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CASE REPORT
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Diagnosis of *Mycobacterium marinum* infection based on photochromogenicity: a case report

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ABSTRACT

A 35-year-old immunocompetent woman from southern China went to the hand surgery clinic with a six-month history of progressive swelling in her right index finger. She had been pinched by a lobster and had received several treatments without any improvement. Pus specimens were taken from the swollen parts of her finger, and the pathology showed granulomatous inflammation. Ziehl–Neelsen staining revealed positive bacillus in the pus specimens. The bacteria grew well on Columbia blood agar. However, the MALDI-TOF MS and 16S rRNA gene sequencing were not able to distinguish between *Mycobacterium marinum* and *Mycobacterium ulcerans* because of their close genetic relationship. Photochromogenicity testing can help differentiate between these species based on the alteration in colony color after light exposure. For our patient, the colonies turned yellow after 18h of incubation in the sun, identifying the species as *M. marinum*. Besides surgical drainage, the patient received rifampicin and clarithromycin for three months, and her symptoms resolved without relapse after six months of follow-up.

KEYWORDS: *Mycobacterium marinum.* Photochromogenicity. Sunlight exposure.

INTRODUCTION

M. marinum is a slow-growing, nontuberculous mycobacterium that is ubiquitously found in aquatic environments. It can be transmitted to humans through contact between fish or contaminated water and broken skin, and it can cause skin and soft tissue infection[1]. Therefore, it is commonly referred to as "swimming pool granuloma" or "fish tank granuloma"[2]. Less commonly, it can infect the lungs, bones, joints, bloodstream, and other body parts[1].

The overall incidence rate of the related disease is increasing[3]. Although MALDI-TOF MS, 16SrRNA gene sequencing, PCR analysis, and high-performance liquid chromatography can be used to identify mycobacteria in samples[4], these methods may not be able to accurately identify *M. marinum*. The following section presents a case in which sunlight exposure was used to assist in the diagnosis of *M. marinum* infection.

CASE REPORT

A 35-year-old immunocompetent woman had swelling in her right index finger and a limited range of motion for a duration of six months. She was otherwise healthy. She is an aquatic product practitioner and sustained an injury to her finger while working with lobsters and oysters. She sought care locally for wound closure and was treated with antibiotics and anti-inflammatory drugs for suppurative

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tenosynovitis, without improvement. Her finger swelling persisted, and she came to our hospital for a second opinion and was admitted for treatment. Upon physical examination, the right finger had an unhealed incision without obvious exudation (Figure 1). The finger's range of motion was limited, and the fingertip was numb. Informed consent was obtained from the patient, and this study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University.

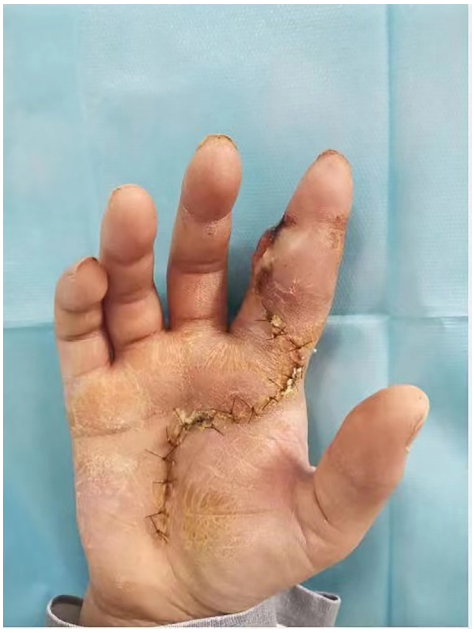

Figure 1 - The right finger had an unhealed incision without obvious exudation.

The results of laboratory tests showed that the patient's monocyte count was 0.63×10^9/L (normal reference range: 0.1–0.6 $\times 10^9$/L), and that her platelet count was 365.9×10^9/L (normal reference range: 125–350 $\times 10^9$/L). All other laboratory tests, including white blood cell count, as well as biochemical and immunological tests, were normal or negative. A histopathology examination revealed fibrous connective tissue hyperplasia, massive inflammatory cell infiltration, and granuloma formation (Figure 2A). Ziehl–Neelsen staining revealed positive bacillus in the pus specimens (Figure 2B).

The pus sample was inoculated in Columbia blood agar, and white bacterial colonies grew after seven days. However, the MALDI-TOF MS and 16S rRNA gene sequencing were not able to distinguish between *M. marinum* and *M. ulcerans* because of their close genetic relationship. Since the antimicrobial therapies for these two bacteria differ, it is important to describe them separately. *M. marinum* and *M. ulcerans* react differently to light: *M. ulcerans* colonies do not change color with light exposure, while *M. marinum* colonies turn from white to yellow. Therefore, we used photochromogenicity to distinguish between the two (Figure 3A). In our case, the colonies turned yellow after 18h of incubation in the sun, which helped identify the species as *M. marinum* (Figure 3B and Figure 3C). The patient was treated with surgical drainage and antimicrobial therapy consisting of rifampicin (0.6 grams orally once daily) and clarithromycin (0.5 grams orally twice daily) for three months. Her symptoms resolved and she remained free of relapse after six months of follow-up.

DISCUSSION

M. marinum is a type of acid-resistant, aerobic, immobile, non-spore-producing bacteria that is photochromogenic[5]. It grows slowly (taking two to six weeks in Lowenstein–Jensen medium), with a preference for temperatures ranging between 30°C and 33°C[6]. When cultured in darkness, the color of *M. marinum* is white, but when exposed to visible light, it turns yellow[5]. This photochromogenicity is due to the crtB gene, which mediates the active production of beta-carotene[7]. Carotenoids are considered to protect cells from photo-oxidation by quenching photosensitizer triplets, oxygen singlets, and other radical species[8]. *M. marinum* is

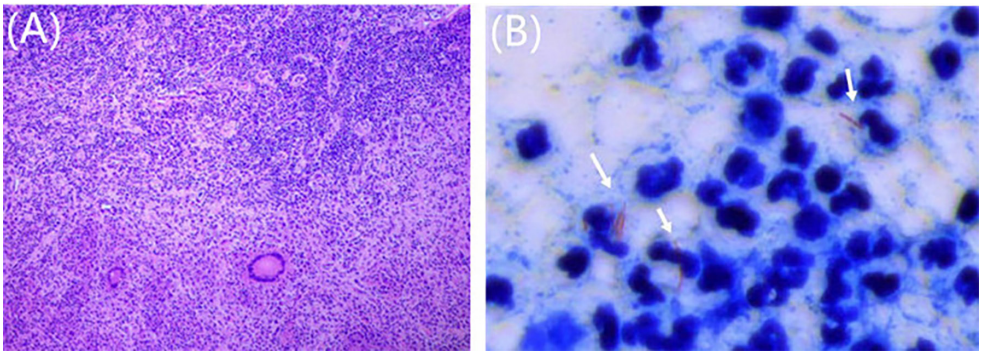

Figure 2 - A) A histopathology examination revealed fibrous connective tissue hyperplasia, massive inflammatory cell infiltration, and granuloma formation; B) Ziehl–Neelsen staining revealed positive bacillus in the pus specimens.

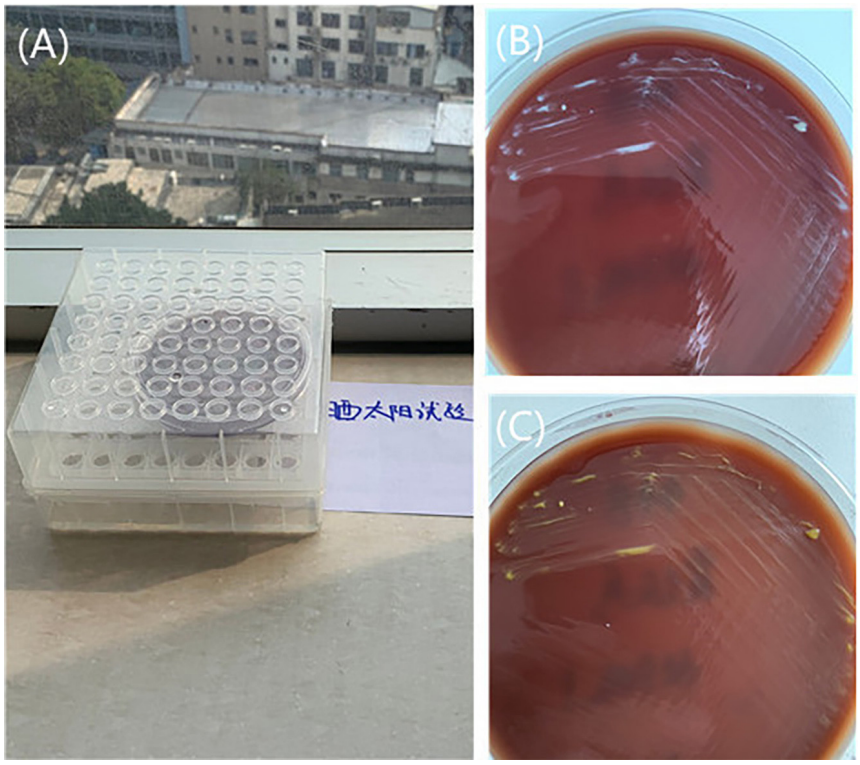

Figure 3 - A) Bacterial colonies were exposed to sunlight for 18 h; B) White bacterial colonies grow on Columbia blood agar; C) Bacterial colonies changed from white to yellow after 18h of sunlight exposure.

able to avoid lethal photo-oxidation through the presence of carotenoids[9].

Based on growth rates and pigment production, Runyon divided nontuberculous mycobacteria into four groups[10]. *M. marinum* contains characteristic photochromogens and is similar to *M. kansasii, M. simiae*, and *M. asiaticum,* which belong to Runyon classification[10]. *M. marinum* can cause single or multiple lesions; single lesions may be nodules or plaques, and multiple lesions are often distributed in beads along the lymphatic vessels. The hands (fingers and back of the hand), wrists, and forearms are most commonly affected[11]. *M. marinum* infection rarely causes systemic dissemination, but deep tissues – such as subsynovial connective tissues, bones, fluid sacs, and joints – can be invaded in patients with severe immunodeficiency[12]. Most patients are chefs, seafarers, fishermen, and marine aquarium staff, etc. People working in these professions frequently suffer pinch wounds and other stinging injuries caused by aquatic animals. Patients with skin trauma may also be infected after exposure to fish culture water, fish, or swimming pool water[13]. Zoonotic disease patterns have been demonstrated experimentally[14]. There are currently neither clinical reports nor relevant laboratory evidence of human-to-human transmission[15,16].

Many mycobacteria can cause chronic granuloma, and the therapeutic schedules for different mycobacteria vary immensely. Thus, it is highly important to accurately identify *M. marinum*. Traditionally, the identification of *M. marinum* has been based on recognized phenotypic and biochemical characteristics. Recently, polymerase chain reaction sequencing, polymerase chain reaction hybridization, MALDI-TOF MS, and high-throughput sequencing have been used to identify *M. marinum*[4]. In addition, 16S rRNA gene sequencing is now used by many scholars to identify bacteria and fungi[17], but this method cannot completely distinguish *M.marinum* from other *mycobacteria*. This suggests that *M. marinum, M. leprae, M. ulcerans,* and *M. tuberculosis* evolved from a common environmental ancestor[11]. In our patient's case, MALDI-TOF MS and 16S rRNA gene sequencing were not able to distinguish between *M. marinum* and *M. ulcerans,* and there were insufficient laboratory resources to distinguish between the two mycobacteria. Thus, we used photochromogenicity to distinguish them. To the best of our knowledge, this is the first report on the use of photochromogenicity to confirm *M. marinum* outside of advanced molecular testing. Therefore, photochromogenicity can be used not only to identify nontuberculous mycobacteria in Runyon's group I, but also use to distinguish *M.marinum* from *M. leprae, M. ulcerans,* and *M. tuberculosis*.

In our case, we placed the bacteria, which grew well on Columbia blood agar, in the sunlight for 18 h, and the color

of the colony changed from white to yellow. Considering that the color of *M. ulcerans* does not change when exposed to sunlight, the bacterium was identified as *M. marinum*. The optimal treatment for *M. marinum* has not been defined due to a lack of comparative studies. The combination of rifampicin and clarithromycin as a dual therapy proved efficacious in our case. Other effective therapies reported in the literature include tetracyclines, trimethoprim-sulfamethoxazole, moxifloxacin, levofloxacin, and azithromycin, as well as various combinations, such as ethambutol plus rifampin[18-20].

CONCLUSION

Although the methods used to identify bacteria and fungi are constantly improving, the identification of *M. marinum* still represents a major challenge for hospitals with insufficient laboratory resources, which may lead to misdiagnosis or cause harm to patients. Based on the patient's occupation and exposure history, the characteristics of the patient's skin lesions, the bacterial growth time, and the results of Ziehl–Neelsen staining, 16S rRNA gene sequencing, and the sunlight exposure test, we can make a diagnosis of *M. marinum* infection and formulate a proper treatment plan. Therefore, as an auxiliary means for the diagnosis of *M. marinum* infection, photochromogenicity has the advantages of simplicity and exceptionally low cost, which make it worth promoting.

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AUTHORS' CONTRIBUTIONS

LLL wrote the manuscript; ML provided the case study information and revised the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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