First Time Isolation of *Mycobacterium hassiacum* From a Respiratory Sample

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ABSTRACT: We describe the first isolation of *Mycobacterium hassiacum*, a rapid-growing, partial acid-resistant mycobacterium, in a respiratory specimen from a patient with exacerbated chronic obstructive pulmonary disease. To provide therapeutic recommendation for future cases, antibiotic susceptibility testing of 3 clinical isolates was performed by broth microdilution. All strains tested showed susceptibility to clarithromycin, imipenem, ciprofloxacin, and doxycycline. The role of *M hassiacum* as a respiratory pathogen remains unclear and needs to be evaluated by future reports.

KEYWORDS: Nontuberculous mycobacteria, respiratory sample, antimicrobial susceptibility testing, diagnosis

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Introduction

At present, more than 170 species of nontuberculous mycobacteria (NTMs) have been described.[1] The increase in isolated species relates to the reliableness of DNA sequencing. The direct sequencing of amplified DNA from the 16S ribosomal RNA (rRNA) gene of *Mycobacterium* species is well described and most widely accepted for species identification.[2] However, the clinical relevance of a newly designated NTM species often remains unclear after their first description.

Members of the genus NTM are widespread in the environment and can be found in ground soil and in various aquatic habitats.[3] At present, diseases caused by NTMs are increasingly recognized worldwide. The clinical symptoms range from harmless asymptomatic infection to severe life-threatening illness. Clinical manifestations of NTM infection are most frequently respiratory symptoms, but disseminated, lymphatic and tissue, diseases are also considerable.[4]

Mycobacterium hassiacum was first isolated in 1997 in a urine specimen. The authors described a rapidly growing mycobacterium (RGM) with a notable high thermos tolerance in comparison with other NTMs.[5] No definite clinical significance was assigned to this new species so far, although *M hassiacum* has been isolated from a patient with peritonitis.[5–7]

The treatment of NTM infections is discussed controversially as isolation of NTMs is not synonymous with the necessity for therapy. The protracted and arduous treatment with multiple antibiotic drugs is associated with unwanted side effects.[4] Therefore, it is essential to consider clinical aspects as well as radiologic findings to distinguish between mere colonization and genuine infection. Among NTM infections, treatment of pulmonary RGM infections is especially challenging because discrepancies between in vitro susceptibility testing and clinical outcome have been reported. Furthermore, certain RGMs such as *Mycobacterium abscessus* demonstrate multiple antibiotic drug resistances thereby limiting therapy options.[4,8]

Phenotypic drug susceptibility testing of RGM by broth microdilution is a standardized method recommended by the Clinical and Laboratory Standards Institute (CLSI).[4,9] The published break point concentrations are at least partly established by clinical studies.

This work describes the first isolation of *M hassiacum*, a NTM in a respiratory sample, and discusses its role as respiratory pathogen. By testing 3 clinical isolates for antibiotic susceptibility, we want to provide guiding information for future cases of infection.

Methods

Patient's clinical course

A 70-year-old man with obstructive pulmonary disease presented at the emergency department with massive cough and mucous expectoration. Further comorbidities were arterial hypertension, type 2 diabetes mellitus, hyperlipoproteinemia, and hepatic steatosis. Physical examination revealed wheezing and attenuated breath noise in the lower left lung. The patient had no fever, and laboratory parameters for inflammation showed slightly increased C-reactive protein values of 13.9 mg/L (normal: <5.00 mg/L) without elevated leukocytes.

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Table 1. Antimicrobial susceptibility testing of *Mycobacterium hassiacum* JZ2014 and reference strains RB1995 and RB0195.

SUBSTANCE	*MYCOBACTERIUM HASSIACUM* JZ2014, μg/mL	*MYCOBACTERIUM HASSIACUM* RB1995, μg/mL	*MYCOBACTERIUM HASSIACUM* RB0195, μg/mL
Ampicillin	16	>16	8
Amoxicillin/clavulanic acid	2/2	>16/2	4/2
Cefuroxime	16	>16	16
Ceftriaxon	>16	>16	16
Moxifloxacin	0.06	0.06	0.06
Ciprofloxacin	0.125	0.125	0.125
Erythromycin	0.125	0.125	0.125
Clarithromycin	0.125	0.125	0.125
Doxycycline	0.25	0.25	0.25
Clindamycin	4.0	2.0	1.0
Bacitracin	2.0	2.0	2.0
Imipenem	0.06	0.06	0.06

Chest radiography showed slight hypoventilation at the left lower lung but no clear proof of pneumonia.

The patient was hospitalized and calculated antibiotic therapy with cefuroxime 2 mg × 500 mg orally was initiated. During hospital stay, 3 sputum samples were sent to our laboratory for microbiological examination. The patient's clinical condition improved slowly and he was discharged at day 6. Antimicrobial treatment was terminated after 8 days in total.

Bacterial strains

We tested in total 3 strains of *M hassiacum* for antibiotic susceptibility. In addition to strain JZ2014 isolated from 1 sputum sample of the present case, 2 further clinical isolates, RB1995 and RB0195, were used for comparison. Although strain RB1995 originates from a urine sample and was presumably used as the reference strain of the DSMZ, origin of the strain RB0195 could not be verified. Both strains were kindly provided by the Institute of Medical Microbiology and Hygiene, Regensburg, Germany.

Culture and drug susceptibility

Sputum samples were decontaminated by the *N*-acetyl-L-cysteine-sodium hydroxide method. Small aliquots were stained with Acridine Orange (Merck Chemicals, Darmstadt, Germany) and used for microscopic examination. Remaining material was used to inoculate mycobacterial growth indicator tube (MGIT; Becton Dickinson, Franklin Lakes, NJ, USA) plus Löwenstein-Jensen and Middlebrook agar. Drug susceptibility testing was performed using the commercial microtiter plate assay Micronaut-S (Merlin, Berlin, Germany). In deviation from manufacturer's recommendations, but according to CLSI recommendation, bacteria were incubated for 5 days until minimal inhibitory concentrations were determined.

16S rRNA amplification and sequencing

Polymerase chain reaction amplification and sequence analysis were performed as described previously.[9] The sequence was compared with known 16S rRNA gene sequences using National Center for Biotechnology Information (NCBI) database. The result was later confirmed by the National Reference Center for Mycobacteria, Borstel, Germany.

Results

Detection and identification of mycobacteria

No acid-fast rods were detected by microscopy in the sputum samples. For one out of total 3 proceeded sputum samples, MGIT indicated growth 4 weeks after inoculation. On Löwenstein-Jensen and Middlebrook agar, small, dry colonies were subcultivated. The 16S rRNA gene sequence analysis allowed species identification as *M hassiacum*. Sequence has been deposited in NCBI GenBank under accession number MG386988.

Antibiotic susceptibility testing

Results of antimicrobial testing are listed in Table 1.

Discussion

Antibiotic susceptibility testing showed high minimum inhibitory concentration (MIC) for all β-lactam antibiotics tested including cephalosporins. Only strain JZ2014 showed low sufficient susceptibility to amoxicillin combined with clavulanic acid. All strains were susceptible to imipenem, clarithromycin,

fluoroquinolones, and doxycycline, whereas varying MICs were obtained for clindamycin depending on the strain tested. Based on this observation, a combination of an aminoglycoside with imipenem, clarithromycin, and doxycycline or a fluoroquinolone, similar to recommendations for other RGM, might be a proper approach for treating pulmonary infections due to *M hassiacum*.[8]

Referring to existing literature, clinical significance of *M hassiacum* is questionable as only 3 reports of isolates from clinical samples exist so far. Although no clinical symptoms were present in the first description of the species, in the second case of isolation, *M hassiacum* caused recurrent cystitis.[5,6] The most recent report described *M hassiacum* as causative pathogen in a case of peritoneal dialysis–related peritonitis.[7]

We present the first isolation of this species from a respiratory sample in a patient with acute pulmonary symptoms. Because RGMs are a notorious subgroup of atypical mycobacteria, well known for causing pulmonary infections in individuals with impaired lung function, our report suggests an acute exacerbation of chronic pulmonary disease due to *M hassiacum*. However, because *M hassiacum* was only determined in one out of 3 sputum samples, diagnostic criteria for NTM lung disease were not matched in our case. Furthermore, no typical signs of pulmonary infection due to mycobacteria, such as small nodulae or fibrocavitary disease, could be obtained by chest radiography.[4,8] In addition, it should be taken into account that treatment of pulmonary NTM infections usually requires long-term antibiotic combination therapy for at least several months and often needs to be complemented with surgical resection.[8] In the present case, calculated monotherapy with cefuroxime was administered but subsequent antimicrobial susceptibility testing showed high MIC for cefuroxime. Nevertheless, general condition of the patient improved and antibiotic treatment was terminated only after 8 days. Thus, it seems unlikely that *M hassiacum* was the true infectious agent in this case and also polymicrobial infection should be taken into consideration.

Therefore, this work cannot clarify, finally, the role of *M hassiacum* as a respiratory pathogen and its true virulence potential. To characterize *M hassiacum* and its clinical relevance, more data from future reports and clinical experience will be required.

Nevertheless, physicians and laboratory staff should be aware of RGM causing infections especially in immunocompromised patients or patients with pulmonary affliction. Our case confirmed sequencing of the 16S rRNA target as a proper approach for detection of rare RGM that should be taken into account more often when medical history raises suspicion of infection with NTMs.

Conclusions

The presented characterization of *M hassiacum* is the first report of a clinical isolate (JZ2014) from a respiratory sample. Antibiotic resistance testing showed susceptibility to macrolides, doxycycline, and fluoroquinolones while high MICs for β-lactam antibiotics including cefuroxime were obtained. Whether the isolated strain was merely colonizing the pulmonary tract or has to be seen as a disease-causing opportunistic pathogen could not be determined finally.

Author Contributions

SDE and SH carried out the experiment and wrote the manuscript. CM are for the patients throughout the treatment. BH, SS and JB specifying the initial laboratory report and BL supervised the project. All authors provided critical feedback.

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