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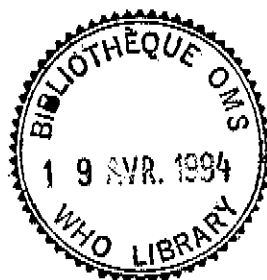
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GUIDELINES FOR SURVEILLANCE OF DRUG RESISTANCE IN TUBERCULOSIS

Tuberculosis Programme, World Health Organization, Geneva

and

International Union Against Tuberculosis and Lung Diseases, Paris



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SUMMARY

These guidelines have been developed mainly to assist national tuberculosis programmes in establishing their own policies for resistance surveillance. The aims of the proposed survey are:

1. To determine the prevalence of initial and acquired drug resistance in the survey area in order to use the levels of drug resistance as a performance indicator for the national tuberculosis programme and to assess whether recommended regimens are appropriate.
2. To establish the foundation for routine surveillance of drug resistance with procedures based on defined guidelines in order to observe trends in drug resistance in the survey area.

In addition, as quality control is essential to achieve these goals, a third aim is:

3. To promote external and internal quality control on laboratory procedures of susceptibility testing, in collaboration with supranational reference laboratories.

The survey should be carried out at country level because decisions on recommended treatment regimens and programme adjustment can only be done at country level, where drug resistance should also be routinely monitored. The director of the national tuberculosis programme identifies a national coordinator for the survey. The coordinator is the link between the laboratory services and the national programme.

It is essential that, in cooperation with a supranational reference laboratory, a system of internal and external quality control of susceptibility testing is established for the central laboratory before starting the survey. Methods of quality control are not described here and need to be developed. To ensure representativeness of the results and avoid serious operational problems the survey should be based on sampling of diagnostic centres.

Patients are eligible for inclusion in the survey if they have been newly registered and found positive on sputum smear microscopy at least once. Prior to beginning of treatment two additional samples are collected at the diagnostic centres and sent to the central laboratory, where cultures are prepared and identification and susceptibility testing is done. The diagnostic centres also must collect information to make it possible to distinguish between new cases, relapses and other previously treated cases.

INTRODUCTION

There is much anecdotal, but little published, evidence that drug resistance is becoming a more important barrier to effective national tuberculosis programmes. The available information suggests there may be high levels of drug resistance, particularly in Asia and parts of Africa (1-6). These guidelines have been developed mainly to assist national tuberculosis programmes in establishing their own policies for resistance surveillance. It is hoped they will become a standard tool, useful for most well established control programmes to indirectly measure performance. Standardized data generated according to these guidelines in different countries will be directly comparable.

The recommendation to use drug resistance tests for monitoring and guiding tuberculosis treatment programmes was made as long ago as 1969 (7). In view of the practical difficulties in collecting comparable data, WHO proposed a global surveillance programme through its collaborating centres for bacteriology of tuberculosis which will function as supranational reference laboratories (8). The proposed programme was based on random sampling of patients reporting to national tuberculosis programmes, with sample sizes weighted according to the reported numbers of cases. Susceptibility testing was to be performed by the reference laboratories according to an agreed technique. As a first step, a regional survey in 10 Latin American countries was carried out (9). Other countries in Asia and Africa undertook national surveys in accordance with the protocol, but regional multicentre surveys were not completed. Meanwhile, several countries, including Tanzania (10) and South Africa (11), had established systematic national surveillance programmes.

Thus, there is need to establish surveillance of drug resistance at country level to obtain data which are standardized and comparable within and among countries. Three principles should be strictly adhered to:

1. Taking patient's history, always differentiate between first and other episodes of tuberculosis (with the intent of differentiating between primary and acquired drug resistance. See definitions below).
2. The laboratory method for testing resistance, albeit not necessarily the same in all countries, should be internationally accepted as an appropriate one.
3. The sample should be representative for the area under study and the size carefully calculated to allow for trend analysis (country/region).

RATIONALE

Causes of drug resistance

Potential causes of drug resistance include inadequate treatment regimens provided by the health services, poor case holding, poor drug supply, poor quality drugs, patient error in following the prescribed regimen, and misuse of antituberculosis drugs in the private sector. However, the most important cause of development of drug resistance may well be the error of health professionals in prescribing correct regimens, particularly the failure to take into account of a high level of primary resistance. High levels of rifampicin resistance, for instance, require adjustments of the

control programme as efficient use of the WHO and IUATLD recommended treatment regimens may not be sufficient to guarantee high cure rates. In addition, the impact of the HIV epidemic on the level of antituberculosis drug resistance is unknown and must be assessed. However, based on the experience in some industrialized countries and because of the extraordinarily high risk of active tuberculosis within a short period of time among co-infected persons, HIV infection can "telescope" an epidemic of drug resistant tuberculosis, permitting its manifestations to be seen in months rather than years.

Surveillance at national level

The overall experience gained in Latin America suggests that a sample survey of drug resistance should be organized for one defined area with unknown levels of resistance, a uniform tuberculosis control system and the infrastructure required for transporting samples. Large failure rates of more than five percent may indicate inadequate routine treatment and high levels of initial resistance, which makes a survey of resistance an urgent priority. If the size of a country makes it unmanageable as a single study area, the survey is organized at various state/province levels with a view to provide data representative for the whole country. One central laboratory should be responsible for surveillance of approximately 25 to 50 million population. Once the proposed survey has shown how the extent of drug resistance at country or state/province level can be assessed with locally established procedures, similar surveys with appropriate modifications may be carried out in other areas.

As the decisions on treatment regimens and programme management can only be done at country level, drug resistance should be monitored primarily at country level. Regional or global surveys including areas with multiple jurisdictions and different tuberculosis control systems proved to be hardly feasible in practice. In addition, data coming out of such surveys are of limited use for national programmes. Survey data from different areas are comparable even without strict standardization of all organisational and technical details. Strict standardization is not essential for assessing programme performance or deciding on revision of routine regimens. Significant changes of already established procedures could preclude the assessment of trends. It is however clear that sampling of strains and drug susceptibility testing must follow accepted standards.

Regional or global estimates on drug resistance, which would require standardization of all methods and a coordinated multicentre study, are not immediate objectives of this survey. As data from different surveys will be comparable, it is hoped that combined analysis of results from several surveys will allow regional or global estimates at a later stage.

DEFINITIONS OF RESISTANCE

Initial resistance

The proposed survey will determine the level of both initial and acquired drug resistance. The level of initial resistance, i.e. of the resistance observed in newly presenting patients, who have not had or do not report prior treatment with tuberculosis drugs, quantifies the extent of the drug resistance problem that the local treatment services will encounter. This definition includes patients with primary resistance as well as those with undisclosed acquired resistance. The level of initial resistance is little sensitive to adjustments of the programme since it is the result of infections that took place in the past, especially when the proportion of

previously treated patients among the newly presenting is small. However, in HIV infected individuals tuberculosis arises both as a result of reactivation of latent infection and from rapidly progressing recently acquired infections. Therefore, initial resistance among HIV-associated tuberculosis patients is an indicator of the efficiency of the control programme in the present as well as in the past.

Primary resistance

The term primary resistance should only be used for strains from patients who have, with certainty, never taken antituberculosis drugs in the past. In control programmes without comprehensive documentation and identification documents for the patients the classification as primary or acquired resistance depends mainly on the histories given by the patients. Newly presenting patients may either not remember prior treatment, refuse to divulge the information on past treatment or were not appropriately asked about the treatment history. The softer definition of initial resistance should be preferred for all patients with seemingly non-acquired resistance, whose previous history is not documented.

Acquired resistance

The level of acquired resistance, i.e. that shown in patients with some record of previous treatment, is a good indicator of programme performance and provides an indirect measure of the recent contribution of the programme to the problem of drug resistance. Acquired resistance arises during the course of treatment, usually as a result of non adherence to the recommended regimen by faulty prescribing or erratic drug taking. Previously treated patients are classified further as relapse cases, if they have been declared cured after treatment in the past, and other retreatment cases. These include defaulters, who discontinued treatment for one month or more, failure cases, who remained or became again smear positive at five months of treatment or subsequently during the course of the previous treatment, and chronic excretors of tubercle bacilli.

LABORATORIES AND DIAGNOSTIC CENTRES

Supranational reference laboratory

The supranational reference laboratory, which may belong to the WHO collaborating centre network, guides and advises the national coordinator during the preparation, implementation and evaluation of the survey. This is done with a view to facilitate ongoing drug resistance surveillance programmes in countries where surveys are carried out. The reference laboratory should be located in the region, preferably in a neighbouring country or in the same country if a state/province is selected as a survey area. However, exceptions can be made to this recommendation if an established relationship already exists between a reference laboratory and a local laboratory. The laboratory must be familiar with all standard methods of culture and susceptibility testing required for the survey. Experienced laboratory staff must be available for visiting the culture laboratories in the survey areas and for retraining of their staff, if required. All reference laboratories should agree on the basic procedures of resistance surveillance as laid down in these guidelines. They ensure equal standards of susceptibility testing by a system of quality assurance, including proficiency testing, which should be established before any one of them assumes responsibility for supervising a central tuberculosis laboratory. That is particularly important when the reference laboratory has the function of central tuberculosis laboratory for the country or the state/province where it is located.

National central laboratory

The national central laboratory, which may be a national reference institution, prepares cultures from the sputum samples and does the identification and susceptibility testing. If there is a peripheral culture laboratory, strains instead of sputum samples can be sent to the central laboratory for testing. The testing is done either following the guidelines provided or, after agreeing with a supranational reference laboratory, following the procedures established nationally. The results of susceptibility tests done by the central laboratories will be validated by external quality control programmes, organized by the supranational reference laboratory. The methods of quality control are not described here and need to be developed by the reference laboratories according to internationally accepted standards.

Diagnostic centre

Diagnostic centres include all institutions where decisions on the diagnosis are taken and suspect tuberculosis patients are registered. Most diagnostic centres in control programmes with limited means are presumably small, non specialized health centres and clinics, run by the government or non-governmental organizations, or outpatient departments of hospitals. Private sector institutions and general practitioners are not included as diagnostic centres, unless their activities are based on some agreement with the national control programme and they are following national guidelines for diagnosis and treatment.

ORGANIZATION AND SURVEY OUTLINE

Suitable survey areas

The country or state/province considered as a survey area should have at least one functioning, central culture laboratory linked by mail or messenger with the majority of tuberculosis diagnostic centres. An existing surveillance system with regular data collection and estimates of levels of drug resistance should not be revised unless the adoption of the guidelines would make the results representative and comparable. It can be expected that a poorly functioning, overburdened programme with limited resources has a high level of drug resistance. The demonstration of this fact may serve as a major impetus for improvement of the programme concerned. Therefore surveys should primarily be carried out in areas with large populations, high prevalence of tuberculosis, high defaulter rates and high rates of failure and retreatment cases, or with unknown indicators of programme performance. Although surveys in such areas will lead to more meaningful results than those from small programmes with good resources, representative and comparable data are needed for all national control programmes.

National coordinator

The director of the national tuberculosis programme will identify a national coordinator, e.g. the head of the central tuberculosis laboratory where the sputum cultures are prepared, or a person designated by him, or alternatively a programme manager situated in the central unit of the control programme. The coordinator is the link between the laboratory services and the national programme. In order to be an effective administrator of the survey, the coordinator needs strong official backing by the authority in charge of health services.

Throughout the intake phase of the survey the national coordinator, together with field officers if required, will closely supervise all diagnostic centres involved in order to ensure good cooperation and promptly identify and correct any operational problems. He will also maintain close contact with the reference laboratory in order to prevent or rectify as early as possible any technical difficulties encountered with the laboratory procedures agreed upon. The national coordinator will collect and prepare for analysis copies of the patients' questionnaires and the laboratory forms.

Preparatory phase

In the preparatory phase of the survey, national coordinator, national tuberculosis programme, central laboratory and supranational reference laboratory will maintain close contact in order to assess jointly the relevant indicators of the national tuberculosis programme, the latest epidemiological data, infrastructure and current laboratory procedures. It is particularly important to review preparation and reading of smears, decontamination of sputum samples, preparation and storage of media, susceptibility testing, and minimal quality requirements for eggs, chemicals and drugs. The nature and extent of technical support by the reference laboratory will depend on the outcome of that assessment. Depending on the local conditions it could be useful to organize an initial pilot trial for a limited time on a limited number of representative diagnostic centres to test out the logistics and then, if necessary, supply additional help.

Quality control

It is essential that, in cooperation with a supranational reference laboratory, a system of internal and external quality control of the laboratory procedures is established before starting the survey. Such a system could include retesting with the same method or a standard method at the reference laboratory of a sample of strains or all strains, initially, from time to time or throughout the survey.

Data analysis

The final data analysis will be done by the national coordinator in cooperation with the reference laboratory with a view to the aims of the survey given in these guidelines. Outside support for the national coordinator and the reference laboratory in preparing the survey, analysing the data and providing additional consultant services must be sought early, if required. Survey results should be published primarily by the national coordinator. The procedures for sampling and susceptibility testing, including the methods of quality assurance, should be clearly presented in the publications, so that results from several surveys using the same methods will be comparable. Co-workers from the central tuberculosis laboratory, the supranational reference centre and the national tuberculosis programme should share authorship according to their contribution to the survey. The guidelines must be duly quoted in any publication on resistance surveys using them. Other financial or technical support should also be acknowledged.

SAMPLING

100% sampling

In theory a 100% sample should avoid all problems that may occur when selecting a group of patients considered to be representative for all patients.

However, severe operational difficulties, which can be expected, and the high costs are disadvantages of this technique. The assumed completeness of the surveyed group of patients is difficult to verify and could probably not be reached in most countries. The logistical problems in involving all diagnostic centres may be relieved by phased collection of patients. Alternatively, patients from only two selected areas are collected for the survey. One area is suspected to have the highest and the other to have the lowest level of resistance with the idea of obtaining a range for each country. It may however be difficult to determine best and worst areas in the absence of reliable data. This approach would also not lead to a countrywide estimate.

Representative sampling

In order to select a representative group of newly registered patients a step of randomization is essential. Simple random sampling of individual patients is not practical in tuberculosis diagnostic centres, mainly because routines usually identical for most patients would be disrupted and consequently compliance of staff and patients would be low and the quality of data poor. Involving all diagnostic centres would also cause serious logistical problems and high costs. Alternatively randomization can take place on the level of diagnostic centres or possibly health districts. By doing so routines are slightly changed for some diagnostic centres, but remain identical for all newly registered smear positive patients in a particular centre.

Sampling acquired resistance

As the proportion of patients with acquired resistance is usually only a small fraction of the total, the confidence intervals around their estimated levels of resistance may be so broad as to make the estimate for them virtually useless. This would suggest that data on the retreatment cases have to be collected over a longer period to increase their proportion of the total. Alternatively, representative sampling could be used in new cases, while 100% sampling is used for retreatment cases.

Trend monitoring

In some national programmes the monitoring of trends, rather than the conclusion that resistance levels are acceptable or not, may be considered the main objective of resistance surveillance. To observe trends, proper sample size estimation is necessary.

The sampling technique is established by the national coordinator and sampling is monitored by the reference laboratory. Additional advice on sampling techniques may be sought through external consultants, if required. Patients of both sexes should be represented. If the area is

multiracial or contains large minority groups of immigrants or transients, it is important to record the ethnic origin as well as the country of origin of the patients. Testing of the HIV status of the patient is not required for inclusion in the survey, but should be recorded if known. Three alternative sampling techniques are discussed in Annex 1.

INTAKE OF PATIENTS

Inclusion criteria

Patients are eligible for inclusion in the survey if they have been newly registered and if they have been found positive on sputum smear microscopy at least once during the specified intake period. Children under the age of 15 who fulfil the admission criteria are also routinely included. For the purpose of this survey, a positive smear is defined as at least five acid fast bacilli detected per 100 immersion fields. There are no specific exclusion criteria.

In some circumstances it may be decided to stratify tuberculosis patients by HIV status in order to obtain separate information on drug resistance among HIV-infected and uninfected patients. However, it is necessary to establish an unlinked method of HIV testing, i.e., a method which does not allow identification of a patient's name. This requires that identifiers which could link one's HIV status to any records be removed. Thus, if a decision is made to test tuberculosis patients for HIV antibody, it is recommended that a very detailed protocol be prepared outlining how unlinked testing will be ensured.

Registration and sputum collection

In addition to the initial sputum sample, the diagnostic centres selected will send to the central laboratory two other sputum samples, e.g. two spot samples or a spot and an overnight sample, of all patients found to be eligible for inclusion. As treatment for any period of time will reduce the chance of a positive culture, the samples must be taken before start of treatment. The centres will also fill in a form giving information about the patient's history. This should make it possible to distinguish between initial and acquired resistance. A sample of the recording form is attached to the guidelines (see Annex 2). Every effort must be made to minimize the risk of falsely classifying a previously treated patient as a new case. If considered necessary, repeated interviews and extensive review of clinical or laboratory records could be included. Each patient attending the diagnostic centre should be assigned a serial number, which will be recorded on the intake forms. The serial number permits identification at the diagnostic centre if a resistant strain has been found and the treatment regimen needs to be altered.

Transport of sputum samples

Sputum should always be treated with care. It should be collected in containers that can be sealed hermetically. This is particularly important if the postal service has to be used. The containers must be rigid to avoid crushing in transit. They should be packed in material that will absorb any leakage caused by accidents. Furthermore, it is advisable that all procedures involving sputum should be carried out in a bacteriological safety box. Particular care needs to be taken when bottles are being opened, closed or shaken and when materials are being centrifuged, which may all lead to the production of infectious aerosols. The transportation of tuberculosis cultures presents special risks in the event of accidents or breakage of the container (see Annex 3).

The sputum samples are not examined by microscopy again, but are sent directly to the central laboratory. Before transport the samples are kept in a cool place, preferably a refrigerator at +4°C. For homogenization of the mucus and organic debris and for decontamination on transit an amount of cetylpyridinium bromide 0.6% or cetylpyridinium chloride 1%, equal to the volume of the sputum, is added if it is anticipated that between collection and processing in the culture laboratory the samples may be exposed to room temperature for more than 48 hours. The patient's serial number in the centre's register and a simple identification for the two successive specimens from the same patient, such as A and B, are written on the container (not on the lid). The two specimens together with the form giving the intake data are sent at once to the central laboratory. A copy of the form is kept in the patient's file at the diagnostic centre. It should be verified that the procedures for sputum smear examinations followed in the microscopy centres are up to an acceptable standard (12). The central laboratory should ensure that the selected diagnostic centres have enough registration forms and the supplies necessary for the collection and transport of the sputum samples.

Replacement

Persons with a positive initial smear reading who fail to return to the diagnostic centre for two further sputum samples, and who cannot be traced during the intake period, are duly registered and later replaced by other patients. Replacement of specimens collected during the intake of patients is restricted to cases in which the samples received in the central laboratory are spoiled on transport or contaminated on both tubes of both samples. No replacement is made in any other case, notably if the patient has been classified incorrectly as smear positive at the diagnostic centre. If replacement is required, the samples are replaced by those of other patients diagnosed in the centre concerned, immediately after the scheduled intake period.

NATIONAL CENTRAL LABORATORY

Cultures

Before processing at the central tuberculosis laboratory, the sputum samples must be kept in a refrigerator at +4°C. Bacteriological examination is carried out as soon as possible. The samples are decontaminated and further homogenized, according to Petroff's method, with sodium hydroxide 4%, for 15 to 30 minutes at the maximum, centrifuged at 3000x for 20 minutes, and the sediment neutralized and washed.

The sediment is inoculated on two tubes of Loewenstein-Jensen medium and one tube of egg medium enriched with sodium pyruvate (14). The cultures are incubated at 37°C until growth of colonies is observed or otherwise for nine weeks. They are first inspected after 48 hours and then weekly, or at least after 21, 28, 42 and 63 days. Each isolate strain will be examined for morphology and pigmentation and the date of appearance of the colonies will be noted. If there is no growth by day 63 or in case of contamination the cultures are discarded and the laboratory forms completed accordingly. All positive cultures are kept until retesting in the reference laboratory has been completed or the strain has been excluded from further testing. The cultures should ideally be stored in a deep freezer at -20°C, but they can also be kept for some time in the refrigerator at +4°C, or even at room temperature.

Identification

Identification of the strains will be based on at least the niacin production test, the nitrate reduction test and the thiophene carboxylic acid hydrazide (2 mg/l) (TCH) resistance test. Mycobacterial strains other than tubercle bacilli will not be further considered for the purpose of the survey.

Susceptibility testing

Drug resistance tests will be performed using preferably the economic variant of the proportion method, although the absolute concentration and proportion methods may also be used (7). The strains' resistance against isoniazid, streptomycin, ethambutol and rifampicin is routinely tested if these drugs are used in the tuberculosis programme, prescribed by private practitioners or freely available. Resistance is expressed as the percentage of colonies that grow on critical concentrations of the substances, i.e. 0.2 mg/l for isoniazid, 2 mg/l for ethambutol, 4 mg/l for dihydrostreptomycin and 40 mg/l for rifampicin. The interpretation will be according to the usual criteria for resistance, i.e. 1% for all drugs. The results of the tests are recorded on the laboratory forms, copies of which are collected by the national coordinator for analysis. A sample of the recording form is attached to the guidelines (see Annex 4).

EVALUATION

Collection of data

During intake all data generated by the central laboratory will be periodically, e.g. every other week, tabulated under supervision of the national survey coordinator. It is important to instruct and motivate the staff for that task and to provide facilities and material before patient intake starts. Based on the tables produced the national coordinator will, for instance every other month, report on the progress of the survey to the head of the national control programme and the reference laboratory. Other information on the field work, such as delays in transport of samples or quality of samples, are included in the progress report. If the data or the comments by the national coordinator suggest some unexpected development with the course of the survey, the reference laboratory, head of the programme and national coordinator should jointly analyze the situation and develop a plan of action as soon as possible.

Consultation among the cooperating parties should in any case take place after approximately half the scheduled intake period for the purpose of evaluating the progress accomplished, the quality of the laboratory procedures as assessed by external quality control, some preliminary results and overall coordination of the survey.

Data analysis should provide indicators of programme performance and allow to assess the appropriateness of the recommended treatment. The following parameters should be included in the final analysis:

1. Total number of diagnostic centres identified in the survey area and listed for random selection, number of sputum smear positive patients registered per month in each centre in the corresponding month of the previous year and total number of patients registered, number of diagnostic centres selected, defined intake period (dates of first and last day), range and expected total number of patients registered per month in the centres selected.

2. Total number of patients/strains evaluated by culture and susceptibility testing with proportion of males and females and age distribution, total number of patients found to be infected with a resistant strain with proportion of males and females and age distribution, distribution over centres selected of all patients evaluated and those infected with a resistant strain.
3. Number and proportion of patients with no previous treatment, cured after treatment, not cured and unknown outcome of previous treatment, number and proportion of patients in each of these categories infected with resistant strains, number and proportion of patients classified as having initial or acquired resistance.
4. Number and proportion of strains resistant to any one of the four drugs tested, of those resistant to two or more drugs except those resistant to isoniazid and rifampicin, of those resistant to at least both isoniazid and rifampicin and all results broken down by initial and acquired resistance.
5. From a public health point of view, the extent of current transmission of drug resistant strains is important. The assessment of the prevalence of initial drug resistance in young age groups has thus higher information value than that among older patients. Among the latter, susceptibility patterns most likely reflect practices years in the past when infection was acquired, while among younger patients practices of the recent past are more likely to be reflected. For the same reason, assessment of trends carries more informative value than data collected in a single survey.

Interpretation of results

The key indicators for programme performance are the proportions of patients having initial or acquired resistance. Levels of initial resistance of less than, say, five percent to any one of the drugs included in the intensive treatment phase suggest that the recommended regimen may not be altered. If higher levels of initial resistance are detected, particularly to rifampicin or isoniazid, strengthening of the routine regimen, for example by adding another drug, should be considered. Initial resistance to rifampicin in particular increases failure and relapse rates. Monitoring of treatment outcome and provision of a powerful retreatment regimen is particularly important. Implications for the current management of the programme are limited, since initial resistance refers to infections that took place in the past. High levels of initial resistance may also indicate a large proportion of patients with inappropriate previous treatment, who have been falsely classified as new cases.

High levels of acquired resistance to any one of the drugs included in the intensive phase of the retreatment regimen will not significantly increase the failure rate of retreatment. However, high levels of acquired resistance would indicate poor programme performance. The proportion of patients with previous treatment of those newly presenting for treatment indicates the burden for the control programme. Even in the absence of results of cohort analysis, corrective action to improve cure rates may be required. Depending on the local situation, the use of inappropriate treatment regimens by the private sector or self medication may have caused high levels of acquired resistance.

Combined resistance, particularly to isoniazid and rifampicin, initial or acquired, is cause for serious concern. Patients would require individualized treatment to have a limited chance of recovery. Without that option patients are practically untreatable. As the development of combined resistance is a stepwise process, serious shortcomings in programme management on several levels are likely. Reorganization of the programme with emphasis on strict adherence to recommended regimens and supervision of treatment is urgently needed.

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ANNEX 1/page 1

SAMPLING TECHNIQUES

Random selection of diagnostic centres

All sputum positive patients newly registered during a defined period of time at a selected diagnostic centre are included in the survey. It can be expected that the level of resistance found varies depending on the site and patient turnover of the diagnostic centre. A defined intake period, identical for all centres included in date of commencement and duration, should result in a balanced sample with different types of centres such as clinics, dispensaries and hospitals represented according to their burden of cases in the control programme.

The number of diagnostic centres, the overall number of patients to be included and the resulting minimum intake period are determined. The centres included in the survey are randomly selected from a list containing all tuberculosis diagnostic centres located in the survey area, including both major referral centres and peripheral health units.

A sample size of at least 600 is needed for the overall survey to be able to detect resistance levels of 3% with a confidence interval of 1% to 5% and a level of confidence of 95%. The resulting intake period will depend on the average number of diagnosed patients per week for the centres selected and the number of centres included. For estimating the sample size published tables can be used (13).

With a population of 50 million in the survey area and one diagnostic centre per 200 000 population, approximately 250 centres have to be identified. A serial number beginning with one is assigned to each of them. Their names with serial numbers, exact addresses and approximate numbers of smear positive cases, registered in each of them in the current month of the previous year, are listed in sequence of the centres' serial numbers. It is important that all diagnostic centres in the survey area are included in the list. With a notification rate of about 100 new cases per 100 000 population and year, approximately 25 000 smear positive cases qualifying for inclusion in the survey can be expected per year. The average number of patients registered per centre is then around 100 per year or two per week. A more precise estimate of the expected number of cases should be derived from the list of diagnostic centres. If there are no data for the previous year, an evaluation of recent records covering a one-month period is sufficient for estimating the average number of patients registered per week. A sample of approximately 10% of all diagnostic centres listed are then selected at random. It can be expected that the 25 centres selected will collect sputum samples for approximately 600 representative patients within three months. By drawing a sufficiently big sample of about 10% of all diagnostic centres on the list the difference between centres of levels of drug resistance should be balanced out and a selection bias is avoided. By defining a limited intake period, identical for all centres selected, small and large centres are represented according to their share of all patients diagnosed.

The main advantage of this technique is its simplicity. The main disadvantage is the risk of missing the largest diagnostic centres resulting in non-representative levels of resistance despite randomization.

ANNEX 1/page 2

To address this, one could stratify based on centre size, placing the largest centres in their own self-representing strata. As the random selection technique is in essence a cluster sample methodology it requires a larger sample size than a method with randomization at the level of individual patients.

Population proportionate cluster sampling

To avoid the risk of missing the largest diagnostic centres when drawing the sample a weighted cluster sampling technique can be used (18). Based on a list of all diagnostic centres with the numbers of newly registered patients per year, a cumulative population list is compiled. Assuming the minimum recommended number of 30 clusters is selected, the total number of patients registered per year in all the centres is divided by 30 to obtain the sampling interval. A random number is picked between one and the sampling interval. This random number determines the first diagnostic centre on the cumulative list to be selected. The sampling interval is sequentially added to the random number to obtain the remaining clusters from the list. If centres are large with twice or three times more patients per year than the sampling interval there may well be more than one cluster per diagnostic centre.

To determine the number of patients per cluster the required total sample size is divided by 30. If there is more than one cluster in a diagnostic centre the number of clusters needed is multiplied by the size of the cluster to calculate the total number of patients needed from that centre. In all selected diagnostic centres consecutive patients are included in the survey until the number required for one or more clusters is reached.

Systematic sampling of diagnostic centres

A list of all diagnostic centres in the country with the numbers of newly registered patients per year in each of them is compiled. Based on this each diagnostic centre is assigned a one-month period during which all newly registered patients are collected for the survey. This should be done in a way that the number of sputum samples sent to the central laboratory for cultures and susceptibility testing is approximately the same each month throughout the year.

This technique involves all diagnostic centres but avoids some of the disadvantages of 100% sampling, such as falsely assumed completeness, high costs and overloading of the central laboratory. Large and small diagnostic centres would be equally represented without applying a complicated sampling method. The phased patient intake gives opportunity to instruct the centres' staff and to correct procedures, which proved to be insufficient. The technique provides an approximately 10% sample of newly registered smear positive patients, which can be augmented if necessary by assigning longer intake periods to the centres.

ANNEX 2/page 1

SURVEILLANCE OF DRUG RESISTANCE IN TUBERCULOSIS

FORM 1: INTAKE, INTERVIEW AND SHIPMENT SHEET/Page 1

Country: Diagnostic Centre:
Code: Code:

A. Patient

Number: Date registered: |___| |___| |___|
Day Mo Yr
Sex: |___| Male |___| Female
Age: Years

Remarks: (e.g ethnic minority, immigrant).....
.....
.....

B. Positive sputum smear

If there are several positive smear results, record the most recent one.

Date collected: |___| |___| |___|
Day Mo Yr

C. Medical record

1. After extensive checking through the Centre's files and other documents, have you discovered that the patient has been registered for tuberculosis treatment before?

|___| No If "Yes", what was the outcome:
|___| Yes |___| Cured after treatment
|___| Not cured
|___| Unknown

2. Result of HIV test?

|___| No If "Yes", record
|___| Yes - Date of test: |___| |___| |___|
Day Mo Yr
- Result: |___| Negative, |___| Positive

ANNEX 2/page 2

SURVEILLANCE OF DRUG RESISTANCE IN TUBERCULOSIS

FORM 1: INTAKE, INTERVIEW AND SHIPMENT SHEET/Page 2

D. History given by patient

1. Previous treatment for tuberculosis? No Yes Don't know
2. If yes, for how long? Months
3. If yes, outcome of treatment? Cured Not cured Don't know
4. Ever tested for HIV? No Yes
5. If yes, result? Negative Positive Don't know

Date completed:
 Day Mo Yr

Responsible Officer:

E. For shipment of samples

Cetylpyridinium bromide/chloride added: No Yes

Date of shipment:
 Day Mo Yr

Responsible Officer:

This form is to be made out in two copies. The original is to be sent to the central laboratory, together with the sputum samples. The copy is kept in the patient's file at the diagnostic centre.

ANNEX 3

SAFE SHIPMENT OF INFECTIOUS MATERIAL

For external quality control of the susceptibility testing in the national central tuberculosis laboratories cultures have to be exchanged between these laboratories and the supranational reference centres. Cultures of *M. tuberculosis* are enriched infectious material containing great numbers of viable organisms that can cause disease in humans. The hazard is compounded when cultures of resistant strains are transported.

Some international organizations, such as the Universal Postal Union, the International Civil Aviation Organization and the International Air Transport Organization, have developed guidelines and procedures designed to facilitate the safe and expeditious shipment of infectious substances while at the same time ensuring the safety of transport personnel and the general public. These organizations have also developed agreed common definitions, and packaging and labelling requirements (15,16). Information on the documentation requirements should be obtained from the appropriate national authorities of the country where the cultures are sent.

Infectious substances and diagnostic specimens likely to contain infectious substances require triple packaging in accordance with the recommendations of the United Nations (16). Cultures of mycobacteria should be shipped on solid medium in screwcap tubes or freeze dried in vials as primary watertight containers. Petri dish cultures and cultures in liquid medium must not be shipped. The primary container should be entirely surrounded by at least two cm of absorptive material and enclosed in a second, durable watertight container. The tissue paper or cellulose wadding in the secondary container must be sufficient to absorb all of the fluid in the specimen in case of leakage of the primary container. Several primary containers may be enclosed in a single secondary container, if the total volume of all the primary containers does not exceed 50 ml (17) and there is no contact between them. Each set of primary and secondary containers should be enclosed in an outer shipping container made of corrugated fibre board, cardboard, wood or other material of equivalent strength.

One copy of the request forms, letters and other information that identifies or describes the specimen should be taped to the outside of the secondary container. Another copy should be sent by air mail to the receiving laboratory and a third retained by the sender. The outer container must bear the infectious substance (biohazard) label. The label should be about 10 cm large and printed in red on a white background. In addition to the sender's and recipient's addresses, the telephone numbers should also be put on the outside of the package.

Compliance with the shipment requirements is the responsibility of the shipper, who must be familiar with the regulations. Failure to comply may result in fines and other penalties. Hand carriage of infectious substances is strictly prohibited by international air carriers, as is the use of diplomatic pouches.

ANNEX 4/page 1

SURVEILLANCE OF DRUG RESISTANCE IN TUBERCULOSIS

FORM 2: RESULTS OF BACTERIOLOGICAL EXAMINATION/Page 1

Country: Diagnostic Centre:
Code: Code:

A. Patient

Number: Date of receipt: |___| |___| |___|
Day Mo Yr

B. Identification

Sample A:

|___| M. tuberculosis
|___| M. bovis
|___| M. africanum
|___| Negative
|___| Contaminated
|___| Other

Sample B:

|___| M. tuberculosis
|___| M. bovis
|___| M. africanum
|___| Negative
|___| Contaminated
|___| Other

ANNEX 4/page 2

SURVEILLANCE OF DRUG RESISTANCE IN TUBERCULOSIS

FORM 2: RESULTS OF BACTERIOLOGICAL EXAMINATION/Page 2

C. Susceptibility of M. tuberculosis

Susceptible to:

Isoniazid
 Rifampicin
 Ethambutol
 Streptomycin

Resistant to:

Isoniazid
 Rifampicin
 Ethambutol
 Streptomycin

Date of recording: / /
Day Mo Yr

Responsible Officer:

This form is to be made out in two copies. The original is to be sent to the diagnostic centre, the copy is filed at the central laboratory.