THE LEVEL AND PATTERN OF ANTI-TUBERCULOUS DRUG RESISTANCE AT THE KENYATTA NATIONAL HOSPITAL

BY

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This dissertation is my original work and has not been presented to any University for a degree.

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LIST OF ABBREVIATIONS

AIDS	: Acquired Immunodeficiency Syndrome
CDC	: Centre for Disease Control
CRDR	: Center for Respiratory Diseases Research
DNA	: Deoxyriboneucleic Acid
DR	: Drug Resistant
D.S.T	: Drug Sensitivity Testing
EMB	: Ethambutol
HIV	: Human Immunodeficiency Virus
INH	: Isoniazid
IUATLD	: International Union Against Tuberculosis and Lung Disease
KEMRI	: Kenya Medical and Research Institute
KNH	: Kenyatta National Hospital
MDR-TB	: Multidrug Resistant Tuberculosis
MTB	: Mycobacterium Tuberculosis
NEJM	: New England Journal of Medicine
NLTP	: National Leprosy and Tuberculosis Programme
N.R.L	: National Reference Laboratory
PAS	: Paraminosalicyc Acid
PTB	: Pulmonary Tuberculosis
RLFP	: Restrictive Length Fragment Polymorphism
RMP	: Rifampicin
SCC	: Short Course Chemotherapy
SDRTB	: Surveillance of Drug Resistance in Tuberculosis
TB	: Tuberculosis
TH	: Thiacetazone
Th2	: T helper 2 cells
TNF	: Tumour necrosis factor
QA	: Quality Assurance
WHO	: World Health Organization
XDR TB	: Extremely Drug Resistant Tuberculosis
ZN	: Ziehl Neelson Staining

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ABSTRACT

Background The Kenyatta National Hospital continues to receive an increasing number of patients who have been diagnosed to have tuberculosis (pulmonary and extra pulmonary) as reflected by the number of new daily registrations at the TB clinic and the overall new entries made at the records department from both the inpatients and outpatient clinical departments. Globally the TB pandemic has been compounded by high levels of anti-tuberculous drug resistance including multidrug resistant TB (MDR - TB). In Kenya the level of total drug resistance is still less than 10%. However certain population groups have been found to have a disproportionately higher prevalence of anti-tuberculous drug resistance including pockets of MDR- TB recently reported in Nairobi . Besides , the city of Nairobi is inhabited by different population groups from both within the country and the neighboring countries. The Kenyatta National Hospital serves as a national referral hospital and a primary health care center for the Nairobi residents. **Objective**: The primary objective was to determine the prevalence of resistance to all the four first line anti-tuberculous drugs and the resistant patterns of the individual drugs as well as to the various combinations of these drugs. The secondary objectives were to determine the demographic and socioeconomic characteristics of those with either of the strains (sensitive or resistant) and the co-morbid factors including HIV infection that may be associated with drug resistance. **Study Design**: A prospective study that involved culture and drug sensitivity testing of the first line anti-tuberculosis drugs (streptomycin, isoniazid, rifampicin, ethambutol) using Lowestein Jensten culture media (conventional method) and resistant ratio method to analyze all sputum samples Our study population consisted of patients with pulmonary tuberculosis who attended the tuberculosis outpatient clinic or were admitted to any of the general wards. The diagnosis for tuberculosis was made either by a positive sputum smear microscopy for Ziehl Neelson staining or positive microbiological (culture) method from 1st June 2007 to 30th September 2007 at the Kenyatta National and Referral Hospital, Nairobi Kenya. **Results:** The following rates of resistance were found in the 81 samples tested: total resistance was 34%, isoniazid mono-resistance was 30%, isoniazid + rifampicin 2.5%,resistance to all the four drugs 2.5%. There was no difference in the demographic and socioeconomic characteristics among those with the resistant and sensitive strains. No difference was found in the HIV status between the two populations (those with the resistant and sensitive strains). Other co-morbid factors associated with the resistant strains were not statistically significant and were considered to be possible incidental associations. **Conclusion:** These data show higher total drug resistance and isoniazid mono-resistance rates than those found at the Kenyatta National Hospital, in previous studies.The trend of MDR-TB has not been well defined. However drug resistance and especially MDR-TB in the context of the high prevalence of HIV in the study population should be closely monitored.

1. LITERATURE REVIEW AND STUDY BACKGROUND

Tuberculosis has been and continues to be the main cause of death by a single infectious agent namely *mycobacterium tuberculosis*. The disease spreads more easily in overcrowded settings and in the conditions of malnutrition and poverty. These characteristics are typical of developing countries.[1] Increasing poverty in overpopulated world has led to lack of attention to tuberculosis services. This coupled with the impact of HIV /AIDS pandemic has led to cases of tuberculosis which are more today than at any previous time in human history.[2] Globally the TB pandemic has been compounded by high levels of anti-tuberculosis drug resistance including multidrug resistance tuberculosis – MDR-TB (resistance to at least isoniazid and rifampicin)[3] Infection of humans with MTB is mainly through inhalation of droplets containing infectious bacilli. Presence of lung cavities in patients with PTB increases the likelihood of transmission. These cavities form as a result of gross tissue necrosis attributable to cytokines secreted by Th2 cells which render infective tissue very susceptible to killing by TNF. Subsequently large lesions containing abundant caseous necrotic tissue are formed. The center is anoxic and acidic and relatively few bacilli are present due to the hostile environment. The final cavities form when the enlarging caseous necrotic tissue erodes into a bronchial tree and the liquified caseous material gets discharged into the bronchial tree . Air enriched with carbon dioxide enters the cavity providing oxygen for the bacilli and neutralizing the cavity.The tubercle bacilli are able to replicate freely and huge numbers line the cavity wall .These bacilli gain access to the bronchi and are expectorated in the sputum , the patient becomes infectious and is said to have open tuberculosis. Post primary tuberculosis may develop directly from a primary lesion (progressive pulmonary tuberculosis). More often there is a latent phase of several years or even decades with a subsequent endogenous reactivation of latent foci of infection.[4]

1.1 Mechanism of Anti-TB Drug Resistance

Clinical drug resistance is defined as a state when MTB organisms are resistant to anti microbial agents at the levels attainable in blood and tissue.[5] This resistance can develop either spontaneously (primary resistance) or under selective pressure .[6] (acquired resistance)

Resistance of *mycobacterium tuberculosis* strains to anti-tuberculosis drug is a man made amplification of a natural phenomenon. Unlike the situation in many bacteria, where drug resistance occurs through one of three basic mechanisms: (the cell wall may become less permeable to antibiotics, bacteria may produce enzymes such as beta lactamases that degrade or inactivate drugs, drug target modifications involving genetic mutation in a key bacterial gene

occurring)[7,8]. Resistance to *mycobacterium tuberculosis* is unique as it develops spontaneously and with a defined frequency as a result of random chromosomal mutation. There is no indication of horizontal gene transfer (acquisition of resistant plasmids or transposons). Anti-tuberculous drugs when used inappropriately ,facilitate the selection of resistant strains which become predominant Drug resistance may also arise as a result of poor compliance during short course chemotherapy [9]
It is anticipated that this happens in a cyclical process where several cycles of interrupted treatment take place .This allows bacterial re-growth and subsequently increases the proportion of resistant mutants leading to the emergence of clinical resistance to anti-tuberculosis drugs[10].
Wild strains of M.tb that have never been exposed to anti-tuberculosis drugs are almost never resistant. A characteristic feature of these mutations is that they're unlinked.Thus resistance to a drug is not associated with resistance to an unrelated drug. Isoniazid inhibits mycolic acid. It quickly kills the rapidly growing tubercle bacilli in the cavity walls. Mechanisms of resistance have remained unclear. Mutations have been found in four genes. Rifampicin interferes with RNA synthesis, streptomycin inhibit protein synthesis ethambutol inhibits cell wall synthesis, pyrazinamide's exact target remains undetermined.[11]
Primary resistance is defined as the presence of drug resistant *mycobacterium tuberculosis* in a patient with no or less than one month of previous anti-tuberculosis drug therapy .
It occurs when transmission of the drug resistant mutant bacilli from the individual who has the acquired resistance to other persons who are HIV negative or positive leads to disease that is drug resistant from the outset. Every active drug against *mycobacterium tuberculosis* is bound to induce resistance and the more active a drug is the more likely it is to induce clinical resistance.[12]
Acquired resistance is defined as resistance to an anti-tuberculosis drug or drugs occurring in a patient who has received at least one month of anti-tuberculosis drugs in the past. This occurs following exposure to a single drug whether as a result of poor adherence to treatment, inappropriate prescription, irregular drug supply or poor drug quality. This exposure to the drug suppresses the growth of bacilli susceptible to that drug but permits the multiplication of pre existing drug resistant mutants.[13]

1.2 Factors associated with development of drug resistance

These include a history of contact with an index case of drug resistance[14], HIV infection,[15, 16, 17] history of previous chemotherapy ,non or poor compliance with treatment, interrupted drug supplies, inappropriate prescribing patterns and poor patient supervision.[18]
Others are factors related to poor or inadequate ventilation and isolation facilities. Socio-economic factors leading to poor compliance include alcoholism, drug abuse, poverty and homelessness.[19]

Genetic mutations resulting in resistance of M.tuberculosis to Rifampicin occur at a rate of 10^{-10} per cell division and lead to an estimated prevalence of 1 in 10^8 bacilli in drug free environments. The rate for isoniazid resistance is approximately 10^{-7} to 10^{-9} resulting in resistance in 1 out of 10^6 bacilli.[20] Bacillary populations larger than 10^7 are common in cavities.[21] This genetic resistance occurs in the absence of anti-microbial exposure but is diluted by the majority of drug susceptible micro-organisms. The presence of anti-microbials provides the selective pressure for resistant organisms to become predominant, especially in patients with a large load of bacilli e.g. those with extensive cavitary disease.[22] Multi drug resistance due to spontaneously occurring mutations is virtually impossible since there is no single gene involved in MDR and mutations resulting in resistance to various drugs arise independently. For example the likelihood of spontaneous mutations resulting in resistance to both INH and RMP is the product of the individual probabilities i.e. 1 in 10^{14} (10^6 x 10^8)[19]. This is the rationale for multi drug regimens in the treatment of tuberculosis.[23] In such situations, treatment with INH and RMP will select strains resistant to both antimicrobials. A similar sequence of events may lead to resistance to other drug combinations, and eventually to all first-line anti-tuberculosis medications.[24]

1.3 Historical Perspective on the Importance of Drug Resistant TB for the Control of The Disease.

Drug resistance appeared soon after the introduction of streptomycin and INH for the treatment of tuberculosis. Canetti, Fox and others realized the importance of adding a third drug, such as PAS or thiacetazone to prevent the selection of INH-resistant bacilli. The use of streptomycin during the initial phase of treatment was also found to be crucial for reducing the total number of bacilli as quickly as possible. During this early period, several surveys reported a low rate of acquired drug resistance.[25] A study in Madras (1960-1963) showed that intermittent chemotherapy with INH and SM twice weekly (S_2H_2) was as effective as INH+PAS or INH + Thiacetazone daily. The implementation of S_2H_2 regimens, either from the outset or after an initial phase of treatment with STH or SPH produced excellent results (a failure /relapse rate of 10 to 15% after 3 years).[26]
However unlike PH regimens, S_2H_2 was accompanied by a two-fold increase in the rate of acquired 'MDR' (before the RMP era the term was applied to simultaneous resistance to INH and SM) Primary anti-tuberculosis drug resistance emerged as an important problem at the end of 1960's. The special treatment of patients with MDR-TB is much more toxic and expensive than the treatment of patients with drug susceptible strains. However, this simple argument is influenced by many additional factors. Under programme conditions, SCC regimens using four drugs result in cure in a majority of patients. Some new patients with low-grade resistance may still respond

clinically.[27] Up to a third of TB patients recover as part of the natural history of the disease.[28] Such an outcome has been documented in MDR- TB[29]

1.4 Association between Clinical/ Demographic Characteristics and the Prevalence of Drug resistance.

The prevalence of drug resistance in younger age groups provides more reliable information on *current transmission* of drug resistant TB than in older people. Age –specific information may further refine our understanding of the dynamics of tuberculosis transmission. On average, patients developing tuberculosis in their 30's will have acquired the infection that led to disease earlier than patients in their twenties. Thus, the age-stratified prevalence of drug resistance may be an indicator of trends of drug resistance over time. Levels of primary drug resistance in France 1962-1970 were less than 8% among patients 50 years or older, but more than 12% among 15 to 19 year olds.[30] However, there are practical limits to gathering sputum for culture in children.

1.5 Migration and other social and political factors in the genesis and interpretation of anti- tuberculosis drug resistance.

Tuberculosis is a classic example of medical conditions intertwined with poverty and other social economic circumstances. The prevalence of drug resistance has been shown to be influenced by geographical locations. Systematic surveillance in the United States has demonstrated dramatic differences from one region to another.[31] Urban and rural settings have differences in the levels of drug resistance as well. MDR-TB is more common in certain social groups.[32,33] Thus, there are gradients in the prevalence of drug resistance, (Country boarders are not necessarily involved) and human movement along such gradients spreads MDR-TB.

1.6 Background

The World Health Organization (WHO) resumed leadership in control of tuberculosis worldwide in the early 1990's.[34] In 1993 the WHO declared TB to be a global emergency.[35] The WHO "Antituberculous Drug Resistance Surveillance Global Project" released the results of the first phase of the anti-tuberculous drug resistance surveillance (1994-1997) in 1997.[32] These confirmed that drug resistance TB was ubiquitous. The Global Project also confirmed the existence of MDR-TB defined as resistance to at least Isoniazid and Rifampicin

1.6.1 The Antituberculosis Drug Resistance Situation in Kenya

TB drug resistance was not considered a major threat in the early part of the 20th century since the incidence of TB had either declined or at least remained stable.

Documented reports from national and subpopulation surveillance of TB drug resistance in Kenya indicate emergence of new resistant patterns including MDR-TB (resistance to at least isoniazid and rifampicin).[36] Previous resistance surveys performed in 1964,1974 and 1984 indicated that initial resistance ranged from 7.1% - 8.9% for H, 0.5% - 1.4% for S and from 1.3% -1.4% for both H and S. Acquired resistance ranged between 16% and 17% for H, and between 2% and 16% for both H and S. There was no resistance to R.[37] In addition, data from a cohort study of patients with TB carried out at the infectious Disease Hospital (IDH) in Nairobi showed an initial resistance to H and S of 9% among patients with HIV-1 infection compared with 5% among non-HIV- infected patients.[38] Two consecutive national surveillance for drug resistant TB have been conducted in Kenya from 1994-1997 and from 1999-2000. The 1995 national surveillance was a cross sectional study that aimed to determine the prevalence of drug resistance in newly diagnosed patients with pulmonary tuberculosis, to determine possible risk factors associated with resistance and to establish standard routine surveillance. The study findings showed that 9.2% had a strain resistant to one or more of the first line drugs for TB treatment. There was a strong association between the previous chemotherapy and resistance. No resistance to either rifampicin or ethambutol was detected.[39]

The 2002 national surveillance was also a cross sectional study that sought to determine the prevalence of drug resistance both in newly diagnosed and previously treated smear positive patients with pulmonary tuberculosis and possible risk factors associated with resistance. A high proportion (6.3%) of Isoniazid mono-resistance in the previously treated group, a new emergence of multidrug resistant strain of MDR -TB (0.83%) and an entry of ethambutol resistance (1.1%) was documented..[40]

1.6.2 Subpopulation surveillance

A surveillance of drug resistant strains and molecular evaluation of transmission of resistant strains (a cross sectional study) in refugee and non refugee populations in north eastern Kenya indicated higher levels of total drug resistance (18.3%) and MDR -TB (2.95%) in the refugee camps than in the non refugee population (OR = 3.7; 95% CI 1.42-9.68; P,0.007).The study also showed evidence of transmission of strains resistant to streptomycin in the refugee population (RFLP and

spoligotyping were used to determine similarities in DNA fingerprinting patterns of the drug resistant isolates).[41]

In another study where the investigators took clinical isolates cultured from specimens of suspected TB patients earlier referred to the Center for Respiratory Diseases Research (CRDR), Kenya Medical Research Institute (KEMRI), which houses the Tuberculosis Reference and Research Laboratory for the public and private health care centers in Nairobi Kenya, the presence of MDR –TB Beijing/W (8.3%) and a possibility of other drug resistant strains was demonstrated.[42] These specimens had been taken there for microscopy, culture and drug susceptibility testing. The investigators did a mutation analysis using a PCR based dot blot hybridization method , DNA sequence analysis and then spoligotyping analysis .

In response to these surveillance findings a task force has been set up to spearhead the implementation of 2nd line anti –tuberculosis drugs for routine use in government hospitals among patients who are infected with the resistant strains . Currently the management of drug resistance tuberculosis is individualized. Definitive randomized or controlled studies have not been performed to determine the best treatment for various patterns of drug resistance.

Regimens have been suggested for use in mono- and poly- resistance.[43]

Risk population surveillance methods recommended by WHO for drug resistance surveillance

The WHO approved the sentinel method of surveillance to supplement the population based periodic surveillance. With this model systems can be designed to collect information continuously (avoiding 3-5 yr intervals). They can be useful for documenting trends. Can offer design flexibility, are useful in detecting outbreaks or localized drug resistant epidemics.

However often they are not population based and may be subject to multiple biases.[44]

Findings of hospital monitoring surveys in other countries

Recent reports from South Africa suggest that the hospital setting may pose a special risk environment for the transmission of XDR TB, which has a high case fatality in HIV infected persons. The prevention of drug resistant TB is of paramount importance for a hospital in a low-income country that cannot afford the high cost of treatment of MDR and XDR-TB. Therefore, nosocomial transmission to both patients and hospital staff need to be monitored. The MTB bacilli has been shown to be readily transmitted to patients with HIV infection and this could lead to hospital outbreaks of drug resistant TB infection. The resistant strain has been associated with a poor prognosis in both HIV negative and positive patients. Patients with the drug resistant TB infection are more likely to default treatment due to long treatment duration and the serious drug toxicity associated with second line antituberculosis therapy.[45]

1.6.3 Antituberculous Drug Resistance Situation at KNH

The study done at the KNH so far was by Dr. Mutie in 1995 on anti-tuberculosis drug resistance pattern . Mbagathi and the city council clinics were also included in this study. The aim was to determine the patterns of anti-tuberculosis drug resistance, compare the results with previous studies and to establish the trends in Nairobi. In this surveillance, the total resistance to the four first line anti-TB drugs was observed in 22.5% of the study population. Initial resistance occurred in 15.3% of the study population while acquired resistance was found in 48.7% of the population which had received prior therapy.[46] The exact magnitude of anti- TB drug resistance in the KNH hospital is currently not well described. The KNH is a government national referral hospital .This implies that the patients visiting the hospital come from all parts of the country including the neighboring East African countries as well as Somalia and Sudan. Besides, no restrictions are put in place to make it a purely referral center. The institution is open to all disease cases ranging from mild to severe illnesses. This may have contributed to the increased burden of diseases including tuberculosis. Initially Mbagathi Hospital was the district center for infectious diseases and an affiliate of KNH . Patients who were diagnosed with tuberculosis would be managed at the Mbagathi hospital. Currently KNH receives patients with tuberculosis who visit the hospital for treatment and does not have a ward that is specially designed for management of infectious diseases . Policy guidelines to initiate control of hospital transmission at the KNH have so far been laid out and indicate that MDR- TB treatment facility at the KNH will be designed in a manner that reduces the risk of transmission of MDR-TB. The key design measures include ensuring adequate ventilation and lighting. Air exhaust systems and the use of Ultra violet radiation may be included if funds allow. In addition, commodities designed to reduce acquisition of this infection by health care workers will be procured. These include N95 respiratory masks and gowns. Infection control measures aimed at reducing the risk of transmission of TB bacilli in health care settings will be vigorously promoted in all health care facilities across the country. [47]

2. STUDY JUSTIFICATION

Data on prevalence and the pattern of anti-tuberculous drug resistance is relevant at the Kenyatta National Hospital, which happens to be located in one of the regions at high risk for high transmission of tuberculosis. Drug resistant tuberculosis poses unique risks related to disease outbreaks in an overcrowded setup, nosocomial infections, and increased mortality in the context of HIV infection. This may frustrate the seemingly successful efforts that were aimed at reducing morbidity and mortality in the HIV/TB pandemic. The registration records at the Kenyatta

National Hospital indicate a high burden of TB morbidity. For instance on the month of September 2006, 93 patients were entered as new sputum smear positive case registrations and 8 as relapses (tested sputum positive after completing treatment). In addition to this the inpatient population consists of patients who are on treatment for pulmonary tuberculosis either as new or retreatment cases. Owing to financial constraints and lack of a readily available diagnostic means within the hospital setting (culture and sensitivity testing facility), a significant population of patients who may be carrying the resistant strain goes undiagnosed. It is therefore important to initiate baseline data on the current drug resistance situation. This will help to establish the trend of anti-tuberculous drug resistance at the hospital and contribute towards policymaking and implementation as well as provide a rationale for control of nosocomial transmission. This would be in keeping with the WHO efforts to help address the drug resistant challenges in the hospital setting as well as provide special alerts in case of a drug resistant outbreak. Also the recently documented presence of MDR-TB in Nairobi should be an indicator to urgently monitor the situation at KNH.

3. RESEARCH QUESTION

What are the rates of anti-tuberculous drug resistance at the Kenyatta National Hospital?

4. AIM OF THE STUDY

To determine and document the level and pattern of anti –TB drug resistance at KNH in order to improve on policy control and management of the disease.

5. OBJECTIVES

5.1 Broad Objective

To determine the level and pattern of resistance to first line anti-TB drugs: streptomycin, isoniazid, ethmbutol and rifampicin among sputum smear and culture positive cases.

5.2 Specific Objectives

i. To determine the prevalence of drug resistance including multidrug resistance for TB.
ii. To determine the social- demographic characteristics of the patients who have the resistant strain.
iii. To determine the co morbid occurrences including HIV status among those with the resistant strains.

6. METHODS

6.1 Case Definitions

New Case

For the purpose of the study resistance among new cases is defined as presence of resistant strains of Mycobacterium tuberculosis in a patient who, in response to direct questioning, denies having had any prior anti-TB treatment (for more than one month) or in a case where hospital records are available there is no evidence of such history.

Previously Treated Case

Resistance in a previously treated patient will be defined as the presence of resistant strains of *Mycobacterium tuberculosis* in a patient who, in response to direct questioning, admits having been treated for TB for 1 month or more or in a case where adequate file documentation is available there is evidence of such history . This will include patients in any of the following four categories:

Treatment Failure – patients who begin treatment for smear positive pulmonary TB and who remain smear positive or become smear positive again at 5 months or later during the course of treatment.

Relapse – patients who become smear positive again after having been treated for TB and declared cured after the completion of their treatment.

Return after default - patients who interrupt their treatment for more than 2 months after having received a total of at least 1 month of anti-TB treatment and who then return with bacteriologically confirmed tuberculosis.

Chronic - patients who continue to be smear positive after the completion of retreatment regimen.

Definition of types of drug resistance

Monoresistance

Resistance to any one single drug.

Polyresistance

Resistance to two or more drugs but excluding concurrent resistance to both Rifampicin and Isoniazid

Multidrug resistance

Resistance to at least rifampicin and Isoniazid

6.2 Sample Size Calculation

Sample size calculation is based on previous resistant rates of isoniazid, which was 2.6%

The following formula for calculation of sample size for prevalence studies has been used.

$$n \geq \frac{z^2 (1-\alpha/2)^2 p (1-p)}{d^2}$$

n = required minimum sample size

p = reported prevalence of resistance to therapy

d = the level of precision = +/- 5%

$z^2 (1 - \alpha /2)^2 = 1.96$ (at $\alpha = 0.05$) or 95% confidence level

P = 2.6

d = 2%

$$n \geq \frac{(1.96)^2 (0.026) (0.974)}{(0.02)^2}$$

n = 243

6.3 Study Site

Nairobi Kenya: Kenyatta National, Referral and Teaching Hospital; TB out patient clinic; all the general wards.

6.4 Study Population

The target population for this survey was all sputum smear and culture positive patients who were registered at the KNH -TB clinic and those admitted to the general wards.

6.5 Study Design

Cross sectional study.

6.6 Clinical Methods

All patients with pulmonary tuberculosis diagnosed by sputum smear microscopy or positive sputum culture were included in the study by consecutive sampling.

Inclusion Criteria

Patients with a first sputum smear positive test for Acid Alcohol fast Bacilli on microscopy or those who the primary clinician found necessary to do a sputum culture which became positive for mycobacterium tuberculosis were eligible if they fulfilled the following: Those who were willing to participate in the study and gave an informed signed consent. Those who provided at least two sputum samples. Those whose sputum smear was collected within the hospital or because of instructions given within the hospital. Those that the diagnosis of TB was done by microscopy for the sputum smears within the KNH laboratories or by culture at the KEMRI-CRDR TB laboratory.

Exclusion Criteria

Those who declined to give consent for recruitment to the study and those unable to give more than one sputum sample were excluded.

Clinical Information

A pre-tested questionnaire was used to get the clinical information from eligible patients. A pre test counseling was done before doing a HIV test in patients with unknown status. A rapid test was done on those who signed an informed consent. However if they declined to do the test they were still included in the study and their status was entered as 'unknown'.

Consent for retaining the patients' sputum samples for future reference (for purposes of internal and external quality control) was also obtained.

The patient's clinical information was obtained from history taking through direct questioning and from information in the patient's medical records.

This was done by a team of 2 study assistants who had been briefed on how to administer the questionnaires and also by the investigator.

The patient's **clinical information** form was used to document the patient's details (see appendix: 7)

Patient's Identification (Appendix: 7 A)

Patient's details entered in the form included the name, TB clinic registration number, date registered, the gender, age, date of sputum collection, HIV status, history of drug abuse, history of previous admission to the KNH, ward number and duration of admission .

History Given By The Patient (Appendix: 7 B2)

The patient was interviewed to establish any history of previous treatment for TB. If none, a standardized history was taken to determine the duration of illness, whether the patient had suffered similar symptoms before, or other symptoms of lung disease (hemoptysis, chest pain, cough), whether they had x-ray examinations before the present illness, or any prior sputum examinations, whether they ever took anti-tuberculous drugs for more than one month and the name of the drugs where applicable and whether they ever had injections for more than a month. A history of chronic illness other than TB was sought.

If the patient was able to remember previous treatment for TB further information was sought about the previous treatment to determine where he was treated, the time of the treatment, how many times the patient was treated, which drugs were used and by whom

was the patient treated? The outcome of the last treatment according to the patient were reported as :" cured " "Not cured" "unknown"

Medical Records (Appendix: 7 C)

Extensive checking through the medical files and other documents e.g. outpatient cards was done to determine whether the patient had been registered for TB treatment before. A response of "No" or "Yes" was entered as appropriate. If the answer was yes the outcome of the last

Course of chemotherapy was entered as "Cured" "Defaulted" "Transferred out" 'Treatment completed" "Failed"

Final Decision (Appendix: 7 D1)

This was made as to whether the patient had been previously treated for TB for more than a month. A response of 'Yes' "No" "Doubtful" was entered as appropriate.

If yes was entered the outcome of the previous treatment was determined as follows:-

Cured/treatment completed, Failed, Defaulted/stopped treatment due to drug complications, Chronic, Relapse/defaulter not distinguishable, Unknown.

6.7 Laboratory Methods

6.7.1 Sputum Collection and Transport Of Sputum Smears

In addition to the initial sputum sample used for diagnosis, a second sputum sample was collected so that the central laboratory had a total of at least two samples (a spot and an overnight sample). Treatment for any period of time would reduce the chance of culture positivity, therefore samples were obtained before treatment was started.

In order to ensure correct collection and transportation of samples to the laboratory. The following guidelines were observed: The patients were given clear instructions on how to collect a good sputum. Aerosols containing *M.tuberculosis* may be formed when the patient coughs, therefore patients were advised to cough either outside in the open air or away from other people and not in confined spaces such as a room in the laboratory or toilets. This information was communicated to the staff who attended to patients suspected to have **PTB**.

Microscopy for identification of positive sputum samples was done at the **KNH** microbiology laboratory. The laboratory staff were requested to retain all smear positive sputum samples for purposes of the study. Before transport sputum samples were kept in a cool place (a refrigerator at +4°C).Suitable rigid containers with a watertight , wide mouthed screw were used for transporting sputum to KEMRI-CRDR. This helped to avoid crushing in transit and to prevent leakage and contamination while transporting sputum from the KNH to KEMRI. The above containers were packed in material that would absorb any leakage caused by accidents. Inpatient number in the KNH file and a simple identification for the two successive samples from the same patient, such as A and B were written on the container (not on the lid). The two samples were sent together with the sputum data form to the culture laboratory. A copy of the form were kept in the patient's file at the KNH.

The sputum shipment form had the following information **(Appendices 5 and 6)** Patient's sputum smear identification: name, date of admission /registration Patient's gender and age

Date of sputum collection indicating different dates for the two smears and results of the smear.

6.7.2 Culturing

Before culturing bacteriological examination were done, the samples were decontaminated and further homogenized, according to petroff's method with sodium hydroxide 4% for 15 to 30 minutes, centrifuged at 3000x for 20 minutes and the sediment neutralized and washed. Acid -fast microscopy was performed on the concentrated samples.

The sediment was then inoculated on two tubes of Lowenstein-Jensen (L-J) egg medium with one of the tubes enriched with sodium pyruvate to optimize growth of *Mycobacterium bovis*. The cultures were incubated at 37 degrees Celsius until growth of colonies was observed or until eight

weeks. Inspection was done after 48 hours and then weekly. Each isolate strain was examined for morphology and pigmentation and the date of appearance of the colonies was recorded. If there was no growth after 56 days (eight weeks) or in case of contamination, the cultures were discarded and the laboratory forms completed accordingly. All positive cultures were kept until after re-testing at the Reference Laboratory had been completed or the strain had been excluded from further testing. The cultures were stored in a deep freezer at –20 degrees Celsius.

6.7.3 Strain identification

Identification of the strain was based on at least the niacin production test, the nitrate reduction test, Paranitrobenzoic acid (PNB) and the thiopene carboxylic acid hydrazide (2mg/l) (TCH) resistance test (WHO/IUATLD, 1997b). If colonial morphology was consistent with M. tuberculosis complex, only one culture per patient was identified. Mycobacterial strains other than *M. tuberculosis* were not further considered for the purpose of the survey.

Susceptibility Testing

Indirect susceptibility testing was performed on only one isolate per patient. Drug resistance test was performed using the resistance ratio (RR) method using Lowenstein-Jensen medium susceptibility testing of MTB isolates to isoniazid (I), ethambutol (E) and rifampicin (R) and dihydrostreptomycin sulphate. The results of the tests were entered on to appropriate laboratory forms and copies forwarded to the survey PI for storage and analysis.

6.7.4 The Bacteriological Examination Form: (Appendix 8)

This contained the patient's particulars, identification of the mycobacterial species in sputum samples A and B and the results of susceptibility of M. tuberculosis was entered as susceptible to or resistant to.

6.7.5 Quality Assurance (QA)

To ensure that results of susceptibility testing were reliable and comparable, internal and external quality control of susceptibility testing were performed during the survey. The results of the tests were entered on to appropriate laboratory forms and copies forwarded to the survey PI for storage and analysis.

6.7.6 Internal quality control

Susceptibility testing was performed on a standard strain H37Rv in each new batch of LJ medium and for each drug. Standardized procedures were followed according to the method used for testing. The quality of the medium was controlled batch by batch and drugs added to the medium were pure drugs obtained from a reputable firm with the percent of potency clearly indicated.

Dilution of drugs and the addition to the medium was performed following standardized acceptable standards.

6.7.7 International Quality Control

This was done by KEMRI in accordance with international standards. Same approach was applied between the NRL and Regional Laboratories, which performed susceptibility testing.

7. DATA MANAGEMENT

Data from the surveillance was continuously entered into computer(s) as it became available using the MS-Access and Excel software. Double entry method (2 data clerks entered the same data for comparison purpose) was used to ensure validity of data. Backups were continuously maintained (into flash disks).

7.1 Data Analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS) software. Prevalence of drug resistance was calculated based on the number of cases with drug susceptibility results available. The number of missing results due to contamination, negative cultures and insufficient growth for susceptibility testing was also analyzed. The following parameters were included in the results: **Analysis of drug resistance patterns**. This included tables describing the proportion of patients with mono resistance to each drug, and to different combinations of drugs including multi-drug resistance among patients with primary resistance and those with acquired resistance. Data was presented based on mutually exclusive categories of resistance, namely, mono and combined resistance; and where necessary stratified or compared by age, gender and type of re-treatment. Statistical difference in the proportion of cases with drug resistance was determined by chi square test (X^2) test .Qualitative variables were analyzed using the chi square test and Fisher's exact test where applicable .A continuous variable was evaluated using student's t-test statistics.

7.2 Analysis of Clinical Characteristics

A student's t- test was used to compare the clinical characteristics of those with resistance and those without.

8. FEASIBILITY OF THE STUDY

The KEMRI laboratory in which the culture and susceptibility testing was done has both internal and external quality control and had the capacity to handle the size of the study sample throughout the study duration .

9. ETHICAL CONSIDERATIONS

The study was conducted after approval by the department of Internal Medicine , University of Nairobi and the Kenyatta National Hospital Scientific and Ethical Review committee.

All patients had received the conventional care for their illnesses and for those who were found to have resistant strains after culture and susceptibility testing their results were communicated to their primary healthcare centers.

Patients who declined to give consent were not denied the usual care.

Patients who gave consent to be recruited were requested to give consent allowing their sputum samples to be stored for future reference.

W.H.O guidelines for prevention of spillage and risk of infection to other patients, members of staff and the survey working team were adhered to.

10. RESULTS

The study period was "between" 1st June to 30th September 2007.

10.1 Study Population

During the period the study was carried out a total of 1035 patients had a diagnosis of PTB (either by clinical and CXR findings or by positive sputum smear). Of these 732 were from the outpatient, while 303 were in the inpatient department. Of the 732 outpatients, 148 were diagnosed with PTB on the basis of sputum smear while 584 had a negative smear. 30 of those who had smear positive results were excluded because they could not provide more than one sputum sample, declined to give consent for recruitment into the study or provided 2 samples but in very

small volumes not adequate for culture. 10 were contaminated and 8 failed to grow even after incubating for the maximum period of 12 weeks. A total of 100 isolates were from the outpatients.

A total of 303 were inpatients 140 had sputum smear positive and 50 had culture positive. 15 were excluded because they could not provide more than 1 sample due to severe illness or unwillingness to be recruited. 5 were excluded because their diagnosis was done outside KNH and treatment commenced for at least more than a month by the time of presenting to the referral hospital.

Another 8 were excluded because the culture was either contaminated or failed to grow. A total of 257 isolates were identified .However due to financial limitations D.S.T was done on a total of 81 isolates belonging to 81 patients whose results are presented . The remaining 177 isolates were preserved at the KEMRI TB laboratory. Selection of the 81 isolates was by consecutive sampling.

10.2 Fig 1: Total Drug Resistance

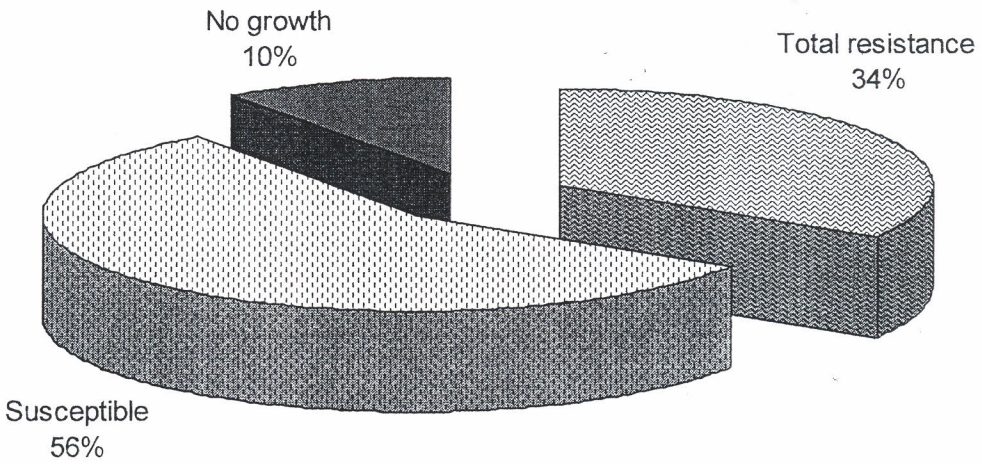

Fig. 1

Shows total resistance

The total drug resistance was 34 % (n = 30). 56 % had susceptible strains and 10 % failed to grow after subculturing for D.S.T

10.3 Fig 2: Combined Drug Resistance Patterns

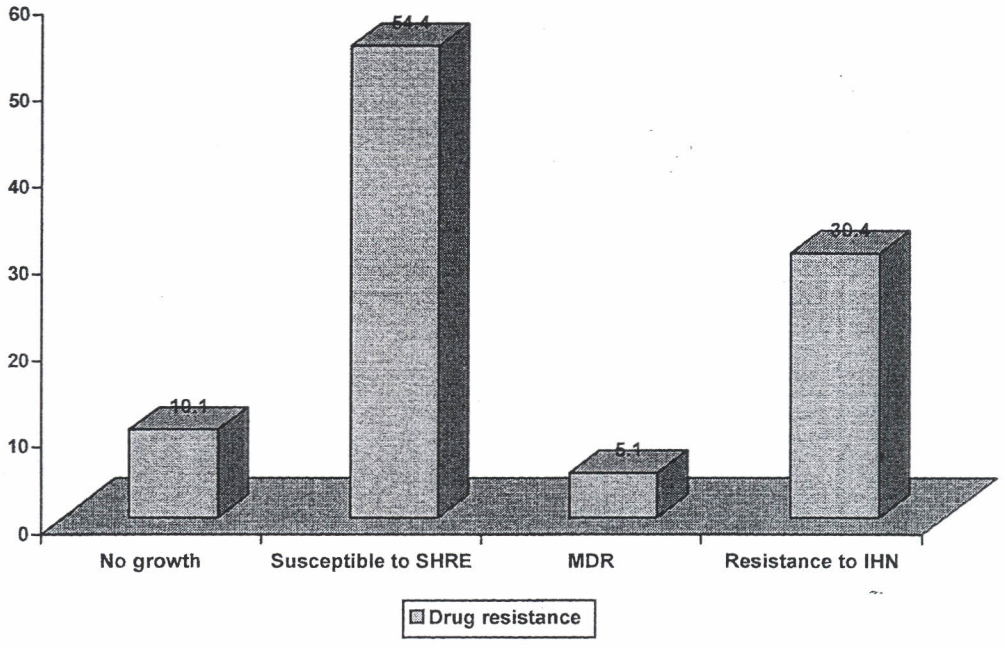

Fig. 2

Shows the resistance by the strict definition of MDR- TB i.e. resistance to at least isoniazid + rifampicin. (2.5%) Which includes resistance to the four first line drugs which are isoniazid + rifampicin + spreptomycin + Ethambutol (2.5%) (n =2) Resistance to only one drug : isoniazid was 30% (n =25) In this case the total MDR-TB was 5% (n = 4).

10.4 Fig 3: Drug Resistance Patterns By Individual Drugs

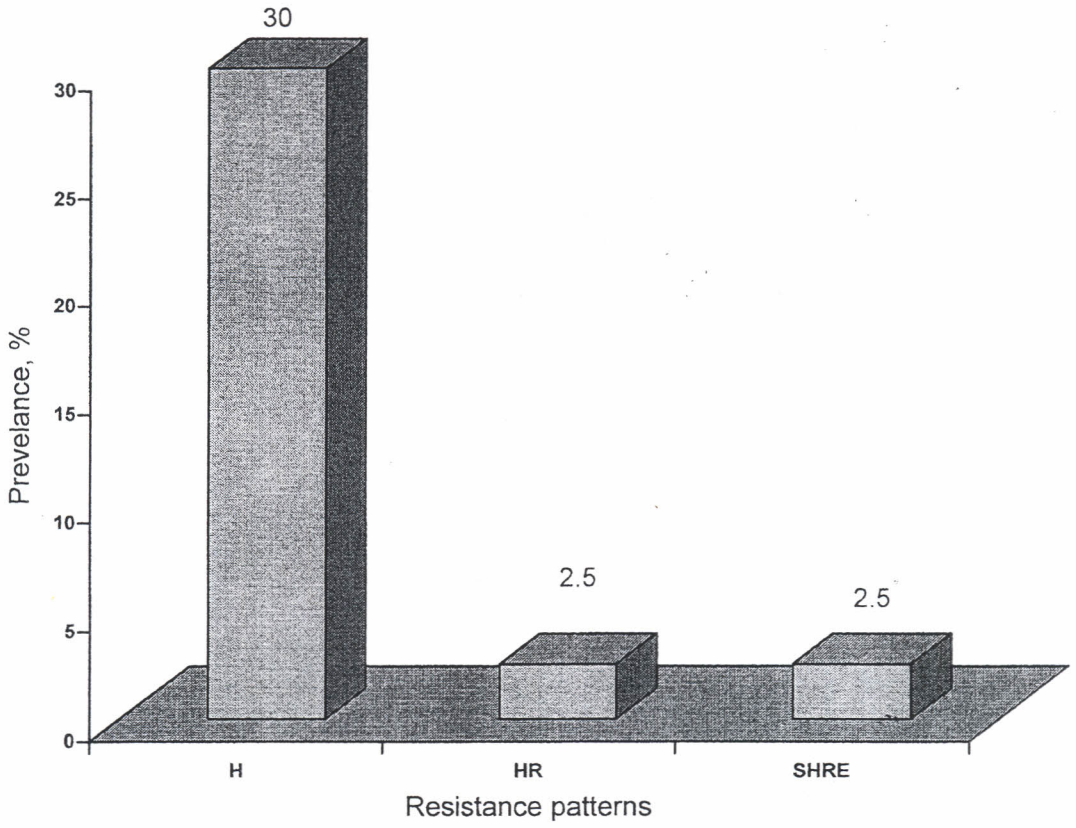

Fig. 3

The graph shows the different resistant patterns presented based on the relative resistance modes, i.e. mono, double, triple and quadruple resistance. The initial and acquired resistance have been combined . In these data monoresistance (resistance to only one drug) was found in Isoniazid - isoniazid monoresistance : 30%. The other three first line drugs did not show a monoresistance pattern. Double resistance (resistant to a combination of 2 drugs) was found in INH + RMP and the rate was 2.5%. It constitutes the definition of MDR TB. Triple resistance (resistance to a combination of 3 drugs) was not observed. Quadruple resistance (resistance to the four drugs) was found : INH + RMP + SM + ETH at a rate of 2.5%. This pattern also defines MDR- TB since it includes at least INH + RMP. There fore the total rate of MDR-TB was 5%.

10.5 Fig 4: Resistance Patterns By Treatment History

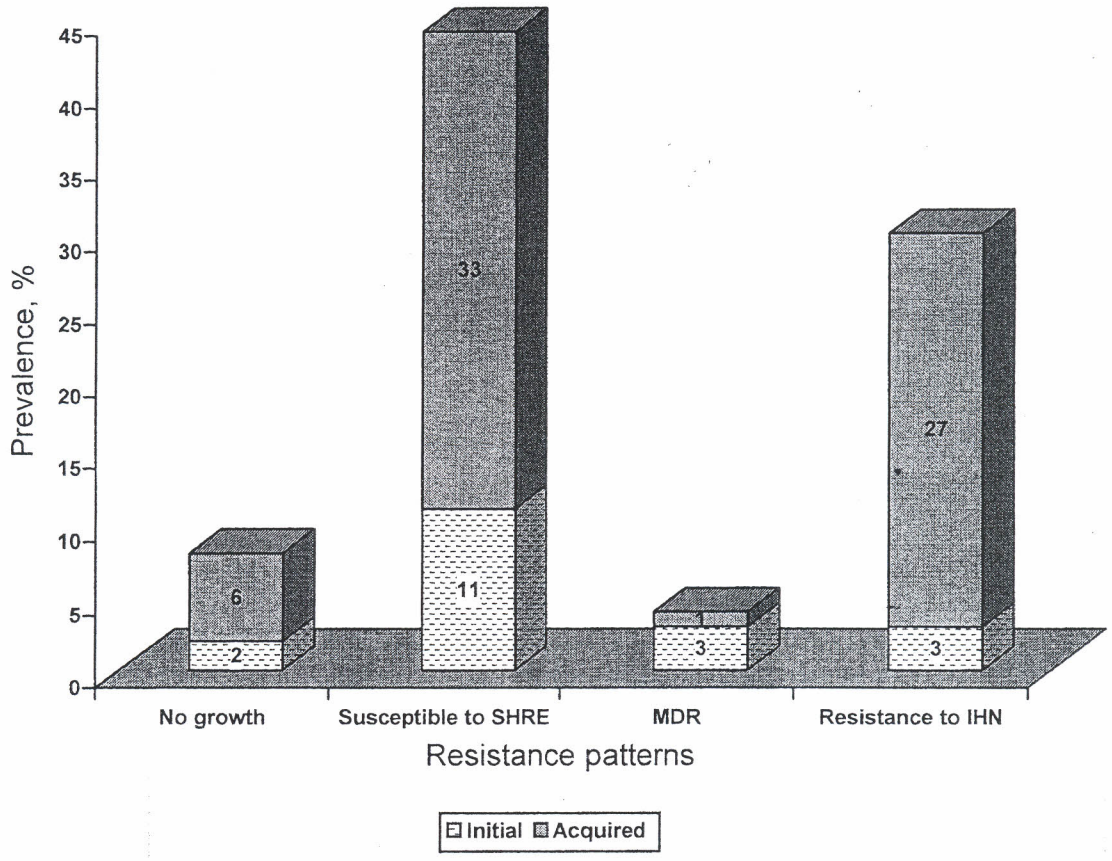

Fig. 4

Shows drug resistance with regard to initial (in patients who were not previously treated) and acquired (in those that were previously treated).

Of those who had INH mono-resistance 3% had initial resistance while 27% had acquired resistance.3 of those with MDR-TB pattern had initial resistance while 1 had acquired resistance.

10.6 DEMOGRAPHIC CHARACTERISTICS

Age group

Age of the study patients ranged from 16 yrs to 87 yrs with a mean age of 34yrs.

Table 1 shows the age categories.

Persons aged between 21 to 40 years constituted 51 out of the 81 patients of the population studied. 19 out of 81 patients who had isoniazid mono-resistance are within the 21-30 and 31-40 age groups (9 and 10 respectively) 3 out of the 4 patients who had MDR-TB fall within 21 to 50 years age range. The age of the fourth patient was not specified. However statistically there was no difference in drug resistance between the various age groups.

Gender

There were 45 male and 36 female patients in the study population.

Drug resistance pattern was equally distributed between males and females who were studied. The males accounted for 44 %(n=11) of the INH mono-resistance while the females accounted for 56 %(n=14).2 of those who had MDR-TB were males and 2 were females.

Marital Status

14 of the individuals studied were single, 27 were married 39 did not specify their marital status, 1 was divorced. 44 %(n=11) of those who had resistance to INH were married while 20% (n=5) were single. The marital status was unspecified in 36 %(n=9) of those who had INH mono resistance.3 out of the 4 individuals who had MDR TB were married while the marital status was unspecified for the remaining 1.

Statistically there is no difference in resistance with relation to marital status.

DEMOGRAPHIC CHARACTERISTICS

Table 1: Distribution of Age Groups By Drug Resistance

Age groups	Susceptible to SHRE (n=44)	MDR (n=4)	Resistance to INH (n=25)	No growth (n=8)	Total (n=81)
<20	5 (11.4%)	0	1 (4.0%)	0	6
21-30	17 (38.6%)	1 (25.0%)	9 (36.0%)	3 (37.5%)	30
31-40	9 (20.5%)	1 (25.0%)	10 (40.0%)	1 (12.5%)	21
41-50	5 (11.4%)	1 (25.0%)	4 (16.0%)	2 (25.0%)	12
51-60	2 (4.5%)	0	0	0	2
Not specified	6 (13.6%)	1 (25.0%)	1 (4.0%)	2 (25.0%)	10

Chi-square= 0.71; d.f. = 12; p= 0.708; n= 81

Persons aged between 21 to 40 years constituted 51 out of the 81 patients of the population studied. 19 out of 81 patients who had isoniazid mono-resistance are within the 21-30 and 31-40 age groups (9 and 10 respectively) 3 out of the 4 patients who had MDR-TB fall within 21 to 50 years age range. The age of the fourth patient was not specified.

Table 2: Drug Resistance By Gender

	Susceptible to SHRE (n=44)	MDR (n=4)	Resistance to INH (n=25)	No growth (n=8)	Total (n=81)
MALE	28 (63.6%)	2 (50.0%)	11 (44.0%)	4 (50.0%)	45
FEMALE	16 (36.4%)	2 (50.0%)	14 (56.0%)	4 (50.0%)	36

Chi-square= 1.60; d.f. = 3; p= 0.658; n= 81

There were 45 male patients and 36 female patients in the study population.

Drug resistance pattern was equally distributed between males and females who were studied. The males accounted for 44 %(n=11) of the INH mono resistance while the females accounted for 56 %(n=14).

2 of those who had MDR -TB were males and 2 were females.

Table 3: Drug Resistance By Marital Status

Marital status	Susceptible to SHRE (n=44)	MDR (n=4)	Resistance to INH (n=25)	No growth (n=8)	Total (n=81)
Single	9 (20.5%)	0	5 (20.0%)	0	14
Married	12 (27.3%)	3 (75.0%)	11 (44.0%)	1 (12.5%)	27
Others	1 (2.3%)	0	0	0	1
Unspecified	22 (50.0%)	1 (25.0%)	9 (36.0%)	7(87.5%)	39

Chi-square= 11.6; d.f. = 9; p= 0.239; n= 81

44 %(n=11) of those who had resistance to INH were married while 20% (n=5) were single. The marital status was unspecified in 36 %(n=9) of those who had INH mono-resistance.

3 out of the 4 individuals who had MDR -TB were married while the marital status was unspecified for the remaining 1.Statistically there is no difference in resistance with relation to marital status.

10.7 SOCIO-ECONOMIC STATUS

Geographical Location

25 individuals in the study came from central province, 15 from Eastern 9 from Nyanza 6 from Western 3 from North Eastern 3 from North Rift 2 from south Rift 1 from Coast and 1 from Nairobi Provinces. 16 individuals did not specify their provinces.

36%(n=9) of those with isoniazid mono-resistance came from central province, 20% (n=5) from Eastern province ,16%(n=4) from Nyanza province and 4% (n=1) each for North Rift, South Rift, and Western Provinces. 2 of the patients who had MDR -TB came from Eastern province 1 came from central and 1 from North Eastern Province.

Current Residence

This was classified into slum area , Middle income residential area , others included rural areas and visitors to the city who are not usual residents. 21 of the studied population were residents in the slums 21 in middle income area 13 were not usual Nairobi residents and 26 did not specify their residential areas. A high proportion of the study population who had INH mono-resistance and MDR-TB came from the slums (INH 28%; n=7), (MDR -TB n=2) and the middle income (INH 32%; n=8), (MDR- TB n=1) residential areas of the Nairobi City.

Education

The level of education was categorized into none, primary , secondary ,college ,university and unspecified. In this study population those who admitted to having no education at all were 9, 8 individuals had unto primary level , 14 up to secondary 4 up to college . Only 1 had university education while 45 patients did not specify their level of education .

Out of the 25 patients who had INH, mono resistance 2 had up to Primary level education 2 Secondary, 1 University while 19 of them did not specify their education level.

Out of the 4 who had MDR- TB 1 had up to Primary level education 1 Secondary level education while 2 of them did not specify their level of education.

Drug resistance pattern by occupation

17 out of the 25 patients (68.0%) who had INH mono-resistance had unspecified occupation. 4 were from the informal sector ,2 were unemployed, 1 was in small scale self employed business and 1was put in the class of others which includes housewife, farmer and housekeeper. This could be explained by the job instability that characterizes this study population. The economical circumstances render these people jobless on and off at unpredictable times. This may have contributed to the poor job specification during the interview.

SOCIO-ECONOMIC STATUS

Table 4: Drug Resistance By Geographical Location (Province)

	Susceptible to SHRE (n=44)	MDR (n=4)	Resistance to INH (n=25)	No growth (n=8)	Total (n=81)
Central	15 (34.1%)	1 (25.0%)	9 (36.0%)	0	25
Nairobi	1 (2.3%)	0	0	0	1
Eastern	6 (13.6%)	2 (50.0%)	5 (20.0%)	2 (25.0%)	15
North Eastern	1 (2.3%)	1 (25.0%)	0	1 (12.5%)	3
Coast	1 (2.3%)	0	0	0	1
North Rift	2 (4.5%)	0	1 (4.0%)	0	3
South Rift	1 (2.3%)	0	1 (4.0%)	0	2
Western	5 (11.4%)	0	1 (4.0%)	0	6
Nyanza	2 (4.5%)	0	4 (16.0%)	3 (37.5%)	9
Unspecified	10 (22.7%)	0	4 (16.0%)	2 (25.0%)	16

Chi-square= 27.5; d.f. = 27; p= 0.438; n=81

36%(n=9) of those with isoniazid mono-resistance came from central province, 20% (n=5) from Eastern province ,16%(n=4) from Nyanza province and 4% (n=1) each from North Rift, South Rift, and Western Provinces. 2 of the patients who had MDR- TB came from Eastern province 1 came from central and 1 from North Eastern Province.

Table 5: Drug Resistance By Current Residence

	Susceptible to SHRE (n=44)	MDR (n=4)	Resistance to INH (n=25)	No growth (n=8)	Total (n=81)
Slum	11 (25.0%)	2 (50.0%)	7 (28.0%)	1 (12.5%)	21
Middle income	10 (22.7%)	1 (25.0%)	8 (32.0%)	2 (25.0%)	21
Others	7 (15.9%)	0	3 (12.0%)	3 (37.5%)	13
Unspecified	16 (36.4%)	1 (25.0%)	7 (28.0%)	2 (25.0%)	26

Chi-square= 5.8; d.f. = 9; p= 0.76; n=81

A relatively high proportion of the study population who had INH mono-resistance and MDR-TB came from the slums (INH 28%; n=7), (MDR -TB n=2) and the middle income (INH 32%; n=8), (MDR- TB n=1) residential areas of the Nairobi City.

Table 6: Drug Resistance By Level Of Education

	Susceptible to SHRE (n=44)	MDR (n=4)	Resistance to INH (n=25)	No growth (n=8)	Total (n=81)
None	5 (11.4%)	0	1 (4.0%)	3 (37.5%)	9
Primary	5 (11.4%)	1 (25.0%)	2 (8.0%)	0	8
Secondary	9 (20.5%)	1 (25.0%)	2 (8.0%)	2 (25.0%)	14
College	3 (6.8%)	0	0	1 (12.5%)	4
University	0	0	1 (4.0%)	0	1
Unspecified	22 (50.0%)	2 (50.0%)	19 (76.0%)	2 (25.0%)	45

Chi-square= 18.9; d.f. = 15; p=0.221; n=81

Out of the 25 patients who had INH, mono-resistance 2 had up to Primary level education 2 Secondary, 1 University while 19 of them did not specify their education level.

Out of the 4 who had MDR- TB 1 had up to primary level education 1 secondary level education while 2 of them did not specify their level of education.

Table 7: Drug Resistance By Occupation

	Susceptible to SHRE (n=44)	MDR (n=4)	Resistance to INH (n=25)	No growth (n=8)	Total (n=81)
Informal	10 (22.7%)	1 (25.0%	4 (16.0%)	1 (12.5%)	16
Formal	3 (6.8%)	0	0	0	3
Unemployed	4 (9.1%)	0	2 (8.0%)	3 (37.5%)	9
Business	3 (6.8%)	0	1 (4.0%)	1 (12.5%)	5
Others	4 (9.1%)	0	1 (4.0%)	0	5
Unspecified	20 (45.5%)	3 (75.0%)	17 (68.0%)	3 (37.5%)	43

Chi-square= 13.8; d.f. = 15; p=0.542; n=81

17 out of the 25 patients (68.0%) who had INH monoresistance had unspecified occupation. 4 were from the informal sector, 2 were unemployed, 1 was in small scale self employed business and 1was put in the class of others which includes housewife, farmer and housekeeper. This could be explained by the job instability that characterizes this study population. The economical circumstances render these people jobless on and off at unpredictable times. This may have contributed to the poor job specification during the interview.

10.8 HIV Status

Out of the 25 who had INH mono-resistance 12(48%) were HIV negative while 8 (32%) were positive. The remaining 5(20%) were not tested for HIV.

Out of the 4 who had MDR -TB 2 were HIV negative while 1 was positive the other 1 was not tested. No significant difference was found with regard to drug resistance between those who tested HIV positive and those who were negative.

Table 8: Drug Resistance By HIV Status

HIV STATUS	Susceptible to SHRE (n=44)	MDR (n=4)	Resistance to INH (n=25)	No growth (n=8)	Total (n=81)
Positive	11 (25.0%)	1 (25.0%)	8 (32.0%)	3 (37.5%)	23
Negative	19 (43.2%)	2 (50.0%)	12 (48.0%)	1(12.5%)	34
Unknown	14 (31.8%)	1 (25.0%)	5 (20.0%)	4 (50.0%)	24

Chi-square= 4.4; d.f. = 6; p=0.610; n=81

Out of the 25 who had INH mono resistance 12(48%) were HIV negative while 8 (32%) were positive. The remaining 5(20%) were not tested for HIV.

Out of the 4 who had MDR-TB 2 were HIV negative while 1 was positive the other 1 was not tested.

11. DISCUSSION

Though our study had a smaller sample size (n=81) that would make it a possible source of sampling error it nevertheless shows an increasing trend in the prevalence of both total drug resistance (34%) and isoniazid mono-resistance (30%) n=81 compared to previous studies. As alluded to in the literature review our study is a sentinel surveillance model, which can be useful for documenting trends and can offer design flexibility.[44] Sample size calculation for our study was based on rates of isoniazid mono-resistance found in previous population based studies. It was anticipated that this would help to prevent multiple biases, which is a common disadvantage of sentinel methodologies since their sample size calculation is often not population based. The drastic reduction of the sample size to n=81 from 243 does not make it too small for interpretation given that our study population was derived from a high risk population (large referral hospital) and therefore has the utility of detecting possible drug resistance outbreak or a localized resistance epidemic. Similar studies with small sample sizes have been done elsewhere and provided useful information on drug resistance epidemics within hospital settings and this is in keeping with the WHO recommendation.[43] However, our study sample size suffers the limitation of a sentinel study bias, which may affect assessment of the drug resistance trend. Data interpretation with the reduced sample size puts into consideration that it is not a population based study and instead looks at a high risk group giving it the advantage of flexibility (the 3-5 year interval required for the population based studies can be avoided and data may be collected continuously to document trends if so desired).With this regard our study meets the objective of forming a basis for information collection at flexible intervals in order to document the drug resistance trends at the hospital. It is important to note that a clear trend of increasing prevalence of both total and isoniazid mono-resistance is observed from our study results compared to previous studies. In 1964,1974 and 1984 it was indicated that initial resistance ranged from 7.1% - 8.9% for H, 0.5% - 1.4% for S and from 1.3% -1.4% for both H and S. Acquired resistance ranged between 16% and 17% for H, and between 2% and 16% for both H and S. There was no resistance to rifampicin.[37] In addition, data from a cohort study of patients with TB carried out at the Infectious Disease Hospital (IDH) in Nairobi showed an initial resistance to H and S of 9% among patients with HIV-1 infection compared with 5% among non-HIV- infected patients.[38] Total drug resistance of 22.5% was documented in 1995 and isoniazid mono-resistance was 17.8%. Initial isoniazid mono-resistance was 7.9% with acquired isoniazid mono-resistance of 9.9%,[48] In the same year Githui et al documented a total drug resistance of 9.2%, initial drug resistance of 6.3% and acquired drug resistance of 37 % (n=46).Initial isoniazid mono-resistance was 5.3%. In a population surveillance, (2002) isoniazid mono-resistance was 6.3%.[40] Our study shows a total drug resistance of 34%.

Initial isoniazid mono-resistance is 3% and acquired is 27% in this study. Rifampicin mono-resistance was not observed in our study. Previous studies have shown low resistance to rifampicin which has been introduced as part of the short course chemotherapy to help achieve the desired high bacillary kill during the intensive phase. This implies that with this regimen (4RHZE/2RH) patients with the isoniazid resistant strain will still get cured. Mutie et al documented 4 out of 191 patients to have MDR-TB in 1995.[48] A population-based surveillance done in the same year did not document MDR-TB in Kenya.[39] However, a sentinel surveillance done at the refugee camps in the same period documented a rate of 2.9% for MDR-TB.[41] The 2002 national surveillance documented a new entry of MDR-TB in Kenya (0.8%) and an entry of ethambutol resistance (1.1%). MDR-TB Beijing/W strain (8.3%) was documented at KEMRI-CRDR in 2004.[42] Our study indicate that 4 out of the 81(5%) patients studied had MDR-TB a pattern reflected both as resistance to rifampicin + isoniazid (2.5%) and as resistance to rifampicin + isoniazid + streptomycin + ethambutol (2.5%).3 out of these 4 patients had acquired MDR-TB resistance while 1 had initial resistance. There is a clear observation that of the four first line drugs isoniazid has been used over a long period for the intensive and continuation phase, rifampicin was recently introduced for continuation phase, streptomycin is not routinely used (useful for retreatment cases) and ethambutol resistance has just been recently documented.

Low rates of initial drug resistance have been documented in the previous studies where the acquired resistance has been found to be higher. It is generally believed that low rates of initial drug resistance are good indicators of a successful treatment programme for patients with smear-positive pulmonary tuberculosis. Success has been strongly associated with implementation of directly observed treatment, short course regimens (DOTS) and with continued improvement in the NLTP's performance optimization of the regimens used.[49] The increasing level of acquired isoniazid mono-resistance observed in this study may be attributed in part to two factors: first is poor bioavailability of the standard regimen used. Poor bioavailability has been shown in single preparations and with combinations of drugs, especially where drugs are not purchased according to good manufacturing practice.[50] In most developing countries where funds are limited and studies are never carried out routinely, there is a need to obtain drug supplies as cheaply as possible. This may lead to supplies, which contain poor quality preparations with subsequent exposure of patients to inadequate doses of drugs, a potential factor for development of resistance. In Kenya, combination drugs of isoniazid with thiocetazone (Thiazina) have been used in the standard regimen for a long time. Other combination drugs like RH and RHZ have been available in limited quantities in private practice. Currently combinations of RH have been introduced into the public health programme. (DOTs).It is anticipated that this will limit the spread of drug

resistance as has been shown in some countries. However, it will be important to monitor the bioavailability of drugs that are in use.

The second factor is the increasing prevalence of HIV/TB coinfection, which has been shown to be associated with a substantial increase of acquired drug resistance.[51]

The true impact of MDR-TB on treatment outcomes of the SCC remains to be determined. Short Course Chemotherapy (SCC) in the current era refers to the 6-month regimen 2HREZ /4 RH. Since a single contagious patient infects an average of one person a month[15] the potential of MDR-TB becoming a serious threat to TB control efforts is readily apparent. Relatively few effective drugs are available against M. tuberculosis, especially in the low-income countries.

No difference in the prevalence of drug resistance between the various provinces was found in this study. Githui et al documented a 3% prevalence of MDR- TB in the refugee camps in the North Eastern Province in 1995-1996.[41] However in this study the observation that 1 out of the 4 persons with MDR- TB was from the North Eastern Province may have been an incidental finding and we were not able to establish whether the person had come from the refugee camp. Among the factors which may influence the geographical distribution of the drug resistance include infrastructure . The central and Eastern provinces which are favoured by the location with relation to the distance covered when accessing the referral hospital contributed a higher proportion of the prevalence of Isoniazid monoresistance as well as MDR-TB. Overcrowding in the slum areas contributes to high transmission rates of tuberculosis, a factor which occurs among the middle income areas as well. Githui et al in 2004 identified 8% MDR- TB from clinical isolates derived from patients visiting various private clinics that are within the Nairobi city which had been taken to KEMRI for culture and drug sensitivity testing.[42] Selection bias may account for the higher proportion of MDR -TB isolates compared to that (n= 4) of this study. While the population studied in 2004 by Githui may be comparable to our study population which comprises of persons visiting the KNH referral hospital who due to health seeking behaviour aspects may choose to visit a private practitioner and later change their mind and come to KNH, it is important to consider that the isolates delivered to KEMRI are mainly from patients who may have failed treatment and therefore there may have been a selection bias . In our study patients who had smear positive and some with smear negative pulmonary tuberculosis were recruited without regard to any previous treatment outcome. Generally the population of patients who seek medical care from the hospital comprises of individuals with a lower than a tertiary education. This is also consistent with the level of employment since it is expected that those with lower or no education are likely to be unemployed. According to these data only a small number of the respondents described their occupation .Age and sex have been associated with drug resistance in a central Haiti study where older age groups were found to be predictors of resistance[52]. Our study did not find any association between age,

sex and resistance.Similar observations have been made before[53] In this data HIV is not associated with a higher prevalence of drug resistance . Data presented in the first and second global reports suggested that HIV infection is not an independent risk factor for the development of drug resistance. Outbreaks of MDR-TB among HIV infected patients were observed in the USA , Argentina , and some European cities . These outbreaks occurring mainly in hospital settings were associated with delays in diagnosis and high case fatality rates[30].The co-morbid conditions identified in this study included 1 patient with scleroderma,1 with lung fibrosis and 1 who had chronic gastritis - the 3 of whom had INH mono- resistance. These were regarded as incidental associations due to the small numbers and could not be analysed. The sample size did not affect this observation since our overall data (prior to sensitivity testing) which has 257 patients culture positive isolates indicate similar low levels of co-morbid conditions. Certain Co-morbid conditions have been associated with drug resistant tuberculosis. The factors that influence development of drug resistance to the *mycobacterium tuberculosis* in-patients with these conditions have been reported in a previous study and include impaired drug absorption leading to suboptimal drug levels and subsequent development of resistance, patients who have undergone gastrectomy and those with malabsorption disorders.[54]

12. STUDY LIMITATIONS

The study was labour intensive and very costly. For instance, culture and sensitivity testing for one patient would require a minimum of eighteen bottles. The recommended Resistance Ratio method used in this study required very dedicated and highly motivated microbiology technologists with appropriate skills and experience. The cost of the bottles and reagents was enormous. The period between specimen collection and results interpretation was a minimum of 12 weeks per individual isolate. Patience and willpower is required if one has to get credible results. A unique social stigma is attached to TB infection and sputum handling and this made it difficult to sustain study assistants. Face to face conversation when interviewing patients who had pulmonary tuberculosis posed a special challenge to the interviewer and sometimes it would be done hurriedly. In this study only sputum specimen was used in diagnosing TB and may cause a bias on the number of patients with drug resistant TB especially if its not pulmonary tuberculosis. In a hospital, setting it would be important to document drug resistant extra pulmonary TB as well since this is common among HIV positive patients.

13. CONCLUSION

There is a high prevalence of isoniazid mono-resistance at the Kenyatta National Hospital. Initial resistance accounts for 3% while acquired resistance is 27%. There is presence of MDR- TB among the KNH patient population whose trend has not yet been established due to factors which could be attributable to the recent emergence of rifampicin resistance and possibly to the health seeking behaviour among the study population. High-density residential areas contribute a significant proportion of the patients with TB in general and those with resistant strains. .Age ,sex, marital , education and employment status were not significant in determining those at risk for developing drug resistance. HIV status and other disease characteristics were not significantly associated with drug resistance.

14. RECOMMENDATIONS

Follow up studies are recommended which may be done at short intervals in order to establish the exact trend of MDR-TB at the Kenyatta National Hospital. These studies need not have population based sample size in order to allow for flexibility. In view of the high prevalence of acquired drug resistance (isoniazid mono-resistance) it will be necessary to carry out drug sensitivity testing for all smear positive patients who have been previously treated. It is necessary for the Kenyatta National Hospital to network with the peripheral health care centers especially those within high-density residential estates to assist them in optimizing TB diagnosis and treatment in order to limit

unregulated patient flow and therefore control hospital transmissions. In this era of SCC, it will be necessary to closely monitor drug resistance to rifampicin since this would pose a special threat given that isoniazid mono-resistance is already rising.

15. APPENDICES

Appendix 1: CONSENT EXPLANATION BEFORE RECRUITMENT

We would like to inform you that we are conducting a hospital based activity which involves participation of adult patients who have a disease called tuberculosis (TB) of the lungs . People acquire this disease when they breathe in droplets of sputum that is coughed out by other people who have the disease .This disease affects both the HIV negative and positive people. It commonly affects the lungs but can spread to other parts of the body as well. It causes cough, fever and weight loss among other symptoms.

We would like you to know that participation in this activity is on a voluntary basis.

If you have understood the information which we have given you and you are willing to participate you will be required to sign a form indicating your willingness to be recruited and a second consent for the retention of sputum cultures for future reference. After that we will ask you some questions whose answers we will indicate in a form. prepared for this activity and then you will give us two samples of sputum produced on the same day one in the morning when you wake up and the other when you reach the hospital. The two will be put in separate plastic containers. Each sample will be coded to ensure confidentiality. This means that your name will not be used to label the sample and therefore you need not worry because we will not expose your results unnecessarily.

The samples will be taken to the Kenya Medical Research Institute (KEMRI) laboratory where they will be subjected to further tests.

The organisms which will grow will be tested with the medicines used to treat TB to check whether they will be eliminated (respond) .Those that do not respond will be considered as resistant and those which respond will be considered sensitive .

if your sputum grows the resistant organisms we will communicate the results to you so that you may be given the appropriate treatment .Since you will be reporting to your primary healthcare centre for treatment, the only way to reach you is to send the

coded results will be sent to your specific centre indicating our drug response findings and the appropriate drug regimen in case a change of treatment is required.

We would like you to understand that you will not be denied treatment or any form of care in this hospital if you are not willing to participate .

We wish to assure you that no information given by you to us will be disclosed without your consent.

Thank you.

Appendix 2: CONSENT FORM

I ..... of ..... have understood the information given to me by..... about a survey "TO

DETERMINE THE LEVEL AND PATTERN OF ANTITUBERCULOSIS DRUG RESISTANCE IN KENYATTA NATIONAL HOSPITAL"

I am willing to participate in this study on a voluntary basis and I do understand that I will be required to reveal some information concerning my illness and also to provide two more samples of sputum in addition to the one I had given for the diagnosis of my disease . I have also been informed that the culture specimen for my sputum sample will be preserved for future reference and I have agreed to this.

I am aware that refusal to take part will not make me get denied clinical care .

Signed / thumb print: (patient).....

Signed: witness (responsible officer).....

Date .....

Appendix 3: HIV COUNSELING FORM

How are you? I am ..... (Name of the counselor)

I would like us to discuss about HIV /AIDS if you do not have any objections.

HIV is the virus that causes AIDS. This is a disease which predisposes the patient to a variety of disease causing infections as well as other diseases such as malignancies among others.

The virus is transmitted through sexual intercourse, exposure to blood and blood products, mother to child during birth and breastfeeding and through use of contaminated needles for example among injection drug users.

For the purpose of this study I would like you to be tested for HIV using a test which involves taking a spot sample of your blood to test it with two methods of which a positive test is the one showing positive by both methods.

I would also like to inform you that refusal to do the test will not make you get excluded from the study and that you will still receive the usual care for all patients with PTB.

We shall communicate the result to you and advise you on how best to remain free of disease if you test negative and on how to live positively and get treatment if you test positive

Thank you.

Appendix 4: CONSENT FORM FOR HIV TESTING

I .....of .....have understood the explanation concerning testing for HIV status and I have voluntarily agreed to be tested. I am aware that refusal to be tested will not make me get excluded from the study or be denied care for my illness.

Signed (thumb print)..... (Patient)

Signed ..... (Counselor)

Date .....

Appendix 5: SPUTUM SHIPMENT FORM

INPATIENT FORM

IDENTIFICATION OF THE PATIENT

Name: .....

District of Origin: .....

Current Residence: .....

KNH Inpatient Number: .....

Date of Admission Day..... Mo ..... Yr. .....

Sex: Male Female

Age ..... Years

Date of Sputum Collection: A..... B.....

Result of Smear .....

Appendix 6: SPUTUM SHIPMENT FORM 2

TB CLINIC FORM

IDENTIFICATION OF THE PATIENT

Name: .....

District of Origin: .....

Current Residence: .....

TB clinic registration number: .....

Date Registered: Day..... Mo..... Yr.....

Sex: Male Female

Age.....Years

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

Result of Smear: .....

Appendix 7: PROFORMA

A: DEMOGRAPHICS

Name: .....

District of Origin: .....

KNH Inpatient Number /TB Clinic Registration Number.....

Date of Admission: Day..... Mo..... Yr..... Ward Name.....

Sex: Male ..... Female.....

Age ..... years Occupation..... Education.....

Date of Sputum Collection: A..... B.....

B1: CLINICAL INFORMATION

a) HIV STATUS: Positive ..... Negative .....

b) History of previous treatment for TB: YES..... NO.....

c) If yes health centre /hospital/ others (specify) where treated .....

d) History of drug abuse: .....

e) History of any other chronic illness (specify) .....

Date of previous admission (if any) 1. Day..... Mo..... Yr.....

2. Day..... Mo..... Yr.....

3. Day..... Mo..... Yr.....

Duration. of admission..... Ward. /Hospital.....

Diagnosis.....

Treatment.....

B2: HISTORY GIVEN BY THE PATIENT

PREVIOUSLY TREATED FOR TB? YES /NO

STANDARDISED HISTORY

a) For how long have you been sick?.....

b) Did you have the same symptoms prior to this episode ? yes..... no.....

c) Did you have other symptoms of lung disease prior to this episode yes ... no.....
(hemoptysis, chest pain, cough)

d) Did you have x-ray examinations prior to this episode? Yes..... no.....

e) Did you have sputum examinations prior to this episode? yes ..... no.....

f) Did you ever take antituberculosis drugs for more than one month
If yes what was the name?.....

g) Did you ever have injections for more than one month? Yes .... No.....
If yes, specify whether were anti TB.

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

No .....Yes.....

If the answer is yes, continue with B3

B3: INFORMATION ABOUT PREVIOUS TREATMENT

Where was the patient treated? Health centre / hospital/private clinic /others specify....

When was the patient treated? (no. of months/days).....

How many times was the patient treated? once/twice/thrice/others specify.....

Which drugs were used for treatment? Anti TBs/other antibiotics/others specify.....

By whom was the patient treated? Doctor/pharmacist/clinical officer/nurse/others specify

Outcome of the last treatment according to the patient.....

Cured .....Not cured .....Unknown.....

C: MEDICAL RECORDS

After extensive checking through the medical files and other documents available in the health center, have you discovered that the patient has been registered for tuberculosis treatment before?

No .....Yes.....

If the answer is yes what was the outcome of the last course of chemotherapy

Cured .....Treatment completed.....

Defaulted ..... Failed.....

Transferred out.....

FINAL DECISION

D1: Patient Has Been Previously Treated For TB For More Than One Month

Yes (answer to question B1 or B2 and /or C was "yes")

No (answer to B1 and B2 and /or C was "no")

D2: If Yes, What Was The Outcome Of Previous Treatment?

Cured / treatment completed.....

Failed .....

Defaulted.....

Chronic.....

Relapse/defaulter not distinguishable.....

Unknown.....

Responsible officer: .....

X-RAY FINDINGS:

X-ray No.: .. ..

1. NORMAL .....

2. CAVITIES SEEN .....

3. PLEURAL EFFUSION .....

4. CONSOLIDATION: underline the appropriate

DIFFUSE / LOBAR / BRONCHOPNEUMONIA /LUNG COLLAPSE

Appendix 8: RESULTS OF BACTERIOLOGICAL EXAMINATION FORM

A: PATIENT

Number: ..... Date of Receipt: .....

B: IDENTIFICATION

Sample A:

— m.tuberculosis

— m.bovis

— M.africanum

— Negative

— Contaminated

— other

Sample B:

— M.tuberculosis

— M.bovis

— M.africanum

— Negative

— contaminated

— other

C: SUSCEPTIBILITY OF M .TUBERCULOSIS

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

Date of Recording: .....

Responsible Officer: .....

Appendix 9: STUDY DURATION

Protocol Presentation	October 26th, 2006
Ethical Committee	November 2006 to April 2007
Data Collection	June 2007 to September 2007
Results Presentation	December 2007

BUDGET

Specimen Sample:	243 @ Kshs. 3,000/-	729,000.00
Stationary:		40,000.00
Printing:		20,000.00
Statistician:		30,000.00
Miscellaneous:		40,000.00
TOTAL:		**Kshs 860,000.00**

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