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Nontuberculous mycobacteria infection among *Mycobacterium tuberculosis* patients attending tuberculosis referral centers in Nasarawa State, Nigeria

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Abstract

Reports of *Mycobacterium tuberculosis* (MTB) infection are increasing probably because of misdiagnosis and incorrect reporting of NTM in the State leading to inappropriate treatment regimens and deaths as a result of NTM infection than MTBC and has become detrimental especially among immune-compromised individuals. Thus, this study is designed to determine the Prevalence of NTM among *Mycobacterium tuberculosis* patients attending Tuberculosis Referral Centers in Nasarawa State, Nigeria. A total of 1380 early morning sputum samples were collected and incubated for the detection of mycobacterial growth, those that were positive for Acid-fast bacilli were further tested (SD Bioline) to detect *Mycobacterium tuberculosis Complex* (MTBC). Growths that were not MTBC but were Acid-Fast Bacilli positive were suggestive of NTM. Of 1380 sputum collected, the overall prevalence of NTM and MTBC were 8.8% and 20.5% respectively in which males had higher prevalence of 9.0% and 20.9% for NTM and MTBC respectively. Patients with No-formal Education showed highest prevalence of 17.6% and 31.3% for NTM and MTBC respectively. In relation to occupation, farmers had higher prevalence of 13.1% and 29.6% for NTM and MTBC respectively. The prevalence of NTM and MTBC was highest among smokers (9,7% and 24.6%) respectively while participants with HIV had higher prevalence of NTM and MTBC than those that were non-reactive (P2=<0.05 and P1=>0.05). Seasonal infections of NTM and MTBC in the State was low during the raining season and higher during the dry season. Strengthening and expansion of clinical and laboratory services to diagnose and manage diseases caused by NTM with MTBC was highly recommended.

Keywords: Non-tuberculous Mycobacterium (NTM); Mycobacterium Tuberculosis Complex (MTBC) Sputum; Patients; Referral Centers; Nasarawa State.

1. Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous, free living, environmental saprophytic organisms known to occupy water systems, soil, and vegetation. They are known to withstand a wide range of environmental temperatures, do not readily grow in standard bacterial culture media and are antibiotic and disinfectant resistant [1]. These group of bacteria as observed by [1] were once thought to be harmless environmental saprophytes and only dangerous to individuals with defective lung structure or the immunosuppressed. However, today, they are now infecting seemingly immune competent children and adults at an increasing rate through pulmonary infection.

Globally, the frequency of pulmonary disease from NTM is reported to be on the increase in places like Europe, North America, Asia, and South Africa [2] others are Taiwan, China, US, etc [3]. The International Union against Tuberculosis and Lung Diseases (IUATLD) also reviewed data from 14 countries and found that the *M. avium* complex (MAC) was the most frequently isolated species in all these countries, which included China, India, and Korea. While *M. fortuitum* was the most frequently encountered species in Belgium (2.1%), the Czech Republic (17.5%), Denmark (5.3%), Finland (6.7%), France (6.5%), Germany (12.2%), Italy (2.5%), Portugal (16.5%), Spain (10.8%), Switzerland (17.5%), Turkey

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(33.9%), and the United Kingdom (6.0%), undoubtedly, environment is the main reservoir of NTM [3] and more alarming is the fact that, there is person-to-person transmission of the highly virulent clonal MABs across the globe [4 and5]. These group of NTM are a significant problem particularly because of very high levels of antibiotic resistance and the disease, a growing problem.

To humans, NTM are considered as opportunistic pathogens and exposure to these organisms in day-to-day life is common. Patients with both genetic and acquired structural lung diseases such as cystic fibrosis, chronic obstructive pulmonary disease, non-cystic fibrosis bronchiectasis, previous pulmonary diseases, tuberculosis and lung cancer [6] and those with immune suppression due to primary immune deficiency syndrome, etc are at high risk of NTM infection [7].

Several factors such as an ageing population with chronic lung disease and advances in radiological diagnostics that have improved the identification of pulmonary abnormalities [8] may contribute to the emergence of NTM-pulmonary disease. Epidemiological and descriptive studies of patients with non-mycobacterial infection are many, but gaps in knowledge still remain. In Nigeria (the top five countries for Tuberculosis) and other Tuberculosis (TB) endemic countries, the chances of missing NTM species are higher because of the higher pre-test probability of TB, scarce resources, limited laboratory capacity and overburdened health systems is of great concern. In addition, with the high prevalence of HIV infection, there is a growing concern that NTM and other infections could be misdiagnosed as pulmonary TB in HIV infected persons. This study therefore, is to determine the prevalence of NTM among TB patients attending TB referral centers in Nasarawa State, Nigeria.

2. Materials and Methods

2.1. Study Area

This study was carried out in Nasarawa State, Nigeria. Nasarawa State is located in the middle belt region of Nigeria (figure 1). The State lies between latitude 7° 45' and 9° 37' East of the Greenwich meridian. The State shares boundary with Kaduna State in the North, Plateau State in the East, Taraba and Benue States in the South, while it shares boundary with Kogi State and Abuja the Federal Capital in the West. Nasarawa State has a total land area of 26,875.59 Square Kilometer and a population of 1,826,883. The State is blessed with good land for agriculture, enjoys 8-9 months of raining season and 4-5 months of dry season and is known for its abundant mineral resources. The people are mostly engaged in farming. Food supply and its closeness to the Federal Capital Territory has always attracted more peoples to the State.

It is important to note that the samples for this study were collected from the three Senatorial zones of the State namely: Northern, Southern and Western Senatorial zones.

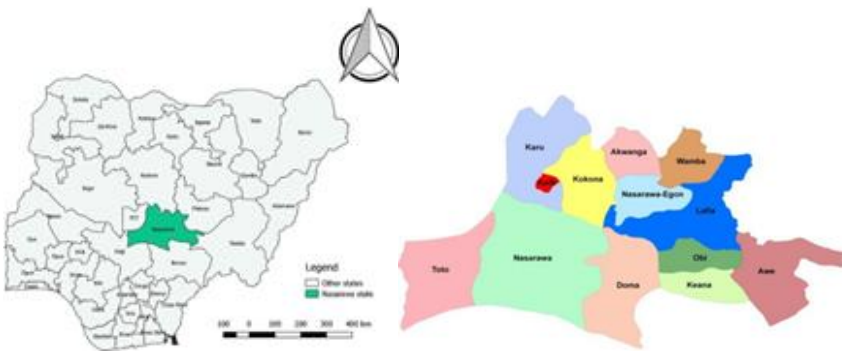

Figure 1 Map of Nigeria showing Nasarawa State and the LGAs in the State

2.2. Ethical Clearance

The protocol for this study was received and approved by the Ethical committee of the Federal Medical Center, Keffi Nigeria, informed written consents were obtained from eligible and willing participants while well-structured questionnaires were administered to each of the participant to obtain relevant sociodemographic information before sputum collection.

2.3. Inclusion and Exclusion Criteria

Consented patients who were recommended and enrolled for TB treatment by medical personnel in the Referral Centers were included for the study while patients who were not recommended and not enrolled for TB treatment were excluded in the study.

2.4. Sample Collection

A total of 1380 early morning, home-collected sputum samples were collected from consented patients that were attending three (3) Referral Centers in the State from June, 2021 to May, 2022 into wide mouthed labelled containers. They were then transported to Zanklin TB Reference Laboratory of Bingham University in cold ice parks for laboratory analyses.

2.5. Smear Microscopy

Smears were made on cleaned grease free microscope slides and allowed to air dried and fixed by gently passing 2-3 times over flame. The slides were then stained following Ziehl-Neelson staining method as described by [9]. For this work, smears were classified as either positive or negative when bacilli were seen or not seen respectively.

2.6. Mycobacterial Culturing and Characterization of Isolates

Five milliliters (5mls) of early morning, home collected sputum was treated with equal volume of NALC-NaOH (Beckton Dickinson Diagnostic systems, Sparks, Maryland, USA) in a tube and vortexed for 20 seconds, allowed to stand for 15minutes and 35mls of sterile 0.067M phosphate buffer (pH 6.8) was added. The tubes were then loaded into centrifuge and spun at 3000rpm. The supernatant was carefully discarded while the sediment resuspended with 2ml phosphate buffer and incubated for inoculation into Lowenstein-Jensen (L-J) medium containing glycerol and incubated at 37^{o}C for 8 weeks.

2.7. SD-Bioline Test (Rapid Diagnostic Bioline)

Colonies from L-J medium was picked and confirmed as AFB by ZN staining technique were then tested with a rapid TB antigen assay (SD-Bioline Ag MPT64 Rapid™ assay; Standard Diagnostics Kyongg-do, Korea) which identifies antigens specific for MTB Complex.

Samples that were confirmed as MTBC isolates using SD-Bioline were further characterized with PCR based genotype MTBC test (Hain lifescience, Nehren, Germany). Cultures that showed growth on LJ media and were AFB but negative for MTBC using SD-Bioline assay were considered as NTM.

Predictive risk factors and Socio-demographic characteristics of the participants were also assessed and compared with NTM and MTB pulmonary infections. We also compared the prevalence of NTM and MTB infections among the patients attending the referral centers. Data were analyzed using statistical SPSS version 9.2. Statistical significance was determined using Chi-square test and two-sided P-values of 0.05 or less ($P \leq 0.05$) were considered statistically significant.

3. Results

Of the 1380 early morning home-collected sputum samples processed for mycobacterial culture, the overall prevalence of NTM and MTBC were 121(8.8%) and 283 (20.5%) respectively in which males had higher prevalence of 20.9% and 9.0% for NTM and MTBC respectively. The prevalence of NTM and MTBC with respect to educational status of participants showed highest prevalence of 17.6% and31.3% respectively among those with non-formal education. Non-tuberculous mycobacterium and MTBC infection in the study showed highest prevalence of 16.4% among ages 41-50years and 40.0% among age less than 10years ($P1 < 0.05$ and $P2 > 0.05$ respectively). The prevalence of NTM and MTBC with respect to marital status revealed that divorced participants had higher prevalence of 17.8% and 36.7% respectively while in relation to the occupation, farmers had higher prevalence in which NTM was 13.1% and MTBC was 29.6% followed by those schooling and house wives respectively. It was also observed that the prevalence of NTM and MTBC was highest among smokers with a prevalence of 9,7% and 24.6% respectively while participants with HIV had higher prevalence of NTM and MTBC than those that were non-reactive (see Table 1).

We also observed the seasonal infection of NTM and MTBC in the State in which there was a close relationship between these two infections and the seasons of the year. In the study, there was a gradual drop in NTM and MTBC infection during the raining season ie from the months of June 11(9.1%), 24(8.5%) to September 2(1.7%), 16(5.9%) for NTM and

MTBC respectively and gradual rise in infection during the dry season ie from November to its peak in the month of February and March (See Figure 2).

Table 1 Prevalence of NTM and MTBC infections with respect to Socio-demographic characteristics and associate risk factors

Characteristics	No. of Sample	MTBC (%)	NTM (%)
1.Gender			
Male	908	190(20.9)	82(9.0)
Female	472	93(19.7)	39(8.3) P1 = <0.05
Total	1380	283(20.5)	121(8.8) P2 = <0.05
2. Educational Status			
Non-formal education	272	85(31.3)	48(17.6)
Primary Education	431	69(16.0)	40(9.3) P1 = <0.05
Secondary Education	536	108(20.1)	20(3.7) P2 = <0.05
Tertiary Education	141	21(14.9)	13(9.2)
Total	1380	283(20.5)	121(8.8)
3.Age			
<10	20	8(40.0)	-
11-20	57	20(35.1)	-
21-30	273	24(8.8)	8(2.9)
31-40	395	47(11.9)	17(4.3) P1 = <0.05
41-50	274	76(27.7)	45(16.4) P2 = >0.05
51-60	293	68(23.2)	31(10.6)
61-above	168	42(25.0)	20(11.9)
Total	1380	283(20.5)	121(8.8)
4. Marital Status			
Married	535	88(16.4)	33(6.2)
Divorced	90	33(36.7)	16(17.8)
Single	574	125(21.8)	51(8.9) P1 = <0.05
Widow	120	25(20.8)	12(10.0) P 2= >0.05
Widower	61	12(19.7)	9(14.8)
Total	1380	283(20.5)	121(8.8)
5. Occupation			
Farming	351	104(29.6)	46(13.1)
House wife	349	82(23.5)	11(3.2)
Civil servants	121	13(10.7)	10(8.3) P1 = > 0.05
Schooling	189	17(9.0)	21(11.1) P2= > 0.05
Business (buying and selling)	370	67(18.1)	33(8.9)
Total	1380	283(20.5)	121(8.8)

6. Facilities			
Alushi Referral Center	465	109(23.4)	45(9.7)
Lafia Referral Center	465	102(21.9)	47(10.1) P1 = <0.05
Keffi Referral Center	450	72(16.0)	29(6.4) P2 = <0.05
Total	1380	283(20.5)	121(8.8)
7.Smoking			
Yes	496	122(24.6)	48(9.7)
No	884	161(18.2)	73(8.3) P1 = > 0.05
Total	1380	283(20.5)	121(8.8) P2 = < 0.05
8. HIV status			
Reactive	580	188(32.4)	80(13.8) P1 = < 0.05
Non-Reactive	800	95(11.9)	41(5.1) P2 = > 0.05
Total	1380	283(20.5)	121(8.8)

NB: P1and P2 = P values for MTBC and NTM respectively

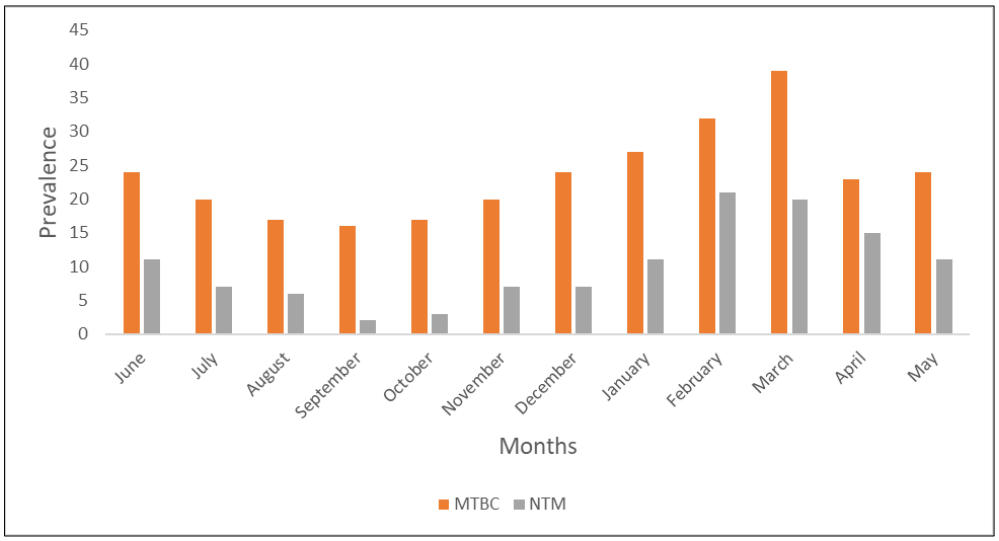

Figure 2 Occurrence of Non-tuberculous Mycobacterium (NTM) and *Mycobacterium tuberculosis* Complex (MTBC) infection in Nasarawa State.

4. Discussion

Pulmonary non-tuberculous mycobacterium (PNTM) disease is a frequently slow and progressive disease, affecting the predominantly already vulnerable patients' populations. It is a fast-global growing health problem that is complicated by many factors observed by [1] as; ubiquitous exposures to the organisms, incomplete understanding of the immune susceptibility to disease, increasing numbers of immune compromised patients, cumbersome diagnostic tests with no prognostic tests and costly multidrug treatment regimens that often fail to cure. Our study revealed an overall NTM prevalence of 8.8% against 20.5% MTBC in which males had 9.0% and 20.9% for NTM and MTBC respectively. This means by implication that a substantial number of patients in the State with NTM infection were incorrectly diagnosed as TB patients, leading to inappropriate treatment, unfavourable outcomes and increased morbidity as observed by [10].

In this study it was observed that educational status and age of participants were important predisposing factors for NTM transmission though statistically not significant (P < 0.05). Participants with no formal education were those with higher NTM (17.6%) prevalence. This agrees with the observations made by [11] in which they stated that education

and poverty are predisposing factors in non-tuberculous mycobacterium pulmonary infections. The prevalence of NTM with respect to age in this study were 16.4% and 11.9% among ages 41-50 and 61-and above, this could be attributed to the level of activities such as farming and other field jobs carried out which exposes these patients to these environmental saprophytes. This was also observed in a work carried out by [8] at Kaduna and Zaria, Nigeria, where they reported higher (58.0%) prevalence of NTM among adult TB suspects. Though MTBC is thought to be an infection related to age, our work reported a high (40.0%) MTBC prevalence among children ages 10 and below. This is of great public health concern and it could be probably be an infection from Parents/Guidance of this children

We also isolated and analyzed the prevalence of NTM in relation to occupations of the participants. The study revealed a high (13.1%) NTM prevalence among the farmers. This, though lower, agrees with the study carried out in Thailand by [12], in which nearly half (46%) of the disseminated NTM infections in HIV-negative cases were associated with farming. This is also related to the prevalence of MTBC (29.6%) and higher seasonal NTM prevalence (see Fig.1) in dry season (February, March and April). This period is usually characterized by heavy amount of dust in the air, low humidity and reduced visibility which predisposes farmers to NTM and MTBC as observed by [8 and 13].

Some of the associated risk factors that could predispose participants analyzed in this study were Smoking and HIV infection. The prevalence of NTM among smokers were higher (9.7%) compared to non-smokers. The prevalence of NTM among HIV positive populations in the study was 13.8% compared to HIV negative individuals with 5.1%. This agrees with what [6] stated that, patients with acquired immunodeficiency syndromes including AIDS and hematological malignancies, hairy cell leukemia, etc are susceptible to NTM infection.

Limitations

This work was hospital-based, hence data for this work were limited to information obtained from patients that presented themselves for Tuberculosis treatment and due to limited resources, laboratory facilities and time frame, we were not able to speciate NTM isolates.

5. Conclusions

In our study, a high prevalence of NTM (8.8%) and MTBC (20.5%) was observed from the three referral centers of Nasarawa State, Nigeria. this by implication means that there could probably be misdiagnosis and incorrect reporting of MTBC leading to inappropriate treatment regimens and deaths as a result of NTM infection than MTBC in the State. This high prevalence which was also statistically significant to the HIV infection, age and occupation of the patients as well as the seasonal increase particularly during the dry season is of great public health importance to health managers, especially when planning for prevention and treatment of MTBC. We observed in this study too that lack of awareness and limited laboratory facilities (our inability to speciate NTM) are the underlying reasons for NTM underreporting in the State and other TB-endemic countries. Several risk factors have now been identified, and it is possible to minimize the NTM disease among these vulnerable patients. For species-specific identification, it is recommended that networks of State, National and Regional laboratories be developed. Molecular methods such as multiplex PCR protocols need to be put in place. Recognition of NTM as an “emerging pathogen” would perhaps elevate the status of NTM for better research funding. Also, development of an animal model would accelerate our understanding of NTM disease and their pathogenesis. Thus, there’s need to strengthen and expand clinical and laboratory services to diagnose and manage diseases caused by NTM with MTBC.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no any competing interest from anywhere.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

Author's Contribution

All the authors conceptualized and designed this research work, collected the samples, analyzed and interpreted the data. We all contributed meaningfully in the drafting and final approval of this manuscript for publication.

Reference

[1] Champa NR, Viviana PL, Andreas K, Denise LD. David WR, Matthew F, Scott CB, Rachel MT and John JM. The rise of non-tuberculosis Mycobacterial Lung Disease. *Front. Immunol.,* 2020; https://doi.org/10.3389/fimmu.2020.00303

[2] Marras TK and Daley CL. Epidemiology of human pulmonary infection with nontuberculous mycobacteria. *Clinics in Chest medicine* 2002; 23: 553-567.

[3] Prevots DR and Marras TK. Epidemiology of human pulmonary infection with nontuberculous mycobacteria. A review. *Clin Chest Med.* 2015; 35:13-34.

[4] Kasperbauer SH, and De Groote MA. The treatment of rapidly growing mycobacterial infections. *Clin Chest Med.* 2015; 36:67-78 doi: 10.1016/j.ccm.2014.10.004

[5] Bryant JM, Grogono DM, Rodriguez-Rincon D, Everall I, Brown KP, Moreno P. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous Mycobacterium. *Science.* 2018; 354:751-7.

[6] Baird TM and Thomas R. Diagnosis, classification and Epidemiology of Pulmonary non-tuberculous mycobacterial disease, In: Chalmers JD, Polverino E, Aliberti S, editors, *Bronchiectasis (ERS Monograph).* European Respiratory Society 2018.

[7] Lake MA, Ambrose LR, Lipman MC, Lowe DM. "Why me now?" using clinical immunology and epidemiology to explain who gets nontuberculous mycobacterial infection. *BMC Med.* 2016; *14:54.* Doi. 10.1186/s12916-016-0606-6

[8] Gambo A; Samer S. K; Alash'le A; Clayton B; Kathleen T. Laura H; and William B. Prevalence of Non-tuberculous mycobacterial infections among Tuberculosis suspects in Nigeria. *Plos One.* 2013; 8(5)e63170.

[9] National Tuberculosis and Leprosy Control Program (NTBLCP). Guidelines on the use of the shorter Regimen and new drugs in the clinical and programmatic management of drug resistant tuberculosis and co-infections in Nigeria 2013.

[10] Henkle E and Winthrop KL. Nontuberculous mycobacteria infections in immunosuppressed hosts. *Clin Chest Med.* 2015; 36:91-9. Doi: 10.1016/j.ccm.2014.11.002

[11] Chalmers JD, Aksamit T; Carvalho ACC; Rendon A, Franco I. Non-tuberculous mycobacterial pulmonary infections. *Pulmonology,* 2018; 24:120-31

[12] Chetchotisakd P, Kiertiburanakul S, Mootsikapun P, Assanasen S, Chaiwarith R, et al. Disseminated nontuberculous mycobacterial infection in patients who are not infected with HIV in Thailand. *Clin Infect Dis.* 2007; 45:421–427.

[13] Lee BY, Kim S, Hong Y, Lee SD, Kim WS. Risk factors for recurrence after successful treatment of *Mycobacterium avium* complex lung disease. *Antimicrob Agents Chemother.* 2015; 59:2972-7