ARTÍCULO ORIGINAL

ISSN 0325-7541
Revista Argentina de Microbiología (2012) 44: 3-9

Disease caused by non-tuberculous mycobacteria: diagnostic procedures and treatment evaluation in the North of Buenos Aires Province

BELÉN IMPERIALE[1]*, MARTÍN ZUMÁRRAGA[2], ANDREA GIOFFRÉ[2], BEATRIZ DI GIULIO[3], ANGEL CATALDI[2], NORA MORCILLO[1]

[1]Laboratorio de Referencia del programa de Control de la Tuberculosis de la provincia de Buenos Aires, Hospital Dr. Antonio Cetrángolo. Italia 1750, Florida, Buenos Aires, Argentina; [2]Instituto Nacional de Tecnología Agropecuaria (CICV/INTA). Los Roseros y Nicolás Repetto, Hurlingham (1686), Buenos Aires, Argentina; [3]Laboratorio de Micobacteriología del Hospital Petrona V. de Cordero. Madero 1218, San Fernando, Buenos Aires, Argentina.
**Correspondence: Email. belen.imperiale@conicet.gov.ar.*

ABSTRACT

Non-tuberculous mycobacteria (NTM) have emerged as pathogens frequently associated to HIV co-infection. The aims of this study were to describe the clinical importance of NTM in patients from the North of Buenos Aires Province and the drug-susceptibility patterns in relation with the therapy used. A total of 23,624 clinical specimens were investigated during the period 2004-2010. Ziehl-Neelsen stain and cultures were used for diagnosis. Molecular and biochemical tests were performed to identify the mycobacteria. TB and mycobacterioses cases were 2 118 and 108 respectively. Sixteen NTM species were found: *Mycobacterium avium* and *Mycobacterium intracellulare* as the main causative agents. Infections produced by more than one species at the same time were confirmed (4 cases). Macrolides and fluoroquinolones were the most active *in vitro* drugs. Treatment evaluation showed that 68.0 % of the cases completed the therapy, 20 % died; and 12 % were relapses. The cases in which the treatment outcome was evaluated received an individual tailor-made therapeutic scheme including those drugs showing *in vitro* activity and presumed *in vivo* usefulness. More than a quarter of the patients had HIV co-infection and the majority of the deaths were associated with this co-infection.

Key words: Non-tuberculous mycobacteria, diagnosis and treatment

RESUMEN

Enfermedad causada por micobacterias no tuberculosas: diagnóstico y evaluación del tratamiento en el norte del Gran Buenos Aires. Las micobacterias no tuberculosas (MNT) emergieron como patógenos frecuentemente asociados a la co-infección con el HIV. EL objetivo del estudio fue describir la importancia clínica de las MNT en pacientes de la región norte de la provincia de Buenos Aires y los patrones de drogo-sensibilidad en relación con la terapia empleada. Se investigó un total de 23.624 especímenes clínicos durante, el período 2004-2010. La tinción de Ziehl-Neelsen y los cultivos se utilizaron para diagnóstico. Las micobacterias fueron identificadas mediante pruebas bioquímicas y moleculares. Los casos de tuberculosis y micobacteriosis fueron 2 118 y 108, respectivamente. Se encontraron 16 especies de MNT, siendo las principales, *Mycobacterium avium* y *Mycobacterium intracellulare*. En 4 casos se confirmaron infecciones producidas por más de una especie al mismo tiempo. Los macrólidos y las fluoroquinolonas tuvieron mayor actividad *in vitro*. La evaluación del tratamiento confirmó que el 68 % de los casos completó la terapia; 20 % murió y el 12 % recayó. Los casos en los que se evaluó el tratamiento recibieron un esquema terapéutico individual incluyendo aquellas drogas que mostraron actividad *in vitro*. Más de un cuarto de los pacientes tuvieron co-infeccion con el HIV y la mayoría de las muertes estuvieron asociadas con esta co-infección.

Palabras claves: Micobacterias no tuberculosas, diagnóstico, tratamiento

INTRODUCTION

In opposition to *Mycobacterium tuberculosis* complex members, non-tuberculous mycobacteria (NTM) are free-living and ubiquitously distributed microorganisms with diverse ability to cause disease in humans. NTM have emerged in the last decades as pathogens frequently associated to the HIV co-infection (11). Nevertheless they can also be found as colonizers of the respiratory tract usually in persons affected by tuberculosis (TB) in the past and having sequel lesions, or they can cause real disease with different localization as lymph adenitis mainly occurring in young children. Patients´ pre-existing conditions such as severe lung infection or past TB, bronchiectasis, emphysema, cystic fibrosis, rheumatoid arthritis and other chronic diseases with pulmonary manifestations can frequently predispose to acquiring a NTM and developing a mycobacterioses (MB). In addition, NTM can cause disseminated disease in severely immunocompromised patients and affect organs such as skin and soft tissues (7, 11).

A large variety of species have been identified as belonging to the mycobacterium genus. Classically, they have been classified into four groups according to the

Runyon system. Groups I to III contain slowly growing mycobacteria. Groups I, II and III represent the photochromogens, the scotochromogens and nonchromogenic mycobacteria, respectively. Group IV group includes the rapid growers (colonies are visibly in agar in 7 days or less), some of which can produce pigment (23, 24).

Another classification is according to the risk of producing infection and disease in humans. Four risk groups have been defined: group I comprising mycobacteria classified as rare pathogens, with low risk to produce infection for the individual and the community; group II includes the potential pathogens or opportunistic organisms which have moderate risk of causing disease; group III comprises strict pathogens with high risk of causing infection in individuals but moderate risk for the community, and group IV composed of more recently characterized species, isolated from human and environmental sources (5).

An increasing number of infections due to NTM had been previously reported. Although MB cases are generally associated to immunocompromised patients or to patients with pre-existing lung disease, the infection may also occur in an immunocompetent person (10, 12, 16).

In case a NTM is recovered from clinical specimens, it is very important to establish whether this mycobacterium is actually the causative agent of the disease. If the NTM is isolated from a sputum sample belonging to a non-immunocompromised host, the mycobacterium must be isolated at least three times for establishing it as the etiological agent of the disease, but if the NTM is isolated once from an immunocompromised patient or from a sample collected from sterile body sites, it is assumed that the NTM is the real causative agent of the MB (2, 11).

Nevertheless, and in order to avoid treatment mismanagement, the diagnosis of TB caused by members of the *M. tuberculosis* complex must be properly studied and dismissed before assuming the MB diagnosis (2, 11).

Measurement of the NTM burden as etiological agents of a disease different from TB has great relevance mainly in health-care centers where the prevalence of the MB is unknown.

The aims of this study were to describe the load and the clinical importance of NTM in patients from the North of Buenos Aires Province and the drug-susceptibility patterns in relation with the therapeutic schemes used, and to evaluate the successful rate of pharmacological treatments.

MATERIALS AND METHODS

Mycobacteria clinical isolates

During the period 2004 to July 2010 a total of 23,624 respiratory and non- respiratory clinical specimens were investigated at the Hospital Zonal Especializado de Agudos y Crónicos Dr. A. Cetrángolo of Buenos Aires Province, Argentina, which is a referral center for lung diseases. This hospital is located in a Northern Buenos Aires City area with almost four million inhabitants and approximately 1400 new TB cases per year. The Hospital Interzonal General de Agudos Petrona V. de Cordero, San Fernando, Buenos Aires, is a general hospital in the same region. This centre works in cooperation with Hospital Dr. A. Cetrángolo for lung diseases and mycobacteriosis cases and contributes with almost 100 new TB cases per year to the total amount of the region. The disease diagnosis was performed by direct smear examination using the Ziehl-Neelsen stain and cultures. To obtain the isolates, specimens from both non-sterile and sterile body sites were processed as previously described (11, 21). Cultures were recovered from solid media in Löwenstein Jensen, Stonebrink and/or Middlebrok 7H11 (M7H11) and liquid media using the M7H9 and the fully automated BACTEC MGIT960 system (BD Argentina) (13, 17, 21).

Patient data recovery

Epidemiological data such as gender, age, site of the lesion, HIV co-infection status and other co-morbidities, previous history of TB or MB treatment, and chemotherapeutic scheme designed for each MB episode were collected. Medical records stored at both Dr. Cetrángolo and P. V. de Cordero Hospitals were used to collect all the epidemiological and clinical information while laboratory records were the source document for microbiological data.

Mycobacteria identification

NTM were identified by biochemical and molecular tests using an algorithm previously reported (18). Biochemical tests included the temperature and time of growth, the cultural characteristics of the colonies, the production of pigment and several enzymatic reactions like niacin production, nitrate reduction, catalase production at room temperature and at 68 °C, telurite reduction and Tween 80 hydrolysis (6).

Molecular identification was performed by PCR-restriction fragment length polymorphism analysis of the *hsp65* gene (PRA) (4, 9). In this work, the gold standard for mycobacteria identification was the combination of biochemical tests and PRA. When the correct identification by PRA was not possible or discordant results were obtained between PRA and phenotypic methods, the 16S-rRNA gene sequencing was performed to properly identify the mycobacterial species (6, 26).

Drug-susceptibility testing (DST)

The indirect proportion method on M7H11 was used to determine the drug-resistance (DR) profile of the isolates to first and second line anti-TB drugs (8, 14, 15, 22).

The microdilution colorimetric method (CMM) using resazurin as vital dye (REMA) was also used in order to determine the minimal inhibitory concentration (MIC) values for the same drugs (22, 25). *M. tuberculosis* H37Rv ATCC 27294 and *Mycobacterium avium* D4 reference strains were used as DST controls.

Table 1 shows the drug concentrations used in both methods as well as the cutoff values proposed to consider the strain susceptible or resistant when tested by CMM and after a comparison with the proportion method. This comparison and the cutoff values obtained by CMM were performed by the ROC curve method (20, 29).

A strain was considered susceptible or resistant to a particular agent when the results obtained by both methods were in full agreement and following the criteria given by the literature (14, 15, 22).

Drugs such as cefoxitin, imipenem, doxycycline and tobramycin frequently used for the treatment of rapid growers, were not properly standardized under our working conditions and therefore were not tested for *in vitro* DST.

Table 1. First and second line drug-concentrations tested in proportion and colorimetric methods

Drug	Drug concentration (µg/ml)	
	PM7H11	CMM (cut-off)
Amikacin	4.0	8.00 to 0.25 (4.00)
Azitrhomycon	10.0	8.00 to 0.25 (2.00)
Clarithromycin	10.0	8.00 to 0.25 (2.00)
Cycloserine	30.0	120.00 to 3.75 (30.00)
Ethambutol	7.5	32.00 to 1.00 (4.00)
Ethionamide	10.0	8.00 to 0.25 (2.00)
Isoniazid	0.2-1.0	1.00-0.03 (0.25)
Kanamycin	6.0	8.00 to 0.25 (4.00)
Levofloxacin	2.0	4.00 to 0.13 (0.50)
Linezolide	1.0	4.00 to 0.13 (0.50)
Ofloxacin	10.0	8.00 to 0.25 (4.00)
P Amino-salycilic acid	8.0	8.00 to 0.25 (2.00)
Rifabutin	0.5	1.00 to 0.03 (0.25)
Rifampicin	1.0	2.00 to 0.06 (0.50)
Streptomycin	2.0	8.00 to 0.25 (4.00)

PAS: para amino-salicylic acid; INH: isoniazid; CS:cycloserine; TTH: ethionamide; RIF: rifampicin; SM: streptomycin; EMB: ethambutol; linezolide; RBT: rifabutine; AMK/KM: amikacin/kanamycin; PM7H11: proportion method on Middlebrok 7H11, CMM: colorimetric microplate method

Study sequence

Given the low frequency of MB in comparison with TB cases in this particular geographical area, each one of the isolates was tested independently at the occurrence moment. For this reason, all the tests leading to the complete characterization of the microorganisms were not carried out simultaneously. DST on M7H11 and the identification by biochemical tests were performed soon after the isolate was grown in culture. The presumptive case caused by a NTM was preliminary reported to physicians on the basis of AFB, culture characteristics, biochemical tests and DST results. After that, the molecular and conclusive identification and MIC determination by CMM were performed. Epidemiological and clinical data were collected at the time of performing the analysis as part of this study.

RESULTS

From a total of 23,624 clinical specimens analyzed, 2,914 (12.3 %) yielded mycobacterial isolates. *M. tuberculosis* and NTM represented 93.9 % (n = 2,736) and 6.1 % (n = 178) of the isolates respectively. The TB cases during the study period were 2,118 while 108 (4.9 %, 108/2226) were confirmed cases of MB.

The main characteristics of the MB cases were: a wide age range from 6 to 81 years old (median: 41.6 years); the majority of the cases occurred in males (n = 70, 64.8 %), the HIV co-infection (HIV+) was present in 27.8 % (n = 30) of the patients, while in 17 patients (15.7 %) no data on HIV status were consigned in medical records. Only 25 (23.1 %) cases had been previously treated for TB and 7 for a previous MB episode.

Mycobacteria identification

A total of 87 out of 108 (80.6 %) isolates were properly identified by PRA and /or sequencing and 16 different NTM species were found. *M. avium* (n = 28, 32.2 %) was the main causative agent found in this study, and *M. intracellulare I* ranked second (n = 23, 26.4 %). In addition, 50.0 % (n = 14) of the cases produced by *M. avium* occurred in HIV+ patients while only 17.4 % (n = 4) of the cases due to *M. intracellulare* belonged to HIV co-infected patients. These findings are in concordance with the literature (1, 5).

Table 2 shows the frequency of different mycobacteria species and their sub-types in relationship with the physical localization of the disease found in this work. *Mycobacterium kumamotonense* and *Mycobacterium sherrisii* were found among all of them.

Infections produced by more than one species at the same time were confirmed in 4 cases. In one case of an HIV+patient, the infection was produced simultaneously by *M. tuberculosis* and *M. avium*; in 2, one HIV+ and the other a non co-infected HIV patient, the infection was produced by *M. tuberculosis* plus *M. intracellulare I*; and the last case was caused by *M. fortuitum* and *M. intracellulare* I in a patient suffering from both diabetes and systemic erythematosus lupus.

In 5 (16.7 %) different HIV+ patients, a first TB episode was confirmed and opportunely treated. However, in a second episode occurring far apart in time, a MB caused by *M. avium* was also diagnosed.

After being properly treated during a year and after at least two consecutive negative cultures, 6 out of 87 (6.9 %) MB cases were reported as relapses, being three of them HIV+ individuals. The etiological agents found were: *M. avium* (n = 3), *M. kansasii I* (n = 1) and *M. intracellulare* (n = 2).

Localization of mycobacteriosis cases

Most of the cases in which the etiological agent was properly identified presented pulmonary localization (68/87, 78.2 %) (See Table 2). The analyzed specimens were sputa, broncho-alveolar and bronchial lavages. Only 26.5 % (18/68) of pulmonary cases presented AFB smear positive by Ziehl-Neelsen stain. Of the 19 (21.8 %) extra pulmonary cases, 9 corresponded to blood and bone marrow, 6 biopsies, 2 cerebrospinal fluid, 1 ascitic liquid, and 1 feces. Only 1 case (1.1 %) had both pulmonary and extra pulmonary localization of the disease. Only 1 extra pulmonary case (5.2 %) showed positive results in the AFB smear examination. Twelve out of 19 (63.0 %) of the patients with extrapulmonary disease had HIV co-infection.

Drug susceptibility testing

Figure 1 shows the percentage of resistant isolates found for each one of the drugs. DST on M7H11 and REMA results showed an important resistance level of the NTM to several first and second line anti-TB drugs. The macrolides [(azithromycin, (AZ), and clarithromycin, (CLA)], the fluoroquinolones, (FQ), [levofloxacin, (LX),

Table 2. Frequency of different mycobacteria species detected by molecular and biochemical tests from 108 cases

NTM species (N: 16)	Disease Localization		Sub-Classification		Total (%)
	P	EP	Type	N	N (%)
M. avium	10	9[1]	*M. avium*	19	28 (32.2)
	1	1	I	2	
	6 1		II	7	
M. intracellulare	6[2]	1[2]	*M. intracellulare*	7	23 (26.5)
	15	1	1	16	
M. gordonae	2	2	*M. gordonae*	4	10 (11.6)
	-	1	III	1	
	2 -		IV	2	
	3 -		V	3	
M. kansasii	5		*M. kansasii*	5	7 (8.0)
	2		I	2	
M. fortuitum	*2*	1[3]	-	3	3 (3.5)
M. flavescens	1	1	-	2	2 (2.3)
M. sherrissi	1	-	*M. sherrissi*	1	2 (2.3)
	-	1	I	1	
M. simiae	1	-	*M. simiae*	1	2 (2.3)
	1	-	IV	1	
M. scrofulaceum	2	-	I	2	2 (2.3)
M. kumamotonense	1	-	*M. kumamotonense*	1	2 (2.3)
	1	-	I	1	
M. haemophilum	-	1	-	1	1 (1.1)
M. lentiflavum	-	14	-	1	1 (1.1)
M. nonchromogenicum	1	-	1	1	1 (1.1)
M. chelonae	1	-	-	1	1 (1.1)
M. vaccae	1	-	I	1	1 (1.1)
M. xenopi	1	-	I	1	1 (1.1)

NTM: non-tuberculous mycobacteria; N: number; P: pulmonary; EP: extra pulmonary; 1: *M. tuberculosis* plus *M. avium*; 2: *M. tuberculosis* plus *M. intracellulare* I; 3: M. *fortuitum* plus *M. intracellulare* I; 4: isolated from both blood and bronchial washing

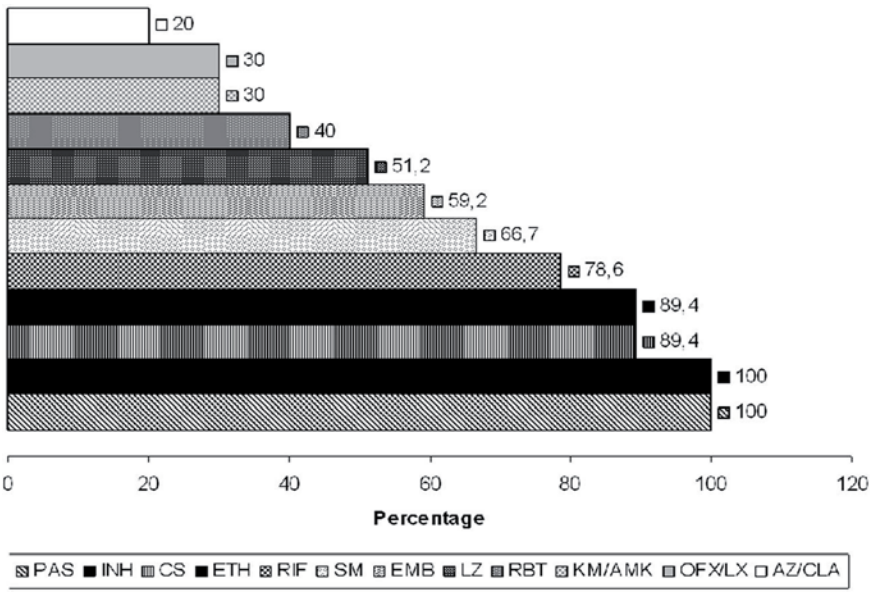

Figure 1. Proportion of drug-resistant non-tuberculous mycobacteria to first and second-line drugs.
PAS: paraamino-salicylic acid; INH: isoniazid; CS: cycloserine; ETH: ethionamide; RIF: rifampicin; SM: streptomycin; EMB: ethambutol; LZ: linezolide; RBT: rifabutine; AMK/KM: amikacin/kanamycin; OFX/LX: ofloxacin/levofloxacin; AZ/CLA: azithromycin/ clarithromycin

Table 3. Drug resistance profile for the main Mycobacterial species

Mycobacteria specie	*M. avium* (n = 28)	*M. intacellulare* (n = 23)	*M. gordonae* (n = 10)	*M.* kansasii (n = 7)
Drug	Resistance (%)			
INH	100.0	100.0	100.0	100.0
RIF	87.5	75.0	95.0	25.0
SM	77.5	66.7	78.0	25.0
EMB	72.5	50.0	75.0	50.0
PAS	100.0	100.0	100.0	100.0
ETH	97.5	75.0	80.0	25.0
CS	50	98.0	75.0	90.0
RBT	45.0	32.5	20.5	17.5
LZ	61.0	90.0	58.0	12.5
AZ	27.5	22.5	30.0	10.0
CLA	17.5	20.0	35.0	12.5
LX	30.0	40.0	35.0	30.0
OFX	15.0	50.0	22.5	35.0
AMK	15.0	20.0	25.0	12.5
KAN	22.5	25.0	30.0	15.0

INH: isoniazid; RIF: rifampicin; SM: streptomycin; EMB: ethambutol; PAS: para amino-salicylic acid; ETH: ethionamide; CS: cycloserine; RBT: rifabutin; LZ: linezolide; AZ: azithromycin; CLA: clarithromycin; LX: levofloxacin: OFX: ofloxacin; AMK: amicacin; KAN: kanamycin; n: number

Table 4. Proportion of drug utilization and its relationship with the treatment success

	Nº patients and treatment evaluation (n = 50)			
Drug	Cured (n = 34)	Death (n = 10)	Relapse (n = 6)	Total (%)
OFX	20	3	-	23 (46.0)
LX	26	1	-	27 (54.0)
AMK	23	4	1	27 (54.0)
KM	10	1	-	11 (22.0)
CLA	19	1	-	20 (40.0)
AZ	26	4	4	30 (60.0)
EMB	28	6	2	34 (68.0)
CS	10	3	-	13 (26.0)
RIF	12	3	-	15 (30.0)

OFX: ofloxacin; LX: levofloxacin; AMK: amikacin; KM: kanamycin; CLA: clarithromycin; AZ: azithromycin; EMB: ethambutol; CS: cycloserine; RIF: rifampicin

and ofloxacin, (OFX)] and the aminoglycosides [amikacin, (AMK), and kanamycin, (KAN)] were the most active drugs tested *in vitro*, exerting their inhibitory action in around 70.0 % of the isolates. On the other hand, isoniazid (INH), para amino-salicylic acid (PAS), cycloserine (CS), ethionamide (ETH) and rifampicin (RIF) showed less than 40.0 % inhibition while streptomycin (SM), linezolid (LZ), and ethambutol (EMB) showed their inhibitory activity in around 50.0 % of the isolates.

Table 3 shows the drug resistance profile for each one of the tested drugs and for the main mycobacterial species found in the study.

Treatment evaluation

A cohort comprising data at the beginning of the therapy, the drug regimens and a final evaluation was obtained from 62 out of 87 (71.3 %) fully identified MB cases. No available data regarding MB treatment were obtained from the remaining 25 MB cases, so they were excluded from the final cohort. A total of 12 (19.4 %) patients were removed from the cohort because 10 of them still remained under treatment at the evaluation time, and the other 2 were lost from the register; therefore the final analysis was performed in 50 cases with complete therapy. All the complete treatments lasted a median of 20 (range: 18-30) months.

A cohort of 50 cases with treatment results was obtained. This evaluation showed that 34 (68 %) cases completed the prescribed therapy and they were considered cured; 10 (20 %) patients (7 HIV+) died; 6 (12 %) were classified as relapses.

Table 4 shows the proportion of drug utilization and its relationship with the treatment results in the 50 cases with complete treatment data. Macrolides (mainly AZ) and FQ (mainly LX) were administered in 100.0 % of the patients. Aminoglycosides (mainly AMK) were used in 76.0 % while EMB was incorporated in 68.0 % of the treatments. RIF and CS were included in 30.0 % and 26.0 % of the treatments, respectively.

Rifabutin (RBT) is not commercially available in Argentina, so although 60.0 % of the isolates were inhibited by it, neither this drug nor LZ, SM and ETH were included in the therapeutic schemes.

In those cases in which *M. tuberculosis* and NTM were isolated, an expanded scheme including INH, RIF, FQ, an aminoglycoside and EMB was established. No cases with drug-resistant *M. tuberculosis* and a NTM were reported.

Twenty two out of 28 patients infected by *M. avium* completed the indicated therapy, 11 (50.0 %) were cured and 3 (13.6 %) died; 10 out of 19 (52.6 %) cases with *M. intracellulare,* were also cured and 2 (10.5 %) died. The remaining eight and seven cases caused by *M. avium* and *M. intracellulare* respectively were still under treatment during the study period. One patient with a mixed infection by *M. tuberculosis* and *M. intracellulare*, 2 with *M. gordonae*, and one with *M. simiae* and another one with *M. lentiflavum* also died.

DISCUSSION

On several occasions, physicians have to assume a case as TB with the sole evidence of the positive AFB smear results until therapy failure and/or when the worsening of the patient's clinical condition pose a doubt about diagnostic accuracy. The evidence of a TB diagnosis based on *M. tuberculosis* identification from clinical samples allows the setting up of a proper treatment and furthermore expect it to be successful. Nevertheless, when a NTM is the causative agent of disease, the currently anti-TB therapy is often worthless with the subsequent patient's health impairment and life - threatening risk. Thus, the detection of NTM as etiological agent is very important still in settings with high incidence rate of TB.

Disease caused by NTM is quite common in the HIV co-infected population, mainly in developed countries. In addition, immunosuppressive therapies are more frequently used not only for cancer therapy but for autoimmune and inflammatory diseases which very often present lung manifestations and can be associated to NTM infection. Therefore, a wider epidemiology of NTM as agents producing MB is increasing in interest and importance (30). In this study period, NTM were reported in 4.9 % of the cases caused by mycobacteria (TB plus NTM) affected patients with and without HIV co-infection; more than a quarter of the cases (26.8 %) were associated to HIV co-infection. Although the North of Buenos Aires is not a region with tropical characteristics, a broad spectrum of species (16) was found. Nevertheless, the main etiological agent was *M. avium*, followed by *M. intracellulare*, *M. kansasii* and *M. gordonae*. Recently described species such as *M. kumamotonense, M. sherrissi* and *Mycobacterium nonchromogenicum I* were also found among these patients. *M. sherrisii* had previously been reported as etiological agent of MB cases in Argentina (3).

Interestingly, mixed infections caused by two different mycobacteria species at the same time were confirmed in 4 (4.6 %) cases.

NTM DST results showed a high resistance level to the main anti-TB drugs, especially INH and RIF. All the isolates were also completely resistant to PAS and almost to CS and ETH. The susceptibility to the studied FQ, macrolides, aminoglycosides (different to SM), and RBT was more than 50.0 %. Nevertheless, between 40.0 to 49.0 % of the isolates showed EMB and LZ MIC values compatible with those of drug-susceptible strains.

The cohort for the evaluation of treatment success comprised almost 50.0 % (patients n = 108) of the original number initially included in this study. The facts that caused this decreased cohort to be effectively evaluated were multiple and mostly related to the underestimation of the proper disease at the diagnostic time. On many occasions, the patients were diagnosed after having received an inadequate standardized treatment for TB for several months. The delay in proper detection of the causative agent as well as no proper treatments in most of those cases, led to the losses in the final cohort. In contrast, HIV+ cases were followed up more closely and deaths occurred in these cases under proper treatment.

The first worldwide publication of *M. kumamotonense,* from clinical isolates was in 2006, in Kumamoto Prefecture, Japan, by Masaki T *et al.* (19). To our knowledge, it is the first time that *M. kumamotonense* is reported in Argentina as an etiological agent.

M. tuberculosis and *M. avium* simultaneous infection was reported in one HIV+ patient who died after starting appropriate therapy. The *M. tuberculosis* isolate was recovered from a solid medium that allowed the discrimination between two different colony morphology which, in the end, belonged to two different species. A mixture culture of both species was obtained in the MGIT 960 liquid medium. This mixed infection findings had previously been reported (27, 28) but it underscores the importance of using more than one culture system when investigating clinical specimens from severe immunocompromised patients. In other 2 cases (1 HIV+ and 1 non co-infected with HIV) the infection was produced by *M. tuberculosis* plus *M. intracellulare I.* The latter case was caused by *M. fortuitum* and *M. intracellulare I* in a patient suffering from both diabetes and systemic erythematosus lupus.

All the cases in which the treatment outcome was evaluated received an individual tailor-made therapeutic scheme including those drugs showing *in vitro* activity and presumed *in vivo* usefulness. Nevertheless, the majority of the deaths caused by NTM were associated to HIV co-infection (83.0 %).

The disease caused by NTM is not currently considered a public health problem in countries where its prevalence is unknown or presumably low. In spite of that, the NTM are causing serious clinical problems and they must be taken into consideration at the time of making a proper diagnosis to provide the best treatment, offering the cure opportunity. In conclusion, a timely detection of the mycobacteria species as the etiological agent is of paramount clinical importance because delays in proper treatment might cause impaired health in people usually affected by other severe illnesses such as HIV co-infection.

Acknowledgment: We thank Dr. Silvina Tártara for providing clinical and epidemiological data from one case; Marcelo Mazza and Daniel Cardinali for their technical assistance. AG and AC are fellows and BI has a fellowship from CONICET, Argentina.

REFERENCES

1. Alvarez J, Garcia IG, Aranaz A, Bezoz J, Romero B, de Juan L, Mateos A, Gómez- Mampaso E, Domínguez L. Genetic Diversity of *Mycobacterium avium* Isolates Recovered from Clinical Samples and from the Environment: Molecular Characterization for Diagnostic Purposes. J Clin Microbiol 2008; 46: 1246-51.

2. American Thoracic Society. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. Am J Respir Crit Care Med 1997; 156:S1–S25.
3. Barrera L, Palmero D, Paul R, López B y grupo de investigación de *M. simiae*. Enfermedad por *Mycobacterium simiae* y *Mycobacterium sherrisii* en la Argentina. Medicina (B. Aires) 2007; 70: 343-6.
4. Brunello F, Ligozzi M, Cristelli E, Bonora S, Tortoli E, Fontana R. Identification of 54 Mycobacterial Species by PCR-Restriction Fragment Length Polymorphism Analysis of the *hsp65* gene. J Clin Microbiol 2001; 39: 2799-806.
5. Cardoso Leao S, Martin A, Mejía GI, Palomino JC, Robledo J, da Silva Telles MA, Portaels F. In: Vanden Broelle, editors. Practical handbook for the phenotypic and genotypic identification of Mycobacteria. 1st Edn. Bruges, Belgium, 2004, pp. 47.
6. Cardoso Leao S, Martin A, Mejía GI, Palomino JC, Robledo J, da Silva Telles MA, Portaels F. In: Vanden Broelle, editors. Practical handbook for the phenotypic and genotypic identification of Mycobacteria. 1st Edn. Bruges, Belgium, 2004, pp. 45-71.
7. Cardoso Leao S, Martin A, Mejía GI, Palomino JC, Robledo J, da Silva Telles MA, Portaels F. In: Vanden Broelle, editors. Practical handbook for the phenotypic and genotypic identification of Mycobacteria. 1st Edn. Bruges, Belgium, 2004, pp. 22.
8. Cynamon, MH, Klemens SP. In: Heifets LB editor. Drug susceptibility testing in the chemotherapy of mycobacterial infections. Chapter 5: Drug susceptibility testing for *Mycobacterium fortuitum* and *M. chelonae*. Boca Raton, Florida, USA: CRC Press, Inc., 1991, pp. 147-59.
9. da Silva Rocha A, da Costa Leite C, Torres HM, de Mirando AB, Pires Lopes MQ, Degrave WM, Suffys PN. Use of PCR-restriction fragment length polymorphism analysis of the *hsp65* gene for rapid identification of mycobacteria in Brazil. J Microbiol Methods 1999; 37:223-9.
10. Debrunner M, Salfinger M, Brändli O, von Graevenitz A. Epidemiology and clinical significance of nontuberculous mycobacteria in patients negative for human immunodeficiency virus in Switzerland. Clin Infect Dis 1992; 15: 330-45.
11. Griffith D E, Aksamit T, Brown-Elliot A, Cattanzaro A, Daley C, Gordon F. An official ATS/IDSA statement diagnosis, treatment and prevention of nontuberculous mycobacterial disease. Am J Respir Crit Care Med 2007; 175: 367-416.
12. Grubek-Jaworska H, Walkiewicz R, Safianowska A. Nontuberculous mycobacterial infections among patients suspected of pulmonary tuberculosis. Eur J Clin Microbiol Infect Dis 2009; 28: 739-44.
13. Hanna B, Ebrahimzadeh A, Elliott B, Morgan M A, Novak S M, Rusch-Gerdes S, Acio M, Dunbar D F, Holmes T M, Rexer C H, Savthyakumar C, Vannier A M. Multicenter evaluation of the BACTEC MGIT 960 system for the recovery of mycobacteria. J Clin Microbiol 1999; 37: 748-52.
14. Heifets L B. In: Heifets L B editors. Drug susceptibility testing in the chemotherapy of mycobacterial infections. Chapter 1: Antituberculosis drugs: antimicrobial activity in vitro. MIC and MBC against *M. avium* Complex. Boca Raton, Florida, USA: CRC Press, Inc 1991; pp. 28-58.
15. Heifets L B. In: Heifets L B editors. Drug susceptibility testing in the chemotherapy of mycobacterial infections. Chapter 4: Dilemmas and realities in drug susceptibility testing of *M. avium-M. intracellulare* and other slowly growing nontuberculous mycobacteria. Boca Raton, Florida, USA: CRC Press, Inc. 1991; pp. 123-46.
16. Henry M T, Inamdar L, O'Riordain, Schweiger M, Watson J P. Nontuberculous mycobacteria in non-HIV patients: epidemiology, treatment and response. Eur Respir J 2004; 23: 741-6.
17. Hillemann D, Richter E, Rüsch-Gerdes S. Use of the BACTEC Mycobacteria Growth Indicator Tube 960 automated system for recovery of Mycobacteria from 9,558 extrapulmonary specimens, including urine samples. J Clin Microbiol 2006; 44: 4014-7.
18. Imperiale B, Morcillo N, Bernardelli A. Identificación fenotípica de micobacterias. Bioquímica y Patología Clínica 2007; 71: 47-51.
19. Masaki T, Ohkusu K, Hata H, Fujiwara N, Iihara H, Yamada-Noda M, Nhung PH, Hayashi M, Asano Y, Kawamura Y, Ezaki T. *Mycobacterium kumamotonense* Sp. Nov. recovered from clinical specimen and the first isolation report of *Mycobacterium arupense* in Japan: Novel slowly growing, non-chromogenic clinical isolates related to *Mycobacterium terrae* complex. Microbiol Immunol. 2006;50:889-97.
20. McNeil B J, Hanley J A. Statistical approaches to the analysis of receiver operating characteristic (ROC) curves. Med Decis Making 1984; 4:137-150.
21. Morcillo N S, Imperiale B R, Palomino J C. New simple decontamination method improves microscopic detection and culture of mycobacteria in clinical practice. Infect Drug Resist 2008; I: 21-6.
22. NCCLS. Susceptibility testing of Mycobacteria, Nocardiae, and other aerobic Actinomycetes; Approved standard. Document M24-A (ISBN 1-56238-500-3). 2003; 31 pp. 19-45.
23. Rogall T, Wolters J, Flohr T, Bötger E C. Towards a phylogeny and definition of species at the molecular level within the genus Mycobacterium. Int J Syst Bacteriol 1990; 40: 323-30.
24. Runyon E H. Anonymous mycobacteria in pulmonary disease. Med Clin North Am 1959; 43: 273-90.
25. Sankar M M, Gopinath K, Singla R, Singh S. In-vitro antimycobacterial drug susceptibility testing of non-tuberculous mycobacteria by tetrazolium microplate assay. Ann Clin Microbiol Antimicrob 2008; 7: 15.
26. Shin S, Kim E C, Yoon J H. Identification of Non-tuberculous Mycobacteria by sequence analysis of the 16S Ribosomal S. Korean J Lab Med 2006; 26: 153-60.
27. Suffys P, da Silva Rocha A, Brandão A, Vanderborght B, Mijs W, Jannes G, Mello F, da Silveira Paro Pedro H, de Souza Fonseca L, Siqueira de Oliveira R, Cardoso Leão S, Féres Saad M.Detection of mixed infections with *Mycobacterium lentiflavum* and *Mycobacterium avium* by molecular genotyping methods. J Med Microbiol 2006; 55: 127-31.
28. Terrones R, de Alarcón A, García-Curiel A,. Luque J A, Arroyo A. Mixed adenitis caused by *Mycobacterium avium* complex and *Mycobacterium tuberculosis* complex in patients with HIV infection. Enferm Infecc Microbiol Clin 1997;15:225-6.
29. Van Der Schouw I T, Verbeek A I M, Ruijs S H J. Guidelines for the assessment of new diagnostic tests. Investigative Radiology 1995; 30:334-40.
30. Winthrop KL, Chang E, Yamashita S, Iademarco MF, LoBue PA. Nontuberculous mycobacteria infections and anti-tumor necrosis factor-α therapy. Emerg Infect Dis 2009; 15:1556-61.

Recibido 24/05/11 – Aceptado 09/11/11