Using diagnostic molecular technique for *mycobacterium marinum* cutaneous infection: A case report

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ABSTRACT:

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Mycobacterium marinum is the most common slow grower non-tuberculous mycobacteria causing skin infection. The characteristic finding includes subcutaneous nodule which can progress into a sporotrichoid pattern. We report a 66-year-old Thai male who presented with erythematous nodule at left wrist for 3 months. The patient had been treated with topical antibiotic and oral ciprofloxacin without improvement. Skin biopsy showed epithelioid granulomatous formation with positive AFB and Fite's acid fast stain. However, culture for mycobacteria yielded negative result. Subsequent16s rRNA sequencing, a molecular technique using for bacterial identification, detected an organism with 99% compatible with *M. marinum*. Clarithromycin, ethambutol and doxycycline were started, and the patient has significant improvement.

Key words: Non-tuberculous mycobacterium, *Mycobacterium marinum*, 16s rRNA sequencing

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ภาควิชาอายุรศาสตร์ โรงพยาบาลจุฬาลงกรณ์ มหาวิทยาลัย

มัยโคแบคทีเรีย มารีนุม เป็นเชื้อมัยโคแบคทีเรียชนิดไม่ก่อให้เกิดวัณโรค ที่ก่อให้เกิดการติดเชื้อบนผิวหนังได้บ่อยที่สุด โดยมักจะมาด้วยตุ่มนูน ซึ่งสามารถพัฒนาไปเป็นผื่นที่มีลักษณะ sporotrichoid pattern ได้ บทความนี้เป็นการรายงานผู้ป่วยชาย อายุ 66 ปี มาด้วยตุ่มนูนแดง บริเวณข้อมือซ้าย มานาน 3 เดือน ผู้ป่วยได้รับการรักษาด้วย ยาฆ่าเชื้อชนิดทา และยารับประทาน ciprofloxacin แต่อาการไม่ดีขึ้น จึงได้ทำการตัดชิ้นเนื้อของผู้ป่วยไปตรวจผลทางพยาธิวิทยา พบว่าเข้าได้กับการติดเชื้อ mycobacteria แต่ไม่สามารถระบุชนิดเชื้อได้ เนื่องจากผลเพาะเชื้อเป็นลบ จึงได้ทำการส่งตรวจ 16s rRNA sequencing และพบว่าเข้าได้กับเชื้อ มัยโคแบคทีเรีย มารีนุม ผู้ป่วยจึงได้รับยาฆ่าเชื้อสำหรับรับประทาน 3 ชนิดได้แก่ clarithromycin, ethambutol และ doxycycline พบว่าผู้ป่วยมีอาการดีขึ้นอย่างเห็นได้ชัด

คำสำคัญ: เชื้อมัยโคแบคทีเรียชนิดไม่ก่อให้เกิดวัณโรค, มัยโคแบคทีเรีย มารีนุม, การจำแนกสปีชีส์ของเชื้อมัยโคแบคทีเรีย

Introduction

Mycobacterium marinum is the commonest slow grower non-tuberculous mycobacterial skin infection in humans. This organism can be found in non-chlorinated fresh or salt water, especially in tank water[1]. An environmental or occupational exposure to injured skin is the main route of entry. Typical manifestations include subcutaneous nodules which progress to granulomatous lesions with lymphocutaneous spreading in a sporotrichoid pattern within weeks to months. Tenosynovitis or osteomyelitis can be found up to 60%[2]. Immunocompromised patients may develop extracutaneous or disseminated diseases [3].

Tissue biopsy and culture are important diagnostic methods for *M. marinum* infection since similar skin lesions can be caused by multiple pathogens such as *Sporothrix spp.*, *Leishmania spp.* and *Nocardia spp*. Nevertheless, the positivity rates for mycobacterial culture remain around 40–60%[4]. Currently, a molecular technique for mycobacterial identification has been successfully developed [5]. Here, we report a case of cutaneous infection caused by *M. marinum* which was diagnosed by 16S ribosomal RNA sequencing from skin biopsy.

Our case

A 66-year-old man presented with chronic wound on his left wrist. The lesion started at 1 month after the patient cleaned his fish tank. A red patch progressed to an erythematous nodule with some papules within 3 months.

Before coming to our hospital, he had been treated with topical antibiotics and oral ciprofloxacin for 1 month without improvement. His underlying diseases included diabetes mellitus, dyslipidemia and history of colonic cancer which was cured after surgery and chemotherapy.

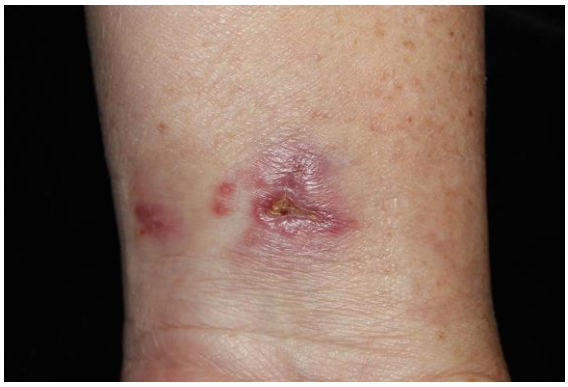
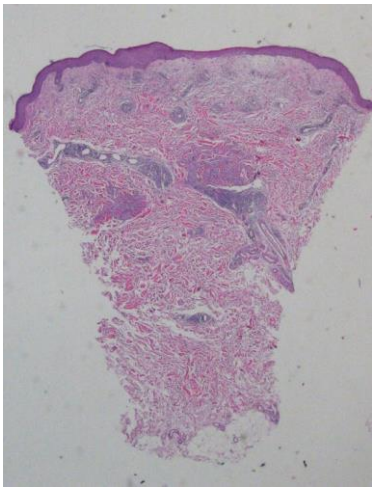


Figure 1 Shows an erythematous nodule with central drainage and a few satellite lesions on the left wrist.

Physical examination revealed an erythematous nodule with central drainage and a few satellite papules on left wrist (figure1). Excisional biopsy revealed a focal epithelioid granulomatous formation with lymphocytic infiltration (figure2). Organisms were found in AFB and modified AFB stain from tissue imprinting and Fite's acid fast stain from skin biopsy specimen (figure3). Culture for mycobacteria at 30°C and 37°C were negative. However, non-tuberculous *Mycobacterium* sp. (NTM) was highly suspected due to negative *Mycobacterium tuberculosis* complex polymerase chain reaction (PCR) and positive NTM PCR from fresh tissue. Subsequently, 16S ribosomal RNA (16S rRNA) sequencing from tissue specimen identified organism with 99% compatible with *M. marinum*. Clarithromycin and ethambutol were prescribed for treatment. The lesion still had draining fluid after 3 months of therapy and markedly improved after doxycycline was added.



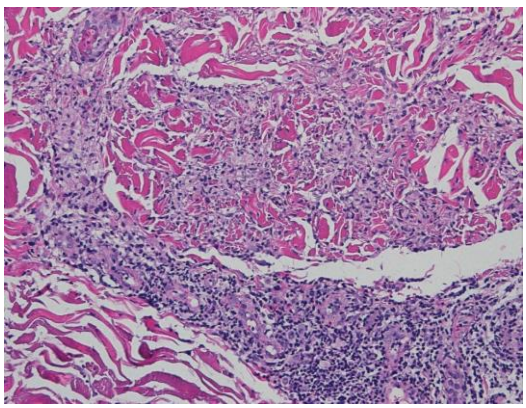


Figure 2 Skin biopsy from left wrist shows epithelioid granulomatous formation with lymphocytic infiltration.

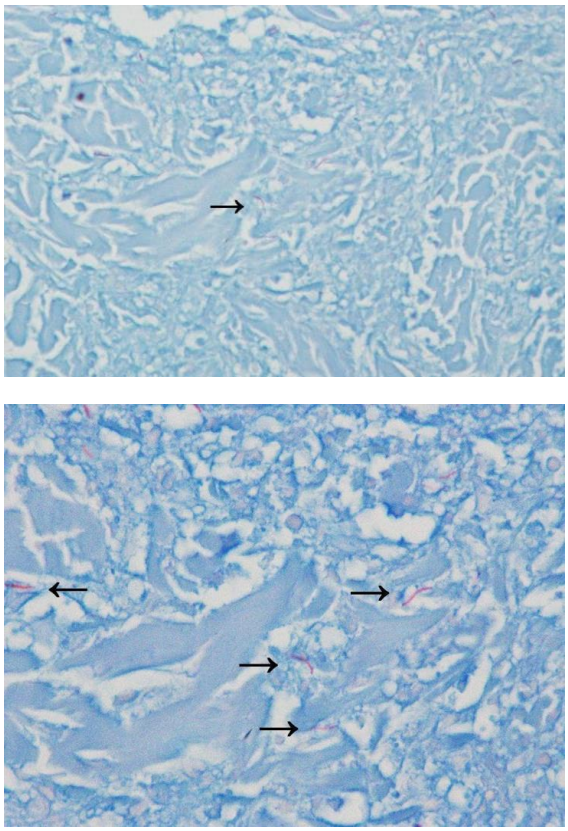


Figure 3 AFB staining shows long and quite large bacilli inside focal granulomatous formation in the dermis (arrow sign)

Discussion

Generally, diagnosis of *M. marinum* infection requires tissue biopsy and culture for mycobacteria. Histopathologic findings in an early phase demonstrates infiltration of neutrophils, monocytes and macrophages, while epithelioid granuloma with lymphocytic infiltration, documented in our case, and epidermal hyperkeratosis represent a late infection[6]. AFB stain positive bacteria of histopathology in our case suggest mycobacterial infection but cannot identified specific type of mycobacteria by AFB in histopathology. Other laboratory examinations such as culture and PCR can identify the specific organism. Culture which remains the gold standard for diagnosis [2] can confirm *M. marinum* infection in only 40-60% of cases due to technical difficulty. Culture for *M. marinum* requires incubation in Lowenstein–Jensen medium at 28 - 30°C which is lower than other mycobacteria and may need duration up to 6 weeks [4]. In our patient, non-tuberculous mycobacterial infection was highly suspected from the history, clinical presentation, positive AFB staining, morphology of the organisms in the histopathologic sections and polymerase chain reaction (PCR) for *Mycobacterium* sp.

Since conventional culture for non-tuberculous mycobacteria is difficult to yield a positive result. Utilization of molecular diagnostic methods in cases of mycobacterial infection has been increasing. Many molecular techniques have been developed for identification of NTM species including DNA–DNA hybridization (DDH) assays, gene amplification assays and 16S rRNA gene sequencing. DDH assays are available in commercial kit which contains 18 major mycobacterial species causing human pathology in the panel. Nevertheless, mycobacterial isolates are required, and the test is difficult to differentiate *M. ulceran* from *M.*

marinum. Gene amplification assay can be used for identification of common pathogenic mycobacterial genomes such as *M. tuberculosis*, *Mycobacterium avium*, *Mycobacterium intracellulare* and *Mycobacterium kansasii* with rapid turnaround time. However, both DDH and gene amplification assays are unable to detect *Mycobacterium* spp. which are not included in the panel. Therefore, negative results in patients with high likelihood of mycobacterial skin infection should be confirmed by 16S rRNA gene sequencings [7].

Genus-specific DNA probes for mycobacteria and PCR of 16S-23S internal transcribed are commercially available for the detection of *Mycobacterium* spp. However, probes may not be able to distinguish mycobacteria which commonly caused skin infections such as *M. haemophilum*, *M. ulcerans* and *M. marinum* [8]. 16S rRNA is a critical component of bacterial cell function. Therefore, gene encoded 16S rRNA is highly conserved among bacterial species and becomes an ideal target for sequencing. This method demonstrates good performance with more than 90% agreement with phenotypic identification [9]. For *M.* marinum, 16S RNA sequencing increases opportunity of detection negative-culture cases up to 60% and contains 99.5 – 100% specificity [10]. In our case, *M. marinum* was identified by 16S rRNA sequencing which confirmed the definite diagnosis despite negative culture result.

In conclusion, molecular method should be considered to assist diagnosis for *M. marinum* infection in addition to conventional culture method especially in patient with highly clinical suspicion.

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