3

Pulmonary Nontuberculous Mycobacterial Infections in the State of Para, an Endemic Region for Tuberculosis in North of Brazil

Ana Roberta Fusco da Costa[1,2], Maria Luiza Lopes[1], Maísa Silva de Sousa[2], Philip Noel Suffys[3], Lucia Helena Messias Sales[4] and Karla Valéria Batista Lima[1]

[1]*Evandro Chagas Institute, Bacteriology Section*
[2]*Federal University of Para, Tropical Medicine Nucleus*
[3]*Oswaldo Cruz Institute, Oswaldo Cruz Foundation*
[4]*Federal University of Para, Department of Integrative Medicine*
Brazil

1. Introduction

Traditionally, *Mycobacterium* species are divided in those belonging to the *Mycobacterium tuberculosis* complex (MTBC), *M. leprae* and the nontuberculous or atypical mycobacteria (NTM), the latter consisting of either rapid (RGM) or slow growing (SGM) species, forming colonies, respectively, within seven days of culture or requiring longer incubation time (Runyon, 1959). Among the RGM are the *M. chelonae* complex, including *M. chelonae*, *M. abscessus*, *M. mucogenicum*, *M. salmoniphilum*, *M. bolletii* and *M. massiliense* (Brown-Elliot & Wallace, 2002; Whipps et al., 2007) and the *M. fortuitum* complex, including *M. fortuitum*, *M. peregrinum*, *M. septicum*, *M. mageritense*, *M. houstonense* and *M. boenikei* (Adékambi & Drancourt, 2004), containing the NTM commonly encountered in human specimens. Clinically important SGM are the *M. avium* complex (MAC), which include *M. avium*, *M. intracellulare*, *M. colombiense* and *M. chimaera* (Tortoli, 2003; Tortoli et al., 2004).

Until now, 130 *Mycobacterium* species, with a considerable variability in pathogenicity have been described, being isolated from natural water reservoirs and drinking water distribution systems in buildings, hospitals, household plumbing, hot tubs, spas, building aerosols, boreal forest soils and peats, acidic, brown-waters swamps, potting soils and metal removal fluid systems (Tortoli, 2009; Euzéby, 2011, Falkinham, 2009). The lack of evidence for person-to-person transmission suggests that the environment is the most likely source of NTM infection (Marra & Daley, 2002).

Unlike the bacterial species that belong to the MTBC, NTM are commonly present in the environment and when isolated from human specimens, may either be (i) contaminants during preparation of sputum cultures in the laboratory, (ii) colonizing organisms of the airways without causing disease or (iii) infectious organisms and causing disease and it is

not always easy to distinguish between these situations (Griffith et al., 2007). In the case of real infection with NTM, clinical syndromes are either lymphadenitis, pulmonary or cutaneous infection or disseminated disease, chronic pulmonary infection being the most common (Katoch, 2004; Piersimoni & Scarparo, 2009; Tortoli, 2009). Such NMT infections are frequently observed in immune-compromised patients in developed nations but also in immune-competent individuals with pre-existing structural pulmonary diseases (Griffith et al., 2007; Jeong et al., 2004; Jarzembowski & Young, 2008; Bodle et al., 2008; Glassroth, 2008; Sexton & Harrison, 2008; Griffith, 2010).

The diagnosis of NTM pulmonary disease is often difficult due to the overwhelming presence of environmental organisms, to the indolent nature of disease and the diversity of, mostly, nonspecific clinical symptoms. Therefore, guidelines and criteria for diagnosis of NTM pulmonary disease have been published (American Thoracic Society, 1997; British Thoracic Society, 2000) followed by the publication of a recent update of more lenient criteria (Griffith et al., 2007). Even so, these recommendations do not seem to be satisfactory as most patients with pulmonary disease due to NTM do not match these criteria (Marras et al., 2007; van Ingen et al., 2009). Also, in endemic countries for tuberculosis (TB), the pulmonary disease form is caused also by infection with organisms of the MTBC, presenting similar clinical symptoms. In addition, diagnostic procedures for pulmonary TB are sputum smear microscopy for acid-fast bacilli (AFB) and X-ray, not differentiating between *Mycobacterium* to the species level. Nonetheless, several case reports and studies on the prevalence of pulmonary disease caused by NTM in North America, Europe and Japan have been published during the last years (Good, 1980; Tsukamura et al., 1988; von Reyn et al., 1993; Falkinham, 2002; Kobashi & Matsushima, 2007; Iseman & Marras, 2008; Billinger et al., 2009; Thomson, 2010; Kendall et al., 2011).

The impact, magnitude and regional dimension of NTM infections in countries where TB is endemic is hardly known (Gopinath & Singh, 2010), such as the case in Brazil, where most cases of infectious NTM have been reported in the southeastern region and, more specifically, in São Paulo (Barreto & Campos, 2000; Ueki et al., 2005; Zamarioli et al., 2008; Pedro et al., 2008). In the Amazon region, North of Brazil, little epidemiological information on this matter is available (da Costa et al., 2009).

We therefore studied the frequency and diversity of NTM isolates, obtained from pulmonary specimens from residents of the Pará State, during a twelve year period.

2. Material and methods

2.1 Study setting and patients

All *Mycobacterium* isolates evaluated were obtained from sputum samples (n = 119) and bronchial washings (n = 9) from individuals with clinical symptoms of pulmonary TB and residents of the State of Pará, North Brazil (Fig. 1). The study included samples of patients from whom NTM was isolated from at least once, and this between January 1999 and December 2010, at the Evandro Chagas Institute, a reference center for the diagnosis of infections with *Mycobacterium*. Patient records were reviewed to assess the frequency of isolation and clinical relevance of the presence of NTM and the diagnosis for NTM lung infection was based on the diagnostic criteria published by the American Thoracic Society (Griffith et al., 2007) (Table 1).

Fig. 1. Geographic localization of the Pará State, Amazon Region of Brazil.

Clinical and radiographic analysis - Pulmonary symptoms that include nodular or cavitary opacities on chest radiograph; multifocal bronchiectasis with multiple small nodules on a high resolution computed tomography (HRCT) scan; lack of abnormalities suggestive for other disease.
Microbiologic analysis - Positive culture from at least two separate expectorated sputum samples, when initial sputum samples are AFB negative, consider repeated sputum AFB smears and cultures or positive culture results from at least one bronchial wash or lavage
Histopathologic analysis - Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM.

Table 1. American Thoracic Society diagnostic criteria on NTM pulmonary disease (Griffith ei al., 2007).

2.2 *Mycobacterium* cultures and isolates

Pulmonary specimens were decontaminated using the N-acetyl-L-cysteine-sodium hydroxide procedure (Webb, 1962; Brasil, 2008), inoculated into Lowenstein-Jensen (LJ) medium (Difco, France) and incubated at 35 to 37°C in the absence of light for at least six weeks or until colonies appeared. Conventional procedures for distinguishing between organisms of the MTBC and of the NTM group included macroscopic analysis of aspect of colonies, which MTBC have a rough aspect resemble breadcrumbs or cauliflowers, detection of cord factor from MTBC by Ziehl-Neelsen stain, and the growth inhibition test in medium containing 0.5 mg/mL para-nitrobenzoic acid, a specific inhibitor of MTBC, all according to Kubica (1973).

2.3 Sequence analysis and phylogenetic analysis

Sequencing of part of the 16S ribosomal RNA (16S rRNA) and 65-kilodalton heat shock protein (*hsp65*) genes was performed as described by previous publications (Kim et al., 2005; Shin et al., 2006). After verification of PCR products on agarose gel Seakem LE 1% (Cambrex, United Kingdom), these were purified using the SNAP TM gel purification kit (Invitrogen). The amplified products were direct sequenced by using both forward and reverse primers of each system and the BigDye Terminator v3.1 cycle sequencing kits (Applied Biosystems, Foster City, CA) and analyzed on an ABI3130 sequencer (Applied Biosystems, Tokyo, Japan).

The 16S rRNA and *hsp65* sequences were aligned using the multiple-alignment algorithm of the Bioedit software (version 7.0.9; Tom Hall [http://www.mbio.ncsu.edu/BioEdit/ bioedit.html]) with the closest relatives retrieved from the GenBank database across of the Basic Local Alignment Search Tool (BLAST, URL: http://www.ncbi.nlm.nih.gov/ BLAST/) and the Ribosomal Differentiation of Medical Microorganisms RIDOM database (RIDOM, URL: http://rdna.ridom.de/). Phylogenetic trees were constructed from the presently-defined 16S rRNA or *hsp65* sequences separately using the neighbor-joining algorithm, including sequences of a selection of NTM-type strains, retrieved from GenBank (accession numbers in parenthesis next to the species names in Figs.3 and 4). For this, we used the Kimura's 2-parameter distance correction model and MEGA software (Version 4.0; Tamura et al. [http://www.megasoftware.net/]). Bootstrap analysis (1,000 repeats) was applied using the *Tsukamurela paurometabola* (KCTC 9821) sequences as an out-group. The GenBank accession numbers for the *Mycobacterium* sequences determined in this study included the following: FJ590454-FJ590472, HM056080- HM056113 for the 16S rRNA, and FJ536235- FJ536253, HM056114- HM056147 for the *hsp65* gene.

2.4 Statistical analysis

Statistical data were derived by using the nonparametric chi-square test and the Fisher exact test, where appropriate. P values less than 0.05 was considered significant. Statistical analysis was performed with the BioEstat software (version 5.0; Ayres et al. [http: www.mamiraua.org.br]).

3. Results

3.1 Patients and NTM isolates

Between 1999 and 2010, *Mycobacterium* isolates were recovered from respiratory specimens of 1,580 patients, that were suspected of having pulmonary TB. Among these, 92% (1,453 cases) were infected with MTBC; from the rest (8%, 128 patients) we obtained 249 NTM isolates. Among the NTM-positive patients studied, 57.5% (n=73) presented at least two positive sputum cultures for the same species, or presented at least one bronchial wash positive culture and suffered therefore from infections as defined by the criteria of ATS (Griffith et al., 2007). The clinical significance of NTM pulmonary isolation among 1999-2010 is shown in Fig.2.

The remaining 55 patients could not be confirmed to suffer from NMT infection because (i) only a single sputum sample was collected and delivered to the laboratory (47 patients); (ii)

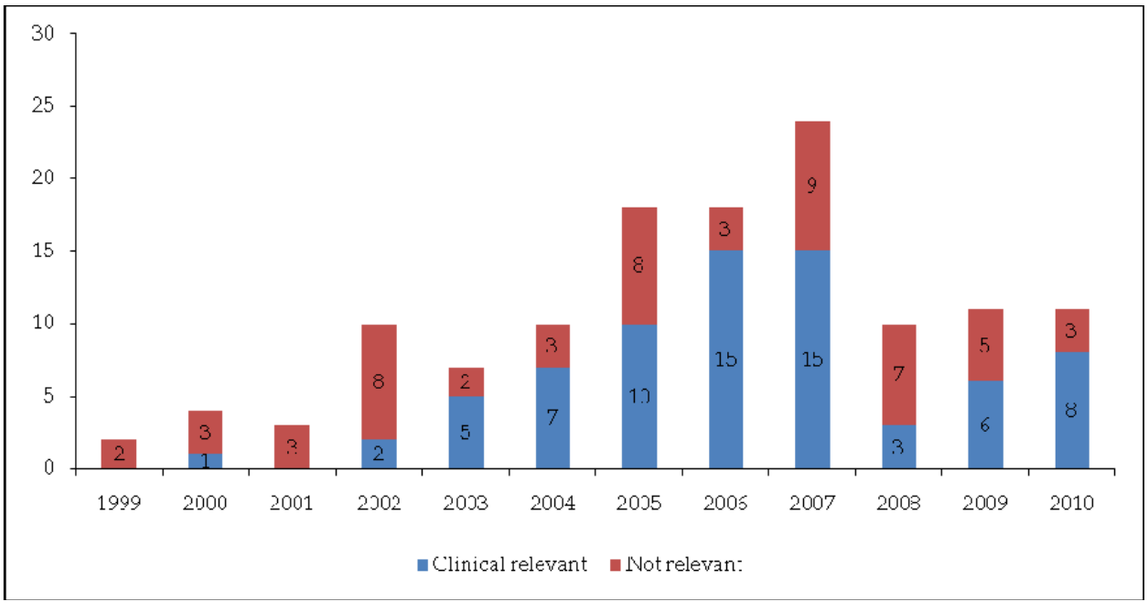


Fig. 2. Frequency of NTM isolation from clinical pulmonary specimens of patients from Pará State, Brazil, 1999-2010.

one sample was culture-positive and the others were culture-negative (five cases) or (iii) NTM were found in some patients who were also TB-positive (three patients). Distribution of species according clinical relevance and years of isolation is show in Tab.2 and Fig.3.

Species	Clinical relevant		Not relevant		Total	
	Patients	Isolates	Patients	Isolates	Patients	Isolates
M. massiliense	20	39	8	8	28	47
M. simiae complex	14	44	5	5	19	49
M. intracellulare	11	32	6	6	17	38
M. avium	10	28	12	12	22	40
M. bolletii	4	14	0	0	4	14
M. abscessus	3	8	1	1	4	9
M. colombiense	3	9	2	2	5	11
M. kansasii	2	3	0	0	2	3
M. simiae	2	4	1	1	3	5
M. fortuitum	1	6	18	18	19	24
M. scrofulaceum	1	3	0	0	1	3
M. szulgai	1	2	0	0	1	2
M. terrae	1	2	0	0	1	2
M. parascrofulaceum	0	0	1	1	1	1
M. smegmatis	0	0	1	1	1	1
Total	73	194	55	55	128	249

Table 2. Clinical significance of NTM isolated in Pará State, Brazil, 1999-2010.

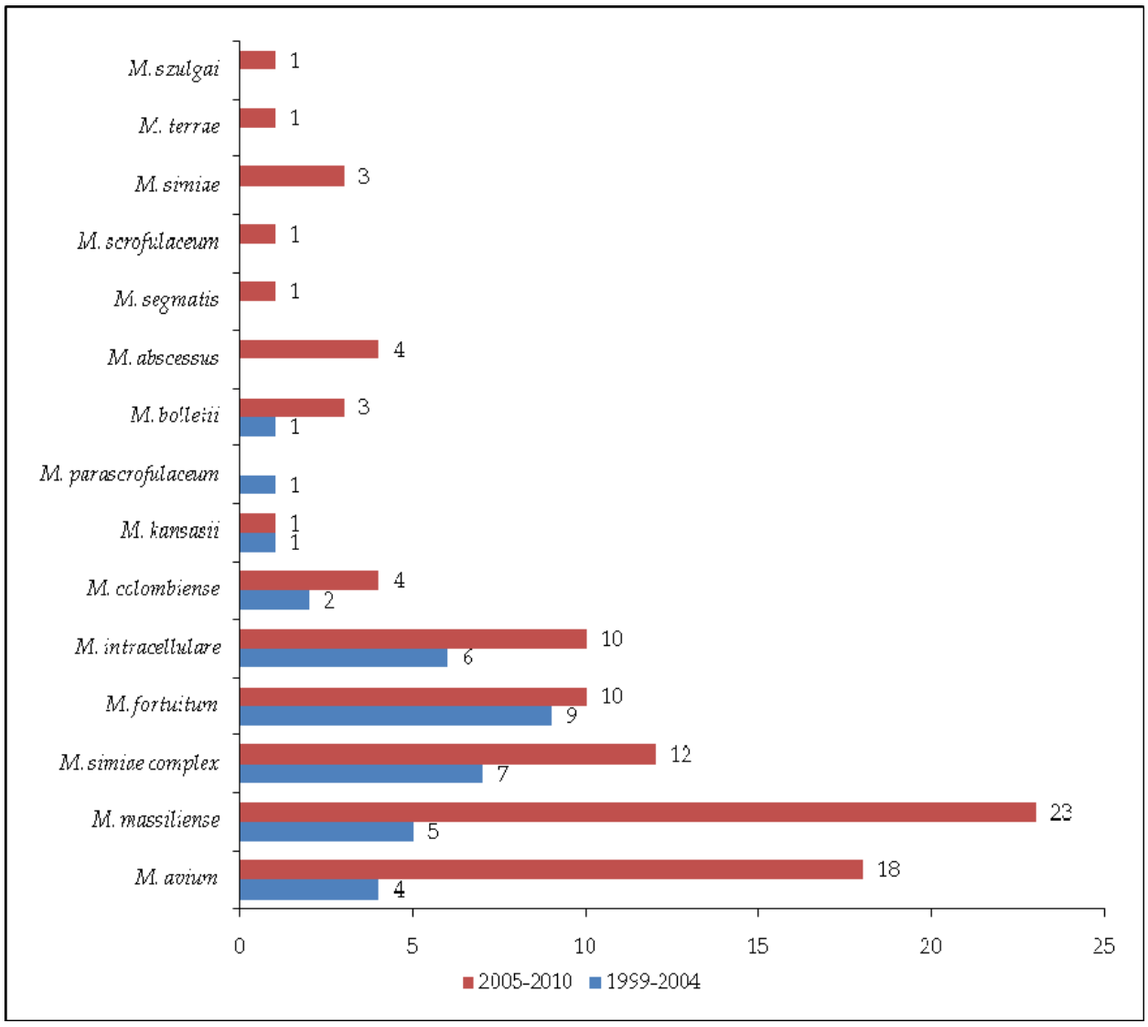


Fig. 3. Frequency of isolation of nontuberculous *Mycobacterium* species between the 1999-2003 and 2004-2010 periods in Pará State, Brazil.

Among the 73 patients with bacteriological ATS criteria for NMT infection, 64.4% (n=47; p = 0,03) were females and more detailed analysis of their treatment history revealed that 72 had previously been unsuccessfully treated for TB, using the first-line multidrug therapy scheme; one patient had been diagnosed as suffering from allergic bronchitis and therefore submitted to corticosteroid therapy. After confirmation of NTM infection, 70 patients were submitted to a daily regimen of clarithromycin (500-1,000 mg) and ethambutol (25 mg/kg) for 12 months. No therapy information was available for the patient infected with *M. fortuitum* and for the two cases with *M. kansasii*. Treatment outcome was not available for all cases but patients infected with members of the *M. simiae* complex did not present clinical improvement and at the end of our study period, one had died due to progression of disease.

All of the patients described above presented respiratory complaints consistent with TB while additional symptoms were observed (Table 3). Bronchiectasis sequelae occasionally associated with hemoptysis in patients infected with *M. abscessus* (n=1), *M. bolletii* (n=2), *M.*

Species	Gender		Clinical characteristics	Total
	Male	Female		
M. massiliense	6	14	chronic cough (20); sputum (20); chest pain (20); hemoptysis (5); dyspnea; loss weight (3)	20
M. simiae complex	4	10	chronic cough (14); sputum (14); chest pain (14) weight loss (14); fever (3); hemoptysis (10); malaise (14); dyspnea (3); down syndrome (1); fatigue (2)	14
M. intracellulare	5	6	chronic cough (11); sputum (11); chest pain (3)	11
M. avium	2	8	chronic cough (10); sputum (10); chest pain (10); HIV (1); gastroesophageal reflux disease (1)	10
M. bolletii	3	1	chronic cough (4); hemoptysis (2); decreased lung volume (1); fever (1); loss weight (1)	4
M. abscessus	1	2	chronic cough (3); sputum (3); hemoptysis (1); dyspnea (1); corticosteroid-immunosuppressed (1)	3
M. colombiense	2	1	chronic cough (3); chest pain (3)	3
M. kansasii	1	1	chronic cough (2); chest pain (2)	2
M. simiae	0	2	chronic cough (2); chest pain (2)	2
M. fortuitum	1	0	chronic cough (1); chest pain (1)	1
M. scrofulaceum	0	1	chronic cough (1); chest pain (1)	1
M. szulgai	0	1	chronic cough (1); chest pain (1)	1
M. terrae	1	0	chronic cough (1); chest pain (1)	1

Table 3. Clinical characteristics of patients with NTM pulmonary infection from Pará State, Brazil, 1999-2010.

massiliense (*n*=5) and *M. simiae* complex (*n*=10) while chronic cough was observed among patients, independent of the infecting *Mycobacterium* species. The interval between the onset of signs and symptoms and a definitive diagnosis of NTM infection was greater than 12 months, being more pronounced in cases with *M. simiae* complex isolates, reporting the presence of symptoms for at least 24 months.

3.2 NTM Identification on the genetic level

Based on 16S rRNA gene analysis, the majority of the NTM species isolated from patients could be grouped into three clades, containing sequences from either *M. avium*, *M. chelonae* or *M. simiae* complexes (Fig. 4).

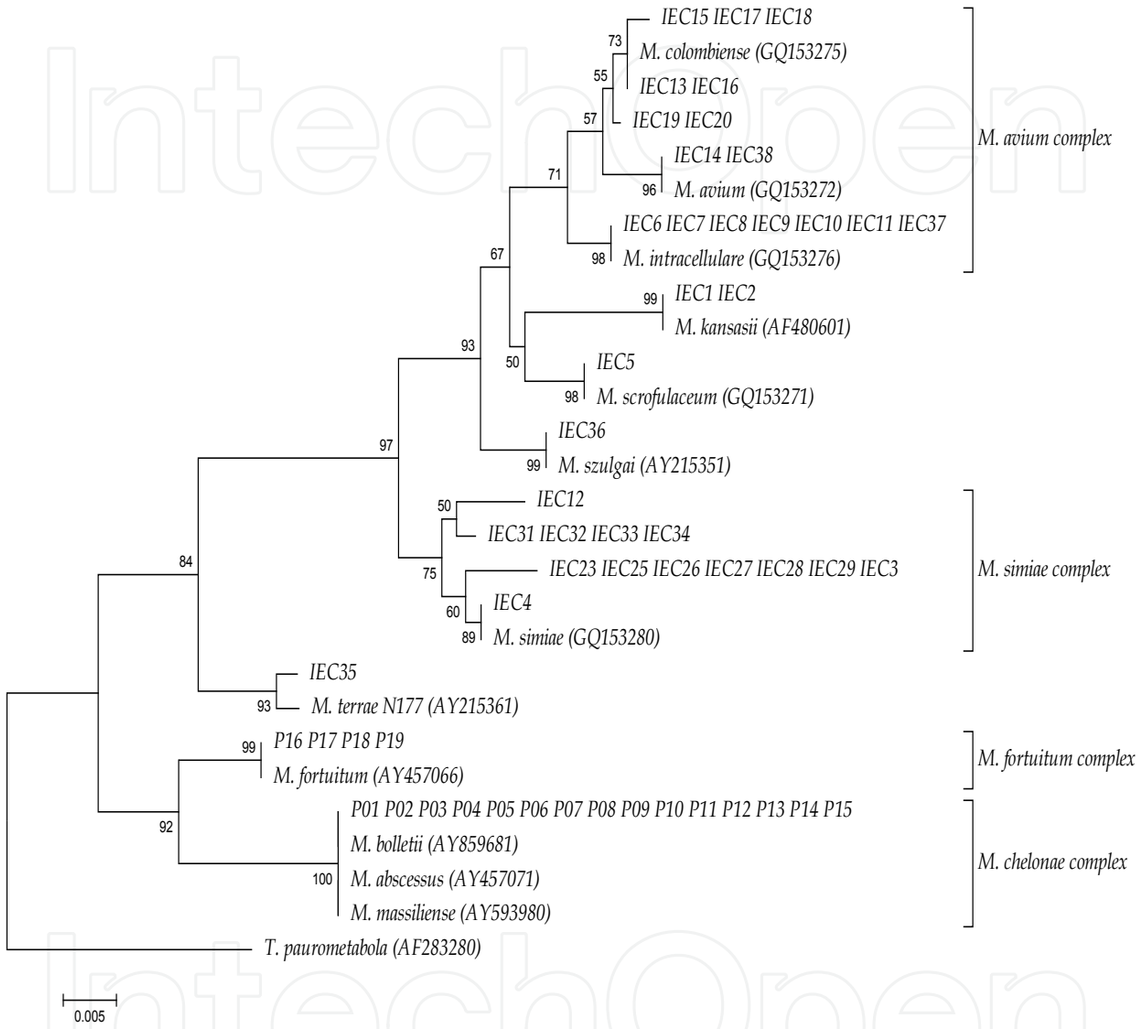


Fig. 4. Relationships between sequences from the type strains and the NTM isolated presently, inferred from partial 16S rRNA gene. Phylogenetic tree was constructed by neighbor-joining method and Kimura's 2-parameter distance correction model. The support of each branch, as determined from 1000 bootstrap samples, is indicated by the value at each node (as a percentage). *T. paurometabola* KCTC 9821 was used as outgroup.

Upon sequence analysis of part of the *hsp65* gene, we observed a higher genetic diversity than that of the 16S rRNA gene; nonetheless, the phylogenetic tree based on *hsp65* gene sequence analysis had the same global topology as that based on 16S rRNA gene (Fig. 5).

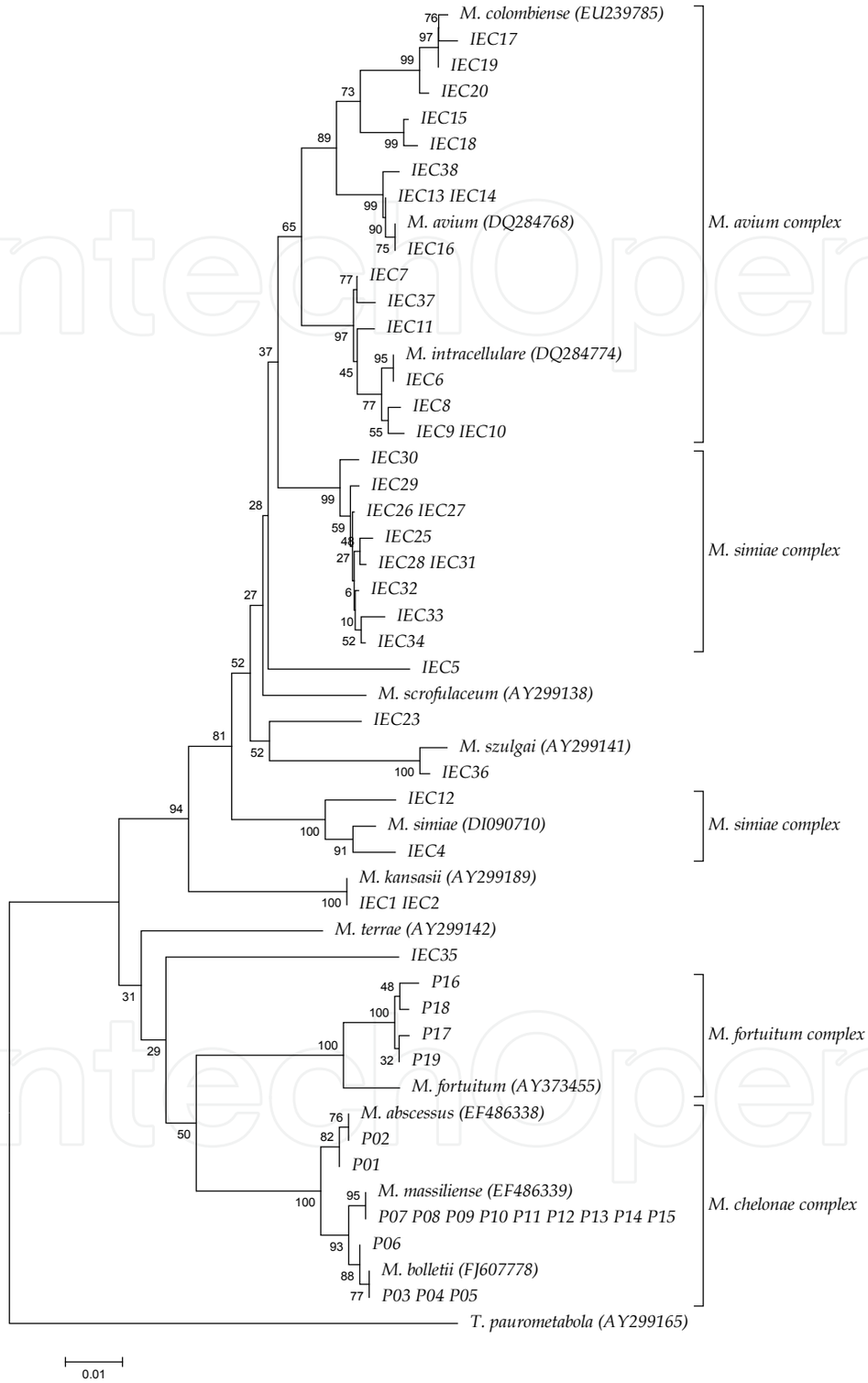


Fig. 5. Relationships between sequences from the type strains and the NMT isolated presentlty, as inferred from partial *hsp65* sequences. Phylogenetic tree was constructed as described above.

Among the 249 infectious isolates, 200 NMT sequences, derived from 108 patients, had already been described elsewhere and characteristic for 14 NMT species, the other 49 sequences derived from 19 cases were unpublished in public databases and all phylogenetically classified into the *M. simiae* complex.

4. Discussion

This study demonstrates that among 1,453 cases that were diagnosed between 1999 and 2010 as suffering from pulmonary TB, presence of NTM was observed in 128 (8%) of these and infection with such species proven to cause disease in 73 cases (5%). It was observed a steady increase in the number of NTM isolates during the study period, which was more pronounced from 2004 on, when an increase in the demand of culture for AFB at the Evandro Chagas Institute was the case. This latter could be due either to the increase of the physicians' sensitivity to occurrence of NTM infections in this region and/or to an increase of infection with NMT of the population.

In this study, significantly more females were infected with NTM, and this is contrary to most published data, presenting males as the major risk group for pulmonary NMT disease (Marras & Daley, 2002). However, some recent reports also demonstrated a female predominance (Freeman et al., 2007; Cassid et al., 2009; Prevot et al., 2010; Wintrop et al., 2010), in concordance with the recent data of Chan & Iseman (2010), describe a higher immune susceptibility of women to NTM pulmonary disease. In addition, when stratifying to the NMT species level, it was observed that gender associated infection was even more pronounced in the case of *M. massiliense* (70% females), *M. simiae* complex (71%) and *M. avium* (66%). Griffith et al. (2003) found a predominance of females (65%) among 154 cases of pulmonary disease by RGM, while descriptions of particular forms of pulmonary disease caused by MAC in women have been reported (Wallace, 1994; Reich & Johnson, 1995). Further studies are needed to elucidate the reasons for female susceptibility. Roughly 40% (n=55) of the patients with NTM-positive cultures did not meet the diagnostic criteria for NTM pulmonary infection but this does not necessarily mean that the presence of NMT is not the cause of disease. Unfortunately, due to lack of follow-up of patients, it cannot confirm this presently. There is little known about the pathophysiology of NTM-related lung disease what makes it difficult to be certain that colonization is not an indolent or even a slowly-progressive infection. Therefore, such cases need to remain under observation and seek expert consultation (Griffith et al., 2007).

Among the cases with confirmation of NMT infection as a cause of disease, mostly, previous diagnosis and treatment of TB was observed, none demonstrating improvement following treatment. Misdiagnosis of NTM infections as caused by members of the MTBC leads to unsuccessful treatment with anti-TB drugs and because clinicians experiment with various TB therapies without considering a culture-based test, there is a considerable delay in detection of NTM. This is even more a matter of concern in high prevalence countries of TB such as Brazil, where mostly, symptomatic patients with sputum smear positive for acid-fast bacilli are treated with anti-TB drugs without being testing for NTM-related disease, except when co-infected with HIV (Brasil, 2005).

In Brazil, it was common until 2009, to start second line treatment without performing culture test for NTM, when no improvement during the first round of TB treatment was

observed. The high level of pulmonary NTM that was misdiagnosed as TB strongly suggests the need for a different strategy of TB control in the state of Para.

In most countries, NTM-related disease, unlike TB, do not need to be reported unless they are healthcare-associated infections. Therefore, information on the frequency and diversity of NTM infections are usually obtained from laboratory records and surveillance studies (Marras et al., 2007; Parrish et al., 2008). To determine the true epidemiological status of NTM pulmonary disease, well designed population-based studies are needed. However, the financial burden on public health care system in developing countries makes it difficult to perform such surveillance studies. Therefore, laboratory procedures such as the introduction of both liquid and solid culture systems and use of molecular methods such as PCR restriction analysis (PRA) in reference laboratories could be an alternative for more knowledge and improvement of diagnose accuracy in those regions.

Sequence analysis has contributed to the recent description of several new *Mycobacterium* species and more precise identification and taxonomy of members of this genus. Genotypic taxonomy is typically based on the detection of highly conserved regions within the genome that harbor hypervariable sequences in which species-specific deletions, insertions, or replacements of single nucleotides are present in 16S rRNA, *hsp65* gene and more recently on a fragment of the gene coding for the beta sub-unit of RNA polymerase (*rpoB*) are also contributing to this field, mostly for RGM (da Costa et al., 2009; da Costa et al, 2010). Several amplification molecular methods, have been proposed to correct NTM identification, including specific DNA probes (AccuProbe: GenProbe, Inc., San Diego, CA, U.S.A) and PRA method based on 16S rRNA (Domenech et al., 1994), 16S-23S rRNA internal transcribed spacer (ITS) (Roth et al., 1998), *hsp65* (Telenti et al., 1993), *rpoB* (Lee et al., 2000), cold-shock protein gene (*dnaJ*) (Takewaki et al., 1994), DNA repair protein gene (*recA*) (Blackwood et al., 2000) and elongation factor Tu gene (*tuf*) (Shin et al., 2009), but all have limitations as the variety of mycobacteria to be identified (da Costa et al., 2010a, b).

Based on the 16S rRNA and *hsp65* nucleotide sequences, we observed that the most frequent NTM isolates from our pulmonary samples were those of the *M. avium*, *M. chelonae* and *M. simiae* complexes. The most common NTM were *M. massiliense*, *M. intracellulare*, followed by *Mycobacterium* sp. from *M. simiae* complex. When compared with reports on NMT infections observed in other studies reported on Brazilian NTM cases, the species diversity and frequency is quite particular to the Para State, suggesting that environmental characteristics as temperature, pH and substrate composition may influence the geographical distribution of species. Our findings are in concordance with the fact that isolates of the *M. simiae* complex are rarely observed in other regions of Brazil.

There are few publications describing NTM in the Amazon region or Brazil. Barreto and Campos (2000) found 35 patients with NTM and showed that isolates of the *M. avium* complex, *M. terrae* and *M. fortuitum* were most common in samples collected between 1994 and 1999 in North of Brazil. A study that evaluated respiratory samples of non-indigenous and indigenous patients from Amazonas State with suspected pulmonary TB identified 19 patients with NTM infection, but the study did not report the identity of the isolates at the species level (Santos et al., 2006). A recent study by da Costa et al. (2009), showed that the *M. chelonae* complex, which includes the *M. massiliense* species, is the most frequent cause of pulmonary infections by RGM in Pará State, Amazon region of Brazil, similar to our

observations. Unlikely, in Brazilian southeast, *M. kansasii* and *M. avium* represented the most frequent type of NTM associated with pulmonary infections between 1991 and 1997 in the state of São Paulo (Ueki et al., 2005; Zamarioli et al. 2008; Pedro et al., 2008).

In contrast to other parts of the world, the species variability found in the present study is different. In countries from Latin America like Colombia, MAC, *M. chelonae* and *M. fortuitum* were the NTM isolated with more frequency (León, 1998), while MAC was most frequently isolated from argentinian HIV patients (Di Lonardo, 1995). MAC and *M. kansasii* were predominant in North America, some countries of Europe and South Africa (Griffith et al., 2007). In Asia, MAC, *M. abscessus* and *M. chelonae* were frequently isolated from pulmonary samples (Simons et al., 2011). The knowledge on diversity and epidemiology of species NTM associated to pulmonary in specific region is important because either: (i) it allows the adequate choice of laboratory methods for diagnosis (ii) it allows to recognize the species associated to disease; and (iii) it supplies information that will serve to improve the organization of health service net to attend these patients.

Perhaps the most important finding of this study was the identification of *M. simiae* complex members as the predominant cause of pulmonary infections. In fact, roughly 20% (*n*=16) of the pulmonary infections were caused by members of the *M. simiae* complex and among these, 14 belonged to an unidentified taxon (n=14). Currently, this taxonomic group is made up of 17 species including *M. simiae, M. genavense, M. intermedium, M. interjectum, M. lentiflavum, M. triplex, M. heidelbergense, M. kubicae, M. palustre, M. montefiorense, M. florentinum, M. sherrisii, M. parmense, M. parascrofulaceum, M. saskatchewanense*, *M. stomatepiae* and *M. europaeum* (Tortoli, 2003, 2006; Tortoli et al., 2010). However, among these species, only *M. simiae* is recognized as a real cause of pulmonary infections, as reported in areas such as the Southwest of the United States, Israel and Cuba (Griffith et al., 2007). It is estimated that 9 to 21% of the *M. simiae* isolates from pulmonary specimens have clinical relevance (Rynkiewicz et al. 1998). The findings of this study suggest that members of this group may have pathogenic potential, but further studies are required to assess the characteristics of these isolates, including details on predisposing conditions from patients, as well as the drug susceptibility these NTM.

5. Conclusion

In conclusion, although our study is not necessarily representative for the whole Amazon region, it clearly demonstrates the importance of NTM pulmonary infections in this region. Our data also show that a variety of NTM species are involved, and that there is need for bacteriologic diagnosis in patients with TB, especially in patients who have failed TB treatment. We have shown that the lack of species identification in a significant subset (8.0%) of patients with a presumptive diagnosis of TB in a regional reference center can lead to misdiagnosis and may be followed by inadequate treatment.

6. Acknowledgment

This work was supported by the Fundação de Amparo à Pesquisa do Estado do Pará, the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, the Conselho Nacional de Desenvolvimento Científico e Tecnológico and the Evandro Chagas Institute, Ananindeua, Pará, Brazil.

7. References

Adékambi T, Drancourt M (2004) Dissection of phylogenetic relationships among 19 rapidly growing *Mycobacterium* species by 16S rRNA, *hsp65*, *sodA*, *recA* and *rpoB* gene sequencing. *Int J Syst Evol Microbiol* 54(Pt 6):2095-105. ISSN 1466-5034.

American Thoracic Society (1997) Diagnosis and treatment of disease caused by nontuberculous mycobacteria. This official statement of the American Thoracic Society was approved by the Board of Directors, March 1997. Medical Section of the American Lung Association. *Am J Respir Crit Care Med* 156(2 Pt 2):S1-25. ISSN 1073-449X.

Barreto AMW, Campos CED (2000) Micobactérias não-tuberculosas no Brasil. *Bol Pneum Sanit* 8(1):23-32. ISSN 0103-460X.

Billinger ME, Olivier KN, Viboud C, de Oca RM, Steiner C, Holland SM, Prevots DR (2009) Non tuberculosis mycobacteria associated lung disease in hospitalized persons in unites states 1998-2005. Emerg Infect Dis 2009; 15(10): 1562-1569.

Blackwood KS, He C, Gunton J, Turenne CY, Wolfe J, Kabani AM (2000) Evaluation of *recA* sequences for identification of *Mycobacterium* species. *J Clin Microbiol* 38(8):2846-2852. ISSN 1098-660X.

Bodle EE, Cunningham JA, Della-Latta P, Schluger NW, Saiman L (2008) Epidemiology of Nontuberculous Mycobacteria in Patients Without HIV Infection, New York City. *Emerg Infect Dis* 14(3):390-396. ISSN 1080-6059.

Brasil (2005) *Tuberculose: guia de vigilância epidemiológica*. 6th ed. Brasília: Ministério da Saúde, pp. 732–756. Retrieved from http://bvsms.saude. gov/bvs/publicacoes/Guia_Vig_Epid_novo2.pdf

Brasil (2008) *Manual Nacional de Vigilância Laboratorial da Tuberculose e outras Micobactérias. Série A. Normas e Manuais Técnicos.* Brasília: Ministério da Saúde, Secretaria de Vigilância em Saúde 2008; pp.1-436. Retrieved from http://portal.saude.gov.br/portal/arquivos/pdf/manual_laboratorio_tb.pdf.(a)

British Thoracic Society (2000) Management of opportunist mycobacterial infections: Joint Tuberculosis Committee Guidelines 1999. Subcommittee of the Joint Tuberculosis Committee of the British Thoracic Society. *Thorax* 55:210-218. ISSN 1468-3296.

Brown-Elliott BA, Wallace RJ Jr (2002) Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. *Clin Microbiol Rev* 15(4):716-46. ISSN 0983-8512.

Cassidy PM, Hedberg K, Saulson A, McNelly E, Winthrop KL (2009) Nontuberculous mycobacterial disease prevalence and risk factors: a changing epidemiology. *Clin Infect Dis* 15;49(12):e124-9. ISSN 1058-4838.

Chan ED, Iseman MD (2010) Slender, older women appear to be more susceptible to nontuberculous mycobacterial lung disease. *Gend Med* 7(1):5-18.

da Costa ARF, Lopes ML, Bahia JRC, Conceição EC, Lima KVB (2010a) Identificação genotípica de membros do complexo *Mycobacterium avium* isolados de infecções pulmonares no Estado do Pará, Brasil. *Rev Pan-Amaz Saude* 1(3):35-42. ISSN 2176-6223.

da Costa ARF, Lopes ML, Furlaneto IP, Sousa MS, Lima KVB (2010b) Molecular identification of nontuberculous mycobacteria isolates in a Brazilian mycobacteria reference laboratory. *Diagn Microbiol Infect Dis* 68(4):390-394. ISSN 0732-8893.

da Costa ARF, Lopes ML, Leão SC, Schneider MPC, Sousa MS, Suffys PN, Corvelo TCO, Lima KVB (2009) Molecular identification of rapidly growing mycobacteria isolates from pulmonary specimens of patients in the State of Pará, Amazon region, Brazil. *Diagn Microbiol Infect Dis* 65(4):358-364. ISSN 0732-8893.

Di Lonardo M, Isola NC, Ambroggi M, Rybko A, Poggi S (1995) Mycobacteria in HIV-infected patients in Buenos Aires. *Tuber Lung Dis* 76(3):185-189. ISSN 0962-8479.

Domenech P, Menendez MC, Garcia MJ (1994) Restriction fragment length polymorphisms of 16S rRNA genes in the differentiation of fast-growing mycobacterial species. *FEMS Microbiol Let* 116(1):19-24. ISSN 0378-1097.

Euzéby JP (1997) List of bacterial names with standing in nomenclature: a folder available on the Internet. *Int J Syst Bacteriol* 47:590–592 List of prokaryotic names with standing in nomenclature. Last full update: July 09, 2011. URL: http://www.bacterio.cict.fr/m/mycobacterium.html.

Falkinham JO 3rd (2002) Nontuberculous mycobacteria in the environment. *Clin Chest Med* 23:529-551. ISSN 1557-8216

Falkinham JO 3rd (2009) Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment. *J Appl Microbiol*107:356-367. ISSN 1364-5072.

Freeman J, Morris A, Blackmore T, Hammer D, Munroe S, McKnight L (2007) Incidence of nontuberculous mycobacterial disease in New Zealand, 2004. *N Z Med J.* 15;120(1256):U2580. ISSN 0028-8446.

Glassroth J (2008) Pulmonary disease due to nontuberculous mycobacteria. *Chest* 133(1):243-251. ISSN 0012-3692.

Good RC (1980) From the Center for Disease Control. Isolation of nontuberculous mycobacteria in the United States, 1979. *J Infect Dis* 142:779-783. ISSN 1537-6613.

Gopinath K, Singh S (2010) Non-tuberculous mycobacteria in TB-endemic countries: are we neglecting the danger? *PLoS Negl Trop Dis* 4:e615, doi:10.1371/ journal.pntd.0000615. ISSN 1935-2735.

Griffith DE (2010) Nontuberculous mycobacterial lung disease. *Curr Opin Infect Dis* 23(2):185–190. ISSN 1473-6527.

Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, Fordham von Reyn C, Wallace Jr RJ, Winthrop K. American Thoracic Society (2007) An Official ATS/IDSA Statement: Diagnosis, Treatment, and Prevention of Nontuberculous Mycobactecterial Diseases. *Am J Respir Crit Care Med* 175:367-416. ISSN 1073-449X.

Griffith DE, Girard WM, Wallace RJ Jr (1993) Clinical features of pulmonary disease caused by rapidly growing mycobacteria. An analysis of 154 patients. *Am Rev Respir Dis* 147(5):1271-1278. ISSN 0003-0805.

Griffith DE (2007) Impact of new American Thoracic Society diagnostic criteria on management of nontuberculous mycobacterial infection. *Am J Respir Crit Care Med* 176:419. ISSN 1073-449X.

Iseman MD, Marras TK (2008) The importance of nontuberculous mycobacterial lung disease. *Am J Respir Crit Care Med.* 2008 15;178(10):999-1000. ISSN 1073-449X.

Jarzembowski JA, Young MB (2008) Nontuberculous mycobacterial infections. *Arch Pathol Lab Med* 132(8):1333-1341. ISSN 1543-2165.

Jeong YJ, Lee KS, Koh WJ, Han J, Kim TS, Kwon OJ (2004) Nontuberculous mycobacterial pulmonary infection in immunocompetent patients: comparison of thin-section CT and histopathologic findings. *Radiology* 231(3):880-886. ISSN 1527- 1315.

Katoch VM (2004) Infections due to non-tuberculous mycobacteria (NTM). *Indian J Med Res* 120(4):290-304. ISSN 0971-5916.

Kendall BA, Varley CD, Choi D, Cassidy PM, Hedberg K, Ware MA, Winthrop KL (2011) Distinguishing tuberculosis from nontuberculous mycobacteria lung disease, Oregon, USA. *Emerg Infect Dis* 17(3):506-9. ISSN 1080-6059.

Kim H, Kim SH, Shim TS, Kim M, Bai GH, Park YG, Lee SH, Chae GT, Cha CY, Kook YH, Kim BJ (2005) Differentiation of *Mycobacterium* species by analysis of the heat-shock protein 65 gene (*hsp65*). *Int J Syst Evol Microbiol* 55:1649–1656. ISSN 1466-5034.

Kobashi Y, Matsushima T (2007) The microbiological and clinical effects of combined therapy according to guidelines on the treatment of pulmonary *Mycobacterium avium* complex disease in Japan – including a follow-up study. *Respiration* 74:394–400. ISSN 1423-0356.

Koh WJ, Kwon OJ, Lee KS (2005) Diagnosis and treatment of nontuberculous mycobacterial pulmonary diseases: a Korean perspective. *J Korean Med Sci* 20:913–925. ISSN 1011-8934.

Kubica GP (1973) Differential identification of mycobacteria. *Am Rev Resp Dis* 107: 9-12. ISSN: 0003-0805.

Lee H, Park HJ, Cho SN, Bai GH, Kim SJ (2000) Species identification of mycobacteria by PCR-restriction fragment length polymorphism of the *rpoB* gene. *J Clin Microbiol* 38(8):2966-2971. ISSN 1098-660X.

León CI (1998) Presencia de las micobacterias no tuberculosas en Colombia. *Médicas UIS* 12: 181-187. ISSN 1794-5240.

Marras TK, Chedore P; Ying AM, Jamieson F (2007) Isolation prevalence of pulmonary non-tuberculous mycobacteria in Ontario, 1997–2003. *Thorax* 62:661-666. ISSN 1468-3296.

Marras TK, Daley CL (2002) Epidemiology of human pulmonary infection with nontuberculous mycobacteria. *Clin Chest Med* 23:553–567. ISSN 1557-8216.

Martin A, Uwizeye C, Fissette K, De Rijk P, Palomino JC, Leão S, Portaels PC, Scarparo C (2009) Extrapulmonary infections associated with nontuberculous mycobacteria in immunocompetent persons. *Emerg Infect Dis* 15:1351–1358. ISSN 1080-6059.

Parrish SC, Myers J, Lazarus A (2008) Nontuberculous mycobacterial pulmonary infections in Non-HIV patients. *Postgrad Med* 120(4):78-86. ISSN 0032-5481.

Pedro HSP, Pereira MIF, Goloni MRA, Ueki SYM, Chimara E (2008) Isolamento de micobactérias não-tuberculosas em São José do Rio Preto entre 1996 e 2005. *J Bras Pneumol* 34(11):950-955. ISSN 1806-3713.

Prevots DR, Shaw PA, Strickland D, Jackson LA, Raebel MA, Blosky MA, Montes de Oca R, Shea YR, Seitz AE, Holland SM, Olivier KN (2010) Nontuberculous mycobacterial lung disease prevalence at four integrated healthcare delivery systems. *AM J Respir Crit Care Med* 182:970-976. ISSN 1073-449X.

Reich JM, Johnson RE (1995) Mycobacterium avium complex lung disease in women. *Chest* 107(1):293-295. ISSN 1931-3543.

Roth A, Fischer M, Hamid ME, Michalke S, Ludwig W, Mauch H (1998) Differentiation of phylogenetically related slowly growing mycobacteria based on 16S-23S rRNA gene internal transcribed spacer sequences. *J Clin Microbiol* 36(1):139-147. ISSN 1098-660X.

Runyon EH (1959) Anonymous mycobacteria in pulmonary disease. *Med Clin North Am*. 43(1):273-290. ISSN 1557-9859.

Rynkiewicz DL, Cage GD, Butler WR, Ampel NM (1998) Clinical and microbiological assessment of *Mycobacterium simiae* isolates from a single laboratory in southern Arizona. *Clin Infect Dis* 26(3):625-30. ISSN 1058-4838.

Santos RMC, Ogusku MM, Miranda JM, Dos Santos MC, Salem JI (2006) Avaliação da reação em cadeia da polimerase no diagnóstico da tuberculose pulmonar em pacientes indígenas e não indígenas. *J Bras Pneumol* 32(3):234-240. ISSN 1806-3713.

Sexton P, Harrison AC (2008) Susceptibility to nontuberculous mycobacterial lung disease. *Eur Respir J* 31: 1322–1333. ISSN 1399-3003.

Shin S, Kim EC, Yoon JH (2006) Identification of nontuberculous mycobacteria by sequence analysis of the 16S ribosomal RNA, the heat shock protein 65 and the RNA polymerase β-subunit genes. *Korean J Lab Med* 26:153–160. ISSN 1598-6535.

Shin JH, Cho EJ, Lee JY, Yu JY, Kang YH (2009) Novel diagnostic algorithm using *tuf* gene amplification and restriction fragment length polymorphism is promising tool for identification of nontuberculous mycobacteria. *J Microbiol Biotechnol* 19(3):323-330. ISSN 1017-7825.

Simons S, van Ingen J, Hsueh PR, Van Hung N, Dekhuijzen PN, Boeree MJ, van Soolingen D (2011) Nontuberculous mycobacteria in respiratory tract infections, eastern Asia. *Emerg Infect Dis* 17(3):343-349. ISSN 1080-6059.

Takewaki S, Okuzumi K, Manabe I, Tanimura M, Miyamura K, Nakahara K, Yazaki Y, Ohkubo A, Nagai R (1994) Nucleotide sequence comparison of the mycobacterial *dnaJ* gene and PCR-restriction fragment length polymorphism analysis for identification of mycobacterial species. *Int J Syst Bacteriol* 44(1):159-166. ISSN 0020-7713.

Telenti A, Marchesi F, Balz M, Bally F, Böttger EC, Bodmer T (1993) Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. *J Clin Microbiol* 31:175–178. ISSN 1098-660X.

Tortoli E (2003) Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. *Clin Microbiol Rev* 16:319–354. ISSN 1098-6618.

Tortoli E (2006) The new mycobacteria: an update. *FEMS Immunol Med Microbiol* 48(2):159-178. ISSN 1574-695X.

Tortoli E (2009) Clinical manifestations of nontuberculous mycobacteria infections. *Clin Microbiol Infect* 15(10):906-10. ISSN 1469-0691.

Tortoli E, Böttger EC, Fabio A, Falsen E, Gitti Z, Grottola A, Klenk HP, Mannino R, Mariottini A, Messinò M, Pecorari M, Rumpianesi F (2010) *Mycobacterium europaeum* sp. nov., a scotochromogenic species related to *Mycobacterium simiae* complex. *Int J Syst Evol Microbiol*. ijs.0.025601-0v1-ijs.0.025601-0. ISSN 1466-5034.

Tortoli E, Rindi L, Garcia MJ, Chiaradonna P, Dei R, Garzelli C, et al (2004) Proposal to elevate the genetic variant MAC-A, included in the *Mycobacterium avium* complex, to species rank as *Mycobacterium chimaera* sp. nov. *Int J Syst Evol Microbiol* 54:1277-1285. ISSN 1466-5034.

Tsukamura M, Kita N, Shimoide H, Arakawa H, Kuze A (1988). Studies on the epidemiology of nontuberculous mycobacteriosis in Japan. *Am Rev Respir Dis* 137: 1280-1284. ISSN 0003-0805.

Ueki SYM, Telles MAS, Virgilio MC, Giampaglia CMS, Chimara E, Ferrazoli L (2005) Micobactérias não-tuberculosas: diversidade das espécies no estado de São Paulo. *J Bras Patol Med Lab* 41(1):1-8. ISSN 1676- 2444.

van Ingen J, Bendien SA, de Lange WC, Hoefsloot W, Dekhuijzen PN, Boeree MJ, van Soolingen D (2009) Clinical relevance of non-tuberculous mycobacteria isolated in the Nijmegen-Arnhem region, The Netherlands. *Thorax* 64:502–506. ISSN 1468-3296.

van Ingen J, Boeree MJ, de Lange WC, Dekhuijzen PN, van Soolingen D (2007) Impact of new American Thoracic Society diagnostic criteria on management of nontuberculous mycobacterial infection. *Am J Respir Crit Care Med* 176:418-419. ISSN 1073-449X

von Reyn CF, Waddell RD, Eaton T, Arbeit RD, Maslow JN, Barber TW, Brindle RJ, Gilks CF, Lumio J, Lähdevirta J, Ranki A, Dawson D, Falkinham JO 3rd (1993) Isolation of *Mycobacterium avium* complex from water in the United States, Finland, Zaire, and Kenya. *J Clin Microbiol* 1993;12:3227-3230. ISSN 1098-660X.

Wallace RJ Jr (1994) *Mycobacterium avium* complex lung disease and women. Now an equal opportunity disease. *Chest* 105(1):6-7. ISSN 1931-3543.

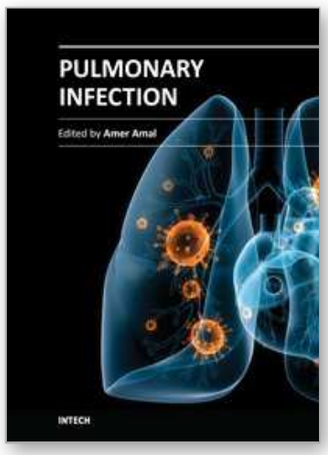
Webb, WR (1962) Clinical evaluation of a new mucolytic agent, acetylcysteine. *J. Thoracic Cardiovascular Surg.* 44:330-343. ISSN: 0022-5223.

Whipps CM, Butler WR, Pourahmad F, Watral VG, Kent ML (2007) Molecular systematics support the revival of *Mycobacterium salmoniphilum* (ex Ross 1960) sp. nov., nom. rev., a species closely related to *Mycobacterium chelonae*. *Int J Syst Evol Microbiol* 57(Pt 11):2525-31. ISSN 1466-5034.

Winthrop KL, McNelley E, Kendall B, Marshall-Olson A, Morris C, Cassidy M, Saulson A, Hedberg K (2010) Pulmonary nontuberculous mycobacterial disease prevalence

and clinical features: an emerging public health disease. *Am J Respir Crit Care Med* 1;182(7):977-82. ISSN 1073-449X.

Zamarioli LA, Coelho AGV, Pereira CM, Nascimento ACC, Ueki SYM, Chimara E (2008) Descriptive study of the frequency of nontuberculous mycobacteria in the Baixada Santista region of the state of São Paulo, Brazil. *J Bras Pneumol* 34(8):590-594. ISSN 1806-3713.



Pulmonary Infection
Edited by Dr. Amer Amal

ISBN 978-953-51-0286-1
Hard cover, 128 pages
Publisher InTech
Published online 14, March, 2012
Published in print edition March, 2012

Pulmonary infections are notorious in causing considerable morbidity and mortality. Caused by bacteria, viruses or fungi, respiratory infections require distinct knowledge of recent advances in pathogenesis. Progress in the understanding of immunopathogenesis of Acinetobacter baumannii infection will explain how an atypical organism establishes infection. The chapter regarding pulmonary nontuberculous mycobacterial infections in the State of Para depicts a unique study in an endemic region for tuberculosis in North of Brazil. The diagnosis and treatment of latent tuberculosis is a formidable challenge. Thus, new developments in diagnosis and treatment of latent tuberculosis are included in this book. Challenging in their diagnosis, nontuberculous mycobacterial pulmonary diseases require special education for management. The problems of respiratory infections in the immunocompromised host are increasing in numbers and in resilience to treatment. Therefore, the chapter describing the host immune responses against pulmonary fungal pathogens comes as a necessary section in this book. The insight brought forth from this book can be valuable for both clinicians and scientists.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen