Revista de Epidemiologia e Controle de Infecção

ORIGINAL ARTICLE

Piauí Central Laboratory of Public Health: frequency of mycobacterial species and epidemiological aspects, 2014-2015

Laboratorio Central de Salud Pública de Piauí: frecuencia de especies micobacterianas y aspectos epidemiológicos, 2014-2015

Laboratório Central de Saúde Pública do Piauí: frequência de espécies de micobactérias e aspectos epidemiológicos, 2014-2015

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ABSTRACT

Background and Objectives: Knowledge about species diversity of non-tuberculous mycobacteria (NTM) and the frequency of tuberculosis (TB) is an important issue in rural-urban regions such as Piauí (northeast of Brazil), of low incidence rate of TB , can help to improve diagnosis and prevention strategies. The aim of this study is to examine some epidemiological aspects and the frequency of *Mycobacterium tuberculosis* (Mtb) and NTM isolated at the central public health reference laboratory, Dr. Costa Alvarenga, Piauí (LACEN-PI). **Methods**: Data records of all mycobacterosis and tuberculosis cases from January 2014 to March 2015 were analyzed. **Results**: Of the 20% (142/706) positive growths, 70% (99) were Mtb and 10% NTM. Remaining clinical samples were inadequate, not allowing the identification of even the suspected NTM. The most frequent clinical form was pulmonary with TB patients younger than those infected with NTM (p = 0.001), the majority living in Teresina (52%). NTMs identified were *M. abscessus* (36%), *M. avium*, *M. intracellulare*, *Mycobacterium sp.* (14% each) and *M. asiaticum*, *M. szulgai*, *M. kansasii* 7% (each). Mtb drug resistance (7.8%) and TB co-infection with the human immunodeficiency virus (HIV-TB) found to be high (49%, 19/39). **Conclusion**: The frequencies of Mtb infection, drug resistance and HIV-TB co-infection are still underestimated and failures in the identification of NTM may decrease the actual frequency of these infections. Therefore, there is a need for improvements in TB control and in the diagnosis of NTMs in Piauí.

Keywords: Mycobacterium tuberculosis. Nontuberculous Mycobacteria. Drug resistance. AIDS Serodiagnosis, Health Services Research.

RESUMO

Justificativa e Objetivos: O conhecimento da diversidade de espécies de micobactérias não tuberculosas (MNT) e a frequência da tuberculose (TB) é uma questão importante em regiões rurais-urbanas como o Piauí (nordeste do Brasil), com baixa incidência de TB, pode ajudar a melhorar o diagnóstico e estratégias de prevenção. O objetivo deste estudo é examinar alguns aspectos epidemiológicos e a frequência de *Mycobacterium tuberculosis* (Mtb) e MNT isolados, no Laboratório Central de Referência em Saúde Pública, Dr. Costa Alvarenga, Piauí (LACEN-PI). **Métodos**: Dados de todos os casos de micobacterioses e tuberculose de janeiro de 2014 a março de 2015 foram analisados. **Resultados:** Dos 20% (142/706), de amostras com crescimento positivo 70% (99) foram Mtb e 10% MNT. O restante era de amostras clínicas inadequadas, não permitindo a identificação inclusive de MNT suspeitos. A forma clínica mais frequente foi pulmonar com pacientes TB mais jovens do que os infectados com MNT (p = 0,001), a maioria morando em Teresina (52%). As MNTs identificadas foram *M. abscessus* (36%), *M. avium*, *M. intracellulare*, *M. sp.* (14%, cada) e *M. asiaticum*, *M. szulgai* , *M. kansasii* 7% (cada). A droga resistência de Mtb (7,8%) e a co-infecção TB e vírus da imunodeficiência humana (HIV-TB) mostraram-se altas (49%, 19/39). **Conclusão:** As frequências de infecção por Mtb, de resistência a medicamentos e co-infecção HIV-TB ainda são subestimadas e as falhas na identificação de MNT podem diminuir a real frequência destas infecções. Portanto, há necessidade de melhorias no controle da TB e no diagnóstico de MNTs no Piauí.

Descritores: *Mycobacterium tuberculosis, Micobactérias não Tuberculosas, Resistência a Medicamentos, Sorodiagnóstico da AIDS, Pesquisa sobre Serviços de Saúde*

RESUMEN

Justificacion y objetivos: Conocer la diversidad de especies de micobacterias no tuberculosas (MNT) y la frecuencia de tuberculosis (TB) es tema importante en regiones rurales-urbanas como Piauí (noreste de Brasil) con baja tasa de incidencia de TB, y puede ayudar a mejorar el diagnóstico y las estrategias de prevenció. El objetivo de este estudio es examinar algunos aspectos epidemiológicos y la frecuencia de Mycobacterium tuberculosis (Mtb) y MNT aislado, en el laboratorio central de referencia de salud pública, Dr. Costa Alvarenga, Piauí (LACEN-PI). **Métodos**: Se analizaron los datos de todos los casos de micobacteriosis de enero de 2014 a marzo de 2015. **Resultados**: Del 20% (142/706), de las muestras con crecimiento positivo el 70% (99) fueron Mtb y el 10% MNT. El resto fue de muestras clínicas inadecuadas, no permitiendo la identificación de MNT incluso sospechosas. La forma clínica más frecuente fue la pulmonar y los pacientes con TB eran más jóvenes que los infectados con MNT (p = 0.001), la mayoría viviendo en Teresina (52%).Los MNT identificados fueron *M.abscessus* (36%), *M.avium*, *M.intracellulare*, *Mycobacterium sp.* (14% cada) y *M.asiaticum*, *M.szulgai*, *M.kansasii* 7% (cada). La resistencia a los medicamentos de Mtb (7,8%) y la coinfección de TB y el virus de la inmunodeficiencia humana (VIH-TB) fueron altas (49%, 19/39)**Conclusión**: Las frecuencias aún subestimadas de resistencia a los medicamentos, coinfección por VIH-TB y fallas de identificaciónidentificación de MNT pueden disminuir la frecuencia real de estas infecciones. Consecuentemente, es necesario mejorar el control y diagnóstico de TB y MNT en Piauí.

Palabras clave: *Mycobacterium tuberculosis, Micobacterias no Tuberculosas, Resistencia a Medicamentos, Serodiagnóstico del SIDA, Investigación sobre Servicios de Salud.*

INTRODUCTION

The importance and usefulness of identifying mycobacterium species, including criteria that may be shared between microbiologists and physicians, have a positive impact on treatment approaches. Although a notable improvement in treating and controlling mycobacterial infections in recent years, tuberculosis, caused by *Mycobacterium tuberculosis* bacilli (Mtb), remains among the highest mortality infectious disease.[1] On the other hand, the species of non-tuberculous mycobacteria (NTM), which can be an environmental opportunistic pathogen, or nonpathogenic in some cases, are increasing as the causal agent of pulmonary mycobacteriosis.[2] Roughly of the 180 NTM species already described one third can be pathogenic. Their frequency and etiopathogenesis still not well described in many geographic regions mainly in developing countries.[3] However, the notification conditions of NTM vary, while in countries of low tuberculosis incidence rate as Australia (5.7–7.7/100.000 inh) this is compulsory based on electronic and laboratory reports for NTM lung disease,[4] in the United States (2.6–3.5/100.000 inh) notification is extra pulmonary NTM.[5,6] In countries of higher tuberculosis incidence rate (34.8/100.000 inh) and treatment discontinuation (10.8%), such as Brazil,[7] NTM notification is not compulsory and available data associated with sporadic and occasional studies. A gap in knowledge about species diversity in NTM infections still persists, especially in regions of rural-urban areas that are common in the state of Piauí. In Piauí tuberculosis incidence rate is low (20.8/100.000 inh) compared to other units of the federation.[7] Therefore, the aim of the present

study is to examine the frequency of some epidemiological and laboratory aspects of Mtb and NTM isolates at referral Laboratório Central de Saúde Pública Dr. Costa Alvarenga, Piauí (LACEN-PI) in a 14-months period.

METHODS

A public laboratory LACEN-PI in Teresina, capital of Piauí, which conjointly the city of Parnaíba is a place of high population density. Linked to the State Department of Health, through the Superintendence of Health Surveillance meets several demands from the Regional Health acting in Epidemiological, Sanitary and Environmental Surveillance activities. The LACEN-PI receives clinical specimens from the state and occasionally neighboring states.

A cross-sectional documentary study carried out in the LACEN-PI from January 2014 to March 2015. The available epidemiological, laboratory test results and clinical records retrieved from the SINAN (Sistema de Informação de Agravos de Notificação), SITETB (Sistema de Informação de Tratamentos Especiais de Tuberculose), Laboratory Environment Manager (GAL) database, the Tuberculosis Laboratory Results Record Book and the Controle de Exames Laboratoriais da Rede Nacional de Contagem de Linfócitos CD4+/CD8+ e Carga Viral (SISCEL) database, at LACEN-PI. For analysis, a structured instrument applied to collect data containing variables related to sex, age, patients living city, body site of mycobacterial infection, HIV status, results of bacterioscopy, culture, GenXpert molecular rapid test, cord factor formation and drug susceptibility test (DST). The Epi-info program version 3.5.4. analyzed the descriptive data.

The slide microscopy stained by Ziehl-Neelsen to examine acid-resistant bacilli presence (AFB) followed by culturing in solid medium Löwenstein-Jensen (LJ) and / or Ogawa-Kudoh (OK) were the methods used in samples sent to LACEN-PI during the study period.[6] For non-sputum clinical specimens, cultivation was at automated BACTEC MGIT 960 SYSTEM (BD Diagnostics, Sparks, MD, USA) according to manufacturer instructions. The real-time polymerase chain reaction (RT-PCR) based assay (GeneXpert TB/Rif), a molecular automatized platform direct to the clinical specimen for Mtb identification and Rifampicin resistance (Xpert, Cepheid, Sunnyvale, CA, EUA). Positive growths in culture media analyzed by the formation of cord factor (CF) by stained BAAR microscopy[8] and for MPT64 immune chromatographic assay, a rapid qualitative Mtb identification. To distinguish between Mtb and NTM positive niacin test, sensitivity to ρ-nitro benzoic acid (PNB) and resistance to thiophene-2-carboxylic acid (TCH) hydrazide tests defined Mtb cultures. Positive results for Mtb undergo MGIT for isoniazid (H), ethambutol (E) and streptomycin (S). Pirazinamide (Z), and second line drugs tested at Laboratório de Referência Nacional para Tuberculose do Centro de Referencia Prof. Hélio Fraga (CRPHF), State of Rio de janeiro. Resistance to isoniazid and rifampicin is the characterization of multidrug resistance (MRTB),

while the only resistance to rifampicin labeled RR-TB. The samples with negative results and suspicions of NTM being analyzed by PCR restriction-enzyme analysis (PRA) of the hsp65 gene, based on the amplification of a 441 bp fragment of the gene after digestion with the restriction enzymes BstEII and HaeIII at TB CRPHF. Data expressed as mean ± SD (standard deviation) or proportion for descriptive analysis. The chi-square test estimated a statistical association between discrete random variables. For expected frequencies less than 5, Fisher's exact test applied, with a 95% confidence interval and significance at $p < 0.05$. The chi-square also estimated the risk and the kappa test evaluates the agreement between the techniques. The Research Ethics Committee of the Piauí State University approved the study (CAAE 39508814.9.0000.5209 - 990.812).

RESULTS

Of 706 suspected clinical specimens of mycobacterial infection sent to LACEN-PI from January 2014 to March 2015, 142 (20%) showed viable growth. Table 1 depicts the available data in the isolates and patients. The most frequently identified species was M. tuberculosis (99, 70%) while 14 were NTM (10%). The remaining isolates (29, 20%), suspected of being NTM, didn't follow the standardized diagnostic routine due to the inadequacy of the clinical samples. Of the identified NTM, M. abscessus predominated (5/14, 36%) followed by 14% (2/14, each) of M. avium, M. intracellulare and Mycobacterium sp., and 7% (1/14, each) of M. asiaticum, M. szulgai, and M. kansasii, respectively.

The majority of the patients came from Teresina (74/142, 52.1%), followed from Parnaíba (21, 14.8%) and other cities of Maranhão state (9, 6.3%) and Pará (1, 0.7%); the pulmonary clinical form was prevalent (128/142, 90.1%), while 6.4 % (9/142) was tuberculous meningitis and the remaineder presented another extra pulmonary clinical form (1, 0.7%, each). Most of them were adults, but those diagnosed with M. tuberculosis infection were younger than that NTM infected (p=0.001) and of male sex (p=0.039). Out of 39/142 (27.5%) patients underwent a serological test for Human Immunodeficiency Virus (HIV), of which 20 (51.3%) were positive with an average CD4/CD8 cell count of 164 ± 223 cells/mm3, ranging from zero to 660 cells/mm3. A massive percentage of HIV positive patients were co-infected with Mtb (95%) while the minority (5%) with NTM. The most frequently NTM species identified in the positive and negative HIV citizens of Teresina and Parnaíba, respectively, were M. abscessus (36%).

Isolates of M. tuberculosis tested for drug-suscetibility was 92.2 % (59/64). Remarkably 4/5 resistant strains showed MR-TB profile, one was extensively resistant (XDR-TB), with resistance profile to quinolone (ofloxacin) plus two second-line injectables (kanamycin and capreomycin), and resistance to S and E; the other

was also resistant to S and the remained isolate were resistant to rifampicin (RR-TB). Given that overall drug resistance of 7.8 % (5/64). Notably is the 6,1% treatment abandonment found.

The GeneXpert rapid molecular test, performed on 65/142 (45.7%) of clinical samples from patients with pneumonia, resulted positive in 46/65 (70.8%) and thus identified Mtb. When comparing these results with those obtained from the in vitro culture medium (n= 40) and microscopy (n= 43), there was complete agreement between the specimens tested for both methods. However, comparing only the microbiological methods the concordance was low (60/82, 73%, k = 0.2241). The cord factor test performed in 107/142 (75.3%) on mycobacterial growths, being present in 73/107 (68.2%) isolates, of which the majority *M. tuberculosis* (68/73, 93.1%) and unexpected one each (1.4 %) were *M. szulgai* and *M. abscessus*, and two (2.7%) *Mycobacterium* sp. not identified at the species level (Table 1).

DISCUSSION

Tuberculosis incidence rate in Piauí state is the lowest in the northeastern states. Even lower when compared to national rates in particular with some states of the federation, such as the Amazon (72.9 / 100,000 inh) and Rio de Janeiro (66.3 / 100,000 inh) in the north and southeast of Brazil, respectively.[7] However, due to poor socioeconomic-sanitary conditions of the population, urban-rural characteristics and the semi-arid tropical climate, to find a higher frequency of NTM infections is not unexpected. Nevertheless, the frequency of clinical specimens sent to LACEN-PI was predominantly pulmonary and infected by the *M. tuberculosis* complex (CMTB). Thus, tuberculosis or co-infections need better prevention strategies in the region.

The diagnosis of TB LACEN-PI is more concentrated in densely populated cities, such as in the capital Teresina and in the city of Parnaíba. Possibly the poor socioeconomic conditions of part of the population and the public health infrastructure, especially in Parnaíba, may be contributing to this situation. In this place the epidemiology of tuberculosis corroborates the literature; affecting young and productive individuals renders them incapacitated to promote family support consequently, their citizenship. Socioeconomic health measures and better control of infected people must be implemented in the region as a TB control tool.

A Brazilian retrospective study based on Sistema de Informação de Agravos de Notificação (SINAN), 2000 to 2005, revealed an underreport of the HIV co-infection at 17.7%. The highest proportion (>35%) registered in Acre state (North region), Alagoas, Maranhão, and Piauí in the Northeast, while the lowest (10%) in São Paulo (Southeast) and Goiás (Center-West) states[9] Therefore,

Table 1. Demographic, clinic and laboratory characteristics of 142 patients diagnosed with mycobacterial infection at Laboratório Central de de Saúde Pública Dr Costa Alvarenga, Piauí (LACEN-PI) from January 2014 to March 2015.

Variables	Number of isolates identified (%)		
	Mtb	NTM	sNTM
Age (mean±SD)	39.3 ±14.91	56.7 ±9.6	57.6 ± 17.6
Male	68 (68.7)2	9 (64.3)	13 (44.8)
Female	31 (31.3)	5 (35.7)	16 (55.2)
Teresina -PI	57 (57.6)	05 (35.7)	12 (41.4)
Parnaiba - PI	14 (14.1)	01 (7.1)	6 (20.7)
Other cities	20 (20.2)	6 (42.9)	9(31.0)
Other state3	8 (8.1)	2 (14.3)	2 (6.9)
Pulmonary Site of infection	89 (90)	13 (93)	26 (90)
Brain Spinal Fluid	5 (5)	1 (7)	3 (10)
Other extrapulmonary sites	5 (5)	-	-
Positive HIV serology	19 (19.2)	1 (7.1)	-
Negative HIV serology	19 (19.2)	-	-
Not done	61 (61.6)	13 (92.9)	29 (100)
Positive Xpert rapid Test	46 (43.5)	-	0 (0)
Not Done	53 (56.5)	14 (100)	11 (37.9)
Positive AFB	67 (67.7)	10 (71.4)	13 (44.8)
Negative AFB	31 (31.3)	4 (28.6)	16 (55.2)
Not done	1 (1)	-	-
Positive culture	84 (84.8)	14 (100)	29 (100)
Negative culture	14 (14.2)	0	0
Not done	1 (1)	-	-
Presence CF	68 (68.7)	3 (21.4)	2 (6.9)
Absent CF	17 (17.2)	11 (78.6)	23 (79.3)
Not done	14 (14.1)	-	4 (13.7)
Mono resistant (RFM)	1 (1)	-	-
MR (H/R)	3 (3)4	-	-
XDR (H/R/quinolona/K/CAPR/E/S)	1 (1)	-	-
Susceptible	59 (59.6)	-	-
Not done	35 (35.4)	14 (100)	29 (100)
Treatment abandonment	6 (6.1)	-	-
Total of isolates	99 (70)	14 (10)	29 (20)
		M. abcessus[5]	
		M. avium[2]	
		M. intracellulare[2]	
		M. sp[2]	
		M. asyaticum[1]	
		M. szulgai[1]	
		M. kansasii[1]	

our study confirms that in Piauí HIV coinfection evaluation still deficient. Of the people living with HIV in Latin America, Brazil corresponds to the largest number and accounts for 49% of all new infections in the region. [10]

Humans may be infected or only colonized with NTM without developing disease since exposure to NTM in the environment is common.[11] One of the main limitations in the records was the LACEN-PI impossibility providing an accurate diagnosis for the suspect NTM cases because of a unique clinical specimen sent by the physicians. Thus, the isolates did not go through the identification routine. Strategies for better interaction between primary care professionals and LACEN are essential so that the collections of clinical samples carried out within the standardized criteria, favoring more conclusive diagnostic results. Officially, American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) states that diagnosis of pulmonary NTM disease relies, besides clinical respiratory and radiological findings (nodular or cavitary opacities on X-rays or multifocal bronchiectasis and multiple small nodules), at least in two positive sputum samples from the same patient to confirm the isolation of NTM as a causal agent.[12] A further limitation was NTM species not identified in isolates obtained from those meeting the criteria for the diagnosis of pulmonary NTM disease, even using the PRA-PCR molecular method. Although this molecular test is valuable, it has some limitations related to operational problems (quality of gel agarose, interpretation of the molecular weights of restriction fragments and of patterns). Additional tools, such as sequencing, may improve resolution.[13] Clearly, the frequency of NTM infection is underestimated and further NTM epidemiological investigation remains an important demand.

Regarding the diagnostic tests available in LACEN-PI,[14] the CF showed 100% agreement identifying *M. tuberculosis*, however, the specificity of the test was lower by the presence of CF in NTM isolates. A variety of recent studies prove the presence of cord factor in some species such as *M. kansasii*, *M. fortuitum*, *M. szulgai*, *M. chelonae*, and *M. marinum* as well as in rough wrinkled colonies of *M. chubuense*, *M. gilvum*, *M. obuense*, and *M. vaccae*, when cultured in a liquid medium.[15] Recently, studies with *M. abscessus* forming rough colonies have described that the mycobacteria of this morphotype have a large cord factor. Because of this factor, the internalization of mycobacteria by macrophages is difficult and there is intense extracellular multiplication and consequent formation of abscess and tissue damage, thus constituting a determinant of virulence.[16,17] But in our study, it was not possible to evaluate the shape of the *M. abscessus* colonies. Mistakenly to identify *M. abscessus* isolates as belonging to the MTBC may lead to false tuberculosis diagnosis and erroneous treatment and conclusion of MR-TB, since *M. abscessus* is not sensitive to drugs used to treat tuberculosis.[18] For sure, a more extensive study in a diversity of MNT species to assess the strength of the presumptive CF test is need. GeneXpert platform high agreement to microbiological methods reinforcing the use of the Xpert molecular rapid test to aid in the faster diagnosis of tuberculosis, as reported by others.[19-21] However, the required equipment maintenance and consumables supplies are intermittent in many health units delaying routine implementation.

Opposite to NTM, DST was available for first line drugs used in the treatment of *M. tuberculosis* and 7.8% of resistance found in Piauí is worrying as it is higher than in other studies, and the estimated globally (3.3%).[1] Celerity in DST is crucial for proper patient treatment, mainly in those infected by drug-resistant isolates, disrupting the transmission chain; meanwhile, LACEN-PI seems to fail to provide this data for all isolates On the other hand, treatment discontinuation is an important cause of the emergence of resistant strains. In 2017, the national rate was 10.8 % while Piauí state recorded 5.4%[22] which is similar to the present study (6 %). Moreover, it is worrying that three of the patients were positive AFB smear, being one MR case and a fourth, although extra pulmonary tuberculosis (meningitis) was an XDR case. This scenario is a challenge to the success of tuberculosis control in the state and represents a risk not only for household contacts but also for its social network. The present study, although with many limitations, among others, the partial identification of NTM and performance of DST and HIV test, contributes to demonstrating that tuberculosis and drug resistance remains a public health problem in Piauí, while HIV coinfection seems underestimated and NTM infection may be emerging. Therefore, there is a need for improvements in control, clinical specimens management and, rapid and effective diagnosis of both diseases.

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AUTHORS' CONTRIBUTION

Mariana O. Santos e Maria Helena Féres Saad, contribuíram para a concepção, delineamento, análise e redação do artigo.

Filipe Aníbal C. Costa e Liline Maria S. Martins contribuíram revisando o artigo.

Maria Helena Féres Saad e Silvia Maria de Almeida, contribuíram para o planejamento, esboço, revisão e apro-vação final do artigo.

Todos os autores aprovaram a versão final a ser pu-blicada e são responsáveis por todos os aspectos do tra-balho, incluindo a garantia de sua precisão e integridade.