

CASE REPORT

Diagnosis of *Mycobacterium marinum* Infection by Metagenomic next-generation sequencing

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SUMMARY

Background: In December 2023, our hospital confirmed a case of finger infection with *Mycobacterium marinum*. The patient sought medical attention at our hospital due to a hard scratch on her left middle finger, which was red, swollen, and ulcerated for one month. Physical examination: A lesion of approximately 1.5 cm x 2 cm in the patient's left middle finger, surrounded by redness and swelling, unclear boundaries, surface rupture, partial scabbing, and no tenderness during compression. She was treated at the previous clinic, common infectious diseases were considered, and was given intravenous infusion treatment: cefotaxime and clarithromycin, and erythromycin ointment was applied externally. She came to our hospital after poor treatment results. The patient has had hypertension for 3 years, no other systemic diseases, no similar medical history among family members, no history of drug or food allergies.

Methods: Clean the wound and remove the scab from the affected area, and use a surgical blade to scrape off necrotic tissue. Send the scraped tissue for pathogen testing: tissue bacterial culture+identification (matrix assisted laser desorption/ionization time-of-flight mass spectrometry, MALDI-TOF), tissue acid fast staining, and tissue metagenomic next-generation sequencing (mNGS). Other auxiliary examinations: blood routine, urine routine, blood fat, liver function, and kidney function.

Results: Tissue bacterial culture+identification: growth of *Mycobacterium marinum*; Acid fast staining of tissue: positive; Tissue mNGS: *Mycobacterium marinum*. Clinical treatment plan: clarithromycin 0.5 g bid po+rifampicin 0.45 g qd po+5-aminolevulinic acid photodynamic therapy (ALA-PDT) qw+boric acid wash wet compress tid. After 14 days of treatment, the area of redness and swelling significantly decreased, and the degree of redness and swelling was significantly reduced compared to admission. The degree of ulcer edge protrusion was also reduced compared to admission. There was a small amount of exudation from the wound, and no necrotic tissue was observed. The patient improved and was discharged.

Conclusions: This article reports a case of finger infection with *Mycobacterium marinum*. *Mycobacterium marinum* was quickly and accurately identified by mNGS, and reasonable treatment measures were adopted clinically. The patient improved and was discharged. This study has important reference significance for the clinical diagnosis and treatment of *Mycobacterium* infection. In addition, mNGS as a novel detection method has considerable prospects for rapid diagnosis of pathogens.

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KEYWORDS

Mycobacterium marinum, metagenomic next-generation sequencing, ALA-PDT

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CASE PRESENTATION

The patient, a 53 year old female, was admitted to the hospital in December 2023 due to a hard object scratch on her left middle finger, redness, swelling, and ulceration for one month. Case characteristics: Acute onset and short course of illness. One month ago, the patient suffered from a scratch on the middle finger of his left hand, and then went to the clinic for treatment. The clinic doctor considered common infectious diseases, intravenous infusion: cefotaxime and clarithromycin, and external application of erythromycin ointment. After 7 days of treatment, the symptoms did not improve significantly, and he went to our hospital for further treatment. Physical examination: A lesion of approximately 1.5 cm x 2 cm on the patient's left middle finger (Figure A), surrounded by redness and swelling, unclear boundaries, surface rupture, partial scabbing, and no tenderness during compression. After admission, debridement and removal of the affected scab were performed, and necrotic tissue was scraped off using a surgical blade. The scraped tissue was sent for pathogen testing, including tissue bacterial culture+identification, acid fast staining, and mNGS. Auxiliary examinations: blood routine, urine routine, liver function, blood fat, kidney function. Inspection results: Auxiliary examination: Blood routine: White blood cell $12.56 \times 10^9/L$, RBC average hemoglobin concentration 324 g/L, monocyte percentage 11.0%, total monocyte count $1.38 \times 10^9/L$, total neutrophil count $7.85 \times 10^9/L$, whole blood high-sensitivity C-reactive protein 48.44 mg/L. Urine routine: urine specific gravity 1.042, protein+, occult blood+, red blood cell count 82.40/ μL , RBC quantification 86.00/ μL by microscopy, urine protein quantification: total protein 0.18 g/L. Blood lipids: triglycerides 2.16 mmol/L, high-density lipoprotein cholesterol 1.28 mmol/L, liver function test: total protein 62.6 g/L, albumin 42.2 g/L, lactate dehydrogenase 272.2 U/L, no abnormalities found in the rest. Tissue bacterial culture+identification (MALDI-TOF): growth of *Mycobacterium marinum* (Figure B, D); Acid-fast staining of tissue: positive (Figure C); Tissue mNGS: *Mycobacterium marinum*. Clinical treatment plan: clarithromycin 0.5 g bid po+rifampicin 0.45 g qd po+ALA-PDT qw+boric acid wash wet compress tid. After 14 days of treatment, compared with admission, the area of redness and swelling is significantly reduced, the degree of redness and swelling is significantly reduced, and the degree of ulcer edge protrusion is also reduced. A small amount of exudation was observed on the wound surface, and no necrotic tissue was observed. The patient improved and was discharged. Discharge advice: 1. Pay attention to wound cleanliness and avoid contamination; 2. Continue anti infection treatment: clarithromycin tablets 0.5 g bid po+rifampicin capsules 0.45 g qd po+boric acid wash wet compress tid; 3. Regularly review liver and kidney function.

DISCUSSION

Mycobacterium marinum is an aerobic positive bacterium and belongs to one of the non-tuberculosis mycobacteria (NTM) [1]. It was first discovered in fish in the 1920s and was first isolated from human skin granulomatous lesions in 1956, confirming that *Mycobacterium marinum* can cause human infections [2]. *Mycobacterium marinum* mainly causes tissue infections, and infections in areas prone to trauma such as palms, elbows, knees, toes, and ankles are the most common [3]. It is clinically also known as "pond granuloma" or "fish tank granuloma" [4]. Seafood sellers, fishermen, chefs, or housewives are the high-risk population [5]. The patient in this case is a middle-aged woman who enjoys cooking various types of food at home. The main reason for this infection may be due to the lack of timely and effective disinfection and treatment after injury, leading to water borne infection while cooking at home.

The incubation period of *Mycobacterium* infection in the sea is 1 - 2 months, and the affected area is more common in the upper limbs. It can also affect the lower limbs, trunk, and face, and is mostly unilateral [6]. The clinical symptoms are mainly skin infections, mainly manifested as painless or painful papules, nodules, granulomas, and very few can rupture to form superficial ulcers. There is currently no evidence of human-to-human transmission. The diagnostic methods for *Mycobacterium* infection include tissue pathological sections, acid fast staining, bacterial culture, and molecular biology identification. The typical pathological description of *Mycobacterium* infection often presents as infectious granuloma, with positive acid-fast staining in the tissue. The gold standard for diagnosis is the detection of *Mycobacterium* infection in the skin lesion tissue. At present, research in Bhandari S, et al. [7] shows that the positive rate of detecting *Mycobacterium marinum* through tissue bacterial culture and acid-fast staining is low, and it takes a long time, making it difficult to achieve early diagnosis of the disease. Molecular identification, as a highly sensitive method, has achieved rapid diagnosis of pathogens, such as mNGS detection, which can shorten the diagnostic time to less than 2 days [8]. In this case, tissue bacterial culture+identification and tissue mNGS were conducted simultaneously. After 96 hours of bacterial growth on a blood agar plate, the results were identified by MALDI-TOF. However, tissue mNGS only took 2 days to obtain results, and the results of the two detection methods were consistent. After receiving the results of mNGS, clinical doctors adopted reasonable treatment measures. This study confirms that mNGS exhibits significant advantages in detection time compared to traditional culture. In clinical practice, *Mycobacterium* infection needs to be distinguished from sporotrichosis. Sporotrichosis is a chronic inflammation of the skin, subcutaneous tissue, and adjacent lymphatic system caused by *Sporotrichosis schenckii*. It often occurs after minor skin trauma and coming into contact with substances contaminated by patho-

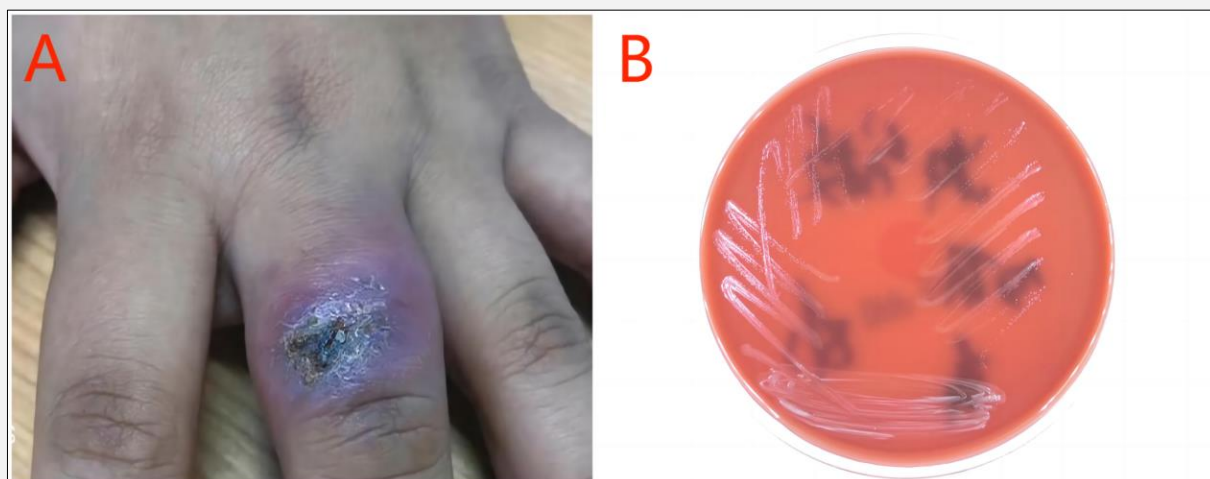


Figure A. A lesion of approximately 1.5 cm x 2 cm in the patient's left middle finger.

Figure B. Growth of *Mycobacterium marinum* on blood agar medium at 35°C, 5% CO₂, 96 hours.

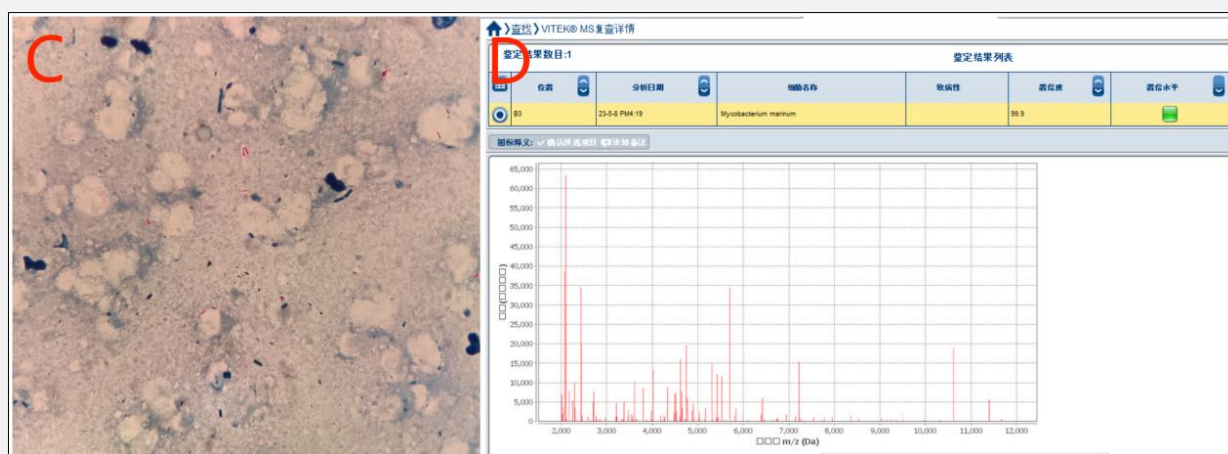


Figure C. Acid-fast staining of tissue × 1000.

Figure D. Identification results of *Mycobacterium marinum* by MALDI-TOF.

gens, so skin lesions are more common in exposed areas. In addition, if *Mycobacterium* infection in marine fish is caused by a single papule or nodular lesion, it can be cured or self-heal within approximately 3 months to 3 years, but sporotrichosis like lesions can persist for a long time [9].

There is no standard for the treatment of *Mycobacterium marinum* infection, and this bacterium is resistant to

most anti tuberculosis drugs [10]. Research by Cascardo CA, et al. [11] shows that the combination of two or more antibiotics is effective. Recommended drugs include clarithromycin, rifampicin, ethambutol, doxycycline tablets, and compound sulfamethoxazole tablets. In addition to antimicrobial therapy, traditional treatment methods such as surgical resection can easily lead to significant tissue damage around the lesion, difficulty

in wound healing and residual scars [12]. Therefore, there is an urgent need for a better, safer, and more convenient treatment method in clinical practice. The report by Chen K, et al. [13] indicates that laser, microwave, photodynamic, and other treatments have good therapeutic effects. In this case, ALA-PDT is currently a commonly used clinical method for treating various skin diseases. The principle is to first allow the photosensitizer Ella (5-aminolevulinic acid) to accumulate in the diseased tissue. Then, when irradiated with a specific wavelength of light, a certain amount of singlet oxygen or other free radicals will be produced, causing changes in the intracellular environment, leading to cell necrosis or inducing apoptosis, effectively killing proliferating active cells. The surrounding normal tissues, due to the less absorption of Ella, have less damage, so the treatment has fewer side effects [14]. This case was treated with clarithromycin 0.5 g bid po+rifampicin 0.45 g qd po+ALA-PDT qd+boric acid wash wet compress tid. After 14 days of treatment, the patient achieved satisfactory therapeutic effects. In this report, the practicality of ALA-PDT as an adjuvant therapy for *Mycobacterium marinum* provides reliable evidence.

The diagnosis of *Mycobacterium* infection is not difficult, but due to the lack of obvious pain symptoms in patients, most of them will ignore it, leading to the gradual worsening of the disease and delayed medical treatment. In this case, the patient went to the clinic for treatment after the middle finger of his lefthand was injured. Without a clear diagnosis, the clinic doctor believed that it was an ordinary infectious diseases caused by trauma and used empirical treatment, so that the condition could not be effectively controlled. There is also regret in this case. The patient's tissue culture results and tissue mNGS detection are both *Mycobacterium marinum*, but no histopathological examination was conducted clinically. Therefore, it is impossible to determine whether there are specific pathological results in tissue. The report by Yao QN, et al. [15] indicates that about 40% of patients with *Mycobacterium marinum* have non-specific histopathological examination, with only lymphocyte and tissue cell infiltration visible, while the remaining 60% of patients have typical tuberculosis like granulomas on histopathological examination.

In summary, this article reports a case of finger infection with *Mycobacterium marinum*. We hoped to improve doctors' understanding of *Mycobacterium marinum* through this case report. In addition, we clarified the practicality of ALA-PDT as an adjuvant therapy. Finally, it was confirmed that mNGS technology has considerable application prospects for diagnosing pathogens.

Ethics Approval and Consent to Participate:

Ethical review and approval was not required for this study. The patient provided written informed consent to participate in this study.

Consent for Publication:

The patient provided written informed consent for study publication.

Availability of Data and Materials:

The original data and materials presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

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Declaration of Interest:

The authors declare no competing interests.

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