

Mycobacterium colombiense and *Mycobacterium avium* Complex Causing Severe Pneumonia in a Patient with HIV Identified by a Novel Molecular-Based Method

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Xiaoling Yu
Wenqian Jiang

Department of Infectious Diseases,
Mengchao Hepatobiliary Hospital of
Fujian Medical University, Fuzhou,
People's Republic of China

Abstract: Non-tuberculous mycobacteria are conditional pathogens that can cause many diseases, among which pulmonary infections are the most common (65–90%). *Mycobacterium avium* and *Mycobacterium abscessus* are non-tuberculous mycobacteria most often associated with lung diseases. Mass spectrometry diagnostic techniques were not effective in *Mycobacterium avium* complex infection. We report a case of *Mycobacterium colombiense* and *Mycobacterium avium* complex causing severe pneumonia in an adult with HIV. Our group developed a novel molecular-based method to identify *Mycobacterium* species. Novel techniques such as molecular cloning which we have described here can make up for the inability of matrix-assisted laser desorption ionization-time of flight mass spectrometry to distinguish the multiple microorganisms present, and may add to the diagnostic toolkit and increase the accuracy and rapidity of diagnosis in the future.

Keywords: non-tuberculous mycobacteria, *Mycobacterium avium* complex, *Mycobacterium colombiense*, human immunodeficiency virus infection, pneumonia

Introduction

Non-tuberculous mycobacteria (NTM) are mycobacterial species other than *Mycobacterium tuberculosis* and *Mycobacterium leprae* that cause tuberculosis and leprosy, respectively.¹ NTM, which are conditional pathogens, can cause chronic skin infection and lymphadenitis, and may further develop into a disseminated disease, of which pulmonary infections are the most common (65–90%). The *Mycobacterium avium* (*M. avium*) complex and *Mycobacterium abscessus* complex is the NTM most often associated with lung diseases.¹ NTM are highly prevalent in the environment; however, non-immunocompromised hosts can withstand infection. Conversely, immunocompromised patients such as organ transplant recipients and people living with human immunodeficiency virus (HIV) are at greater risk of NTM infection.² Herein, we report a case of severe pneumonia caused by a *Mycobacterium colombiense* (*M. colombiense*) and *M. avium* complex (MAC) in a patient with HIV. The blood culture of the patient was positive, but the pathogenic cultures could not be identified through matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS). Sanger sequencing results of 16S rDNA polymerase chain reaction (PCR) of the cultured pathogenic

Correspondence: Xiaoling Yu
Department of Infectious Diseases,
Mengchao Hepatobiliary Hospital of
Fujian Medical University, Fuzhou 350025,
Fujian, People's Republic of China
Tel/Fax +86 591 88116019
Email xiaolingyu82@163.com

microorganisms showed double peaks which indicated the existence of more than one pathogenic microorganism. MAC are difficult to decipher by re-sequencing. Using molecular cloning and 16S rDNA sequencing, multiple microorganisms can be distinguished and identified separately.

Case Description

A 62-year-old man presented with anhelation, fatigue and anorexia for two months before hospitalization. The patient was initially seen in a medical centre in Fuzhou, Fujian, China with no medical history and was a smoker for 35 years. Due to the patient's history of multiple sexual encounters with various female partners as well as his severe condition at presentation, he was tested for chronic viral infections including HIV. The patient's examination results were as follows: body weight, 40 kg; malnourished, emaciated, weight loss in the last 2 months, 10 kg; body temperature, 37 °C; respiratory rate, 23 breaths/minute; heart rate, 96 beats/min; blood pressure, 116/61 mmHg; T lymphocyte subgroup detection, CD4+ T-cell (absolute value) count of 15 cells/ μ L [reference range: 410–1590 cells/ μ L]; high-sensitivity quantitative HIV nucleic acid detection, 2.48×10^5 copies/mL. The initial complete blood count (CBC) showed the following: white blood cell count, 11.63×10^9 cells/L (90.8% neutrophils, 5.0% lymphocytes, 2.7% monocytes, 1.0% eosinophils and 0.5% basophil); haemoglobin, 97 g/dL [reference range: 130–175 g/dL]; and platelets, 286×10^9 cells/L. The erythrocyte sedimentation rate was 79 mm/hr [reference range: 0–15 mm/hr], and the C-reactive protein level was 119.39 mg/L [reference range: 0–8 mg/L]. Initial chest digital radiography (DR) showed extensive bilateral lung lesions involving almost all pulmonary lobules (Figure 1). Blood gas analysis (FiO₂: 63%) showed the following: pH, 7.46; PCO₂, 23 mmHg; PO₂, 161 mmHg; and oxygenation index, 256. The β -d-glucan (β DG) test was 159.2 pg/mL [reference range: negative < 70 pg/mL; grey area = 70–95 pg/mL; positive > 95 pg/mL]. Due to the low CD4+ T-cell count, the patient was subsequently diagnosed with HIV infection via Western blot testing. Additionally, lung CT showed an interstitial pattern at the inferior lobe of the left lung with diffuse pulmonary bullae indicating emphysema. The patient was then treated speculatively with fluconazole and ceftazidime for possible fungal and bacterial infections, respectively. As the patient did not improve after ten days, he was referred to the infectious service of Mengchao Hepatobiliary Hospital of Fujian Medical University.



Figure 1 Chest X-ray. Chest diagnostic radiograph showed a slight increase and thickening of the texture of both lungs, with increased diffuse patch density. The boundary was fuzzy, and no obvious consolidation shadow was seen in the rest of the lungs.

Meropenem, sulfamethoxazole and caspofungin were administered due to suspicion of bacterial or pneumocystis pneumonia and other fungal infections. During treatment, the patient experienced chills and had fever reaching 40 °C with low blood oxygen saturation at 80%. He was sedated and methylprednisolone was administered as an anti-inflammatory medication. After treatment, his chills improved, and blood oxygen saturation increased to 90% in room air.

On the second day after admission, the patient's temperature was 37 °C and he again experienced chills. Intravenous immunoglobulin and linezolid were administered to enhance immune function and to further target gram-positive bacteria, respectively. Four days after admission, the patient's condition deteriorated. The family members of the patient required automatic discharge from the hospital. The doctor informed them that the diagnosis and treatment of the condition might be delayed after discharge, resulting in unpredictable consequences. They expressed understanding, but still insisted on discharge.

Materials and Methods

The results of blood culture showed that there was microbial growth, but the microbial species could not be

identified by mass spectrometry. The blood sample was then inoculated on Colombian blood agar for bacterial culture. MALDI-TOF-MS (VITEK MS, BioMerieux SA, BioMerieux Inc., France) was used to identify the cultured pathogenic microorganisms.

For the molecular identification of microorganisms, we decided to use PCR. The genome of the isolate was extracted by a one-step genome extraction kit (Weiyin, China), and 16S rDNA PCR was carried out with 16S universal primers (27F: 5' AGAGTTTGATCCTGGCTCAG 3', 1492R: 5' AGAGTTTGATCCTGGCTCAG 3'). Then, the PCR product was sequenced using the ABI 3730xl DNA Sequencer.

The 16S rDNA PCR product was purified by a GeneJET Gel extraction kit (Thermo, USA). Clones were constructed using the pMD19-T Vector Cloning kit (Takara, Japan) and transformed into DH10B *Escherichia coli*. Positive clones were then screened by ampicillin and sequenced with the ABI 3730xl DNA Sequencer.

Results

We obtained a 1427 bp sequence, aligned to the NCBI nucleotide collection (nr/nt) database using BLAST. This resulted in the identification of *M. avium* (Accession: CP046507.1), with a query cover of 100%, an E value of 0.0, and a percent identity of 97.05%. MALDI-TOF-MS however did not successfully identify the microorganism, since the sequencing results showed two sequencing peaks (Figure 2). Therefore, we suspected that the isolate contained more than one microorganism.

We then decided to ligate the PCR product into a T vector for further identification and selected 11 positive *Escherichia coli* transformants for Sanger sequencing. Of these transformants, 9 were identified as *M. avium* and 2 as

M. colombiense. These results further confirmed our suspicion that two microorganisms were present in the isolate.

Discussion

Here, we report a case of a late-stage HIV-infected adult with respiratory symptoms for two months. In accordance with other NTM-infected patients who experience relative emaciation, this patient had lost 10 kg of body weight.³ A chest CT scan indicated emphysema, while blood and sputum cultures could not identify the pathogenic culprits through MALDI-TOF-MS, and double peaks on Sanger sequencing indicated the existence of more than one pathogenic microorganism. As MAC infection was suspected but could not be distinguished, we used molecular cloning to isolate species and identify their genomes.

The MAC contains a group of heterogeneous, slow-growing mycobacteria, including *M. avium*, *Mycobacterium intracellulare* (*M. intracellulare*), and *M. colombiense*.⁴ MAC infections are mostly caused by *M. avium* and *M. intracellulare*, while *M. colombiense* is extremely rare. A handful of cases of *M. colombiense* have been previously reported, but concurrent *M. avium* and *M. colombiense* causing pulmonary infection has not yet been encountered in the literature (Table 1).

M. colombiense is a slow-growing *Mycobacterium* that was first isolated in 2006, identified from blood and sputum samples of a person living with HIV in Colombia.⁵ *M. colombiense* may be ingested or may directly invade the body through skin injury, causing the development of local lymph node lesions.⁶ A recent report showed that amikacin, clarithromycin, ciprofloxacin, moxifloxacin and rifabutin are effective against *M. colombiense* infection.⁷ In our case, although the

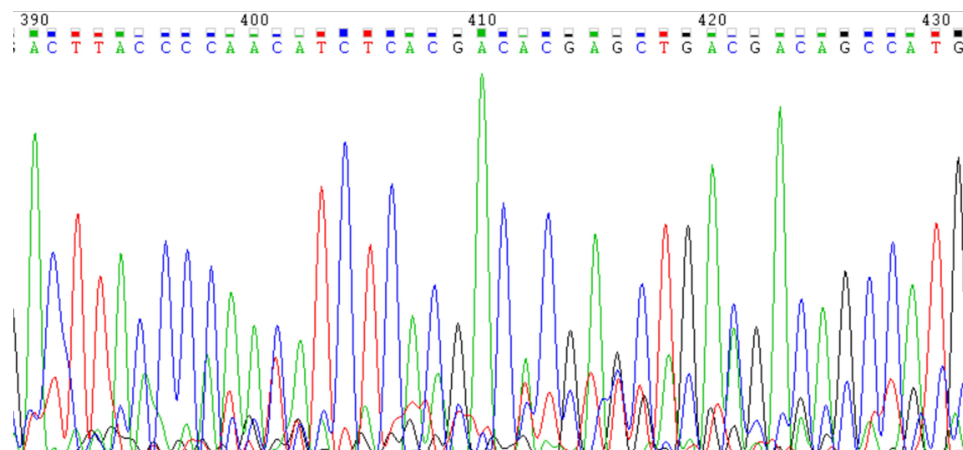


Figure 2 Partial Sanger sequencing peak map of the 16S rDNA PCR product of the isolate sequenced with the 1492R primer.

Table 1 Comparison of Case Reports of *Mycobacterium colombiense* Infections in Patients with and without HIV

Description	Age	Country	Infected Tissue Location	Treatment	Comorbidities	Outcome	Year of Report
<i>M. colombiense</i> infection with pseudotuberculous lymphadenopathy ¹⁰	25 months	France	Right subclavicular lymph node.	Surgical excision.	None.	Not described.	2009
Acquired immunodeficiency due to <i>M. colombiense</i> and cytomegalovirus coinfection ¹¹	49 years	Canada	Right superior lobar bronchus.	Piperacillin-tazobactam, vancomycin, rifampicin, clarithromycin, ethambutol, doripenem, tobramycin, and ciprofloxacin.	Multi-resistant <i>Pseudomonas aeruginosa</i> associated pneumonia.	Multi-organ failure and septic shock leading to death.	2013
<i>M. colombiense</i> associated pulmonary disease ¹²	49 years	Brazil	Right lung (all lobes), left upper lobe.	Rifampicin, ethambutol, clarithromycin, streptomycin and amikacin.	Pulmonary tuberculosis.	No improvement following treatment, death reported at 3-month follow-up.	2016
<i>M. colombiense</i> associated cervical lymphadenitis in an HIV-infected patient ¹³	36 years	USA	Right submandibular lymph node.	Levofloxacin, ethambutol, azithromycin, clarithromycin, clofazimine and amikacin. Surgical resection for sinus tract.	HIV on antiretroviral therapy (Truvada, Prezista and Norvir) and persistent right level IV sinus tract.	Healing of sinus tract, lost to 9-month follow-up.	2017
Disseminated <i>M. colombiense</i> infection in an HIV patient ¹⁴	47 years	Portugal	Lymph node, epiploon, liver	Clarithromycin, rifabutin and ethambutol.	HIV on antiretroviral therapy, lymphoproliferative disorder, immune reconstitution inflammatory syndrome.	Following 20 months of treatment, clinical and imagological resolution.	2019

sequencing results of the pathogenic microbial culture were positive, MALDI-TOF MS could not identify the strain. Therefore, it was not possible to perform drug sensitivity tests and to administer antibiotics in a targeted manner. Antiretroviral therapy (ART) was not used due to the rapid worsening of the patient's condition. Furthermore, before the sequencing results from the blood culture were obtained, the patient elected to be discharged home on November 15, 2019, and died a week later.

MALDI-TOF MS is a mass spectrometry-based analysis technology that was developed in the mid-1980s. This method has become the gold standard for clinical

laboratory microbial identification but has the disadvantage of not being able to identify complex infections.⁸ Moreover, the Vitek MS IVD 2.0 database contains data for *M. avium* but no data for *M. colombiense*. In this case, the identified species were MAC consisting of *M. avium* and *M. colombiense*. MAC are difficult to decipher by re-sequencing, and as such, MALDI-TOF MS cannot identify them. Thus, MALDI-TOF MS is not an effective method in such cases of complex bacterial infections, including two or more microorganisms.

In addition to molecular cloning, molecular identification of some MAC members at the species level can be performed by sequencing the genes *hsp65*, *rpoB*, *sodA*,

dnaJ, *gyrB* and *recA*.⁴ Members of the MAC can also be identified by next-generation metagenomic sequencing. However, the cost is greater than that of Sanger sequencing.⁹ In a clinical setting, next-generation sequencing (NGS) is typically used for the identification of complex microorganisms. Furthermore, the mycolic acid composition of the cell wall of *Mycobacterium* is critical for identifying *Mycobacterium* species.⁴ We showed that using molecular cloning and 16S rDNA sequencing allowed the identification of more than one microorganism causing infection in this patient.

Conclusion

We reported for the first time a case of severe pneumonia caused by concurrent *M. avium* and *M. colombiense* in a newly diagnosed patient with late-stage HIV with a very low CD4+ T-cell count. MAC infection is common in such patients, however, MALDI-TOF MS was not able to distinguish the multiple microorganisms present. Thus, we resorted to molecular techniques to confirm the presence of more than one pathogenic organism. In future cases where multiple pathogenic organisms cannot be identified by mass spectrometry, novel techniques such as molecular cloning which we have described here, may add to the diagnostic toolkit and increase the accuracy and rapidity of diagnosis.

Abbreviations

NTM, non-tuberculous mycobacteria; HIV, human immunodeficiency virus; *M. avium*, *Mycobacterium avium*; *M. colombiense*, *Mycobacterium colombiense*; *M. intracellulare*, *Mycobacterium intracellulare*; MAC, *Mycobacterium avium* complex; CBC, complete blood count; β dg, β -d-glucan; DR, digital radiography; MALDI-TOF-MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry; PCR, polymerase chain reaction; ART, antiretroviral therapy; NGS, next-generation sequencing.

Ethics Approval and Informed Consent

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Ethical approval for this study was provided by the Institutional Ethical Committee of the Faculty of Medicine, Mengchao Hepatobiliary Hospital of Fujian Medical University, Fujian, China (No. 2019_057_01).

Written informed consent was provided by the patient's father, his consent included consent to publish the case details and any accompanying images.

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Disclosure

The authors report no competing interests in this work.

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