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Misidentification of *Mycobacterium smegmatis* as *Mycobacterium fortuitum* by DNA line probe assay

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ABSTRACT

Optimal management of infection with mycobacterial species requires accurate identification down to complex/species level due to variations in outcomes. Over the last few decades, there have been significant advances in laboratory diagnostics with development of newer and rapid molecular methods. Here we describe a case of *Mycobacterium smegmatis* that was misidentified as *Mycobacterium fortuitum* by DNA line probe assay.

1. Introduction

Accurate and timely identification of mycobacterial species is crucial for optimal management of patients since treatment and outcomes vary depending on the mycobacterial species identified. Older biochemical tests have now been replaced by newer diagnostic methods including matrix associated laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and molecular methods including line probe hybridization assays, as well as 16S ribosomal RNA gene, *rpo*B, and *hsp*65 PCR. DNA line probe assays provide rapid means of identification and currently there are two commercially available assays including INNO-LiPA MYCOBACTERIA v2 assay (Fujirebio Europe, Ghent, Belgium) and GenoType assays (Hain Lifescience GmbH, Nehren, Germany) however neither is currently approved by the FDA and therefore use is largely restricted to the public health laboratories in United States. Studies utilizing these lines probe assays have reported satisfactory sensitivity and specificity [1–5]. Here we present a case of *Mycobacterium smegmatis* that was misidentified as *Mycobacterium fortuitum* by a DNA line probe assay.

A 69-year-old female was admitted to our facility with right elbow arthroplasty infection. Two months prior to hospitalization she sustained a fall outdoors and had a distal humeral open fracture. She underwent debridement followed by total elbow arthroplasty two weeks later. Five weeks after arthroplasty she developed increasing right elbow redness and swelling and was seen in another institution where she underwent multiple occurrences of surgical debridement. Multiple operative cultures grew an acid-fast bacilli. These were sent to a reference laboratory and were identified as *M. fortuitum* complex by INNO-LiPA MYCOBACTERIA v2 line probe assay (Fujirebio Europe, Ghent, Belgium). The patient was subsequently transferred to our facility for definitive surgical management and underwent resection arthroplasty. Surgical cultures showed growth on sheep blood agar after 48 h of incubation. Gram stain showed beaded Gram-positive bacilli. MALDI-TOF MS (Bruker MALDI Biotyper 3.1 using Mayo Clinic's Custom Library Database) was done with organism identified as *Mycobacterium smegmatis* (score value 2.4). We additionally obtained the isolate previously identified as *M. fortuitum* from the outside institution which was also identified as *Mycobacterium smegmatis* by MALDI-TOF MS. We then performed 16S 500 bp rRNA gene sequencing on both isolates which yielded a 466 base pair long fragment with 100% match with *M. smegmatis* (ATCC® 14468) using Mayo Clinic's custom library version # MAA2018. Broth microdilution was performed using Sensititre™ RAPMYCO AST Plate as per CLSI guidelines. showed inducible macrolide resistance and intermediate susceptibility to cefoxitin with MIC of 32 mcg/mL by broth microdilution and susceptibility to amikacin, ciprofloxacin, doxycycline, imipenem, linezolid, minocycline, moxifloxacin, tobramycin, and trimethoprim sulfamethoxazole (TMP-SMX). The patient was started on intravenous imipenem, doxycycline, and trimethoprim-sulfamethoxazole with plans to do total of 3–6 months of total therapy depending on clinical response.

Here we describe a case of *M. smegmatis* prosthetic joint infection likely resulting from traumatic inoculation during the initial fall. Among

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the rapidly growing NTMs, the majority of reported infections are secondary to *M. fortuitum*, and *M. abscessus-chelonae* complex. *M. smegmatis* is an environmental saprophyte and has rarely been identified as a cause of infection in humans [6–10]. Identification in the lab typically involves MALDI-TOF MS, sequencing or DNA probes. INNO-LiPA is a line probe assay which identifies 16 mycobacterial species including *M. smegmatis* and *M. fortuitum.* This assay is based on amplification of 16S-23S ribosomal RNA spacer region followed by reverse-hybridization with probes immobilized on the nitrocellulose strips. The positive result is depicted by a color change with each line corresponding to a specific mycobacterial species. Per manufacturer's instructions with *M. fortuitum,* probe line 23 (MFO) is positive in addition to the control line and the *Mycobacterium* genus line. With *M. smegmatis* line 23 (MFO) and line 24 (MSM) are both positive however in this case only line 23 was positive. On literature review we found one previous report of such misidentification with INNO-LiPA assay [11]. In this case *M. smegmatis* was misidentified as *M. fortuitum* which was also due to lack of reaction with line 24. In another study of INNO-LiPA line probe assay all 5 *M. smegmatis* isolates were correctly identified [12]. There have been previous reports of misidentification of *M. smegmatis* as *M. fortuitum* as well as *M. fortuitum* as *M. smegmatis* by another line probe assay, GenoType assay [5,13]. The management in our case did not change related to this misidentification as susceptibility panel tested was same for both organisms with use of same treatment regimen however this situation created some degree of confusion for the treatment team. Both *M. fortuitum* and *M. smegmatis* are typically tested for inducible macrolide resistance which was performed in this case and inducible resistance was seen. One concern with such misidentification is the errors in the post-analytic phase as isolation of non-tuberculous mycobacterium species from clinical isolate does not always suggest mycobacterial infection and results are interpreted based on the underlying clinical scenario, pathology findings and individual patient risk factors. As such, *M. fortuitum* may be more likely to be implicated as a pathogen if identified from a clinical specimen as it is known to cause a wide spectrum of clinical syndromes compared to *M. smegmatis* which is rarely identified as a cause of infection. However, in our case, multiple surgical cultures were positive for *M. smegmatis* without any other organism identified. Correct identification is necessary to report in the literature as well which will help with establishing hosts at risk, location of infection and pathogenesis in human infections and management strategies and how these may differ from other rapid growers.

Here we have presented a 2nd case of *M. smegmatis* misidentified as *M. fortuitum* by a DNA line probe assay. More studies are required to identify the sequence variations associated with such misidentification.

CRediT authorship contribution statement

Madiha Fida: Data curation, Writing – original draft, Writing – review & editing. **Elena Beam:** Writing – review & editing. **Nancy L. Wengenack:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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