Prevalence of MDR-TB Based on Demographic Factors Among Patients Attending Nauth and St Patrick’s Hospital Mile 4 Abakaliki in Southeast Nigeria

Chinenye Esther Okoro-Ani1, *, Chima Innocent Ugbor2, Stellamaris Ojiuzor Ibhawaegbele2, Iniekong Philip Udoh3, Chukwuma Paulinus Igweagu4, Ogechukwu Calista Dozie-Nwakile3, Chima Gabriel Ezeah1

1Department of Medical Microbiology, Medical Laboratory Services, Enugu State University Teaching Hospital, Enugu, Nigeria
2Department of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Nigeria
3Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus, Enugu, Nigeria
4Department of Community Medicine, Enugu State University Teaching Hospital, Parklane, Enugu, Nigeria

Email address: chinenyeokoro07@gmail.com (C. E. Okoro-Ani)
*Corresponding author

To cite this article:
Chinenye Esther Okoro-Ani, Chima Innocent Ugbor, Stellamaris Ojiuzor Ibhawaegbele, Iniekong Philip Udoh, Chukwuma Paulinus Igweagu, Ogechukwu Calista Dozie-Nwakile, Chima Gabriel Ezeah. Prevalence of MDR-TB Based on Demographic Factors Among Patients Attending Nauth and St Patrick’s Hospital Mile 4 Abakaliki in Southeast Nigeria. Chemical and Biomolecular Engineering. Vol. 6, No. 1, 2021, pp. 11-16. doi: 10.11648/j.cbe.20210601.12

Received: August 28, 2020; Accepted: September 18, 2020; Published: March 22, 2020

Abstract: This study was designed to determine the prevalence of multi-drug resistant tuberculosis (MDR-TB) among pulmonary tuberculosis patients attending Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi and St Patrick’s Hospital Mile 4 Abakaliki demographically. Patients with persistent cough for over two weeks were screened by Ziehl-Neelsen ZN technique for the presence of acid fast bacilli (AFB) in their sputum and a total of 103 patients with AFB positive sputum samples were recruited. The positive sputum samples were subjected to Xpert MTB/RIF assay (GeneXpert®, Cepheid USA) and culture on Lowestein Jensen medium for 42 days at 37°C. Drug susceptibility testing was done on the isolates using the nitrate reduction assay (NRA). Eighty-three (83) (80.6%) of the isolates were obtained from culture after suspected colonies were subjected to morphological, biochemical, and immunological tests and out of the 83 (80.6%) samples analysed by Xpert MTB/RIF assay 45 (67.2%) were rifampicin resistant. Age group 26-35 years showed the highest proportion of positive culture results (33.7%) followed by age group 18-25 (28.8%) years. Demographically, age group 26-35 years had a high prevalence rate of MDR-TB (50.0%) and female gender also showed high prevalence rate of MDR-RB (48.5%). Strikingly, educational status was significantly associated with MDR-TB (P=.020). St Patrick’s hospital had a high prevalence rate of MDR-TB (46.94%) when compared with NAUTH (38.9%) and these indicates that there is high prevalence of MDR-TB among patients with pulmonary TB in these sites. The demographic results of this study calls for urgent and serious intervention as MDR-TB prevalence is increasing even in the face of intense national TB control program.

Keywords: Prevalence, MDR-TB, Demographic Factors, Patient

1. Introduction

Tuberculosis, one of the oldest recorded human afflictions, is still one of the biggest killers among the infectious diseases, despite the worldwide use of a live attenuated vaccine and several antibiotics. Mycobacterium tuberculosis is the causative agent of tuberculosis in humans. Other tuberculosis complex organisms are Mycobacterium bovis (causes tuberculosis in cattle and humans, as well as other carnivores) and M. africanum (the causative agent of human tuberculosis in tropical
Africa) [1]. It is the leading cause of death from a curable infectious disease and approximately 9 million new cases of TB are identified per year, with almost 2 million deaths related to TB, making Mycobacterium tuberculosis the single greatest cause of mortality due to a bacterial pathogen [2]. Multi-drug resistant tuberculosis (MDR-TB) is a type of TB that is resistant to Rifampicin and Isoniazid, two of the first line anti-TB drugs [3]. Of great concern is the fact that MDR-TB which is caused by Mycobacterium tuberculosis strains that do not respond to standard therapies not only poses problems for the treatment of individuals but also for the control of TB in populations as it represents lapses in public health [4]. Studies both locally and globally have been done to assess the burden of MDR-TB within the population. In Nigeria, Lawson et al., [5] in an effort to ascertain the prevalence and risk factors associated with MDR-TB reported 13% prevalence rate of MDR-TB among studied population. According to the American Lung Association (ALA), 9.2% of cases of TB were resistant to Isoniazid, and 1.3% resistant to both Isoniazid and Rifampicin in the United States, although these cases had no history of previous treatment with TB drugs [6]. World Health Organisation (WHO) estimates that 26% of patients with TB infection in Nigeria are HIV positive [7].

Global surveillance programme showed variation in the magnitude and trends of drug resistance in different countries. Migration of population has also been reported to strengthen the transmission dynamics of TB as well as antimicrobial drug resistant organism [8]. However, the implementation of Directly Observed Therapy (DOTs) strategy in Nigeria since 1993 has achieved a case detection rate of 30% and treatment success rate of 79% which is still below the global target of 70% detection and 85% cure rate respectively [9]. This implies that majority of active cases are still not detected within the communities and this will continue to transmit TB infection [8]. It has been observed that MDR-TB has reached alarming levels worldwide with the emergence of strains that are virtually untreatable with the existing drugs and it has been indicated that MDR-TB is likely to be more prevalent in Africa than previous reports indicated [7, 10]. Therefore this study assessed the demographic prevalence of MDR-TB among pulmonary tuberculosis patients attending Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi and St Patrick’s Hospital Mile 4 Abakaliki and the findings calls for urgent and serious intervention as MDR-TB prevalence is increasing even in the face of intense national TB control program.

2. Materials and Methods

2.1. Study Area

This study was conducted at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi and St Patrick’s Hospital, Mile 4 Abakaliki. Nnewi is the second largest city in Anambra State and is home to nearly 388,805 residents. Abakaliki is the capital of Ebonyi state and has a population of 149,683 persons [12]. NAUTH is a tertiary health institution and serves as a site for treatment and management of both TB and HIV patients. It is also a referral centre for both cases. St Patrick’s Hospital, Mile 4 Abakaliki is a faith-based health facility and offers both antiretroviral therapy and TB care to patients.

2.2. Sample Size

Minimum sample size was calculated using the formula stated by [13] and a total of 103 sputum smear positive AFB samples were collected for the study.

2.3. Ethical Approval

Ethical approval for this study was obtained from NAUTH research and ethics committee. Consent was obtained from each participant and participants’ confidentiality was maintained throughout the study. Participants received no financial motivation for their involvement in the study. Participants were free to withdraw from the study at any point and their withdrawal would not affect their treatment. This study was conducted between January 2015 and September 2016.

2.4. Sample Collection and Analysis

Consenting, eligible participants were screened for presence of AFB in their sputum. Two sputum samples (spot and early morning) were collected in sterile screw-cap universal containers from each participant on 2 consecutive days and stained by Ziehl-Neelsen’s method.

Progressively, early morning mucoid or mucopurulent sputum specimen was collected from each participant with smear positive AFB test result into a sterile screw-cap universal bottle. The specimen was then stored in the refrigerator until transported to the TB reference laboratory of Dr Lawrence Henshaw Memorial Hospital (DLHMH) in Calabar, Cross River State. Transport was done within 72hrs of collection.

After appropriate sample preparation, two Lowestine Jensen (LJ) medium slants were cultured for each sample. Tubes were loosely capped and incubated as such at 37°C for one week in a slanted position to ensure even distribution and absorption of inoculum. After 1 week, tubes were incubated upright for up to 6 weeks and the caps tightened. An in-house strain H37RV and an uninoculated tube were used as positive and negative control respectively.

After Colonies was confirmed by Ziehl-Neelsen (ZN) staining for acid-fastness, nacian test was carried out on each inoculated and control tubes. The formation of a yellow colour was interpreted as positive reaction; absence of colour was regarded as negative reaction for production of Nacian. Catalase test, p-Nitrobenzoic Acid (PNB) and TB Ag MPT64 Rapid Test was carried out in this study and M. tuberculosis identification was based on its slow growth rate, no pigmentation, no growth on Lowestine Jensen (LJ) medium containing p-nitrobenzoic acid, nacian production, catalase negative at 68°C and positive Ag MPT 64 test.

Drug susceptibility testing (DST) was carried out on all confirmed M. tuberculosis colonies and nitrate reduction
assay (NRA) method was used [11].

GeneXpert MTB/RIF assay for detection of Rifampicin Resistance was carried out on the sputum samples of the participants. Sputum sediments were mixed with sample buffer in a ratio of 1:3 in a screw cap tube and screwed tightly. The tube was vortexed for 20 seconds. Sample was incubated at room temperature for 10mins. After 10mins the sample was vortexed again for 20 seconds and incubated at room temperature for 5mins. After incubation, 2ml of sample was inoculated into the genexpert cartridge. Cartridge was scanned into the GeneXpert machine and allowed to run for 2hrs. After 2hrs the test result was read off the screen of the GeneXpert machine monitor.

### 2.5. Data Analysis

Data was statistically analyzed using statistical package for social sciences SSPS for windows version 20.0 software. A standard questionnaire was completed for each recruited patient to collect demographic parameters. Frequencies were calculated as percentages. Comparison of categorical variables and significance testing was done with χ2 test. P-value of less than 0.05 (P<0.05) was considered statistical significant.

### 3. Results

Demographically, table 1 showed the culture positivity result of the study. Age range 26-35 years had the highest culture positivity prevalence (33.7%). It shows also that male (59.0%) was more infected with TB than female (41.0%). Table 2 showed that educational status was statistically associated with MDR-TB and the study had a prevalence rate of 44.8%. In Figure 1, mile 4 Abakaliki had MDR-TB prevalence of 46.94% compared to 38.89% from NAUTH.

#### Table 1. Culture Positivity with respect to Socio-Demographic Factors.

| Variable          | Number in the Study | Number Culture Positive | %   |
|-------------------|---------------------|--------------------------|-----|
| Age (years)       |                     |                          |     |
| 18-25             | 30                  | 23                       | 28.8|
| 26-35             | 35                  | 28                       | 33.7|
| 36-45             | 19                  | 18                       | 21.7|
| 46-55             | 11                  | 10                       | 12.0|
| 56-65             | 5                   | 3                        | 3.6 |
| >65               | 3                   | 1                        | 1.2 |
| Gender            |                     |                          |     |
| Male              | 61                  | 49                       | 59.0|
| Female            | 42                  | 49                       | 41.0|
| Employment Status |                     |                          |     |
| Civil servants    | 7                   | 7                        | 8.4 |
| Self employed     | 71                  | 57                       | 68.7|
| Student           | 17                  | 13                       | 15.7|
| Unemployed        | 8                   | 6                        | 7.2 |
| Educational Status|                     |                          |     |
| None              | 14                  | 11                       | 13.3|
| Primary           | 32                  | 26                       | 31.3|
| Secondary         | 46                  | 36                       | 43.4|
| Tertiary          | 11                  | 10                       | 12.0|
| Location/Residence|                    |                          |     |
| Rural             | 62                  | 52                       | 62.7|
| Semi-urban        | 9                   | 5                        | 6.0 |
| Urban             | 32                  | 26                       | 31.3|
| Marital Status    |                     |                          |     |
| Married           | 61                  | 53                       | 63.9|
| Single            | 41                  | 30                       | 36.1|
| Widow/Widower     | 1                   | 0                        | 0.0 |

#### Table 2. Prevalence of MDR-TB based on Demographic Factors.

| Variable          | Resistant Isolates | MDR Isolates | Prevalence rate (%) | P-value |
|-------------------|--------------------|--------------|---------------------|---------|
| Age (years)       |                    |              |                     |         |
| 18-25             | 21                 | 9            | 42.9                | .464    |
| 26-35             | 21                 | 11           | 50.0                | .600    |
| 36-45             | 15                 | 6            | 40.0                | .548    |
| 46-55             | 7                  | 2            | 28.6                | .464    |
| 56-65             | 2                  | 2            | 100                 | .548    |
| >65               | 1                  | 0            | 0.0                 | .000    |
| Gender            |                    |              |                     |         |
| Male              | 34                 | 14           | 41.2                | .000    |
| Female            | 33                 | 16           | 48.5                | .000    |
| Employment Status |                    |              |                     |         |
| Civil servants    | 5                  | 3            | 60.0                | .000    |
| Variable          | Resistant Isolates | MDR Isolates | Prevalence rate (%) | P-value |
|-------------------|--------------------|--------------|---------------------|---------|
| Self employed     | 47                 | 21           | 44.7                | .776    |
| Student           | 11                 | 2            | 45.5                |         |
| Unemployed        | 4                  | 1            | 25.0                |         |
| Edu. Status       |                    |              |                     |         |
| None              | 10                 | 8            | 80.0                |         |
| Primary           | 20                 | 8            | 40.0                | .020    |
| Secondary         | 30                 | 9            | 30.0                |         |
| Tertiary          | 7                  | 5            | 71.4                |         |
| Location/Residence|                    |              |                     |         |
| Rural             | 41                 | 20           | 48.8                |         |
| Semi-urban        | 4                  | 2            | 50.0                | .625    |
| Urban             | 22                 | 8            | 36.4                |         |
| Marital Status    |                    |              |                     |         |
| Married           | 43                 | 20           | 48.8                |         |
| Single            | 24                 | 10           | 41.7                | .702    |

**Figure 1.** Prevalence of MDR-TB in each Site.

**Abbreviations.**
NonMDR-TB: Non multidrug resistance Tuberculosis.
MDR-TB: Multidrug resistance Tuberculosis.
NAUTH: NnamdiAzikiwe University Teaching Hospital.

**4. Discussion**

Multidrug-resistant tuberculosis (MDR-TB) has continued to be a challenge for tuberculosis control globally [14]. It has been observed that the main new barrier that challenges the control of TB is high burden of multidrug-resistant TB (MDR-TB). MDR-TB is a man-made problem due to poor management and quality of antituberculosis drugs and can be minimized by making tight identification of its predictors [15]. Though the major contributing factor identified for the spread of MDR-TB is poor infection control [7]. The result of this study showed high culture positivity rate demographically and this agrees with the report of [16] that stated 65.7% culture positivity rate in South-West Nigeria. Otu et al., [17] reported that 100 out of 120 sputum samples were positive for M. tuberculosis on culture in a study in Calabar. This rate is however higher than the 33% culture positivity rate reported by [8]. In India, Guade et al., [18] reported a lower culture positivity rate of 44%.

The high rate of TB culture positivity in this study could
be attributed to the pooled effect of analyzing samples from two centers with high population of pulmonary TB patients including referrals. Importantly, standard procedures were followed to avoid distortion of the organism; therefore samples were maintained in cold chain from collection until analysis at the laboratory. Samples were promptly couriered within 72 hrs to the testing laboratory. As a result, more of the acid fast bacilli contained in the samples were still viable at the time of culture and this reflected in the increased number of organisms isolated. Furthermore, the high rate could be due to the high proportion of the participants yet to commence treatment.

In patients receiving treatment, organisms may have lost their ability to grow on culture media; patients being treated with a rifampicin containing regimen often become culture negative by about the third week of treatment although they may still be sputum smear positive [18]. Lukoye et al., [23] reported a 90.5% TB culture positivity in a national survey done in Uganda. In this survey also, 90.7% of patients enrolled were treatment naive.

Demographically, age group 26-35 years had the highest proportion of positive results followed by age group 18-25. This age distribution of the culture positive results agrees with [8]. They reported that 65% of the culture positive results in their study at Nnewi were from age group 21-40 years. Also [16] reported that 45.8% of the culture positive results were from age group 25-34 years. Tuberculosis has been known to affect the economic (productive) age group [19]. The males in this study had a higher TB positivity rate than the females. This agrees with [8], who reported a statistically significant higher proportion of males with TB positive culture results than females in a study at NAUTH Nnewi. Also [17] reported that out of 100 cultures positive results in a study at Calabar 53 were from males. Das et al., [20] reported that 72% of the culture positive results were from the males in a study in Odisha, India. This could be because men engage in more active lifestyle habits and are more to be seen in congregate settings like prisons.

The prevalence rate of MDR-TB in this study is similar to that reported by [21] in Georgia and [22] in Tanzania. It is however lower than that reported by [16] in South West Nigeria and [8] stated a lower rate of MDR-TB in Nnewi. The result of this study showed that level of education had a significant association with MDR-TB and this agrees with [18]. They reported that illiteracy contributed significantly to development of MDR-TB in India. Worthy of note, Illiteracy is always associated with ignorance and this could affect drug compliance. For instance many persons in our environment even in this century still believe that TB is a supernaturally acquired disease. They believe that TB is either due to spiritual invocations or as a result of harmful fetish practices of an aggrieved enemy. These set of individuals are more interested in assessing spiritual, herbal or traditional remedy. Even when they commence treatment in the hospital they do not hesitate to abandon their regimen for alternative unorthodox solutions and this could lead to treatment failure and exacerbation of illness.

5. Conclusion
Conclusively, MDR-TB is a major public health problem and mainly affects economically productive age group of the population as Age group 26-35 years showed the highest proportion of positive culture results (33.7%) followed by age group 18-25 (28.8%) years. The alarming increase in the prevalence of MDR-TB calls for intensified national TB control program and regulated treatment plan due to the fact that educational status was significantly associated with MDR-TB.

References
[1] McGrath M, Geyvan Pi Hius N. C, Van Helden P. D, Warren R. M and Warner D. F (2013) Mutation rate and the emergence of drug resistance in Mycobacterium tuberculosis. Journal of Antimicrobial Chemotherapy; 205: 1-11.
[2] McBryde E. S, Meehan I. M, Doan T. N, Ragonnet R, Marais J-B Guernier V, Trauer J. M (2017). The risk of global epidemic replacement with drug resistant Mycobacterium tuberculosis strains. International Journal of Infectious Diseases; 2856: 1-7.
[3] Ricks P. M, Farai M, Surbhi M, Rosalia I, Abbas Z, Lauren A. L, DeLuca N, Krashin J. S, Nakashima A. K and Holtz T. H (2012). Characteristics of multidrug-resistant tuberculosis in Namibia. BMC Infectious Diseases; 12: 385-393.
[4] Sergeev R., Colijin C., Murray M., Cohen T. (2012). Modelling the dynamic relationship between HIV and drug resistant tuberculosis. Translational Medicine; 4: 135-148.
[5] Lawson L., Habib A. G., Okobi M. I., Idiong D., Olajide I., Emenyonu N., Omoja N., Cuevas L. E., Ogiri S. O (2010). Pilot study on multidrug resistant tuberculosis in Nigeria. Annals of African Medicine; 9: 184-187.
[6] American Lung Association (ALA) (2013). Multiple Drug-Resistant Tuberculosis (MDR-TB) Fact Sheet. Retrieved from: http://www.lung.org/lung-disease/tuberculosis/factsheets/multidrug-resistant.html
[7] World Health Organization (2013). Global tuberculosis report 2013: http://www.who.int/tb/publications/global_report/en/
[8] Uzoewulu N. G., Ibeh I. N., Lawson L., Goyal M., Umenyonu O., Ofiaeli R. O., and Okonkwo R. (2014). Drug resistant Mycobacterium tuberculosis in tertiary hospital southeast, Nigeria. Journal of Medical Microbiology and Diagnosis; 3: 141-148.
[9] (FMH, 2010) Federal Ministry of Health (2010) Department of public health guidelines for community tuberculosis care Stop TB national Tuberculosis and Leprosy control programme Nigeria 6-9.
[10] Gandhi, N. R., Moll, A., Sturm, A. W., Pawinski, R., Govender, T., Lalloo, U., Zeller, K., Andrews, J. and Friedland, G. (2006). Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet, 368: 1575-1580.
[11] Manual on Technical SOPs for TB Labs (2012): Nigeria Federal Ministry of Health, Department of Public Health Abuja.
Federal Republic of Nigeria Official Gazette (2009). National Population Commission Abuja, Nigeria.

Charan J. and Biswas T. (2013). How to calculate sample size for different study designs in medical research. *Indian Journal of Psychological Medicine;* 35 (2): 121-126.

Solomon Weldegebreal Agedom, Mebrahtu Teweldemedhin, and Hailay Gebreyesus. Prevalence of Multidrug-Resistant Tuberculosis and Associated Factors in Ethiopia: A Systematic Review. *Journal of Pathogens,* 2018: 8.

World Health Organization, “WHO report 2013,” Global Tuberculosis control, WHO, Geneva, Switzerland, 2013.

Olusoji D and Osman E. (2011). Prevalence and risk factors associated with drug resistant TB in South West, Nigeria. *Asian Pacific Journal of Tropical Medicine;* 17: 148-151.

Otu A., Umoh V., Habib A., Ameh S., Lawson L., Ansa V. (2013). Drug resistance among pulmonary tuberculosis patients in Calabar, Nigeria. *Pulmonary Medicine;* 2013: 76-84.

Gaude G. S, Hattiholli J, Kumar P. (2014) Risk factors and drug-resistance patterns among Pulmonary tuberculosis patients in Northern Karnataka region, India. *Nigerian Medical Journal;* 55 (4): 327-332.

World Health Organisation (2012). Nigeria Tuberculosis Fact Sheet. WHO January 2012.

Das D., Prakasini S, Biswanath M. (2016). First Line Anti-TB Drug Resistance in an Urban Area of Odisha, India. *Journal of Clinical and Diagnostic Research.* 10 (11): DC04-DC06.

Vashakldze L., Salakala A, Shubladze N., Cynamon M (2009). Prevalence and risk factors for drug resistance among hospitalized pulmonary tuberculosis patients in Georgia. *International Journal of Tuberculosis and Lung diseases;* 13 (9): 1148-1153.

Range, N, Henrik F, Said M, Pascal M, John C, Andrew K, Apolinary M and Aase B. A (2012). Anti-tuberculosis drug resistance pattern among pulmonary tuberculosis patients with or without HIV infection in Mwanza, Tanzania. *Tanzania Journal of Health Research;* 14 (4): 1-9.

Lukoye D, Ssengooba W, Musisi K, Kasule G. W, Cobelens F. G. J, Joloba M and Gomez G. B (2015). Variation and risk factors of drug resistant tuberculosis in Sub-Saharan African: a systematic review and meta-analysis. *BMC Public Health;* 15: 1-13.