A novel *Mycobacterium cosmeticum*-like bacterium as a cause of ear infection

Jeanette W.P. Teo\(^1\)*, Janet Cheng W. S\(^1\), Roland Jureen\(^1\) and Raymond T.P. Lin\(^1\)

\(^1\) National University Hospital
Department of Laboratory Medicine,
Microbiology Unit,
Singapore 119074
Republic of Singapore

* Corresponding author: Jeanette_Teo@nuhs.edu.sg.
Tel: +65-67724856

Email addresses of authors:
Janet_Cheng@nuhs.edu.sg
Roland_Jureen@nuhs.edu.sg
Raymond_Lin@nuhs.edu.sg
Abstract

Background: Otitis externa can have a bacteriological agent associated with the condition, typically *Pseudomonas aeruginosa* and *Staphylococcus aureus* being most commonly implicated. However the condition has never been attributed to *Mycobacterium* spp.

Findings: Here, we describe the phenotypic and molecular characterization (16S rRNA, 16S-23S internal transcribed spacer (ITS), partial *rpoB* gene and *hsp65* gene sequencing) of a novel non-tuberculous mycobacterium (NTM), which is the causative agent of chronic otitis externa in an adult male patient. Genetically, the bacterium is most closely related to *Mycobacterium cosmeticum* however biochemical features indicate that it is distinctly different.

Conclusions: We highlight for the first time an atypical NTM as a cause of ear infection.

Keywords: ear infection, non-tuberculous mycobacterium, gene sequencing, phylogeny

Background

Nontuberculous mycobacteria (NTM) are known albeit uncommon causes of otic infections (otitis media and otomastoiditis). Members of the *Mycobacterium abscessus* complex are typical causative agents [1,2] although on rare occasions *M. avium* [3] and *M. fortuitum* [4] have also been isolated from patients with disease. Likewise, otitis externa can have a bacteriological component associated with the condition, with *Pseudomonas aeruginosa* and *Staphylococcus aureus* being most commonly implicated [5,6] however the condition has never been attributed to *Mycobacterium* spp.
Findings

In this report, we describe the isolation of a novel *M. cosmeticum*-like bacterium (NTM165). The isolate was obtained from an ear swab of a 39-year-old male patient with chronic otitis externa and is believed to be the causative agent. As this was a referral sample from a local polyclinic, we were unable to obtain further clinical details with regards to history and treatment. Ziehl-Neelsen staining indicated that the bacilli were acid-fast. After 3 days of growth at 37°C on Middlebrook 7H10 agar plates, NTM165 colonies were smooth and off-white color. No growth was observed at 42°C. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF MS) identification of NTM165, with full protein extraction procedures was performed as described by the manufacturer (Bruker Daltonics, Bremen, Germany). A result score of 1.727 matching *Mycobacterium cosmeticum* was generated however this was an inadequate score to confidently define the species. The isolate was sent to a local reference tuberculosis laboratory for further identification by high-performance liquid chromatography (HPLC) analysis. Evaluation of the mycolic acid pattern by HPLC indicated that NTM165 had a profile similar to the *M. fortuitum* complex, but provided no further identification. Mycobacterial commercial DNA probe assays, GenoType Mycobacterium CM/AS (Hain Lifescience GmbH, Nehren, Germany) and INNO-LiPA MYCOBACTERIA v2 (INNOGENETICS N.V., Gent, Belgium), yielded no identification for the NTM.

For molecular identification, the mycobacterial DNA was extracted according to the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) protocol for gram-positive bacteria. The full length 16S rRNA, 16S-23S internal transcribed spacer (ITS), partial *rpoB* gene and *hsp65* genes [7,8] were amplified and sequenced. The gene sequences were compared to those available in the GenBank database using BLASTN and an approximate phylogenetic affiliation was determined for NTM165. Phylogenetic trees were built using MegAlign software (DNASTAR, Madison, WI). Trees were drawn by bootstrap analysis with 1000 resamplings and 111 seeds. The full 16S rRNA gene sequence of NTM165 showed highest level of sequence similarity (1440/1456 nt, 99%)
to *M. cosmeticum* type strain LTA-388 (NCBI Reference Sequence NR_025787) (Fig. 1). It has been proposed that a genotypic difference of >1% is sufficient to be named as a new species [9] and where there are distinct phenotypic differences between the related species, the separation as a new species is even more strongly warranted. BLAST sequence analysis showed that NTM165 *hsp65* had a 99% (411/414 nt) DNA identity to *M. cosmeticum* (GenBank accession number AY449731.1) whilst the closest match to the *rpoB* sequence was *M. canariasense* (GenBank accession number KJ720535.1) (677/696 nt, 97%) (Fig. 1). The ITS sequence was also searched against the GenBank database and the closest result was a 94% identity to *M. neoaurum* (GenBank accession number CP006936.2). The absence of *M. cosmeticum* and *M. canariasense* ITS sequences in the GenBank database likely accounted for *M. neoaurum* as the best match.

Biochemical characterization of NTM165 was performed as described by Metchock et al. [10]. The biochemical features were compared with other closely related mycobacteria (Table 1). NTM165 was phenotypically distinct from related species judging by its morphology, single carbon source utilization and nitrate reduction tests. Susceptibility testing was performed and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. The isolate was susceptible to amikacin, cefoxitin, ciprofloxacin, moxifloxacin, clarithromycin (up to 14 days of incubation), doxycycline, imipenem, linezolid and sulfamethoxazole.

**Discussion**

The first reported strains of *M. cosmeticum* were obtained from cultures of a sink drain in a nail salon and from a granulomatous lesion of a female mesotherapy patient in Venezuela [12]. The pathogenicity of *M. cosmeticum* was further demonstrated in additional cases whereby the bacterium was responsible for pulmonary disease, catheter-associated bacteremia and most recently as a colitogenic agent [13, 14]. *M. canariasense*, akin to *M. cosmeticum*, is an opportunistic pathogen linked to bacteremia in patients with underlying malignant diseases [15-17]. Therefore, it is not
inconceivable that NTM165, being related to these two species, has also pathogenic potential.

Conclusion

In this report, we describe for the first time a novel *M. cosmeticum*-like NTM as a causative agent of chronic otitis externa. We highlight the importance of the use of a multiple-pronged approach for the identification and characterization of the microorganism. MALDI-ToF provided the first clues that this organism was highly similar to *M. cosmeticum* but subsequent molecular analysis of 16S rRNA, *hsp65* and *rpoB* gene sequences and comparative biochemical assays unambiguously identified NTM165 as being dissimilar to other related *Mycobacterium*. Our experience with HPLC identification parallels previous reports where it has been shown the technique is inadequate in differentiating species with shared patterns, particularly amongst the fast growers [15, 17].

The 16S rRNA, *hsp65*, *rpoB* and ITS sequences of NTM165 have been deposited in GenBank under the respective accession numbers of KP012254, KP012255, KP012256, KP012257.

List of abbreviations: None

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

JT was responsible for conception and design, molecular experiments, data analysis, interpretation, results elaboration and manuscript writing. JC was responsible for biochemical and phenotypic characterization of the isolate. RJ and RL were responsible
for data collection and manuscript revision. All authors read and approved the final manuscript.

Acknowledgments

None

References


Fig. 1. Neighbour-joining trees of full-length 16S rRNA (A) and rpoB (B) sequences of NTM165 and closely related rapidly growing mycobacterial species. Bootstrap values are displayed on branch nodes. GenBank accession numbers are given in parenthesis. ATCC, American Type Culture Collection; CCUG, Culture Collection, University of Göteborg, Sweden; DSM, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures; NCTC, National Collection of Type Cultures, UK; CIP, Collection de l’Institut Pasteur, France.
Table 1. Phenotypic characteristics of NTM165 and closely related rapidly growing mycobacteria

<table>
<thead>
<tr>
<th></th>
<th>NTM165</th>
<th>M. cosmeticum†</th>
<th>M. canariasense†</th>
<th>M. brisbanense † (M. fortuitum complex)</th>
<th>M. neoaurum†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth on MacConkey agar</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth on 5% NaCl</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reductase</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>Tween hydrolysis</td>
<td>–</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
<td>V</td>
</tr>
<tr>
<td>Arylsulfatase (3 days)</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-mannitol</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>Dulcitol</td>
<td>–</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

† Phenotypic characteristics of the *M. cosmeticum*, *M. canariasense*, *M. brisbanense* and *M. neoaurum* isolates were obtained from their respective publications [12, 15, 18, 19]
V, variable
NA, data not available