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Rapidly growing mycobacteria in TB/HIV co-infection: a report of two cases focusing on difficulties in diagnosis and management

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SUMMARY

Recent reports indicate an increase in rates of infection and disease due to rapidly growing mycobacteria (RGM) in patients with pre-existing chronic lung disease. Studies have described difficulties in correctly identifying closely related species, even when proper methodologies are adopted, and several different gene targets have been proposed.
We describe two cases of RGM infection in a 29-year-old HIV-1 positive Congolese man and a 19-year-old HIV-1 positive Liberian woman, respectively, both with bronchiectasis due to previous *Mycobacterium tuberculosis* (MTB) infection. *Mycobacterium porcinum* and *Mycobacterium bolletii* were identified in bronchoalveolar lavage fluid and sputum, respectively.
After starting the patients on antiretroviral treatment and primary prophylaxis against non-tuberculous mycobacteria (NTM), and ensuring that they adhered to their prescribed regimen, we observed an improvement in their clinical condition and mycobacteria cleared from their respiratory specimens.
Management of RGM respiratory infection in immunocompromised patients has to be evaluated on a case-by-case basis, taking into account the patient's pulmonary sequelae, adherence to multiple treatments and immune profile.

KEY WORDS: RGM, TB/HIV coinfection, Diagnosis

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CASE REPORT

As the size of the vulnerable population increases, non-tuberculous mycobacteria (NTM) disease is also likely to become more frequent (Prevots *et al.,* 2010). Literature shows that there is a marked geographic variability in the prevalence and distribution of NTM species (Marras *et al.,* 2002). As is the case with detection of most bacterial species, and particularly in cases of rapidly growing mycobacteria (RGM), it is important to define their clinical significance (Leao *et al.*, 2009; Parrish *et al.,* 2008).
Although symptomatic disease is an emerging condition in HIV negative patients with structural lung disease, the clinical interpretation of RGM detected in biological samples from HIV positive patients appears to be questionable, particularly regarding their implications in disease progression and the timing of antiretroviral therapy (ARV) effects on viral suppression and viral recovery (Alvarez-Uria *et al.,* 2009; Schinsky *et al.*, 2004).
The clinical outcomes and microbiological data of two HIV positive patients with a history of pulmonary tuberculosis (TB) and RGM isolation are reported herein.
The first patient was a 29-year-old HIV-1 positive Congolese man who was referred to us because of

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cough and weight loss. He had received six months of anti-TB treatment about eleven years previously. He was being treated for HIV with ARV therapy but his adherence was poor.

On admission, his CD4 count was 34 cells/ml and viral load 5.4 $\log_{10}$ copies/ml. A chest computed tomography scan showed bilateral fibrotic pulmonary lesions associated with bronchiectasis. Microscopic examination of a bronchoalveolar lavage specimen using the Ziehl-Neelsen (Z-N) stain showed acid-fast bacilli (AFB). Clinical sample yielded non-pigmented rapidly growing AFB on liquid (MGIT, Becton Dickinson, Sparks, MD, US) and solid (Lowenstein–Jensen, Becton Dickinson, Sparks, MD, US) culture media that were subsequently identified as *Mycobacterium porcinum* by 16S rRNA gene sequencing. The 16S rRNA genes were amplified by PCR (Perkin-Elmer Gene-Amp PCR System 2720) using the universal primers 27F 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R 5'-TACGGYTACCTTGTTACGACTT-3' (d'Azevedo PA *et al.*, 2010). PCR products were purified and sequenced using the BigDye® Terminator v1.1 Cycle Sequencing kit (Applied Biosystems Warrington, UK) and the ABI PrismTM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, US). 16S rRNA was sequenced twice to ensure sequence data accuracy. Sequence data were compared with NCBI GenBank entries using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST) and showed 97% similarity with sequences from *M. porcinum* strain CIP 105392 (Gen Bank accession no. AY 262737).

Antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2003). The isolate proved to be susceptible to third generation cephalosporins, fluoroquinolones, imipenem, linezolid, minocycline, amikacin and

TABLE 1 - *Antimicrobial susceptibility of Mycobacterium spp isolates.*

Antimicrobial agent	*M. bolletii* *Minimum inhibitory concentration (MIC) (µg/mL)*	*M. porcinum* *Minimum inhibitory concentration (MIC) (µg/mL)*
Amoxycillin clavulanate*	>256	>256
Cotrimoxazole† (disk 1.25/23.75 µg)	>8	>8
Cefotaxime† (disk 30 µg)	>64	32
Cefoxtin*	>256	>256
Levofloxacin† (disk 5 µg)	>4	<4
Ciprofloxacin‡	32	16
Imipenem*	0,75	0,75
Amikacin‡	32	16
Tobramycin† (disk 10 µg)	>8	>8
Clarithromycin‡	32	16
Teicoplanin*	>256	>256
Vancomycin*	>256	>256
Linezolid† (disk 30 µg)	<8	<8
Minocyclin† (disk 30 µg)	>64	<4

*E-test (AB Biodisk); ‡Broth microdiluition; †Disk diffusion

macrolides, but resistant to tobramycin, netylmicin, glycopeptides and sulfamethoxazole-trimethoprim (Table 1).
One week after admission, the patient refused further hospitalization and was followed up at our outpatient facility.
Although we considered prescribing antimycobacterial drugs, the patient's poor compliance with treatment forced us to focus our efforts on ARV therapy and prophylaxis regimens, which included primary prophylaxis against NTM with clarithromycin 500 mg po bid.
During follow-up, his respiratory symptoms improved and periodic respiratory specimen cultures tested negative. His viral load was undetectable after six months of ARV therapy.
The second patient was a 19-year-old HIV-1 positive Liberian woman who was referred to us because of cough and fever. Her medical history indicated that she had received antitubercular treatment about seven years previously. On admission, her CD4 count was 300 cells/ml and viral load 3.5 $\log_{10}$ copies/ml. Chest radiograph showed bilateral apical fibrosis, parenchymal calcification and bronchiectasis.
The patient refused bronchoscopy and two sputum samples were collected on consecutive days. Z-N stain was negative and the patient was discharged. ARV treatment was recommended and she was referred for outpatient follow-up. Subsequently, culture results were positive for RGM colonies by the previously reported methods, and the *Mycobacterium* spp. isolate showed 100% identity with *M. bolletii* (GenBank accession no. AY859681).
Susceptibility testing was performed as previously reported, and the strain proved to be susceptible to imipenem and linezolid, but resistant to beta-lactams, fluoroquinolones, aminoglycosides, macrolides, glycopeptides, sulfamethoxazole-trimethoprim and minocycline (Table 1). Meanwhile, the patient appeared to be in good health and no specific treatment was given to eradicate RGM.
In the subsequent three months of follow-up, sputum samples were collected biweekly and only the first two samples tested were positive.
At present, the patient is regularly attending our Day Care Unit to check her adherence to therapy. Her last CD4 count was 450cells/ml and viral load undetectable.

DISCUSSION

Two cases of RGM respiratory infection in HIV patients have been here described. Both the mycobacterial species isolated from the two patients, *M. porcinum* and *M. bolletii,* are new designations. Indeed, a recent report suggests that *M. bolletii* may be a subspecies of M. *abscessus*, while *M. porcinum* has recently been included within the *M. fortuitum* third biovariant complex (Leao *et al.*, 2009).
Among the *M. abscessus* related species, *M. bolletii* has been described in patients with chronic lung disease (Adékambi *et al.,* 2009). Recently, *M. porcinum* has been isolated from human specimens (McGrath *et al.,* 2008). These species can be responsible for severe disease in patients with underlying risk factors, such as pulmonary TB, HIV infection or co-infections.
Several scientific societies, including American Thoracic Society, Infectious Diseases Society of America and British Thoracic Society, have published guidelines specifying criteria to define lung infections due to RGM (McGrath *et al.,* 2008; Bicmen *et al.*, 2010). As a general rule, isolation from several or, alternatively, sterile biological samples, is required, as well as radiological findings and a compatible clinical picture. However, differentiating colonization from infection is not straightforward, so it is not uncommon to start antibiotic treatment to assess therapeutic response.
In our first patient, both clinical data and a positive microbiological result on a respiratory sample obtained by bronchoscopy suggested that *M. porcinum* could have an etiological role in pulmonary involvement. An appropriate ARV regimen, the patient's adherence to it and weight gain were the key strategies for eradicating RGM.
However, it is also possible that the *M. porcinum* isolate's susceptibility to clarithromycin had a role in the favorable course of the infection. On the contrary, our second patient highlights different clinical concerns.
The case met microbiological criteria for RGM respiratory infection as more than two separate sputum cultures were positive for *M. bolletii*, but the patient's favorable clinical course after a short period of hospitalization argued in favor of a case of RGM colonization rather than a respiratory

tract infection. The patient's poor compliance towards further investigations prevented us from gaining more information on bronchiectasic lesions. Interestingly, neither patient developed immune reconstitution inflammatory syndrome, despite undergoing ARV therapy (Murdoch *et al.,* 2008).

Both patients had bronchiectasis, which is known to be a local risk factor for NTM infection, even in the non-HIV population (Parrish *et al.,* 2008; Matthiessen *et al.,* 2010).

These patients require a thorough evaluation, especially when they are experiencing pulmonary exacerbations, to assess their role in the decline of lung function and timeliness of targeted antimicrobial therapy. When treating HIV positive patients, more attention should be given to this issue, because the boundary between infection and colonization depends upon the immunological recovery of the patient.

Moreover, literature shows that there is a marked geographic variability in the prevalence and distribution of species responsible for NTM-related disease (Buijtels *et al.,* 2009; Tortoli *et al.,* 2009). The increasing number of chronically ill patients coming from countries where RGM are likely more commonly found in environmental reservoirs, such as municipal water systems or soil, could lead to overdiagnosis of RGM disease and, in some cases, to inappropriate treatment of these infections.

However, as RGM colonizing the respiratory tract may contaminate the hospital environment, in higher prevalence settings the issue of prevention and control of potential healthcare-associated disseminations of multidrug resistant mycobacteria should be taken into account (Lai *et al.,* 2006). Our report highlights that:

1) immigration from different geographic and socio-economic settings and new diagnostic tools are increasingly providing opportunities to detect new opportunistic pathogens;
2) the patient's lifestyle can impair adherence to treatment for multiple chronic infections;
3) the potential nosocomial diffusion of multidrug resistant mycobacteria should be taken into account;
4) the microbiology laboratory can helpfully contribute to patients' diagnosis and management, emphasizing the crucial role of cooperation between microbiologist and clinician.

REFERENCES

Adékambi T., Drancourt M. (2009). *Mycobacterium bolletii* respiratory infections. *Emerg. Infect. Dis.* **15**, 302-305.

Alvarez-Uria G., Falcó V., Martín-Casabona N., Crespo M., Villar Del Saz S., Curran A., et al. (2009). Non-tuberculous mycobacteria in the sputum of HIV-infected patients: infection or colonization? *Int. J. STD. AIDS.* **20**, 193-195.

Bicmen C., Coskun M., Gunduz A.T., Senol G., Cirak A.K., Tibet G. (2010). Nontuberculous mycobacteria isolated from pulmonary specimens between 2004 and 2009: causative agent or not? *New Microbiol.* **33** (4), 399-403.

Buijtels P.C., Van-der-Sande M.A., De-Graaff C.S., Parkinson S., Verbrugh H.A., Petit P.L., Van-Soolingen D. (2009). Nontuberculous mycobacteria, Zambia. *Emerg. Infect. Dis.* **15**, 242-249.

D'Azevedo P.A., Comin G., Cantarelli V. (2010). Characterization of a new coagulase-negative Staphylococcus species (Staphylococcus pettenkoferi) isolated from blood cultures from a hospitalized patient in Porto Alegre, Brazil. *Rev. Soc. Bras. Med. Trop.* **43**, 331-332

Lai C.C., Lee L.N., Ding L.W., Yu C.J., Hsueh P.R., Yang P.C. (2006). Emergence of disseminated infections due to nontuberculous mycobacteria in non-HIV-infected patients, including immunocompetent and immunocompromised patients in a university hospital in Taiwan. *J. Infect.* **53** (2), 77-84.

Leao S.C., Tortoli E., Viana-Niero C., Ueki S.Y., Lima K.V., Lopes M.L., et al. (2009). Characterization of mycobacteria from a major Brazilian outbreak suggests that revision of the taxonomic status of members of the *Mycobacterium chelonae-M. abscessus* group is needed. *Journal of Clinical Microbiology.* **47**, 2691-2698.

Marras T.K., Daley C.L. (2002). Epidemiology of human pulmonary infection with non tuberculous mycobacteria. *Clin. Chest. Med.* **23**, 553-567.

Matthiessen W., Schmidt C., Rüsch-Gerdes S., Schönfeld N. (2010). Significance of local and general risk factors for the pathogenesis of pulmonary, non-tuberculous mycobacteriosis in non-AIDS patients. *Pneumologie.* **64**, 281-290.

McGrath E., McCabe J., Anderson PB. (2008). American Thoracic Society; Infectious Diseases Society of America. Guidelines on the diagnosis and treatment of pulmonary non tuberculous mycobacteria infection. *Int. J. Clin. Pract.* **62**, 1947-1955.

Murdoch D.M., Venter W.D., Feldman C. and Van Rie A. (2008). Incidence and risk factors for the immune reconstitution inflammatory syndrome in HIV patients in South Africa: a prospective study. *AIDS.* **22** (5), 601-610.

CLSI (2003). Susceptibility testing of mycobacteria,

nocardiae, and other aerobic actinomycetes; approved standard. Document no. M24-A. Clinical Laboratory Standards Institute, Wayne, PA.

Parrish SC, Myers J., Lazarus A. (2008). Nontuberculous mycobacterial pulmonary infections in Non-HIV patients. *Postgrad. Med*. **120**, 78-86.

Prevots D.R., Shaw P.A., Strickland D., Jackson L.A., Raebel M.A., Blosky M.A., et al. (2010). Non tuberculous Mycobacterial Lung Disease Prevalence at Four Integrated Healthcare Delivery Systems. *Am. J. Respir. Crit. Care Med*. **182**, 970-976.

Schinsky M.F., Morey R.E., Steigerwalt A.G., Douglas M.P., Wilson R.W., Floyd M.M., et al. (2004). Taxonomic variation in the *Mycobacterium fortuitum* third biovariant complex: description of *Mycobacterium boenickei* sp. nov., *Mycobacterium houstonense* sp. nov., *Mycobacterium neworleansense* sp. nov. and *Mycobacterium brisbanense* sp. nov. and recognition of *Mycobacterium porcinum* from human clinical isolates. *Int. J. Syst. Evol. Microbiol.* **54**, 1653-1667.

Tortoli E. (2009). Clinical manifestations of nontuberculous mycobacteria infections. *Clin. Microbiol. Infect.* **15**, 906-910.

Wallace R.J. Jr., Brown-Elliott B.A., Wilson R.W., Mann L., Hall L., Zhang Y., et al. (2004). Clinical and laboratory features of *Mycobacterium porcinum*. *J. Clin. Microbiol.* **42**, 5689-5697.