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*Case Report*

# Successful diagnosis of *Mycobacterium marinum* infection by mNGS in a patient with Human Immunodeficiency Virus: a case report

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## Abstract

Introduction: *Mycobacterium marinum* infection rarely occurs and has atypical symptoms. It is challenging to distinguish disseminated *M. marinum* infection from multifocal dermatosis caused by other factors clinically.

Case Presentation: Herein, we reported a 68-year-old male patient with Human Immunodeficiency Virus (HIV) who presented redness and swelling in his left hand after being stabbed by marine fish for over 2 months. *Mycobacterium tuberculosis* infection was considered according to biochemical and pathological examinations, while empirical anti-infection treatment was ineffective.

Result: The metagenomic next-generation sequencing (mNGS) detected a large amount of *M. marinum* sequences, and the patient was finally diagnosed with *M. marinum* infection. After one month of combination therapy with ethambutol, rifabutin, moxifloxacin, and linezolid, the swelling disappeared significantly. In this case, the successful application of mNGS in diagnosing and treating *M. marinum* infection has improved the understanding of the microbe both in the laboratory and clinically, especially in patients with HIV.

Conclusions: For diseases with atypical symptoms or difficulty in determining the pathogens, mNGS is suggested in clinical procedures for rapid and accurate diagnosis and treatment.

**Key words:** Human immunodeficiency; *Mycobacterium marinum*; infection; metagenomics next-generation sequencing (mNGS).

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## Introduction

*Mycobacterium marinum* is one of the nontuberculosis mycobacteria (NTM) that lives mainly in the aquatic environment. Previous studies have shown that 87% of patients infected with *M. marinum* had exposure histories to fish, shrimp, or seawater prior to the onset . Although *M. marinum* infection is rarely detected, it poses a higher risk of disease in immunocompromised patients [3].

Clinical detection of *M. marinum* is based primarily on bacterial culture and histopathologic analysis. However, it often takes 2–3 weeks, sometimes up to 6 weeks, to identify the species . Another reason for delayed diagnosis is its atypical characteristics of causing superficial infections and granulomas . Therefore, rapid detection is essential for optimal diagnosis and treatment of *M. marinum* infections, especially in immunocompromised patients. In this case, we reported a case using mNGS technology for rapid diagnosis and treatment of *M. marinum* infection in an HIV patient complicated with type II diabetes.

## Case presentation

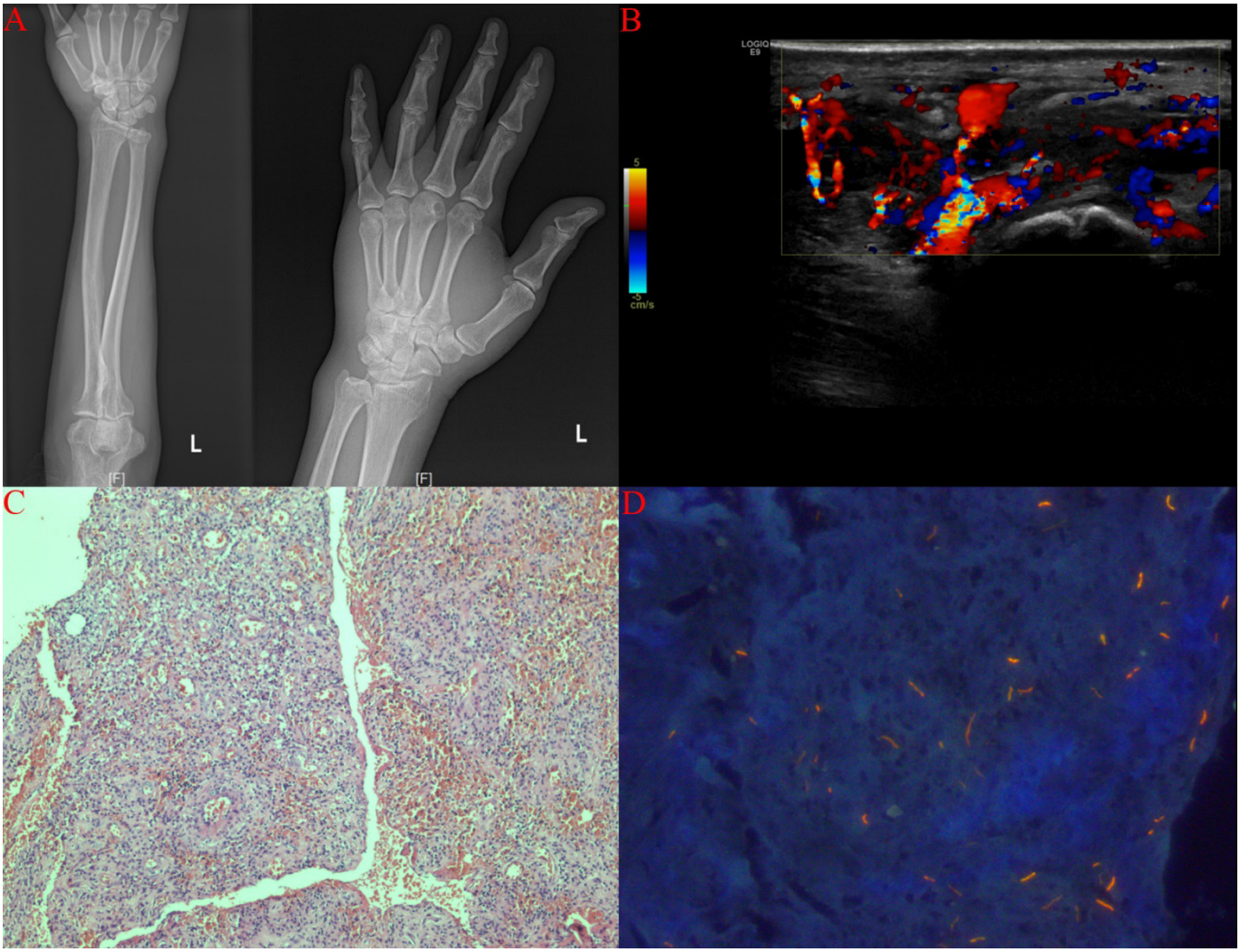
A 68-year-old man presented to our infection clinic on November 27th, 2021, with a red and swollen left hand that had present for 2 months. Two months earlier, his left hand was stabbed by a fish and deteriorated to a persisting red swelling. After initial treatment with a wet dressing of Chinese medicine and broad-spectrum antibiotics for about two weeks, the patient felt more swollen and tender. Therefore, the patient went to a local hospital for inpatient treatment, where erysipeloid of left hand was diagnosed. At the same time, cefoperazone sodium, levofloxacin, and imipenem were administered as anti-infection treatment, and tetanus antitoxin was injected for tetanus prevention. However, the redness and swelling of the left hand recurred during his hospitalization. In addition, he has been taking genvoya (elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide tablet) for antiviral therapy of HIV for four years. During his 4-year history of type II diabetes, the

patient used gliclazide to maintain stable glycemic status. No other prior medical and family history was known at presentation.

No cough or fever was reported after admission to our hospital. Physical examinations revealed high temperature and mild tenderness of the local skin which was peeling, presented evident redness and swelling, and appeared soft and fluctuant to touch (Supplementary Figure 1). Routine laboratory tests are shown in Supplementary Table 1. The C-reactive protein and procalcitonin were significantly elevated (CRP: 18.09 mg/L; PCT: 0.068 ng/mL), and the level of interleukin-6 was above the limit value (38.21 pg/mL). Soft tissue swelling was evident on radiographs of the left hand. Color ultrasound of both hands showed thickening of the subcutaneous tissue of the left hand and abnormal blood flow (Figure 1). The lesion was considered of infectious nature, then linezolid and ornidazole were administered from November 27th to December 2nd for empirical anti-infection treatment. Follow-up tests showed positive for antigen A (ESAT-6) and antigen B (CFP-10) in the T-cell spot of tuberculosis test (T-SPOT.TB), so *M. tuberculosis* infection was suspected. However, a microbial culture of both blood and tissue was negative. Meanwhile, the therapeutic regimen was altered to linezolid, moxifloxacin, and isoniazid on December 2nd. No improvement was observed in the following week.

On December 8th, a debridement was performed. Tissue samples were collected for pathologic examination and mNGS analysis. The mNGS analysis

**Figure 1.** Results of pathological and radiographic examinations. **A.** Radiographs of the left hand showed soft tissue swelling. **B.** Color ultrasound of both hands showed that the soft tissue thickness of the left hand was 16 mm, and that of the right hand was 5 mm. The blood flow signals were abundant in the swollen area of the left hand. **C.** Histopathological slide of the left hand showed chronic epithelioid granulomatous inflammation and inflammatory granulation tissue hyperplasia. **D.** Acid-fast fluorescence staining showed many gram-positive bacilli.



was completed within one day and revealed 27,829 sequences of *M. marinum* (Supplementary Figure 2). Five days later, histopathologic results showed chronic epithelioid granulomatous inflammation with hemorrhagic necrosis. Moreover, acid-fast staining showed the presence of numerous slender red-stained positive bacilli (Figure 1). Finally, *M. marinum* infection was confirmed.

According to the guidelines [6,7], and considering the obvious digestive tract reactions of the patient, such as nausea, vomiting, and poor appetite, a combination of ethambutol, rifabutin, moxifloxacin, and linezolid was used as therapy against *M. marinum*, and prevention for other infections may occur. To avoid drug-drug interactions, antiretroviral medication was changed to lamivudine and dolutegravir. The lesions had significantly subsided and discharge was allowed after one month. After this period, the patient is still under treatment of antiretroviral, and his condition is stable during follow-up. And the changes in the inflammatory factors of the patient over the whole medical course was shown in Supplementary Table 2.

## Discussion

*M. marinum* can infect humans and cause skin lesions, while a disseminated infection would occur in severely immunocompromised patients [2]. However, the diagnosis of *M. marinum* infection is often significantly delayed as it is difficult and time-consuming to isolate the bacterium in the laboratory [4,5]. Clinically, T-SPOT.TB and acid-fast bacilli are methods widely used to identify mycobacterial infection, and T-SPOT.TB positivity is considered to have high specificity of *M. tuberculosis* [8]. Nevertheless, NTMs such as *M. marinum* and *Mycobacterium kansasii* may induce false positive T-SPOT.TB [9,10], making it difficult to identify *M. marinum* accurately. In this case report, the positive T-SPOT.TB and acid-fast bacilli mislead the diagnosis and treatment of *M. marinum,* because *M. tuberculosis* can also cause similar skin and soft tissue infections [11]. Therefore, for patients with positive T-SPOT.TB and acid-fast bacilli test and with no available clinical etiological results, in addition to considering *M. tuberculosis* infection, the possibility of *M. marinum infection* caused by exposure to aquatic organisms should be adequately clarified.

mNGS has been applied to identify *M. tuberculosis* and some NTMs [12]. Since many mycobacteria are slow-growing or unculturable in artificial media and treatment regimens for different mycobacteria vary, timely diagnosis of specific pathogenic microorganisms is critical for correct treatment. Clinical identification of *M. marinum* is mainly through isolation of the bacterium from infected tissues or fluids, while the growth rate is low . Recently, molecular methods such as next generation sequencing (NGS) have been introduced into detecting *M. marinum* infection [13,14], confirmed by culture after a couple weeks after incubation, which exhibited excellent performance in early diagnosis of slow-growing or unculturable bacteria such as *M. marinum*. In this case report, the use of mNGS technology also showed advantages in identifying pathogens more accurately and quickly.

Currently, there is no explicit guideline for the optimal treatment of *M. marinum* infections, and there are successes and failures with almost all anti-mycobacterial regimens [4]. According to the lately ATS/IDSA guideline, the combination of clarithromycin and rifampin, moxifloxacin, or levofloxacin is effective in most patients with skin infections, and treatment should be continued for 1–2 months after all lesions have resolved [6,7], and doxycycline, rifabutin, rifampicin, ethambutol, azithromycin, minocycline, clarithromycin and other antibiotic were used in combinations for different cases [13,15,16]. In this case report, considering the severe digestive tract reactions (nausea, vomiting, and poor appetite) of the patient, ethambutol and rifabutin rather than clarithromycin were administered for the treatment of *M. marinum* infections, and moxifloxacin and linezolid were used in combination considering the clinical manifestations, and the expected treatment performance was achieved by the combined medication.

In conclusion, the successful diagnosis and treatment of *M. marinum* infection identified by mNGS in an HIV patient complicated with type II diabetes have provided referential experiences for treating other similar cases. As a culture-independent method, mNGS can significantly shorten the diagnosis time of *M. marinum*, offering strong technical support for symptomatic clinical treatment. It is challenging to distinguish marine infections from other diseases based on skin symptoms, especially in immunocompromised patients. Clinically, if the patient has skin lesions with a history of exposure to aquatic organisms, it is suggested to consider *M. marinum* infection, which requires further identification through molecular methods, such as mNGS, to apply appropriate and fast diagnosis and treatment for the patient.

### Acknowledgements

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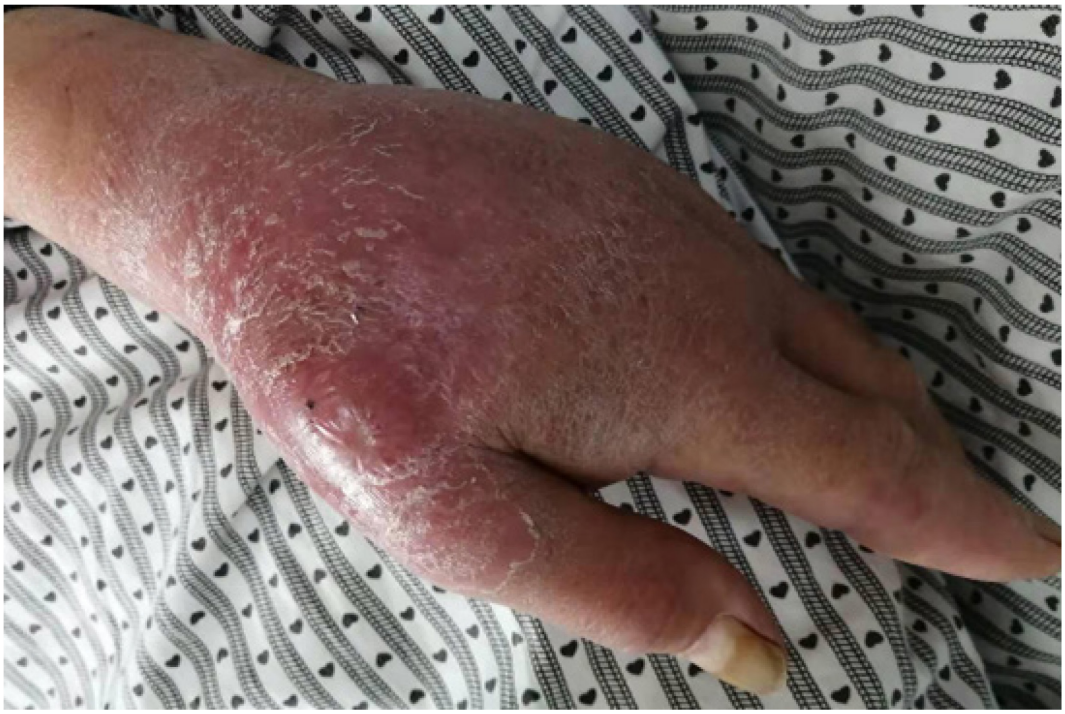
**Conflict of interests:** No conflict of interests is declared.

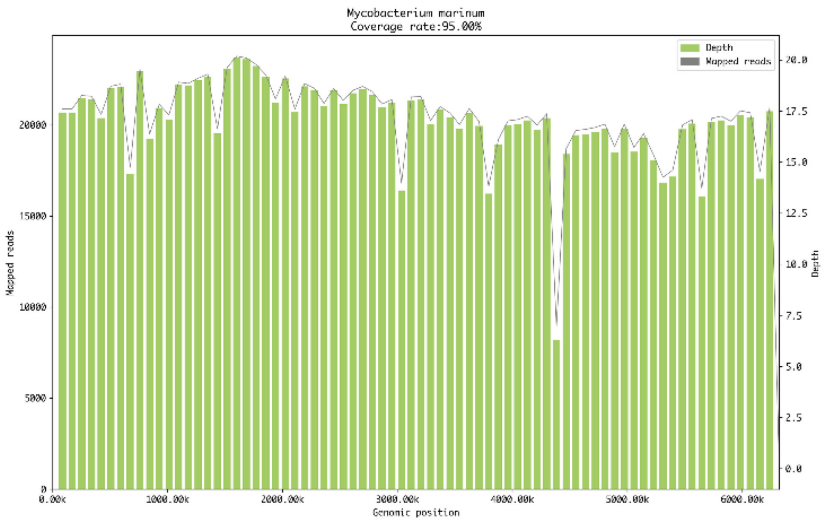
## Annex – Supplementary Items

**Supplementary Figure 1.** The patient's left hand was swollen, and the skin was dark red, with a few skin desquamations, when admitted.

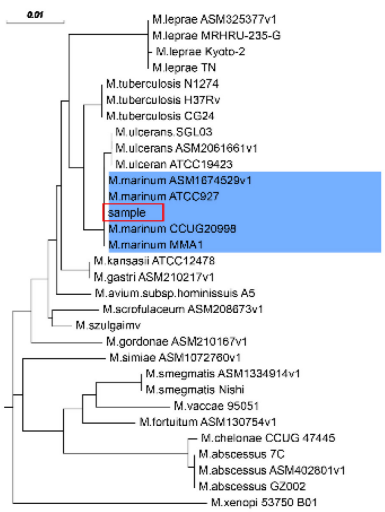


**Supplementary Figure 2.** Results of mNGS analysis. A. The mNGS of the tissue sample achieved 27,829 reads of *M. marinum*, the genome coverage was 95%. B. 16S phylogenetic analysis, the 16S sequencing information of the pathogen detected in the patient's sample was consistent with the sequence of *M. marinum*, indicating that the pathogen belonged to this mycobacterial species.

A




B



**Supplementary Table 1.** The examination results when the patient was admitted.

| | Items | Test values | Reference values |
|---|---|---|---|
| **Biochemical tests** | White blood cell count | $8.07 \times 10^9$/L | 3.5–9.5 |
| | C-reactive protein | 18.09 mg/L | < 10 |
| | Procalcitonin | 0.068 ng/mL | < 0.05 |
| | Interleukin-6 | 38.21 pg/mL | 0–5.30 |
| | Erythrocyte sedimentation rate | 23 mm/h | 0–15 |
| | Glycolic acid | 4.27 ug/mL | 0–2.7 |
| | CD4 T lymphocyte count | 178 cells/uL | 550–1440 |
| | T helper/suppressor lymphocytes（CD4+/CD8+） | 0.2 | 1.4–2.0 |
| **Pathological tests** | Hepatitis B and C antibody-antigen detection | Negative | — |
| | HIV-1 RNA Quantitative Detection | < 20 copies/mL | < 20 copies/mL |
| | EB Virus DNA Fluorescence Detection | < 500 copies/mL | < 500 copies/mL |
| | Quantitative detection of giant cell DNA | < 500 copies/mL | < 500 copies/mL |
| | Tuberculosis DNA Fluorescence Detection | < 500 copies/mL | < 500 copies/mL |
| | Galactomannan antigen detection (GM test) | 0.32 | < 0.5 |
| | Fungal D-glucan detection | ≤ 37.5 pg/mL | < 95 |
| | Gram-negative bacilli endotoxin detection | 0.03 EU/mL | < 0.11 |
| | Cryptococcus capsular antigen detection | Negative | — |
| | T-SPOT.TB Test | Antigen A(ESAT-6) 31<br>Antigen B(CFP-10) > 50 | 0–6<br>0–6 |
| | Tuberculin test | Negative | — |
| | Talaromyces marneffei antigen detection | Negative | — |
| | Systemic lupus erythematosus antibody test | Negative | — |
| | Cell culture | Negative | — |

**Supplementary Table 2.** The change of inflammatory factors over the medical course.

| **Date** | **Nov 27 2021** | **Dec 2 2021** | **Dec 9 2021** | **Dec 24 2021** | **At discharge** |
|---|---|---|---|---|---|
| **Treatment** | **Linezolid and ornidazole** | **Linezolid, moxifloxacin, and isoniazid** | **Ethambutol, rifabutin, moxifloxacin, and linezolid** | | |
| **Inflammatory factors** | | | | | |
| WBC ($\times 10^9$/L) | 8.07 | — | 4.99 | 3.66 | 4.29 |
| CRP (mg/L) | 18.09 | — | < 10 | < 10 | < 10 |
| PCT (ng/mL) | 0.068 | — | — | 0.077 | — |
| ESR (mm/h) | 23 | — | — | 48 | — |

WBC: white blood cell count; CRP: C-reactive protein; PCT: procalcitonin; ESR: erythrocyte sedimentation rate.