

**Determination of the prevalence of tuberculosis  
With drug-resistant strains of Mycobacterium tuberculosis in  
Khartoum, Gazira and camps for displaced people, Sudan**

**Muna Obied Ali**

**Supervisor:  
Gunner Bjune, M. D., Ph. D., Professor**

**Co supervisor:  
Per Sandven, Ph. D.**

**Thesis submitted as partial completion of the Master  
of Philosophy Degree in International Community Health**



**Department of General Practice and Community Medicine,  
The Faculty of Medicine, University of Oslo  
Oslo, Norway, May 2002**

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## ACKNOWLEDGEMENT

I wish to give honuor and dignity to all the **patients** who volunteered for this study

My gratitude to my supervisor **Professor Gunner Bjune**, the Department of General Practice and Community Medicine, the Faculty of Medicine, University of Oslo, Norway, for supervising me through out the study. I can never thank you enough for your wonderful scientific approach, sense of humanity and trust and possibility to develop new approach and thinking in science.

I owe my sincere gratitude to my supervisor **MD Per Sandven**, the Department of Bacteriology, National Institute Of Public Health, Oslo, Norway, for supervising my laboratory work and for his valuable discussions.

I wish to thank **Elisabet Ronnild**, **Anne Kristin** and **kgersti Haugum** the Department of Bacteriology, National Institute Of Public Health, Oslo, Norway, for their technical assistance and valuable knowledge and experience I have gained.

My thanks to **MD Asma Elsony**, National Tuberculosis Programme, Sudan for her advises and support during my field work.

My great thanks go to **Einar Heldal**, Head of the National Tuberculosis Register, Oslo, Norway, for reading my papers and valuable comments.

My sincere thanks to all my colleagues in the National Tuberculosis Programme, Sudan for their assistance and support, and my warmest thanks to my field work assistance, **Nigud El Khair** for being helpful and enthusiastic.

I wish to thank all the people in the National Tuberculosis Reference Laboratory, Khartoum, Sudan, especial thanks go to **MD Nageeb Suleiman** for his valuable advises and discussions and to **Yasser Tumsah** for his skillful technical assistance.

I can never thank enough my **family** for everything they have done for me. Trust and believe. Thank you for support of my decisions, tolerance and love.

I wish to thank all my teachers, the programme co-ordinators, my colleagues and friends in the Department of General Practice and Community Medicine, the Faculty of Medicine, university of Oslo for their support and help of making my stay easy far away from home.

My warmest thanks go to **Aasve Nesland**, my classmate for her concern, help and love.

## ABSTRACT

**TITLE:** Determination of the prevalence of tuberculosis with drug-resistant strains of *Mycobacterium tuberculosis* in Khartoum, Gazira and camps for displaced people, Sudan

**RESEARCHER:** Muna Obied Ali

**SUPERVISORS:** M.D., Ph.D., Professor Gunnar Bjune; M.D. Per Sandven

The financial support was provided by (NORAD) Norwegian Agency for Development Cooperation

### DESCRIPTION OF THE STUDY

**SETTING:** Khartoum, Gazira and camps for displaced people

**OBJECTIVES:** To find the extent of anti-tuberculosis drug resistance in Sudan and to estimate the association between drug resistance-TB and proportion of new cases and previously treated cases. And to identify medical, social and demographic factors associated with the development of drug-resistant TB

**DESIGN:** Strains isolated from 144 patients with pulmonary tuberculosis were studied for susceptibility to anti-tuberculosis drugs by the BACTEC method.

Data collection forms were filled, to identify factors associated with drug resistance.

**RESULTS:** Twenty-seven strains (50%) were resistant to at least one anti-tuberculosis drugs. Thirty-one (22%) were multi-drug resistant. With exception of only 2 cases, all MDR were found among previously treated cases. The highest rate of mono-drug resistance was observed for streptomycin in both groups of patients (new and previously treated patients). 22 (23.6%) strains collected from new and 8 (15.6%)

of strains collected from previously treated patients were resistant to streptomycin respectively. Resistance to ethambutol was only seen in multi-drug resistance strains. With the exception of only one strain, all strains resistant to rifampicin were multi-drug resistant.

In Khartoum 24 (26.4%) were multi-drug resistant, in Gazira 4 (16%) and in the camps for displaced people 3 (10.7%) were multi-drug resistant.

A history of previous treatment for tuberculosis, being more than 40 years of age, having long duration of symptoms, low weight and household contact of a TB patient were significantly associated with resistance to at least one anti-tuberculosis drug and multi-drug resistance.

**CONCLUSIONS:** There is a high prevalence of *M. tuberculosis* strains resistant to streptomycin also in new patients; drug resistance except for streptomycin among new cases is a rare phenomenon in Sudan, which indicates a low rate of transmission of resistant strains. Drug resistance among previously treated patients is present at an alarming level.

## ABBREVIATIONS

|        |   |
|--------|---|
| AIDS   | Acquired immune-deficiency syndrome                       |
| BCG    | Bacille Calmette Guerin                                   |
| CAT    | Category  |
| CI     | Confidence interval                                       |
| DOTS   | Directly-Observed Treatment, Short course                 |
| DR     | Drug resistance   |
| DST    | Drug susceptibility tests                                 |
| E      | Ethambutol  |
| H      | Isoniazid   |
| HIV    | Human immunodeficiency virus                              |
| IUATLD | International Union Against Tuberculosis and Lung Disease |
| LHL    | Norwegian Lung and Heart Association                      |
| MDR    | Multi-drug resistance                                     |
| Mtb    | <i>Mycobacterium tuberculosis</i>                         |
| NAD    | Nicotinamideadenine dinucleotide                          |
| NIPH   | National Institute of Public Health                       |
| NRL    | National Reference Laboratory                             |
| NTP    | National Tuberculosis Programme                           |
| OR     | Odds ratio  |
| PAS    | Para-aminosalicylic acid                                  |

|      |                                 |
|------|---------------------------------|
| PHC  | Primary Health Care             |
| QC   | Quality control                 |
| R    | Rifampicin                      |
| RNA  | Ribonucleic acid                |
| rpob | B-subunit of the RNA polymerase |
| S    | Streptomycin                    |
| TAD  | Treatment after default         |
| TB   | Tuberculosis                    |
| TBMU | Tuberculosis management unit    |
| TH   | Thioacetazone                   |
| WHO  | World Health Organization       |
| Z    | Pyrazinamide                    |

## INTRODUCTION

### **Tuberculosis global problem**

The revision of the epidemiology of TB in the world shows a decline in the rate of the infection since the discovery of anti-tuberculosis drugs up to 1984 when it has been noticed that the incidence of TB infection had not only stopped to decline in many developed countries but was actually increasing. It was realized that the disease was out of control across most of the poorest countries of the world. That is why World Health Organization (WHO) declared TB to be a global emergency in 1993 (1).

It is estimated that one-third of the global population (1.7 billion people) is infected with *M. tuberculosis* and that 7 to 8 million new cases of TB occur each year. TB kills an estimated 2 to 3 million people each year.

Most of the TB cases and deaths from TB occur in developing countries. Increases in TB incidence have been observed in developed countries as well. A combination of social, economic, historical and factors including urban homelessness, intravenous drugs abuse, alcoholism, poor nutrition, immigration from endemic areas, growing neglect of tuberculosis control programmes, and mostly the AIDS epidemics, are thought to be responsible for this reversal (2). If TB control will not be further strengthened nearly 1 billion people will be newly infected, 200 million people will

get sick, and 35 million will die from TB between 2000 and 2020 (3). Around 6% of all deaths worldwide are attributed to TB (4, 5). It is expected that TB will remain one of the 10 leading causes of mortality and morbidity in the world.

The resurgence of TB has been accompanied by increasing rate of drug resistance. The spread of *M. tuberculosis* with drug-resistant and MDR strains is one of the most actual problems in infectious diseases. World community is anxious about the possibility of the development of dangerous MDR TB that is resistant to at least R and H.

The MDR TB can cause many deaths. Patients infected with resistant especially MDR strains, are less likely to be cured, especially if they are co-infected with human immunodeficiency virus.

## **TB epidemiology in Sudan**

### **Introduction:**

Sudan is the largest country in Africa covering about one million sq. miles. A federal government system was adopted in 1992 where the country was divided into 26 states, 120 provinces and 674 localities.

TB is one of the major health problems in Sudan and considered as one of the top health priorities for the government of Sudan. The estimated annual risk of infection is 1.8%, which gives an incidence of 90/100.000 smear positive cases that puts Sudan

among the high prevalence countries for TB in the East Mediterranean Region. The total number of cases in Sudan is estimated to 45,000 cases. The increased number of refugees from neighboring countries and the increased number of internally displaced population from war-affected areas add to TB burden in Sudan.

The NTP was established in 1986. Now the programme is implemented in 73 provinces in 22 states.

NTP has a central unit under the umbrella of Directorate General of Preventive and Social Medicine at the Federal Ministry of Health. There is a coordinator in each state and province (that are implementing the programme) including laboratory supervisors. For more strengthening of the programme and implementation within the primary health care system, NTP and national programme for control of leprosy have joined together in August 2001.

NTP is receiving support (drugs, reagents, supplies, logistics) mainly from government of Sudan, LHL and WHO. Since the adoption of directly observed treatment strategy, centers are established to cover 75% of the population (493 DOTS centers of which 235 are TBMU/DOTS centers). The targets of the programme are to achieve DOTS all over by the end of 2005, a cases detection rate of at least 70% and a cure rate of at least 85%.

NTP and National Health Laboratory (NHL) established jointly a National Reference Laboratory in 2000. Renovation of the NRL is taking place now.

A senior clinical microbiologist, three junior microbiologists and five technologists constitute the staff of the NRL. The staff has received training in national and regional courses on culture and susceptibility testing, QC for microscopy centers and training of microscopists.

Regarding the national microscopy network for TB, 237 laboratories are established (out of national target to establish 280 in order to have one laboratory for each 100.000 population). There is a laboratory coordinator in each of the 22 states who is responsible for supervision and training of state microscopy centers.

Regarding studies on DR in Sudan there is no national surveillance done. Limited studies were conducted since 1960s, but they do not reflect the current situation of DR in Sudan.

**Background:**

The total number of smear positive newly diagnosed TB cases reported from 20 states out of the 26 states in the year 2000 was 12,248, the number of relapse cases was 2,081 during the same year. The total number of smear negative cases was 6,264 and extra-pulmonary was 3,716.

Conversion rate was 82% in DOTS areas in comparison with 59.9% in non-DOTS areas. Cure rate was 73%.

The recommended treatment regimens in Sudan for different categories are as follows:

- CAT (1) 2 SHRZ

6 TH (for new smear positive pulmonary TB patients)

- CAT (2) 2 SHRZE/ 1 HRZE

5 HRE (for smear positive previously treated TB patients)

- CAT (3) 2 STH

10 TH (for smear negative pulmonary TB and extra-pulmonary patients)

#### **Impact of on the NTP:**

Main objectives of the national tuberculosis programme are case finding and management.

Implementation of DOT strategy, (if done correctly) has been effective in curing up to 85% of all TB cases under programme condition.

DR TB is a significant threat to TB control because few effective drugs are available against *M. tuberculosis* (6). In particular the spread of strains resistant to the most important drugs, isoniazid and rifampicin, could have serious changes in the epidemiology and control of TB. Not only are patients infected with strains resistant

to multiple drugs are less likely to be cured, but second-line treatment is much more toxic and expensive than treatment of patients with susceptible organisms (7).

From a public health point of view, attempts to introduce second-line drugs for MDR – TB in a setting that is unable to guarantee acceptable cure rates of drug-susceptible TB cases, will most likely lead to disastrous consequences. Drug-resistance to second-line drugs will emerge rapidly, resulting in greater harm than benefit (2).

The WHO, IUATLD and several worldwide partners in 1994 launched global project on anti-TB DR surveillance, the main objectives of that project were:

- To improve the performance of the NTP through policy recommendations
- To improve the diagnostic capacity of laboratories
- To revise policy on anti-TB treatment based on the analysis of the results
- To assess the impact of migration on the prevalence of anti-TB DR.

Data from 35 geographical settings confirmed that DR TB was significant. MDR-TB existed in all countries surveyed. Some of these settings had a prevalence of MDR-TB in new cases exceeding 4% (8).

Exact situation of TB drug resistance in Sudan is unknown, data available from NTP regarding the 2000 treatment result' report were as follow:

- The total number of cases was 12.248 in the year 2000

- The number of cured patients was 6419 (52.4%)
- The total number of deaths was 493 (4%)
- The total number of failure cases was 214, the failure rate is 1.7 %
- The total number of defaulters was 1415 (11.6%)

It is expected that a large proportion of the failure cases are resistance.

Failure to respond to treatment may also be due to:

- Non-compliance
- False positive smear
- Chronic excretors of sensitive strains

In a study done in northern Sudan from August 1961 to January 1965, tests of sensitivity to H, S and para-aminosalicylic acid were done on 401 strains of *M. tuberculosis*. Resistance to one or more drugs was found in 28% of 181 strains from patients who claimed not to have received previous chemotherapy. Of the 180 strains from patients with a definite history of previous chemotherapy, 85% were resistant. The proportion of resistant strains in all 401 patients was 54% indicating the magnitude of the therapeutic problem facing the physicians (9).

We can observe the high rate of drug resistance in this study although it had been conducted after few years since the introduction of the anti-TB chemotherapy.

**CHAPTER I**

**LITERATURE REVIEW**

## **Global TB epidemiology**

Increase in TB incidence occurs all over the world, but most alarmingly in the developing countries where it is still one of the major causes of deaths. In industrialized countries, more than 80% of individuals infected with *M. tuberculosis* are over the age of 50. By contrast in the developing countries, over 75% of TB cases are found in individuals below the age of 50, the most economically productive age group (2).

After the discovery of the chemotherapy there was steady decline in TB morbidity, but in the last 20 years the number of infection is increasing each year (i.e. in excess of what would have been observed had the previous rate of decline continued (3, 10, 11).

A combination of social, economic, historical and demographic factors including urban homelessness, intravenous drugs abuse, alcoholism, poor nutrition, growing neglect of tuberculosis control programmes and mostly the AIDS epidemics, are thought to be responsible for this reversal (12).

Demographic factors have played a major role in the global re-emergence of TB. The populations size, mostly of poor countries has increased, the highest incidence of TB across the world are in central Africa and Southern Asia, particularly in India, where

the population increase is known to be the most rapid. Current annual population growth in these countries is about 100 million, which means that global TB incidence in absolute numbers will continue to increase by around 100.000 cases every year (2).

An important factor associated with increase TB infection in many countries is HIV and acquired immune deficiency syndrome (AIDS). It is known that co-infection with HIV infection increases the risk of TB infection developing into disease by a 100 fold. TB patients infected with HIV become severely ill and the rate of death among them is higher compared with non-HIV infected patients. In addition HIV infection elevates the risk of development of the disease from the primary infection. So HIV increases the incidence of TB especially in young and middle- aged adults (2, 13, 14). It is difficult to diagnose tuberculosis in HIV- infected patients, and the disease rapidly leads to death if not treated (15).

Prior to the onset of the AIDS epidemic the incidence of TB in the United States, and most other industrialized countries, had declined steadily for almost 40 years. During the period 1984-1991, over 39.000 excess cases of the disease were identified in the United States by the Center of Disease Control (CDC) in Atlanta (3), and the majority attributed to infection with HIV (16). Similar trends were reported in several European countries, and TB in young adults is now considered as a sentinel disease for AIDS in this settings (17).

In developing countries the situation is even worse. Nearly 80% of the million people in the world, believed to be annually infected with HIV and *M. tuberculosis*, are living in Africa, especially sub-Saharan Africa (1, 18). It has been estimated that 230.000 of the 305.000 excess cases of TB attributable to HIV in 1990 occurred in Africa (19).

Social and economic factors have played a major role in the epidemiology of TB in the world. Inequity and poverty in the poor countries contribute to the higher prevalence of the disease in those countries. It is known that TB rate is higher in lower socio-economic groups where the bad living conditions like unemployment, overcrowded living conditions and hunger play an important role for spread of infection and development of the disease (2, 13, 20).

TB control programmes have failed to reduce TB transmission. Lack of political commitment and inadequately funded programmes increase the pool of chronic cases. Due to improper health facilities TB patients who have interrupted their treatment, transferred from a curable patients to failure patients, which is the critical situation for development of DR strains, and transmission of that strains among the population (13).

**Drug-resistant TB:**

Primary resistance refers to the resistance observed following infection with an isolate of *M. tuberculosis* that is already resistant to a given anti-tuberculosis agent, where as secondary, or acquired, resistance corresponds to drug resistance emerges during treatment. The latter is generally the result of poor compliance on the part of the patient, or poor conception of the regimen and inadequate supervision on the part of the physician (15, 21).

Adult patients can be infected with primary drug-resistant strains or acquire resistance to anti-tuberculosis drugs during the treatment. Usually children have primary resistance, as they get infected from an adult source with drug resistant TB (22, 23).

WHO and International Union Against Tuberculosis Lung Disease recommended to use the terms drug resistance among new cases and drug resistance among previously treated cases. That is because the terms acquired drug resistance and primary drug resistance suggests that the exact causative nature of drug resistance is known. But sometimes patients do not disclose prior treatment for TB due to several reasons. On the other hand, patients who failed to be cured by ordinary anti-TB treatment may do so because their strain was initially resistant to anti-TB drugs and not because it acquired resistance during the treatment (24).

Drug resistance among new cases (formerly: primary drug resistance) is the presence of drug-resistant strain of *M. tuberculosis* in a newly diagnosed patient who never received anti-TB drugs or has received them for less than one month (24).

Drug resistance among previously treated cases (formerly: acquired drug resistance) is that found in a patient who has previously received at least one-month therapy with anti-TB drugs (24).

The emergence of strains of *M. tuberculosis* that are resistant to anti-mycobacterial agents are a worldwide problem whose global magnitude is not well described. The WHO and the IUATLD reviewed 63 surveys of resistance to anti-TB drugs that were performed between 1985 and 1994. Rates of primary resistance to H, administered as a single agent, ranged from 0 to 16.9% (median rate, 4.1%); to S, 0.1%-23.5% (median, 3.5%); to R, 0-3.0% (median, 0.2%); and to E, 0-4.2% (median, 0.1%). The rates of acquired resistance to these agents, which were higher than those of primary resistance, were as follows: H, 4.0%-53.7% (median rate, 10.6%); S, 0-19.4% (median, 4.9%); R 0-14.5% (median, 2.4%); and E, 0-13.7% (median, 1.8%). The highest rates of MDR have been reported in Nepal (48.0%), Gujarat, India (33.8%), New York City, (30.1%), Bolivia (15.3%), and Korea (14.5%) (25, 26).

Resistance of mycobacterial strains to anti-TB drugs was noticed since the introduction of S in 1943, when used as mono-therapy for the treatment of TB. It is easy to understand why, as the frequency of mutation leading to S resistance is now

known to be  $10^{-6}$ . It is not uncommon for patients with pulmonary disease to harbor such numbers of tubercle bacilli in a single lesion (27). Consequently, regimens were developed in which S was combined with another drug, as it was correctly reasoned that the S-resistant mutants present in the population would remain susceptible to the second drug (28). So the development of multi-drug regimens since 1950s offered a way to overcome the problem. The frequency of the transmission of DR organisms was thought to be low until the early 1990s when outbreaks of MDR TB were reported in patients with HIV infection in the United States and Europe, and the problem received inter-national attention (5, 20, 29-31).

#### **Multi-drug resistance (MDR):**

One of the most alarming consequences of dual infection with HIV and *M. tuberculosis* has been the emergence of MDR strains, which cause a potentially untreatable form of TB (32, 33). Small epidemics of the transmission of MDR-TB in institutions, such as hospitals, HIV clinics and prisons, involving both HIV- infected individuals and staffs, were reported in USA and in a much smaller scale in some European countries (34-36). The mortality rates were extremely high (70-90%), and the median survival time from diagnosis of MDR-TB was less than 16 weeks (34).

The current definition of MDR TB, involves resistance to at least H and R, the key components of short course chemotherapy, although resistance to other anti-TB agents is not uncommon in certain areas (21, 37).

Outcomes of MDR TB is usually poor with high mortality rate. Persons with MDR TB were reported to have alveolar infiltrates, cavity pulmonary lesions, reticular interstitial infiltrates and respiratory insufficiency than those infected with susceptible strains (38).

DR is more prevalent among refugees and immigrants from countries with a poorly functioning control programme. For instance, in a study conducted in California, it was found that the case rate of DR disease was 30 out of 100.000 amongst immigrants from South-East Asia (39). Whereas in UK, the few cases of MDR-TB which have been recorded were amongst Kurdish refugees (40, 41) or immigrants from the Indian subcontinent (42).

MDR TB should be suspected in the following cases:

- Patients in hospitals or prisons known to have had outbreaks of MDR TB.
- Patients from geographic areas where MDR TB is common.
- Patients known to be at high risk for MDR TB, those infected with HIV or having AIDS, intravenous drug users or homeless.
- Patients who have relapsed after prior treatment or having a history of previous treatment for TB (43, 44).

### **Drug resistance in developing countries:**

DR in developing countries is a good indicator of the efficacy of the national TB programme. Resistance was common in countries applying substandard regimens but decreased as short course chemotherapy was more widely implemented. For instance, in Algeria the level both of primary and acquired resistance declined by 3-4 folds during the period 1965-1990 (44), as the result of the rational deployment of resources and improved control programmes. Reliable figures from longitudinal studies of resistance levels in developing countries are not available, although it is generally believed that the situation is deteriorating due to the impact of the HIV epidemic, deteriorating socioeconomic conditions and instable drug supply (45). In a recent report, increased prevalence of MDR-TB was described in the republic of Djibouti, where the situation was compounded by war, famine and HIV (46).

### **Molecular basis of drug resistance:**

*M. tuberculosis* is naturally resistant to many anti-biotics; particularly to those belonging to the B-lactam, macrolide or tetracycline families. This may be due to its highly lipophilic cell envelope acting as an efficient permeability barrier (47, 48).

Drugs active against *M. tuberculosis* are classified into two groups: first and second-line. There are currently five first-line drugs and, with the exception of E they are all bactericidal and display low levels of toxicity.

Second line drugs are generally less efficacious and levels of toxicity are generally higher and reserved for the treatment of cases with resistance to first-line agents.

Resistance of *M. tuberculosis* to anti-TB drugs is a man made amplification of a natural phenomenon. Wild strains of *M. tuberculosis* that have never exposed to anti-TB drugs are almost never resistance, though natural resistance to specific drugs e.g. pyrazinamide has been documented for *M. bovis*.

However, regarding DR development the interest focuses on the random process of genetic mutations that leads to the emergence of clinical resistance to anti-TB treatment (49). The mechanisms are chromosomal caused by one or more mutations in independent genes.

During bacterial multiplication, resistance develops through spontaneous mutations at different gene loci at a low but defined frequency in the wild type strain. Mutations resulting in resistance of *M. tuberculosis* to R occur at a rate of  $10^{-10}$  per cell division

and lead to estimated resistance prevalence of 1 in  $10^8$  bacilli in drug-free environments. The rate for H and S is  $10^{-7}$  to  $10^{-9}$ , resulting in resistance in 1 in  $10^6$  bacilli (50). Thus resistant organisms (or mutants) evolve in the absence of anti-microbial exposure, but they are diluted within the majority of drug-susceptible *M. tuberculosis*.

When two or more drugs are administered together the value becomes the product of the individual probabilities to each drug. The mutation rate for resistance to more than one drug is calculated by multiplying the rates for the individual drugs. For example the likelihood of spontaneous mutations resulting in resistance to both H and R is the product of individual probabilities, i.e. 1 in  $10^{14}$  ( $10^6 \times 10^8$ ) The probability of MDR development is dependent on the number of mutant bacilli (23, 51, 52). This is in fact one of the essential reasons for the use of multi-drug regimens in the treatment of TB (53, 54).

An untreated cavity has  $10^7$ - $10^{10}$  organisms, many of which are resistant to single drug. Exposure to a single drug, due to: irregular drug supply, poor drug quality, inappropriate prescription and/or poor adherence to treatment suppresses the growth of bacilli susceptible to that drug but permits the multiplication of drug-resistant organisms. If the single anti-TB therapy is replaced by another effective drug, the second drug will kill bacilli sensitive to the second drug but the small number of mutants resistant to the second drug will survive. As a result mutants resistant to two drugs are selected, resulting in acquired drug resistance. Transmission of such bacilli to other persons may lead to disease, which is drug resistance from the outset, a

phenomenon known as primary resistance. Every drug active against *M. tuberculosis* is found to select for resistance (55-57).

### **Resistance to isonized:**

Together with R, H comprises the backbone of the current short course chemotherapy regimen used to control TB. It shows remarkable specificity being active only against members of the *M. tuberculosis* complex (*M. africanum*, *M. bovis* and *M. tuberculosis*).

Its action as anti-TB agent is mediated by the haem-containing enzyme, catalase peroxidase, encoded by the *katG*, (58, 59) which is also implicated as a mycobacterial virulence factor (59, 60). There is now a large body of evidence, both biochemical and genetic demonstrating that H undergoes a peroxidative reaction catalyzed by catalase peroxidase, in which it is transformed into an exquisitely potent bacterial derivative (59, 61-63). The precise action of this compound remains obscure. Although it has been proposed that H may be converted into isonicotinic acid, an analogue of nicotinic acid the precursor for nicotinamideadenine dinucleotide (NAD) synthesis. It seems that its principal site of action is mycolic acid biosynthesis (63). A new gene, *inhA* was isolated recently which encodes an enzyme involved in mycolic acid production, however as all mycobacteria examined contain *inhA* and produce mycolic acids, it is conceivable that there may be another target, confined to *M. tuberculosis*, as this would explain the specificity of H.

Shortly after introduction of H, resistant mutants of *M. tuberculosis* were isolated (62, 64). These often lack catalase-peroxidase, or produced weak activity, and displayed reduced virulence in the guinea-pig model. It is now known that this can result from deletion or mutation of the *katG* gene, and that lowering enzyme activity results in DR (59, 65). When there is an over production of catalase- peroxidase, susceptibility to H is usually increased. Roughly 60% of H resistant mutants have mutations in the *katG* locus, whilst 20% have undergone modifications of their *inhA* genes (66). Some highly resistance strains have mutations at both sites and others have no known defects, suggesting that a third resistance mechanism may also exist.

**The potential mechanisms of resistance to isoniazid include:**

- Mutations that inhibit the mycolic acid biosynthesis.
- Mutations in the biosynthesis pathway that would inhibit the assembly of the catalase-peroxidase of *M. tuberculosis* to an active compound.
- Inactivation of an inhibitor of NAD glycohydrolase, which depletes intracellular concentration of NAD.

## **Rifampicin**

R interferes with transcription and elongation of ribonucleic acid (RNA) by binding to the deoxyribonucleic acid (DNA)-dependent polymerase (67). Development of resistance to R follows a “single-step” high-level resistance pattern (68), with mutants arising spontaneously in strains not previously exposed to the antibiotics at a frequency of  $10^{-8}$  (69). Resistance to R is associated with specific mutations involving a core region of 27 amino acids in the  $\beta$ -subunit of the RNA polymerase (70-73). The majority of the mutations ( $> 75\%$ ) affects only two positions, His-526 and Ser-531. Complementation studies have shown that such mutations results in R resistance: a plasmid carrying a mutated rpoB of *E.coli* conferred resistance to a susceptible strain (67), and a plasmid carrying the wild type rpoB of *M. tuberculosis* or *M. leprae* partially restored susceptibility to R in a resistant mutant of *M. smegmatis* (47).

Several groups have confirmed that mutations in rpoB are present in  $>97\%$  of more than 200 R resistant clinical isolates of *M. tuberculosis* investigated so far (66, 71, 73).

Resistance to R is rarely found without associated resistance to other drugs. Most importantly, R resistance predicts both resistance to H and poor outcome of therapy (37, 68, 69). Together with the possibility of targeting a single region of the genome for diagnostic purposes, in contrast to the multiple genes involved in resistance to H

and to other drugs, makes R a valid surrogate marker for MDR-TB. So detection of resistance to R could be used as a surrogate marker for the presence of MDR-TB.

### **Streptomycin:**

S, a broad-spectrum aminoglycoside, was the first antibiotic available for TB control and its use in mono-therapy soon led to the emergence of resistant strains. S resistance in *M. tuberculosis* represented an easy target for molecular genetic analysis. S interferes with protein synthesis in *mycobacteriae* by binding to the 16S ribosomal RNA causing misreading of the genetic code and inhibition of translation. The principal site of mutation is the *rps1* gene, encoding ribosomal protein S12, whose primary structure has been highly conserved during evolution, where as alterations to functionally constrained loops of the 16S ribosomal ribonucleic acid (rRNA) that interact with the S12 protein represent a secondary site (43, 47, 74).

### **Pyrazinamide**

There is less knowledge on the mechanism of action of Z than for any other anti-mycobacterial agent (70). Susceptible *M. tuberculosis* strains produce the enzyme pyrazinamidase, which converts Z to pyrazinoic acid. It is thought that the action of pyrazinoic acid is the combined effect of its specific activity and the ability to lower the pH below the limits of tolerance of the target organism. There is imperfect agreement between loss of pyrazinamidase activity and resistance to Z.

## **Ethambutol**

The mode of action of E has not yet been elucidated. Initial reports described the binding of E to the cell wall. Later inhibition of arabinogalactan synthesis that is the component of the cell wall was presented as the most relevant target. Genetic explanation of E resistance is mutation in embCAB gene cluster (74). More recently the inhibition of glucose conversion into the precursors used for the synthesis of cell wall polysaccharides such as arabinogalactan, arabinomannan and peptidoglycan has been proposed. A non-contiguous genomic region has been cloned and presented in a preliminary report as containing determinants, which encode the putative target of E.

## **Risk factors for the development of drug resistance**

The emergence of drug resistance TB in a population has been associated with:

### **Health systems:**

- Lack of drug availability
- Poor management of TB patients due to lack of, or inadequate treatment guidelines.
- Frequent or prolonged shortage of drug supply in areas with inadequate resources or political instability and usage of drugs of unproven quality.

**Health providers:**

- Incorrect management of individual cases. The most common error is addition of a single drug to a failing regimen.
- Improper selection of the appropriate chemotherapeutic regimen due to lack of recognition of prior treatment and ignorance of the importance of standardized regimens (37, 64). In addition, providers might not monitor patients appropriately while on therapy.
- Unavailability of drugs.

**Patient's non – adherence to prescribed treatment**

- Social/cultural/political factors such as population movements, rural/urban immigration.
- Social stigmatization, gender differentials and lack of health education.
- Unstable situation due to war, imprisonment or poverty (75).

Previous treatment for TB, non-compliance to treatment or failure to complete a curative course of therapy are considered to be risk factors for the development of acquired drug resistance or resistance among previously treated cases.

Previous treatment for TB is considered by many authors to be an important risk factor for the development of drug resistance (23, 38, 52, 71, 72).

Non-adherence is difficult to predict from demographic or social characteristics but is less likely to occur if directly observed therapy (DOT) is in use (76). Some potential

barriers to successful treatment include the need for long term (6 to 12 months) and complicated drug regimens, the cost in time and money, long wait in crowded public health facilities, contradictory expectations and beliefs between patients and health care providers, communication difficulties and transportation. Homelessness, alcoholism, drug addiction and substance abuse can predict non-compliance (23, 52).

Finally a crucial element in the emergence of DR is the lack of a properly organized system to ensure prompt diagnosis and effective treatment (77). For this reason, the level of anti-TB drug resistance in a population is an indicator of the effectiveness of a NTP.

**Risk factors for development of resistance among new cases:**

Contact with a patient who has infectious, drug-resistant TB is a significant factor for primary resistance. Transmission of infection depends on several factors like the infectiousness of the possible source and the closeness and intensity of the exposure. Any person who shares the air space with a patient with MDR TB for a relatively prolonged time (e.g., household member, hospital room mate) is at a higher risk for infection than those with a brief exposure to a MDR TB patient, such as a one-time hospital visitor. Exposure of any length in a small, enclosed, poorly ventilated area is more likely to result in transmission than exposure in a large well-ventilated space.

HIV infection is another important risk factor for development of primary drug resistance (38, 43, 78). Persons with HIV infection are more likely to be infected with TB if exposed. The HIV epidemic may have a significant effect on the spread of primary drug-resistance in communities with co-existent HIV and DR TB since contacts of HIV infected people with *M. tuberculosis* are more likely to result in active disease and more quickly compared with contacts of infected immune-competent people. This explains the high level of primary DR TB when combination of TB, inadequate treatment and HIV infection is observed (48, 71, 72).

Immuno-compromised conditions other than HIV infection are also important risk factors for development of MDR TB. They include conditions requiring prolonged high dose corticosteroid therapy and other immunosuppressive therapy, organ transplantaion, chronic renal failure, some haematological disorders e.g., leukemia and lymphoma and systemic disease of connective tissues.

## **OBJECTIVES OF THE STUDY**

### **General:**

To find the extent of anti-TB DR in Sudan

### **Specific:**

- To estimate the proportion of DR TB in new cases
- To estimate the proportion of DR TB in previously treated cases
- To identify medical, social and demographic factors associated with the development of DR TB

## **CHAPTER II**

### **MATERIAALS AND METHODS**

### **Patients and bacterial strains**

The study was performed in 3 different districts in Sudan (Khartoum, Gazira, and camps for displaced population) on patients with pulmonary TB diagnosed and treated in the 3 different districts.

Population at risk was 7.800.000 inhabitants of Khartoum, Gazira states and camps for displaced population (according to the last estimation made on 1997)

Target population consisted of TB patients from Khartoum, Gazira states and camps for displaced population.

Study sample included patients from Khartoum, Gazira states and camps for displaced population with new and previously treated patients diagnosed in the 3 different areas was selected according to the inclusion criteria.

### **Inclusion criteria**

1. Stains collected from patients with new smear positive pulmonary TB during a five months period (July -December) 2001 in above-mentioned sites.

2. Strains collected from patients on re-treatment regimen diagnosed as failure, relapsed or treatment after default during a five months period (July – December) in 2001 in above-mentioned sites.

1. All strains were collected from patients before the start of treatment with anti-TB drugs.

### **Study type**

We performed a case control study in the NTP's clinics in the chosen areas in Sudan, where suspected patients were identified as pulmonary TB patients by smear microscopy testing. Culture of the sputum specimens was performed in the RNL in Sudan; drug susceptibility testing was performed in the NIPH, Oslo, Norway

A case control design was chosen because this type of study is relatively simple and economical to carry out and increasingly used to investigate risk factors of disease development – DR TB in our case. The study included patients with the disease of interest, DR TB and a control group, patients with drug susceptible TB.

### **Sample size**

According to WHO and IUATLD recommended strategies for sample collection for a survey on the prevalence of anti-TB DR, the calculation of an appropriate sample size should be based on the following:

Expected prevalence of resistance to R or to the drug of the lowest known level of resistance, from previous available data or based upon data available from the NTP.

Precision should be as accurate as possible but calculation needs to ensure a sample that is logistically feasible to obtain.

The recommended level of confidence around the estimated prevalence is 95%

The calculated sample size needs to be increased by 5%-20% to account for expected losses. These include patients whose culture are contaminated or does not grow and patients whose susceptibility testing does not give interpretable results.

We used formula (student formula) for sample size calculation  $N = Z^2 pq/d^2$

N – number of observations needed

P – estimated prevalence

Z=1.96

d = allowable error (0.05-0.08)

q=1-p

Since there is no recent study conducted in Sudan regarding the prevalence of *M. tuberculosis* strains resistant to anti-TB drugs I have based my estimation upon the results of the study done in Ethiopia in 1997, assuming that it has the similar situation as in Sudan regarding TB prevalence.

In that study 107 isolates were identified as *M. tuberculosis* had been investigated for drug susceptibility, it was found that, 44% strains were resistant to H, 28% were resistant to streptomycin, 12% were resistant to R and 2% were resistant to E (79).

According to the WHO and IUATLD recommendations we calculated our study sample taking R to base our calculation on.

According to data available from the study, we estimated that  $p=0.12$  for any type of resistance and we would like to have  $\alpha = 0.05$ .

Number of observations needed is;

$$N = (1.96)^2(0.12 \times 0.88) / 0.05^2 = 162$$

The calculated sample size was increased by (5%-20%) to account for expected losses. The final sample size consisted of 170 patients.

### **Collected sample**

A total of 170 *M. tuberculosis* strains isolated from patients with pulmonary TB in the 3 chosen areas in the study during July – December 2001 were cultivated on Lowenstein-Jensen media and forwarded to the NIPH, reference laboratory for tuberculosis, for further analysis. Twenty-six strains were either contaminated or died during transportation. The final sample size consisted of 144 strains.

### **Representativeness of the samples:**

We ensured representativeness of the target population by including in the sample all newly diagnosed patients who fulfill the inclusion criteria.

During the planned period of data collection all patients with newly diagnosed pulmonary TB and all patients with previously treated pulmonary TB diagnosed in the NTP clinics of the 3 different areas included in the study.

The above-mentioned states was chosen accordingly: Khartoum State as a city with a large number of inhabitants (4.600.000) with a higher rate of patients who interrupted their treatment and many failure cases (problem of capital city with a lot of demographical changes)

Gazira State as an example of stable Population City with 1.200.000 inhabitants  
Camps for displaced populations where they represent around 2 millions inhabitants coming from the southern and western parts of Sudan (for war, political instability and employments reasons)

The sample size was collected during fixed period of time as it is described in the inclusion criteria. Strains were collected from smear positive cases of pulmonary TB. Collection of sputum for smear examination was performed routinely for all patients when suspected for TB. Collection of sputum for culture examination was done for the study though it was not performed routinely for all TB patients.

### **Data collection**

Data collection forms of the 144 patients were filled in order to identify factors associated with the development of DR. (Appendix)

Social factors such as the marital status and smoking habit, medical factors such as the history of previous treatment with anti-tuberculosis treatment and demographic factors such as the age and gender were analyzed.

## **Variables**

### **Dependent variable:**

Drug resistance

Definitions: Resistance of *M. tuberculosis* strains to anti-TB drugs according to susceptibility test by BACTEC method

Scale of measurement:

1. Susceptible
2. Resistance

### **Independent variables:**

1. Patients categorization according to the results of previous treatment
  1. New case

2. Relapse
3. Failure
4. Treatment after default

2. Age (Expressed in years)

3. Gender

1. Male
2. Female

4. Address

1. Khartoum
2. Gazira
3. Camps for displaced population

5. Origin of patients (Sudanese people are culturally divided geographically according to their regions of origin into 4 groups: north, south, east, west and central part of Sudan)

1. North
2. South
3. West
4. East
5. center

6. Weight (measurement of weight)

Expressed in kilograms

7. Height (measurement of height)

Expressed in centimeters

8. Occupation

1. Student

2. Labor

3. Governmental employee

4. Running small business

5. Running big business

6. Employee in private sector

7. Un-employee

8. Housewife

9. Marital status (Defined in terms of legal status)

1. Single

2. Married

3. Widowed

4. Divorced

10. Education

1. Illiterate (level of education)
  2. Literate
- 
11. Crowded living conditions (Mean number of persons per room in housing condition)  
(Number of persons per room)
- 
12. Smoking
    1. Smoker
    2. Non-smoker
- 
13. Contacts with TB patient (TB among relatives or persons living or working together)
    1. Household contact
    2. Community contact
    3. Unknown
- 
14. HIV status (When TB patients are not adequately treated and level of acquired resistance is elevated, co-existence of HIV could be responsible for the rapid spread of tuberculosis )
    1. Positive
    2. Negative

15. BCG vaccination

1. Present

2. Absent

16. Symptoms of the disease

1. Present

2. Absent

17. Duration of the symptoms

Expressed in weeks

### **Bacteriological methods used in the study**

Zeil-Neelsen staining (acid-fast staining procedure) was used for smear microscopy to identify smear-positive pulmonary tuberculosis cases.

We used Lowenstein-Jensen (L-J) culture medium.

Identification of the strains was based on the niacin production test and nitrate reduction test.

For strain susceptibility testing radiometric drug susceptibility test, BACTEC (Becton Dickinson Diagnostic System, Towson, MD, USA) was performed.

**Procedure for Zeil-Neelsen staining:**

The numbered slides were placed on a staining rack in batches (maximum 12), ensuring that slides do not touch each other.

The entire slide was flooded with Zeil-Neelsen carbolfuchsin, which has been filtered prior to use, or each slide was covered with a piece of filter paper if unfiltered carbolfuchsin was used.

The slide slowly was heated until it was steaming, Avoiding boiling. The steaming was maintained for three to five minutes using low or intermittent heat. each slide was rinsed individually in a gentle steam of running water until all free stain was washed.

The slide was then flooded with the decolourising solution for a maximum of three minutes.

The slide was thoroughly rinsed with water. Excess water was removed from the slide and the slide flooded with counter-stain for 60 seconds and rinsed thoroughly with water. Excess water was drained from the slide and the smear allowed to air dry (80).

**Procedure of culture using Lowenstein-Jensen medium**

We used Sodium hydroxide (NaOH) (modified Petroff) method for homogenization and decontamination.

Procedure:

To 1ml of sputum, we added 2ml of 4% NaOH, tightened cap of container and shaken to digest, let it stand for 15 minutes at room temperature with occasional

shaking, centrifuged for 15 minutes, poured off the supernatant, added 15ml sterile saline or distilled water and resuspended the sediment, centrifuged at 3 000 x g for 15 minutes, decanted the supernatant and inoculate onto culture medium immediately (80).

### **Identification tests:**

#### **Niacin production test:**

All mycobacteria produce niacin, however, only *M. tuberculosis*, *M. simiae* and occasional strains of *M. africanum*, *M. bovis*, *M. marinum* and *M. chelonae* lack the enzyme necessary to further convert the niacin to niacin ribonucleotide. Thus the determination of whether niacin has accumulated in the culture medium is a valuable differential test in identifying these species of mycobacteria, particularly *M. tuberculosis*. Reagent-impregnated filter paper strips have been developed to replace cyanogens bromide, a highly toxic substance. The development of a yellow color in the test medium incubated with a reagent strip is indicative of niacin accumulation and a positive test (81).

#### **Nitrate reduction test**

Only a few species of mycobacteria, notably *M. tuberculosis*, produce nitroreductase, which catalyzes the reduction of nitrate to nitrite. The development of a red color on addition of sulfanilic acid and N-naphthylethylene-diamine to an extract of the unknown culture is indicative of the presence of nitrite and a positive test (81).

### **Strain susceptibility testing**

We tested susceptibility of *M. tuberculosis* strains towards four of the first line anti-TB drugs: H, R, S and E. Z was excluded because it is difficult to measure susceptibility to it using the BACTEC method and it is thus excluded from the drugs that recommended by WHO/IUATLD to estimate DR in the surveyed regions.

Susceptibility test to anti-TB drugs was performed in the NIPH using radiometric drug susceptibility test, BACTEC Becton Dickinson Diagnostic System, Towson, MD, USA) (82, 83).

These drugs were chosen because they have been widely used through out the world and their susceptibilities can be reliably measured by standardized BACTEC method. In 1975 radiometric detection of metabolic products of *M. tuberculosis* was reported. The principle was thereafter used for the detection of growth of tubercle bacilli in selective media containing  $^{14}\text{C}$  -labelled-substrate. The resulting release of  $^{14}\text{C}$   $\text{CO}_2$  was measured automatically using an ionization chamber (BACTEC).

The system is a modification of the conventional proportion method. Instead of an agar base the test uses middlebrook 7H12 broth with a radiolabeled fatty acid substrate. BACTEC 460 method uses standard sputum concentration techniques and a combination of polymyxin B, amphotericin B, carbenicillin and trimethoprim to minimize contamination by non-mycobacterium organisms. This decontamination treatment allows the specimen to be inoculated directly into middlebrook 7H12 broth media containing  $^{14}\text{C}$ -labeled palmitic acid.

Growth of *M. tuberculosis* in the media releases  $^{14}\text{CO}_2$  in the bottle that is then measured radiometrically. The amount of growth, indicated by changes in the growth index ( $\Delta\text{G1}$ ) in the media with known drug concentrations compared to that in the control bottle, has been correlated to the presence or absence of resistance of >10% of the inoculums. If an isolate grows beyond a specific growth index compared with the control it is considered resistant to that specific agent (83, 84).

The concentrations of the anti-TB drugs used for the test were as follows:

H, 0.2  $\mu\text{g/ml}$ ; R, 2.0  $\mu\text{g/ml}$ ; E, 7.5  $\mu\text{g/ml}$ ; and S, 6.0  $\mu\text{g/ml}$

## **Statistical analysis**

Data sorting and patients distribution into case and control groups was performed after laboratory testing of all collected *M. tuberculosis* strains.

SPSS was used for all the statistical analysis.

Association between categorical variables was assessed by the  $\chi^2$  test. Differences between groups were expressed as Odds ratio (OR) with 95% confidence intervals (95% CI). Student t-test was used to test for differences in means of continuous variables. P value of  $< 0.05$  was chosen as the level of significance.

## **Definitions**

**Cases** were patients whose strains of *M. tuberculosis* are resistant to at least one of the first line anti-TB drugs

**Controls** were patients included in the study according to the inclusion criteria, whose strains of *M. tuberculosis* are susceptible for anti-TB drugs.

**New cases** were patients who have not been previously treated for TB or treated for less than one month.

**Previously treated cases** were patients with a history of previous anti-TB treatment for at least one month.

Resistance to each of the first line anti-TB drugs was defined according to the results of susceptibility tests by BACTEC method.

The first line anti-TB drugs:

Isoniazid (H), Rifampicin (RH), Pyrazinamide (Z), Ethambutol (E), Streptomycin (S)

**Any kind of resistance:** was defined as resistance to at least one of the first line anti-TB drugs.

**Poly-resistance** was resistance to more than two first line anti-TB drugs.

**MDR** was defined as resistance to both H and R with or without resistance to other agents.

**Drug resistance among new cases:** (formerly acquired drug resistance) was defined as presence of a resistant strain of *M. tuberculosis* in a patient who, in response to direct questioning, denied having had any prior anti-TB treatment (for more than one month) and where there was no documented evidence of such treatment.

**Drug resistance among previously treated cases** (formerly acquired drug resistance) was defined as resistance of a *M. tuberculosis* strain to anti-TB drugs in a previously treated patient.

**Previously treated cases:** Are patients with a history of previous anti-TB treatment for at least one month. This includes patients in one of the following groups:

- Patients who become smear positive again after having been treated for TB and declared cured after the completion of their treatment (relapse cases).
- Patients who are having treatment for smear positive pulmonary TB and who remain or become smear positive again at five month or later during the course of treatment (treatment failures).
- Patients who have interrupted their treatment for more than two months after having received a total of at least one month of anti-TB treatment and who return with bacteriologically confirmed TB (return after default).

- Patients who continue to be smear positive after the completion of a re-treatment regimen (chronic cases).

# **CHAPTER III**

## **RESULTS**

### **Drug resistance of the *M. tuberculosis* strains**

A total of 170 strains collected from patients with smear positive pulmonary tuberculosis in the 3 different places in Sudan (Khartoum state, Gazira state and camps of displaced population) were identified as *M. tuberculosis*. Twenty-six strains were excluded from the susceptibility testing since they were either contaminated or failed to grow when sub-cultured. Out of the remaining, 93 strains were from new cases and 51 strains were from previously treated cases. All strains were tested by the BACTEC method for susceptibility to the first line anti-tuberculosis drugs: H, R, S, and E. The susceptibility pattern to H, R, S and E of the 144 *M. tuberculosis* strains is represented in (Table1).

It is important to mention that the strains were collected mainly from the chest referral hospitals, where most chronic cases are usually admitted, like Abu-Anga hospital in Khartoum. This is known as a TB hospital and patients from all over the country are usually referred to that hospital when they have failed to be cured by ordinary anti-TB treatment. 29 of the 144 patients were from Abu-Anga hospital (20%)

Seventy- two (50%) of the strains were susceptible to at least one drug, and 72 (50%) were resistant. Mono-resistance to S was present in a high number of both new and previously treated patients. When we excluded S resistance, we found that 100 (69.4%) of the strains were susceptible to at least one of the other remaining drugs, and 44 (30.6%) were resistant.

The highest rate of mono-drug resistance was observed for S in both groups of patients (new and previously treated patients). 22 (23.6 %) strains collected from new and 8 (15.6 %) of strains collected from previously treated patients were mono-resistance to S (Table 1).

**Table 1:** Drug susceptibility pattern of H, R, S and E of the *M. tuberculosis* strains isolated from 144 patients with pulmonary TB in Khartoum, Gazira and camps for displaced population.

|   |   |   |   | NO. PREVIOUSLY TREATED CASES |    |   |     |    |       |
|---|---|---|---|------------------------------|----|---|-----|----|-------|
| H | R | S | E | NO. NEW CASES                | F  | R | TAD | T  | TOTAL |
| R | R | R | R | 0                            | 10 | 3 | 8   | 21 | 21    |
| R | R | S | S | 0                            | 2  | 1 | 0   | 3  | 3     |
| R | R | R | S | 2                            | 2  | 1 | 2   | 5  | 7     |
| R | S | R | S | 8                            | 0  | 0 | 1   | 1  | 9     |
| R | S | S | S | 1                            | 0  | 0 | 0   | 0  | 1     |
| S | R | R | S | 1                            | 0  | 0 | 0   | 0  | 1     |
| S | S | R | S | 22                           | 0  | 1 | 7   | 8  | 30    |
| S | S | S | S | 59                           | 1  | 1 | 11  | 13 | 72    |

S: susceptible

R: resistance

F: failure

R: relapse

TAD: treatment after default

T: total of previously treated patients

Resistance to four drugs was detected in 21 strains (15%). Mono-drug resistance to H and S was detected in 1 and 30 strains respectively. Resistance to only two drugs was found in 3 strains (for H and R), 1 strain (for S and R) and 9 strains (for H and S). Resistance to 3 drugs was detected in 7 strains. 31 strains (22%) were MDR. Twenty-one strains (14.6%) were resistant to E, resistant to E was only seen in MDR. With the exception of only one strain, all strains resistant to R were MDR.

With exception of 2 new patients, all MDR cases were found among previously treated cases. Most MDR strains (90%) were also resistant to S and 68% of them were resistant to E.

Strains were collected from 93 (64.6 %) newly diagnosed patients, and 51 (35.4 %) previously treated patients (table 2).

Among the 93 strains collected from new cases, 34 (36.5%) strains were resistant to at least one drug. Out of the 51 strains collected from the previously treated cases 38 (74.5%) were resistant to at least one drug.

The highest rate of drug resistance was observed for S and H in both groups of patients (new and previously treated patients), with 33 (35%) and 35(69%) of strains collected from new and previously treated patients were resistant to S respectively.

Eleven (12 %) and 30 (59%) collected from new and previously treated patients were resistant to H respectively (Table 2).

**Table 2.** Drug resistance pattern of H, R, S and E of the *M. tuberculosis* strains according to first or previous treatment.

| Resistance to: | Number of new cases | Number of previously treated cases |
|----------------|---------------------|------------------------------------|
|                | Total 93 (%)        | Total 51 (%)                       |
| H              | 11(11.8)            | 30 (58.8)                          |
| R              | 3 (3.2)             | 29 (56.8)                          |
| S              | 33 (35.4)           | 35 (68.6)                          |
| E              | -                   | 21 (41.1)                          |
| Single drug    | 34 (36.5)           | 38 (74.5)                          |
| MDR            | 2 (2.1)             | 29 (56.8)                          |

Strains were collected from three different areas in Sudan: Khartoum state, which is the capital city of Sudan (population of 4.600.000) Gazira State (population of 1.200.000) and camps for displaced population (population of 2 millions). In Khartoum state 49 (53.8%) out of 91 strains were resistant to any of the tested drugs while in Gazira state 14 (56%) out of 25 strains were resistant and in the camps for displaced population 9 (32%) out of total 28-tested strains were resistant to any of the four tested anti-tuberculosis drugs (table 3).

In Khartoum state 24 (26.4%) were MDR, in Gazira state 4 (16%) were MDR and in the camps for displaced population 3 (10.7%) were MDR. (Table 3).

**Table 3:** Drug resistance pattern of the tested *M. Tuberculosis* strains according to patients' address

|                | Khartoum   | Gazira   | Camps    | Total 144 |
|----------------|------------|----------|----------|-----------|
|                | Total 91   | Total 25 | Total 28 |           |
| R. To any drug | 49 (53.8%) | 14 (56%) | 9 (32%)  | 72        |
| MDR            | 24 (26.4%) | 4 (16%)  | 3(10.7%) | 31        |

**Factors associated with infection with drug-resistant and multi-drug resistant strain of *M. tuberculosis***

To identify factors associated with the development of DR in the three chosen districts in Sudan, demographical, social and medical factors were collected from patients were studied in relation to susceptibility patterns of *M. tuberculosis* strains.

Patients were categorized according to history of previous treatment with anti-TB medications. Ninety-three of patients in the study were new cases. Fifteen patients were positive for AFBs at 5 months of treatment (failure), and other thirty previously

treated patients had interrupted their treatment and six patients had relapsed (Figure 1).

Resistant to any kind of drug was present in all patient categories but MDR was common among patients treated previously.

Patients previously treated for TB were having a high risk of infection with DR and MDR. Thirty-eight of the 72 (52.8%) patients infected with drug-resistant strain had an evidence of previous treatment for TB. Thirteen out of the 72 (18.1%) patients infected with susceptible strain were treated previously. Previous treatment significantly associated with the development of the disease with drug-resistant strain ( $P < 0.0001$ ) (table 4)

Only two among new patients (2.1%) were MDR. Fourteen of the 15 (93.3%) failure cases were MDR. Four of the 6 (66.7%) relapse patients were MDR. For those who had interrupted their treatment 11 out of 30 (36.7%) patients were MDR.

A total of 29 of the 31(93.5%) patients infected with MDR strains had an evidence of previous treatment for TB. Twenty-two of the 113 (19.5%) patients infected with non-MDR strains were treated previously. Previous treatment significantly contributed to development of the disease with MDR strains ( $P < 0.0001$ ) (table 5)

The age distribution in the study ranged from 13 to 70 years with the mean age of 40. There were 83 patients with age of 40 years or above.

Patients in the age group (> 39 years) were having a high risk of infection with both DR and MDR strains. Fifty-six of the 72 (77.8%) patients infected with DR strains were 40 years or above. Forty-two out of the 72 (58.3%) patients infected with susceptible strain were in this group. Above 39 years was a significant factor associated with development of TB with resistant strains.  $P = 0.012$  (Table 4).

Twenty-five of the 31(80.6%) patients infected with multi-drug resistant strain were 40 years old and above. Fifty-eight of the 113 (51.3%) patients infected with non-MDR strain were in the same group. Above 39 years was significantly contributed to development of TB with a MDR strain.  $P= 0.003$  (table 5).

To explore a possible association between symptoms' duration and the presence of DR we selected the duration of cough as cough is of major importance for transmission. We found that the mean duration of cough for patients with MDR TB was 51.16 weeks, while the mean duration of cough for TB patients with non-MDR strains was 12.45.

Patients with long duration of TB symptoms were having a high risk of infection with DR and MDR strains. Thirty out of the 72 (41.7%) patients infected with drug resistant strains were having long duration of cough (more than 20 weeks). Five out of the 72 (6.9%) susceptible patients were having long duration of cough. Having long duration of cough was highly associated with infection with drug resistant strains.  $P < 0.0001$  (table 4).

Twenty-three out of the 31 (74.2%) patients infected with MDR strains were having long duration of cough (>20 weeks). Twelve of the 113(10.6%) patients infected with non-MDR strains had long duration of cough. Having long duration of cough was a significant risk risk factor for infection with a MDR strain.  $P < 0.0001$  (table 5).

Contact with another TB patient was defined as a known TB patient living in the same house, or a known community contact (work place or neighborhood) or no known contact. Thirty-five patients had a history of household contact, 24 had history of community contact and 85 patients had no history of any kind of contact.

Contact with TB patient was a strong risk factor for infection with DR and MDR strains. Twenty- five out of the 72 (34.7%) patients infected with DR strains had a history of household contact with a TB. Ten out of the 72 (13.9%) patients infected with susceptible strain had a history of household contact with a TB patient. History of a known TB patient living in the same house was significantly associated to infection with a DR strain.  $P = 0.003$  (table 4).

Seventeen out of the 31 (54.8%) patients infected with MDR strains had a history of household contact. Out of the 113 patients infected with non-MDR strains, 18 (15.9%) had history of household contact with a TB patient. History of household contact with TB patient was strongly associated with infection with MDR strains.  $P < 0.0001$  (table 5)

The weight of the patients in the study ranged from 30 to 70 kilograms. The mean weight is 50 kilograms. Forty-two patients weighed 40 kg and less.

Low weight of patients was strong risk factor for infection with DR and MDR strains. Twenty-eight out of the 72 (38.9%) patients infected with resistant strains were weighing 40 kg or less. Among the 72 patients infected with susceptible strain, 14 (19.4%) were weighing 40 kg or less. Having low weight (40 Kg and less) was a significant risk factor for infection with resistant strains.  $P = 0.01$ (table 4)

Sixteen out of the 31(51.6%) patients infected with MDR strains, were weighing 40 kg and less. Twenty-six out of the 113 (23.0%) patients infected with non-MDR strains were weighing 40 kilograms or less. Having low weight was a strong risk factor associated with infection with MDR strains.  $P = 0.002$  (table 5)

Body mass index was calculated to estimate type of body constitution. BMI of the patients in this study ranged from 12.4 to 30.61, with a mean of 20.1. Ninety-nine (68.75%) patients were under-weight, that is BMI less than 20.0. Forty-one (28.4%) had a normal weight, that is BMI between 20.0 and 24.9. Three (2.08%) of the patients were overweighted, that is BMI between 25.0 and 29.9. One (0.7%) of the patients was obese, that is BMI more than 30.

Among the 144 tested TB patients 98 (68%) of them were males and 46 (32%) were females (Figure 2). There was no significant difference in risk of DR (table 4) and MDR (Table 5) according to gender of patients.

Regarding marital status of the patients, we classified them according to the legal status in the country into four different groups: married, divorced, widowed and unmarried. Eighty-nine patients were married, 46 were unmarried, 8 were divorced and one patient was widowed. (Figure 3)

Among the 56 single, 28 (38.9%) out of 72 patients infected with DR strains, were resistant to any drug and 28 (38.9%) out of the 72 patients infected with drug susceptible strains, were susceptible.  $P = 1.000$  (Table 4)

Eight (25.8%) out of the 31 patients infected with MDR strains, were single.

Out of the 113 patients infected with non-MDR strains, 48 (42.5%) were singles.

$P = 0.092$  (Table 5).

Among the 144 patients, 87 were illiterate and 57 were literate. (Figure 4)

Forty-six (63.9%) out of the 72 patients infected by resistant strains were illiterate.

While out of the 72 infected by susceptible strains, 41 (56.9%) were illiterate.

$P = 0.394$ . (Table 4)

Twenty (64.5%) out of the 31 patients infected with MDR strains were illiterate.

While 67 (59.3%) out of the 113 patients infected with non-MDR strains, were illiterate. MDR was relatively higher among illiterate but the difference was not significant.  $P = 0.598$ . (Table 5).

Residence of patients was classified as urban or rural. Ninety-five patients were urban and 49 were rural.

Among the 72 patients infected by drug resistance strains, 48 (66.7%) were urban. Forty-seven (65.3%) were urban, out of the 72 patients infected by susceptible strains.  $P = 0.579$ . (Table 4).

Among the 31 patients infected by MDR strains, 24 (77.4%) were urban. Seventy-one (62.8%) were urban out of the 113 patients infected with non-MDR strains. Being a rural or urban resident was not a risk factor for infection with MDR strains.  $P = 0.297$  (Table 5).

Occupation was classified as governmental employee (23), student (15), laborer (35), running small business (17), running big business (0), unemployed (18) employee in private sector (5) and housewife (31). Drug resistant was found in about the same percentages for all occupations except for those who were employees in private sector. (Figure 5)

Among the 72 patients infected by DR strains 4 (5.6%) were employed in private sector. While 1 (1.4%) patient was employed in private sector among the 72 patients infected with drug susceptible strains.  $P$  value = 0.172 (Table 4).

Three (9.7%) out of the 31 patients infected by MDR strains, were employees in private sector, and 2 (1.8%) were employed in private sector among the 113 patients infected by non-MDR strains.  $P = 0.033$  (Table 5).

The origin of the patients was classified into 5 groups, northern Sudan 29 patients, southern Sudan 35 patients, western Sudan 53 patients, eastern Sudan 7 patients and central Sudan 20 patients. (Figure 6)

People from west Sudan trended to have a higher rate of resistant to any drug and MDR compared to patients from other regions.

Among the 72 patients infected by DR strains, 29 (40.3%) were from west of Sudan.

Within the 72 patients infected with susceptible strains, 24 (33.3%) were from west of Sudan.  $P = 0.388$ . (Table 4).

Out of the 31 patients infected by MDR strains, 11 (35.5%) were from west of Sudan.

Forty-two (37.2%) were from west of Sudan out of the 113 patients infected by non-MDR strains ( $P = 0.863$ ). (Table 5)

Patients housing conditions were classified according to the number of people per room. The number of people per room ranged from 1 to 10 with the mean number of 3 and the mode was 3. Those living in crowded households with 3 or more persons per room were 101. There was no significant differences regarding risk of resistant to any drug ( $P=0.363$ ), (table 4). Or MDR ( $P=0.909$ ), regarding patients crowded or non-crowded housing conditions. (Table 5)

Smoking was not a risk factor for development of resistant to any drug or MDR.

Among the 144 patients, 19 were smokers and 125 were non-smokers. Eleven (15.3%) were smokers out of the 72 patients infected by resistant strains where as 8 (13.2%) out of the 72 patients infected by susceptible strains, were smokers.  $P=0.460$

Four (12.9%) were smokers among the 31 patients infected by MDR strains. Among the 113 patients infected by non-MDR strains, 15 (13.3%) were smokers.  $P=0.957$

Thirty-nine patients were BCG vaccinated and 105 were non-BCG vaccinated. Out of the 72 patients infected with DR strains, 13 (18.1%) were BCG vaccinated and 59 (81.9) were non-BCG vaccinated. Among the 31 MDR strains 4 (12.9%) were BCG-vaccinated and 27 (87.1%) were not. Non BCG vaccination was significantly associated with development of DR  $P= 0.045$  and MDR strains  $P=0.015$

**Table 4:** Demographic and medical characteristics of 144 patients with pulmonary TB in relation to infection with resistant and susceptible strains of *M. tuberculosis*

|                           | Infection with drug resistant strains<br>N=72 (%) | Infection with drug susceptible strains<br>N=72 (%) | P. Value |
|---------------------------|---|---|----------|
| Males                     | 48 (66.7%)  | 50 (69.4%)  | 0.633    |
| Age                       | 56 (77.8%)  | 42 (58.3%)  | 0.012    |
| Marital status            | 28 (38.9)   | 28 (38.9)   | 1.000    |
| Education                 | 46 (63.9%)  | 41 (56.9%)  | 0.394    |
| Previous treatment for TB | 38 (52.8%)  | 13 (18.1%)  | < 0.0001 |
| Contact with TB pts       | 25 (34.7%)  | 10 (13.9%)  | 0.003    |
| Duration of cough         | 30 (41.7%)  | 5 (9.7%)  | < 0.0001 |
| Origin of patients        | 29 (40.3%)  | 24 (33.3%)  | 0.388    |
| Low weight                | 28 (38.9%)  | 14 (19.4%)  | 0.01     |
| Residence<br>Urban/Rural  | 48 (66.7%)  | 47 (65.3%)  | 0.579    |
| Occupation                | 4 (5.6%)  | 1 (1.4%)  | 0.239    |
| Housing conditions        | 48 (66.7)   | 53 (73.6%)  | 0.363    |
| Smoking                   | 11 (15.3%)  | 8 (13.2%)   | 0.460    |

TB = tuberculosis OR = odd ratio 95% CI = 95% confidence interval

**Table 5:** Demographic and medical characteristics of 144 patients with pulmonary TB in relation to infection with MDR and non-MDR strains of *M. tuberculosis*

| Characteristics              | Infection with multi-<br>drug resistant<br>strains<br>N=31 (%) | Infection with non-<br>multi-drug resistant<br>strains<br>N=113 (%) | P. Value |
|------------------------------|--|---|----------|
| Males                        | 20(64.5%)  | 78(69%)   | 0.633    |
| Age                          | 25(80.6%)  | 58 (51.3%)  | 0.003    |
| Marital status               | 8 (25.8%)  | 48 (42.5)   | 0.092    |
| Education                    | 20(64.5%)  | 67 (59.3%)  | 0.598    |
| Previous treatment for<br>TB | 29(93.5%)  | 22 (19.5%)  | < 0.0001 |
| Contact with TB pt           | 17(54.8%)  | 18 (15.9%)  | < 0.0001 |
| Duration of cough            | 23(74.2%)  | 12 (10.6%)  | < 0.0001 |
| Origin of patients           | 11(35.5%)  | 42 (37.2%)  | 0.863    |
| Low weight                   | 16 (51.6%)   | 26 (23%)  | 0.002    |
| Residence<br>Urban/Rural     | 24 (77.4%)   | 71 (62.8%)  | 0.297    |
| Occupation                   | 3 (9.7%)   | 2 (1.8%)  | 0.168    |
| Housing conditions           | 22 (71%)   | 79 (69.9%)  | 0.909    |
| Smoking                      | 4 (12.9%)  | 15 (13.3%)  | 0.957    |

TB = tuberculosis OR = odd ratio 95% CI = 95% confidence interval

# **CHAPTER IV**

## **DISCUSSION**

In this study we described the prevalence of TB DR among new and previously treated patients in Khartoum, Gazira state and camps for displaced population, and we identified some of the risk factors associated with the development of DR TB.

Drug susceptibility was tested by the BACTEC method in the National Institute of Public Health, Tuberculosis Laboratory in Norway, one of the four DST methods, which have been standardized and are widely used throughout the world to measure DR of *M. tuberculosis* (85, 86).

### **Prevalence of drug-resistance TB in the three chosen areas in Sudan**

The extent of the problem of DR TB in Sudan is not known since there is no national surveillance done. Limited studies were conducted since 1960s, but they do not reflect the present situation of drug resistance in Sudan. A major challenge now a days for the NTP and the Tuberculosis Reference Laboratory is to establish a national surveillance to determine the resistance pattern to the first line anti-TB drugs in Sudan. This study is an explorative pilot study to base the national surveillance on.

Our study showed that resistance to at least one drug among new and previously treated cases was 34 (36.5%) and 38 (74.5%) respectively. MDR among new and previously treated cases was 2 (2.1%) and 29 (56.8%) respectively. High level of DR among previously treated cases is probably due to the fact that most of our strains were collected from chest referral hospitals in Khartoum and Gazira states, where

most TB patients who had taken anti-TB drugs and failed to be cured end up. (Figure 7)

MDR was present in 24 (53.8%) strains in Khartoum state, 4 (16%) strains in Gazira state and 3 (10.7%) in the camps for displaced population. We can observe that the highest level of MDR was found in Khartoum State. Fewer cases of MDR in camps for displaced people reflect the good treatment outcome, where the DOT strategy is vigorously applied. The difference was not significant, but it might be so if we increase the sample size.  $P=0.161$

Mono-resistance was found at a high rate for S. 22 (23.7%) and 8 (15.7%) of the strains were resistant to S among new and previously treated patients respectively. This is not unexpected since S was the first anti-TB drug introduced in Sudan and it was widely used by physicians as mono-therapy for the treatment of TB.

Mono-resistance was at a very low level for H, which has only been used in combination with other anti-TB drugs.

Mono-resistance to E or R was not detected. Resistance to R predicted resistance to H and served as a marker of MDR. The fact that resistance to R is rarely observed without associated resistance to other drugs was confirmed by other studies ((87, 88).

### **Risk factors for the development of drug-resistant TB**

High rates of DR among failure and relapse cases were observed in our study. DR TB and MDR-TB were significantly associated with a history of a prior treatment period. This association might be due to initial infection with DR strains, with unsuccessful treatment being the result. However it is more likely that previous treatment was the cause of MDR-TB. Those who had a history of previous treatment may have received non-standard regimens or have interrupted treatment. The selection of resistant mutants takes place after several regimens have been administered in which cycles of killing (when drug is taken) and re-growth (when the drug is stopped) occur (47, 89). As the cycles are repeated, there is a stepwise selection favoring the resistant strains relative to the susceptible ones. This cyclic process creates an imbalance, causing resistance to one drug and later to other drugs and ultimately to MDR. Not surprisingly, any DR and MDR-TB were significantly associated with previous treatment cases. Previously treated cases, including failures is the richest source of DR bacilli in the community (90, 91). They are also an important source of infection, since their infectiousness is usually longer than that of new cases (92). However, the absolute number of MDR-TB cases is so far small and should not constitute a public health threat, provided the TB control programme keep up high cure rates among new cases.

The administration of SCC under DOT is the cornerstone of curing new cases with active disease, and thus to reduce recruitment to the pool of previously treated cases. On the other side, if SCC is ineffectively used, an increase in the pool of infectious cases, and often DR, will take place (93).

All patients in our study were HIV negative. The data presented in the WHO and IUATLD report on drug resistance surveillance suggested that HIV is not an independent risk factor for DR, or MDR-TB. Although evidence from different settings suggests that HIV-infected TB patients are no more likely to develop drug resistance than HIV-negative TB patients (94, 95). Nevertheless, when TB patients are not adequately treated and levels of acquired resistance are elevated, co-existence of HIV could be responsible for the rapid spread of primary drug-resistant TB. This is because HIV infects CD4 + T-cells exclusively. CD4 + T-cells produce interferon – gamma, which activates macrophages to inhibit intracellular growth of *M. tuberculosis*. As HIV progresses, CD4 + T-cells count drops leading to increased risk of primary infection and development of primary TB from primary infection.

Long duration of cough, an indicator of long duration of TB symptoms, was found to be strongly associated with MDR and resistance to any drug. Patients might have received non-standard regimens or have interrupted treatment prior to their proper management. Long duration of cough is a serious situation, since it means long duration of infectiousness of patients with resistance strains and that adds to the impact of spread and transmission of the disease.

In this study low weight of patients was strongly associated with MDR and resistance to any drug. That is probably an effect of MDR rather than a primary risk factor since long duration of symptoms of the disease and chronic illness are the main causes of the loss of weight.

Resistance was higher in known household contact of TB patients than in patients with community contacts or without known contacts. Socioeconomic conditions and hygiene are amongst the strongest factors influencing favourably the course of the TB epidemic in the community (96). Since TB is associated with poverty (97), improvements of the social conditions of people should lead to a reduction in the incidence of active disease.

In this study, education and male gender trended to be associated with the development of DR but the association was not significant. If this reached significant when we increased the sample size, it may be explained by that educated people in the Sudanese society represents to some extent a higher or a middle class in the society, so they are the ones who are most likely to afford access to the health services, and thus to drugs.

There was a trend towards an association between origin in the west of Sudan and development of DR. If this reaches significance in further study it may be explained by the movements of people from west of Sudan to Khartoum due to war and famine. This is the problem of migration in increasing the risk of irregularity in treatment.

There is no significant association between the developments of drug resistance or MDR and the marital status of the patients.

This may reflect the Sudanese culture taking much care of singles. Being a single in Sudan is not a risk factor for developing DR TB.

There was a trend toward an association between patients working in private sector and the development of DR. If that association should be proved significant with a larger sample size, it may reflect the difficulties of accessibility to the health services related to the financial situation of the patients since those who are employees in private sector are more likely to afford access to the health services.

We did not observe any association between the development of DR and the crowded living conditions. This may be due to the fact that people in Sudan sleep in big yards outside their rooms and the rooms are constructed with a wide space between the roof and the ground, windows and doors are usually kept open with a good ventilation inside the rooms.

## **Future perspectives**

The results of this study underline the necessity of establishing a National Reference Laboratory for TB in Sudan, in order to establish a national surveillance on anti-tTB DR in the whole country. Susceptibility testing for those who have interrupted their treatment or failed to be cured by ordinary anti-TB treatment is important in order to avoid errors in case management. Treatment of patients infected with DR *M. tuberculosis* must be prescribed according to the most likely susceptibility pattern established through national surveillance. For those infected with susceptible *M. tuberculosis*, treatment must be prescribed according to standard treatment regimens, which will eventually lead to cure of the patients.

High prevalence of DR and MDR in previously treated patients might indicate poor patients' and/or doctors' compliance. Poor compliance causes accumulation of mutants resistant to anti-TB drugs. Spread and transmission of such strains will increase the problem of drug resistance in the community. This will cause serious problems for health services since second line treatment is expensive and have toxic effects. Drug sensitivity surveillance is thus a helpful tool for planning further anti-TB activities in Sudan.

In Sudan all health workers in the NTP are well trained and educated for the management of TB.

Based on the analysis of these results NTP may already revise its policy on anti-TB treatment. For instances, high prevalence of drug resistance to S could initiate a discussion of whether to change the regimen by replacing S by E.

Also patients who have interrupted their treatment are treated with the new patients' treatment category (CAT 1). High prevalence of MDR and resistance to any drug among patients who have interrupted their medication could be avoided by treating them within the category for the previously treated patients (CAT2)

## **CONCLUSIONS AND RECOMMENDATIONS**

### **CONCLUSIONS:**

1. There is a high prevalence of *M. tuberculosis* strains resistant to S also in new patients
2. Drug resistance except for S among new cases is a rare phenomenon in Sudan, which indicates a low rate of transmission of resistant strains.
3. Drug resistance among previously treated patients is present at an alarming level
4. Resistance to two or three drugs is not a common phenomenon.
5. Resistance to R predicts resistance to H and can serve as a valid marker of MDR
6. Prevalence of MDR and drug resistance is associated with previous anti-TB treatment, being more than 40 years of age, long duration of disease symptoms (cough), low weight and household contact with a TB patient.

**RECOMMENDATIONS:**

1. It is important to have a well functioning National Reference Laboratory in Sudan and reliable laboratory facilities to monitor/survey drug resistance in time.
2. NTP should use all efforts to ensure proper cure of TB patients.
3. S should be replaced by E in the first 2 months of the treatment.
4. TAD patients should have been given CAT2 (3SRZE, 5RZE) initially.

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# **APPENDIX**

DATA COLLECTION FORM

Patient identity

Card number:

Date: D | M | Y |

Name: \_\_\_\_\_

Sex:  M  F

Age:

Marital Status:  Married  Unmarried  Divorced  Widowed

Residence:  Urban  Rural

Education:  Illiterate  Literate

Number of years completed:

Primary (1-6)  Intermediate (7-9)

Secondary (10-12)  Post Secondary  Others

Occupation:  Government employee  Student  Labor

Running small business  Running big business

Unemployed  Employee in private sector

House Wife

No of people per room:

Address: \_\_\_\_\_

Tribe: Northern Southern  
Western Eastern

Medical history

Weight: (.....) kg.

Height: (.....) cm.

BCG Scar: Present Absent.

HIV status: Positive Negative

Patient' categorization: New case Failure Relapse TAD

Smoking: Present Absent Duration

Main Complains Of Suspect:

Cough: Present Absent Duration (.....)

Weight loss Present Absent Duration (.....)

Chest pain: Present Absent Duration (.....)

Shortness of breath Present Absent Duration (.....)

Fever: Present Absent Duration (.....)

Night sweat: Present Absent Duration (.....)

Tiredness: Present Absent Duration (.....)

Hemoptosis: Present Absent Duration (.....)

Laboratory data:

Smear microscopy: Positive Negative

Culture: Positive Negative

Result of susceptibility test (BACTEC):

S  Susceptible Resistance

|                        |                                      |                                     |
|------------------------|--------------------------------------|-------------------------------------|
| H                      | <input type="checkbox"/> Susceptible | <input type="checkbox"/> Resistance |
| R                      | <input type="checkbox"/> Susceptible | <input type="checkbox"/> Resistance |
| E                      | <input type="checkbox"/> Susceptible | <input type="checkbox"/> Resistance |
| Resistance to any drug | <input type="checkbox"/> Susceptible | <input type="checkbox"/> Resistance |
| MDR:                   | <input type="checkbox"/> Yes         | <input type="checkbox"/> No         |

