



INNO-LiPA DNA line probe assay misidentification of *M. smegmatis* as *Mycobacterium fortuitum* complex

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ABSTRACT

Seven weeks after being kicked in the face by a cow, a 34-year-old male patient developed a posttraumatic mycobacterial lymphadenitis. A rapidly growing mycobacterial isolate cultured from a surgically drained lymphadenitis pus specimen was identified as *Mycobacterium smegmatis* by matrix-assisted laser desorption/ionization mass spectrometry and a combination of ITS-, *hsp65*-, and 16S rRNA-DNA sequence analysis, but as *Mycobacterium fortuitum* complex using the commercial INNO-LiPA Mycobacteria v2 line probe assay. As it is unclear if the misidentification of this strain is an exception, more research is required.

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1. Introduction

The rapidly growing mycobacteria (RGM) are a distinctive subset of the nontuberculous mycobacteria (NTM) and are generally defined as mycobacteria that show mature colonies after subculture on solid media within 7 days under optimal conditions (Griffith et al., 2007). RGM that cause disease in humans generally belong to the *M. fortuitum* complex, the *M. chelonae*-*M. abscessus* group, and the *M. smegmatis* complex. Of these, *M. fortuitum*, *M. abscessus*, and *M. chelonae* are most frequently isolated from human samples in clinical microbiology laboratories (Hoefsloot et al., 2013). The *M. smegmatis* group consists of the closely related *M. smegmatis*; *M. goodii*; and, according to some authors, also *M. wolinsky* (Brown et al., 1999). The RGM are opportunistic pathogens that produce disease in a variety of clinical settings, such as localized cutaneous infections, disseminated disease, and pulmonary disease. Epidemiology, types of clinical disease,

and intrinsic antimicrobial resistance patterns differ among individual species of RGM (Brown-Elliott and Wallace, 2002). Identification of mycobacteria in high-resource countries has become increasingly accessible and fast by introductions of a DNA line probe assay, nucleic acid sequencing techniques, and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) (van Ingen, 2015). Thereby, identification-based guidance of diagnosis and treatment has become feasible.

2. Case presentation

A 34-year-old male without significant prior medical history presented at the department of emergency medicine after having been kicked in the face by a cow. He had an infraorbital soft tissue laceration and comminutive os zygoma fracture requiring open reduction and internal fixation. The surgical follow-up was uncomplicated, and he was treated with amoxicillin/clavulanic acid for 7 days.

About 7 weeks after the initial event, he presented with a submental nodular enlargement of approximately 3 cm in diameter. Ultrasound-guided fine needle aspiration and pathological examination confirmed the clinical suspicion of a necrotic lymph node. In the subsequent week, the lymph node enlarged and was surgically drained, and pus was sent for microbiological investigations. A drain was left in situ,

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and no antibiotic treatment was initiated. The patient's symptoms improved. Gram stain of a smear preparation of the drained pus revealed many polymorphonuclear leukocytes but no organisms; specifically, no ghost bacilli were observed. On the third day of culture, a smooth colony type growth was noted on blood agar (trypticase soy agar, 5% sheep blood, Beckton Dickinson, BD). By MALDI-TOF MS (Microflex, using MALDI biotyper real time classification v3 software and the Biotyper database V3.3.1.0_4110–4613, Bruker, Germany), the isolate was identified as *Mycobacterium smegmatis* (score value 2.0). In contrast, species determination via INNO-LiPA Mycobacteria V2 line probe assay (Innogenetics, Belgium, Europe, performed according to manufacturers' instructions) identified the colony as *M. fortuitum* complex. To exclude a mixed culture as the source of this discrepancy, a subculture from a single colony was prepared. When this pure subculture was reinvestigated by MALDI-TOF MS and INNO-LiPA Mycobacteria v2 line probe assay, the same discrepant results remained. DNA sequence analysis using *Mycobacterium* spp. specific primers for 16S rRNA, yielded a 469–base pair (bp) fragment with 100% identity to the 16S rRNA sequence of *M. smegmatis* NCTC 8159^T genome sequence (Genbank Sequence ID LN831039.1, nucleotides 3902792–3902324 and 5035500–5035032, for the two genomic copies of the 16S rRNA sequence) and only 444/468 (95%) identity with *Mycobacterium fortuitum* strain ATCC 49404^T (Sequence ID: AF480581.1). Also, the sequences of the amplicons of the 65-kDa heat shock protein-encoding gene (*hsp65*) and the 23S-5S internal transcribed spacer (ITS) showed the best match with *M. smegmatis* NCTC 8159^T (Genbank sequence ID LN831039.1; *hsp65*: 99% sequence identity over 427 bp, nucleotide numbers 3902530–3902719, ITS: 100% sequence identity over 251 bp, nucleotide numbers 3902798–3902321, respectively). As with the 16S rRNA sequences, *hsp65* and ITS sequences differed significantly from sequences from several other mycobacterium type strains of, e.g., *M. fortuitum*. In summary, our isolate clustered closely with the 16S rRNA, *hsp65*, and ITS sequences from the *M. smegmatis* type strain and only distantly with those from *M. fortuitum* type strains.

Susceptibility testing by broth microdilution according to CLSI guidelines [CLSI, document M24-A2] showed inducible macrolide resistance; intermediate susceptibility to ceftioxin (MIC = 32 mg/L); and susceptibility to imipenem, amikacin, doxycycline, linezolid, ciprofloxacin, and trimethoprim–sulfamethoxazole.

Following lymph node drainage, no further therapy was started, and no recurrence of lymph node infection or swelling was noted during a 1-year follow-up.

3. Discussion

Here we describe a case of traumatic inoculation and infection with *M. smegmatis* resulting in necrotizing lymphadenitis, requiring surgical treatment but no antibiotic treatment. Traumatic inoculation is thought to be the most common mode of transmission of this environmental organism (Ciubotaru et al., 2004; Griffith et al., 2007; Newton et al., 1993). Correct mycobacterial species identification is important because it may point to specific clinical diagnoses and lead to antibiotic tailoring while awaiting results of in vitro susceptibility testing.

The introduction of the DNA line probe assay technology accelerated the identification of many mycobacterial species in comparison with biochemical and phenotypic identification methods. The INNO-LiPA Mycobacteria v2 line probe assay was designed to amplify the mycobacterial ITS, which has been documented as more discriminative than the 16S rRNA gene (Frothingham and Wilson, 1994). In this patient's isolate, 16S rRNA-, *hsp65*-gene as well as ITS sequencing and MALDI-TOF MS results concordantly identified the isolate as *M. smegmatis*, whereas the INNO-LiPA Mycobacteria v2 line probe assay misidentified the isolate as *M. fortuitum* complex. The package insert of the INNO-LiPA Mycobacteria v2 line probe assay states that “Probe line 23 (MFO) displays a positive reaction for isolates belonging to the *M. fortuitum*–*M. peregrinum* complex. *M. smegmatis* also reacts positive with this

probe, but is clearly distinguished from this complex by a positive MSM probe on line 24.” Therefore, according to the insert, the problem with this misidentification is not the reaction with the *M. fortuitum* complex probe but, instead, the absence of a visible reaction with the *M. smegmatis* specific probe. We repeated INNO-LiPA identification of the isolate with the same results, making human operational errors unlikely. Multiway alignment of the 23S-5S ITS sequence of our *M. smegmatis* isolate clustered with ITS sequences from the *M. smegmatis* (type) strains but much less with sequences from *M. peregrinum* or *M. fortuitum* type strains. We therefore determined that the “MSM” probe from the INNO-LiPA Mycobacteria V2, “line 24” does not adequately identify all *M. smegmatis* isolates. Misidentification of RGM as *M. fortuitum* complex using the line blot assay has been well described, but to our knowledge, this is the first case with *M. smegmatis* being the true species identity (Tortoli et al., 2010; Van Ingen et al., 2010). In 2005, the Dutch reference laboratory for tuberculosis identified 1 *M. smegmatis* isolate (by *rpoB* sequencing, 16S sequencing, and the Genotype Mycobacterium AS kit (Hain)) that was misidentified as *M. fortuitum* by INNO-LiPA v2, caused by nonreactivity of the MSM probe on line 24. This isolate was recovered, and further analysis by MALDI-TOF MS and *hsp65* sequencing reconfirmed this isolate's identity as *M. smegmatis*. Previously, 5 *M. smegmatis* isolates all reacted with the MSM probe in the INNO-LiPA V2 (Tortoli et al., 2003).

M. smegmatis is an infrequently encountered RGM in clinical microbiology laboratories but was clinically significant in this case of necrotizing lymphadenitis after traumatic inoculation from the cows' kick. Remarkably, in 2004, a French case of *M. smegmatis* lymphadenitis was also found in association with traumatic inoculation by a cow (Ciubotaru et al., 2004). Similar to our case, surgical debridement has been necessary for most patients, and additional antibiotic therapy should be considered (Griffith et al., 2007). The use of the INNO-LiPA Mycobacteria v2 line probe assay rapidly identifies mycobacteria (Padilla et al., 2004; Tortoli et al., 2010). Nevertheless, *M. fortuitum* identification by INNO-LiPA V2 may be interpreted with some caution. Further assessment of the true identity of such an isolate might be warranted, e.g., in the case of an aberrant clinical response or a deviant antibiotic susceptibility profile. In such isolates, *hsp65* or *rpoB* sequencing or MALDI-TOF MS could correct such misidentifications (Alcaide et al., 2018).

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Conflict of interest

The authors declare that they have no conflicts of interest.

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