Case Report: Disseminated Infection by *Mycobacterium sherrisii* and *Histoplasma capsulatum* in an African HIV-Infected Patient

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Abstract. *Mycobacterium sherrisii* is a new species of opportunistic, slow-growing, non-tuberculous *Mycobacterium* closely related to *Mycobacterium simiae* that can currently be identified with the sequence of 16S rARN gene and the heat-shock protein 65. Few cases of patients infected by this *Mycobacterium* have been reported and all of them were associated with human immunodeficiency virus or other immunosuppressive conditions. Clinical management is complex, because there is not a clear correlation between the *in vitro* antibiotic susceptibility testing and the patient's clinical outcome.

INTRODUCTION

Mycobacterium sherrisii has been recently recognized as a new species mainly associated with pulmonary or disseminated disease in immunocompromised patients. A new case of disseminated infection by *M. sherrisii* in an African human immunodeficiency virus (HIV)-infected patient is described. The patient was simultaneously affected by a disseminated form of *Histoplasma capsulatum* infection. We discuss the clinical aspects of the *M. sherrisii* infection, the complexity of the diagnosis and clinical management, and review the literature.

CASE REPORT

A 35-year-old man born in Ghana was admitted to our hospital in 2009 with severe asthenia, fever, and non-productive cough. On examination he was cachectic, had disseminated lymphadenopathy, and hepatosplenomegaly. The chest x-ray showed a faint interstitial pattern, and hilar and mediastinal enlarged lymph nodes with a pulmonary ground-glass pattern was observed in the thoracic computed tomographic scan. A fiberoptic bronchoscopy detected multiple ulcerated bronchial and tracheal lesions suggestive of fungal infection, which was confirmed by cultures of bronchial aspirate (BAS), bronchoalveolar lavage (BAL), blood culture, and bone marrow aspirate. All samples were positive for *H. capsulatum*. A diagnosis of HIV was simultaneously made, with a CD4 cell count of 26 cells/mm³ and an HIV viral load of 79,000 copies/mL. The patient was treated with lipidic amphotericin 3 mg/kg/day for 4 weeks followed by itraconazole 400 mg/day as a secondary prophylaxis. The patient also started antiretroviral therapy (HAART) with darunavir, ritonavir, tenofovir/emtricitabine (DRV/RTV/TDF/FTC), which resulted in clinical and immunological improvement. Mycobacterial cultures (VersaTREK system, Thermo Fisher, USA) of sputum, BAS, BAL, urine, and feces were negative. However, a non-tuberculous, non-chromogenic *Mycobacterium* was isolated in the blood culture; although, the species could not be determined by the Geno Type-CM/AS reverse line dot assay (Hain Lifescience, Germany). The strain was sent to a reference laboratory for identification and for antibiotic susceptibility testing (AST).

Two months later, the patient was admitted with acute respiratory failure. A miliary pattern was observed on the chest x-ray. The CD4 count was 96 cells/mm³ and the HIV viral load 458 copies/mL. The patient had adhered to the HAART and antifungal drug regimens. A new fiberoptic bronchoscopy showed complete healing of previous endobronchial lesions. The fungal cultures of BAL and BAS were negative; but the mycobacteria cultures were positive for a non-tuberculous *Mycobacterium* strain. Several samples of sputum and feces were also positive.

The strain isolated in the first blood culture was ultimately identified as *M. sherrisii*. The sequencing of the 16S ribosomal RNA gene showed the same sequence as the GenBank accession no. AY353699 (*M. sherrisii*) and 4 bp differences with *M. simiae* (GenBank accession no. AY604042). Moreover, the non-chromogenic colonies isolated were more consistent with *M. sherrisii* than with *M. simiae*. No biochemical or chromatographic analyses were performed because of their poor capacity to differentiate *M. simiae* from related strains.^{1,2} The AST was performed using the broth microdilution system by the Mycobacteria Growth Indicator Tubes (Becton Dickinson, Sparks, MD) following the standardized methodology by the Clinical Laboratory Standards Institute. The following AST was obtained: rifampicin resistant (4 µg/mL), amikacin resistant (> 5 µg/mL), streptomycin resistant (> 6 µg/mL), clarithromycin resistant (> 8 µg/mL), linezolid resistant (> 2 µg/mL), ethambutol susceptible (< 8 µg/mL), and moxifloxacin susceptible (< 1 µg/mL).

Based on previous case reports, the patient initiated treatment with levofloxacin (500 mg/daily), ethambutol (800 mg/daily), and azithromycin (500 mg/daily), despite resistance to clarithromycin. Three months later viable mycobacteria were still isolated in the patient's feces but the pulmonary miliary pattern was visibly improved. The HAART therapy was modified to abacavir, lamivudine, and raltegravir (ABC/3TC/RAL) to prevent drug–drug interactions. Secondary antifungal prophylaxis with itraconazole and antimycobacterial therapy was continued. The patient was lost to follow-up because he went back to Ghana 10 months after the initial diagnosis of histoplasmosis and 8 months after starting antimycobacterial treatment, with a CD4 count of 254 cells/mm³ and a viral load under 20 copies/mL.

Twelve months later the patient returned to our hospital. He had maintained good adherence to HAART and his viral load was undetectable. Despite stopping the antifungal and

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TABLE 1
Mycobacterium sherrisii infection in HIV-infected patients

Patient	1	2	3*	4	5*	6	7	8	9	10
Year Author	2005 Gämperli and others ³	2007 Loulergue and others ⁴	2007 Tortoli and others ⁵	2009 Crump and others ⁹	2009 Crump and others ⁹	2010 Barrera and others ⁷	2010 Barrera and others ⁷	2010 Barrera and others ⁷	2012 Ho and others ⁸	2013 Taján and others ⁸
Age (y)	47	53	7	49	36	49	30	29	36	35
Gender	Male	Male	Male	Male	Male	Male	Male	Male	Female	Male
African descent	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes
Lung disease	Yes	Yes	No	No	No	No	No	No	No	Yes
Dissemination	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Positive samples	Sputum BAL	Sputum Peritoneal fluid Blood culture	Peritoneal fluid Lymph node Blood culture Gastric aspirate	Blood culture	Blood culture	Sputum	Sputum	Sputum BAL Blood culture	Blood culture Hepatic biopsy	Sputum BAS Feces
Treatment	Clarithromycin Moxifloxacin Rifabutin Sulfamethoxazole	Clarithromycin Moxifloxacin Rifabutin Ethambutol Amikacin	Clarithromycin Ciprofloxacin Ethambutol Amikacin	Azithromycin Ethambutol	Azithromycin Ethambutol	Unknown	Clarithromycin Ciprofloxacin Ethambutol	Clarithromycin Ciprofloxacin Ethambutol	Clarithromycin Rifampicin Ethambutol	Azithromycin Levofloxacin Ethambutol
CD4/CD4%	19/6	19/3,2	72	13	31	230	89	20	58	26/3
HIV viral load	595,000 (baseline)	460 (on therapy)	1,088,000 (baseline)	Unknown	Unknown	Unknown	Unknown	Unknown	963,500 (baseline)	79,000 (baseline)
HAART	Start AZT/3TC/ RTV/LOP	D4T/3TC/NVP	Start D4T/3TC/ RTV/LOP	AZT/3TC/ABC	D4T/3TC/NVP	Unknown	Unknown	Unknown	TDF/FTC/EFV	Start TDF/FTC/ RTV/DRV
Immune reconstitution	Probably	Probably	Probably	No	No	Unknown	Unknown	Unknown	Yes	Yes
Time (weeks) to clinical symptoms	Four		Some						Four	Four
Outcome	Cured	Improved	Cured	Improved	Negative blood cultures	Lost to follow-up	Died	Died	Cured	Cured
Other conditions		TBC		TBC					Cerebral Toxoplasmosis	Disseminated Histoplasmosis

*Previously identified as *M. simiae*.

AZT = Zidovudine; 3TC = Lamivudine; d4T = Stavudine; NVP = Nevirapine; LOP = Lopinavir; ABC = Abacavir; EFV = Efavirenz; TDF = Tenofovir; FTC = Emtricitabine; RTV = Ritonavir; DRV = Darunavir.

antimycobacterial treatments, he did not present any symptoms of the previous disseminated infections.

DISCUSSION

Mycobacterium sherrisii is a new species of opportunistic, slow-growing, non-tuberculous *Mycobacterium* closely related to *M. simiae*. It was first characterized by Selvarangan and others¹ in 2004 however it was not recognized as a novel species until 2011.² The first strains were isolated from HIV-infected patients born in Africa or who had resided there for a long time before moving to other countries.^{3–5} New cases appeared later in Italy,⁶ Argentina,⁷ and recently Singapore,⁸ not related to an African source.

There are only a few reported cases. All patients except one are men, and all are immunosuppressed by HIV or other diseases. Clinical and epidemiological data of all cases are summarized in Tables 1 and 2.

Two distinct clinical forms of this infection exist: the first, with an indolent course, affects older people with previous pulmonary tuberculosis or some non-HIV immunosuppressive condition. Here, *M. sherrisii* acts as a respiratory tract colonizer rather than a pathogen; and the second, associated with HIV-infection, where *M. sherrisii* disseminates and can cause the patient's death.

The main limitation for the laboratory diagnosis of *M. sherrisii* is that this strain is not included in the commercial reverse line dot assays available. In addition, biochemical tests are insufficient to identify these isolates, because they are similar to closely related mycobacteria. Indeed, the first report showed its similarity to *M. simiae* and *Mycobacterium triplex*¹; currently, the sequence of 16S rARN gene and the heat-shock protein 65 are the reference identification methods.² Until *M. sherrisii* was characterized, some of the strains were misdiagnosed as *M. simiae* or *Mycobacterium lentiflavum*^{5,6,9} and retrospectively identified as *M. sherrisii* by sequencing techniques. Consequently, any isolation of a non-tuberculous strain identified as *M. simiae* or other closely related species with commercially available methods should be confirmed by 16S rRNA sequencing.

Another important issue is the management of these patients. There seems to be a poor correlation between the *in vitro* AST and the clinical outcome^{3,6,9}; the recommended empirical treatment includes macrolides and quinolones based on *M. simiae* cases.¹⁰ Ethambutol and rifampins could be added however the susceptibility patterns vary among isolates.

The AST performed in a *M. sherrisii* strains collection² showed resistance to quinolones (ciprofloxacin, gatifloxacin, and moxifloxacin), linezolid, rifampicin, sulfamethoxazole, ethambutol, and streptomycin. Amikacin showed a variable susceptibility pattern among isolates. All strains, except one, were susceptible to clarithromycin and all were susceptible to rifabutin. The results are similar to the strain collection from Argentina⁷ and other cases^{3,4}; our strain differs from these results because it is resistant to clarithromycin and susceptible to ethambutol and moxifloxacin.

Despite the AST results, most cases were treated with drugs that showed moderate susceptibility or resistance, with successful outcomes. We suggest two possible explanations: first, the initiation of HAART treatment in the HIV cases improves the immunological status of the patient; and second, the combination of antibiotics could act synergistically improving antimycobacterial drug activity. Indeed, two studies showed *in vitro* synergy in non-tuberculous mycobacteria, which included *M. simiae* and related species^{11,12}; an alternative to the classic AST could be the synergy study of the drugs used.

In summary, the best empirical treatment should probably include rifabutin, clarithromycin, and ethambutol. Moxifloxacin or levofloxacin could be used as alternative drugs.

Recently, the first case of disseminated *M. sherrisii* associated with immune reconstitution inflammatory syndrome was reported⁸. In our patient, *M. sherrisii* dissemination occurred after the initiation of HAART treatment. Although the blood culture positive for *M. sherrisii* came from the first admission, clinical evidence of infection with respiratory failure and milary pattern occurred after 8 weeks on HAART, when CD4 rose to 90 cells/mm³. Most likely, the patient immune reconstitution highlighted the clinical signs of mycobacterial dissemination. As seen in Table 1, the literature shows that in patients' 1, 2, 3, and 9 and in our patient, a variable period of time exists between starting HAART and clinical evidence of

TABLE 2
Mycobacterium sherrisii infection in non-HIV-infected patients

Patient	1*/†	2*	3	4	5
Year Author	2007 Tortoli and others ⁶	2007 Tortoli and others ⁶	2010 Barrera and others ⁷	2010 Barrera and others ⁷	2010 Barrera and others ⁷
Age (years)	58	53	65	72	65
Gender	Male	Male	Male	Male	Male
African descent	No	No	No	No	No
Lung disease	Yes	Yes	Yes	Yes	Yes
Dissemination	Pleural	No	No	No	No
Positive samples	Pleural biopsy	Sputum	Sputum	Sputum	Sputum
Treatment	Isoniazid Rifampicin Ethambutol Pirazinamide	No Unclear clinical value	Clarithromycin Ciprofloxacin Ethambutol	Clarithromycin Ciprofloxacin Ethambutol	Clarithromycin Ciprofloxacin
Outcome	Lost to follow-up	Unknown	"Cronification" Positive sputum persists	"Cronification" Positive sputum persists	"Cronification" Positive sputum persists
Cause of immunosuppression	Reumatoid Arthritis		TBC (before)	TBC (before)	Silicosis

* Retrospective identification (samples from 1996 and 2006).

† Previous identification *Mycobacterium lentiflavum*.

infection. We therefore agree with Ho and others⁸ that this new non-tuberculous *Mycobacterium* should be included in the list of opportunistic diseases to prevent when immune reconstitution inflammatory syndrome is suspected in HIV-infected patients, particularly when some relationship with Africa exists.

Received September 13, 2012. Accepted for publication January 11, 2013.

Published online February 18, 2013.

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REFERENCES

- Selvarangan R, Wu WK, Nguyen TT, Carlson L, Wallis CK, Stiglich SK, Chen YC, Jost KC Jr, Prentice JL, Wallace RJ Jr, Barrett SL, Cookson BT, Coyle MB, 2004. Characterization of a novel group of mycobacteria and proposal of *Mycobacterium sherrisii* sp. nov. *J Clin Microbiol* 42: 52–59.
- van Ingen J, Tortoli E, Selvarangan R, Coyle MB, Crump JA, Morrissey AB, Dekhuijzen PN, Boeree MJ, van Soolingen D, 2011. *Mycobacterium sherrisii* sp. nov., a slow-growing non-chromogenic species. *Int J Syst Evol Microbiol* 61: 1293–1298.
- Gamperli A, Bosshard PP, Sigrist T, Brandli O, Wildermuth S, Weber R, Mueller NJ, 2005. Pulmonary *Mycobacterium sherrisii* infection in a human immunodeficiency virus type 1-infected patient. *J Clin Microbiol* 43: 4283–4285.
- Loulergue P, Lamontagne F, Vincent V, Rossier A, Pialoux G, 2007. *Mycobacterium sherrisii*: a new opportunistic agent in HIV infection? *AIDS* 21: 893–894.
- Tortoli E, Galli L, Andebirhan T, Baruzzo S, Chiappini E, de Martino M, Brown-Elliott BA, 2007. The first case of *Mycobacterium sherrisii* disseminated infection in a child with AIDS. *AIDS* 21: 1496–1498.
- Tortoli E, Mariottin A, Mazzarelli G, 2007. *Mycobacterium sherrisii* isolation from a patient with pulmonary disease. *Diagn Microbiol Infect Dis* 57: 221–223.
- Barrera L, Palmero D, Paul R, Lopez B; Grupo de Investigacion de M. simiae, 2010. Disease due to *Mycobacterium simiae* and “*Mycobacterium sherrisii*” in Argentina. *Medicina (B Aires)* 70: 343–346.
- Ho J, Balm M, Huggan P, Chew N, Venkatachalam I, Archuleta S, 2012. Immune reconstitution inflammatory syndrome associated with disseminated *Mycobacterium sherrisii* infection. *Int J STD AIDS* 23: 369–370.
- Crump JA, van Ingen J, Morrissey AB, Boeree MJ, Mavura DR, Swai B, Thielman NM, Bartlett JA, Grossman H, Maro VP, van Soolingen D, 2009. Invasive disease caused by nontuberculous mycobacteria, Tanzania. *Emerg Infect Dis* 15: 53–55.
- Valero G, Moreno F, Graybill J, 1994. Activities of clarithromycin, ofloxacin, and clarithromycin plus ethambutol against *Mycobacterium simiae* in a murine model of disseminated infection. *Antimicrob Agents Chemother* 38: 2676–2677.
- van Ingen J, Totten SE, Helstrom NK, Heifets LB, Boeree MJ, Daley CL, 2012. In vitro synergy between clofazimine and amikacin in nontuberculous mycobacterial disease. *Antimicrob Agents Chemother* 56: 5324–5327.
- van Ingen J, Totten SE, Heifets LB, Boeree MJ, Daley CL, 2012. Drug susceptibility testing and pharmacokinetics question current treatment regimens in *Mycobacterium simiae* complex disease. *Int J Antimicrob Agents* 39: 173–176.