NATIONAL DRUG-RESISTANT TUBERCULOSIS PREVALENCE SURVEY REPORT NIGERIA

August 2012

PREFACE

At present the NTBLCP has adopted the combined use of rifampicin, isoniazid, pyrazinamide, ethambutol and streptomycin as first-line anti-tuberculosis treatment regimen administered as a fixed dose combination. Of these fixed dose combination anti-TB drugs, rifampicin and isoniazid are the most potent currently available globally. When there is resistance to these two drugs a grave condition termed **Multi-Drug Resistant Tuberculosis (MDR-TB)** is said to have emerged. In addition to resistance to these two drugs there may be resistance to the other drugs in the regimen.

Multi-drug resistant TB (MDR-TB) has emerged as a major problem in the world, the estimated incidence is 440,000 annually[1] and the world prevalence is estimated to be 2-3 times the incidence. There are documented cases of MDR-TB in 90 countries in the world and is seen as a major clinical and public health threat in the world. It is found mostly among the poor and the middle class, MDRTB is developed when a TB patient is poorly managed (interrupted drug supply, poor drug quality, poor adherence to treatment, inappropriate prescription) causing the mycobacterium tuberculosis to develop resistance against the two major first line anti-Tuberculosis drug (Rifampicin and Isoniazid). The laboratory diagnosis is sophisticated and requires much expertise, the drugs are not readily available and a lot of financial and human resources are required to manage an MDR-TB patient.

The Imperative for a Drug Resistance Survey

The management of drug susceptible TB requires the daily use of the above mentioned anti-TB drugs for 6 months or more, compared to a lot of other prevalent infectious diseases, this is very demanding to the affected persons as well as to the health system being also health and economic resources depleting. The management of DR-TB is even more resource depleting, complicated, expensive, and challenging. A successfully managed case of MDR-TB will cost averagely 300 times more than a drug susceptible TB. MDR-TB cases require 20-24 month of daily combination therapy, the drugs are far more expensive and potentially more toxic. In the light of the above it becomes imperative to determine the presence, prevalence and pattern of drug resistant TB in Nigeria. This will inform programmatic interventions (including the developing routine surveillance) policy and planning for the efficient and effective control of anti-TB drug resistance. Furthermore data generated from this survey will form a baseline for the monitoring of trends and continued surveillance of anti-TB drug resistance.

One case of sputum positive TB can potentially infect 10-15 new cases averagely in a year and this index case can possibly be a DR-TB case. Programme and clinical experience have shown that there are MDR-TB cases in Nigeria. In the light of the above it becomes imperative to quantify the magnitude of this problem.

The Survey

The National Drug Resistant Tuberculosis Prevalence Survey was cross-sectional, prospective and facility-based. A standard scientifically appropriate methodology i.e. a 30-cluster sampling technique was used. The survey methodology and entire protocol met all requirements and obtained full clearance of the National Health and Ethical Research Committee of Nigeria and IRB approvals of CDC Atlanta USA. Preparatory to the

main survey a time and site limited Pilot survey was conducted to test the survey tools, instruments, methods and logistic arrangement planned for the main survey, the findings of which informed the conduct of the main survey. Eligible patients were admitted into the survey in the normal course of routine operation of the TB treatment centers (DOTS centers). The survey did not constitute a deviation from standard routine functioning of the treatment centers. Patients were totally free to decline at no penalty and participation was free of any cost to all consenting patients. The survey did not involve the administration of any medications or test procedure to the patients other than the usual drugs and test they normal would routinely be administered for the care of their TB condition. Sputum samples were routinely collected from TB suspects. Samples collected from eligible consenting suspects, were confirmed positive for TB bacilli by AFB sputum smear examination. These were then subjected to Line Probe Assay and drug susceptibility testing to determine resistance patterns. Blood samples taken from patients were screened for HIV following national guidelines. The skill building, infrastructural support and hands-on re-training preparatory to the survey further enhanced the capacity of the TB treatment facility centers and workers.

This National Drug Resistant Tuberculosis Prevalence Survey sought to determine the prevalence, nature and pattern of TB drugs resistance among TB patients in the country as well as the prevalence of HIV co-morbidity among them. The outcome of this survey will inform proper programme planning and strategic direction for a comprehensive and effective TB control in Nigeria and thus greatly improve treatment outcome and reduce morbidity and mortality among TB and HIV patients as well as reducing TB transmission in the general population.

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Dr C. Onyebuchi Chukwu

Honourable Minister of Health

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Abbreviations

AFB	-	Acid Fast Bacillus
AIDS	-	Acquired Immune Deficiency Syndrome
ASM	-	American Society of Microbiologist
CDC	-	Centers for Disease control and Prevention
CPT	-	Cotrimoxazole Preventative Therapy
DOT	-	Direct Observation of Treatment
DOTS	-	Directly Observed Treatment Short-course
DR-TB	-	Drug Resistant Tuberculosis
DST	-	Drug Susceptibility Testing
FCT	-	Federal Capital Territory
FGN	-	Federal Government of Nigeria
FMOH	-	Federal Ministry of Health
GDF	-	Global TB Drug Facility
GFATM	-	Global Fund to Fight AIDS, Tuberculosis and Malaria
H	-	Isoniazid
HBC	-	High Burden Countries
HIV	-	Human Immunodeficiency Virus
HMIS	-	Health Management Information System
ILEP	-	International Federation of anti-Leprosy Associations
INH	-	Isoniazid
IPT	-	Isoniazid Preventative Therapy
IUATLD	-	International Union Against Tuberculosis and Lung Disease
KNCV	-	Royal Netherlands Tuberculosis Foundation

LGA	-	Local Government Area
LPA	-	Line Probe Assay
MDR-TB	-	Multi-Drug Resistant Tuberculosis
M.tb	-	Mycobacterium Tuberculosis
MTBC	-	Mycobacterium Tuberculosis Complex
NGO	-	Non-governmental Organization
NIMR	-	Nigerian Institute for Medical Research
NTRL	-	National Reference Laboratory
NTBLCP	-	National Tuberculosis and Leprosy Control Programme
NTBLTC	-	National Tuberculosis and Leprosy Training Centre
NTM	-	Non-tuberculous Mycobateria
PHC	-	Primary Health Care
PLHIV	-	People Living with HIV and AIDS
QA/QC	-	Quality Assurance/ Quality Control
R	-	Rifampicin
RIF	-	Rifampicin
SRL	-	Supra-National Reference Laboratory
TB	-	Tuberculosis
TB/HIV	-	Tuberculosis and Human Immune-deficiency Virus co-infection
TBCTA	-	Tuberculosis Coalition for Technical Assistance
TBL	-	Tuberculosis and Leprosy
USAID	-	United States Agency for International Development
WHO	-	World Health Organization
XDR-TB	-	Extensively Drug Resistant Tuberculosis
ZRL	-	Zonal Reference Laboratories

Executive Summary

TBD after review is completed

Executive Summary (contd.)

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INTRODUCTION

Multidrug resistant TB is said to occur when *M. tuberculosis* bacilli, the bacilli responsible for tuberculosis, are resistant to Rifampicin and Isoniazid; the 2 most potent anti-tuberculosis drugs. Other than these key drugs a mosaic of resistance to any or combinations of component drugs of the standard anti-TB regimen have also been document in global efforts to control and cure TB in human populations. Extensively drug resistant TB (XDR-TB) is said to occur when in addition to MDR-TB resistance the bacilli strains shows resistance to any of the second-line injectable anti-TB drugs (Kanamycin, Amikacin or Capreomycin) and any of the floroquinolones. Drug resistance TB portends a very grave danger and particularly challenging to treatment interventions. XDR-TB is virtually untreatable and should by all means possible be avoided

Nigeria is within the top ranking high TB burden countries of the 22 high TB burden countries in the world and ranks 2nd in Africa. Annually Nigeria detects approximately 93,000 TB cases estimated at about 30% of total estimated TB cases in the country. The WHO estimates MDR-TB prevalence to be currently at 2.2% and 9.4% among new and retreatment cases respectively. With the high case notifications and the epidemiologic significance of occurrence of MDR-TB strains, it becomes imperative that a true and accurate determination of the MDR-TB burden and the pattern of anti-TB mono- and poly drug resistances in Nigeria be done to inform programme case management and overall planning of TB control activities.

OBJECTIVES

The objective of the National drug resistance prevalence survey in Nigeria is to determine the prevalence of MDR-TB among new and retreatment smear-positive TB cases in Nigeria. Using internationally recommended standards and guidelines[2], this survey determined the national cross-sectional prevalence rate of primary and acquired anti-tuberculosis drug resistance (including M/XDR-TB) in Nigeria. It is hoped that this will form a baseline for continued surveillance to determine trends of MDR-TB in Nigeria.

Data derived from this survey can be used for evidence-based program planning and implementation, and for identifying and characterizing the epidemiology of MDR -TB and for planning interventions to reduce related morbidity and mortality. It also estimates the current prevalence of HIV among AFB sputum positive TB patients.

Secondary Objectives:

Additional objectives of this survey included

- To provide program-level data regarding the prevalence and pattern of drug resistant TB and MDR -TB in various populations (new TB cases, re-treatment TB case,[a] HIV-infected TB cases)
- To provide evidence bases upon which recommendations can be made to the NTBLCP for the controlling drug-resistance and MDR -TB in Nigeria
- To determine the prevalence of HIV among AFB sputum-smear positive TB respondents

[a] Re-treatment cases will be further classified by previous treatment outcome: treatment failure, relapsed, return after default, chronic as per WHO guidelines.

METHODOLOGY

Survey Protocol

A survey protocol detailing the justification, aims and objective of the survey and the methodology was developed and subjected to peer and institutional review board (IRB) reviews. The protocol was approved by the National Health Research and Ethics Committee of the Federal Ministry of Health and IRB of CDC HQ Atlanta. Due to the nature of the survey requiring transfer of samples between Nigeria and USA, a Materials Transfer Agreement was also signed between the CDC and government of Nigeria.

Survey Design

This survey is a national cross-sectional survey of adult patients with sputum smear positive TB attending DOTS clinics in Nigeria. The study was designed to ensure that sampling adequately represents all pulmonary TB patients in the six geo-political zones of the country. All the states in each zone had equal chance of representation as determined by their disease burden.

A modified weighted 30-cluster sampling technique proportional to the size of new smear positive TB cases was used.**Error! Bookmark not defined.** A national sampling frame of all TB DOTS clinics in the country with a cumulative list of all sputum smear positive TB cases by diagnostic facility in year 2007 notification was compiled; beginning with the facility that had the highest notification returns. A sampling interval was obtained by dividing the cumulative sputum smear positive population by 30. This sampling interval was applied to identify 30 clusters from the list of the country's TB diagnostic centers. See the 'Sampling Procedure' section

Survey Population

The study population was drawn from two broad groups of patients:

Group 1: New AFB sputum smear-positive patients registered at designated study sites that had never been treated for TB. (i.e. TB treatment category 1 patients)

Group 2: AFB sputum smear-positive patients registered at designated study sites who have been treated previously with anti-TB drugs for more than one month and were classified in any of the following groups:

cases of treatment failure, relapses, return after default (RAD) and Chronic cases (i.e. TB patients who have failed category II treatment and are not on second line anti-TB drugs).

Survey Areas

To obtain a representative national sample, the study was carried out in 30 clusters selected across Nigeria (this expectedly was across all the six geo-political zones that included the Federal Capital Territory (FCT)).

Fig 1: Nigeria DR-TB Survey – selected clusters

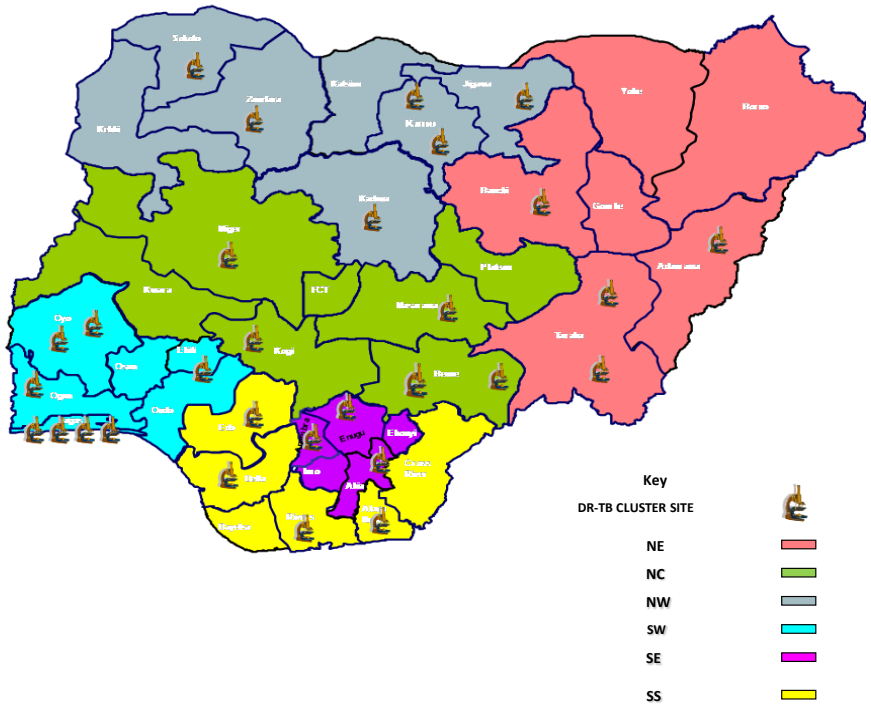


Fig 1: Nigeria Drug resistance TB survey - selected clusters (PPS sampling)

Survey Centers

The study centers/clusters included peripheral, secondary, and tertiary health facilities where TB is diagnosed as per national guidelines (i.e. DOTS treatment sites); these centers comprise of public and private chest clinics, hospitals and TB microscopic centers where TB patients are diagnosed and managed across the country using the DOTS strategy.

For the purpose of this study, a cluster is defined as a health facility or a group of contiguous health facilities that notify at least twenty new smear positive cases per month.

Sampling Procedure

Sampling was done by population-proportionate (weighted) 30-cluster sampling technique covering all the 6 geo-political zones. A list of all microscopy and DOTS centers in each zone was compiled with the numbers of new and retreatment sputum-smear positive TB cases seen in the year 2007. A cumulative population of all new and retreatment smear positive TB patients registered in 2007 was also compiled. The sampling interval was determined by dividing the cumulative population by thirty (30) thus:

New smear positive cases registered by the NTBLCP in 2007 were 47,298. The sampling intervals for the 30-cluster sampling was therefore 47298/30 = 1,576.6

Using the sampling interval calculated, the thirty clusters were selected using MS Excel random function. The cluster boundary selected was considered as a focal cluster site. Due to the limited number of re-treatment cases in the program and the practice of 'referral centre' for the treatment of Cat. 2 cases in a number of state TB programs, sites with high number of retreatment cases (often Cat. 2 referral sites) reported in 2007 were added to the focal sites. If the patient population of the focal site and the high retreatment site meet our cluster population definition of a minimum of 240 sputum positive cases in 2007 report, such sites were retained as the cluster survey sites. If the focal site and the high retreatment site patient population were below our cluster definition, further random sampling is carried out using Epi-info within the cluster until the target population is met. By this selections total of 72 sites in 24 states in the 6 geopolitical zones were involved in the survey.

Sample Size

Sample Size (New Cases)

The sample size was calculated using the formula:

$$n\ (SRS) = \frac{N*Z^2*P*(1-p)}{D^2*(N-1) + Z^2*P*(1-p)}$$

Where

n (SRS) = sample size based on simple random sampling or complete sampling

N = smear-positive cases for 2007

Z = z-value that corresponds to the desired Confidence interval (CI)

For a 95% CI, z = 1.96

D = absolute precision, 1%

P = expected prevalence of MDR, 1.9%.

n (cluster-sampling) = Sample size for cluster sampling

Design effect = 2

	New TB Cases	Retreatment TB Cases
N	42,545	4,753
Z (95%)	1.96	1.96
D	1%	5%
P	1.9%	9.3%
Design effect	2	2
Drop out rate	10%	10%
Sample size (SRS)	1547	277

$$n\,(SRS) = \frac{42{,}545 \times 1.96^2 \times 0.019(0.981)}{(0.01)^2 \times (42{,}545) + 1.96^2 \times 0.019 \times 0.981}$$

$$= 702.798\ (\cong 703)$$

For cluster sampling, n (cluster – sampling) = n (SRS) x design effect

= 702.798 x 2

= 1405.596 (≅1406)

Plus 10% drop out rate = 1406 +141

Calculated sample size = 1547

Number of clusters = 30

Number of patients that each cluster was expected to contribute was 1547/30 ≅ 52 (51.56)

Sample Size (Re-treatment Cases, RAD, Relapses, Chronic Cases)

A 9.3% prevalence of drug resistant TB was assumed among this group based on reports from WHO estimates for Nigeria in the literature.

The sample size for this group is therefore calculated using the formula:

$$n\,(SRS) = \frac{N^* Z^2 * P^* (1-p)}{D^2 * (N-1) + Z^2 * P * (1-p)}$$

$$n\,(SRS) = \frac{4{,}753 \times 1.96^2 \times 0.093 \times 0.907}{(0.05)^2 \times (4{,}753) + 1.96^2 \times 0.093 \times 0.907}$$

$$= 126$$

For cluster sampling, n (cluster – sampling) = n (SRS) x design effect

$$= 126 \times 2$$

$$= 252$$

Plus 10% drop out rate = 252 + 25 = 277

Calculated sample size = 277

Number of clusters = 30

Number of patients averagely per cluster: 277/30 = 9

Total expected sample enrollment = 1547 (new cases) + 277 (retreatment cases) = 1824

Survey Enrolled respondents:

At the conclusion of this survey a total of 1723 survey respondents were successfully enrolled. This consisted of 1414 new cases, 305 retreatment cases and 4 unknown/unclassified treatment statuses. These were from across all the thirty (30) clusters in the survey; every geopolitical zone of the country was therefore sampled.

Patient Intake/ survey management

Consequent to the lessons learnt from the experience of the pilot survey and due to the limitation of availability of only 2 reference laboratories with capacity to analyze sputum samples as per the protocol for this survey, the implementation of the survey roll out was staggered/phased such as to avoid all survey cluster sites starting enrollment concurrently to avoid deluged of sputum sample beyond the infrastructural capacity of the laboratories to cope. This also allowed for increased proficiency in the laboratory experience with regards to the management of this survey.

Patient's intake was carried out by the general health worker at the DOTS clinic throughout the duration of the study. Enrollment of respondents into the survey was based on satisfaction of the inclusion criteria for the survey and continued in a consecutive sampling approach until the required sample size for that centre/cluster is attained. Each center in a cluster contributed the number of patients that is proportional to the center's AFB positive case contribution to the cluster population in 2007 case notification that was used in designing the National sampling frame. All TB suspects that met the inclusion criteria were registered for the survey.

Inclusion Criteria

Patient eligibility for this study had the following criteria:

- Strongly AFB sputum-smear positive TB cases of grades +, ++, and +++ who have not received any previous anti-TB treatment or have received for less than four weeks.
- Respondents with AFB sputum smear grades of 'scanty' were considered eligible when such respondent were HIV positive persons or has been declared 'TB treatment failure cases' and being re-registered for TB retreatment.
- Sputum-smear positive TB cases that have been on treatment for upwards of 5-months and still smear AFB positive or that qualify for re-treatment according to National TB guidelines.
- Patients who have failed re-treatment

- Patients should be 15-years old and above.
- Patients who have given informed written consent to participate in the study
- Patients who decline HIV testing will still be included in the survey.

Sputum Collection:

Diagnosis of tuberculosis was done as per national guidelines. All patients who presented at the study clinics with suspected active tuberculosis were instructed on how to produce sputum specimens of suitable quality. Each patient was instructed to produce and submit 3 sputum specimens within two consecutive days. The specimens were collected in standard screw-capped leak-proof sputum containers with specific clinic identification number. The first sputum specimen were obtained on the first contact with the center (spot specimen) while the second specimen was an early-morning specimen produced at home after cleaning the mouth with water. The third specimen is another "spot" specimen produced at the study clinic when the early morning specimen is submitted.

The three sputum specimens were processed at the same time. Survey eligible patients that were registered for the survey were drawn from those whose two or all the sputum specimens were found to be strongly AFB smear positive (as defined in the eligibility criteria). The objectives of the survey were clearly explained to these TB smear positive persons and their consent for participation sought. Only those who give informed written or oral consent are enrolled for the study in each study clinic. This informed consent also informed that all patients enrolled in the study that were determined to be resistant to TB treatment, were eligible for appropriate 2nd-line TB treatment at the designated treatment center.

Upon confirmed eligibility for inclusion and informed consent, the patients are interviewed to collect information on their bio-data, clinical history and any previous treatment received (Form 2). The patient's history is carefully obtained and any available medical records reviewed to clearly ascertain whether or not the patients had received previous anti-TB treatment. Classification of patients as never treated or previously treated will be a critical issue for distinction between primary and acquired drug resistance.

As in national guidelines, all patients diagnosed with TB, were offered HIV counseling and testing. Healthcare professionals in the TB units were trained to administer counseling for both pre- and post-HIV testing, receipt of results, and referral to appropriate services. Blood samples of those consenting to be tested for HIV were collected in the health unit and sent to the designated HIV diagnostic laboratory. The laboratory procedures used were the standard testing procedures used by health providers throughout the country as per national HIV testing algorithm. As is standard practice, the HIV test results were sent by the laboratories to the soliciting health units/study centers.

The HIV test results were registered in the Patient Clinical Investigation Form as well in the routine patient records of the NTBLCP. A copy of the questionnaire was kept in the health records of the patient. Accepting an HIV test after appropriate counseling was NOT compulsory in order for the patient to be accepted for participation in the drug resistance survey. Patients that declined HIV testing were recorded as such and analyzed and presented as a group.

Patient Registration

Each patient meeting the inclusion criteria was enrolled and assigned a serial referral number which were pre-printed on adhesive labels which were placed on the intake forms. This number shows the geo-political area code, state code, the cluster number, study/health centre code and the patient's number. For example:

Patient Survey ID: *'SWZ – LA –17- LA1 – 0001'.*

Implies:

Geo-political zone:	SWZ	-	South Western Zone
State:	LA	-	Lagos
Cluster Code:	17	-	Cluster Center Code (options are Between 1 - 30)
Study Site:	LA 1	-	Mainland Hospital Yaba
Serial Patient Num:	0001	-	Respondent Number 0001 enrolled

These codes in a set form the unique survey respondents' ID. Respondents were registered into the routine TB treatment register as is normally done routinely in the TB Control programme. A facility survey register was provided that recorded each survey respondents' survey as well as routine TB programme identifications for the respondent. This register was used for tracking of respondents who were tested positive for MDR-TB to enable enrollment into a MDR-TB treatment centre.

At the study center, the following three standard forms were utilized:

Form 1 - Sputum Request/Shipment Form (Annex 2)

Form 2 - Patient Clinical Information Form (Annex 3)

Form 3 - Bacteriological Examination Results Form (Annex 4).

Form 1 – Sputum Request/Shipment Forms

This form is routinely used in the TB program for requesting sputum examination. It was completed for each enrolled patient at the study site. The information includes identification of the patient, basic demographic information, dates of collection of the sputum and the results of the smear examination at the study clinics. Each TB suspect that satisfied the survey bacteriological inclusion criterion, in addition to consenting for the study was enrolled into the survey and survey identification stickers (all bearing same ID for each respondent) were applied unto the respondent's forms. For each enrolled patient, the essential information in the sputum request form were transferred into the AFB result section of the Bacteriological Examination Result Form and made into triplicate.

Form 2 – Clinical Examination Form

This form was completed for each enrolled patient at the point of registration (i.e. study site). The form consists of four sets of data: patient identification (survey ID number) and other demographics, patient history, and documented data on previous treatment episodes and final decision on whether the patient has been previously treated or not. To help the patient remember any previous TB treatment a minimal set of questions

(reflected in this form) were asked of all patients indicating no previous TB treatment. This form was completed at the clinical interview of respondents and duplicated (photocopied) once reviewed by the site survey supervisor. A copy was retained at the survey site while the other was returned to the survey secretariat by the supervisors.

Form 3 –Bacteriological Examination Results Form

This form was initiated for each enrolled patient at the point of enrollment (i.e. survey site) with the completion of the AFB results. It consisted of three sets of data/sections in addition to enrolled patient survey ID: AFB smear results for each of the sputum cups, Line Probe Assay (Hain assay) result and a section on culture results. The last two tests (Hain assay and culture) were done in the national reference laboratories. This form was completed in triplicate. Two copies were sent along with the sputum specimen to the national reference laboratories (one of these eventually to the designated supranational reference laboratory along with the Hain assay report if MDR-TB was observed or if such sample was among the randomly selected for rechecking). The original copy was kept in the patient's record file at the study clinic. At the end of the survey, these original forms were sent from the study clinics to the survey secretariat.

Patient Replacement

Patients who met the inclusion criteria but could not be included in the study such as patients who failed to return to the study clinic to submit subsequent sputum specimens within twenty-four hours were replaced by continued consecutive sampling.

Patient care for those identified with drug-resistant TB:

For this survey MDR-TB treatment took place in University College Hospital Ibadan. This survey supported the NTBLCP to report TB treatment resistant findings to the respective patients' TB diagnostic center and to the treatment center. Using local treatment supporters, each identified patient was followed-up and provided with referral information to TB treatment center. The treatment center has trained staff, treatment protocols, and second-line anti-TB drugs. Patients initiated on second-line TB treatment were initiated on an 18 - 24 month treatment regimen as per national guidelines.**Error! Bookmark not defined.**

COLLECTION AND ANALYSIS OF SAMPLES

Sputum Specimen Collection/Preservation

The sputum specimens were collected, at all the designated study clusters, in rigid, wide-mouthed, screw capped sputum containers (sputum pots). The patient's serial referral numbers as well as sample identification for the three consecutive samples from the same patient (A, B and C) were pasted *on the side of the container*. No identification was written on the lid of the sputum containers to avoid any possible mix-up of specimens. The outer part of the sputum pots may have been contaminated with sputum during expectoration. These sputum pots were wiped with cotton wool soaked in 5% phenol solution, or comparable disinfectant, immediately after receipt to effect decontamination. TB suspects that were enrolled for the survey had their survey ID stickers pasted on sides of the sputum pots (same ID on each sticker). These were then packaged by 'triple packaging bio-safety techniques' for shipment to the national reference laboratories under cold chain conditions to arrive within maximum of four days of expectoration.

LABORATORY PROCEDURES

Microscopy at the Study Site

Loopfuls of sputum specimens were smeared on clean, grease-free, dry slides and dried. The methods for staining the smears for AFB in this survey were the standard Ziehl-Neelsen (ZN) and/or fluorescent staining techniques either, as may be in use at the different TB diagnostic centers.

Sputum specimens found to be AFB smear positive at the study site were appropriately transferred to the National TB Reference Laboratories (NTRL) for processing of the sputum and the Hain assay and culture. These samples were transported in safe sandwich boxes with accompanying forms to the NTRLs by a trained health worker or designated Courier Company skilled in handling of biologicals. Before transportation, the sputum specimens were kept in a cool place, preferably a refrigerator at +4ºC. It was transported in a properly

maintained cooler with ice packs. Specimens were delivered at the national laboratory within 4 days of expectoration.

Hain assay

The AFB sputum smear positive specimens underwent a Line Probe (Hain) assay to determine the MDR Mycobacterium strains. This assay determined the samples susceptibility to rifampicin (RIF) and isoniazid (INH) anti-TB drugs as well as confirmation as a MTBC strain or otherwise. The following procedures were used to carry out the above listed investigations.

Two most positive smear specimens per patient were forwarded to a National TB Reference laboratory (NTRL) for further testing. All sputum specimens received at the NTRL were properly documented on receipt, each specimen was digested, decontaminated and concentrated using the NALC-NaOH standard method, and then tested by Line Probe assay (Hain) assay to detect bacilli of the *Mycobacterium tuberculosis* complex (MTBC) and whether the strain has mutations that were associated with resistance to INH and RIF. Specimens that are positive for the MTBC and have no mutations (susceptible to INH and RIF) were reported as sensitive with no culture performed. Specimens that were found to be resistant to either or both drugs, or with Hain assay results that were un-interpretable (ie MTBC member with mutations not clearly defined) or negative for MTB were inoculated onto solid media to detect growth, and when growth is obtained, the isolate was sent to CDC Atlanta (the supranational laboratory) to undergo first and second-line drug testing. The following procedures were used to carry out the above listed investigations.

Culture

In this survey, solid (e.g. Lowenstein-Jensen (LJ)) media was used in-line with CDC guidelines for sputum culture. For primary cultures, a plastic pipette was used to transfer a 0.2mL portion of the sputum sediment to the surfaces of two slopes of LJ medium. All the slopes were incubated at 37º C and observed on days three, five, and seven to screen for rapid growing organisms, and then examined weekly for 8 weeks. Some *Mycobacteria* grow very slowly and extended incubation beyond 8 weeks may be beneficial in some cases. Any

tubes showing obvious contamination (e.g. growth of molds) were discarded. Slopes showing confluent growth or isolated colonies of a buff, yellow or orange color were closely examined to determine if TB colonies were present and in some cases, mixed with a non-tuberculous mycobacterial species. Colonies from all resultant growth were examined for growth rate, morphology, pigmentation and acid-fast properties. The date of appearance of the colonies was recorded on the laboratory work sheet. Once the growth has been confirmed as a slow-growing, non-pigmented AFB, one LJ slope was kept for storage at the NTRL and one was shipped in a three capped safe containers for infectious specimen according to international standard by designated courier service to CDC Atlanta TB Supranational Reference Laboratory (SRL) for identification and drug susceptibility testing. All cultures were stored in a deep-freezer at -20ºC until retested or excluded from further testing. Any processed specimen remaining was stored at 2-8°C for at least the duration of the study. Any residual remaining from Hain assay testing was stored at 2-8^{o} C overnight to allow repeat PCR and hybridization if required.

Identification of Isolates

Pure cultures of the isolates are identified at the NTRL and at the SRL based on cultural characteristics and the results of the Hain assay or the Capilia antigen detection assay. Colony appearance, consistency, rate of growth (slow and rapid growers) will be noted. Although preliminary examination of the cultures may suggest that the organisms are tubercle bacilli, it is necessary to carry out confirmatory tests. Isolates suggestive of *M.Tb* will be sent by the NTRL to the SRL for drug susceptibility testing (DST) along with appropriate documentation (Forms 1 and 3).

Drug Susceptibility Tests

At the Supra-national Reference Laboratories, drug resistance tests to isoniazid, streptomycin, rifampicin, ethambutol, select flouroquinolones, amino glycosides, and capreomycin were performed on all colonies identified as belonging to the MTBC. The drug susceptibility tests were carried out by the proportion method using both 7H10 Middlebrook medium and the Hain assay assay. The 7H10 media were prepared at CDC Atlanta according to standard methods.

Quad plates with drug-containing media were inoculated with 0.1 ml of 10^{-3} and 10^{-5} dilutions of the even cell suspensions of each isolate. Control medium without drugs were also inoculated with the same concentration and volume of cell suspensions. All the inoculated plates were sealed with parafilm to prevent drying and incubated at 37ºC and examined after 3 to 4 weeks. The number of colonies on the control and drug-containing media were counted. The critical proportion was taken at 1% for all the drugs. Any isolate showing more than 1% bacterial population growing on the drug-containing medium as compared to the control medium was considered resistant.

Results were recorded on *Anti-tuberculosis Drug Resistant Results* form (Annex 5). A copy of each completed form and an electronic dataset of all results (in Ms Excel) was sent back to the national survey secretariat.

Survey Center Roles and Responsibilities

The activities at these TB diagnostic clinics (i.e. study sites/clusters) were focused on patient intake and sample collection in accordance with the adopted sampling strategies. The main activities at this level comprise of the following:

- Consent taking for enrollment into the survey and completion and signing of consent form
- Registration of designated number of new cases (Category I) of TB presenting within the period of the study.
- Registration of re-treatment cases of TB that have received treatment but were still smear-positive.
- Interview of patients, collection of clinical information and completion of relevant forms.
- Collection of sputum samples from suspects and subsequent cases registered for the survey.
- Proper labeling/identification of the sputum samples (both spot and early morning), proper storage/preservation of samples.
- Accurate direct AFB sputum-smear microscopy for TB diagnosis as per national guidelines.
- Proper transportation of all sputum-smear AFB-positive samples to the national reference laboratories.

- Proper documentation and maintenance of all data within the clinic records for each patient registered for the study.
- Appropriate treatment for all TB cases identified as a result of this survey. This was carried out by the National TB (DOTS) control program.
- Appropriate treatment for cases of drug-resistant TB that were identified as a result of this survey. capacity for this treatment is very limited in the National TB Control Programme. For the purposes of this survey UCH MDR-TB Treatment centre was used. The identified patients were admitted for the first 6 months of treatment or till sputum conversion if it goes beyond that. The feeding and hospital expenses were borne by the program. The Green light committee had approved the 2nd line drugs that the patients identified were treated with. Strict compliance with the National MDR-TB and TB infection Control guidelines was observed.

National Reference Laboratory Responsibilities):

The primary responsibility of the National reference laboratories was to receive, process, confirm the presence of M.tb by Hain assay,[b,3,4] culture all MDR-TB positive by Hain assay specimens and other (10%) randomly selected non-MDR-TB samples and dispatch (through a certified courier) the isolate from these to the Supranational Laboratory for drug susceptibility testing (DST).

Itemized, the main activities of the National Reference Laboratories were:

- Adequate documentation of all specimens received from the cluster laboratories.
- DNA strip – Hain assay of all AFB positive specimens for Identification of MDR-TB
- Culture of specimens that are HAIN positive
- Transportation of isolates to the Supranational laboratory

[b] The PCR-assay proposed for this survey, will be the Genotype Hain assay developed by Hain Lifescience for the molecular genetic identification of *M. tuberculosis* complex and its resistance to rifampicin and/or isoniazid by examining mutations in the *rpoG* and *katG/inhA* genes respectively.

- Readiness to provide HAIN Susceptibility report to the Supranational laboratories if requested
- Reporting of MDR-TB patients identified to the survey secretariat for identification of the appropriate cluster, facility and respondent for treatment in the designated facilities (MDR-TB treatment centre)
- Proficiency testing between the National Reference Laboratories and the Supranational Reference laboratories
- Proper documentation of information and data generated for each patient.
- Reporting of drug-resistant cases identified to the survey coordination committee.

Supranational Reference Laboratories

The primary responsibilities of the supranational reference laboratories were to receive and process isolates from the National reference laboratories for strain identification and drug susceptibility testing confirmation. The Supranational Laboratory was also responsible for appropriate recording and reporting, quality assurance and proficiency testing of samples it analyzed. The shipping of isolates to the Supranational Reference Laboratory (SRL) was done following the International Air Transport Association (IATA) regulations and international standards for shipping of infectious materials.[5,6]

Itemized, the main activities at the supranational reference laboratories level comprised of the following:

- Adequate documentation of all specimens received from the national laboratories.
- Sub-culturing of all primary cultures received.
- Identification/characterization of strains of *M.tb* isolates.
- Drug susceptibility testing of *M.tb* isolates.
- Reporting of drug-resistant cases identified to the National Drug Resistance Survey secretariat
- Proficiency testing between the National Reference Laboratories and the Supranational Reference laboratories.

QUALITY ASSURANCE

A system of internal and external quality control was put in place for the survey. This system of control focused on each essential component of the survey namely: sampling (selection of the patients included in the study), obtaining accurate clinical information (distinction between never treated and previously treated patients), recording and reporting, and sputum transport and laboratory techniques both internally and externally. . Standard operating procedures (SOPs) were developed to enhance quality control as per the protocol. Specific areas of QA/QC focus were:

Sampling/Patient Intake

Monthly supervisory visits were made by the representatives of the central coordinating team or designated staff to the study clinics to ensure that patients were enrolled according to the sampling method adopted and that data and samples were being sent to the zonal reference labs as per the protocol. Since consecutive patients were to be enrolled, this was checked during these supervisory visits by comparing the TB Register of the chest clinic and the patients included in the survey.

Interview for Clinical/Patient Information

To help ensure adequate quality assurance at regular intervals during the survey, the collected interview forms were checked carefully for deficiencies. Also the reliability of the information recorded was assessed. Training was provided to clinic staff prior to the survey initiation to ensure an understanding of the survey, interview, and reporting processes.

Laboratory Techniques

At the study clinics, the process of sputum collection (including sputum quality and quantity), sputum transportation, storage and dispatch of sputum shipment forms was supervised at regular intervals by the State

TBL control officer, LGA TB supervisor, the WHO TB National Program Officer (NPO), and the central lab coordination team. To ensure that results of susceptibility testing are reliable and comparable, a system of quality assurance and SOPs were put in place.

Internal Quality Control of Susceptibility

Standardized procedures for AFB smear staining, susceptibility testing and mycobacterial culture were developed and followed. Quality assured Hain assay kits were commercially obtained from Lifesciences® which were used for all specimen testing done in-country. Also SOPs for the conduct of line probe assay was developed and followed strictly. Equally, the standardized procedure for the formulation of media was followed and appropriate SOPs developed for this and strictly adhered to.

International (External) Quality Control of Susceptibility Test

International Quality Control of susceptibility testing was carried out by exchanging samples of *M. tb* in two directions: from the Supranational Reference Laboratory to the National Reference Laboratory and from the National Reference Laboratory to the Supranational Reference Laboratory.

Prior to the initiation of the survey, the designated Supranational Reference Laboratory tested for the sensitivity, specificity and reliability of the Hain assay of the national laboratories. The results obtained at the National Reference Laboratory were compared (sensitivity, specificity, reliability) to those obtained at the Supranational Reference Laboratory. The procedure was double-blinded. The minimum required agreement was defined for each drug and was 100% for Isoniazid and Rifampicin.

A sample of the strains isolated during the survey was sent to the Supranational Reference Laboratory to be retested. The results were compared for agreement with respect of each drug. At the end of the study 100% of resistant strains and 10% of the susceptible strains were sent to the Supranational Reference Laboratory for validation.

Sensitivity, specificity and accuracy of susceptibility testing was calculated for the national reference laboratory and for each of the drugs tested. The operational definitions of the parameters measured were:

Sensitivity – the ability to detect true resistance

Specificity – the ability to detect true susceptibility

Concordance (Efficiency or Accuracy) – the ratio between the number of correct results and the total number of results

Predictive value for resistance – the rate of true resistance to total resistance

Predictive value of susceptibility – the rate of true susceptibility to total susceptibility

Data Collection and Reporting

At regular intervals (of about 2–3 months) during the intake period the survey coordinator and the survey technical team tabulated all data produced by the study sites and the zonal laboratories. The survey coordinator and survey technical team made regular reports, based on these tables, to the key stakeholders. These reports included information on field work, such as enrolment of patients, quality of clinical information collected, transport or logistic problems, and contamination of samples. If the data or comments suggested that a significant problem has occurred or was a potential threat to the survey, the coordination team and The National Multi-Drug Resistant TB Committee in collaboration with key stakeholders analyzed the situation and developed a corrective plan of action.

Training and Personnel

Before the commencement of the actual survey, a 3 day training workshop was organized for all personnel to be involved in the study.

- All cadres of health workers (medical officers, TBL supervisors, data entry clerks, laboratory scientists/technicians, statisticians, community health extension workers) who were to be involved in the survey underwent training on overall survey, general roles and their specific roles.
- Health workers who were to be in-charge of intake of patients and intervals at the diagnostic centers involved in the study were identified and trained.
- Refresher training on preparation and reading of smears, decontamination of sputum samples, storage and transport of samples and proper registration at the study sites was done for all laboratory staff involved in the survey.

The broad training workshop focused on the following areas for the appropriate staff:

- The standardized criteria for enrolment of patients into the survey, the sampling methods, standardized modes of interviews for completion of the relevant forms.
- Standardized procedures for obtaining reliable and comparable data on previous treatment.
- Proper methods of sample collection, preservation and transportation to the reference laboratory.
- Standardized techniques for sample concentration and smear microscopy.
- Standardized techniques for culture, isolation and identification.
- Standardized techniques for drug susceptibility testing.

Prior to the start of the survey, laboratory staff from the 30 study cluster centers received refresher training in AFB smear microscopy and procedures necessary for recording patient and test information. Lab personnel were required to demonstrate proficiency in AFB microscopy techniques prior to their involvement in the survey. In addition, laboratory staffs at the National Reference Laboratories were trained and had to demonstrate proficiency in the processing of sputum using the NALC-NaOH procedure and specimen culture techniques. They were also required to demonstrate proficiency in smear-microscopy, culture, PCR-line probe assay techniques prior to their involvement in the survey.

DATA MANAGEMENT AND ANALYSIS

Data Collection

At monthly intervals, during the period of patients' intake, all data generated through the survey questionnaires (Patient Clinical Information forms) at each participating study site were collated by the designated LGA coordinator, Zonal program officer, and WHO national professional officer (NPO) and sent to the national survey secretariat. Laboratory data were collated at each of the National Reference Laboratories. The individual laboratory data were collated at these laboratories into an electronic dataset in Ms Excel software using similar capture templates.

Data Management

The laboratory datasets from each of the national reference laboratories were each subjected to 100% data check and validation comparing with initial data records (sputum request forms, Line Probe assay and sputum culture registers). Data from the supranational laboratory (CDC Atlanta) was collected into a summary electronic format in Ms Excel software

All the patient clinical information forms (survey questionnaire) collected from study sites as well as the laboratory datasets were entered using a double entry technique into an electronic database in Epi-Info version 6 (for windows), compared for discrepancies and validated against actual paper records. Thus a single overall survey database was created which was subsequently analyzed.

Data Analysis

Data analysis was done using Epi-Info version6 (for windows), comparative queries were also done in STATA and SPSS. Analytic queries (program) were done to determine the demographic characteristics, resistance rates, geographic distribution of resistance strains, and distribution of Mycobacterium species across various independent variables.

The point prevalence of drug resistance for Rifampicin and Isoniazid was calculated using the number of cases for whom Line Probe assay result were performed as denominator (see Fig 2). However, number of missing result e.g. due to contamination, negative cultures or insufficient growth for susceptibility testing, were also be reported. Drug resistance for Ethambutol, Pyrazinamide, streptomycin and second-line anti-TB drugs, were determine through culture and drug sensitivity testing (DST), resistance rates for these were therefore determined using the number of cases for whom DST was performed as denominator.

Analysis of drug resistance patterns to determine proportion of isolates resistant to each of the first and second-line drug tested, proportion that are multiple drug resistant (MDR)-TB, proportion that are XDR-TB and comparison of the pattern of resistance between HIV positive, HIV negative, and HIV unknown patients amongst other queries were also done. Further comparisons based on age, gender, geographic location, type of treatment case etc, were done

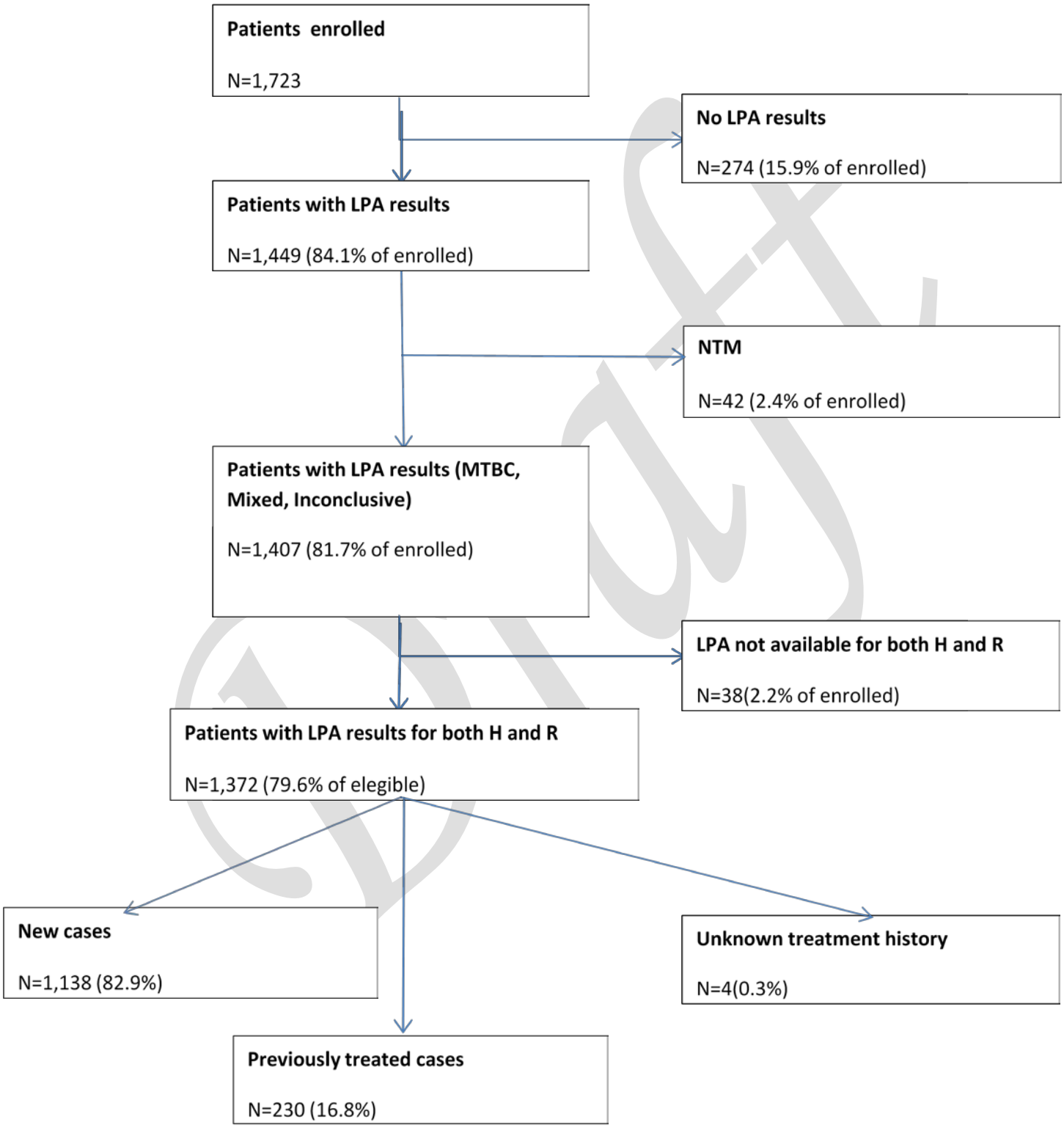
A summary table, describing proportion of patients with MDR-TB, mono-resistant to each drug, and to different combination of drug resistance was generated (see table 8); data were also stratified by HIV status to determine the relationship between TB drug resistance and HIV. Analysis for other potential factors associated with TB drug resistance was also done using the data collected.

Weighting and Imputation

The survey design assumed a hypothetical situation in which there will be equal number of survey respondents (52 new cases) in the enrollment for each cluster, however in reality some cluster met or slightly surpassed this target while some cluster will not have as much TB suspects reporting for diagnosis and treatment. A statistical approach (weighting) to correct for this differential in enrollment experience so that a more accurate estimate of drug resistance may be obtained was adopted in calculating prevalence values among new cases. A statistical weighting factor was generated for each cluster reflective of the enrollment experience in that cluster as a factor of the statistically calculated sample size target. The statistical software package with appropriate commands applied such factor in the computation of prevalence values for new cases.

As is natural in most survey some results, values or data may be missing either due to non response from respondent, loss/inadequacy of sample collected, non recording by survey staff etc. It could be argued that such missing values had they been present could have affected findings differently. To address such concerns, a statistical technique of 'Imputation' for missing values was done using various modeling scenarios. Missing values were imputed for and the results obtained after imputation were not different from those obtained without imputing for missing values.

Fig.2 Flow Chart of Survey Participants



RESULTS AND FINDINGS

All clusters successfully enrolled new and retreatment TB cases as respondents in this survey. There were 4 respondents whose treatment status was not recorded. Sampling expectations per cluster was designed as 52 new cases and 'take all' for retreatment cases. Variable enrollment of new cases required a weighting factor to be applied in the analysis of data on new cases.

Table 1: Enrollment by cluster and treatment category

CLUSTER	New cases	Retreatment cases	TOTAL
1	60	19	79
2	51	12	63
3	53	8	61
4	49	6	55
5	16	4	21
6	33	8	41
7	52	10	62
8	49	16	65
9	40	4	44
10	43	7	50
11	50	10	60
12	29	10	39
13	59	7	66
14	54	9	63
15	53	13	66
16	51	13	64
17	60	9	69
18	29	6	35
19	49	8	57
20	44	4	48
21	55	6	61
22	45	13	58
23	23	8	31
24	50	13	65
25	68	13	81
26	57	9	66
27	57	23	81
28	50	11	61
29	48	16	64
30	37	10	47
TOTAL	**1414**	**305**	**1723**

Table 1 shows the enrollment pattern of smear positive respondents from the thirty clusters. Most clusters were able to contribute the expected number of respondents.

A total of 1,723 respondents with smear positive tuberculosis were enrolled (Table 2). Of these 1, 127 (65.4%) were males, 581 (33.7%) females while the gender of 15 (0.9%) respondents was not reported. There were 391 respondents less than 25 years (22.7%), 1,187 (68.9%) were 25 - 54 years old while 8% were 55 years and above. The ages of 7 respondents (0.4%) were unknown. Three hundred and forty-five respondents (20%) had no formal education, 455 (26.4%) had primary education while 900 (52.2 %) had at least secondary education. There were 23 respondents (1.3%) whose educational level was unknown.

Consent for testing was obtained from 1,452 respondents (84.3%). Of this number, 206 (14.2%) were HIV positive while 1,246 (85.8%) were negative. This represents 72.3% and 12.0% of all enrolled respondents respectively (Table 2). There were 271 respondents with unknown HIV status. Of the HIV positive respondents, 164 (13.7 %) were new cases while 42 (16.7%) were retreatment cases.

Table 2: Characteristics of TB respondents by history of treatment

	New cases		**Previously treated cases**		**Unknown history of treat.**		**Total cases**		**p- value**
	n	%	n	%	n	%	n	%	
Total	1,414	100.0	305	100.0	4	0.2	1,723	100.0	
Sex									0.0506
Male	913	64.6	212	69.5	2	50	1,127	65.4	
Female	492	34.8	87	28.5	2	50	581	33.7	
Unknown	9	0.6	6	2.0	0	0	15	0.9	
Age group, years									0.000
≤24	336	23.8	55	18.0	0	0.0	391	22.7	
25-34	527	37.3	120	39.3	2	50.0	649	37.7	
35-44	276	19.5	65	21.3	0	0.0	341	19.8	
45-54	161	11.4	36	11.8	0	0.0	197	11.4	
55-64	77	5.4	21	6.9	0	0.0	98	5.7	
>65	34	2.4	6	2.0	0	0.0	40	2.3	
Unknown	3	0.2	2	0.7	2	50.0	7	0.4	
Education									0.008
None	285	20.2	59	19.3	1	25.0	345	20.0	
Primary	382	27.0	72	23.6	1	25.0	455	26.4	
Secondary	564	39.9	119	39.0	0	0.0	683	39.6	
Post secondary	167	11.8	50	16.4	0	0.0	217	12.6	
Unknown	16	1.1	5	1.6	2	50	23	1.3	
HIV status*									0.0001
Negative	1,036	73.3	210	68.9			1,246	72.3	
Positive	164	11.6	42	13.8			206	12.0	
Unknown	214	15.1	53	17.4	4	100	271	15.7	

History of TB as given by respondents

Among enrolled respondents, the mean duration of illness prior to seeking medical care (Table 3) was 15.7 weeks for females and 16.4 weeks for males. History of previous TB treatment was obtained from 329 (19.1%) respondents. Seven hundred and three (40.8%) of the respondents had a previous history of symptoms related to lung diseases (hemoptysis, chronic cough and chest pain). There were 540 (31.3%) respondents with history of having chest x-rays and 634 (36.8%) had sputum examination done prior to the current episode of illness. About 33 (19.3%) respondents had anti-TB drugs for more than one month. Based on the history of TB, a total of 332 (19.3%) respondents used anti-TB drugs for one or more months. Of these persons, 255 (76.8%) had a previous history of use of first-line anti-TB drugs, while 77 (23.2%) had streptomycin injection in addition to other anti-TB drugs.

Table 3: History of TB as given by respondents

History	n = 1,723	%
Previously treated for TB	329	19.1
Similar symptoms prior to current episode	623	36.2
Symptoms of lung disease	703	40.8
x-ray done prior to current illness	540	31.3
Sputum examination done prior to current illness	634	36.8
Anti TB drugs for more than 1 month	332	19.3
Drug use for >1 month		
Oral First line anti – TB drugs	255	76.8 (n = 332)
Streptomycin	77	23.2 (n = 332)
Mean duration of illness before seeking care		
Females	15.7 weeks	
Males	16.4 weeks	

Table 4 shows that 20 (6.8%) respondents reported treatment for less than 1 month, 145 (49.4%) received anti-TB treatment for greater than 7 months. The mean duration of treatment was 6 months (24.3 weeks)

Table 4: Duration of Treatment (among previously treated respondents):

Duration (in months)	Frequency	Percentage	Cumulative Percentage
< 1	20	6.8	6.8
1-3	62	21	27.9
3 - 5	39	13.3	41.2
5 - 7	28	9.5	50.7
> 7	145	49.4	100

Mean duration of treatment = 24.3 weeks

Table 5 shows verifiable history of treatment outcome, 57.5% had favorable treatment outcome.

Table 5: Verified Outcome of Treatment (among retreatment cases):

Outcome	Frequency	Percentage (%)
Cured	78	51.0
Completed treatment	10	6.5
Defaulted	36	23.5
Failed	28	18.3
Unknown	1	0.7
Total	153	100.0

Table 6 shows that rifampicin, Isoniazid, Pyrazinamide and ethambutol were used in almost equal frequencies implying use of fixed drug combinations; streptomycin usage is a proxy indicator of respondents who received a previous category 2 anti-TB treatments. The 301 respondents who gave a history of use of other anti-TB drugs most were pyridoxine (vitamin B6). There was no second line anti-TB drugs in the list of drugs reported as 'others'

Table 6: Verified history of previous anti-TB drug usage (among respondents treated for > 1 month)

Type of Drugs taken	History of usage	Verified usage	Percentage (%)
Rifampicin	305	270	88.5
Isoniazid	305	260	85.2
Pyrazinamide	305	256	83.9
Ethambutol	305	256	83.9
Streptomycin	304	88	28.9
Others	301	0	0

Table 7 shows that out of the 1,723 respondents enrolled for the survey, 1,414 (82.1%) were new cases of tuberculosis while 305 (17.9%) were previously treated cases. The classification of 4 of the respondents could not be determined from the information provided. For respondents who gave a history of previous treatment of TB, verification of outcome of previous treatment was done by reviewing medical files and other documents available in the study centers. Of these 305 respondents 139 (45.6%) were cured, 83 (27.2%) defaulted from treatment, 22 (7.2%) failed treatment, 38 (12.5%) completed treatment while for 4 respondents (1.3%), treatment outcome was not documented so they could not be classified.

Table 7: Respondents' Classification (with respect to history of previous TB treatment)

Classification	**Number**	**Percent (%)**
New Case	1414	82.1
Retreatment cases	305	17.7
Retreatment after completion	38	12.5
Retreatment after cure	139	45.6
Retreatment after treatment failure	22	7.2
Retreatment after default	83	27.2
Chronic Tuberculosis	23	0.75
Unclassified	4	0.2 (n = 1723)
TOTAL	**1,723**	**100.0**

Table 8 shows that of 1723 respondents enrolled, 1449 sputum samples were subjected to Line Probe Assay, 70 were found to be MDR-TB, giving a crude (general) prevalence rate of 4.8% (CI: 3.8 – 6.1 %) across all treatment categories, stratified by treatment category, these was a prevalence of 2.9% (weighted) (CI: 2.1 – 4.0%) among new TB cases and 14.3% (CI: 10.2 – 19.3%) among retreatment TB categories.

Forty-five, 3.1% (CI: 2.3 – 4.2%) of all respondents tested had Rifampicin mono-resistance (without concomitant INH resistance), stratified by treatment category, these were a 1.4% (CI: 0.9 – 2.2%) and 10.6% (CI: 7.1 - 15.2%) prevalence of Rifampicin mono-resistance among the new and retreatment TB categories respectively.

Sixty-nine (4.8%; CI: 3.7 – 6.0%) of all respondents tested had Isoniazid mono-resistance (without concomitant RIF resistance), stratified by treatment category, these were a 4.3% (CI: 3.3 – 5.5%) and 5.7% (CI: 3.2 – 9.4%) prevalence of Isoniazid mono-resistance among new and retreatment TB categories respectively

Streptomycin mono-resistance was found among 37 (30.6%; CI: 22.5 – 39.6%) of all respondents (n=121) whose sputum sample were subjected to liquid and solid culture DST, stratified by previous treatment, these were a 25.6% (CI: 16.6 – 36.4%) and 41.0% (CI: 25.6 – 57.9%) prevalence of resistance among new and retreatment categories respectively.

Any resistance to Rifampicin (not considering concomitant resistance to Isoniazid or to any other drug) was found in 115 (7.9%; CI: 6.6 – 9.5%) among all cases (generally) tested by Line Probe Assay. Stratified by treatment category there were a prevalence rate of 4.4% (CI: 3.4 – 5.6%) among new TB cases and 24.9% (CI: 19.6 – 30.9%) among retreatment TB cases.

Any resistance to Isoniazid (not considering concomitant resistance Rifampicin or to any other drug) was found among all cases (generally) in 139 (9.6%; CI: 8.1 – 11.3% of) respondents tested by Line Probe Assay. Stratified by treatment category there were a prevalence rate of 7.2% (CI: 6.0 – 8.8%) among new TB cases and 20.0% (CI: 15.2 – 25.6%) among retreatment TB cases.

Table 8: Drug resistance patterns among survey respondents

Drug resistance patterns	All cases			New cases (weighted)			Retreatment cases		
	N	Freq	%	N	Freq	%	N	Freq	%
MDR-TB	1449	70	4.8	1388	41	2.9	245	35	14.3
RHZE*	121	4	3.3						
RHS**	121	15	12.4						
Mono drug resistance patterns									
Rifampicin	1449	45	3.1	1388	20	1.4	245	26	10.6
Isoniazid	1449	69	4.8	1388	60	4.3	245	14	5.7
Ethambutol	65	13	20						
Pyrazinamide	121	11	9.1						
Streptomycin	121	37	30.6	82	21	25.6	39	16	41
Any resistance to Rifampicin or Isoniazid									
Rifampicin	1449	115	7.9	1388	61	4.4	245	61	24.9
Isoniazid	1449	139	9.6	1388	100	7.2	245	49	20

*Of the 121 respondents who had DST performed 4 respondents had concomitant Ethambutol and Pyrazinamide resistance, as well as being RH resistant (MDRTB positive).**There were 15 respondents who had concomitant streptomycin resistance as well as being RH resistant (MDRTB positive

Fig 3: From the survey results, MDR-TB occurrence is distributed across all the zones of the country, although the SWZ has a significantly higher prevalence.

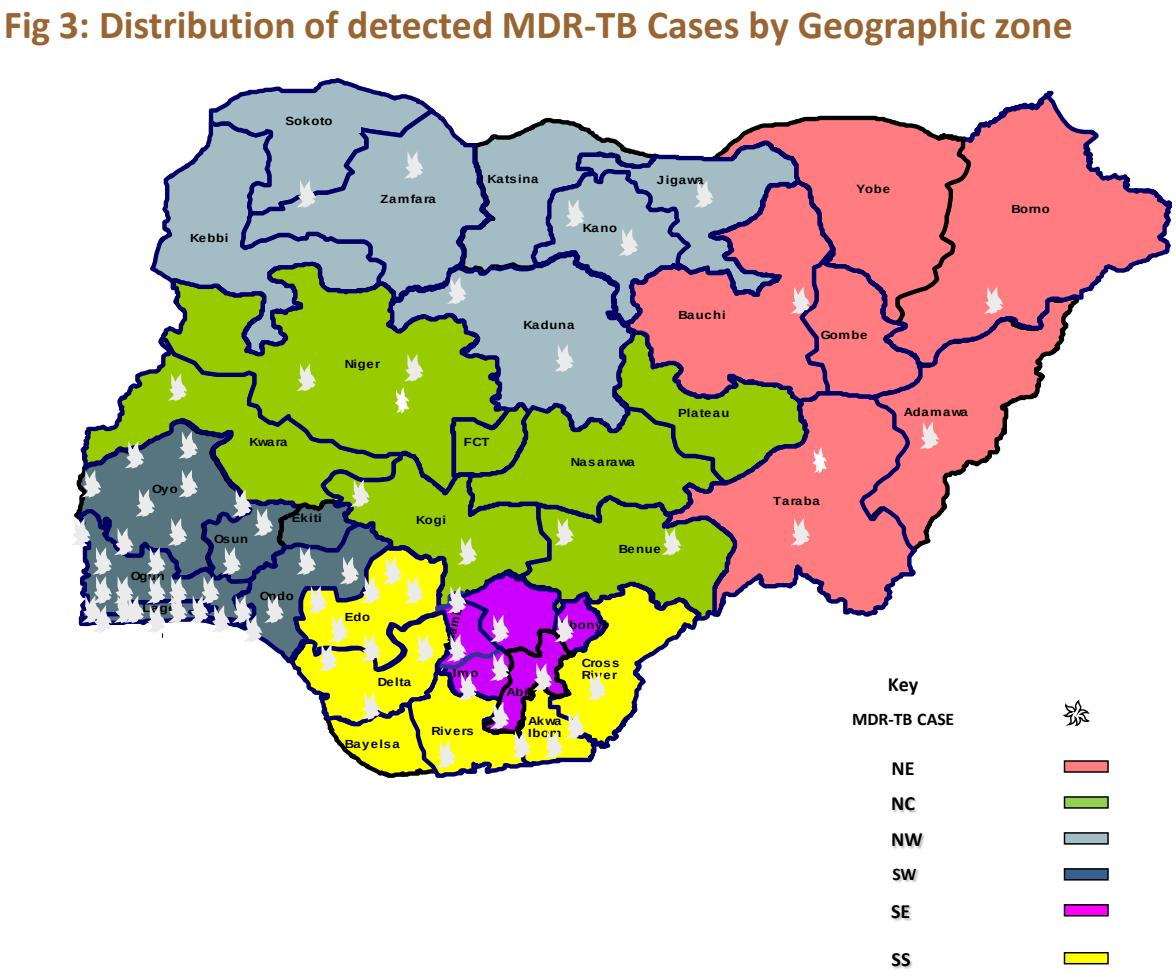


Fig 3: Distribution of detected MDR-TB cases by geographic zone

Table 9 shows that there is no statistically significant difference in the occurrence of MDR-TB with regards to gender, age, educational level and HIV status of respondents. However there is a very strong association between retreatment and the occurrence of MDR-TB (p <0.000).

Table 9: Association between MDR-TB and characteristics of respondents

		MDR-TB			
		Yes	No	Total	p-value
Gender	n= 1435				
	Female	22 (4.4%)	478	500	
	Male	46 (4.9%)	889	935	0.6587
Age category	n=1444				
	<25	12	328	340	
	25-34	23	514	537	
	35-44	16	268	284	
	45-54	9	154	163	
	55-64	8	81	89	
	>64	1	30	31	0.2243
Educational Level	n=1430				
	No formal education	11 (4.2%)	250	261	
	Primary	17 (4.5%)	361	378	
	Secondary	27 (4.4%)	582	609	
	Post-secondary	13 (7.1%)	169	182	0.4097
Treatment category					
	New cases	34	1166	1200	
	Retreatment cases	35	210	245	0.0000
HIV Status	n=1248				
	Positive	5	170	175	
	Negative	61	1012	1073	0.1212

Table 10 shows that previous anti-TB treatment (p <0) is the strongest predictor of MDR-TB in a multivariate analysis of other possible health outcome determinants.

Table 10: Logistic Regression on Predictors of MDR-TB

Term	Odds Ratio	95%	C.I.	Coefficient	S. E.	Z-Statistic	P-Value
Education (2 vs 1)	1.4774	0.624	3.4981	0.3903	0.4398	0.8874	0.3748
Education (3 vs 1)	1.0614	0.477	2.3619	0.0596	0.4081	0.1461	0.8838
Education (4 vs 1)	0.9498	0.4442	2.0307	-0.0516	0.3877	-0.133	0.8942
Age (2 vs 1)	1.5123	0.8837	2.5881	0.4137	0.2741	1.509	0.1313
HIV	0.4325	0.1676	1.1161	-0.8382	0.4837	-1.7328	0.0831
Previous treatment	5.1954	3.0968	8.7164	1.6478	0.264	6.2416	0.0000
Sex (M/F)	0.8919	0.5126	1.5519	-0.1144	0.2826	-0.4047	0.6857
CONSTANT	*	*	*	-3.5061	0.3985	-8.7971	0

Education Key 1: No Formal Education, 2: Post-Secondary Education, 3: Primary Education, 4: Secondary Education.
Age category key 1= ≤30yrs olds, 2= >30yrs old

Table 11 shows that there is a very low resistance to the second-line anti-TB drugs in Nigeria. Only 1 case shows resistance to ciprofloxacin & ofloxacin (same respondent). This case does not have a concomitant resistance to rifampicin and or isoniazid. Six cases (5%) have ethionamide resistant TB bacilli.

Table 11: Prevalence and pattern of 2nd line anti-TB drug resistance

	Resistance		Total
Drugs	Yes	No	
Kanamycin	0 (0%)	121	121
Capreomycin	0 (0%)	121	121
Ciprofloxacin	1 (0.8)	120	121
Ofloxacin	1 (0.8)	120	121
Ethionamide	6 (5%)	115	121
PAS	0 (0%)	115	121

Of the seventy cases who are MDR-TB positive none was found to have drug resistance strains to any injectables (kanamycin, amikacin and capreomycin) and floroquinolones (ciprofloxacin and ofloxacin) second-line anti-TB drugs

Table 12 shows that out of 1449 respondents' tested by Line Probe Assay, 1366 (94.3%) were found to be strains of mycobacterium tuberculosis complex (MTBC), while 42 (2.9%) were non-tuberculous mycobacteria (NTM) strains and samples from 32 (2.2%) respondents yielded mixed growth (MTBC and atypical strains). Nine respondents' samples were inconclusive.

Table 12: Prevalence of Atypical Mycobacteria (NTM) Among Respondents

Speciation	Frequency	Percent (%)
MTBC	1366	94.3
NTM	42	2.9
Mixed strains	32	2.2
Inconclusive	9	0.6
Total	1449	100.0

Table 13 shows that there is no statistically significant association between the occurrences of MTBC and NTM across HIV status. .

Table 13: Association of MTBC and NTM with HIV status in co-infected respondents
p value = 0.5673

	HIV STATUS		
MYCOBACTERIAL SPECIES	POSITIVE	NEGATIVE	TOTAL
MTBC	168 (96%)	1039 (96.8%)	1207
NTM	7 (4%)	34 (3.2%)	41
Total	**175**	**1073**	**1248**

p value = 0.5673

Table 14a shows that of the 70 samples detected as resistant to Rifampicin in Nigeria only 1 was found to be sensitive in Atlanta, while of 133 samples with sensitive results for Rifampicin in Nigeria, 11 had discordant results in Atlanta. For rifampicin testing, there was 94.1% concordance {(69+122)/203} between the Nigerian National Reference Laboratories' Line Probe Assay results and those of the Supranational Reference laboratory in Atlanta.

Sensitivity: 69/80 = 0.863 (86.3%)
Specificity: 122/123 = 0.992 (99.2%)
Positive predictive value: 69/70 =0.986 (98.6%)
Negative Predictive value: 122/133 = 0.917 (91.7%)

Table 14a: International Comparison of Rifampicin Assay

		Supra-National Reference Lab		
		Resistance	Sensitive	Total
Nigeria Reference Lab	Resistance	69	1	70
	Sensitive	11	122	133
TOTAL		**80**	**123**	**203**

Table 14b shows that of 63 samples detected as resistant to Isoniazid in Nigeria only 3 were found to be sensitive (discordant) in Atlanta, while of the 141 samples with sensitive results for Isoniazid in Nigeria, 10 had discordant results in Atlanta. For Isoniazid testing, there was 93.6% concordance {(60+131)/204} between the Nigerian National Reference Laboratories' Line Probe Assay results and those of the Supranational Reference laboratory in Atlanta.
Sensitivity: 60/70 =0.857 (85.7%)
Specificity: 131/134 = 0.978 (97.8%)
Positive predictive value: 60/63 =0.952 (95.2%)
Negative Predictive value: 131/141 = 0.929 (92.9%)

Table 14b: International Comparison of Isoniazid Assay

		Supra-National Reference Lab		
		Resistance	Sensitive	Total
Nigeria Reference Lab	Resistance	60	3	63
	Sensitive	10	131	141
TOTAL		**70**	**134**	**204**

Table 15c shows that of 203 samples identified as MTBC in Nigeria only 1 was found to be NTM (discordant) in the Supra-national Reference Laboratory in Atlanta. Three samples identified as NTM in Nigeria, were all identified as MTBC Atlanta. For mycobacterial identification, there was 98.1% concordance {(202+0)/206} between the Nigerian National Reference Laboratories' Line Probe Assay results and those of the Supranational Reference laboratory in Atlanta.
Sensitivity: 202/205 =0.985 (98.5%)
Specificity: 0/1 = 0 (sample size very small)
Positive predictive value: 202/203 =0.995 (99.5%)
Negative Predictive value: 0/3 = 0 (sample size very small)

Table 14c: International Comparison of Mycobacterial identification

		Supra-National Reference Lab		
		MTBC	NTM	Total
Nigeria Reference Lab	MTBC	202	1	203
	NTM	3	0	3
TOTAL		**205**	**1**	**206**

Table 15a shows that of 37 samples identified as RIF resistant (R+) by LPA, 7 were found to be sensitive (R-) by culture DST (discordant) in the Supra-national Reference Laboratory in Atlanta. Of 83 samples identified as sensitive (R-) to RIF by LPA, one was identified as resistant (R+) by culture DST.
Sensitivity of LPA technique: 30/31 =0.968 (96.8%)
Specificity: 82/89 = 0.921 (92.1%)
Positive predictive value: 30/37 =0.811 (81.1%)
Negative Predictive value: 82/83 = 0.988 (98.8%)

Table 15a Comparison of Hain Assay with Culture DST for RIF (Atlanta)

		RIF Culture DST		
		R+	R-	**Total**
RIF Line Probe Assay	R+	30	7	37
	R-	1	82	83
TOTAL		**31**	**89**	**120**

R+: Resistant, R- : Sensitive

Table 15b shows that of 32 samples identified as INH resistant (R+) by LPA, one was found to be sensitive (R-) by culture DST (discordant) in the Supra-national Reference Laboratory in Atlanta. Of 88 samples identified as sensitive (R-) to INH by LPA, 11 were identified as resistant (R+) by culture DST.
Sensitivity of LPA technique: 31/42 =0.738 (73.8%)
Specificity: 77/78 = 0.987 (98.7%)
Positive predictive value: 31/32 =0.969 (96.9%)
Negative Predictive value: 77/88 = 0.875 (87.5%)

Table 15b Comparison of Hain Assay with Culture DST for INH (Atlanta)

		INH Culture DST		
		R+	R-	**Total**
INH Line Probe Assay	R+	31	1	32
	R-	11	77	88
TOTAL		**42**	**78**	**120**

R+: Resistant, R-: Sensitive

Table 16 shows that there was no statistically significant difference in the gender, HIV status and age of respondents who had Line Probe Assay compared those who did not. There appears to be a higher proportion of persons with primary school or less education not having their sputum tested for Line Probe Assay.

Table 16 Association between Gender, Age, HIV status and Educational level amongst respondents who had or did not have Line Probe Assay

		LPA			
					p-value
Gender	n= 1708	Yes	No	Total	
	Female	500	61	581	
	Male	935	192	1127	0.0982
Age category n=1716					
	<25	340	51	391	
	25-34	537	112	649	
	35-44	284	57	341	
	45-54	163	34	197	
	55-64	89	9	98	
	≥65	31	9	40	0.138
Educational Status n=1700					
	≤Primary	639	161	800	
	≥Secondary	791	109	900	0
HIV	n= 1452				
	Positive	175	31	206	
	Negative	1073	173	1246	0.6560

DISCUSSIONS

This survey revealed that there were more male respondents than females with a ratio of about 2:1. This is in keeping with findings in other studies involving pulmonary diseases.[7,8,9,10] The male preponderance is similar to finding in the routine national TB control program[11]. The reason for this gender difference is not clear but different explanations have been put forward. It may also be due to differences in health seeking habits of people arising from the stigma associated with tuberculosis. Women tend to have a more far reaching psycho-social consequences of stigmatizing ailments and may consequently avoid self exposure inherent in presenting at the health centers. The societal roles of men and cultural habits[9] that influence risk of exposure have also been implicated as possible reasons. Social factors such as smoking, alcoholism and poor nutrition may also play a part[9]. This difference goes beyond prevalence to disease progression. A fifteen year study in India showed that among tuberculosis infected respondents, more males progressed to tuberculosis disease than females[10].

This survey also confirms a known fact that TB disease is prevalent among the economically active age group. In this study, these groups encompass about 70% of the respondents. This further buttresses the fact that TB disease has a grave economic consequence to a national economy. A good health care system should provide health care delivery for the workforce in order to maintain a viable economy.

The literacy level of the respondents enrolled in this survey is reflective of the national literacy level and gives further evidence that TB is a wide spread disease across all educational levels. The mean duration of illness before seeking health care was found to be 4 months across all educational level and age ranges. This may be a reflection of the poor awareness of the general public about the symptoms of tuberculosis and may also be due to the stigma associated with tuberculosis.

The HIV positivity rate (see Table 2) among the overall pool of respondents was 12%, although 15.7% of respondents' HIV status was 'unknown' (declined and non-documentation). Among respondents who consented to being tested this amounts to a prevalence of 14.2%. The TBHIV co-infection rate in this survey is thus lower than the rate among tuberculosis patients as generally reported (25 – 27%) by the TB control programme in the country.[11] The lower TBHIV co-infection rate found in this survey as compared to the reported co-infection rate in the TB control programme could be due to the established observation of most TBHIV co-infected patients being smear negative as most of the tuberculosis respondents who are HIV sero-positive will have pauci-bacillary or smear negative disease and by that fact would not have qualified for the survey. Nevertheless this survey shows that the prevalence of HIV infection in TB patients is much higher, almost four-folds, than in the general population (the National HIV prevalence is 4.1% in general population), further accentuating the fact of TBHIV co-morbidity.

From the findings, in this survey the proportion of respondents that did not complete their treatment among previously treated is higher than the proportion that completed treatment. The mean duration of treatment was 6 months (24.3 weeks) in a scheduled 8 month regimen. According to WHO[12] attainment of 85% treatment success rate is linked to reducing the proportion of those that did not complete treatment to 10%. Reports from other studies demonstrated that factors such as duration of treatment and hence completion rate is a measure of

the quality of care, which is related to provider, respondents, as well as health system factors. The non completion of treatment may therefore be attributable to the attitude of health workers, inadequate knowledge and skill of the provider, distance of treatment sites, poor drug management, cost of treatment (especially in private health facilities), inadequate respondent counseling including knowledge about treatment duration and side effects of drugs were associated with treatment non-completion[13,14,15,16]

A key target of TB control is to attain a treatment success rate of at least 85%. This will among others restore the quality of life and productivity; prevent deaths from active TB or its late effect, relapse, reduce transmission of TB to others as well as prevent the development and transmission of drug resistant strains.[17] From the results shown above, the treatment success rate is relatively low in comparison to the global targets. There are a high proportion of respondents who failed treatment (18.3%). Overall, the treatment outcomes were unsatisfactory. This could be a proxy indicator of the quality of care provided by the health care system. Other specific causes could, among others be: inadequate training and supervision, inadequate documentation and non-adherence to the National TB control programme protocol (Kelly, 2001) and drug stock out. The poor treatment outcomes, especially among the previously treated respondents constitute high risk for the development of drug resistant TB (WHO, 2009). To improve the outcome of TB treatment, there is need to enhance supervision and monitoring, improve counseling during the intensive and continuation phases of treatment and home visits to increase the motivation of respondents (Belay et al, 2009).

According to the WHO in 2008, the 27 high MDR-TB burden countries refer to those member states estimated to have at least 4000 MDR-TB cases arising annually and/or at least 10% of newly registered TB cases with MDR-TB. According to the Global TB Control, WHO report 2011, the estimated prevalence of MDR-TB in Nigeria amongst new cases is 2.2% and in retreatment cases is 9.4%. This national survey showed a prevalence 2.9% (CI: 2.1 – 4.0%) among new cases and 14.3% (CI: 10.2 – 19.3%) for retreatment cases. The findings with imputation were not different from the survey results. These findings are within the trends for Africa. In WHO TB Report for 2011, the proportion of MDR-TB among TB respondents in Africa is 3.9 - 5.0% in new cases and 0.0- 16.7% in retreatment cases.

This survey shows a very strong association between the occurrence of MDR-TB with previous treatment ($p = 0.0000$). When a multivariate analysis of relevant health determinants was done previous treatment remained the strongest predictor of MDR-TB. Prior exposure to anti-TB drugs is a well-established risk factor for DRTB as shown by surveys and surveillance system world-wide[18]**.** This high level of acquired resistance points to poor program performance which could have stemmed from poor adherence, inappropriate prescriptions, irregular drug supply and or poor drug quality. The finding from this survey underscores the need for the TB program to strengthen all systems for the delivery of DOTS so as to ensure cure at first presentation as new cases bearing in mind the increased risk for DR-TB in retreatment.

With reference to age, the survey reports that MDR-TB peaks amongst the age groups 45-64. In the 13 countries of Central & Eastern Europe, the frequency of MDR-TB peaked in young adulthood.[19] There was no difference in the prevalence of MDR-TB between the sexes in this survey. Overall combining data from 38 countries and 3 territories providing drug resistance surveillance data stratified by sex, and using the robust standard errors

approach, the odds ratio of harboring MDR-TB strains for female TB cases compared with male TB cases was 1.1 (95% CI: 0.9 – 1.4), showing no overall association between MDR-TB and sex of the patient as similarly found in this survey. There is statistically no significant association between educational level and MDR-TB in this survey report, though post-secondary level of education has a higher rate of incidence.

Of the 1248 respondents with documented HIV status 175 were found to be HIV positive with only 5 co-infected with MDR TB giving an MDRTB-HIV co-infection rate of 6.7% (Table 10). Also only 70 respondents were found to have MDR-TB giving a crude MDR-TB rate of 4.8% (Table 8). A primary criterion for recruitment into the survey was strong sputum smear positivity. TBHIV co-infected patients usually present with pauci-bacillary and smear negative TB and as such a large percentage of this group of patients could have been excluded from this survey. A logistic regression on some probable predictors of MDR-TB in the study (Table 11) showed a weak association between MDR-TB and HIV ($p = 0.0831$). This weak association could be argued to be due to low numbers of smear positive HIV co-infected TB patients resulting in fewer co-infected MDR-TB patients found in the survey and consequent insufficient power in analysis.

Other African studies demonstrated no association between MDR-TB and HIV[20,21,22,23] although a more recent preliminary results of a survey conducted in Mozambique in 2007 found a significant association[24] . Also most studies from Eastern European countries and Ukraine showed increased risk for MDR-TB among HIV positive patients. [18,24] Several reasons were found to have accounted for this association: social vulnerability including injection drug users (IDU), lack of proper care resulting to poor adherence and suboptimal treatment. These observed confounders associated with MDR-TB HIV co-infection are found in special situations such as IDU which is not prevalent in our setting, hospitalized patients and prison inmates, a group that were not recruited in this survey.

Though, HIV infection and tuberculosis are intimately linked, there is presently a limited evidence supporting an association between MDR-TB and HIV outside institutional outbreaks[18]. Co-existence of both DR-TB and HIV results in higher morbidity and mortality among co-infected patients.[25] Gaining more insight into how HIV impacts on the MDR-TB epidemiology will demand a robust routine surveillance system for DR-TB among TBHIV co-infected patients.

The resistance pattern to first-line anti-TB drug was found to be varied in this survey ranging from 7.9% for any resistance to rifampicin to as high as 30.6% for streptomycin (Table 8). The highest resistance of 30.6% found for streptomycin could be explained by its use in retreatment cases, treatment of other disease condition and cross resistance[26] with other aminoglycosides such as gentamycin (a commonly used drug in our setting). This high resistance is very alarming considering the fact that streptomycin is used during the first 2 months in the standard retreatment regimen in our TB program and as such is being used for increasing number of patients that already may have streptomycin resistant bacilli strains.

Among the two most important first line anti-TB drugs, rifampicin and isoniazid, any resistance for isoniazid (9.6%) was found to be higher than that to rifampicin (7.9%). Isoniazid is used with rifampicin in intensive phase of TB treatment and used with ethambutol in continuation phase of treatment in 8 month treatment regimen within the TB program and as such TB patients are exposed more to INH than Rifampicin which could account for this greater resistance. The result of national MDR TB survey in Rwanda published in 2007 showed a similar trend, with an INH resistance of 6.2% and Rifampicin 3.9%[27].This rifampicin and isoniazid resistance rate calls for vigilance with regards to the adoptions of 6 months regimen to ensure strict DOT.

Among 121 cases that had DST performed, concomitant resistance to RHZE was found in 4 cases (3.3%). The resistance pattern was not segregated into new and re-treatment categories due to the nature of sampling of specimen subjected to DST. RHZE is administered as a fixed dose combination (FDC) during the intensive phase of treatment of TB and increased resistance will render this proven bed-rock of TB treatment useless. Also concomitant streptomycin resistance among the MDR TB cases (RHS resistance) was found in 15 (3.3%) cases of 121 cases subjected to DST (Table 8).

This survey showed very low resistance to the second-line anti-TB drugs in Nigeria. Only a case showed cross-resistance to ciprofloxacin and ofloxacin (same respondent). Of some concern is resistance to ethionamide: 6 (5%) of 121 cases subjected to DST had ethionamide resistant TB bacilli. It is important to note that of the 70 MDR-TB cases, none was found to have any resistance to injectables (kanamycin, amikacin and capreomycin) and fluoroquinolones (ciprofloxacin and ofloxacin), and thus no case of extensively drug-resistant TB (XDR-TB) was found in this survey.

The performance of LPA directly from smear-positive sputum correlated very highly with culture and DST performed on MGIT 960. Overall, an acceptable proportion of valid results were obtained. This study demonstrated high sensitivity and specificity of the LPA method, relative to conventional DST using MGIT 960 and agar proportion method for rifampicin (96.8%) and a positive predictive value of 81.1%. LPA testing for INH was found to be relatively less sensitive (73.8%) as has been documented in other studies but with a very high positive predictive value (96.9%).[28]

The study highlights the efficiency of rapid diagnostic tests for early identification of DR-TB cases minimizing the risk of disease progression and amplification of drug resistance. In addition, groups such as health care workers, prisoners, and HIV-positive individuals—would likely benefit from rapid DST which would reduce the opportunity for nosocomial transmission and the morbidity associated with delayed diagnosis of DR-TB. The rapid screening for DR-TB would permit expeditious initiation of appropriate regimen without unnecessary exposure to second-line drugs. This will also contribute to prevent the transmission of DRTB in the community.

A sample of the strains isolated during the survey was sent to the Supranational Reference Laboratory in Atlanta to be retested. The results were compared for agreement with respect of each drug. At the end of the study 100% of resistant strains and 10% of the susceptible strains were sent to the Supranational Reference Laboratory for validation. Sensitivity, specificity and predictive values for resistance and susceptibility was calculated for the national laboratories with respect to each of the drugs (rifampicin, isoniazid) tested and mycobacterial

identification. For rifampicin sensitivity was found to be 86.3%, specificity 99.2%, predictive value for resistance 98.6% and predictive value susceptibility 91.7% (Table 15a). For isoniazid sensitivity was found to be 85.7%, specificity 97.8%; predictive values for resistance and susceptibility were 95.2% and 92.9% respectively (Table 15b). Comparison of the mycobacterial identifications by Line Probe Assay showed a sensitivity of 98.5% and a positive predictive value of 99.5%. There was no sample identified as NTM by the National reference laboratory amongst the samples sent for retesting to the Supra-national laboratory. The supranational reference laboratory however identified one sample as NTM amongst the lot. This sample size of NTM is thus too small for calculations of specificity and negative predictive values (Table 15c).

CONCLUSION AND RECOMENDATIONS

CONCLUSION

This study has confirmed concerns about the presence, and quantified the magnitude of MDR-TB and other first line anti-TB drug resistance in Nigeria. The prevalence of multi-drug resistance tuberculosis in Nigeria, from the findings of this survey, is higher than the WHO estimates for both new and retreatment TB cases in Nigeria. This study has demonstrated the urgent need for the observance of measures (including DOT) to limit acquired resistance to first line anti-TB drugs as well as the need for measures to maintain vigilance in the use of rifampicin and isoniazid due to the confirmed mono-resistance to this drugs especially as the country transits to six-month (RH based) anti-TB regimen. There was no case of XDR-TB found in Nigeria in this study and resistance to second-line anti-TB drugs is very low, measures should be adopted to keep this so.

Conducting this survey - the first ever DR-TB survey in Nigeria and a nationwide survey in a populous country with a huge landmass - has been very challenging for the TB Control programme but nevertheless very successful. It has required huge investments of financial, material and human resources especially in the development of laboratories with sufficient bio-safety level for mycobacterial culture, huge logistics costs of sputum transportation under cold chain for long distances in a setting already challenged by inadequate transportation infrastructure and electricity, and extraordinary commitment to achieve on the part of all survey team members. Consequently it is strongly recommended that an efficient routine drug resistance surveillance system be developed within the TB programme which will then be complemented by periodic survey possibly every 5 years. This study has also been the first study, globally, to use the Line Probe (Hain) assay on a national scale in addition to culture DST and has demonstrated a very high concordance between both procedures.

RECOMMENDATIONS

1. Due to the very high cost and lengthy process of drug resistance survey, an efficient and accurate routine anti-TB drug resistance surveillance should be developed which will be periodically complemented with national DRS.
2. Measures be deployed, informed from these findings, to facilitate identification and placing on treatment cases of MDR-TB and thereby further curtail the spread of primary forms of DR-TB.
3. From the findings of this survey, most of DR-TB is acquired resistance, consequently measures to improve quality of care including, but not limited to DOT, uninterrupted drug supply of quality assured anti-TB drugs, defaulter tracing and ensuring cure on fist treatment, be intensified in the TB control programme.

4. Due to the already high RIF and INH resistance the adoption of 6-month anti TB regimen (RH based regimen) should be strictly with daily DOT and non-conversion after intensive phase should be followed with immediately DST
5. Considering the high drug resistance rates to streptomycin, rifampicin and isoniazid, the TB control program will need to highly prioritize developing diagnostic capacity for DST and make this more proximal to TB patients, such that routine DST will be available at Cat.1 intensive phase non-conversion
6. Measures should be evolved to avoid the abuse of second-line anti-TB drugs and keep resistance to these drugs very low as has been found in this study.

ANNEX

Case Definitions:

New Case:

- Active TB respondent who has never before been treated for tuberculosis, or treated for less than one month

Previously Treated Cases:

- **Retreatment after prior cure**: had been declared cured after treatment completion and re-diagnosed with active TB
- **Retreatment after prior treatment failure**: Completed prior treatment and re-diagnosed with active TB
- **Retreatment after interruption in prior treatment**: Stopped during prior treatment for two months or more and still having active TB
- **Retreatment after prior treatment failure**: Treated for more than one month, but remained with active TB or became sputum positive again.
- **Chronic TB**: Remains sputum smear or culture positive after having completed a supervised treatment regimen

Unclassified: History of previous anti-TB treatment not available for analysis.

Cured: Sputum smear positive respondent at diagnosis, who completed 6-8 months of treatment and who is smear negative at the end of 6^{th} or 7^{th} month of treatment, and at least on one previous occasion.

Completed treatment: 1. Any respondent who was smear-positive at diagnosis, and who completed treatment but in whom smear examination results are not available at the end of treatment. 2. All smear negative and extra-pulmonary TB respondents who completed treatment

Defaulted: Any respondent who has interrupted treatment for 8 consecutive weeks or more after the last date of last attendance during the course of treatment

Failed: Respondents who remained smear positive, or became smear positive again at the end of fifth month or later during chemotherapy

Transferred out: A respondent who was transferred to another treatment centre in another state, and whose treatment outcome is not known

Annex 2: Sputum Examination Request Form (Form 1)

Specimen Examination Request Form

I. Site Identification

Survey ID (*Geopolitical zone code-LGA code-Cluster Code-Study Site-Serial number of patient*):

☐☐☐-☐☐☐-☐☐-☐☐-☐☐☐ **SURVEY ID STICKER**

Date shipped (dd/mm/yyyy) : _____/_____/_____

II. Patient Identification Information

Name :…….
Sputum Identification number……...
Local Government TB Identification Number : ……..
Date Registered into TB program (dd/mm/yyyy) : _____/_____/_____

SEX: ☐ M ☐ F AGE (years):……..
Date of Birth (dd/mm/yyyy) ……/……/…….

Type, date, and results of sputum collected and shipped:

□ A. Spot-Date collected (dd/mm/yyyy) : ____/_____/_____ CPB/CPC added: □ Yes□ No
□ Negative □ Scanty (____AFB/100 HPF) □ 1+ (10-99 AFB/100 HPF) □ 2+ (1-9 AFB/1 HPF)
□ 3+ (>10 AFB/1 HPF)

□ B. Morning-Date collected (dd/mm/yyyy) : ____/_____/_____ CPB/CPC added: □ Yes□ No
□ Negative □ Scanty (____AFB/100 HPF) □ 1+ (10-99 AFB/100 HPF) □ 2+ (1-9 AFB/1 HPF)
□ 3+ (>10 AFB/1 HPF)

□ C. Spot-Date collected (dd/mm/yyyy) : ____/_____/_____ CPB/CPC added: □ Yes□ No
□ Negative □ Scanty (____AFB/100 HPF) □ 1+ (10-99 AFB/100 HPF) □ 2+ (1-9 AFB/1 HPF)
□ 3+ (>10 AFB/1 HPF)

ORIGINAL TO STAY AT DIAGNOSTIC CENTER
TWO COPIES TO BE SENT TO REGIONAL MIRCOSCOPY CENTER WITH SPUTUM SPECIMEN(S)

Annex 3: NATIONAL MULTI DRUG RESISTANCE TUBERCULOSIS SURVEY

Patient Clinical Information Form

(Make duplicate copy when completed)

Site identification

□□□-□□□-□□-□□-□□□ **Sticker**

1. Date of form completed (dd/mm/yyyy): □□/□□/□□□□
2. Sputum Identification Number: □□□□□□□□

Socio demographic information

3. Sex: □*M* □*F*
4. Date of birth *(dd/mm/yyyy)*:□□/□□/□□□□
5. Age as of last birthday *(years)*: □□□
6. Level of Education: □*No formal education* □*Primary* □*Secondary* □Post secondary

History of TB disease given by the patient

7. Have you been previously treated for TB?: □*Yes* □*No* □*Don't know*
8. For how long have you been sick *(indicate time in weeks)*? : □□
9. Did you have the same symptoms prior to this episode?: □*Yes* □*No* □*Don't know*

10. Have you had other symptoms of lung disease prior to this episode *(coughing blood, prolonged cough, chest pain)*? : □*Yes* □*No* □*Don't know*
11. Have you had lung X-rays done prior to this episode?: □*Yes* □*No* □*Don't know*
12. Have you had sputum examinations prior to this episode?: □*Yes* □*No* □Don't know
13. Have you taken TB drugs for more than one month? □*Yes* □*No* □*Don't know*
14. If "Yes", what drug(s))? : □*Rifampicin* □*Isoniazid* □*Pyrazinamide* □*Ethambutol* □*Streptomycin* □*Others (specify)* _____ □*Don't know*

15. If "Yes", for how long was your last treatment? : ☐*Less than 1 month*

☐More than 1 month

16. Have you had injections for more than one month? ☐*Yes* ☐*No* ☐*Don't know*

17. If "Yes", what drug(s))? : _____

18. Did the patient remember having had previous TB treatment after answering questions 8 to 17? : ☐*Yes* ☐*No* ☐*Don't know*

(If "Yes", answer questions 19 to 25; if no go to question 26)

History of previous TB treatment

19. When was the patient treated *(dd/mm/yyyy)*? : ☐☐/☐☐/☐☐☐☐

21. In which health unit did the patient receive prior treatment *(name of unit)*? :

_____ ☐*Don't know*

22. How many treatment episodes have you had (*Specify number of times*)? _____

23. Based on the patient response select from the list below the drugs used for treatment:

☐*Rifampicin* ☐*Isoniazid* ☐*Pyrazinamide*

☐*Ethambutol* ☐*Streptomycin*

☐*Others (specify)* _____ ☐*Don't know*

24. How long was the patient treated during the last epiosode? : ☐☐ (*weeks*)

25. According to the patient, what was the outcome of the last treatment?

☐*Cured* ☐*Not Cured* ☐*Unknown*

Information Taken from Patients' Medical Records

26. Do you have access to the patient past medical record: ☐*Yes* ☐No

(If yes answer questions 29 and 30. If no go to question 31)

27. After extensive review of medical files and other documents available in the health facility, have you discovered that the patient has previously been treated for TB?:

☐ *Yes* ☐ *No*

29. If "Yes to question 27", what was the outcome of the last treatment?

☐ *Cured* ☐ *Defaulted* ☐ *Transferred out*

☐ *Completed treatment* ☐ *Treatment failed*

Summary of TB treatment history

30. Has the patient been previously treated for TB for more than one month? *(Please mark only one of the following options)*: ☐ *Yes* ☐ *No* ☐ *Doubtful*

31. If "Yes to question 30", what was the outcome of the last treatment?

☐ *Cured/ Treatment completed* ☐ *Treatment failed*

☐ *Chronic – Completed re-treatment and still has active TB*

☐ *Relapse/defaulter not distinguishable* ☐ *Defaulted*

☐ *Transferred out* ☐ *Unknown*

Classification of Patient for the survey

32. Based on the above information, indicate classification of the TB patient:

☐ New Case (never treated OR treated for less than one month)

☐ Retreatment after prior cure (declared cured after treatment completion and re-diagnosed with active TB)

☐ Retreatment after completion of prior treatment (completed prior treatment and re-diagnosed with active TB)

☐ Retreatment after interruption in prior treatment (stopped during prior treatment for two months or more and still having active TB)

☐ Retreatment after prior treatment failure (treated for more than one month, but remained TB active OR became sputum positive again)

☐Chronic (remains sputum OR culture positive after having completed a supervised treatment regimen)

Laboratory data:

Sputum sample collection and smear result:

33. Date "First Spot" collected *(dd/mm/yyyy)*: ☐☐/☐☐/☐☐☐☐

34. Test result of "First Spot": ☐*1+* ☐*2+* ☐*3+* ☐scanty ☐negative

35. Date "Morning sample" collected *(dd/mm/yyyy)*: ☐☐/☐☐/☐☐☐☐

36. Test result of "Morning sample": ☐*1+* ☐*2+* ☐*3+* ☐scanty ☐ negative

37. Date "Second Spot" collected *(dd/mm/yyyy)*: ☐☐/☐☐/☐☐☐☐

38. Test result of "Second Spot": ☐*1+* ☐*2+* ☐*3+* ☐scanty ☐ negative

HIV test information:

41. Date of HIV test *(dd/mm/yyyy)*: ☐☐/☐☐/☐☐☐☐

42. HIV test result: ☐*Positive* ☐*Negative* ☐*No Data*

TB treatment information:

39. Date patient registered in TB program *(dd/mm/yyyy)*: ☐☐/☐☐/☐☐☐☐

40. Date patient start TB treatment *(dd/mm/yyyy)*: ☐☐/☐☐/☐☐☐☐

_____ _____

Interviewer's Name and signature Reviewer's name and signature

Annex 4: Request for Bacteriological Examination

Request for Bacteriology examination form

Survey ID No: ---------- Date: ----------

Date of Birth: ---------- Sex (mark one) M ---------- F ----------

RESULTS (To be completed at the smear microscopy laboratory)

Smear results

Date collected	Specimen	Laboratory Specimen No	Appearance	Results (mark one) Negative	Scanty	1+	2+	3+

* visual appearance of sputum (blood stained, mucopurulent, salivary

No AFB	0%
1-9 AFB per 100 HPF	scanty (exact no)
10-99 AFB per 100 HPF	1+
1-10 AFB per HPF	2+
>10 AFB per HPF	3+

Date: ----------

Examined by (signature): ----------

Culture results (To be completed at the reference laboratory)

Date collected	Specimen	Laboratory Specimen No	0%	Results (mark one) neg	1 +	2+	3+	Contaminated

No growth reported	0%
Fewer than 10 colonies	Report no of colonies
10-99 colonies	1+
100 - 199 colonies	2+
200 - 500 colonies	3+
>500 colonies	Confluent growth

Date: ----------

Examined by (signature): ----------

MTB DR plus (To be completed at the reference laboratory)

Date tested	Specimen No.	Identification as *M. tuberculosis*	Rifampin: list mutations if present	Rifampin: interpretation (Resistant or Susceptible)	Isoniazid: list mutations if present	Isoniazid: interpretation (Resistant or Susceptible)	Comments:

Date: ---------- Examined by: ---------- Signature: ----------

Annex 5: Anti-tuberculosis Drug Resistant Results form (a completed example)

Centers for Disease Control and Prevention
Centers for Global Health
Division of Global HIV / AIDS
International Laboratory Branch
TB / OI Unit

Mycobacteriology Culture Final Report

Submitter	**Country Name**	Nigeria		
	POC	Abiola Tubi		
	Laboratory name	CDC-Nigeria		
	Study name	MDR TB Survey		
Date Received:	7/23/2010	**Date Reported:**	5/24/2011	
Submitter Identifier: SEZ-AB-01-AB1-0010		CDC Specimen ID: 2011486591		
Final Identification: Mycobacterium tuberculosis complex				

Identification method: Immunochromatographic

Molecular Drug Susceptibility Testing - Line Probe Assay

Drug	Result / Interpretation
Isoniazid	Resistant
Rifampin	Resistant

Hain Lifesciences Genotype MTBDRplus

Phenotypic Drug Susceptibility Results

BD MGIT 960 First Line Drug Susceptibility Testing Method

Drug concentration	Interpretation	Drug concentration	Interpretation
Streptomycin 1.0 μg/ml	Susceptible	Rifampin 1.0 μg/ml	Resistant
Isoniazid 0.1 μg/ml	Resistant	Ethambutol 5.0 μg/ml	Susceptible
Isoniazid 0.4 μg/ml	Resistant	Pyrazinamide 100 μg/ml	Susceptible

Indirect Agar Proportion Drug Susceptibility Method, 7H10 agar plates

Drug concentration	Interpretation	Drug concentration	Interpretation
Isoniazid 0.2 μg/ml	Resistant	Rifabutin 2.0 μg/ml	Susceptible
Isoniazid 1.0 μg/ml	Resistant	Ciprofloxacin 2.0 μg/ml	Susceptible
Isoniazid 5.0 μg/ml	Susceptible	Kanamycin 5.0 μg/ml	Susceptible
Rifampin 1.0 μg/ml	Resistant	Ethionamide 10.0 μg/ml	Susceptible
Ethambutol 5.0 μg/ml	Susceptible	Capreomycin 10.0 μg/ml	Susceptible
Streptomycin 2.0 μg/ml	Susceptible	PAS 2.0 μg/ml	Susceptible
Streptomycin 10.0 μg/ml	Susceptible	Ofloxacin 2.0 μg/ml	Susceptible
		Amikacin 4.0 μg/ml	Susceptible

Susceptibility is defined as < 1% resistance and is calculated as the number of colonies on the drug containing media compared to the number of colonies on drug free media

Comments:

Reviewed by: Heather Alexander, PhD
Signature: HA Date: 5/25/11
Phone: 404 639 5331 Fax: 404 639 1287
1600 Clifton Road, MS F08, Atlanta, GA 30333

Date Printed: 25 May 2011

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