

Final Report

IRAQ FIRST NATIONAL ANTI-TUBERCULOSIS
DRUG RESISTANCE SURVEY 2013-2014

August 2015

By: National Tuberculosis Control Program

In collaboration with WHO

Funded by GF & MoH

Contents

Acknowledgments.....	iii
Summary.....	iv
List of Acronyms.....	v
DRS team that commissioned the study.....	v
National Central Coordination team, NTP, MOH Iraq	v
Background	Error! Bookmark not defined.
TB Epidemiology	Error! Bookmark not defined.
Goal and objectives.....	Error! Bookmark not defined.
Overall goal	Error! Bookmark not defined.
General objective.....	Error! Bookmark not defined.
Specific objectives.....	Error! Bookmark not defined.
Materials and Methods.....	Error! Bookmark not defined.
Study design.....	Error! Bookmark not defined.
Sample size determination	Error! Bookmark not defined.
Sampling strategy.....	Error! Bookmark not defined.
Inclusion and exclusion criteria.....	Error! Bookmark not defined.
Intake of patients.....	Error! Bookmark not defined.
Lab procedures	Error! Bookmark not defined.
Diagnostic procedures at the DTCs (BMUs).....	Error! Bookmark not defined.
Diagnostic procedures at Governorate TB Clinic (GTCs).....	Error! Bookmark not defined.
Culture at the GTCs:.....	Error! Bookmark not defined.
Logistics of DRS	Error! Bookmark not defined.
Training	Error! Bookmark not defined.
Supervisory visits	Error! Bookmark not defined.
Study implementation	Error! Bookmark not defined.
The data collection for the study began in November 2013 and completed in October 2014.	Error! Bookmark not defined.

Data management and analysis.....	Error! Bookmark not defined.
Data management (data collection, verification and entry).....	Error! Bookmark not defined.
Data analysis	Error! Bookmark not defined.
Results.....	Error! Bookmark not defined.
External Quality assessment results	Error! Bookmark not defined.
Drug Resistance Results	Error! Bookmark not defined.
Discussion.....	Error! Bookmark not defined.
Limitations of the study	Error! Bookmark not defined.
Conclusion and Recommendation	Error! Bookmark not defined.

Acknowledgments

Summary

List of Acronyms

AFB	Acid-fast bacillus/bacilli
AIDS	Acquired immunodeficiency syndrome
DOT	Directly observed treatment
DOTS	Directly observed treatment, short-course
DRS	Drug resistance survey
DST	Drug-susceptibility testing
EPT	Extra-pulmonary tuberculosis
EQA	External quality assessment
GDF	Global Drug Facility
GF	Global Fund to Fight AIDS, Tuberculosis and Malaria
HIV	Human immunodeficiency virus
MDR	Multidrug-resistant
MTB	Mycobacterium tuberculosis
MOTT	Mycobacteria other than TB
M&E	Monitoring and evaluation
NTP	National TB Control Programme
CPHL	Central Public Health Laboratory (NRL instead)
PMDT	Programmatic Management of Drug-Resistant TB
RIF	Rifampicin
SRL	Supranational TB Reference Laboratory
SS	Sputum smear
TB	Tuberculosis
WHO	World Health Organization
XDR	Extensively drug-resistant

DRS team that commissioned the study

National Central Coordination team, NTP, MOH Iraq

NTP Manager

NRL Manager

OR Focal person

MDR Focal person

Head of surveillance

WHO – Technical Assistance from WHO HQ. EMRO and country office

Dr. Mohammad Abdel Aziz

Dr. Samiha Baghdadi

Dr. Matteoignol

Dr. Anna Dean

Dr. Ali Akbar

Dr. Novera Ansari

Data entry and Data cleaning,

NTP MOH

DQA

SNRL Egypt

Data analysis- WHO HQ

Final report

Funding agency

GFATM

MOH

1. Background

Iraq has an estimated 32,249,932 inhabitants. The country is administratively divided into 18 governorates with varying population sizes ranging from around 800 thousand to 7 million. The Ministry of Health (MoH) has established the National Tuberculosis Control Programme (NTP) in 1989, and introduced the DOTS strategy in 1998. By 2000, the DOTS strategy was implemented at all the Governorate Respiratory and Chest Disease Clinics – except for the three northern governorates of Kurdistan Iraq – assuming 100% population DOTS coverage regardless of the actual number of people who have real access to that clinic. By 2008, the three northern governorates had started DOTS implementation.

The Government of Iraq considers TB as a major public health problem, and keeps TB control high in the national health agenda. Currently, many health reforms are ongoing including the expansion of the DOTS services into the Basic Package of Health Services to be delivered through the nationwide network of Primary Health Care Centers (PHCCs). The Government of Iraq invests strongly in the rebuilding of the health infrastructure that was damaged during the war and encourages recruitment of qualified health personnel. The NTP enjoys the full support of the Government of Iraq. In addition to the strong political commitment to the National TB Control Program, the government also supports the procurement of first and second-line anti TB drugs and the strengthening of the storage and distribution infrastructure.

The overall responsibility for TB control rests with the NTP within the MoH. NTP is responsible for policy and strategy formulation, coordination with partners, as well as planning, implementation and monitoring of control activities. The organizational structure of NTP is based on 4 levels:

1. The National TB Institute (NTI) or Chest & Respiratory Diseases Specialized Center at the national (central) level in Baghdad: the NTI is responsible for training of staff, implementation of the national TB control plan and supervision of activities in governorates.
2. 19 Governorate Respiratory and Chest Disease Clinics at the governorate (intermediate) level: the governorate clinics are responsible for diagnosis and registration of TB cases detected in their geographic areas. These clinics provide treatment to their patients or refer them to the district TB Management Unit for treatment follow up.
3. There are 124 health districts in Iraq (district level). The health district's core organizational entity is the TB Management Unit (TBMU)/ District TB Coordinator (DTC). In each one of these districts, the main Primary Health Care Center has a TBMU which ensures TB diagnosis and treatment. The TBMUs also have TB register and reporting facilities. TBMUs are also responsible for the referral of TB patients to a PHCC that is the nearest to the patient's residence – if available – and for treatment follow-up of patients. The TBMU is the core organizing principle of NTP functions. There are currently 85 TBMUs which perform diagnostic and follow-up services for TB patients, and 39 TBMUs which perform follow-up services only. Under this strategy the NTP envisions that all TBMUs provide diagnostic services, and that their service delivery is further upgraded to include: 1) Follow up with patients; 2) Registration of data on TB suspects, patients and contacts as well as laboratory results through the regular recording and reporting system and the Electronic Nominal Registration System (ENRS); 3) forward data to higher levels; 4) implement web-based TB surveillance (WTBS; pilots are already under trial in 6 governorates); 5) supervise geographically related PHCCs; 6) implement PPM

activities such as registration of TB patients among prisoners, supervise TB services in prisons in the district, coordinate with focal points in public and private hospitals and private clinics in the district, participate in quarterly TB review meetings as members of the local TB review committees.

4. There are 2331 Primary Health Care Centers (PHCCs) throughout Iraq (1100 PHCCs are directed by Doctors and 1231 PHCCs are directed by paramedical staff) in addition to 22 main PHCCs with training functions (peripheral level). The role of PHCCs, other than main centers, is limited to the monitoring of patients' treatment intake under DOT. Currently only 1247 PHCCs, or 53% of the total number of PHCCs, are involved in the DOTS program at the peripheral level. The expansion of DOTS services to include the remaining PHCCs is planned by the Ministry of Health. Under this plan DOTS services were integrated into the Basic Package of Health Services and were implemented through all PHCCs. The Ministry of Health envisions for the near future that approximately 300 health facilities (TBMUs, PHCCs, hospitals) which currently have laboratory facilities and serve around 80-100 thousand population be upgraded to provide TB microscopy services.

TB Epidemiology

Based on the current epidemiological situation, Iraq ranks 99 out of 212 countries and territories by estimated number of cases on the global level. It is considered among the 7 high TB burden countries in the Eastern Mediterranean Region (EMR), contributing to 3% of the total cases. There are an estimated 20,000 TB cases in Iraq. The estimated deaths due to TB are more than 4000 annually.

Table 1: TB burden, rank of Iraq in EMR

	Country	Incidence rate		Country	Incidence rate
1	Djibouti	620	12	Tunisia	25
2	Somalia	286	13	Bahrain	23
3	Pakistan	231	14	Syria	20
4	Afghanistan	189	15	Egypt	18
5	Sudan	119	16	Saudia Arabia	18
6	Morocco	91	17	Iran	17
7	Iraq	64	18	Lebanon	17
8	Yemen	49	19	Oman	13
9	Kuwait	41	20	Jordan	5.4
10	Libya	40	21	West Bank & Gaza Strip	4.9
11	Qatar	38	22	UAE	3.1

During the period 2000-2003 there was a steady increase in the number of notified TB cases, which was interrupted during the period of war and internal conflict 2003-2007. Since then, with the relative improvement in the security situation, and because of external support from the GFATM Round 6 and Round 9 grants (2008-2015), improvement in case notification was observed. The case detection rate of SS+ TB cases has improved from 43% in 2008 to 48% by the end of 2010 (all new forms and relapse). NTP maintained a high treatment outcome (above 85%) since the start of DOTS in 2000 even though the number of defaulters and transferred patients increased in the years 2003-2006 because of the war.

Figure 1: Case notification by treatment category in 2011

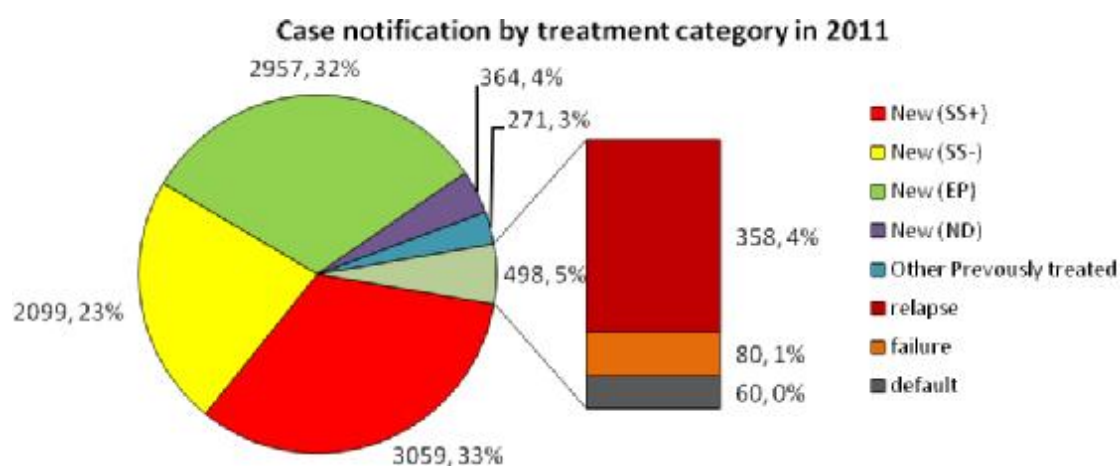


Figure 2: Treatment success and failure rates, 2000-2010.

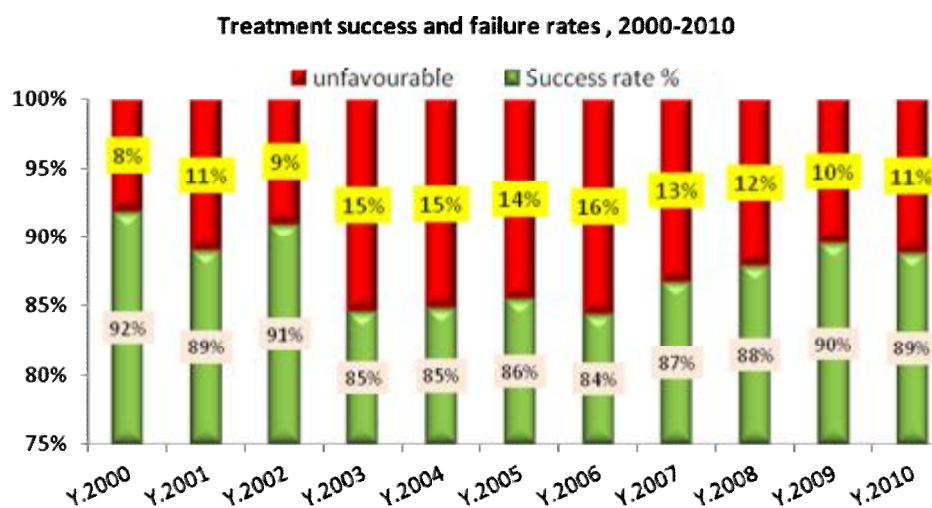


Figure 3: TB notifications per 100.000 population for new (SS+), and (all types + relapse) rates/100.000 population.

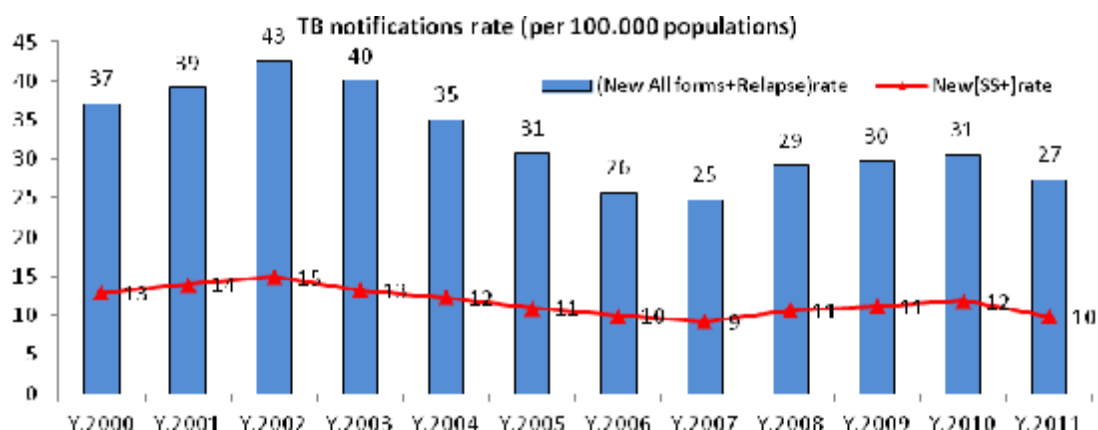


Figure 4: Number of new (SS+) cases detected during 2000-2011.

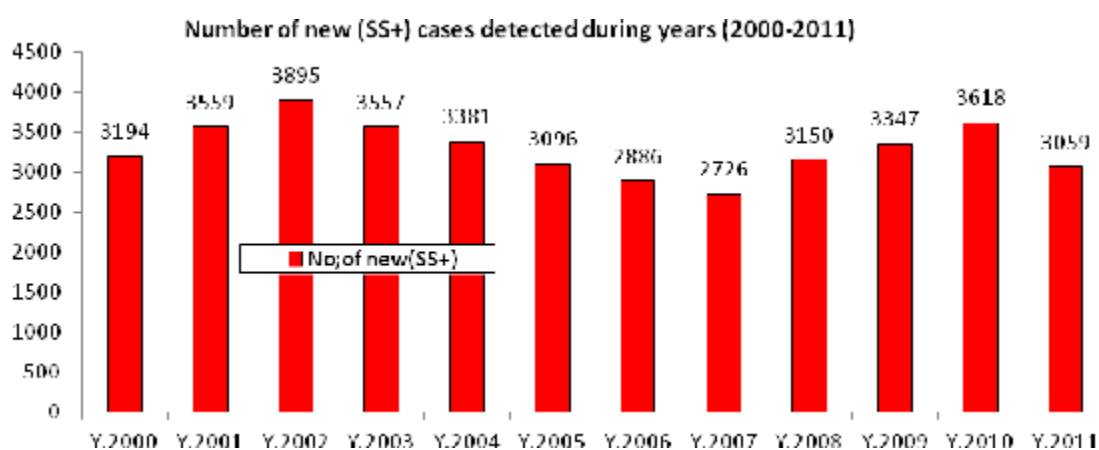
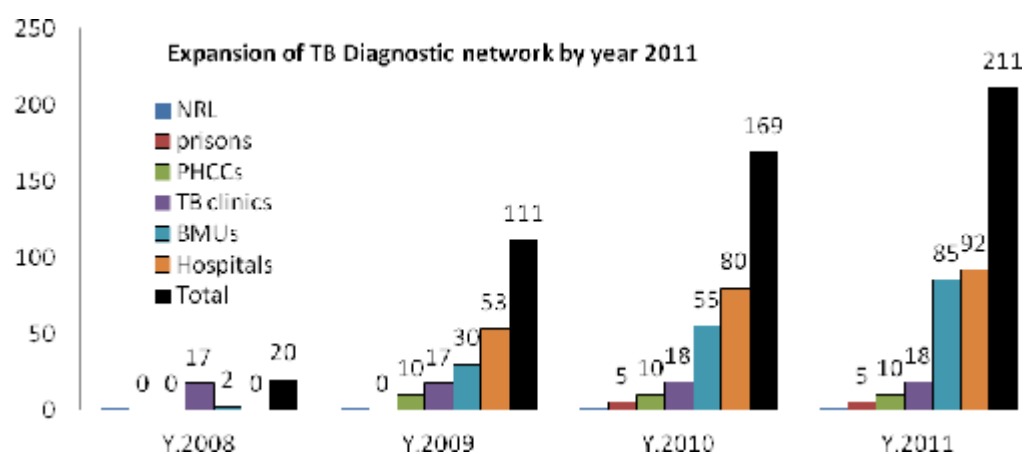


Figure 5: Diagnostic laboratories network in 2011

The characteristics by age and sex remained unchanged during the years 2009-2011 (Figures 6-8). High incidence is generally found in the age group 15-44, with the highest incidence being specifically among the most productive age group of 25-34 years. More cases among males (particularly, aged 15 to 45 years) compared to females are observed for any form of TB infection, especially in SS+ cases. However, the incidence rate is highest in the eldest age group (>65years) compared to any other age group.

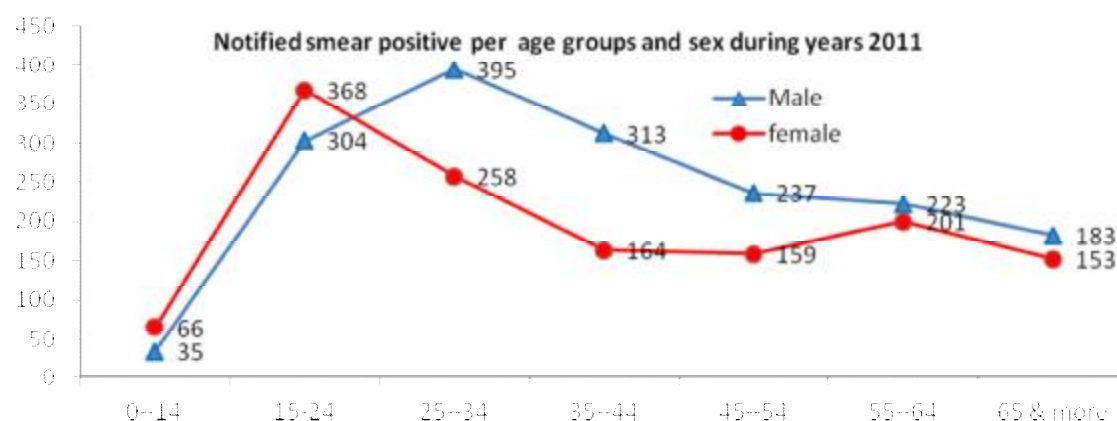
Figure 6: Distribution of new TB cases by age and sex, 2011

Figure 7: Notified new smear-positive cases per age groups and sex (rate per 100,000 population), 2011.

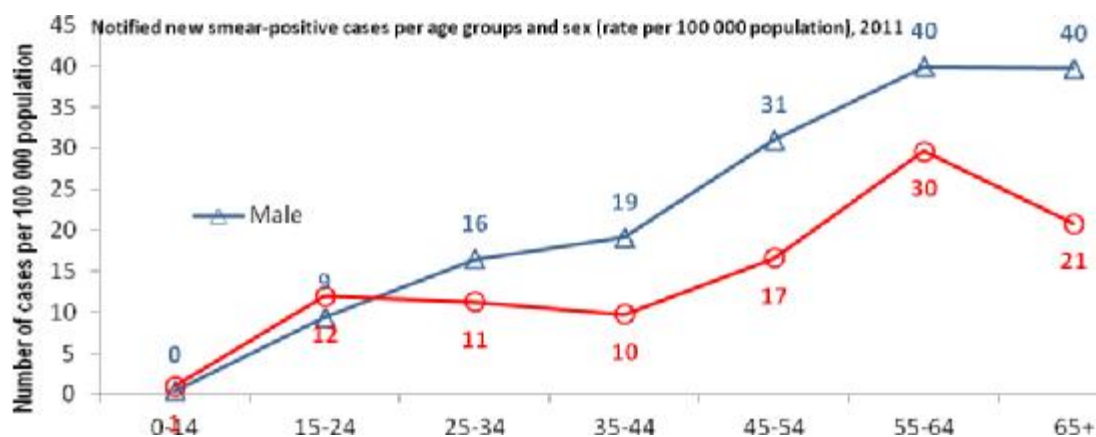
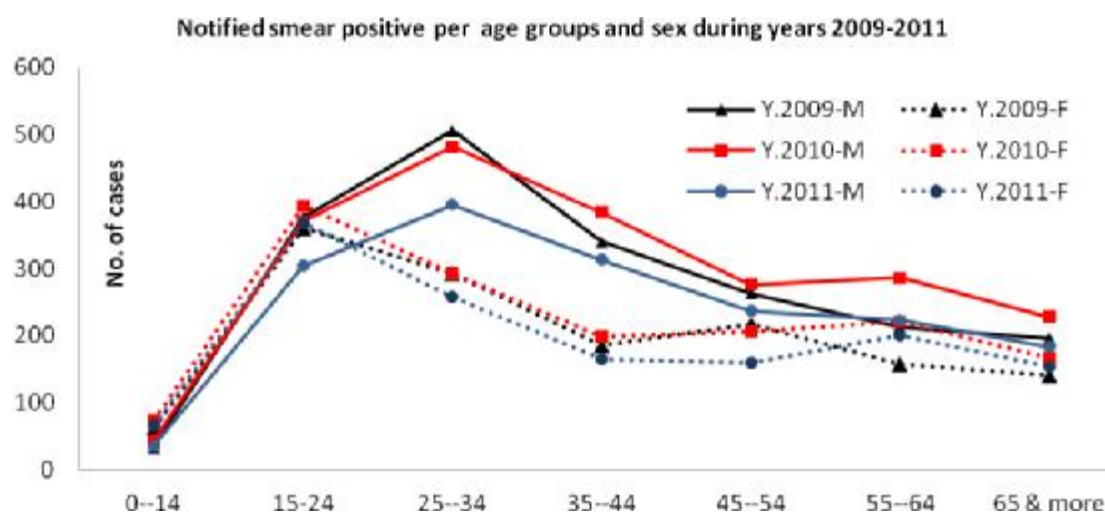


Figure 8: Distribution of new TB cases by age and sex, 2009-2011.



Goal and objectives

Overall goal

The overall goal is to have a more precise overview for drug resistant TB epidemiology in Iraq.

General objective

- Measure the prevalence of drug resistance among new and previously treated smear positive PTB cases in Iraq

Specific objectives

1. Estimate the frequency of resistance to the first-line anti-TB drugs (isoniazid, rifampicin, ethambutol, streptomycin,) among new and previously treated sputum smear-positive pulmonary TB cases.
2. Estimate the prevalence to both INH and RIF in both study groups (new and retreated)
3. Highlight risk factors associated with anti-TB drug resistance.

2. Patients and Methods

Study design

This study was a longitudinal study targeted all new smear-positive TB patients and all pulmonary smear positive cases retreated for the first time from randomly selected clusters.

Sample size determination

The sample size was determined as follow: In 2011 a total of 3059 new smear-positive cases were notified countrywide. The proportion of rifampicin resistance among newly diagnosed patients was assumed to be 2.2%. It is expected to measure a difference in proportion of 1.1% (change of 2.2%) with a 95% confidence. To detect this change a sample size of 559 smear-positive patients is required (obtained using EPI 7 Statcalc programme). Since a cluster sampling strategy was employed, the sample size would be doubled to 1117 to accommodate an estimated design effect of 2. Taking into account the expected losses due to culture contamination or/and patients whose susceptibility testing should not yield interpretable information, the sample size was increased by another 15%. It was estimated that a sample size of 1314 new sputum smear-positive patients would be required (annex ?).

- a. The total sample should therefore include 1314 new smear-positive patients. In addition, specimens were collected and tested from all previously treated cases registered in the selected DTC during the enrolment period for new cases. It is expected that around 226 previously treated cases to be enrolled (annex ?). As per the 2010 data retreatment cases may be too few to make a conclusion with statistical significance.

Sampling strategy

The sampling strategy proposed in this survey is weighted cluster sampling method in which clusters are selected with probability-proportional-to-size (PPS) and in each cluster a fixed number of new patients was included. The primary sampling unit in the survey was the District TB Coordinator unit (DTC). The cluster sampling method is appropriate in Iraq because of the logistic difficulties to cover all DTCs in the country. Within each cluster, a consecutive number of eligible patients were enrolled in the study. A total of 50 clusters have been chosen in this study. In each cluster a total of 26 new smear-positive patients was expected to enroll as well as all retreatment smear-positive cases registered during the intake period. The following methodology was used to select the clusters. A list of all DTCs in the country (from 19 health directorates belonging to the eighteenth governorates of Iraq) with the numbers of new notified smear positive patients for the year 2011 was compiled. From this list a cumulative patient population of new smear positive cases was derived. A random number (21) has been picked between zero and the sampling interval. The sampling interval of 61 equals the number of total smear-positive cases (3 059) divided by the number of clusters (50). The random number determines the first DTC to be selected from the cumulative list. The sampling interval was sequentially added to the random number to identify the remaining clusters from the cumulative list. From this procedure a total of 46 DTCs were selected for the survey.

Given that five DTCs are not expected to reach half the target cluster size of 26 new smear positive cases according to notification data from 2011 additional 7 DTCs with similar demographical characteristics were selected. A total of 53 out of 126 DTCs in the country were enrolled in the survey constituting the 50 clusters (annex ?).

Inclusion and exclusion criteria

Cases were included in the study after having provided informed consent were:

Inclusion criteria:

- b. Newly registered sputum smear-positive cases according to the WHO/International Union Against TB and Lung Disease (The Union) criteria (i.e. no previous treatment history for more than one month);
- c. Children under the age of 15 who satisfy the definition of a smear-positive TB.
- d. Previously treated cases (relapse, failure of Category I, return after default of Category I) presenting to the diagnostic centre during the intake period.

Exclusion criteria:

- Newly registered cases with sputum smear negative pulmonary TB;
- Patients with extra-pulmonary TB;
- Other TB cases (e.g. those that have received already at least one retreatment regimen).

Intake of patients

Based on the number of new sputum smear-positive pulmonary TB cases in 2011 and the number of clusters at each diagnostic center, the estimated intake period is ≤ 16 months in 50 healthcare facilities (Annex 2). Taking into account the expected workload for the eleven diagnostic laboratories at governorate level and the supranational laboratory, the study duration was limited to 18 months and due to logistic difficulties and budget shortage patients' enrollment was limited to only 12 months risking with sample size fulfilment. All newly diagnosed smear-positive

patients should be enrolled in the survey until the required number is reached in each cluster or until the 12-months enrollment period is completed.

Patients meeting inclusion criteria but who cannot be included in the survey for various reasons should be replaced by other patients diagnosed in the same center according to the sampling procedure described. The selected centers should also be required to submit sputum specimen of previously treated patients in addition to sputum specimen of new cases during the intake period of new cases.

Lab procedures

At the start of the DRS the following were in place

- Supervisory visits to all laboratories involved in DRS were conducted to determine the situational conditions at the Laboratories involved in the study, and to detect requirements. Specifically the following were checked during visit: equipment and reagents, procedures, infection control, & IQA;
- Training of laboratory technicians;
- An incentive system for all lab technicians involved in the DRS was identified to ensure continuation of assignment of same lab technicians throughout the period of the study;
- Establish specimen transportation system;
- Procurement of requirements;
- Printing questionnaires, forms, registers.

Diagnostic procedures at the DTCs (BMUs)

- The two sputum samples were smeared and stained using Z.N stain.
- Available reagents and materials:
 - Basic fuchsin,

- Ethyl alcohol,
- Phenol crystals,
- Distilled water,
- Methyl alcohol,
- Sulphuric acid or Conc. HCl (fuming 37%),
- Methylene chloride blue,
- Consumables for DSM (cup, wooden stick, marker pen, diamond pencil, slide ,slide box, waste receptacles, mask burner, gloves, surgical towelsetc),
- Equipments (microscope, timer, UV light, fan extractor, etc.)
- Disinfectants (70% alcohol ,5% phenol or home bleaching)
- Samples transportation consumables

Diagnostic procedures at Governorate TB Clinic (GTCs)

- If culture test was available at governorate chest clinic, then cultures on LJ were done for all smear positive specimens included in the survey. A stained smear was done to confirm positivity of samples. The positive culture was sent to the NRL for DST.
- If there was no availability to conduct culture, then the samples were sent to the nearest culture lab, or to the NRL twice per week (preconditions: samples transportation, consumables, & preservatives).
- The following reagents needed to be sufficiently available throughout the study:
 - NaOH-HCl-phenol red (indicator)-distilled water-NALC powder-Na₂HPO₄-KH₂PO₄-Mg SO₄-Mg citrate-Asparagine-Glycerine-Malachite green-Eggs.
 - Decontamination of sputum specimens by equal amount of 4% NaOH (Petroff's method).

- Culture equipments (BSC class II, Autoclave, oven, incubator, shaker, UV light, & Distillatory)

Culture at the GTCs:

- Decontamination of sputum specimen according to the Petroff method (equivalent volumes of the sample & 4 % NaOH solution are mixed in a screw-capped tube, incubated at 37° C for 15 minutes with shaking, then centrifuged at 2000- 3000 g for 15 minutes. Sediment is then neutralized and washed, the total contact time between NaOH and the sample should not exceed 30 minutes unless the sample is strongly contaminated. The sediment is inoculated on two tubes of LJ medium and incubated at 37° C for 8 weeks or until growth of colonies is observed. All positive cultures are kept until retesting at the SNL has been completed. Ideally they should be stored in a deep-freezer at 20° C but they can also be kept for some time in the refrigerator at + 4° C.
- CPC 1 % or CPB 0.6% can't be used as decontaminant except if used at room temperature or in case of applying liquid media. Never use CPC or CPB if specimen is put in refrigerator, as it may be crystallized and can't protect specimens from contamination and would inhibit MTB growth.
- Decontamination with NALC-NaOH is done for automated detection culture systems. However, fast specimen transport is a prerequisite to minimize contamination.
- Results appear in 2-8 weeks.

At the National Reference Laboratory (Central Level)

- All positive cultures were submitted to identification and DST.
- Identification is based on:

- Niacin production in niacin strip test
- Catalase production at room temperature in catalase test
- Inhibition of MTB growth on LJ containing PNBA in a conc. of 500 mg/L.
- All positive cultures after identification were kept in deep freeze (-70 °c) for genotyping.
- DST is done by Proportion method:
 - ✓ Depends on comparing the number of colonies grown on antibiotic containing media to those grown on antibiotic free media.
 - ✓ Concentration of antibiotics in LJ:
INH 0.2 mg/L E 2 mg/L.
R 40 mg/L S 4 mg/L.
- Reagents that should be available are: Antibiotic powders in addition to culture reagents.
- Reading DST results after 4 weeks:

$$\% \text{ resistance for any antibiotic} = \frac{\text{No. of colonies on drug-containing media}}{\text{No. of colonies on control tubes}} \times 100$$

Table 2: Evaluation of DST results:

Antibiotic	% resistance
INH	< 1% sensitive
R	< 1% sensitive
E	< 1% sensitive
S	< 1% sensitive

I.Q.A at survey lab:

- Rates of contaminated and false –ve cultured are monitored in TB labs involved in the DRS and feedback based on this monitoring is given to survey coordinator. Corrective actions or deletion of these cases should be done.

Contamination rate = $\frac{\text{No. of contaminated culture tubes}}{\text{Total no. of tubes inoculated}} \times 100$ (Shouldn't be > 3%)

- False negative rates applied only to new smear positive patients and smear as denominator, with those for which all or at least one tube stayed negative and none were positive as nominator.
- Lab technicians monitored culture positive results and resistant results by cluster for evidence of cross-contamination.

DST, including rechecking:

Test performed was WHO – recommended to eliminate variability of results arising from different techniques.

- Prior to the DRS, the participating labs proved evidence of proficiency by participating in at least one round of DST proficiency testing with SRL. The lab had – established system of QA.
- NRL used its standard lab results forms to record the results of Culture and DST, with any modifications needed for the DRS.

Results were sent to coordination team and diagnostic centre.

Logistics of DRS

Organization of laboratory services for the survey

All selected district TB coordinating units collected sputum during a specified time period vary from 6-12 months according to patient load and concurrent security condition (in hot areas in Iraq). Collected samples were transported to the respiratory and chest clinic at the same governorate. Then if there is not capacity to perform culture then samples are transported to the nearest governorate chest clinics that perform culture. Governorates that have respiratory and chest clinics that are able to perform culture are Baghdad (NRL), Erbil, Babil, Najaf, Kerbala, Thiqr, Misan and Basra. Then culture samples transported to national reference laboratory in Baghdad.

Collection of specimens

Patients presenting to a healthcare facility with features of pulmonary TB underwent sputum examination. Clinic staff instructed patients on how to best produce a deep sputum specimen. Sputum specimens should be collected away from others in a well ventilated area, in an outdoor setting.

Two sputum specimens collected from each PTB suspect as per NTP policy: a 'spot' sample and a morning sample on consecutive days. Specimen collection jars should be wide mouthed with a well-fitting screw-top lid and with an external label on the outside of the jar (screw caps). Collection jars should be well labeled with points of identification (name of patient, name of district and name of governorate), date of sample collection, as well as a unique numerical identification number that include coding. Cellophane tape should be used to seal the labeling.

Smears should be made and examined by ZN staining at the microscopy centre normally assigned to that clinical facility and only samples with positive smears for AFB are transported (enrolled).

Before enrolment a clear description of the survey goals and procedures should be given and informed consent for each patient should be obtained. Patients were carefully classified based on treatment history as new or previously treated TB cases. A sample of Clinical Information Form is given in **Annex 4**.

Transport of specimens to Provincial Laboratories

Transport of sputum samples (2 for each smear positive patient), from coordinating TB district units (Basic management Units) to the provincial laboratory (at provincial chest and respiratory disease clinic) occurred at least twice weekly. Attention was paid to transport logistics in order to minimize the transport time, exposure to temperature extremes and to prevent breakage and contamination. Sputum samples are transported in cool boxes. The Sputum Shipment Form (**Annex 6**) and Clinical Information Form (**Annex 4**) accompanied the sputum samples. It was recommended that each patient's specimens be placed in a sealed plastic bag. This packaging also provided some protection to the specimen. Provincial DRS coordinators were responsible for establishing efficient and secure transport methods. Sealed in plastic bags; culture specimens were transport in cool box to national reference lab for DST monthly.

Transport of specimens from Provincial Laboratories to SRL

Around 10% of sputum samples cultured for DST and all samples diagnosed with MDR-TB to anti-TB were transported by means of DHL to the SRL in Egypt. Packaging conformed to IATA guidelines and was performed by officers with

appropriate training. Shipment on ice boxes was done. Shipments of samples were expected to be three; after 4 months from starting DSR, after another 3 months, and after another 3 months but actually what happened is all required samples were shifted at the end of field work (after finalization of DST testing for all patients) due to semantic obstacles

Transport time of sputum samples: targets

Following collection and examination, sputa for DRS studies should be:

- refrigerated / cooled with ice as soon as possible;
- transported to Provincial lab or NRL within 48 hour from collection (minimum twice weekly shipments);
- Cultures were sent in monthly pattern to the national reference lab.
- One shipment of study DST cultured samples was sent to SNL in Egypt after the whole study finalized.

Infection Control Measures:

All health facility and laboratory infection control protocols were followed during the study and all personal (masks, gowns, and surgical gloves) and workplace (proper ventilation, exhausts, UV light sources, disinfectants, etc.) and environmental protection measures (as for autoclave for consumables disinfection) were provided.

Quality Control Measures:

- All lab work participants were previously trained.
- District TB coordinators monitored specimen transportation quality and timeliness to intermediate laboratories.

- Governorate coordination teams monitored patients' enrollment and clinical form filling at district level as well as monitored specimen transportation quality and timeliness to national reference laboratory.
- Central coordination teams monitored specimen transportation quality and timeliness to national reference laboratory.
- Central supervisory visits checked the validity of filled clinical forms, specimen transportation, transportation system, cultures, and filling laboratory part of data forms.
- Routine internal and national level external quality assurance measures continually and vigorously implemented to ensure high quality findings of the study.
- All of resultant MDR samples and 10% susceptible samples after DST were retested externally by the supranational laboratory in Egypt.

Training

First training for trainers was conducted in Baghdad for the national team and the central NTP coordination team of twenty people during August 2013. This was followed by five workshops in clusters held in Erbil, Basra, Najaf and Baghdad during October 2013. Each training workshop was conducted for two days and covered aims, eligibility, logistics, and forms filling. The total numbers of staff trained were approximately 140 field staff from NTP/MOH undertaking the field work in the 50 sampled districts in the study. The main groups of nominees were from below categories:

1. Chest clinic that provide culture for the survey sample group and the
2. Chest clinic staff that do not provide culture;

3. Laboratory technicians responsible for monitoring sputum sample transportation in governorates with and without culture;
4. District TB coordinators in health districts involved in the survey;
5. Laboratory technicians responsible for direct smear microscopy during the survey;
6. Laboratory technicians responsible for doing culture for sputum samples during the survey ;
7. Laboratory technicians responsible for doing anti-TB drug susceptibility testing for sputum sample during the survey;
8. Staff responsible for preparing the Excel & SPSS statistical package forms related to the survey;
9. Supervisors for data input during the survey;
10. Staff responsible for data input during the survey

Supervisory visits

Governorate TB Coordinators were responsible for monitoring the survey implementation in their Governorates and to report on progress to the Coordinating Team on monthly basis. All DTCs involved in the survey should be visited by the Governorate TB Coordinator at least once every month.

Routine NTP supervisory visits for lab work and regular semiannual supervisory visits were employed for checking procedures, verifying that all eligible patients are included (based on the laboratory or treatment register), monitoring completeness and quality of clinical information forms. A short standardized monitoring reports were produced (**annex** ?). Central supervision was in coordination with WHO technical assistance in 4 monitoring missions. To the

extent possible problems identified were addressed on the spot which is clear during study monitoring missions (**annex**).

Study implementation

The data collection for the study began in November 2013 and completed in March 2015 including patients' enrollment from November 2013 to end of October 2014.

Data management and analysis

Data management (data collection, verification and entry)

Soon after receiving the questionnaire (clinical forms, lab results for DSM with/without culture results-according to availability of culture testing-) from the Governorates, all relevant data were entered in a dedicated data entry database (Excel format). The coordinating team was responsible for this task. Supervision of data entry was sustainably available, in addition, the technical officer for the study affiliated by WHO had randomly checked at least 10% of entered forms at different three occasions (documented in monitoring missions' reports-**annex**)

Data analysis

Data were analyzed initially (crude analysis) by NTP OR focal person using SPSS format yielding crude estimates for the proportions of drug resistance for new and retreated patients. Then at WHO Headquarter office in Geneva, under WHO technical assistance and using STATA format, further and detailed statistical analysis was done. This analysis took into consideration the following issues:

- 1- Weighted analysis taking into consideration the weight of each case in presenting this study findings.

- 2- Intra-cluster and inter-cluster variation using random effect analysis for inter-cluster analysis, and robust SE for intra-cluster analysis during estimating proportions of drug resistance.
- 3- The number of missing results, (due to contamination, negative cultures and insufficient growth for DST) were compensated with imputation method to increase the power of the study by regaining the actual sample size during estimating proportions of drug resistance.

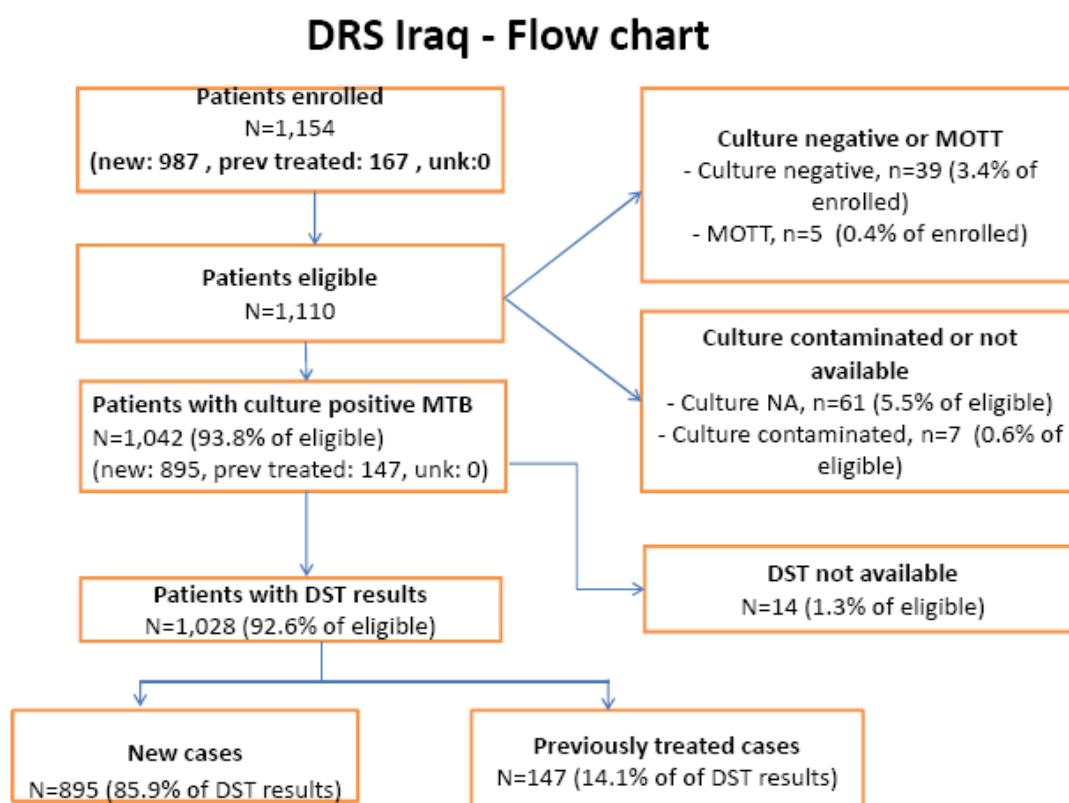
Studied factors then tested with univariate logistic regression analysis to find out risk factors associated with anti-TB drug resistance.

Results

A) Crude Analysis:

Total enrolled patients were 1110 SS+PTB patients including 987 new patients (75.1% out of planned sample size) and **167** retreatment cases (73.9% out of planned sample size) (figure 9).

Figure 9: Results of enrollment and laboratory testing:



39 patients had negative culture results (3.4% out of enrolled) and five patient were having mycobacteria other than TB (MOTT) (0.4%), 61 patients were not associated with known culture results (5.5%), in addition, 7 cultures were contaminated (0.6%) (figure 9).

DST was requested for 1042 patients where results appeared only for 1028 patients; 895 (85.9%) new & 147 (14.1%) retreated patients.

Male to female ratio was 1.2:1 in both study groups (table 3) and dominant age group among new patients was 15-24 year (23.9%) and among retreated patient 25-39 year (27.9%). More than two thirds of selected patients from urban settings (table 3).

Analysis of missing values regarding age group, sex and place of residence showed no significant contribution to observed analysis of these variables ($P > 0.05$, table 4).

Regarding first line anti-TB drug resistance among new PTB patients: Around 96.4% were susceptible to all of INH, RIF, Ethambutol and Streptomycin. Any resistance to INH was encountered in 8.8% of the sample and any resistance to RIF was found in 6.3% of the sample. Ethambutol resistant found in 6% of the sample while resistance to Streptomycin was found in 21.7%. Monoresistance, poly resistance & MDR were found as prevalence as 19.7%, 9.1% & 1.1% respectively (table 5).

Table 3: Distribution of sampled SS+PTB patients according to treatment history and to demographic characteristics:

Variables	New cases		Previously treated cases		Total cases	
	n	%	n	%	n	%
	956	86.1	154	13.9	1,110	100.0
Sex						
- Male	526	55.0	85	55.2	611	55.1
- Female	429	44.9	69	44.8	498	44.9
- Missing	1	0.1	0	0.0	1	0.1
Age group (y)						
- 0-14	21	2.2	0	0.0	21	1.9
- 15-24	228	23.9	26	16.9	254	22.9
- 25-34	191	20.0	43	27.9	234	21.1
- 35-44	162	17.0	26	16.9	188	16.9
- 45-54	124	13.0	18	11.7	142	12.8
- 55-64	118	12.3	28	18.2	146	13.2
- ≥65	108	11.3	12	7.8	120	10.8
- Unknown	4	0.4	1	0.7	5	0.5
Place of residence						
- Urban	664	69.5	115	74.7	779	70.2
- Rural	260	27.2	36	23.4	296	26.7
- Other	26	2.7	3	2.0	29	2.6
- Missing	6	0.6	0	0.0	6	0.5

Table 4: Distribution of missing values encountered during analysis:

Variables	%	P value
Treatment history		0.384
- New patients	7.1	
- Previously treated patients	9.1	
Sex		0.777
- Male	7.9	
- Female	6.8	
- Missing	0.0	
Age group, years		0.311
- 0-14	0.0	
- 15-24	6.3	
- 25-34	10.7	
- 35-44	6.4	
- 45-54	8.5	
- 55-64	6.2	
- ≥ 65	5.8	
- Unknown	20.0	
Place of residence		0.075
- Urban	7.1	
- Rural	8.1	
- Other	3.1	
- Unknown	33.3	

Regarding to anti-TB resistance among retreatment group:

Half the sample was susceptible to all tested first line drugs (INH, RIF, Ethambutol & Streptomycin). Any resistance to INH was found as 26.5%, to RIF as 29.3%, to Ethambutol as 15.6%, and to Streptomycin as 29.9%. Monoresistance, Polyresistance & MDR to anti-TB were found as 19.7%, 6.8%, and 20.4% respectively (table 5).

Table 5: Prevalence of anti-TB resistance in the two study groups:

Resistance Type	New		Retreatment	
	N=895	%	N=147	%
Susceptible for all	621	69.4%	74	50.3%
INH resistant	79	8.8%	39	26.5%
Rifampicin resistance	56	6.3%	43	29.3%
Ethambutol resistant	54	6.0%	23	15.6%
Streptomycin resistant	194	21.7%	44	29.9%
Monoresistance	176	19.7%	29	19.7%
Polyresistance	81	9.1%	10	6.8%
MDR	10	1.1%	30	20.4%

B) Advanced analysis:

After using STATA format for advance analysis taking into consideration, weighing of cases, intra- & inter- cluster variation, and compensation for missing values of DTS; the final estimate for MDR-TB prevalence among SS+PTB cases was 1.1% in new cases and 19.7% in retreated cases (Table 6). And the proportion of susceptible patients is 71.3% in new cases and 52.9% in retreated cases. The proportion for INH resistance in new & retreated cases was 7.6% & 27.9% respectively while the proportion for RR in new and retreated cases was 5.9% & 24.3% respectively (table 7).

Table 6: Estimated prevalence of MDR-TB in the two study groups using different analytic methods:

Method of Analysis	Estimated Proportions of MDR-TB					
	New Patients			Retreatment Group		
	%	L	U	%	L	U
Crude	1.1	0.5	2.1	20.0	13.7	27.6
Weighted	0.9	0.5	1.7	---	---	---
Robust SE	0.9	0.3	1.5	20.0	12.8	27.2
Random effect	1.1	0.4	1.8	20.0	13.4	26.6
Imputation+ Robust SE	1.1	0.3	1.8	19.7	12.5	26.8

Table 7: Proportions and 95% confidence intervals for INH and to RIF:

Drug-resistance pattern	<u>New</u> % [95% CI]	<u>Previously treated</u> % [95% CI]
Susceptible to all drugs	71.3 [66.1- 76.0]	52.9 [44.3 - 61.3]
Any drug resistance	28.7 [24.0 - 33.9]	47.1 [38.7 - 55.8]
- Any resistance to H	7.6 [5.4 - 10.7]	27.9 [20.6 - 36.1]
- Any resistance to R	5.9 [4.4 - 8.0]	24.3 [17.4 - 32.2]

Among studied factors (age, sex, treatment history and residence); Only previous treatment history was a significant risk factor for MDR-TB according to this study ($P < 0.05$, table 8).

Table 8: Univariate logistic regression for study factors as factors contributing to anti-TB drug resistance:

Variables	Tested (N)	MDR (%)	OR	95%CLs		P value
				Upper	Lower	
Sex						
- male	563	3.1	---	---	---	---*
- female	464	2.8	0.9	0.4	1.8	0.742
Age group, years						
- 0-24	259	2.1	---	---	---	---*
- 25-34	209	3.5	1.7	0.5	6.1	0.397
- 35-44	176	2.3	1.1	0.3	4.1	0.886
- 45-54	130	4.3	2.1	0.5	7.9	0.279
- 55-64	137	4.5	2.1	0.5	8.6	0.261
- ≥65	113	1.1	0.5	0.1	3.1	0.445
History of treatment						
- New cases	888	0.9	---	---	---	---*
- Previously treated	140	15.2	19.3	7.5	50.0	0.000
Place of residence						
- Urban	724	0.3	---	---	---	---*
- Rural	272	0.3	1.0	0.5	2.2	0.982
- Other	28	0.3	0.8	0.1	6.7	0.856
*Background level						

- **Challenges during implementation**

- This workload is witnessed for the first time by NTP lab network and was a test for the intermediate lab capabilities in particular since they took the responsibility for culture for more than one governorates' samples.
- It is the first time to use such an active, prompt and regular samples transportation at a national level and in different weather conditions.
- At month six of patient enrolment for the study, security events broke through that affected the middle part of Iraq and disconnected northern areas affecting patients' enrolment. Anyhow;
 - Compensation was done through increasing sample size at more stable areas.
 - Conflict areas unexpectedly kept sending samples until the land road was cut 100%
- Delay in transfer cost release in some instances, summer heat waves and power shortage damaged some sputum & culture samples and contributed to further losses in effective size.
- NTP team feels proud for continuing the study in such critical security situation and approaching more than 75% of planned sample size, continuing the heavy lab work, finalizing the field work of the study, and submitting database within the planned schedule. NTP core and DRS steering committee are deeply bound to all study field staff for their self-neglect and outstanding conduct in such subnormal work conditions.

Limitations of the study

- Losses of eligible participants
 - Logistic considerations and financial support limitations has reduced the period of patients enrollment from 18 to 12 months which affected the final sample size of the study.
 - Due to the security breakthrough in month six, this study enrolment experienced the inability to continue enrollment in some governorates (12/50 clusters) rather than non-participation, or inability to transport sputum/culture samples.
- Implications for representativeness
 - For the above reason, this study cannot be considered fully national study.
 - Mostly affected governorates (clusters' size < 50% of estimated size at governorate level) are Erbil and Dohuk. If we exclude them, then this study will be representative for the remaining 16 governorates.

Conclusion and Recommendation

- Presence of MDR in Iraq is low (1.1%). However, it needs to be monitored because:
 - H resistance is relatively high (7.6%)
 - R resistant is relatively high (5.9%)
- Previous treatment with anti-TB is the main risk factor for MDR-TB in Iraq.

Table: Enrollment of TB patients in drug resistance survey:

Governorate	District	New SS+PTB			Cat 2 SS+PTB		
		Target	Enrolled	% enrolled	Target	Enrolled	% enrolled
الرصافة-بغداد Baghdad-Resafa	Sadr	78	91	116.7	21	19	90.5
	Baladiat	52	55	105.8	17	14	82.4
	Resafa	26	18	69.2	6	7	116.7
	Shaab	26	28	107.7	5	6	120.0
	Madain	26	14	53.8	5	5	100.0
<i>Subtotal</i>		208	206	99.0	54	51	94.4
الكرخ-بغداد Baghdad-Karkh	Krakh	26	3	11.5	5	1	20.0
	Kadhmia	26	26	100.0	8	4	50.0
	Ilam	26	7	26.9	5	2	40.0
	Mahmoodia	26	19	73.1	7	7	100.0
	Amil	26	19	73.1	5	4	80.0
<i>Subtotal</i>		130	74	56.9	30	18	60.0
البصرة Basrah	Basrah-1	26	28	107.7	12	4	33.3
	Basrah-2	26	24	92.3	8	3	37.5
	Abo-Alkhaseeb + Al-Zubair	26	20	76.9	4	5	125.0
	Hartha + Qurna	26	24	92.3	7	1	14.3
<i>Subtotal</i>		104	96	92.3	31	13	41.9
نينوى Ninawa	Ayser	26	28	107.7	5	1	20.0
	Sinjar	26	9	34.6	0	0	0
<i>Subtotal</i>		52	37	71.2	5	1	20.0
ميسان Misan	Amara	26	26	100.0	4	4	100.0
الديوانية Diwania	Diwania-1	26	21	80.8	4	3	75.0
	Shamia	26	13	50.0	4	2	50.0
	Diwania-2	26	26	100.0	5	3	60.0
<i>Subtotal</i>		78	60	76.9	17	8	47.1
ديالى Diala	Baquba	26	34	130.8	0	1	
	Khalis	26	5	19.2	3	0	0.0
	Miqdadia	26	21	80.8	3	5	166.7
<i>Subtotal</i>		78	60	76.9	6	6	100.0
بابل Babil	Hilla 1 + Hilla 2	52	31	59.6	4	5	125.0
	Hashmia+ Mahweel	26	10	38.5	4	3	75.0
<i>Subtotal</i>		78	41	52.6	8	8	100.0
الأنبار Anbar	Ramadi-1	26	4	15.4	4	0	0.0
	Falluja	26	5	19.2	4	0	0.0
<i>Subtotal</i>		52	9	17.3	8	0	0.0
كربلاء المقدسة Karbala	Karbala-center	26	26	100.0	1	5	500.0
	Hindia	26	26	100.0	1	2	200.0

Subtotal		52	52	100.0	2	7	350.0
كركوك	Kirkuk-1 + Kirkuk 2	26	19	73.1	5	0	0.0
Kirkuk	Haweeja-2+ Haweeja1	26	8	30.8	7	1	14.3
Subtotal		52	27	51.9	12	1	8.3
واسط	Al-Hay	26	13	50.0	0	0	
Wasit	Kut-2+kut 1	26	25	96.2	8	6	75.0
Subtotal		52	38	73.1	8	6	75.0
ذي قار	Nasirya-2	26	26	100.0	0	0	
Thiqar	SuqAlshuyukh	26	22	84.6	1	1	100.0
	Rifai	26	26	100.0	3	3	100.0
Subtotal		78	74	94.9	4	4	100.0
المثنى	Simawa+Rumaitha	26	4	15.4	3	1	33.3
صلاح الدين	Biji +Shirgat	26	15	57.7	8	2	25.0
Salahelden	Balad	26	2	7.7	3	2	66.7
Subtotal		52	17	32.7	11	4	36.4
النجف الاشرف	Najaf Shimali	26	26	100.0	4	1	25.0
Najaf	Najaf Janoobi	26	26	100.0	6	6	100.0
Subtotal		52	52	100.0	10	7	70.0
اربيل	Suran +Shaqlawaw+Qwisinjaq+Erbil Centre	26	6	23.1	9	0	0.0
دهوك	Shikhan + Duhok + Aqraa+Baradarash	26	17	65.4	4	0	0.0
السليمانية	Sulaimania-center	26	31	119.2	3	3	100.0
Sulaimania	Chamchamal	26	0	0.0	4	1	25.0
	Kalar	26	1	3.8	1	1	100.0
Subtotal		78	32	41.0	8	5	62.5
Grand Total		1300	928	71.4	230	144	62.6

NB) Districts with red fonts were not planned to be a source of sampled patients but added after month six of enrollment after the security break through by ISIS to overcome sampling deficiency.