Scientific Research Publishing

Journal of Tuberculosis Research, 2023, 11, 95-108
https://www.scirp.org/journal/jtr
ISSN Online: 2329-8448
ISSN Print: 2329-843X

Characterization of Mycobacterium Species Circulating among Tuberculosis Patients in Bayelsa State, Nigeria

Smart Enoch Amala[1], Goodluck Silas[2], Amba Ollor Ollor[1], Vivian Nkemkanma Agi[1]

[1]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria
[2]Bayelsa State Ministry of Health, Yenagoa, Nigeria
Email: smrtamala@gmail.com

How to cite this paper: Amala, S.E., Silas, G., Ollor, A.O. and Agi, V.N. (2023) Characterization of Mycobacterium Species Circulating among Tuberculosis Patients in Bayelsa State, Nigeria. *Journal of Tuberculosis Research*, **11**, 95-108.
https://doi.org/10.4236/jtr.2023.113010

Received: March 2, 2023
Accepted: July 28, 2023
Published: July 31, 2023

Abstract

Introduction: Tuberculosis is caused by infection with *Mycobacterium tuberculosis*. Looking at the evolution of the bacterium gene due to mutation is crucial to identify species circulating among patients in an area. WHO speculated that tuberculosis is caused by *M. tuberculosis* (MTB), but identification of the strains of MTB circulating in a particular area is important for the management of MTB and to identify pulmonary infections caused by non-tuberculosis mycobacterium. Contact tracing of drug resistant MTB in circulation in an area is also an important procedure of MTB therapeutic choice. **Aim:** This study aimed to isolate and identify *Mycobacterium* species circulating in Bayelsa State, Nigeria. **Materials and Methods:** A total of 102 sputum samples collected from MTB patients were cultured in Lowenstein Jensen (LJ) solid media. Isolates on LJ media were confirmed using Zeihl Nelseen staining method for AFB and Standard Diagnosis Bioline TB Ag MPT64 Rapid test kit. The 16s rRNA gene amplification, agarose gel electrophoresis, and gene sequencing were conducted. Phylogenic analysis and evolutional distances of the strains are computed using the Juke-cantor method. **Result:** Out of 102 sputum samples examined 15 (14.7%) had growth of *Mycobacterium* species (AFB positive). The extracted DNA of MTB amplified on agarose gel electrophoresis aligned horizontally at lanes 1 - 15 showing 16S gene band (1500 bp). Two 2 (2.0%) are non-tuberculosis *Mycobacteria* species, while 13 (12.7%) were *M. tuberculosis*. The non-tuberculosis *Mycobacterium* species isolated are *Mycobacteriode abscesses* and *Mycobacterium kansassii* strain FDAARGOS 1534. The tuberculosis strains are *Mycobacterium tuberculosis* MG003 and R2092 but the predominant strain was MG003. The degree of the genetic evolution of the non-MTB *Mycobacterium kansassii* strain

FDAARGOS 1534 was 75.4%. **Conclusion:** The two major strains of *Mycobacterium tuberculosis* (MTB) circulating in Bayelsa State are MTB MG003 and MTB R2092; MTB MG003 was predominant. The non-tuberculosis species are *Mycobacteriode abscesses* and *Mycobacterium kansasii*.

Keywords

Tuberculosis, Non-Tuberculosis, Strains, Circulating, Bayelsa

1. Introduction

Tuberculosis, a disease caused by *Mycobacterium tuberculosis* (MTB) was discovered on 24th of March 1882 by Dr. Robert Koch as the causative agent of tuberculosis (TB) [1]. The discovery contributed much to the management of tuberculosis globally. The date was chosen yearly as world tuberculosis day. The global fight to eliminate tuberculosis gained momentum over the years. Tuberculosis is an infectious disease that affects the lower respiratory tract, and may also affect other parts of the body. *M. tuberculosis* has survived for centuries. It has existed for over 70,000 years, infecting about 2 billion people worldwide [1].

Globally, about 10.4 billion new cases of tuberculosis emerge every year. It was recorded that one-third of the world population carries *M. tuberculosis* in latent stage which may result in active tuberculosis subsequently. Tuberculosis has a high motility rate and it is responsible for about 1.4 million deaths worldwide [2].

Understanding the genetic finger print of *M. tuberculosis* may be a tool in the fight against tuberculosis globally. Genetic fingerprinting will show the genetic constitution of *M. tuberculosis* and expose the genetic mutation of the bacterium. *M. tuberculosis* phylogeny comprises four major important lineages such as L1-Indo-Oceanic, L2-East-Asian, L3-East African-Indian, and L4-Euro-American. They all vary in their inclination, natural tendency to spread, and the ability to cause infection [3].

In high-burden settings such as Nigeria, inter-patient transmission is a source of MDR-TB infection. Studies have used WGS to infer transmission of *M. tuberculosis* by combining genetic distances (as a measure of single nucleotide variants), epidemiological data, and phylogenetic data. The Single Nucleotide Variants (SNV) threshold for inferring transmission varies within locations and diversities of data set. It was observed in 2013 that isolates from epidemiologically related patients which differ in a few to 5 Single Nucleotide Variants (SNVs) indicate inter-patient transmission. Studies have limited transmission inference to isolates that differ with 1 to 3 SNVs. Guerra-AssuncËao and colleagues inferred transmission from epidemiologically related cases between isolates differing up to 10 SNVs [4].

A theme that had emerged from the studies of the genetics of bacterial population are frequently biomedical and ecological correlates of population struc-

ture. In other words, groups of related bacterial genotypes tend to behave non-randomly. It was correct for species (such as *M. tuberculosis*) in which horizontal gene transfer contributes little to genomic diversity. The availability of large databases containing IS6110 typing data for 6000 isolates allowed the determination of whether isolates from all three groups were equally present among the genotypes causing clusters of tuberculosis [5].

The Houston *M. tuberculosis* database contain typed information for the 850 strains recovered for a period of 2-year in a comprehensive population-based survey. Detailed epidemiological data, including contact surveys are available to all patients from which these organisms were isolated. Interestingly, 25 of the 26 clusters of cases involving 5 or more patients were by genotypic organisms from clusters 1 and 2. This striking association was confirmed by analysis of strains recovered in metropolitan New York zone. Twenty one clusters of new cases in the New York area are caused by genotypic organisms from groups 1 and 2. The non-random association of organisms from groups 1 and 2 with clustered cases was independent of the drug susceptibility phenotype of the strains [6].

Critically, the dominance of group 1 and 2 organisms as causes of clusters were not due to the formal though trivial explanation that group 3 organisms do not occur in the various localities. The variation in the behavior of pathogens was probably the result of two processes. Nucleotide changes which can result in the alteration of amino acids in proteins or the alteration of regulatory sequences that may cause an increase or decrease in the expression of proteins. Data shows that single nucleotide change or amino acid changes can significantly alter *M. tuberculosis* virulence in guinea pig and other pathogens. A second possibility not yet fully explored is the IS6110 chromosomal integration site or other mobile elements [7].

In recent years, studies using whole genome sequencing (WGS) of isolates of *M. tuberculosis* have demonstrated its value in understanding modes of transmission, recurrent tuberculosis (TB), the development of drug resistance, and the bacterium evolution [4].

At the same time, improvement in the next-generation sequencing platforms and library preparation workflows makes it possible to determine the genome sequence from bacterial DNA samples within a week. The re-culture of isolates for DNA isolation was considered a necessary step in published WGS studies. The cultivation of slow growing bacteria, such as *M. tuberculosis*, takes one to several weeks and it is a time-consuming process in WGS projects [8]. In a study conducted in Abuja, Nigeria twenty of 222 isolates were suspected to be NTM. NTM identification with Inno-Lipa Mycobacteria v2 identified *Mycobacterium intracellulare* (sequevars Min A, B, C and D) in four isolates. *Mycobacterium fortuitum*; *Mycobacterium peregrinum* complex in three; *Mycobacterium avium*; *Mycobacterium paratuberculosis*, and *Mycobacterium silvaticum* in two. *M. avium* complex in one *Mycobacterium chelonae* complex (group III, *Mycobacterium abscessus*) in one; *Mycobacterium* genus in four; and five were negative

for *Mycobacterium* [9].

The prevalence of *M. africanum* had decreased in recent times due to its reduced virulence [10]. According to previous reports from Nigeria, *M. africanum* was detected with a prevalence of 33% and 13% [11] [12]. In current times, only 3% of TB in Nigeria was shown to be due to *M. africanum*. On the other hand, the low recovery of *M. africanum* might be explained by difficulties in isolating this species in routine practice [13]. The analysis of the geographic distribution of the agent of human TB in West Africa showed the prevalence of *M. africanum* was stumpy in Ivory Coast, Niger, and Guinea, and on the way out in Cameroon. Furthermore, *M. africanum* was missing in Chad. In comparison, a high proportion of *M. africanum* was reported in countries such as Gambia, Mali, Burkina Faso, and Ghana, and in some parts of Nigeria. It showed that there was no apparent pattern of *M. africanum* geographical distribution across West Africa. Thus, specific features of the national TB control programs and ethnic background may play a role in determining the dissemination blueprint of *M. africanum* [13].

The common difficulty with TB epidemiological research is miniature sample size. Thus, the distribution of *M. tuberculosis* lineages observed may be a random snapshot of lineage compositions in different countries. In this sense, it presents the current state of facts that needs further studies [14]. The information on the prevailing MTBC strains circulating among LW and cattle across three different states of Nigeria using molecular tool was reported. There was high prevalence of Uganda I strain, a rare lineage not reported in the country including Uganda I, LAM_CAM10 of *M. tuberculosis* and SB0944 of *M. bovis* were predominant. Other families obtained were TB family of *M. tuberculosis* and SB0300, SB1026, SB1027 and SB1439 of *M. bovis*. [14] The finding of the big cluster of 29 isolates could indicate recent and probably rapid transmission and may therefore be alarming.

This work is aimed to determine the prevalence of mycobacterium and non-mycobacterium tuberculosis species circulating in Bayelsa State, Nigeria, the prevalence by local governments, gender, and phylogenic relationship of isolates.

2. Materials and Methods

This study was conducted in Bayelsa State, Nigeria. Bayelsa State is located in the South-South region of Nigeria. Samples were obtained from the eight local government areas of the State. Bayelsa has a population of approximately 1.7 million people and most the people live in Yenagoa and Southern Ijaw. The State is situated between latitude 4'15' North and 5'23' South longitude, 5'22' West and 6'45' East. It borders with Delta State in the north, Rivers State in the east, and the Atlantic Ocean in the west and south. The population is mainly the indigenous Ijaws and some non-indigenous residents in Yenagoa, the state capital.

A facility-based cross-sectional study design was used. Samples were collected from participants who had previously been diagnosed with tuberculosis from

both untreated (new cases) and those undergoing treatments. Samples were collected from those who consented and were able to produce sputum up to 2ml and above. They were recruited and followed up in the tuberculosis program from February 2020 to September 2022.

2.1. Ethical Approval

Application for ethical clearance was sent to the Bayelsa State Health Research Ethical Committee (BSHREC). This committee is under Bayelsa State Ministry of Health Yenagoa, Nigeria. A written approval was conveyed on the 10th of November 2020. The ethical approval was presented at each sample collection centers.

2.2. Sample Size Determination

The minimum sample size was determined statistically with the formula:

$$N = z2(pq)$$

where $q = 1 - p$

$$e2N = z2p(1-p)e2$$

where N = minimum sample size.

Z = Standard normal deviant curve at 95% = 1.96

P = Accepted proportion in population (=93=0.93

e = Precision/error margin = 5% (0.05)

$N = z2p(1-p)e2$

N = (1.96) 2 0.89

N = (3.84)

N = 3.84 × 26.04

N = 99.9 approximately 100 minimum sample size. The sample size was increased to 102 in other to admit more willing participant [15].

2.3. Collection of Samples

Consent was obtained from each of the subjects before collecting demographic data and samples. Sputum samples of about 2 - 5 ml were collected from the participants between the age ranges of 16 to 90 years. A total of 102 samples were collected in a falcon tube container after each patient had been instructed on the procedures of collecting sputum specimens as prescribed by the World Health Organization. Homogenization and decontamination of sputum samples with sodium hydroxide N-acetyl cysteine (NaOH/NALC-Na) citrate solution were carried out.

2.4. Cultivation of Samples

Each Lowenstein Jensen (LJ) solid culture media was inoculated with 200 µl of the already processed sputum sample. The inoculums were spread evenly on the entire surface of the medium. The LJ medium cap was corked ensuring that

there were no sputum droplets around the rim. The outside of the tubes were cleaned with paper towel soaked in tuberculocidal disinfectant. The tubes were left in a slant position with cap loosened until inoculums were absorbed (about a week), then tighten securely and incubated in upright position at 37˚C (±1˚C). Examinations for growth were conducted weekly, for about 8 times. The growth of *M. tuberculosis* on LJ solid medium was confirmed by ZN-staining technique and a biochemical test using Standard Diagnosis Bioline TB Ag MPT64 Rapid test was carried out according to the manufacturer's instructions.

The 16S gene of the LJ positive growth were amplified and verified with 1% agarose Gel Electrophoresis. Sequencing of the 16S gene was done using the Big Dye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing were carried out at a final volume of 10 ul. The component includes 0.25 ul Big Dye® terminator v1.1/v3.1, 2.25 ul of 5× BigDye sequencing buffer, 10 uM Primer PCR primer, and 2 - 10 ng PCR template per 100 bp. The sequencing temperatures were as follows, 32 cycles at 96˚C for 10 s, 55˚C for 5 s and 60˚C for 4 min.

The 16S rRNA region of the rRNA gene of the isolates was amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'CGGTTACCTTG TTACGACTT-3' primers on a ABI 9700 Applied Biosystems thermal cycler, at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5 uM and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95˚C for 5 minutes; denaturation, 95˚C for 30 seconds; annealing, 52˚C for 30 seconds; extension, 72˚C for 30 seconds for 35 cycles and final extension, 72˚C for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light trans illuminator.

Sequences obtained were edited using the bioinformatics algorithm trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0. The bootstrap consensus tree inferred from 500 replicates was taken to represent the evolutionary history of the taxa analysis [10]. The evolutionary distances were computed using the Jukes-Cantor method [14].

3. Results

(**Figure 1(a)** and **Figure 1(b)**) The growth of *Mycobacterium* species showing the phenotypic appearance of *Mycobacterium* species on Lowenstein Jenson medium with yellowish buff, rough and tough.

3.1. Prevalence TB Species by LGA

The prevalence of TB by Local Government Area showed Brass and S/Ijaw were 2 (13.3%) each respectively. Kolga and Ogbia were 1 (6.7%) each respectively,

(a) (b)

Figure 1. (a) = Positive growth on LJ solid medium; (b) = No growth on LJ medium.

and Yenagoa 9 (60.0%). The prevalence of TB species by LGA were Brass, Kolga and S/Ijaw *M. tuberculosis* MG003 was 1 (6.7%) each respectively, and Yenagoa 6 (40.0%). The prevalence of *M. tuberculosis* R20922 was 1 (6.7%) each in Brass and S/Ijaw respectively, and in Yenegoa 2 (13.3%). The prevalence of *M. abscessus* was 1 (6.7%) in Ogbia, and *M. kansasii* 1 (6.7%) in Yenegoa respectively as shown in Table 1.

3.2. Prevalence of TB Species by Gender

The prevalence of *Mycobacterium* species among male are *M. tuberculosis* MGOO3 5 (33.3%) and *M. tuberculosis* R2092 2 (13.3%) respectively, while the prevalence among female were *M. abscesus* 1 (6.7%), *M. kansasus* 1 (6.7%), *M. tuberculosis* MGOO3 4 (26.7%) and *M. tuberculosis* R2092 2 (13.3%) respectively. The overall prevalence among male was 7 (46.6%) and female 8 (53.3%) respectively as shown in Table 2.

3.3. Gene Sequencing

The results of the amplified 16S gene of the isolates on agarose gel electrophoresis were shown in Figure 2 below. The isolates were numbered 1 - 15 while G represents the DNA ladder which serves as a control. They were at 1500 and 100 base pair respectively. The extracted DNA of each culture was placed on Agarose Gel Electrophoresis as shown in Figure 2. The isolates were cultivated on Lowenstein Jensen solid culture media to scientifically select organisms the genes were to be sequenced. The presence of 16S gene of bacterium was confirmatory for bacteria. The line 1 - 15 in Figure 2 was the already amplified DNA of each organism, which G was the 100 bp DNA ladder of 1500 bp. The alignment of all the bands on the same lane horizontally was a good indicator of the present of 16S gene. This showed that each of the isolates were successful extracted and amplified. This procedure was carried out before forwarding the amplified DNA for sequencing.

Table 1. Percentage occurrences of TB species by local government area in Bayelsa.

	M. abscessus	*M. kansassii* strain PDAARGOS1534	*M. tuberculosis* MGOO3	*M. tuberculosis* R2092	Total
Brass	–	–	1 (6.7)	1 (6.7)	**2 (13.3)**
Ekeremo	–	–	–	–	**0 (0.00)**
Kolga	–	–	1 (6.7)	–	**1 (6.7)**
Nembe	–	–	–	–	**0 (0.00)**
Ogbia	1 (6.7)	–	–	–	**1 (6.7)**
S/ijaw	–	–	1 (6.7)	1 (6.7)	**2 (13.3)**
Sagbama	–	–	–	–	**0**
Yenegoa	–	1 (6.7)	6 (40.0)	2 (13.3)	**9 (60.0)**
Total	**1 (6.7)**	**1 (6.7)**	**9 (60.0)**	**4 (26.7)**	**15 (14.7)**

Numbers in parenthesis = percentages.

Table 2. Prevalence of *Mycobacterium* species by gender in Bayelsa state.

	M. abscessus	*M. Kansassii* strain FDAAGOS1534	*M. tuberculosis* MG003	*M. tuberculosis* R2092	Total
Male	–	–	5 (33.3)	2 (13.3)	**7 (46.7)**
Female	1 (6.7)	1 (6.7)	4 (26.7)	2 (13.3)	**8 (53.3)**
Total	**1 (6.7)**	**1 (6.7)**	**9 (60.0)**	**4 (26.7)**	**15 (14.7)**

Numbers in parenthesis = percentages.

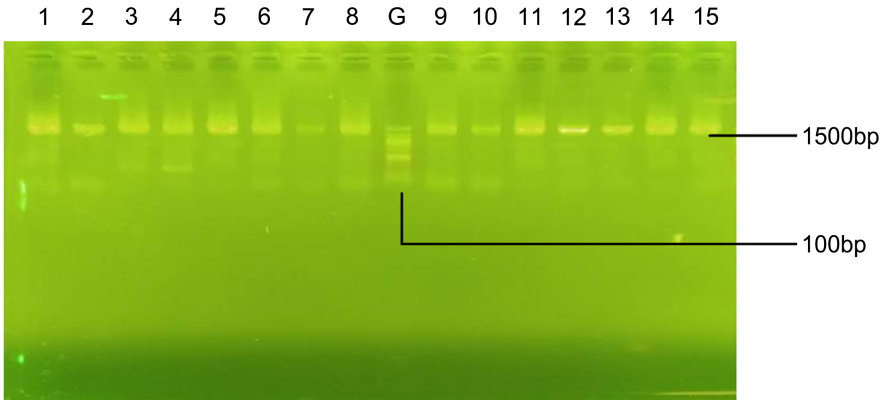

Figure 2. Agarose Gel Electrophoresis showing 16s gene of bacteria, Lane 1 - 15 represents the 16s gene bands (1500 bp). Lane G represents the 100 bp DNA Ladder of 1500 bp.

3.4. Phylogenic Analysis

The phylogeny tree (**Figure 3**) showed the evolutional distance between the sequenced isolates which were represented as S1-S15 and the one already existing at the gene bank. The isolation of non-tuberculosis *Mycobacterium* species was

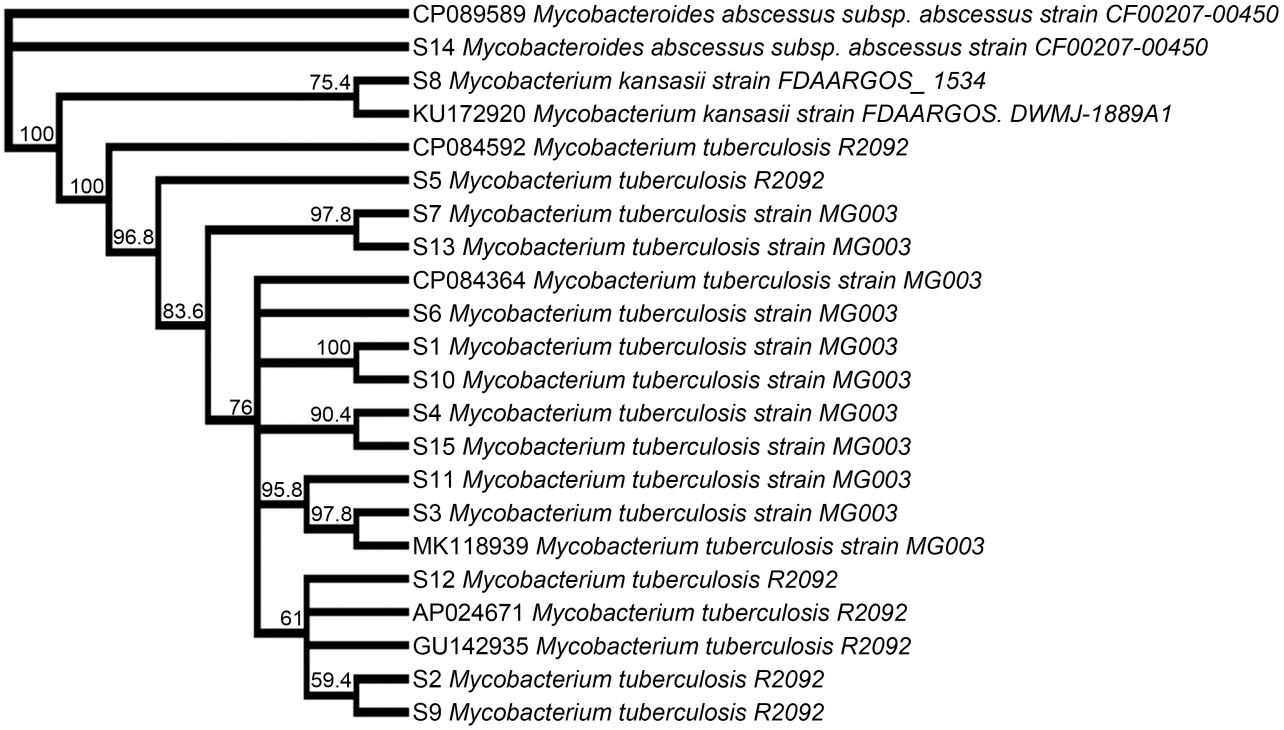

Figure 3. Phylogeny tree of *Mycobacterium* species.

obtained by profiling the isolates from phenotypic culture method. Among the samples cultured 15 (14.7%) out of the 102 subject had growth of *Mycobacterium* species. The negative control had no growth. The isolates on LJ media were subjected to additional confirmatory test by DNA sequencing. It was observed that all the 15 bacteria were acid fast bacilli (AFB) but two are non *Mycobacterium tuberculosis* species.

4. Discussion

Ziehl-Nelson (ZN) staining technique for identification of *M. tuberculosis* is approved for diagnosing, treatment monitoring, and management of tuberculosis cases. The confirmation of AFB by ZN-staining technique is an evidence for positive MTB infection [8]. Most health facilities in Nigeria rely on ZN-staining method for detection of TB before commencing treatment for tuberculosis. The practice or procedure is encouraged to close the gap between testing and management of tuberculosis infection. This may have setback if the treatment for tuberculosis is direct at a non-tuberculosis species.

Mycobacteroide abscessus was one of the mycobacterium species identified by this research and it was isolated from 41 years old male subject resident in Yenagoa. The culture result had a physiological appearance simulating MTB on LJ solid media. The confirmatory test on LJ slop also was positive, depicting MTB but the genetic profiling revealed *M. abscessus* (MBA) complex. MBA complex is responsible for most chronic non-tuberculosis lung disease. Song's finding was in agreement with this result that MBA causes cutaneous infections and it is a

leading microorganism associated with drug resistance [16]. *M. abscessus* was also isolated in Federal Capital, Abuja with other mycobacterium species that were not isolated in Bayelsa such *M. bovis*; *M. africanus*; *M. intracellulare* (sequevars Min A, B, C and D); *M. fortuitum*; *M. peregrinum*; *M. avium*, *M. paratuberculosis*, *M. silvaticum*; *M. avium* and *M. chelonae* [14]. The reason might be because Abuja as the Federal Capital of Nigeria comprises of people from the 32 states of the country working, doing businesses and foreigners from different countries.

It is important to identify the DNA genetic fingerprint of the bacterium that is associated with lung infection to aid the initiation of effective treatment, management and to drastically reduce or eliminate the possibility of drug resistance strains that may complications lungs disease. *M. abscessus* might easily resist the drugs used for treatment of *M. tuberculosis* because it's a non-tuberculosis species and may be a brainteaser for TB in suspected patients. This organism is mostly classified as non-tuberculosis bacterium because it can be found in soil and other surfaces but its ability to cause lung disease with symptoms simulating that of tuberculosis may lead to misdirected treatment [16].

MBA lung infection has always been associated with persons with compromised immunity like HIV-positive patients. There are possibilities for healthy individuals with no secondary infection to be exposed *M. abscessus* infection. *M. abscessus* is one of the organisms known to mutate easily [16].

Mycobacterium kansassii (MBK) strain FDAARGOS 1534 was identified in a subject aged 62 years (female) who was resident in Yenagoa. *M. kanasassi* (MBK) and *M. abscessus* may have been imported because both patients had lived in the USA as migrants where the two TB species are predominately reported as *Mycobacteria* species responsible for non-tuberculosis pulmonary infections. Changes in environmental conditions, conditions of living and reduced immune status due to poor economic conditions of living in Nigeria may stimulate the latent non-tuberculosis species to become active and infectious. Both bacteria may be associated with drug resistance and mycobacterium treatment failures. Identification of TB strains involved in lung infections should be considered as an additional probe before commencing treatment to reduce the development of drug resistance strains and their transmission which may be of public health concern. MTB was not the only cause of pulmonary infection in Bayelsa State. Over the years, MTB had been seen as the only cause of lung disease in Bayelsa but the phenotypic and genotypic identification of MBK had further provoked the need to consider molecular identification of pulmonary acid-fast bacteria as an additional tool to employ in TB investigations. These two organisms were commonly reported in Europe and America [17]. This was the first time non-tuberculosis *Mycobacterium* specie was reported among tuberculosis patients in Bayelsa State.

The most prevalent species of *Mycobacterium* identified in Bayelsa was *M. tuberculosis*. Two major strains of *M. tuberculosis* circulating Bayelsa based on

the sequenced result are *Mycobacterium tuberculosis* MG003 and *Mycobacterium tuberculosis* R2092. The strains are different from the strains circulating in Jos [18], and strains in Abuja, Ibadan, and Nnewi in Nigeria because these strains were resistant to fluoroquinolones [19]. The strains isolated from patients in Bayelsa were sensitive to fluoroquinolones and showed no mutation on the gyr gene. Strain MG003 was more predominant than strain R2092 in the sampled population. Most of the strains recovered from other regions in Nigeria were not associated with tuberculosis in Bayelsa. A similar study conducted in South West Nigeria [20] also revealed different MTB genotypes compared with the strains isolated in Bayelsa.

Verification of the genetic DNA fingerprinting of *M. tuberculosis* is an important tool required for the production of anti-mycobacterial agents. Prediction of antimicrobial resistance is important and will be based on the results of the phylogenetic analysis. There were some degrees of variation between the strains in the gene bank and the strains isolated from the patients recruited for this study. Some were 97.8% similar in their genetic makeup, while others were not so closely related.

Mycobacterium kansasii strain FDAARGOS51534 identified in the research was 75.4% similar to *Mycobacterium kansasii* strain FDAARGOS in the gene bank. *M. tuberculosis* strain MGOO3 labeled S7 was 97.8% similar to *M. tuberculosis* strain MGOO3 labeled S13 both isolates were recovered from the sampled population. This showed that organisms of the same strains might be genetically different to certain degree due to mutation.

The most predominant cluster (DNA fingerprinting) of mycobacterium strain circulating in Bayelsa was the *Mycobacterium tuberculosis* strain MGOO3. This was followed by strain R2092 as shown in the phylogenetic analysis. The non-tuberculosis species isolated are *M. absecessus* and *M. kansasii*. The treatment and management of tuberculosis in Nigeria are supervised by the National Tuberculosis and Leprosy Control Program. The treatment guideline for tuberculosis (National Guidelines) did not consider other strains (variations) across the different geographical locations in the country, it's therefore necessary to determine guidelines to accommodate strain diversities. There was distinction in the clusters of tuberculosis infections. It is necessary to determine the genetic variant of mycobacterium from suspected patient to enable the prediction of a likely drug- resistant strain.

Various genetic profile studies in different parts of the world have revealed great diversity in the genetic evolution of *Mycobacterium*. A study in Ethiopia revealed the existence of extensive mycobacterium drug-resistant MTB. This was achieved by the phylogenic and spoligotyping profile of various isolates of *M. tuberculosis* [21] [22]. Drug-resistant patternshave been linked to the clusters of *M. tuberculosis* strains predominant in particular region [23] [24]. It was observed in this study that the cluster of *M. tuberculosis* strain MGOO3 was the most common strain associated with pulmonary infections in Bayelsa State.

5. Conclusion

The predominant species of mycobacterium associated with lung infections in Bayelsa State are *M. tuberculosis* MG003 and *M. tuberculosis* R2092. The non-tuberculosis species, *M. abscessus* and *M. kansasii* are aliens to Bayelsa. The fight against drug resistance in tuberculosis will achieve good success if MTB investigation is trailed to DNA genetic level.

Limitation of Study

The study could not cover the entire Niger Delta region to determine MTB and non-tuberculosis species associated with pulmonary infections in the geographical terrain. The DNA sequencing conducted were not from the slides but from the cultures only.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Barberies, N.L., Bragazzi, L., Galluzzo, F. and Martaini, M. (2017) The History of Tuberculosis: From the Historical Records to the Isolation of Koch's Bacillus. *Journal of Preventive Medicine and Hygiene*, **58**, 9-12.

[2] Luca, S., Morris, M. D., Quezada, L., Bhat, P., Moser, K., Smith, J., Perez, H. and Mihaescu, T. (2013) History of BCG Vaccine. *Medical*, **8**, 53-58.

[3] Gagneux, S. (2012) Host-Pathogen Coevolution in Human Tuberculosis. *Philosophical Transaction of the Royal Society of London Biological Science*, **367**, 850-859. https://doi.org/10.1098/rstb.2011.0316

[4] Guerra-Assunção, J.A., Crampin, A.C., Houben, R.M.G.J., Mzembe, T., Mallard, K., Coll, F., Khan, P., Banda, L., Chiwaya, A., Pereira, R.P.A., McNerney, R., Fine, P.E.M., Parkhill, J., Clark, T.G. and Glynn, J.R. (2015) Large-Scale Whole Genome Sequencing of *Mycobacterium tuberculosis* Provides Insights into Transmission in a High Prevalence Area. *eLife*, 4, e05166. https://doi.org/10.7554/eLife.05166

[5] Srinand, S.P., Kathryn, E.S., Nancy, D.C., Barry, N.K., Thomas, S.W. and James, M.M. (1997) Restricted Structural Gene Polymorphism in the *Mycobacterium tuberculosis* Complex Indicates Evolutionarily Recent Global Dissemination. *Proceedings of the National Academic of Sciences of the United States of America*, **94**, 9869-9874. https://doi.org/10.1073/pnas.94.18.9869

[6] Feyzioglu, B., Dogan, M., Sanli, O.O., Ozdemir, M. and Baykan, M. (2014) Comparison of the Performance of TK System with LJ and MGIT Methods in the Diagnosis of Tuberculosis. *International Journal of Clinical Experimental Medicine*, **7**, 1084-1088.

[7] Audu, E.S., Gambo, M.S. and Yakubu, A.A. (2017) Rifampicin Resistant *Mycobacterium tuberculosis* in Nasarawa State, Nigeria. *Nigerian Journal of Basic Clinical Sciences*, **14**, 21-25. https://doi.org/10.4103/0331-8540.204075

[8] Global Laboratory Initiative (2014) Mycobacteriology Laboratory Manual Processing Sputum for Smear Microscopy and Qualitative Culture. First Edition, Otsuka

Pharmaceutical Co, Ltd., Otsuka, Japan, 19-50.

[9] Saitou, N. and Nei, M. (1987) The Neighbor-Joining Method: A New Method for Reconstructing Phylogenetic Trees. *Molecular Biology and Evolution*, **4**, 406-425.

[10] Mbugi, E.V., Katale, B.Z., Siame, K.K., Keyyu, J.D., Kendall, S.L., Dockrell, H.M. *et al.* (2015) Genetic Diversity of *Mycobacterium tuberculosis* Isolated from Tuberculosis Patients in the Serengeti Ecosystem in Tanzania. *Tuberculosis*, **95**, 170-178. https://doi.org/10.1016/j.tube.2014.11.006

[11] Ani, A., Bruvik, T., Okoh, Y., Agaba, P., Agbaji, O., Idoko, J. and Dahle, U.R. (2010) Genetic Diversity of *Mycobacterium tuberculosis* Complex in Jos, Nigeria. *BMC Infectious Diseases*, **10**, Article No. 189. https://doi.org/10.1186/1471-2334-10-189

[12] Lawson, L., Zhang, J., Gomgnimbou, M.K., Abdurrahman, S.T., Le Moullec, S., Mohamed, F., *et al.* (2012) A Molecular Epidemiological and Genetic Diversity Study of Tuberculosis in Ibadan, Nnewi and Abuja, Nigeria. *PLOS ONE*, **76**, e38409. https://doi.org/10.1371/journal.pone.0038409

[13] Molina-Moya, B., Abdurrahman, S.T., Madukaji, L., Gomgnimbou, M.K., Spinasse, L., Gomes-Fernandes, M., Gomes, H.M., Sarah, K., Russell, D., Bimba, J.S., Lovett, L., Christophe, S., Luis, E.C. and Dominguez, J. (2018) Genetic Characterization of Mycobacterium Tuberculosis Complex Isolates Circulating in Abuja, Nigeria. *Infection and Drug Resistance*, **11**, 1617-1625.

[14] Jenkins, A.O., Cadmus, S.I., Venter, E.H., Pourcel, C. and Hauk, Y. (2011) Molecular Epidemiology of Human and Animal Tuberculosis in Ibadan, Southwestern Nigeria. *Veterinary Microbiology*, **151**, 139-147. https://doi.org/10.1016/j.vetmic.2011.02.037

[15] Charm, J. and Biswas, T. (2013) How to Calculate Sample Size for Different Study Designs in Medical Research. *Indian Journal of Psychological Medicine*, **35**, 121-126. https://doi.org/10.4103/0253-7176.116232

[16] Felsenstein, J. (1985) Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution*, **39**, 783-791. https://doi.org/10.2307/2408678

[17] Jukes, T.H. and Cantor C.R. (1969) Evolution of Protein Molecules. In: Munro, H.N., Ed., *Mammalian Protein Metabolism*, Academic Press, New York, 21-132. https://doi.org/10.1016/B978-1-4832-3211-9.50009-7

[18] Song, J.Y., Sohn, J.W., Jeong, H.W., Cheong, H.J., Kim, W.J. and Kim, M.J. (2006) An Outbreak of Post-Actipuncture Cutaneous Infection Due to *Mycobacterium abscessus*. *BMC Infectious Diseases*, **6**, 118-120. https://doi.org/10.1186/1471-2334-6-6

[19] John, L.J. and Jerrold, J.E. (2006) Tuberculosis and Atypica Mycobacterial Infection. Second Edition, Churchill Livingstone, London, 1-65.

[20] Ani1, A.T.B., Yetunde, O.P.A., Oche, A., John, I. and Ulf, R.D. (2010) Genetic Diversity of *Mycobacterium tuberculosis* Complex in Jos, *Nigeria. Biomedical Central of Infectious Diseases*, **10**, Article No. 189. https://doi.org/10.1186/1471-2334-10-189

[21] Lawson, L., Zhang, J., Gomgnimbou, M.K., Abdurrahman, S.T. and Le Moullec, S. (2012) A Molecular Epidemiological and Genetic Diversity Study of Tuberculosis in Ibadan, Nnewi and Abuja, Nigeria. Nigeria. *Public Library of Science*, **7**, e38409. https://doi.org/10.1371/journal.pone.0038409

[22] Gashaw, F., Berhanu, E., Yalemtsehay, M., Bazezew, Y., Misikir, A., Balako, G. and Gobena, A. (2021) Phenotypic and Genotypic Drug Sensitivity Profiles of *Mycobacterium tuberculosis* Infection and Associated Factors. *BMC Infectious Diseases*, **21**, Article No. 261. https://doi.org/10.1186/s12879-021-05961-8

[23] Senghore, M., Out, J., Witney, A., Gehre, F., Doughty, E.L. and Kay, G.L. (2017) Whole-Genome Sequencing Illuminates the Evolution and Spread of Multidrug-Resistant Tuberculosis in Southwest Nigeria. *Public Library of Science*, **12**, 184-510. https://doi.org/10.1371/journal.pone.0184510

[24] Li, Y., Xinrui, C., Shiming, L., Hao, W., Jianlin, W., Peng, L., Jing, W., Zhi, Z., Huixia, G., Machao, L., Kanglin, W. and Erhei, D. (2016) Characterization of *Mycobacterium tuberculosis* Isolates from Hebei, China, Genotypes and Drug Susceptibility Phenotypes. *BMC Infectious Diseases*, **16**, Article No. 107. https://doi.org/10.1186/s12879-016-1441-2