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RESEARCH ARTICLE

Non-tuberculous mycobacteria isolation from presumptive tuberculosis patients in Lambaréné, Gabon

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Abstract

Objective: The prevalence of clinical cases of pulmonary non-tuberculous mycobacteria (NTM) is increasing worldwide. The aim of this study was to determine the proportion and the NTM species isolated from presumptive tuberculosis patients in Lambaréné, Gabon.

Method: From January 2018 to December 2020, sputum samples from presumptive TB patients were analysed at the tuberculosis reference laboratory of the Centre de Recherches Médicales de Lambaréné. Two sputum samples were collected per patient, and culture was performed using Bactec MGIT 960. The GenoType Mycobacterium CM/AS was used for NTM isolates confirmation and species differentiation.

Results: Among 1363 sputum samples analysed, 285 (20.9%) were Auramin acid fast bacilli (AFB) smear-positive. NTM were isolated in 137/1363 (10%) of the samples. The most prevalent NTM species was *Mycobacterium intracellulare* ($n = 74$; 54%).

Conclusion: These results show the presence of NTM among presumptive TB patients in Gabon, which could potentially complicate TB diagnosis. This presents a new public health challenge, and emphasises the need to consider NTM in planning the prevention and management of tuberculosis control.

KEYWORDS
CM/AS genotype, Gabon, *Mycobacterium intracellulare*, non-tuberculous mycobacteria, prevalence

INTRODUCTION

Non-tuberculous mycobacteria (NTM), also referred to as atypical mycobacteria, mycobacteria other than tuberculosis (MOTT), or environmental mycobacteria, are defined as mycobacteria which do not cause tuberculosis or leprosy. Recently, the prevalence of clinical NTM, particularly pulmonary NTM, has increased worldwide [1]. Consequently, NTM have been studied intensely and become a major medical interest [2]. One driving factor behind the changing epidemiology (or recognition) of NTM is the emergence of the Human Immunodeficiency Virus (HIV) [3,4]. More than 150 species of NTM have been identified in recent years. It has been reported that some NTM species are associated with a wide range of human infections such as lung, skin, and disseminated diseases,

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cystic fibrosis, bronchiectasis, history of tuberculosis and immunosuppression [4,5]. In areas of high TB and HIV co-endemicity, the prevalence of NTM varies significantly between countries [6,7].

In high-income countries (HICs), the epidemiology of NTM infections has been well described [7,8]. Some recent studies show the increase of NTM-related disease in Europe and the United States of America [8,9]. The incidence rates of NTM are currently estimated between 1.0 and 1.8 per 100,000 people per year [4]. Perhaps this is due to the use of better laboratory tools for diagnosis, and guidelines for treatment in case of infection [4].

Data on prevalence, diagnosis and treatment from sub-Saharan Africa (SSA) are scarce; to the best of our knowledge, there are no specific African treatment guidelines, and American Thoracic Society (ATS) guidelines apply. As summarised by Okoi et al [10], only a few countries conducted studies to date which reported systematic isolation and frequency rates of NTM from presumptive TB patients. South Africa reported an NTM rate of 6% [11] from 2001 to 2005; in Nigeria and Uganda NTM frequency was 4.3% in both 2011 and 2009 [12,13]; in Tanzania NTM frequency was 9.7% [12] from 2012 to 2013. Unfortunately, many cases remain undiagnosed, due to the low capacity of laboratories in SSA, where TB is highly endemic. In these countries, TB diagnosis is often based on microscopy [11,12]. Microscopy does not differentiate NTM from MTBC. The consequence of such a diagnostic strategy is that a number of presumptive TB patients, confirmed with microscopy, are wrongly treated with anti-tuberculosis drugs. As NTM are commonly resistant to rifampicin, isoniazid, para-aminosalicylic acid, ofloxacin and levofloxacin [14], presumably many of these cases would be considered 'treatment failures'; which could have dire consequences for patient outcome, misinterpretation of drug susceptibility patterns indicating drug-resistant *M.tb.*, and TB control [13,15].

Like other sub-Saharan countries, Gabon has no data for NTM prevalence, but has established a very high TB incidence of 521 cases per 100.000 inhabitants [16]. There is an urgent need to determine the real NTM prevalence, which will contribute to creating awareness and guidelines for NTM diagnosis and treatment, and that way optimising TB control in the country.

The main objective of this study is to determine the proportion of NTM and identify the different NTM species isolated from presumptive TB patients in the Lambaréné area and beyond, in order to generate baseline data regarding NTM-related lung disease in Gabon.

METHODS

Ethics approval

The study protocol was approved by the Institutional Ethics Committee of the Centre de Recherches Médicales de Lambaréné (CERMEL). Written informed consent was obtained from all volunteers prior to study enrolment for prospective sampling, while an agreement was obtained from the ethics committee for the use of the pre-existing data.

Study design, site and duration

This was a mixed retro- and prospective cross-sectional study, conducted at the CERMEL TB laboratory in Lambaréné, Gabon from January 2018 to December 2020.

Data collection

Demographic data, clinical signs and symptoms indicative of presumptive pulmonary TB, previous history of TB treatment, and HIV status were collected from the patients using a structured questionnaire. Study data were collected and managed using REDCap (Research Electronic Data Capture) [17,18], a secure, web-based software designed to support data capture for research studies hosted at CERMEL.

Sample collection

All sputum samples were referred from the major hospitals in our region: Albert Schweitzer Hospital, Georges Rawiri regional hospital, HIV clinic, TB clinic (Base d'épidémiologie) and CERMEL. We did not perform BAL or induced sputum in this study. Actually, we did not perform any invasive methods for the collection of sputum. Two sputum samples were collected from patients individually during two consecutive days and sent to the CERMEL TB laboratory to be processed.

Smear microscopy

All samples were processed through a microscopy smear to assess their AFB positivity; two auramine-stained smears were prepared from each specimen.

Mycobacterial culture, differentiation between MTBC and NTM

All samples were subjected to culture. The sputum samples were treated with BD MycoprepTM (Beckton Dickinson Diagnostic Systems) and then incubated in the automated BACTEC MGIT 960TM machine (Becton Dickinson Diagnostic Instrument Systems). Positive samples from the MGIT were inoculated on blood agar to check for contamination, ZN smear stains were performed to confirm the presence of AFB, and also tested either with the rapid

Id TB test (Capilia TB Neo) or SD BIOLINE TB Ag MPT64 (Standard Diagnostic). A positive culture with a positive rapid Id TB test was considered as MTBC. A positive culture with negative rapid Id TB test was considered as potential NTM. MTBC and NTM were differentiated by the rapid ID TB test which can detect MPB 64, a mycobacterial protein secreted by *M. bovis*. MPB 64 is similar to MPT 64 the protein produced by MTBC and not by NTM [19]. Samples with no growth after 42 days of incubation in MGIT were declared negative.

Identification of NTM by genotype Mycobacterium CM

The suspected NTM isolate was processed further by Genotype Mycobacterium CM/AS version 2. This procedure was done in three steps according to the manufacturer's instructions [20]; DNA extraction was carried out using the Genolyse kit (Bruker Hain Lifescience).

Data analysis

Data analysis was performed using R version 4.0.1. Categorical variables were summarised as proportion while continuous variables were summarised by median and range. Categorical variables were compared using the Chi square test.

RESULTS

Study population characteristics

We analysed 1363 sputum samples collected during between January 2018 and December 2020. The median patient age was 40 years (range 27–54 years), and 701 (51.4%) were males. Of 1363 presumptive TB cases, 378 (27.7%) were positive for MTBC, and 137 were positive for NTMs (10%). Of the 137 NTM cases, 4 (2.9%) had MTBC/NTM mixed infections (Figure 1). The baseline characteristics of the study population are summarised in Table 1. HIV co-infection

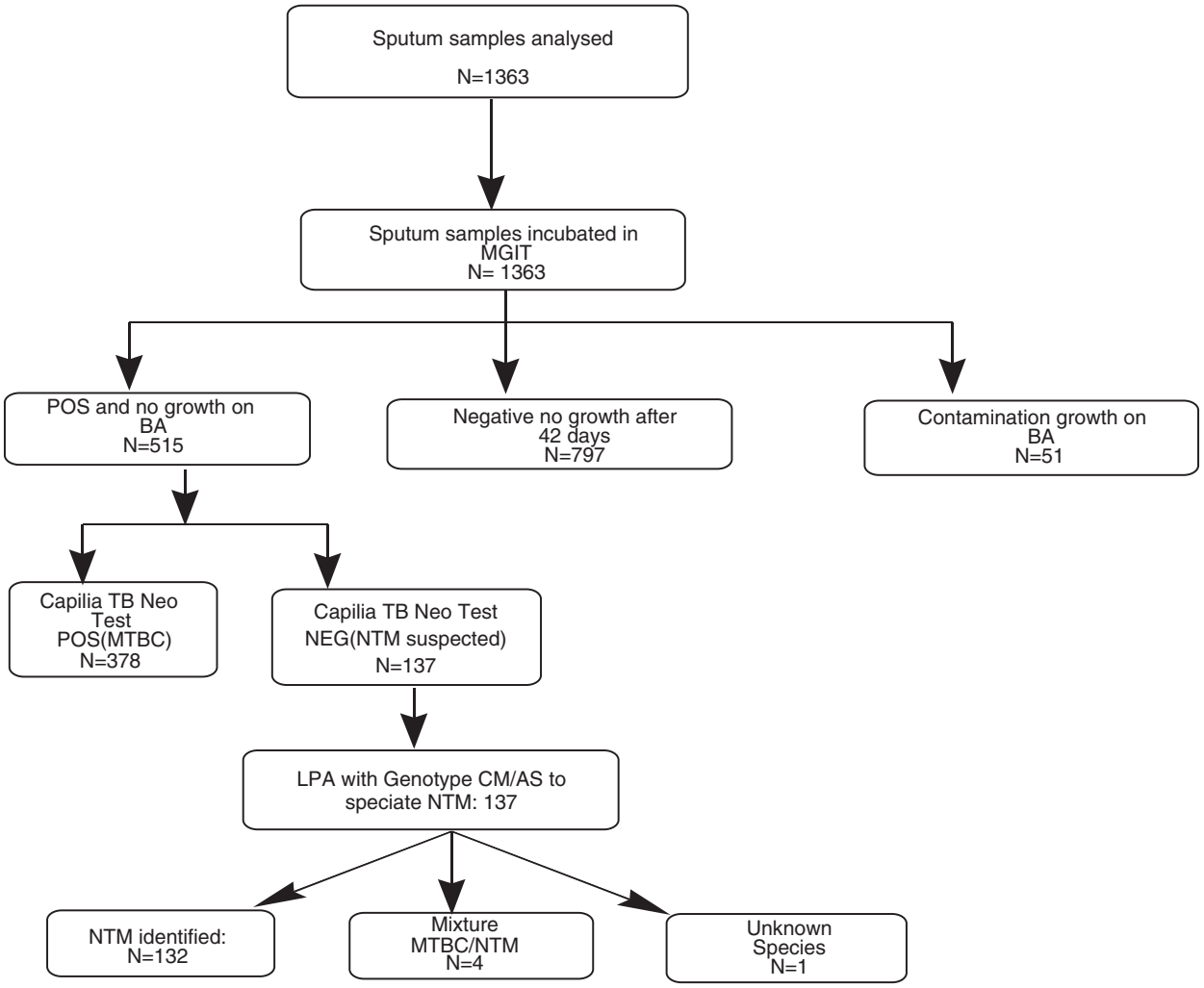

FIGURE 1 Flowchart showing samples analysed and test results. MTBC, mycobacteria tuberculosis complex. NTM, non tuberculous mycobacteria; LPA, line probe assay; POS, positive; NEG, negative; BA, Blood Aaar

TABLE 1 Demographic and clinical characteristics of the study population (n = 1363)

Characteristic	MTBC n = 378	NTM n = 137	Negative n = 848	Total n = 1363
Median age (range)	40 (27–54)			
Sex				
Male	210 (55.6%)	84 (61.3%)	407 (48%)	701(51.4%)
Female	168 (44.4%)	53 (38.7%)	441(52%)	662 (48.6%)
HIV infection				
Negative	147 (38.9%)	100 (73%)	249 (29.4%)	496 (36.3%)
Positive	90 (23.8%)	36 (26.2%)	173 (20.4%)	299 (22%)
Unknown	141 (37.3%)	1 (0.8%)	426 (50.2%)	568 (41.7%)
TB history				
No	226 (59.8%)	100 (73%)	493 (58.1%)	819 (60.08%)
Yes	68 (18%)	32 (23.3%)	98 (11.6%)	198 (14.6%)
Unknown	84 (22.2%)	5 (3.7%)	257 (30.3%)	346 (25.4%)

Abbreviations: MTBC, Mycobacterium tuberculosis complex; NTM, Non-tuberculous mycobacteria.

was found in 90/378 (23.8%) of patients with MTBC, and in 36/137 (26.2%) of patients with NTM. 32/137 (23.3%) NTM cases and 68/378 (18%) MTBC cases had a history of TB. The most frequent clinical charateristic of patients with NTM were HIV infection, history of TB, fever, cough, and chest pain (Table 2).

Mycobacteriological test results

Of 137 NTM positive patients, 118 (86%) were smear-negative. Of 378 MTBC culture-positive patients, 266 (70%) were smear-positive; 19/137 (14%) of NTM cases were misdiagnosed as having MTBC based on smear microscopy (Table 3).

Identification of NTM species among presumptive TB cases

Speciation of the suspected 137 NTM with the Genotype® Mycobacterium CM/AS assay yielded 132 NTM strains, four mixed NTM/MTBC infections, and one (0.73%) unidentifable NTM. The most frequently isolated NTM species were *M. intracellulare* (74 isolates; 54%), followed by *M. fortuitum* (30 isolates; 21.9%), *M. abscessus* (9 isolates; 6.6%) and *M. avium* (7 isolates; 5.1%) (Figure 2).

Discussion

This is the first study to determine NTM proportion in presumptive TB patients in Gabon. We found NTM in 10% of our suspected TB patients, which is comparable to the prevalence reported in Tanzania (9.7%) by Hoza et al., but less than the 15% prevalence reported in Zambia [21,22]. In our study, 14% (19/137) of NTM infected patients with a positive AFB smear were misdiagnosed as TB patients. This result is in line with those reported by Adikaram et al., who estimated that in Africa, 4.5% to 15% of patients with NTM pulmonary symptoms have been erroneously diagnosed as being infected with one of the MTBC agents [23,24].

TABLE 2 Clinical characteristics of patients with NTM infection

Variable	NTM
HIV status	
Negative	100 (73.0%)
Positive	36 (26.3%)
Unknown	1 (0.7%)
History of PTB	
No	100 (73.0%)
Yes	32 (23.4%)
Unknown	5 (3.6%)
Hemoptysis	
No	116 (84.7%)
Yes	21 (15.3%)
Fever	
No	98 (71.5%)
Yes	39 (28.5%)
Cough	
No	32 (23.4%)
Yes	105 (76.6%)
Weight loss	
No	29 (21.2%)
Yes	108 (78.8%)
Night sweats	
No	69 (50.4%)
Yes	68 (49.6%)
Chest pain	
No	24 (17.5%)
Yes	113 (82.5%)

In many countries in SSA, sputum microscopy remains the only diagnostic tool available for tuberculosis detection and treatment initiation. Due to this diagnostic shortcoming, some patients with NTM presenting with similar symptoms to TB will not be properly diagnosed and consequently will not be treated correctly. Therefore, NTM patients could potentially be mismanaged as MTBC. This leads to two major consequences; (1) these patients may have an unfavourable response to anti-tuberculosis treatment, because NTM are naturally resistant to first-line anti-tuberculosis drugs; and (2) this may lead to these individuals being misclassified and treated as MDR patients, which can lead to a risk of selection of resistant tubercle bacilli mutants [13,24,25]. Using microscopy alone as the primary diagnostic tool for tuberculosis in endemic countries constitutes both a public health and a diagnostic problem. Knowing that conventional biochemical methods that identify mycobacterial species as a result of culture are tedious to perform and hence increase the time to report results, WHO encourages the use of molecular methods, which also allows to identify various NTM species.

Our results showed that the significant symptoms most frequently associated with having NTM were cough, fever, history of confirmed TB, chest pain and HIV/AIDS co-infection. In Mali in 2012, Maiga et al. reported that 12% of positive culture results for NTM were among HIV-positive patients [26]. Over the past few years, there has been an emergence of NTM, especially in countries with a high TB and HIV/AIDS prevalence [27,28]. Tuberculosis combined with NTM in HIV patients pose an additional challenge in TB management.

In this study, the frequency of NTM isolated was 10% and the most frequent species was *Mycobacterium intracellulare* 74/137 (54%) from the MAC complex. Our findings are similar to those of South Africa [29], Zambia [21], and also in Botswana [30]. This could be explained by the fact that *M. intracellulare* is more pathogenic than *M. avium* [31], generally associated with pulmonary symptoms, lymphadenitis and disseminated infections in immunocompromised people but also in immunocompetent people.

Another species we found is *Mycobacterium fortuitum*. This species is most often found in Europe, as reported in the study on the inventory of non-tuberculous mycobacteria in the European Union [32]: 5.3% in Denmark, 6.7% in Finland, 10.8% in Spain and 16.5% in Portugal. *M. fortuitum* has been mostly accociated with cutaneous infections, but, when isolated from respiratory samples, is thought to be a causative agents for pulmonary disease and lymphadenitis [4]. However, in the normal host or in people with underlying lung infections, it is most often considered a coloniser [33].

This study had limitations, including the lack of radiological data to allow differentiation between colonisation and clinical significance according to the criteria by the American Thoracic Society and the Infectious Diseases Society of America.

TABLE 3 Identification of samples with non-tuberculous mycobacteria

	Microscopy AU		Culture	
	Positive	**Negative**	**Positive**	**Negative**
MTBC	266 (70.37%)	112 (29.63%)	378 (100%)	0
NTM	19 (13.87%)	118 (86.13%)	137 (100%)	0

Abbreviations: AU, auramine; MTBC, *Mycobacterium tuberculosis* complex; NTM, Non-tuberculous mycobacteria.

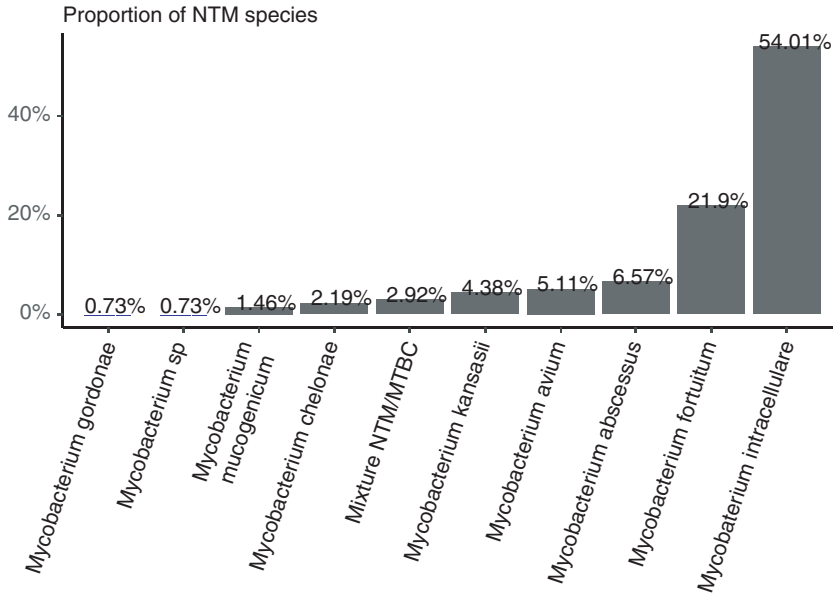

FIGURE 2 Frequency of NTM species by GenoType Mycobacterium CM/AS

Conclusion

Our data show, for the first time in Lambaréné, Gabon, an NTM prevalence of 10% among presumptive TB cases. *M. intracellulare* is the most common NTM species. On the one hand, our results show that many patients may be at risk of receiving inappropriate treatment and being managed as TB patients, due to the use of microscopy as the only diagnostic tool for mycobacteria. On the other hand, this work stresses the importance of mycobacterial culture, molecular tests and identification to allow for accurate and increased detection rates; though at a relatively higher cost. In view of our findings, TB diagnostic laboratories and national TB control programmes urgently need to integrate the diagnosis of NTM to improve the control and management of tuberculosis.

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