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Case Report

A clinical case and a review of *Mycobacterium fortuitum* infections direct diagnosis approach and treatment in a patient with leg fractures

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Abstract

Mycobacterium fortuitum infections of the musculoskeletal system are commonly missed, given their rarity and the absence of systemic symptoms. In this study, we isolated the *M. fortuitum* from the skin sinus tract of a traffic accident patient's right medial knee surgical incision (over the open fracture wound), and confirmed by Morphological analysis, MALDI-TOF MS, 16S rRNA gene sequencing, and mNGS. Then we adjusted the treatment plan and treated the patient with cefoxitin, amikacin, and doxycycline. At three months follow-up review, his wound had completely healed. This report may provide a reference for the clinical treatment of *Mycobacterium fortuitum* infection in patients with open fractures.

Key words: Femur amputation; *M. fortuitum*; infections.

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Introduction

Non-tuberculous mycobacteria (NTM), excluding *Mycobacterium tuberculosis* and *Mycobacterium leprae,* is an opportunistic pathogen, widely present in natural environments such as water, soil, dust, vegetables, domestic animals, and dairy products [1]. In recent years, the incidence of NTM infections is on the rise worldwide. NTM can cause lung, skin, foot, or lymphatic infections in immunocompetent patients [2]. *Mycobacterium fortuitum* (*M. fortuitum*) belongs to one of the NTM [3], is mainly isolated from patients with skin and soft tissue infections, and is rarely reported in open fracture infections [4]. When a patient's musculoskeletal system is infected with *M. fortuitum*, timely diagnosis may be difficult due to the culture and growth of specimens [5], and delayed diagnosis may result in joint destruction and functional impairment.

With the improvement in the laboratory and diagnostic equipment for the characterization of microorganisms [6], we rapidly identified *M. fortuitum* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) [7,8], 16S rRNA gene sequencing, and metagenomic next-generation sequencing (mNGS). We present a rare case study of a patient with *M. fortuitum* infection in a car accident amputee, including its diagnosis and treatment.

Case presentation

A 66-year-old male patient was admitted to the Department of Ankle Surgery on October 10, 2020, due to a rupture of an open wound about the inner side of the mid-shaft tibia-fibula right lower leg in September. Two months before admission, the exposed puncture wound on the mid-shaft right tibia-fibula of the patient was contaminated, with a small amount of sediment-like pollutants. On August 7, 2020, he was treated operatively with open reduction and internal fixation with intramedullary nails [9,10]. Given the wound infection could be present at the time of surgery (PATOS) [11], one week before admission, the healed wound at the medial lower right leg and the wound at the right knee joint surgery incision began to bleed with ulceration and pus. At the time of admission, the patient's body temperature was 36.4 ℃, his pulse was 82 beats/minute, the respiration rate was 20 times/minute, blood pressure was 110/72 mmHg, he had no co-morbid conditions and wasn't immunocompromised. The patient's distal right knee joint skin temperature was higher than the opposite

side, and there was no significant knee and ankle movement restriction. There was slight ulceration on the anterior and medial side of the right knee surgical incision with a small amount of purulent discharge when squeezed. The skin sinus tract was found on the medial right knee joint surgery incision (on the wound of the open fracture) and many purulent secretions were found during extrusion. The skin around the wound was red and significantly swollen. However, the sense of touch and the blood supply to the distal right lower limb were good. An auxiliary examination at the time of admission yielded the following findings: white blood cell count, 6.09×10^9/L; neutrophil percentage, 67%; C-reactive protein, 39 mg/L; urea nitrogen, 4.24 mmol/L; creatinine, 49 µmol/L; fasting blood glucose, 5.5 mmol/L; procalcitonin, < 0.05 ng/mL; erythrocyte sedimentation rate, 99 mm/hour. On November 25, 2020, distal right femur amputation was performed with a wire saw [12]. On December 12, 2020, the patient underwent debridement and Vacuum Sealing Drainage (VSD) procedures of the wound and obtained a sample of a tuberculosis-like cold abscess which was gray and cold, with no obvious odor. Then, we examined the pus sample for smear and Acid-Fast stain [13], and the primary smear may show Acid-Fast-positive bacilli.

Meanwhile, samples were inoculated on blood agar and chocolate agar and cultured in an atmosphere containing 5% CO_2. After 48 hours, needle-tip-sized colonies were observed, and after 72 hours, grayish-white colonies formed on the agar. The colonies were initially identified by Acid-Faststain [14]. We performed a real-time polymerase chain reaction (PCR) of *Mycobacterium tuberculosis* for Acid-Fast stain-positive colonies [15] and found that the bacteria were not *Mycobacterium tuberculosis*. Using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), the colonies were identified as *M. fortuitum*. The colonies were transported to Beijing Ruiboxingke Biotechnology Co., Ltd. for 16S rRNA gene sequencing identification, which confirmed the result obtained using MALDI-TOF-MS. On December 17, fresh tissue was collected from the wound and transported to Jiangsu Centron Medical Diagnostics Co. for metagenomic next-generation sequencing (mNGS), confirming the presence of *M. fortuitum*. The patient with the lower right limb was then treated with the debridement and

Figure 1. *M. fortuitum* direct diagnosis and treatment workflow. (**A**) Sinus tract found in the medial surgical incision; (**B**) Tuberculosis-like abscess.

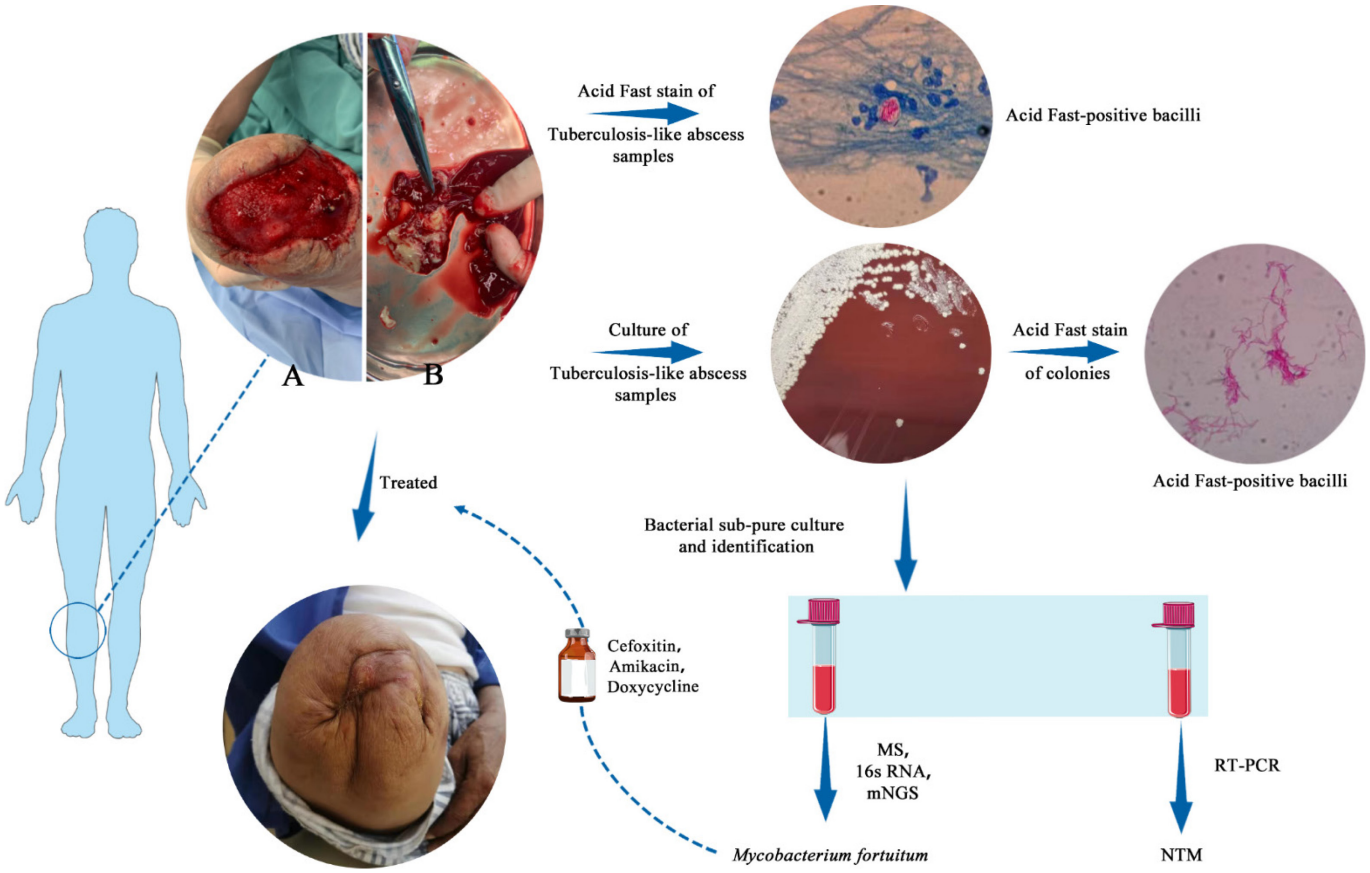

Table 1. Biochemical parameters at critical times.

Biochemical parameters	White blood cell count ($\times 10^9$/L)	Neutrophil percentage (%)	C-reactive protein (mg/L)	Urea nitrogen (mmol/L)	Creatinine (μmol/L)	Procalcitonin (ng/mL)	Erythrocyte sedimentation rate (mm/h)	Fasting blood glucose (mmol/L)
Initial admission	6.09	67	39	4.24	49	< 0.05	99	5.5
Pre-amputation	5.15	65.8	37	3.86	50	0.05	65	/
Pre-treatment	4.09	67	25	3.71	43	/	78	/
Post-treatment	3.76	56.6	4	3.7	53	< 0.05	15	/

Normal reference range: White blood cell count ($\times 10^9$/L): 4.0-10.0; Neutrophil percentage (%): 39.8-70.5; C-reactive protein (mg/L): 0.0-5.0; Urea nitrogen (mmol/L): 3.1-9.5; Creatinine (μmol/L): 57-104; Procalcitonin (ng/mL): < 0.05; Erythrocyte sedimentation rate (mm/h): 0.0-15.0; Fasting blood glucose (mmol/L): 3.5-11.1.

VSD [16] technique fifteen times during hospitalization. At the same time, the secretion from the wound of the lower right leg was sampled eight times for bacterial culture, Gram staining [17], and Acid-Fast staining. The bacterium was isolated on December 15, 2019. Between October and January, the patient was treated with intravenous cefazolin and levofloxacin successively and oral rifampin, isoniazid, pyrazinamide, ethambutol, and clindamycin [18], all of which were ineffective. After identifying the infectious pathogen, the patient was treated with intravenous cefoxitin (12 g/12 hours) and amikacin (0.2 g/12 hours) for up to 6 weeks according to The Sanford Guide to Antimicrobial Therapy (48th edition) guidelines [19], and the infection was resolved. After the drugs were replaced by oral doxycycline, the wound significantly improved. After identification and treatment, the patient recovered (Figure 1) and serum infection markers such as C-reactive protein and erythrocyte sedimentation rate improved gradually (Table 1). Then he was discharged from the hospital on April 12, 2021. He was instructed to continue oral doxycycline for six months, and after discharge, the patient was followed up once a month for three months and recovered well.

Discussion

M. fortuitum, a rapidly growing mycobacterium, is a relatively uncommon pathogen [20-22]. NTM causes infections that primarily affect the lungs (pulmonary disease), skin, soft tissues, and lymph nodes, but are also responsible for surgical wound infections, and implant-associated and catheter infections [23]. According to research [24], the *M. fortuitum complex* includes the species *M. fortuitum*, *M. peregrinum, M. senegalense, M. setense, M. septicum, M. porcinum, M. houstonense, M. boenickei, M. brisbanense*, and *M. neworleansense*. A comprehensive literature search of the PubMed and Web of Science databases was conducted for articles published until May 2022. The key search terms were "open fracture" and "*Mycobacterium fortuitum*". The language was restricted to English. The literature review showed 6 cases of *M. fortuitum complex* associated with open fracture infection (Table 2) [1,4, 25-28]. Osteomyelitis is a known complication of open fracture, but *M. fortuitum* infections are very rare. *M. fortuitum* infections of the musculoskeletal system are commonly missed due to their rarity and the absence of systemic symptoms [1]. For patients infected with *M. fortuitum*, the pathogen can be accurately identified, and timely surgical debridement combined with appropriate antimicrobial therapy can effectively avoid infection recurrence. This case reports the diagnosis and treatment of *M. fortuitum*, which may provide a reference for clinical diagnosis and treatment of *M. fortuitum* infection.

Infectious diseases are a leading cause of morbidity and mortality worldwide and spread quickly. As the first line of pathogen detection, the microbiology laboratory plays an important role in infection control

Table 2. Review of literature of reported cases of open fracture to *M. fortuitum.*

Case report	Gender	Age	Pathogen	Presentation	Diagnosis approach	Antimicrobial	Outcome
Eriko Kashihara (2022)	Male	37	*Mycobacterium farcinogenes*	fracture	MALDI-TOF MS, 16S rRNA sequence	levofloxacin, amikacin, and rifampin	No recurrence
Khai Phang Wong (2020)	Male	55	*Mycobacterium fortuitum*	open complex fracture dislocation	no report	cefoxitin, clarithromycin and doxycycline	No recurrence
Lei Tian (2019)	Male	68	*Mycobacterium houstonense*	humeral fracture	molecular sequencing	levofloxacin, amikacin,	No recurrence
K Kwan (2010)	Male	35	*Mycobacterium chelonae, Mycobacterium fortuitum*	open fracture	no report	amikacin, clarithromycin	No recurrence
Toïdi Adékambi (2006)	Female	31	*Mycobacterium conceptionense sp*	open tibia fracture	16S rRNA sequence	no report	No report
Glenn L (1993)	Male	25	*M.fortuitum*	open fracture	no report	ciprofloxacin,	No recurrence

by employing microscopic examination, culture, identification, drug sensitivity, and so on. With the completion of the human genome project in the early twenty-first century and the rapid development of sequencing technology, high-throughput and low-cost second-generation sequencing technology emerged [29]. In recent years, multiple molecular detection methods came into our view, including MALDI-TOF MS, 16S rRNA gene sequencing, and mNGS [30-33]. Whether it can be the next routine pathogen identification tool has become a topic of concern. In this case, using MALDI-TOF MS, 16S rRNA gene sequencing, and mNGS, we identified *M. fortuitum* in the purulent secretions of skin sinuses in patients with open fractures. Due to the difficulty in growing the organism in culture and the lack of typical clinical symptoms [1,34], *M. fortuitum* infections are often misdiagnosed. This is the reason why the patient's condition repeatedly worsened without effective medication during the pre-treatment. However, when treating patients with severe abscess of the unknown pathogen, physicians need to identify the causative pathogen as early as possible and make an accurate diagnosis to provide targeted treatment [33]. MALDI-TOF MS, 16S rRNA gene sequencing, and mNGS minimize the time to diagnose *M. fortuitum* condition and the course of the disease.

Regarding treatment, *M. fortuitum* infection's distinguishing feature is its resistance to almost all traditional anti-tuberculous medications and many antibiotics. Thus, these require a prolonged course of treatment and even wound debridement to eliminate these infections [35]. On the other hand, treatment strategies are different depending on the remote sample, which complicates the clinical treatment of *M. fortuitum* infections [36]. After the pathogen was identified, the patient's therapeutic regimen requires careful consideration of the choice of antimicrobial agents according to official treatment guidelines [37]. In this report, the patient was treated with cefoxitin, amikacin, and doxycycline. At three months follow-up review, his wound had completely healed, and his flap was clean.

We already know that *M. fortuitum* ubiquitously exists in natural environments such as water, soil, and dust, and is an opportunistic pathogen [1]. In this case, the clinician performed open reduction and intramedullary nailing of the fracture along the proximal right calf and the knee joint cavity at the patient's surgical site. One week after the first surgical procedure, the patient's exposed fracture wound had mild redness and exudation, which improved with antibiotic treatment and dressing changes. However, the infection was not completely controlled, and over time, the infection gradually spread to the proximal right calf and the knee joint cavity. on the other hand, the patient's wound was an exposed puncture wound on the mid-shaft right tibia-fibula, and there was a small amount of sediment-like pollutants, which was a contaminated wound at the time of admission. Taken together, we believe that infection during surgery and contamination by ground pollutants during a car accident is the most likely sources of infection in this case.

To the best of our knowledge, we report the first case of incident mycobacterial infection confirmed by a combined analysis of MALDI-TOF MS, 16S rRNA sequencing, and mNGS in China. We aim to highlight the rare cause of such diseases and demonstrate that these infections can be treated successfully by accurately identifying the culprit pathogens and may provide novel insights for the clinical treatment of *M. fortuitum*.

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