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Postsurgical Wound Infection Caused by *Mycobacterium conceptionense* Identified by Sequencing of 16S rRNA, *hsp65*, and *rpoB* Genes in an Immunocompetent Patient

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Rapidly growing mycobacteria are ubiquitous in the environment and are increasingly being recognized as opportunistic pathogens. Recently, a new species, *Mycobacteium conceptionense*, has been validated from the *Mycobacterium fortuitum* third biovariant complex by molecular analysis. However, there are few reports, and postsurgical wound infection by this species is rare. We report a case of postsurgical wound infection caused by *M. conceptionense* in an immunocompetent patient that was identified by a sequencing analysis of 16S rRNA, *hps65*, and *rpoB* genes. **(Ann Clin Microbiol 2014;17:23-27)**

Key Words: Gene sequencing, *Mycobacterium conceptionense*, Wound infection

INTRODUCTION

Rapidly growing mycobacteria (RGM) are defined as non-tuberculous mycobacteria (NTM) that grow within 7 days on solid media. They are ubiquitous in the environment and often can be isolated from tap water. Increasingly, they are being recognized as opportunistic pathogens [1]. The major important RGM are *Mycobacterium abscessus*, *Mycobacterium chelonae*, and *Mycobacterium fortuitum* complex. The *M. fortuitum* complex is composed of *M. fortuitum*, *Mycobacterium peregrinum*, and *M. fortuitum* third biovariant complex. Recently, a few new species of *M. fortuitum* group have been found exploiting advancements of molecular analysis [2-4]. *Mycobacterium conceptionense* was validated but there are few reports, and wound infection is rare [5-7]. We report a case of postsurgical wound infection in an immunocompetent patient that was caused by *M. conceptionense.*

CASE REPORT

A 66-year-old man presented with pus and a skin defect on his lower back for one month at previous operative site. He was operated on by foraminotomy with a device for intervertebral assisted motion (DIAM^{TM}) insertion, which was a H shaped interspinous spacer consisting of silicone core, poly ester mesh, fixation cables and titanium crimps, as a result of spinal stenosis eight months earlier. The lesion had started as erythematous nodule with swelling one month before and then burst. Physical examination revealed that soft tissue was exposed with yellow pus over the wound. He did not complain of tenderness or local heat. No specific medical or family histories were reported, and his general condition was good. Laboratory tests including complete blood count, liver function tests, and C-reactive protein showed no significant abnormalities. A Blood Quantiferon-TB

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test was negative. No active pulmonary disease was detected by chest radiography. Skin biopsy of the lesion showed a denuded epidermis with granulation tissue and granuloma with central neutrophilic aggregation, appearing as a fungal or mycobacterial infection. The patient initially received empirical anti-mycobacterial treatment with isoniazid, rifampicin, pyrazinamide, and ethambutol (HREZ) and wound dressing daily.

Non-pigmented strain was cultured on egg-based solid media

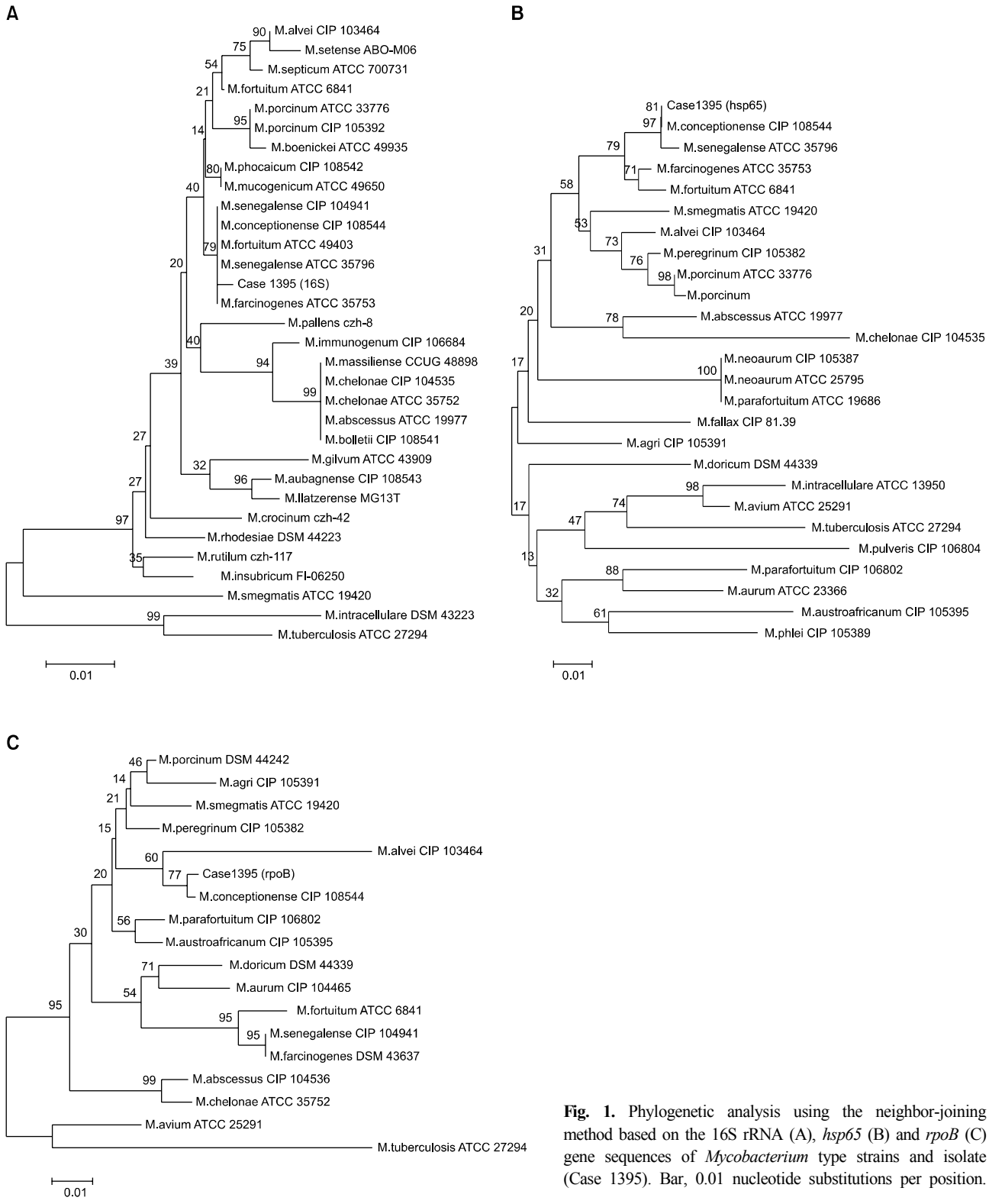

Fig. 1. Phylogenetic analysis using the neighbor-joining method based on the 16S rRNA (A), *hsp65* (B) and *rpoB* (C) gene sequences of *Mycobacterium* type strains and isolate (Case 1395). Bar, 0.01 nucleotide substitutions per position.

in 5 days at 36°C from tissue on skin-defect. Acid fast stain using Ziehl-Neelsen method showed positive results. However, the isolate from tissue culture was not identified by PCR-restriction fragment length polymorphism (RFLP) analysis based on the *rpoB* gene. Fungal culture was negative. After two months, the patient complained of continuous drainage of yellow pus from the wound, and the anti-mycobacterial medication was stopped. The same NTM was cultured on solid medium for mycobacteria from pus. Gam stain and acid-fast bacillus stain directly from clinical specimen were negative and this isolate was also unidentified by RFLP method. Antimycobacterial susceptibility tests were performed according to the Clinical and Laboratory Standards Institute (CLSI) M24-A, retrospectively. The results were interpreted according to the criteria for rapidly growing mycobacteria. The strain was susceptible to amikacin, ciprofloxacin, clarithromycin, doxycycline, and imipenem, however, it was intermediate to cefoxitin, and tobramycin.

We performed 16S rRNA gene sequencing to identify NTM as previously described [8], but the isolates was not identified because the sequence was indistinguishable, showing 99.38% homology with *M. fortuitum* (GenBank Accession No. AY457067), 99.58% with *Mycobacterium farcinogenes* (GenBank AF055333), 99.79% with *Mycobacterium senegalense* (GenBank AF480596) and *M. conceptionense* (GenBank EU191913). The isolate was further investigated by sequencing using *hsp65* and *rpoB* [9]. The *hsp65* sequences of the strain showed 99% similarity with *M. senegalense* (GenBank AY684045) as well as 99.5% similarity with *M. conceptionense* (GenBank EU191920). Sequencing analysis based on *rpoB* showed 99.4% homology with *M. conceptionense* (GenBank AY859695), and the second closest match was *Mycobacterium porcinum* (GenBank AY544955), with 98.3% homology. This isolate was finally identified as *M. conceptionense* by 16S rRNA, *hsp65*, and *rpoB* genes sequencing and phylogenetic analysis using MEGA version 4 (Fig. 1).

After recovering of NTM from solid agar media, the anti-mycobacterial treatment changed to minocyclin, ofloxacin, and clarithromycin and wound dressing continued daily. After 2 weeks, oral medication was stopped because the patient developed dizziness. The patients refused to take anti-mycobacterial medication anymore. In spite of dressing of the wound daily, the amount of discharge around the wound increased, and local pain developed. Eventually, he was re-operated on and a new DIAM installed, and the patient is progressing well.

DISCUSSION

M. conceptionese is a non-pigmented RGM belonging to the *M. fortuitum* group that was recently distinguished from *M. porcinum*. New species of the *M. fortuitum* group have steadily increased following the advancement of molecular methods [2-4]. Generally, the 16S rRNA gene has been used for the identification of unusual mycobacterial isolates. However, sequence analysis of partial 16S rRNA gene cannot discriminate between closely related RGM species. Thus, additional genes such as *sodA*, *hsp65*, and *rpoB* were proposed for sequence analysis for the molecular identification of mycobacteria because these genes showed greater interspecies and intraspecies sequence divergence than 16S rRNA gene [10]. In this case, we identified the isolate performing sequence analysis of *rpoB* and *hsp65* genes as well as the 16S rRNA gene. The isolate shared more than 99.5% sequence similarity of the 16S rRNA gene with *M. senegalense*, *M. farcinogenes*, and *M. conceptionense*, whereaas for *hsp65* analysis, the isolate showed 99.5% sequence similarity of *M. conceptionense* and 99.0% of *M. senegalense*. Also, *rpoB* gene sequence of the isolate shared 99.4% similarity with *M. conceptionense* and 98.3% with *M. porcinum*. The results supported the view that *rpoB* sequencing is the primary tool for the molecular identification of RGM, and alternative DNA targets such as *hsp65* provide better resolution at the species level [10,11]. However, there are no validated interpretative criteria for the identification of RGM by sequence analysis except 16S rRNA gene [12].

The *M. fortuitum* groups are considered common etiologic pathogens for postsurgical wound infection [13]. Various medical or surgical procedures related to wound infection by RGM include cardiac bypass surgery, augmentation mammoplasty, and subcutaneous needle injections or liposuction for cosmetic purposes [14]. Typically, the wound infection related to RGM occurred as a late postoperative complication [15]. Clinical manifestations usually start with watery discharge, wound breakdown, and low fever after 1 to 12 months. Thus, non-healing of a surgical site or dehiscence of a previously healed incision should be an important trigger to suspect mycobacterial infection [14,16,17]. Most reported patients were generally healthy. Risk factors for the surgical site infection were trauma and contamination of surgical supplies and antiseptic solution [14]. In the initial report of skin and soft tissue infection related to *M. conceptionense*, this microorganism was isolated from post-traumatic osteitis that occurred after exposure to a river [5].

Water has been mentioned as an important source because of the ubiquitous environmental distribution of RGM. However, the sources of infection in the subsequent cases were not revealed [6,7]. Our patient denied any contact with contaminated water, trauma, or acupuncture before the onset of symptoms. It could be assumed that the DIAM was infected, although the source in our case remains unknown. The patient underwent a surgical operation at other hospital, so we could not investigate the possibility of nosocomial infection caused by a surgical equipment contamination.

The optimal antibiotic therapy for *M. conceptionese* infection has not been established. In previous reports, most patients were treated with a combination of surgery and antimicrobial agents according to the antimicrobial susceptibility of the isolates [5-7]. Especially in plastic surgical sites infected by RGM such as *M. fortuitum*, it is important to use adjuvant antimicrobial treatment in addition to a prompt surgical approach because patients treated with surgery alone had relapses within 4 to 6 weeks [13]. The duration of antimicrobial therapy in wound infections was diverse, ranging from 3 to 18 months [7]. In our case, initial antimicrobial therapy including minocyclin, ofloxacin, and clarithromycin lasted only for 2 weeks because of side effects. Then he denied appropriate anti-mycobacterial treatment and incision and drainage at skin defected region. The only treatment was daily site dressing. It leaded to be the progression of the infection by *M. conceptionense*. The amount of discharge around the wound started to increase, and expanding pain developed around the wound. Eventually, he was treated by reoperation and the infection related symptoms were relieved.

We describe a case of surgical site infection in an immunocompetent patient caused by *M. conceptionese*. We identified the isolate performing sequence analysis of the *rpoB* and *hsp65* genes in addition to 16S rRNA gene. In view of the limited reports of *M. conceptionese* infection, further investigation is needed to establish the predisposing factors, treatment, and prognosis.

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=국문초록=

16S rRNA, *hsp65*, 및 *rpoB* 염기순서분석으로 동정한 *Mycobacterium conceptionense*에 의한 면역능이 정상인 환자에서 발생한 수술후 창상감염

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신속발육항산균은 자연환경에서 흔히 검출되는 균으로, 기회감염병원체로 인체감염의 주요 원인균으로서 인식되고 있다. *M. conceptionense*는 분자역학적 분석을 통해 *M. fortuitum*의 세번째 생체변이종에서 분리되어 새로운 종으로 명명되었다. 그러나 이 균에 의한 감염 보고는 많지 않고 특히 수술후 창상 감염에 대한 보고는 거의 없다. 이에 저자들은 16S rRNA, *hsp65* 및 *rpoB* 유전자 염기순서분석을 이용하여 동정한 *M. conceptionense*에 의한 수술 후 창상감염을 보고하고자 한다. [Ann Clin Microbiol 2014;17:23-27]

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