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Case Report

Challenging diagnosis of *Mycolicibacterium cosmeticum/canariasense* infection: A case report and literature review

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ABSTRACT

We present the case of an immunocompromised child with *Mycolicibacterium cosmeticum/ canariasense* infection. Our case highlights the difficulty in adequate speciation. Most isolates described in the literature were identified using 16 s-*rRNA* PCR, which if performed on our sample would at best be inconclusive. Misidentifications could have a real impact on the body of evidence collected on these isolates thus far.

1. Introduction

Rapidly growing Mycobacteria (RGM) are mostly environmental microorganisms of low virulence to the human host that belong to the non-tuberculous mycobacteria group (NTM) [1,2]. Over the last decade, an increase in RGM infections has been reported worldwide [3]. This increase may be attributed to the emergence of these pathogens, the growing population of immunosuppressed patients as well as to the advancement in molecular diagnostic techniques [4–5].

RGM detection requires an appropriate index of suspicion but standard microbiological methods may be inadequate for RGM identification. The recent introduction of matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) to routine microbiology has significantly improved NTM species identification but certain uncommon species remain a diagnostic challenge. Furthermore, MALDI-TOF identifications must be validated per species, which complicates the correct identification of rare species. Additional challenges are the rapidly changing taxonomy of this group [6] and the specialization required for performing antimicrobial susceptibility tests for RGM [7,8].

Endovascular infections involving RGM are rare and mainly associated with indwelling vascular catheters in immunocompromised patients. Most cases of RGM endovascular infections are due to *mycobacterium fortuitum* (recently reclassified as *Mycolicibacterium fortuitum*), *mycobacterium abscessus* and *mycobacterium chelonae* [9] albeit cases involving other rare species have anecdotally been reported. We present a case of endovascular infection in a pediatric immunosuppressed patient involving a rare and difficult-to-identify RGM species and discuss several lessons learned.

2. Case presentation

A 42-month old male was diagnosed with metastatic N-myc negative neuroblastoma in October 2017. A port-a-cath was inserted in mid-November 2017 and treatment with Rapid Cojec protocol (cisplatin, vincristine, carboplatin, etoposide and cyclophosphamide) was started [10]. In January 2018 a second line treatment with TVD (Topotecan-Vincristine-Doxorubicin) [11] was initiated due to an inoperable abdominal mass.

In April 2018, the child was hospitalized due to febrile neutropenia without an apparent infectious focus and was treated with piperacillin-tazobactam and amikacin until counts recovery. A week later, blood culture obtained from the central line yielded bacterial growth on MacConkey agar (after 72 h of incubation at 36℃) presumptively identified as Gram-negative non-fermentative bacilli. The patient was readmitted; he was asymptomatic, afebrile and not neutropenic;

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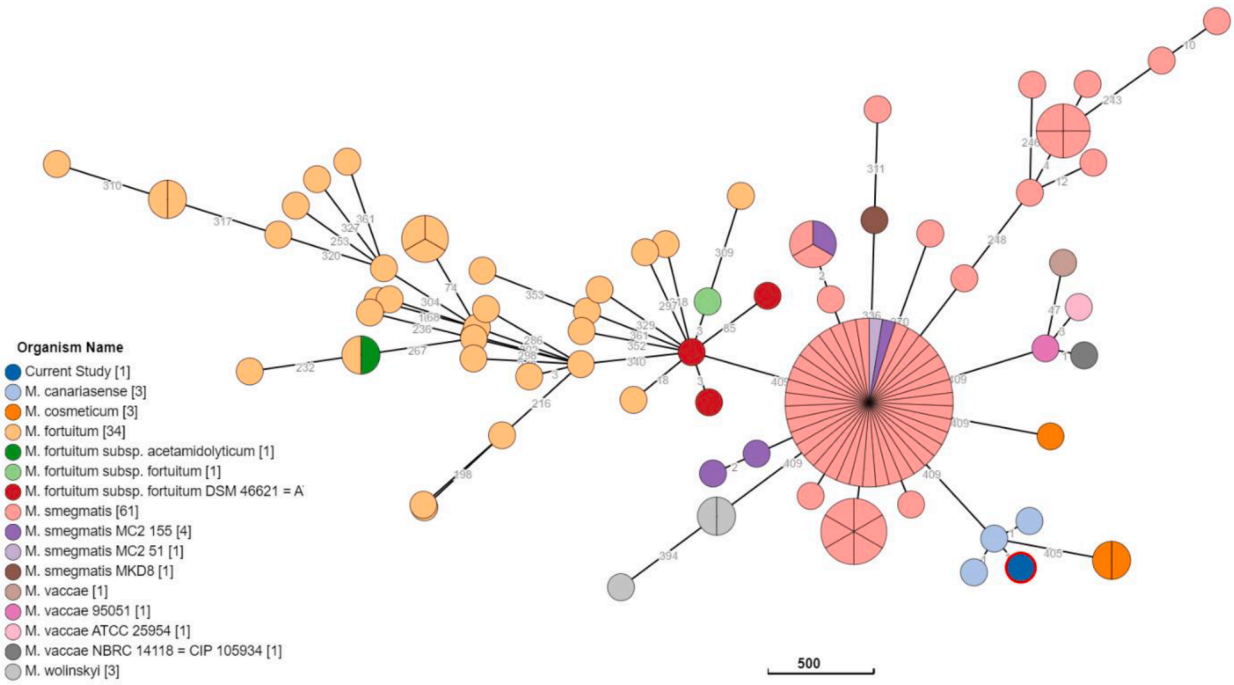

Fig. 1. A minimum spanning tree of an ad-hoc cgMLST schema for the isolate in the current report and related NTM species.

empirical treatment was started with piperacillin-tazobactam. Six additional blood cultures from both central and peripheral blood (taken over the following days) yielded similar growth.

On day 6 of admission the child developed fever, the port-a-cath was removed and cervical ultrasound doppler showed large thrombus in the right internal jugular vein. Trans-thoracic echocardiography revealed no vegetations.

Initial testing using the VITEK2 with the GNB ID cards as well as MALDI-TOF MS using VITEK MS system (Biomérieux, France) failed to identify the bacterial isolates. Standard Ziehl-Neelsen (ZN) staining indicated the presence of acid-fast bacilli (AFB). The isolate was submitted to the National Mycobacterium Reference Laboratory of the Israeli Ministry of Health for further analysis. GenoType Mycobacterium CM and GenoType Mycobacterium AS (Hain Lifescience Nehren, Germany) Line Probe Assays (LPA) were initially used for routine rapid identification of AFBs. Both assays identified the isolate to the genus level (*mycobacterium spp*) but failed to identify it to the species level. Analysis using the BioTyper MALDI-TOF MS (Bruker Daltonics, Germany) identified the isolate as *M. canariasense_cosmeticum* with a score of 2.14 which is considered reliable for species-level identification in mycobacteria [12]. Since this was one of the first isolates to be identified as *M. canariasense_cosmeticum* in the reference laboratory, a specific validation for the species was not performed.

This mass spectrum of *M. canariasense* and *M. cosmeticum* constitutes the best match with *M. canariasense_cosmeticum* (*M. canariasense* DSM 44828 T DSM b), NCBI:txid228230. Susceptibility testing for antimycobacterial drugs was performed using E-test and was interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The isolate was found to be susceptible to amikacin (MIC = 2 μg/ml), cefoxitin (MIC = 3 μg/ml), doxycycline (MIC = 0.125 μg/ml), trimethoprim/sulfamethoxazole (MIC = 0.002 μg/ml), imipenem (MIC = 0.38 μg/ml), ciprofloxacin (MIC = 0.75 μg/ml) and moxifloxacin (MIC = 0.032 μg/ml).

The fever resolved five days after catheter removal and antimycobacterial treatment initiation with a combination of imipenem, clarithromycin and amikacin. Treatment was continued for 4 weeks followed by long term oral treatment with clarithromycin and ciprofloxacin. Follow up ultrasonography at 4 weeks showed significant thrombus diameter reduction with full resolution two months later. Serial follow up blood cultures were negative.

In order to resolve the identification of the implicated RGM, the initial isolate was subject to whole genome sequencing (WGS) using Illumina MiSeq Reagent Kit v2 (500-cycles) following library preparation using the Nextera DNA Flex protocol (Illumina, CA, US). The raw sequence was assembled using Shovill (v1.0.4) with SPAdes (v3.13.1) (https://github.com/tseemann/shovill). Sequence deposited to SRA as bioproject PRJEB62811.

Species identification was performed *in silico* using several different approaches: (1) BLAST search of the 16S *rRNA* against several databases, including NCBI (https://blast.ncbi.nlm.nih.gov/), RDP (release 11; https://rdp.cme.msu.edu/), WGS assembly was analysed with GTDB-Tk (v2.1.0; https://github.com/Ecogenomics/GTDBTk)

and leBIBI (https://umr5558-bibiserv.univ-lyon1.fr/lebibi/lebibi.cgi); (2) average nucleotide identity (ANI) search using fastANI (v1.1; https://github.com/ParBLiSS/FastANI); (3) MASH analysis using MinHash (v2.0; https://github.com/marbl/Mash); (4) digital DNA-DNA hybridization usingGGDC (https://ggdc.dsmz.de/). The search showed mixed results, with NCBI providing *M. cosmeticum* as the top hit and RDP and leBIBI providing *M. canariasense* as the top hit. All other strategies clearly indicated *M. canariasense* as the top match (ANI 99.8 vs. 92.7, GGDC 98.1% vs. 19.5%, matching hashes 924/1,000 vs. 109/1,000).

cgMLST analysis methods:

Genome assemblies (n = 119) for the species *M. canariasense*, *M. cosmeticum*, *M. fortuitum*, *M. smegmatis*, *M. wolinskyi*, and *M. vaccae*, were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/; last accessed 202304). An ad hoc cgMLST schema were generated using chewBBACA (v2.6.0) [25] (with a Prodigal [26] training file for the genome *M.cosmeticum strain DSM 44829* (GCF_000613185.1), and including loci with 95% genome presence), including all 119 genome assemblies and the WGS assemblies of this study's isolate, producing ad hoc cgMLST schema consisting of 409 loci. A minimum spanning tree (MST) was generated (with MSTreeV2) and visualized from the ad hoc cgMLST schema using GrapeTree [27] (Fig. 1).

Table 1
Published cases of human infection with *M. cosmeticum* and *M. canariasense*.

Outcome	Catheter removal	Antibiotic Treatment	Antibiotic susceptibility	ID technique	Infection site	Clinical presentation	Immunocompromised host	Age	No. of cases	Author	
***M. cosmeticum* cases**											
–	–	–	ciprofloxacin, amikacin, tobramycin, cefoxitin, clarithromycin, doxycycline, sulfamethoxazole and imipenem	16S PCR, HPLC, NGS	Skin	SSTI	No	Adult	1	Cooksey RC, 2004 [16]	**1**
Cure	Yes	No									
	–	16S	Central line	Fever	No	77 yr	3	Cooksey RC, 2007 [17]	**2**		
Cure	Yes	No	–	16S	Central line	No symptoms	Yes (lymphoma)	43 yr			
Death	–	TMP/SMX	–	–	Sputum	Fever, Dyspnea	Yes (AIDS)	36 yr			
–	–	–	–	–	Skin	SSTI	–	–	1	Beer K, 2009 [14]	**3**
Cure	–	rifampicin, isoniazid, pyrazinamide and ethambutol	–	PCR	Ascites	Abdominal distention, weight loos, night sweets	No	63 yr	1	Addley J, 2010 [13]	**4**
–		–	–	–	Colon	Colitis	No	32 yr	1	Boschetti, 2011 [15]	**5**
Cure	–	Amikacin, Azithromycin	Amikacin, Ciprofloxacin, cefoxitin, clarithromycin, imipenem, linezolid, TMP/SMX								
	16S	Blood	–	No	0 yr (Neonate)	1	Koay W, 2015 [18]	**6**			
Cure	–	Moxifloxacin, TMP-SMX, clarithromycin + Joint replacement	Ciprofloxacin, Moxifloxacin, linezolid, TMP-SMX, imipenem, amikacin	–	Prosthetic joint	Arthritis	No	67 yr	1	Vutescu E, 2017 [20]	**7**
–		–	–	16S	Sputum	No symptoms	Yes (AIDS)	39 yr	3	Varghese B, 2017 [19]	**8**
–	–	–	–		Sputum		Yes (lung transplantation)	48 yr			
–	–	–	–		Skin	No symptoms	No	59 yr			
***M. canariasense* cases**											
Cure	Yes	Ceftazidime, vancomycin, amikacin +	Amikacin, tobramycin, imipenem, doxycycline, cefoxitin, ciprofloxacin, clarithromycin	16S, *hsp65*	Central line	Yes	Yes (Multiple myeloma)	43yr	12	Campos-Herrero et al. 2006 [21]	1
Cure	Yes	Ceftazidime, vancomycin, amikacin	Amikacin, tobramycin, imipenem, doxycycline, cefoxitin, ciprofloxacin, clarithromycin	16S, *hsp65*	Central line	Yes	Yes (Leukemia)	40yr			
Cure	Yes	Clarithromycin, amikacin, cefoxitin, imipenem	Amikacin, tobramycin, imipenem, doxycycline, cefoxitin, ciprofloxacin, clarithromycin	16S, *hsp65*	Central line	Yes	Yes (Leukemia)	58yr			
Cure	Yes	Amikacin, imipenem	Amikacin, tobramycin, imipenem, doxycycline, cefoxitin, ciprofloxacin, clarithromycin	16S, *hsp65*	Central line	Yes	Yes (Multiple nyeloma)	59ye			
Cure	Yes	Imipenem	Amikacin, tobramycin, imipenem, doxycycline, cefoxitin, ciprofloxacin, clarithromycin	16S, *hsp65*	Central line	Yes	Yes (Leukemia)	57yr			
Relapse	No	Clarithromycin, ciprofloxacin	Amikacin, tobramycin, imipenem, doxycycline, cefoxitin, ciprofloxacin, clarithromycin	16S, *hsp65*	Central line	Yes	Yes (Leukemia)	40yr			

(*continued on next page*)

Table 1 (*continued*)

Cure	Yes	Ceftazidime, teicoplanin	Amikacin, tobramycin, imipenem, doxycycline, cefoxitin, ciprofloxacin, clarithromycin	16S, *hsp65*	Central line	Yes		Yes (Leukemia)	59yr		
Cure	No	Ceftazidime, amikacin	Amikacin, tobramycin, imipenem, doxycycline, cefoxitin, ciprofloxacin, clarithromycin	16S, *hsp65*	Central line	Yes		Yes (Lymphoma)	49yr		
Cure	Yes	Ciprofloxacin, amikacin	Amikacin, tobramycin, imipenem, doxycycline, cefoxitin, ciprofloxacin, clarithromycin	16S, *hsp65*	Central line	Yes		Yes (Leukemia)	50yr		
Cure	Yes	Ciprofloxacin, amikacin	Amikacin, tobramycin, imipenem, doxycycline, cefoxitin, ciprofloxacin, clarithromycin	16S, *hsp65*	Central line	Yes		Yes (Leukemia)	37yr		
Death	No	Ciprofloxacin, amikacin	Amikacin, tobramycin, imipenem, doxycycline, cefoxitin, ciprofloxacin, clarithromycin	16S, *hsp65*	Central line	Yes		Yes (Lymphoma)	63yr		
Relapse	No	Clarithromycin	Amikacin, tobramycin, imipenem, doxycycline, cefoxitin, ciprofloxacin, clarithromycin	16S, *hsp65*	Central line	Yes		Yes (solid tumor)	48yr		
Cure	Yes	-	-	MALDI-TOF MS, PCR	Central line	Fever and chills	Yes		42yr	1	Paniz-Mondolfi et al. 2014 [22]	2
	Cure	-	Central line	16S		Fever	Yes		69yr	1	Tagashira et al. 2015 [23]	3

SSTI- skin and soft tissue infection, HPLC- high performance liquid chromatography, NGS- next generation sequencing, TMP/SMX- trimethoprim sulfamethoxazole, ID - identification, MALDI-TOF MS- matrix assisted laser desorption ionization time of flight mass spectrometry.

3. Discussion

We present an immunocompromised child with an indolent persistent bacteremia due to *M. cosmeticum/M. canariasense* caused by an infected thrombus related to venous central catheter. The patient had a favorable outcome after catheter removal and a combination anti-mycobacterial treatment followed by long term suppressive treatment. Of note is that optimal treatment was delayed due to the inconclusive identification of the isolate, and the paucity of literature regarding treatment regimens and clinical success.

A review of the current literature revealed 12 reported cases of human clinical infections with *M. cosmeticum* [13–20] and 14 reported cases with *M. canariasense* [21–23] (Table 1). *M. canariasense* is almost exclusively an opportunistic pathogen in severely immune compromised, while *M. cosmeticum* also infects normal hosts. In only 3 of the *M. cosmeticum* cases, was the organism isolated from blood cultures, unlike *M. canariasense* cases, in which all of the isolates were grown from blood, mostly in relation to central venous catheters in cancer patients. Antimicrobial susceptibility patterns of *M. cosmeticum* seem to vary between the few published cases, while *M. canariasense* appeared to be universally susceptible to anti-mycobacterial drugs.

Our case highlights the difficulty of achieving speciation of RGM using conventional laboratory identification techniques. Not only could RGM masquerade as non-fermentative Gram-negative rods, but even after their recognition, accurate identification may be inconclusive or misleading using standard techniques. The speciation of RGM is important, especially as the susceptibility profile of environmental isolates of *M. cosmeticum* seems to be changing, raising a concern regarding the potential emerging resistance [24].

Furthermore, reviewing the reported cases shows that most of the isolates of both *M. cosmeticum* and *M. canariasense* were identified to the species level using 16 s *rRNA* PCR, which if performed on our current sample would at best be inconclusive, with a potential for misidentification. With less than 40 cases published for both mycobacteria, misidentifications as such could have a real impact on the body of evidence collected thus far.

An important limitation to our report is the lack of susceptibility testing using borth microdilution.

With the increasing availability of WGS at the clinical microbiology frontline, accurate identification of NTM could be expedited leading to improved diagnosis, better choice of empiric antimicrobial treatment and a better epidemiological picture in cases of outbreaks or when attempting to implicate the source of an infection with environmental bacteria such as RGM.

4. Funding, conflicts of interest, and ethical approval

The authors have no affiliation with any organization with direct or indirect financial interests in the subject matter discussed in the manuscript. No funding was received for this study. Ethical approval was not required or sought.

CRediT authorship contribution statement

Daniel Grupel: Writing – original draft, Writing – review & editing. **Motro Yair:** Formal analysis, Data curation. **Jacob Moran-Gilad:** Conceptualization, Writing – review & editing. **Dana Danino:** Supervision, Conceptualization, 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jctube.2023.100393.

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