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Non tuberculosis mycobacteria isolates among new and previously treated pulmonary tuberculosis patients in Nigeria

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ABSTRACT

Objective: To determine the prevalence of non tuberculosis mycobacteria (NTM) among new and previously treated tuberculosis (TB) patients in Nigeria. **Methods:** It was a retrospective study. A total of 102 sputum smear positive samples/culture isolates from pulmonary TB patients (41 new smear positive and 61 smear positive retreatment cases) were sent to the Institute of Tropical Medicine, Antwerp Belgium between 2007-2009. Data on patients´ characteristics were retrieved from their treatment cards. **Results:** Among the 102 samples, 25 isolates results (20 were culture negative while 5 were contaminated) were excluded from the study. Data were available for 77 mycobacterium isolates. 70 (90.9%) were identified as *Mycobacterium tuberculosis* and 7 (9.1%) as atypical mycobacteria. Among the atypical mycobacteria, three of them were *Mycobacterium fortuitum*, two *Mycobacterium intracellulare* and two *Mycobacterium chelonae*. Of the seven isolates with atypical mycobacteria, 4 (57.1%) were from previously treated patients, while 3 (42.9%) were new sputum positive patients. There was no statistically significant difference in NTM infection between new and previously treated pulmonary TB patients (P=0.97). **Conclusions:** The study shows the involvement of atypical mycobacterium in pulmonary infection in both new and previously treated TB patients. Therefore, there is a need to carry out culture and drug susceptibility testing in all pulmonary TB patients especially those who had failed conventional DOTS treatment to rule out NTM infections.

1. Introduction

Over 95 species of the genus *Mycobacterium* have been described in the literatures[1]. Most of human diseases have largely been due to *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*. Aside these, some *Mycobacterium* species have been described to have pathogenic role in humans[2]. These species have been described with several nomenclatures such as environmental mycobacteria, atypical mycobacteria, mycobacterium other than tuberculosis (MOTT) or non tuberculous mycobacteria (NTM). While there has not been an international consensus on the nomenclature, the American Thoracic Society (ATS) has endorsed the name NTM[3,4]. The NTM are thought to live as saprophytes in water or soil and infrequently cause disease in humans but with the advent of HIV/AIDS, NTM have increasingly become an important cause of morbidity and mortality in humans[2]. NTM have been observed to cause pulmonary disease that is very similar to *Mycobacterium tuberculosis* especially when such cases showed no clinical improvement to conventional anti-tuberculosis (TB) treatment in addition to isolation of the NTM organism during culture.

The diagnosis of NTM is not possible using AFB microscopy which is the main stay of TB diagnosis in several developing countries. As a result, many of the pulmonary diseases caused by NTM are not identified but rather treated with conventional anti-TB treatment which eventually fails because majority of the NTM are resistant to conventional anti-TB treatment[5,6]. The prevalence of NTM and NTM-

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associated hospitalization has been on the increase in several industrialized countries[7-9]. Some of the countries report a NTM prevalence rate as high as 50% among cultured mycobacteria[7-9]. However, there is paucity of data from developing countries largely due to the lack of laboratory infrastructure for culture and specie identification. This study was therefore embarked upon to identify the common NTM and examine the clinical implications of NTM infection in Nigeria.

2. Materials and methods

The study was a retrospective study of patients from Oyo and Osun States in Nigeria. They were diagnosed as pulmonary TB and had culture and drug susceptibility test results from the Institute of Tropical Medicine Antwerp between 2007 and 2009.

Three sputum samples were obtained from each patient for routine diagnostic purpose in the field, but for the study purpose we obtained another two sputum specimens from the positive ones. The two sputum specimens obtained from each patient with a positive AFB results were collected and kept in 50 mL screw cap centrifuge tube (falcon). 5-7 mL of sputum with an equal volume of 1% cetylpyridinium chloride (CPC) and NaCl was added to the sputum to inhibit the growth of fungi and other bacteria. The containers were securely capped and shacked by hand until specimens were liquefied. The caps were properly sealed and specimens were sent to Antwerp. The average period from collection to deliver to the Supranational Lab was 5-7 days by express couriers.

At the Supra National Laboratory in Antwerp Belgium, liquefaction and decontamination were carried out with the modified Petroff method. The specimens were inoculated onto 3 Lowenstein-Jensen (LJ) slopes containing 0.5% pyruvate, glycerol and 500 mg/L p-nitro benzoic acid (PNB), and incubated at 37 ℃ for up to 8 weeks. *Mycobacterium tuberculosis* was confirmed by colony morphology, absence of pigment and failure to grow in PNB. MOTT were identified by rapid growth within 7 days and growth in PNB[12].

The treatment cards and TB registers at their respective treatment centres were reviewed to extract the personal characteristics of the patients. The data were analyzed using EPI INFO 2002 statistical software.

3. Results

The sputum samples of 102 TB patients (41 new smear positive and 61 smear positive re-treatment TB patients) aged 25-80 years were sent to the Supranational Laboratory of the Institute of Tropical Medicine Antwerp, Belgium.

Of the 102 patients sputum positive samples sent to the Institute of Tropical Medicine Antwerp, Belgium, the sputum samples of 25 patients were excluded (20 patients sputum samples were negative and 5 were contaminated) from this study. Since the results of the culture for the 2 samples for each patient are the same, one patient sputum sample is equivalent to a patient. Of the 77 patients samples with culture result, 70 (90.9%) were *Mycobacterium tuberculosis* isolates and 7 (9.1%) were atypical mycobacterium. Of the 70 *Mycobacterium tuberculosis* isolates, 44 (62.9%) were derived from previously treated patients and 26 (37.1%) from new patients who have not been previously treated with anti-TB drugs. Among the seven atypical mycobacteria, three were *Mycobacterium fortuitum* (*M. fortuitum*), two were *Mycobacterium intracellulare* (*M. intracellulare*) and two were *Mycobacterium chelonae* (*M. chelonae*). Of the seven, 4 (57.1%) were previously treated patients with anti-TB drugs who were categorized as category II failure while 3 (42.9%) were diagnosed as new patients who had actually started anti-TB treatment based on the positive smear result. There was no statistically significant difference in NTM infection between new and previously treated pulmonary TB patients (P=0.97).

4. Discussion

This study reported a prevalence rate of atypical mycobacterium among pulmonary tuberculosis patients to be 9.1%. This is comparable though slightly lower than the 11% earlier reported in Lagos, also in the south west region of Nigeria[10] but much lower to reports of between 23.1%-26.6% in northern Nigeria[11,12]. Whether there is geographic difference in the prevalence of NTM in the country is not very clear and further research is needed to ascertain and validate this finding. However, bovine TB has been reported to be more prevalent in the Northern compared with the Southern part of the country[11].

The increasing frequency of pathogenic NTM has become important especially with the advent of HIV/AIDS. It has been observed that HIV/AIDS patients with severe immunosuprression are at risk of NTM[13] which can cause localized or disseminated infections as in *Mycobacterium intracellulare*[14]. The National HIV prevalence rate in Nigeria has been on the increase since 1991 and currently it has been estimated at 4.6%. While relating the HIV prevalence rate to the country's population of over 140 million people, Nigeria has the third largest number of HIV infected people in the world-an estimated 3.5 million people[15]. Thus, it is expected that the prevalence of NTM among HIV infected patients may be on the increase in the country. Therefore, there is a need to quantify the burden of the disease and plan for appropriate management.

4 (57.1%) of the seven atypical mycobacterium isolated, were from previously treated patients who had failed conventional first line anti-TB treatment without any clinical improvement. These patients were classified as category 2 failure and drug resistant tuberculosis suspects thus missing the diagnosis of NTM infection in these patients. Similar observations were reported in some other studies[16-18]. The difficulty in making appropriate diagnosis of NTM by clinicians is because the clinical and radiological features of NTM infected patients are similar to that of *Mycobacterium*

tuberculosis patients. Thus NTM infected patients are not properly managed. This places unnecessary economic and social burden on the patient and their family members and can lead to eventual death of the patient.

The study observed that *Mycobacterium tuberculosis* was still the most common causative agent of smear positive pulmonary TB infection which is similar to what has been described in earlier studies in Nigeria[10–12]. The most frequent atypical mycobacterium isolated from the sputum specimen were *M. fortuitum*, followed by *M. Chelonae* and *M. intracellulare*. These organisms have been reported in literature as significant NTM that are responsible for pulmonary TB–like infection in humans[19]. In older studies in Nigeria[10–12], *M. Chelonae* and *M. intracellulare* were not described. The description of these species may be due to the improved quality of culture and specie identification techniques in the Supranational Laboratory in Antwerp, Belgium where the samples were processed.

In conclusion, the study demonstrates the involvement of NTM in human pulmonary infections in Nigeria and that newer variants were described in Nigeria which had not been reported in previous studies. There is a need for all cases of pulmonary tuberculosis to undergo sputum culture and specie identification. In areas like developing countries where the appropriate laboratory infrastructure is not readily available and accessible to all pulmonary tuberculosis patients, priority should be given to previously treated TB patients to undergo sputum culture and drug susceptibility testing. There is also a need for a national survey to quantify the burden of NTM in the country.

Conflict of interest statement

We declare that we have no conflict of interest.

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