Nontuberculous mycobacterial infections in a Brazilian pediatric population: a seven-year survey

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ABSTRACT

The aim of this study was to describe the frequency of nontuberculous mycobacteria (NTM) isolation and their related outcomes among pediatric patients of a Brazilian university hospital from 2012 to 2019. NTM were identified in different clinical samples by microbiological culture and molecular-based methods. NTM were isolated from 14 patients, out of whom four (27%) were infected and were treated accordingly. Two were infected with *Mycobacterium avium* complex (MAC), two with *M. abscessus* complex (MABSC) and one with *M. intracellulare*. Two patients had cystic fibrosis-related lung disease and improved after successful NTM eradication. One patient was HIV-positive and died. One patient had severe combined immunodeficiency (SCID)-related pneumonia and is currently being followed-up. We conclude that NTM frequency in our center was low among pediatric patients. Whether this is inherent to Brazilian patients, due to the broad coverage of the Bacille Calmette-Guérin (BCG) vaccine in Brazil, or a result of underdiagnosis remains to be elucidated.

KEYWORDS

Nontuberculous mycobacteria; children and adolescents; epidemiology; opportunistic infection; BCG

Introduction

Nontuberculous mycobacteria (NTM) are a neglected pathogenic group with more than 170 species [1]. Those are commonly classified into slow-growing mycobacteria, the main of which are the *Mycobacterium avium* complex (MAC) and *M. kansasii*, and rapid-growing mycobacteria, which mainly include the *M. abscessus* complex (MABSC) and the *M. fortuitum* complex [2]. Most NTM have low pathogenicity and act as opportunistic pathogens in patients with immunodeficiency or preexisting conditions such as cystic fibrosis (CF) [3,4]; however, an increased incidence of healthcare-associated infections in immunocompetent individuals has been reported in the last decades, which is likely due to improved diagnostic methods [5].

Still, such data mainly concern adult populations and a few studies have looked to the incidence of NTM in children and adolescents. Broad epidemiological studies in Europe and North America shows incidence rates varying from 0.6 to 3.3 cases of NTM disease per 100,000 children, and lymphadenitis accounts for most cases [6–9]. Still, no studies have supported up or downward changes in prevalence, which makes it difficult to understand the true impact of NTM infections among the pediatric population, especially in South America, where studies on NTM are lacking [10].

Our study aimed to describe the frequency of NTM isolation in microbiological cultures of clinical specimens of patients aged 21 years old or less in a Brazilian university hospital, as well as to assess clinical and treatment-related outcomes in patients who had at least one NTM-positive microbiological culture in a period of seven years.

Patients and methods

Study design

This was an observational, descriptive, retrospective, clinical series study carried out at the University of Campinas Teaching Hospital (HC Unicamp), a regional reference center in the diagnosis and treatment of tuberculosis. The study enrolled patients aged up to 21 years old with different clinical syndromes and/or comorbidities who had at least one NTM isolation in microbiological culture of clinical samples from January 2012 to March 2019. Definition of NTM infection relied on the joint criteria of the American Thoracic Society and the Infectious Diseases Society of America [11].

Mycobacterial culture

Clinical samples were cultured at the HC Unicamp Laboratory of Microbiology. Sputum samples were pre-treated with sodium hydroxide (NaOH) 4% to eliminate

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any possible interfering bacteria, and all samples underwent Ziehl-Nielsen staining for detection of acid-fast bacilli. Mycobacterial growth was performed in Middlebrook 7H9 liquid culture medium with a maximum incubation period of 42 days, and the automated MGIT® System (Becton Dickinson, New Jersey, NY, United States) was used for primary isolation.

Molecular identification

Differentiation between NTM and *M. tuberculosis* and species identification were made using Multiplex Polymerase Chain Reaction (PCR) and PCR-Restriction Enzyme Analysis (PRA-PCR), respectively, both of which relied on a previously described method, with minor modifications [12].

Statistics

Data analysis was made by basic descriptive statistics, using Microsoft Excel® 2016 (Microsoft, Redmond, WA, United States). Variables were described as frequencies and percentages.

Ethics

As only medical records were assessed, and not the patients directly, the study was exempted from informed consent.

Results

During the study period, 34,760 mycobacterial cultures were requested in the HC Unicamp, and 712 cultures of

456 patients gave a positive result to NTM (Figure 1). Among the pediatric population, NTM were recovered from samples of 14 patients (22 positive cultures) (Figure 1), among whom 10 (71%) were male, with median age of 17.0 years old (range: 2.0–20.8). Tracheobronchitis and pneumonia were the most frequent clinical syndromes, comprising 10/14 (71%) cases (Table 1). No growing trend in the NTM frequency was observed in seven years.

MABSC was the most isolated microorganism group, being recovered from five (36%) samples, followed by MAC (four samples; 29%), *M. intracellulare* (three samples; 21%), *M. goodii*, *M. peregrinum*, and *M. lentiflavum* (one sample each). Only one isolate (recovered from one sample) was not identified to the species level (Table 1). Most patients did not undergo specific mycobacterial treatment as subsequent follow-up indicated transient colonization with NTM, without significant clinical outcomes. Four patients were regarded as being infected and were treated, two of whom had CF-related lung infection – one with MABSC and other with MAC – both patients improved after treatment and further microbiological cultures were NTM-negative. One patient with severe combined immunodeficiency (SCID) was diagnosed with *M. intracellulare* pneumonia. This patient improved after treatment and is currently being followed-up. One patient was HIV-positive and passed away after a MAC-related pneumonia. After reviewing his medical record, we found that he did not have a proper adherence to MAC treatment (Table 1).

Treatment of MABSC infection included oral clarithromycin and inhaled amikacin for three months. Treatment of MAC infection basically consisted of oral clarithromycin and ethambutol. The CF patient with MAC infection

Figure 1. NTM-positive microbiological cultures at the HC Unicamp from January 2012 to July 2019. One culture of colon biopsy was positive for *M. goodii*, but it turned out to be a contamination. Abbreviations: MTBC (*Mycobacterium tuberculosis* complex).

Table 1. Patients seen at the HC Unicamp from January 2012 to July 2019, from whom NTM was isolated at least one time in clinical samples. Patients' age, gender, isolated species, isolation samples, clinical conditions, preexisting diseases, treatments and outcomes.

Patients/ Gender	Age (years)	Microorganism/num- ber of isolations	Isolation sample	Clinical condition	Preexisting disease	Treatment	Outcome
1/M	2.0	<i>M. peregrinum</i> /1	BAL	Pneumonia	HIV/AIDS	Not treated	Colonization/ improvement
2/M	2.7	<i>M. intracellulare</i> /1	Gastric lavage	Pneumonia	SCID	Clarithromycin, ethambutol, rifampicin (ongoing)	Infection/ Improvement
3/M	2.7	<i>Mycobacterium</i> sp./1	Gastric lavage	Immunosuppression	IBD/	Malnutrition	Not treated
4/M	8.7	<i>M. avium</i> /1	Skin injury	Celulitis	Trauma	Not treated	Colonization/ improvement
5/M	8.9	<i>M. intracellulare</i> /1	Sputum	Pneumonia/TST conversion	Asthma	Not treated	Colonization/ improvement
6/F	13.3	<i>M. abscessus</i> /2	Sputum	Tracheobronchitis	CF	Oral clarithromycin and inhaled amikacin (3 months)	Infection/ improvement/eradication
7/M	15.8	<i>M. abscessus</i> /1	Sputum	Tracheobronchitis	CF	Not treated	Colonization/ improvement
8/F	18.2	<i>M. intracellulare</i>	Sputum	Tracheobronchitis	CF	Not treated	Colonization/ improvement
9/M	18.4	<i>M. abscessus</i> /1	Sputum	Pneumonia	Tuberculosis	Rifampicin, isoniazid, pyrazinamide, ethambutol (TB)	Colonization/ improvement
10/F	18.7	<i>M. abscessus</i> /1	Sputum	Tracheobronchitis	CF	Not treated	Colonization/ improvement
11/M	20.4	<i>M. avium</i> /6	Sputum	Tracheobronchitis	CF	Clarithromycin, ethambutol, rifampicin (6 months)	Infection/ improvement/eradication
12/M	20.7	<i>M. avium</i> /4	Sputum	Pneumonia/LPPS/ Consumptive syndrome	HIV/AIDS	Clarithromycin and ethambutol (bad adherence)	Infection/death
13/F	20.8	<i>M. lentiflavum</i> /1	Sputum	TST conversion/ Occupational exposure	None	Not treated	Colonization/ improvement
14/M	20.8	<i>M. avium</i> /1	Sputum	Tracheobronchitis	CF	Not treated	Colonization/ improvement

Subtitles: **BAL** (Bronchoalveolar Lavage), **SCID** (Severe Combined Immunodeficiency), **TST** (Tuberculin Skin Test), **LPPS** (Lymphoproliferative Syndrome), **IBD** (Inflammatory Bowel Disease), **CF** (Cystic Fibrosis), **SCID** (Severe Combined Immunodeficiency).

was also given oral rifampicin, with all medications being administered for six months. One patient had transient *MABSC* colonization; however, this patient also had tuberculosis and was treated for it (Table 1).

Discussion

We found a low frequency of NTM isolation in pediatric patients, without growing trending over seven years. As commonly reported [11], NTM isolation was linked to preexisting conditions, although most isolates merely suggested colonization, without requiring specific treatment (Table 1).

An immediately interesting finding from our study was the case of *M. intracellulare* infection in a patient with SCID (Table 1), which is rarely reported in the literature. SCID predisposes to infections with several opportunistic pathogens and, while the role of *M. intracellulare* in SCID is unknown, there are several cases reports of disseminated infection with *Mycobacterium bovis*, also known as Bacille Calmette-Guérin (BCG). Therefore, it is recommended that BCG infection be presumed in patients diagnosed with SCID who are vaccinated with BCG until proven otherwise, and treatment should be initiated even in the absence

of clinical manifestations [13–19]. Our case continues to be monitored.

The fact that most patients addressed in this study had CF is explained by a systematic screening for NTM colonization/infection started in 2014 at the HC Unicamp CF Reference Center [20], due to the increasing concern about NTM as potential pulmonary pathogens among this population [3,4]. Clinical improvement of two patients after treatment indicates both the presence of NTM infection and successful eradication therapy, which relied on the current guidelines and is usually effective [11,21].

The finding of a single case of NTM infection in HIV (Table 1) adds information to what is not so frequently reported in the literature, as HIV-related pneumonia has become rare after the advent of the Highly Active Antiretroviral Therapy (HAART) and is most commonly found in patients with advanced immunodeficiency state [13], as was our patient's case. As our patient had a poor adherence to treatment, this is likely to be the cause of such impaired immunological status.

Although the HC Unicamp Microbiology Laboratory is a regional reference in mycobacteriology, it is not clear whether our findings truly represent a low NTM frequency or if such low frequency is due to underdiagnosis. In the

lung disease setting, for instance, the worldwide adherence to clinical guidelines for NTM is considered suboptimal [14], and, in the HIV/AIDS setting, NTM are not regarded as a priority in microbiological investigations, unlike *M. tuberculosis* [15]. When it comes to emergent countries, it is suggested that the lack of facilities and expertise makes NTM infection overlooked at the peripheral level by clinicians [16]. Comparison between our setting and other emergent countries is difficult, as NTM data normally result from studies assessing the burden of pulmonary tuberculosis in children from whom NTM were isolated in respiratory specimens [17–20]. Among pediatric populations of developed countries, lymphadenitis is the most common infection caused by NTM [5]; however, we did not find any such case in our study. On the other hand, the low NTM frequency in our center may possibly be explained by the broad coverage of the BCG vaccine, which is routinely applied in Brazilian neonates, can cross-react with *MAC* and *MABSC* [22] and may therefore provide our patients with a protective response against NTM.

Our study has limitations, such as a reduced cohort (selection bias) and lack of a systematic search of NTM cases in the diagnostic routine of immunosuppressed patients, especially those with HIV/AIDS. The latter is more evident as positive cultures (characterizing either colonization or infection) were predominantly found in CF patients, who have been systematically screened for NTM for five years. Additionally, our retrospective design makes the study susceptible to the quality of medical records.

Conclusion

Overall, the frequency of NTM infection in pediatric patients seen at our hospital is low and suggests epidemiological features that are inherent to Brazilian patients and are possibly explained by the broad BCG coverage, although underdiagnosis due to a low suspicion rate among clinicians cannot be ruled out. We also report a rare case of *M. intracellulare* infection in a patient with SCID. The efforts for diagnosis of NTM infection in patients with predisposing clinical syndromes and the awareness of NTM among clinicians should be improved. Ultimately, the follow-up of patients with confirmed NTM isolation or who have been diagnosed with NTM infection should be done more thoroughly.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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