicators were significantly decreased after standard treatment (Figure 3).

The fatality rate of cutaneous anthrax is up to 20% if left untreated and only 1% if treated.[18] The key to reducing mortality is early diagnosis and appropriate antibiotic treatment. The judgment of professional infectious doctors and sensitive diagnostic methods are particularly critical. mNGS is a rapid, precise, and specific method for identifying

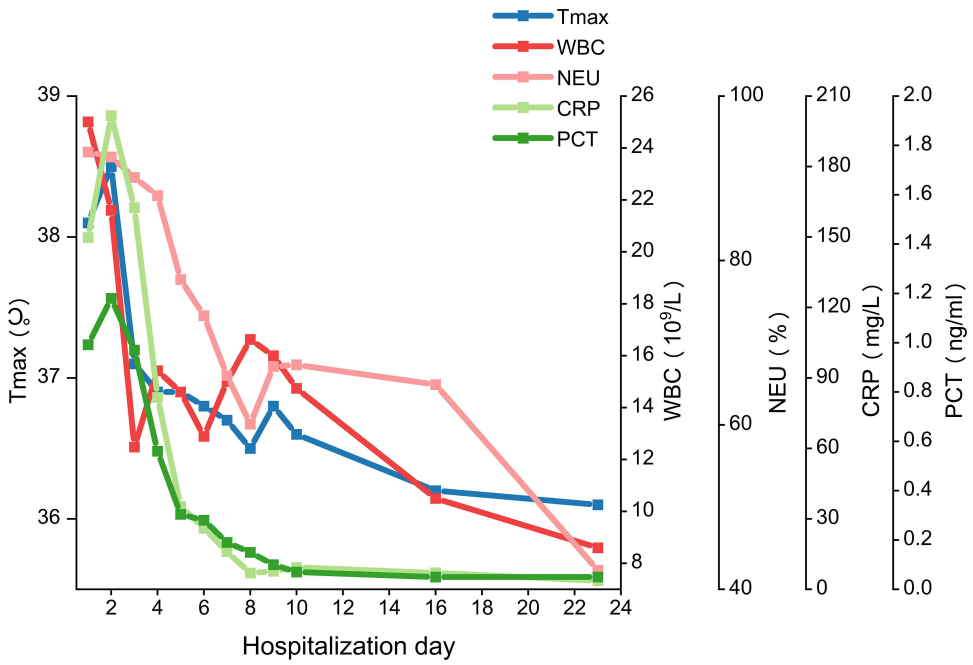

Figure 3 Dynamic change of temperature, WBC count, NEU%, CRP and PCT levels.

infections with specific pathogens.[8,19–23] However, any pathogen test can only be used as an adjunct to an infection and the final diagnosis must be clinically based. Detection of mNGS can be a diagnostic tool for microorganisms that are difficult to find in the regular environment, such as *B. anthracis*. mNGS provides clinicians with an effective diagnostic tool. In conclusion, the patient had an epidemiological history of anthrax as well as typical cutaneous anthrax lesions, and the DNA of *Bacillus anthracis* was also detected by mNGS. We ultimately considered this as a clinically confirmed case of anthrax or suspected case according to the criteria of the CDC. We successfully diagnosed a case of cutaneous anthrax by mNGS for the first time. The incidence of anthrax has gradually decreased for decades. Not all doctors can timely identify the disease by the characteristics of skin lesions. mNGS provides us with an accurate diagnostic tool for pathogen detection. The patient finally established a clinical diagnosis and received timely treatment. More importantly, a public health event could be averted. We suggest that mNGS can be used to assist clinicians in the diagnosis of specific pathogens.

Reporting Checklist

The authors have completed the CARE reporting checklist.

Ethical Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. Publication of case details did not require institutional approval. No specific ethics committee approval was required for this study. This study was conducted under biosafety level 3 condition.

Funding

National Natural Science Foundation of China (82070641) Key research and development project of Shaanxi Province (2018ZDXM-SF-037) Project of Shaanxi Provincial Clinical Medical Research Center for Infectious Diseases (2020LCZX-01) Clinical Research Center for Infectious Diseases, the First Affiliated Hospital of Xi'an Jiaotong University (No. XJTU1AF2021CRF-006, 2022-XKCRC-04).

Disclosure

All authors have completed the ICMJE uniform disclosure form. The authors have no conflicts of interest to declare.

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