The Problem of Resistance in *Mycobacterium tuberculosis* may be Underestimated in Africa

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**Abstract**

**Background:** The true burden of tuberculosis (TB) and particularly multidrug-resistant (MDR) TB in Sub-Saharan Africa has remained underestimated. **Methods:** We investigated drug susceptibility profile and genetic diversity of *Mycobacterium tuberculosis* isolates collected from confirmed tuberculosis patients in Ibadan, southwestern Nigeria. **Results:** We confirmed that from 74 randomly selected *Mycobacterium tuberculosis* isolates available for drug susceptibility testing in Ibadan, in 2011, 13.5% of them were MDR-TB. **Conclusions:** This figure is obviously above the national and World Health Organization figures.

**Keywords:** Africa, drug resistance, Nigeria, tuberculosis

**INTRODUCTION**

The World Health Organization (WHO) not so long ago released its yearly, highly appreciated report on the developments in tuberculosis (TB) control.\(^1\) However, the data on Africa are unfortunately scarce, but based on what is available in recent years using the WHO estimates, the problem of resistance does not seem as bad on this continent as observed in the former Soviet Union states and large parts of Asia (including India and China) as these areas accounted for about 47% of the 600,000 multidrug-resistant (MDR) TB cases in 2016.\(^1\) The WHO has also alerted that the MDR-TB crisis is globally on the rise, with five countries including India, China, the Russian Federation, Indonesia, and Nigeria accounting for the shortfall in a number of patients eligible for MDR-TB treatment in 2015.\(^2\)

Nigeria currently ranked 4th among countries with the highest burden of TB and still grapples with challenges related to diagnosis and tracking of the disease, and this is further exacerbated by the increasing problems of drug resistance (DR) among population at risk. Unfortunately, as in most developing countries, TB diagnosis in Nigeria is mainly based on sputum smear microscopy with only about 50% sensitivity and obviously less reliable than molecular tests. In Nigeria, data on the prevalence of resistance are only available from the most recent survey in 2010,\(^3\) in which 2.9% of the cases were reported as MDR-TB. This is similar to what (4.3%; 3.2–5.4) was reported by the WHO.\(^2\) In this letter, however, we like to express our concern on the validity of these data in the WHO reports regarding the current situation in Africa [Figure 1].

**METHODS**

In Nigeria, culture is not routinely performed, but on the basis of 74 randomly selected *Mycobacterium tuberculosis* isolates available from representative sampling in Ibadan (Southwestern Nigeria) in 2011, we performed the classical phenotypic resistance testing using Canetti method.\(^4\)

A cross-sectional study was conducted in eight directly observed treatment short-course (DOTS) centers in Ibadan.\(^4\) Sputum samples were collected from confirmed TB patients based on the accepted algorithm of the National TB Program (i.e., patients with pulmonary TB were diagnosed...
based on sputum smear microscopy and/or radiological findings) with the support of health officers at the various DOTS centers. Following good laboratory best practices, sputum samples were processed and decontaminated using the N-acetyl-L-cysteine–sodium hydroxide as earlier described.\(^{[5]}\) Thereafter, the supernatant was carefully poured off and the pellet obtained was inoculated on media.

Drug susceptibility test was based on direct proportion method with the phenotypic test performed on Löwenstein–Jensen (LJ) slopes following earlier described methods.\(^{[6]}\) Pellet obtained from decontaminated sputum was inoculated directly on drug-containing medium using the LJ slopes (rifampicin [RMP]: 40 mg/L, isoniazid [INH]: 0.2mg/L, ethambutol [EMB]: 2 mg/L, and streptomycin [SM]: 4 mg/L), incubated at 37°C, and monitored for growth at 4 and 6 weeks.

**Quality control**

Reference strains of fully susceptible H37Rv (ATCC 27294) and MDR (ATCC 35838) of *M. tuberculosis* were used as controls and served as susceptible and resistant controls, respectively.

**Genotype database comparison and analysis**

Genotyping results were analyzed based on the comparison from the international spoligotyping (SITVIT) database of the Pasteur Institute of Guadeloupe (http://www.pasteur-guadeloupe.fr: 8081/SITVITDemo)\(^{[6]}\) and the revised publicly available international multimarker database (SITVITWEB).\(^{[7]}\) Assignment of mycobacterial lineages and families to the genotyped isolates and spoligotypes was analyzed with TB lineage (http://tbinight.cs.rpi.edu/about_tb_lineage.html) and Spotclust (http://tbinight.cs.rpi.edu/run_spotclust.html), respectively. Using standard signatures provided in SpolDB4 and SITVITWEB\(^{[6,7]}\) and those of Gagneux and Small,\(^{[8]}\) relevant families and lineages were confirmed.

**RESULTS**

To our surprise, 13.5% of the isolates were found MDR, while 25.7% of the isolates were resistant to RMP, 31.1% to INH, 25.7% to SM, and 17.6% to EMB. In addition, 47.3% of the isolates revealed pan-susceptibility to all drugs. Total resistance to at least one of the drugs was detected in 52.7% of the isolates, while total resistance to INH only was observed in 5.4% of them. Again, total resistance to INH (whether associated or not with resistance to other drugs) was observed in 31.1% of the isolates. For quality control purposes, the proportion method of drug susceptibility testing (DST), which is a universally accepted method, was used. In addition, reference susceptible and resistance strains of H37Rv and ATCC27294 were used as quality control to validate the DST results of isolates studied.

We also applied spoligotyping to a subset of the isolates, and this revealed that majority of the isolates were from the Euro-American lineage (i.e., Lineage 4) and clustered into two genotype families (LAM10_CAM=12 and T1 = 7)\(^{[6‑8]}\) and that there was no correlation between phylogenetic features of the *M. tuberculosis* isolates and resistance [Table 1]. Moreover, all the patients were subjected to HIV testing (26 were HIV-positive and 48 HIV negative), and no correlation between HIV infection and resistance was observed, indicating that the problem of resistance is not restricted to particular risk groups.

**DISCUSSION**

Importantly, we found MDR-TB in 13.5% of our tested patient population. Back in 2011, the problem of resistance in just a city in Nigeria may, therefore, have been much higher than reflected in the latest official report of the WHO. This is confirmed by a newly published report on the prevalence of DR-TB in Nigeria which indicated that the burden of DR in the country was high (prevalence rate of any DR among new TB cases was 32.0%; 95% confidence interval: 24.0 ± 40.0%; 734/2892)\(^{[9]}\) It is also pertinent to note that majority of those TB cases were in the active transmission, low-aged group. This age group was found to harbor 80% (8/10) of the reported MDR-TB cases [Table 1], a factor that may play an additional role in the further transmission of these *M. tuberculosis* strains, which may equally be exacerbated by coinfection with HIV and poor patient care. This assertion has been earlier alluded to by findings of Ogbudebe et al.\(^{[10]}\) that implicated high rate of TB in urban slum settings (which are normally uninvestigated as seen in most African countries) as an indicator of high underlying TB and HIV prevalence.

Notably, we confirmed that strains of LAM10-CAM family were responsible for most TB transmission in Ibadan during the study period. Significantly, as in other studies in Nigeria,\(^{[11,12]}\) we found diverse strains of *M. tuberculosis* (i.e., SIT 838, 580, 403, 373, 71, 53, and 61) [Table 1]. Coincidentally, these SIT strains have also been reported in earlier studies where most of them clustered into the LAM10_CAM and T1 families.\(^{[11,12]}\)

Some of the limitations of this study were the small sample size used and that we did not further subject the isolates to

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**Figure 1:** Trend of multidrug-resistant tuberculosis in Nigeria and Africa based on the World Health Organization estimates from 2010 to 2017.
Table 1: Distribution of the isolates according to sex, age, HIV status, spoligotype patterns, octal designation, spoligotype international type, and family profiles

| Isolates (lab number) | Sex   | Age  | HIV status | Spoligotypes                                      | Octal designation | SIT   | Family       | Drug resistant  |
|-----------------------|-------|------|------------|--------------------------------------------------|-------------------|-------|--------------|----------------|----------------|
| 1 (M86)               | Female| 54   | Negative   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 53    | T1           | MONO-P         |
| 2 (H102/7)            | Female| 78   | Positive   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 373   | T1           | MONO-R         |
| 3 (H133/6)            | Female| 40   | Positive   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 61    | LAM10_CAM    | POLY-S and E   |
| 4 (H125/7)            | Male  | 53   | NA         | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 61    | LAM10_CAM    | MDR            |
| 5 (T176/4)            | Male  | 60   | Positive   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 61    | LAM10_CAM    | MONO-I         |
| 6 (M87)               | Male  | 22   | Negative   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 61    | LAM10_CAM    | POLY-S and E   |
| 7 (H98/6)             | Female| 38   | Negative   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 1580  | T1           | MONO-R         |
| 8 (C95/5)             | Male  | 40   | Negative   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 53    | T1           | MONO-I         |
| 9 (AMH48/7)           | Female| 32   | Positive   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 771   | T1           | PS             |
| 10 (EGB68/8)          | Male  | 27   | Positive   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 403   | LAM10_CAM    | MDR            |
| 11 (AMH9/10)          | Female| 38   | Negative   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 403   | LAM10_CAM    | POLY-S and E   |
| 12 (C12/4)            | Female| 27   | Positive   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 403   | LAM10_CAM    | MONO-R         |
| 13 (AMH48/7)          | Female| 32   | Positive   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 1580  | T1           | PS             |
| 14 (H96)              | Female| 38   | Positive   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 1580  | T1           | POLY-I, S and E |
| 15 (UCH006)           | Female| 25   | Positive   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 1580  | T1           | PS             |
| 16 (T35/7)            | Female| 34   | Positive   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 403   | LAM10_CAM    | PS             |
| 17 (IW2)              | Male  | 49   | Negative   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 838   | LAM10_CAM    | POLY-R and S   |
| 18 (M69)              | Male  | 35   | Negative   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 403   | LAM10_CAM    | MONO-E         |
| 19 (H38/8)            | Female| 38   | Positive   | gggggggggggggggggggggggggggggggccccggggggg       | 77777777760771    | 403   | LAM10_CAM    | POLY-S and E   |

MDR: Multidrug resistant, POLY-S and E: Poly resistance to streptomycin and ethambutol, POLY- I, S and E: Poly resistance to isoniazid, streptomycin, and ethambutol, MONO-R: Mono-resistance to rifampicin, POLY-R and S: Poly-resistance to rifampicin and streptomycin, MONO-I: Mono-resistance to isoniazid, MONO-S: Mono-resistance to streptomycin, MONO-E: Mono-resistance to ethambutol, PS: PAN susceptible, SIT: Spoligotype international type
mycobacterial interspersed repetitive unit – variable number of tandem repeats in addition to spoligotyping. This would have helped to rule out laboratory cross-contamination. Despite these, our findings provide reasons to substantiate underestimation of DR-TB in Nigeria and by extension, Africa.

**Conclusion**

Our findings confirm the underestimation of DR-TB in Nigeria. We advocate active case finding and resistance testing with modern laboratory tools to correctly estimate the true burden of TB and DR-TB in endemic countries of Africa. This will require more concerted regional and technical efforts at regular surveillance and use of modern diagnostic tools to determine the problem of resistant TB in Nigeria and other African countries, where the disease has remained unabated.

**Acknowledgment**

We sincerely appreciate the support of Dr Wole Lawal of the Oyo State Hospitals Management Board, Ministry of Health, Oyo State and the Tuberculosis Field Supervisors at the different DOTS centers where this study was conducted in Oyo State.

**Financial support and sponsorship**

This research was partially funded by an African Doctoral Dissertation Research Fellowship award offered by the African Population and Health Research Center (APHRC) in partnership with the International Development Research Centre (IDRC) and Fogarty International/NIH grant (No. D43TW007995).

**Conflicts of interest**

There are no conflicts of interest.

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