SURVEILLANCE PROTOCOL

Entitle:
STUDY OF PRIMARY DRUG RESISTANCE IN TUBERCULOSIS, BHUTAN
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   Monggar Regional Referral Hospital,
   Mongar.

   Sangay Wangchuk, DMLT
   Central Laboratory
   Gelephu Regional Referral Hospital,
   Gelephu.
III. Roles and Responsibilities of Study Investigators:

Mr. Sonam Wangchuk will serve as team leader and responsible for planning, preparation and administration of the protocol. He will provide all technical guidance for the study. He will guide the protocol through Bhutan Health Research Council and Ethical Review Board (ERB) in the Ministry. He will be responsible for the writing report and publication to this study.

Mr. Karchung Tshering will be responsible for DST testing in the laboratory. He will manage and guide laboratory technicians involved in culture, identification, DST in Mycobacteriology section, PHL. He will make supervisory visit to western region hospital labs if there is problem with specimen quality, storage and transportation. He will contribute to the report writing and publication related to this study.

Mr Binay Thapa, will serve as focal person in the Eastern regional responsible for sample transportation to PHL. He will make supervisory visit to Eastern region hospital labs if there is problem with specimen quality, storage and transportation.

Mr Sangay Wangchuk will serve as focal person in the Central regional responsible for sample transportation to PHL. He will make supervisory visit to Central region hospital labs if there is problem with specimen quality, storage and transportation.
1. Background

Tuberculosis is a major public health concern in the South East-Asia region contributing to 2/3rd of total global tuberculosis burden. Tuberculosis is also a public health problem in Bhutan and to combat the tuberculosis in the country, the national tuberculosis control program (NTCP) was established in 1976. Based on ARTI 1.5% (ARTI survey conducted 1991), the current estimated incidence of tuberculosis infection is 159/100,000 population (WHO report 2008) and new smear positive cases 75/100,000 populations (WHO report 2008); which is equivalent to 516 new smear-positive cases taking the baseline of country population at 6, 50,000.00 (population and housing census conducted in 2004) with escalation growth rate 1.5% annually. Bhutan was one of the first countries in the region to introduce DOTS in 1997 which was adopted by WHO as global strategies to fight against the tuberculosis. Despite the nationwide DOTS coverage above 95% from 2000-2007 (NTCP cohort data), no significant reduction in tuberculosis disease burden was not accomplished (NTCP cohort data for last 2002–2007). The overall case detection and cure rate marginally remained same from 69-72% and 75-78% respectively in 9FYP against the target set at 75% case detection and 85% cure rate.

In 2008, the detection rate remained more or less same at 70.9% but drastic increased in cure rate 93.9% was observed with mortality rate round 3% (NTCP cohort report 2008). However, 46 relapse and 18 failure cases were reported, the highest number reported till date. The MDR cases registered for 2008 was 8 numbers but only two were laboratory confirmed.

The HIV cases are also on rise every year since the detection of first case in 1993 in the country. However, the current detection rate is still low as per the estimates based on sexual risk behavior of the population and youth population which is estimated to be 40% at the moment. Though the incidence of tuberculosis infection among HIVAIDS in the country is not known but studies done in the other countries have showed increase tuberculosis cases with HIV epidemic since Mycobacterium tuberculosis being one of the first opportunistic to infect immuno- suppressed in HIV infected patients. The studies have also reported high incidence of MDR cases among HIV patients.
2. Rationale

The prevalence rate of primary anti-tuberculosis drug resistance in the country is not yet established at the moment which is one of the critical indicators for program evaluation. The periodic laboratory base surveillance study is necessary to know the rate of drug resistance in the country against base line to guide program to strategies the necessary intervention

The development of tuberculosis drug resistance strains among the infected patients are the natural phenomenon and cannot be avoided but the inefficient tuberculosis control program will invariably increase the drug resistance cases in the population. The tuberculosis drug resistance is caused mainly due to poor patient compliance and other associated factors like poor case holding, improper prescription of treatment regimens, failure to follow up the patients and inadequate and poor quality of drug supply.

The National Tuberculosis Control Program has started long course chemotherapy after its inception in 1976 and in 1994, NTCP introduced short course chemotherapy nationwide followed by DOTS in 1997. After 32 years of NTCP inception, no drug resistance study was conducted to find out the prevalence of primary ATT drug resistance in the country. There was no drug resistance or multi-drug resistance (MDR) cases reported either clinically or laboratory tested in the country until 2000. As of December 2008, 93 MDR cases have been registered in TB unit JDWNRH base on clinical finding and put under second line ATT drugs (Table 1, NTCP cohort data). Most declare MDR cases were treatment failure cases against CAT I and CAT II but no clear information are available with program to make more cases analysis. In recent years NTCP has recorded the increase number of relapse and failure cases (NTCP cohort data 2005-2008); an indication of presence of drug resistance among patients.

The rising number of HIV cases every year in the country further demands the urgency to conduct MDR study to determine the prevalence rate of drug resistance or MDR to monitor the incidence of MDR among general population and HIV infected patients. Also, the availability of quality DST data has become an eligible criterion for any country applying for the procurement of second line tuberculosis drugs to green light committee (GLC) funded by Global Fund.
Table 1: MDR cases from 2000 -2008

<table>
<thead>
<tr>
<th>Year</th>
<th>2000</th>
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<th>2003</th>
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<th>2006</th>
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<th>2008</th>
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<tbody>
<tr>
<td>MDR</td>
<td>13</td>
<td>13</td>
<td>05</td>
<td>09</td>
<td>20</td>
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<td>12</td>
<td>06</td>
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Note: Only 4 cases were laboratory confirmed from Vellore, India and Bangkok, Thailand.
In 2008, 2 were laboratory confirmed (PHL and NTRL, Bangkok, Thailand)

3. Objectives

1. Determine the prevalence rate of primary drug resistance pattern among new diagnosed sputum positive cases in the country
2. Determine the prevalence rate of previously resistance pattern among previously treated sputum positive cases in the country

4. Materials and Methods

1. Sampling strategy
100% sampling strategy will be used for the study. Country has 29 hospital/BHU-I laboratories performing sputum microscopy distributed across 20 districts. The locations of 29 labs are geographically representative and strategically located to catch all smear positive cases for the study if case holding is good in all hospitals. The only problem perceived with 100% sampling strategy is the sample transportation from hospitals to PHL due to transportation constraint in the country. However, data shows that from 29 hospitals, 60% of smear positive cases are reported from a national referral, two regional referral, Phuentsholing, Samtse and Trashigang hospitals from where samples transportation to PHL is fairly established. Samples transportation from other hospital labs could be easily worked out with movement of ambulance since very less sample
sized is expected. Two regional referral hospitals will be used as transient point for sample transportation to PHL.

2. Sample size

The estimated smear positive as per the country population for 2009 is 516 new smear-positive base on ARTI 1.5% but PHL expects to detect around **450 smear positive cases** as per the trend and NTCP annual cohort data analysis for past 4 years (Table: 2). The estimated total samples to be processed for culture, identification and DST by PHL is around 900 samples. On average PHL will be processing 75 samples per month and 4 samples per day during one study period (July 2009 to June 2010).

Table 2: Total number of sputum positive cases detected annually (2005-2008)

<table>
<thead>
<tr>
<th>Year</th>
<th>Total smear positive patients detected</th>
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<tr>
<td>2008</td>
<td>420</td>
</tr>
<tr>
<td>2007</td>
<td>381</td>
</tr>
<tr>
<td>2006</td>
<td>372</td>
</tr>
<tr>
<td>2005</td>
<td>353</td>
</tr>
</tbody>
</table>

3. Intake of patients

3.1 Inclusion criteria

A patient registered as a smear positive case (**new* or previously treated**) according to WHO/UATLD definition of smear positive should be included. Children under the age 15 years who meet the inclusion criteria should also be included.

Definition:

* **New cases**: for the purpose of surveillance, resistance among new cases is defined as the presence of resistant strains of *M. tuberculosis* in a patient who, in response to direct questioning denies having had prior anti-TB treatment (for more than 1 month), OR, has no evidence of such history from adequate documentation if available with patients or hospitals.

**Previously treated cases**: for the purpose of surveillance, resistance in a previously treated patient is defined as the presence of resistant strains of *M. tuberculosis* in a patient who, in response to direct questioning admits having been treated for tuberculosis for 1
month or more, OR, has evidence of such history from adequate documentation if available with patients or hospitals.

**Case definition “new cases”**
Patients with at least two sputum smears positive for AFB under direct microscopy who has no history of past tuberculosis infection

OR

One sputum specimens positive for AFB and radiographic abnormalities consistent with PTB who in response to direct questioning denies having had any prior (more than one month) or adequate document available for whom there is no evidence of previous TB infectious or treatment history.

### 3.2 Sputum collection and transport

The hospitals selected for the study will send two sputum samples in addition to the initial sputum sample used for diagnosis so that the PHL will have a total of at least two samples preferably overnight and spot sample or two spot samples. Correct collection of samples is critical to ensure accurate and reliable results. Patients should be given clear instructions with demonstration to produce sputum in open air or away from other people in suitable container supplied. *Treatment for any period of time will reduce the chance of culture positivity, therefore samples must be obtained before treatment is started.*

Samples should be kept in refrigerator at 4°C after collection and shipped to PHL in proper cold chain within 48 hours. However, if samples cannot be transported with 48 hours, 1% cetylpyridinium chloride (CPC); equal to the volume of the sputum should be added and mixed to homogenize and decontaminate samples for further storage and shipment to PHL weekly from nearby hospitals and twice a month from far places. *Never refrigerate (stored at 4°C) after adding CPC because of the likelihood of crystallization at cool temperature which cannot protect sample from contamination and inhibit the growth of M. tuberculosis.* The patient’s identification number for two successive samples must be labeled on container (not on the lid). The samples should be sent with sputum shipment form to PHL. A copy of the form must be retained in respective hospital labs. Before shipping samples to PHL, each container should be packed with absorbent
paper and packed into the small biohazard plastic bag to absorb and protect any leakage caused by accident during transportation. The samples should be transported in shipment /cold box without icepack if mixed with CPC.

**Note:** Cold places especially in winter where temperature falls below \(<10^\circ\text{C}\), 1% cetylpyridinium chloride (CPC) should not be used as preservatives for transportation to PHL. Hospitals must make an effort to transport within 48 hours. **Hospitals far from NTRL must store samples at 0 \(^\circ\text{C}\) till they are transported.**

### 3.3 Registration
For surveillance study, three forms will be used – the clinical form, the sputum form, and the laboratory form. Each patient meeting inclusion criteria should be assigned a serial number which should be recorded on the intake form (site identification number will be provided for each hospital during study period to avoid duplication number).

#### i. Clinical form
The main objective of the clinical form is to correctly identify the patient as new case (never treated or treated in the past for less than 1 month) or as previously treated for TB). The form consists of four information.

- patient identification
- patient history
- documented data on previous treatment episodes
- final decision on history of previous treatment

The clinical form (Annexure: 1) is design to collect accurate information about the patient history of past treatment if any which is critical and has important implications for subsequent data analysis and interpretation. Especial efforts are therefore needed from clinicians/medical officers during the surveillance to ensure the reliability of clinical data. Where ever necessary two medical officers should interview patient independently to validate clinical data collected. A copy of clinical form should be sent to PHL separately along with samples.
ii.  **Sputum shipment form**

The sputum shipment form includes the following information:

- identification of patient
- date of sputum collection
- result of the smear examination at the diagnostic centre
- Date of sample shipment

This form should accompany the sputum sample to PHL and copy to be kept at the respective hospital labs.

iii. **Laboratory results form (this form will be filled by NTRL, PHL)**

The Laboratory results form includes the following information:

- patient identification
- results of identification of M. tuberculosis in the two samples sent to PHL
- Results of susceptibility testing done on only one sample.

4. **Laboratory Methods**

4.1 **Sample decontamination**

The aim of deconatmiantion is to kill as much of the containing flora as possible while harming as few mycobacteria as possible. The decontamination will be done by modified Petroff method exposing mycobacteria up to 2% of NaOH for sputum samples that are being shipped to PHL without CPC. Samples containing CPC need not decontaminate by Petroff method since CPC decontaminates and homogenized the sputum sample.

4.2 **Cultures**

After decontamination by modified Petroff method the sediment is inoculated on two tubes of LJ medium. Sample containing CPC should be directly centrifuge at >3000 g for
15 minutes and discard it. Add distilled water on to sediment to (equivalent to original volume) to wash CPC and re-centrifuge. Inoculate the sediment on two tubes of LJ medium. The cultures are incubated at 37\(^{0}\)C for 8 weeks or until growth of colonies is observed. The first reading should be done after 48 hours and then weekly. Each culture is examined for morphology and pigmentation and the week of appearance of the colonies is noted. If there is no growth by 8\(^{th}\) week or in case of contamination, the cultures are discarded and the laboratory form completed accordingly. All positive culture will be stored in deep-freezer at -20\(^{0}\)C for retesting if required and cross-checking with supranational reference laboratory (SRL).

4.3 Identification
Preliminary identification of strains will be based on acid-fastness and cord formation. However, definitive identification based on biochemical tests is necessary at least para-nitrobenzoic acid (PNB) susceptibility test, thiophene carboxylic acid hydrazide (TCH) resistance test, nician product test, nitrate reduction test, and catalase test. If colonial morphology is consistent with \textit{M. tuberculosis complex}, only one culture per patient needs to be process for DST. Mycobacterial strains other than \textit{M. tuberculosis} will not be processed for DST for the purpose of surveillance.

4.4 Drug Susceptibility Testing (DST)
Drug susceptibility tests will be performed using variant of the proportion methods using LJ medium. Resistance will be expressed as the percentage of colonies that grow on critical concentrations of the substance i.e. 0.2mg/l for isoniazid, 2mg/l for ethambutol, 4mg/l for dihydrostreptomycin sulfate, and 40 mg/l for rifampicin. The interpretation will be based on the usual criteria for resistance 1% for all drugs. To rule out non-tuberculosis mycobacteria p-nitrobenzoic acid medium will be used. The results of the tests will be recorded on the lab form and data will be complied for analysis.

4.5 Quality Assurance
4.5.1 Internal quality control at PHL
Drug and drug free media will be prepared based on requirement twice a month. Every new batch of LJ medium and each drug media prepared will be tested for contamination and susceptibility on the standard H$_3$Rv strain. Every batch of DST processed on samples should have control H$_3$Rv KK11-20, *M. tuberculosis* SM & RFP resistance, *M. tuberculosis* INH & EBM resistance, and *M. fortuitum* ATCC 6841 stains inoculated on both free and drug LJ media. In case of any discrepancies results with control strains on free and drug media; the whole batch of DST samples should be considered as invalid and repeat the test.

### 4.5.2 International quality control with SRL

National Tuberculosis Reference Laboratory, Ministry of Public Health, Bangkok, Thailand is the WHO regional Supra Reference Laboratory identified for National Tuberculosis Reference Laboratory in PHL. The SRL will be responsible for assessing quality of DST and provide all technical assistance required by NTRL, PHL to strengthen and maintain quality at par with reference lab at all time through following mechanisms:

1. SRL will send 30 panel test samples to assess the standard of DST of NTRL, PHL biannually (Annexure 5).
2. SRL will make supervisory visit annually to NTRL, PHL to conduct onsite evaluation.
3. NTRL, PHL will send 20% of total samples strain to SNRL quarterly for cross-checking.
4. NTRL, PHL will send samples to SRL if confirmation is required
5. NTRL, PHL will collaborate with SRL for research studies.

### 5. Data management and Analysis

#### 5.1 Data Collection
Data will be sent to PHL by hospital labs weekly/twice a month (based on ambulance movement) along with the samples for DST. The data will be screened out before compilation to monitor the patient enrollment for the surveillance study, quality of clinical information collected by the medical doctors and quality of samples collected and transported to PHL. PHL will assign code number for each district hospitals for surveillance study.

5.2 Data Management

WHO software programme (4th version) for Surveillance of Drug Resistance in Tuberculosis (SDRTB will be used for data compilation and management). To ensure accuracy, data will be entered twice by two different lab personnel in PHL.

5.3 Data Analysis

Prevalence of drug resistance will be calculated based on number of cases available for DST from the total patient enrolled during study period after exclusion of culture contamination, negative result cultures and insufficient culture growth for susceptibility testing. The proportion of patients with resistance to individual drugs and to different combination of drugs among the patients will be determined using tabulation form Annex 4. Data analysis for other parameters/variables will be done based on the information required.

References
   WHO/TB/2003.320
2. Guidelines for surveillance, drug resistance in tuberculosis, 1st edition
3. Interim recommendations for the surveillance of drug resistance in tuberculosis,
   WHO/HTM/TB/2007.385

Annexure: 1
A. IDENTIFICATION OF THE PATIENT

Name: .................................................................

TB district number: .................. Date registered: Day..... Month....... Yr........

Sex: Male/ Female Age: ......... years

Date of sputum collection: A ........ B ........

B. HISTORY GIVEN BY THE PATIENT

B1 Previously treated for TB? No [___] Yes [___]

If the answer is no, go to B2, if yes, go to B3.

B2 Standardized history

• For how long have you been sick? ........................................
• Did you have the same symptoms prior to this episode? ..............
• Did you have other symptoms of lung disease prior to this episode (haemoptysis, chest pain, cough)? .......
• Did you have X-ray examinations prior to this episode? ..............
• Did you have sputum examinations prior to this episode? ..............
• Did you ever take tuberculosis drugs for more than one month? ........
  If yes, what was the name? ........................................
• Did you ever have injections for more than one month? ..............
• Have you ever been incarcerated? ........................................
• Did you ever test yourself for HIV? ......................................
  If yes, what was the test result? ........................................

Did the patient remember previous treatment for TB after these questions?

No [___] Yes [___], If the answer is yes, continue with B3
B3 Information about previous treatment

• Where was the patient treated? .................................................................

• When was the patient treated? .................................................................

• How many times was the patient treated? ..................................................

• Which drugs were used for treatment? ....................................................... 

• By whom was the patient treated? ............................................................

• Outcome of the last treatment according to the patient: Cured [ ] Not cured [ ] Unknown [ ]

C MEDICAL RECORDS

After extensive checking through the medical files and other documents available in the health centre, have you discovered that the patient has been registered for tuberculosis treatment before? No [ ] Yes [ ]

If the answer is yes, what was the outcome of the last course of chemotherapy: Cured [ ] Treatment Completed [ ] Defaulted [ ] Failed [ ] Transferred out [ ] Unknown [ ]

Did you find out that the patient has had HIV testing according to the record? No [ ] Yes [ ]

If yes, when was the test done according to the record: (DD/MM/YY): ____ / ____ / ____ ; and what was the outcome according to the record? Negative [ ] Positive [ ]

D FINAL DECISION

D1 Patient has been previously treated for TB for more than a month:

Yes [ ] (answer to question B1 or B2 and/or C was 'yes')
No [_____] (answer to B1 and B2 and/or C was ‘no’)

Doubtful [____]

D2 If yes, what was the outcome of previous treatment?

Cured/treatment completed [____]

Failed [____]

Defaulted [____]

Chronic [____]

Relapse/defaulter not distinguishable [____]

Unknown [____]

Responsible Medical Officer: .................................................................

Annexure: 2

SPUTUM SHIPMENT FORM
IDENTIFICATION OF THE PATIENT

Name: .................................................................................................................................

TB district number: ....................... Date registered: (DD/MM/YY) ........... / ........... / ...........

Sex: Male/ Female Age: ........... years

Date of sputum collection: A: ....../ ....../ .......... B: ....../ ....../ ..........

Result of smear: A: ......................................................... B: .........................................................

CPB/CPC added: Yes/ No

Date Sample shipped: _____ / _____ / _____

________________________________________

Public Health Laboratory Use Only

Date samples received in Laboratory: ...... / ....../ .............

Date of culture: ...... / ...... / .............

Laboratory Specimen Number: .................................................

Annexure: 3

RESULTS OF BACTERIOLOGICAL EXAMINATION FORM

District: .........................................................
Hospital: .......................................................... Code: ......................................................

A  PATIENT

Number: ........................................... Date of receipt: Day..... Month............. Yr........

B  IDENTIFICATION

Sample A:                          Sample B:
  |___| M. tuberculosis               |___| M. tuberculosis
  |___| MOTT                           |___| MOTT
  |___| Negative                       |___| Negative
  |___| Contaminated                   |___| Contaminated
  |___| Other                          |___| Other

C  SUSCEPTIBILITY OF M. TUBERCULOSIS

Susceptible to:  Resistant to:
  |___| Isoniazid                    |___| Isoniazid
  |___| Rifampicin                   |___| Rifampicin
  |___| Ethambutol                    |___| Ethambutol
  |___| Streptomycin                  |___| Streptomycin

Date of recording: |____|____|_____| Day Month Yr

Responsible Officer: ........................................................................................................

Annexure: 4

ANTI-TUBERCULOSIS DRUG RESISTANCE RESULTS FORM

<p>| NEW | PREVIOUSLY TREATED |</p>
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<th></th>
<th>No.</th>
<th>%</th>
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**Annexure: 5**

**PROFICIENCY TESTING REPORT FORM**

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