



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



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**Prevalence of Rifampicin Resistant Among Pulmonary
Tuberculosis by Using GeneXpert Technique in River Nile
State- Sudan**

A thesis Submitted for partial fulfillment of the Msc Degree in Medical Laboratory
Sciences in (Microbiology)

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الآية

بسم الله الرحمن الرحيم

قال تعالى:

﴿ وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِّنْ

الْعِلْمِ إِلَّا قَلِيلًا ﴾

صدق الله العظيم

سورة الإسراء - الآية (85)



Dedication

To all whom I love and respect

My parents

My Brothers and Sisters

My teachers.....

My Friends and Colleagues

I can't forget to congratulate some one

Who stood behind me?

To achieve my success

Acknowledgment

All thanks to Allah from the start to the end.....

I am grateful to all those who contribute to help me to make this research as accurate and as useful as possible .

I would like to post bond my gratitude to my supervisor

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For his continuous guidance and help to make me produce this research in a proper way,

My thanks also extended to my colleagues & my friends, specially, Alwaleed Elfadil

Who encouraged me all the time

List of Abbreviation

Abbreviation	Term
ART	Anti Retroviral Treatment
CT	Cycle Threshold
CPT	Cotrimoxazole Prophylaxis
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
DOT	Directly Observed Therapy
DST	Drug Susceptibility Testing
E	Ethambutol
EPTB	Extrapulmonary Tuberculosis
EQA	External Quality Assessment
F	Fluvastatin
FQs	Fluoroquinolones
H	Isoniazid
HIV	Human Immunodeficiency Virus
IRIS	Immune Reconstitution Inflammatory Syndrome
LPA	Line Prop Assay
MDR	Multidrug Resistance
MTB	Mycobacterium Tuberculosis
NTM	Non Tuberculosis Mycobacterium
NTP	National TB Control Program
PTB	Pulmonary Tuberculosis
Q	Quinidine
RIF or R	Rifampicin
RR-TB	Rifampicin Resistance Tuberculosis
RT-PCR	Real Time Polymerase chain Reaction
S	Streptomycin
TB	Tuberculosis
UVGI	Ultraviolet Germicidal Irradiation
WHO	World Health Organization
XDR	Extensive Drug Resistance
Z	Pyrazinamide

Abstract

This Study was prospective laboratory base descriptive study, the study was conducted in Central Diagnostic Laboratory in Atbara , received specimens for diagnosis and identification of suspected cases of pulmonary tuberculosis and resistant bacilli collected from different centers distributed in different locality of the state .

150 sputum samples were obtained during the period between January to June-2018. The patients referred to central lab either suspected new cases of pulmonary tuberculosis or suspected multidrug resistance patients . The average age were ranged from 2-90 years. The mean age was 46 year. The PCR screening test was revealed 66.6% (100/150) positive for Mycobacterium tuberculosis.

Tuberculosis was higher in males 75 (75%) than females 25 (25%) male /female ratio was (3:1), most of the cases encountered in the age group from 17 to 35 years 49% (49/100), the frequency of tuberculosis was more in married individual 54 (54%) than singles, patients from rural area were 67 (67%) while 33(33%) were from urban area, the frequency of patients symptoms in the study were cough for 2 weeks or more 55% , hemoptysis(bloody sputum) 29% , weigh loss 15% , and night sweat 1%. The patients were diagnosed after appearance of symptoms 56% within weeks, 43% within months ,and 1% within days, 97% of patients with unscreened for HIV, the screened patients 1% was HIV seropositive ,and 2% were negative, superimposed diseases such as diabetes and hypertension were encountered in 28% of the patients included in the study , the study revealed that recurrent rate was 59%(59/100) due to failure, relapse or lost of follow up, MDR for tuberculosis was 10%(10/100). The study reaveled there was a significant difference between new cases of tuberculosis and recurrent tuberculosis patients, p.value = 0.000.

ملخص البحث

أجريت هذه الدراسة علي نمط دراسته أستطاعيه وصفيه في المعمل التشخيصي بمدينة عطبرة الذي يستقبل العينات للتشخيص والتعرف علي العصيات القابلة والمقاومة للعلاج التي تم جمعها من مراكز الدرن المختلفة في الولاية.

150 عينة بلغم تم الحصول عليها في الفترة ما بين شهر يناير وحتى يونيو 2018م ، المرضي المحولين إلي المعمل المركزي إما حالات جديدة لمرض الدرن أو مرضى مقاومين للعلاج . و تتراوح أعمارهم ما بين (2-90) سنة ومتوسط العمر 46 سنة .

أظهر تفاعل البوليمرز المتسلسل وجود 100 عينة بلغم موجبه لعصيات الدرن .

مرض الدرن أعلي في الذكور بنسبة بلغت 75% من الإناث بنسبة 25% أي بمعدل 3:1 ، أغلب المرضي

تتراوح أعمارهم ما بين 17-35 سنة بنسبه بلغت 49% ، يزيد تردد مرض الدرن عند المتزوجين بنسبة

54% مقارنة مع غير المتزوجين ، تزيد نسبة مرض الدرن في المرضي القادمين من الريف بنسبة 67%

مقارنة مع مرضي الحضر بنسبة 33% ، تتكرر الأعراض للمرضي في صورة كحه لمدة أسبوعين أو أكثر

بنسبة 55%، بلغم مصحوب بدم بنسبة 29%، نقصان وزن 15%، وتعرق ليلي بنسبة 1% ، تم تشخيص

المرضي بنسبة 56% في خلال أسابيع، 43% في خلال أشهر ، 1% في خلال أيام وذلك من ظهور

الأعراض عليهم ، 97% من مرضي الدرن لم يسبق لهم فحص فيروس الأيدز ، أما الدين قاموا بفحص

الفيروس 2% سلبي و 1% إيجابي ، 28% من مرضي الدرن في هذه الدراسة يعانون من أمراض أخرى

كالضغط والسكر ، أظهرت هذه الدراسة أن معدل تكرار المرض 59% نسبة لفشل العلاج ، أنتكاسه أو نتيجة

لفقد المتابعه ، 10% من المرضي في هذه الدراسة من مرضي الدرن المقام للعلاج . كما أظهرت هذه الدراسة

وجود أختلاف دو أهمية ما بين الحالات الجديده لمرض الدرن والحالات المكررة .

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Chapter One

**Introduction,
Justification,
Objective**

Introduction

Tuberculosis:

Tuberculosis (TB) is an infectious disease caused by the mycobacterium tuberculosis (MTB).

Tuberculosis generally affects the lungs, but can also affect other parts of the body. Most infections do not have symptoms, in which case it is known as latent tuberculosis.

About 10% of latent infections progress to active disease which if left untreated kills about half of those infected.

The classic symptom of active TB are a chronic cough with blood containing sputum, fever, night sweats, and weight loss.

Treatment requires the use of multiple antibiotics over a long period of time. Antibiotic resistance is a growing problem with increasing rates of multiple drug resistant tuberculosis (MDR-TB).

Gene Xpert MTB/RIF:

The xpert MTB/RIF is cartridge based nucleic acid amplification test, automated diagnostic test that can identify Mycobacterium tuberculosis (MTB) DNA and resistance to rifampicin (RIF) by nucleic acid amplification test ⁽¹⁾.

1.2 Rationale

These study is done to evaluate the role of gene expert for diagnosis of cases of Rifampicin resistant in order to see the percentage of cases of Rifampicin resistant.

This study targets TB patients , TB is most common problem in sudan .

1.3 Objectives

1.3.1 General objective:

To determine the Prevalence of Multi Drug Resistant Among Pulmonary Tuberculosis by Using GeneXpert Technique in River Nile State.

1.3.2 Specific objectives:

- To identify the important of gene expert for diagnosis of cases of TB that resist Rifampicin.
- To identify the other lines of treatment in cases of Rifampicin resistant.

Chapter Two

Literature Review

2-Literature Review

2-1 Tuberculosis

Presumptive TB refers to a patient who presents with symptoms or signs suggestive of TB (previously known as a TB suspect).

Case definitions

- **A bacteriologically confirmed TB case** is one from whom a biological specimen is positive by smear microscopy, culture or rapid molecular-based methods (such as Xpert MTB/RIF).
- **A clinically diagnosed TB case** is one who does not fulfill the criteria for bacteriological confirmation but has been diagnosed with active TB by a clinician or other medical practitioner who has decided to give the patient a full course of TB treatment. This definition includes cases diagnosed on the basis of X-ray abnormalities or suggestive histology and extrapulmonary cases without laboratory confirmation.

*Clinically diagnosed cases subsequently found to be bacteriologically positive (before or after starting treatment) should be reclassified as bacteriologically confirmed.

Bacteriologically confirmed or clinically diagnosed cases of TB are also classified according to:

1. anatomical site of disease.
2. history of previous treatment.
3. drug resistance.
4. HIV status.

Classification based on anatomical site of disease:

Pulmonary tuberculosis (PTB)

- Refers to any bacteriologically confirmed or clinically diagnosed case of TB involving the lung parenchyma or the tracheobronchial tree.
- Miliary TB is classified as PTB because there are lesions in the lungs.
- Tuberculous intra-thoracic lymphadenopathy (mediastinal and/or hilar) or tuberculous pleural effusion, without radiographic abnormalities in the lungs, constitutes a case of extrapulmonary TB
- Patient with both pulmonary and extrapulmonary TB should be classified as a case of PTB.

Extrapulmonary tuberculosis (EPTB) refers to any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs, e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges

Classification based on history of previous TB treatment (patient registration group)

New patients have never been treated for TB or have taken anti-TB drugs for less than 1 month. Previously treated patients have received 1 month or more of anti-TB drugs in the past. They are further classified by the outcome of their most recent course of treatment as follows:

- **Relapse patients** have previously been treated for TB, were declared cured or treatment completed at the end of their most recent course of treatment, and are now diagnosed with a recurrent episode of TB (either a true relapse or a new episode of TB caused by reinfection).

- **Treatment after failure patients** are those who have previously been treated for TB and whose treatment failed at the end of their most recent course of treatment.
- **Treatment after loss to follow-up patients** have previously been treated for TB and were declared lost to follow-up at the end of their most recent course of treatment. (These were previously known as treatment after default patients.)
- **Other previously treated** patients are those who have previously been treated for TB but whose outcome after their most recent course of treatment is unknown or undocumented.
- **Patients with unknown previous TB treatment history** do not fit into any of the categories listed above.

New patients have never had treatment for TB, or have taken anti-TB drugs for less than 1 month. New patients may have positive or negative bacteriology and may have disease at any anatomical site.

Classification based on drug resistance:

Cases are classified in categories based on drug susceptibility testing (DST) of clinical isolates confirmed to be *M. tuberculosis*:

- **Monoresistance:** resistance to one first-line anti-TB drug only.
- **Polydrug resistance:** resistance to more than one first-line anti-TB drug (other than both isoniazid and rifampicin).
- **Multidrug resistance:** resistance to at least both isoniazid and rifampicin.
- **Extensive drug resistance:** resistance to any fluoroquinolone and to at least one of three second-line injectable drugs (capreomycin, kanamycin and amikacin), in addition to multidrug resistance
- **Rifampicin resistance:** resistance to rifampicin detected using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs. It includes

any resistance to rifampicin, whether monoresistance, multidrug resistance, polydrug resistance or extensive drug resistance.

- **Rifampicin resistance:** resistance to rifampicin detected using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs. It includes any resistance to rifampicin, whether monoresistance, multidrug resistance, polydrug resistance or extensive drug resistance.

2-1-1 MDR:

Resistance to tuberculosis (TB) drugs is a formidable obstacle to effective TB care and prevention globally. Multidrug-resistant TB (MDR-TB) is multifactorial and fuelled by improper treatment of patients, poor management of supply and quality of drugs, and airborne transmission of bacteria in compromising treatment options available to patients infected with these strains. Since then, clinicians in some settings have reported patients infected with strains in which virtually all treatment options have been exhausted. This rapidly evolving landscape is a clarion call to policy-makers and practitioners to respond with improvements in care delivery and introduction of innovative tools and approaches. In 2009, the 62nd World Health Assembly urged WHO Member States to provide universal access to care for drug-resistant TB patients. In that resolution, it was acknowledged that national TB programme managers, clinicians, nurses, all care providers and affected people themselves need guidance on how best to bring together different elements of health systems and services needed to effectively address the MDR-TB challenge. In 2014, the 67th World Health Assembly passed a resolution approving the new post-2015 Global TB Strategy, the *END TB strategy*, with its ambitious unprecedented targets and with its vision of ending the TB – as an epidemic disease – by 2035. Therefore, this Handbook has been developed for the purpose of describing ways to implement established WHO policies relevant for the management of MDR-TB. These WHO policy recommendations have been

produced using the GRADE methodology for evidence assessment, as adopted by WHO in 2008.

While drug-resistant TB is today a major threat worldwide – and in some settings up to one third of new cases are multidrug-resistant at first diagnosis – it is important to remember that most patients are infected by drug-susceptible strains and can be cured with the standard six-month first-line regimen. Therefore, besides focusing on care for drug-resistant TB, the programmatic management of MDR-TB is premised upon keeping the number of cases with drug resistance to a minimum and treating those that have the condition with the best possible means available.

We trust that you will find this publication useful. WHO will continue working to keep pace with scientific advances in diagnostics, therapeutics and care delivery options so that it can respond effectively and in a timely manner to the needs of TB patients with drug resistance in all countries. ⁽⁹⁾

Epidemiological data and drug resistance surveillance:

The epidemiological context of TB and drug resistance must be taken into account to optimize case detection strategies for drug-resistant TB. Data from drug resistance surveillance and surveys are crucial to help determine the probability (or risk) of an individual patient or groups of patients having drug-resistant TB, which is needed for establishing effective strategies for targeted DST.

All programmes should have representative drug resistance surveillance data on at least rifampicin, isoniazid and second-line drugs (from the groups of fluoroquinolones and secondline injectable drugs) most commonly used in the country. These data should be stratified by patient-group and TB treatment histories – new patients, different categories of retreatment patients (patients who failed a new regimen with first-line anti-TB drugs, patients who failed a retreatment regimen with first-line anti-TB drugs, patients who relapsed or

returned after loss to follow-up) and high-risk groups (including for example, drug-resistant TB patient contacts or prisoners). In countries where routine drug resistance surveillance data are not available, are outdated or are not representative of the population or sub-groups of TB patients, a drug resistance survey should be organized periodically every four to five years.

While rifampicin resistance is a reliable proxy for MDR-TB in patient groups in many countries, country-specific data should be obtained on the frequency of concomitant isoniazid resistance when rifampicin resistance is present. For patients who are diagnosed with rifampicin resistance using Xpert MTB/RIF (which is not able to detect isoniazid resistance), understanding the frequency of MDR-TB among rifampicin-resistant patients may be useful for designing initial treatment regimens with or without isoniazid. Rifampicin monoresistance is managed in the same way as MDR-TB, with the exception of including isoniazid; Drug resistance surveillance data also enable programmes to estimate the number of patients who could be detected and enrolled in the drug-resistant TB programme, which in turn greatly facilitates strategy planning and long-term drug procurement.

High-risk groups and targeted DST:

Ideally, testing for drug resistance should be provided for all identified TB patients before the start of TB treatment, so that the most appropriate therapy for each individual can be determined. However, the goal of universal access to DST has not yet been realized for most of the world's TB patients, and in many areas only patients considered at high risk for drug-resistant TB have access to DST. Targeted DST allows for the most drug-resistant TB patients to be detected with the resources available. This requires careful risk assessment of patients.

Risk for drug-resistant TB is determined by patient history combined with epidemiological data from drug resistance surveillance or surveys, which can help identify high-risk groups.

Recommendations regarding conventional and molecular DST for drug-resistant TB detection.

Establishing the presence of drug-resistant TB is done through DST using conventional (phenotypic) or molecular (genotypic) tests.

The best strategy for detection of drug-resistant TB, and the WHO recommended strategy, is to use rapid DST. The WHO 2011 update of *Guidelines for the programmatic management of drug-resistant tuberculosis*, specifically states:

Rapid drug susceptibility testing (DST) of isoniazid and rifampicin or of rifampicin alone is recommended over conventional testing or no testing at the time of diagnosis of TB, subject to available resources (conditional recommendation, very low quality evidence) ⁽¹⁰⁾

Using rapid DST, patients can be started on an appropriate treatment regimen sooner and infection control measures implemented if needed, improving treatment outcomes while also decreasing transmission of infection to others.

Interpreting rifampicin resistance results from molecular testing WHO-recommended molecular testing methods (Xpert MTB/RIF and line probe assays) have been found to have a high sensitivity and specificity for detection of rifampicin resistance. Molecular methods do not have perfect concordance with phenotypic culture-based DST methods and patient details such as treatment history and risk factors for drug-resistant TB should always be taken into account when interpreting laboratory results.

WHO-recommended molecular methods detect mutations in the *rpoB* region of *M. tuberculosis* DNA, which are responsible for >95% of rifampicin-resistant strains. Given the resultant high sensitivity of molecular methods, a negative result generally excludes rifampicin resistance and no further testing to confirm negative results is required. In rare instances, when a patient is strongly presumed to have

RR-TB even after a negative molecular test, follow-up testing using phenotypic culture-based DST may be used to test for rifampicin resistance resulting from a small number of mutations occurring outside the *rpoB* region.

WHO-recommended molecular methods also have very high specificity for detection of rifampicin resistance. Nevertheless, any diagnostic test with a specificity of less than 100% when used in a population with a low prevalence of the condition can result in a lower positive predictive value, which means that a number of false-positive results are present among those diagnosed as having the condition. Increasing evidence, however, is showing that the occurrence of ostensibly false-positive rifampicin resistance detected by Xpert MTB/RIF compared to phenotypic culture-based DST methods may be linked to the detection by molecular methods of strains that are truly resistant to rifampicin, yet are not detected by culture-based DST. Such strains appear to have clinically-relevant mutations in the *rpoB* region conferring resistance to rifampicin, causing disease that is likely to fail first-line treatment. A recent study has shown that an epidemiologically-significant proportion (close to 10%) of rifampicin-resistant strains in first failure and relapsed patients are missed by phenotypic DST.

The interpretation of molecular results and follow-on steps will depend on the result itself as well as on the patient group from which the patient originated. All patients identified by molecular methods should be initiated on an appropriate WHO-recommended treatment regimen as soon as possible. Prompt treatment initiation will have a positive effect on patient outcomes, while the treatment regimen can be refined when additional testing results become available. When a molecular method detects rifampicin resistance, the decision on further steps depends on the patient's risk of having drug-resistant TB:

- In patients originating from a group at high risk of MDR-TB, a WHO-recommended regimen for MDR-TB with the addition of isoniazid should be

initiated (when susceptibility to isoniazid is not known). The patient should be registered as having bacteriologically confirmed RR-TB, and another sputum sample (taken immediately, prior to treatment onset) should be sent for phenotypic or another genotypic DST to isoniazid. The sample should be subjected to phenotypic DST against fluoroquinolones and second-line injectable agents. Confirmatory testing of rifampicin resistance using another technology is not necessary in such cases. When DST results are available, the MDR-TB treatment regimen should be refined based on the results and patient registration updated accordingly.

Treatment modifications may include dropping isoniazid if resistance was shown, adding an appropriate fluoroquinolone or second-line injectable agent or, in case of XDR-TB, placing the patient on an appropriately designed regimen including Group 5 drugs. Accordingly, the registration of the patient should be modified reflecting this new information and be notified as per national regulations.

- In patients originating from a group at low risk of MDR-TB, this result may be considered unexpected and further follow-up is required. While this unexpected result may be attributed to test specificity not being 100% for detection of rifampicin resistance, it may also result from the non-systematic or random errors at pre- or post-analytical stages of the testing that are relatively frequent even in quality-assured laboratories. These include clerical errors when recording specimen information or test results, or administrative errors that result in specimens being mixed up, etc. While not addressing the test's specificity, an immediately repeated test on a fresh sample can be useful in improving a clinician's confidence when deciding on the treatment to be prescribed. When the result of a second test shows rifampicin susceptibility (an unsurprising result in an individual at low risk of MDR-TB), a WHO-recommended first-line regimen should be prescribed, and the patient should be registered as having susceptible,

bacteriologically confirmed TB. When the result of a second test is in accordance with the initial finding of rifampicin resistance, a WHO-recommended regimen for MDR-TB with the addition of isoniazid should be started without any further delay. Such a patient should be registered as having bacteriologically confirmed RR-TB, and an additional sample should be taken for phenotypic or genotypic DST to confirm resistance to rifampicin and also test for susceptibility to isoniazid, fluoroquinolones and second-line injectable agents. When DST results become available, the MDR-TB treatment regimen and patient registration should be refined, if appropriate.

Treatment modifications may include dropping isoniazid from the regimen if resistance was found, adding an appropriate fluoroquinolone or second-line injectable agent or, in the case of detection of XDR-TB, placing the patient on an appropriately designed regimen including Group 5 drugs. Accordingly, the registration of the patient should be modified to reflect this new information and be notified as per national regulations. In case of discordance in rifampicin resistance results between a molecular method and phenotypic DST or another molecular method, the available culture isolate should preferably be referred for DNA sequencing in a reference laboratory, and in the meantime a clinical decision should be made on whether the MDR-TB regimen should be continued. Emerging data show that Xpert MTB/RIF detects some rifampicin-resistant strains that are susceptible on phenotypic DST. Sequencing of these discordant results usually resolves in favour of Xpert MTB/RIF, and patients with rifampicin resistance missed by phenotypic DST often harbour mycobacteria with clinically relevant mutations in the region conferring resistance to rifampicin, causing disease that is likely to fail first-line treatment.

Molecular methods are not suitable for monitoring of treatment response. Results can stay positive for *M. tuberculosis* by detection of DNA in dead organisms after

viable bacteria have been eliminated, resulting in false-positive results. Therefore, culture remains the preferred method for monitoring patient response to drug-resistant TB therapy.

Definitions of Treatment outcomes for TB patients

All bacteriologically confirmed and clinically diagnosed TB cases should be assigned an outcome from this list ⁽¹⁾

Outcome	Definition
Cured	A pulmonary TB patient with bacteriologically confirmed TB at the beginning of treatment who was smear- or culture-negative in the last month of treatment and on at least one previous occasion.
Treatment completed	A TB patient who completed treatment without evidence of failure BUT with no record to show that sputum smear or culture results in the last month of treatment and on at least one previous occasion were negative, either because tests were not done or because results are unavailable.
Treatment failed	A TB patient whose sputum smear or culture is positive at month 5 or later during treatment. Also included in this definition are patients found to harbour a multidrug-resistant (MDR) strain at any point of time during the treatment, whether they are smear-negative or –positive
Died	A TB patient who dies for any reason before starting or during the course of treatment.
Lost to follow-up	A TB patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more.
Not evaluated	A TB patient for whom no treatment outcome is assigned. This includes cases “transferred out” to another treatment unit as well as cases for whom the treatment outcome is unknown to the reporting unit.
Treatment success	The sum of cured and treatment completed.

Outcomes for RR-TB/MDR-TB/XDR-TB patients treated using second-line treatment Outcome Definition ⁽¹⁾

Cured	Treatment completed as recommended by the national policy without evidence of failure AND three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase.
Treatment completed	Treatment completed as recommended by the national policy without evidence of failure BUT no record that three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase.
Treatment failed Treatment	terminated or need for permanent regimen change of at least two anti-TB drugs because of: <ul style="list-style-type: none"> • lack of conversion by the end of the intensive phase, or • bacteriological reversionb in the continuation phase after conversion to negative, or • evidence of additional acquired resistance to fluoroquinolones or second-line injectable drugs, or • adverse drug reactions (ADRs).
Died	A patient who dies for any reason during the course of treatment. Lost to follow-up A patient whose treatment was interrupted for 2 consecutive months or more.
Not evaluated	A patient for whom no treatment outcome is assigned. (This includes cases “transferred out” to another treatment unit and whose treatment outcome is unknown)
Treatment success	The sum of cured and treatment completed

2-2 Characteristic of mycobacterium tuberculosis bacillus:

Mycobacteria are rod – shaped bacilli that can cause variety of diseases in humans.

There are three main groups:

Mycobacterium tuberculosis complex this group include M. tuberculosis, M. bovis, M. africanum, M. microti and M. Canetti .they all can cause tuberculosis in humans. The vast majority of tuberculosis is cause by M. tuberculosis.

Mycobacterium leprae causes leprosy.

The bovine bacillus (*Mycobacterium bovis*) caused much infection in cattle. Infection was often passed on to man through contaminated milk.

Bovine TB in milk can be killed by boiling the milk, and bovine tuberculosis rarely occurs where this is the practice. The extent of the transmission of bovine tuberculosis to humans is difficult to determine because of technical problems in isolating the organisms. One important difference is the resistance to pyrazinamide in M. bovis.(3unionClinical Tuberculosis).

Non tuberculosis mycobactira NTM: this group include all other mycobacteria that can cause diseases in the humans.NTM sometimes can cause clinical manifestation (in the lung, lymph node, bone, skin) similar to those of tuberculosis .most NTM exist in the environment, are not usually spread from person and are often non pathogenic in person with intact immune system and intact lung tissue.

M. tuberculosis is strictly aerobic bacterium it there for multiplies better in pulmonary tissue (in particular at the apex where oxygen concentration is higher).⁽¹⁾

Tuberculosis is usually spread from person-to-person through the air by **droplet nuclei** (<5 microns) that are produced when a person with pulmonary or laryngeal tuberculosis coughs, sneezes or talks.

Droplet nuclei may also be produced by aerosol-producing investigations such as sputum induction, bronchoscopy and through manipulation of lesions or processing of tissue or secretions in the laboratory. Droplet nuclei, which are small particles 1 to 5 μm in diameter containing 1-5 bacilli, are highly infectious. ⁽²⁾

Droplets nuclei can remain suspended in the air for several hours depending on the environment. ⁽¹⁾

Three factors determine the likelihood of transmission of M. tuberculosis:

- The number of organisms expelled into the air.
- The concentration of organisms in the air, determined by the volume of the space and its ventilation.
- The length of time an exposed person breathes the contaminated air one cough can produce 3,000 droplet nuclei and a sneeze up to a million droplet nuclei; the infectious dose of tuberculosis is 1 to 10 bacilli. The most infectious cases are those with smear positive pulmonary TB, with (+++) on smear microscopy being the most infectious than (+). Smear negative pulmonary TB cases are much less infectious. Extra-pulmonary cases are almost never infectious, unless they have pulmonary tuberculosis as well. Individuals with latent tuberculosis infection are not infectious, as they do not have replicating bacteria and cannot transmit the organism.

Transmission generally occurs indoors, in dark, poorly ventilated spaces where droplet nuclei stay airborne for a long time. Direct sunlight quickly kills tubercle bacilli, but they can survive in the dark for several hours.

Close contact and prolonged exposure increases the risk of transmission. Once infected, the progression to active disease is dependent on the immune status of the individual.

In those with normal immunity, 90% will not progress and only 10% will develop active disease (half of these now and half later on in life).

The risk is highest in the first two years after infection, when half the cases will occur. Those most at risk include children <5 years of age and the elderly. ⁽²⁾

The length of time required for TB patients to become non infectious after starting TB therapy is not exactly known, however once an effective TB therapy is started, as long as the patients follow the prescribed treatment regimen, there is considerable evidence showing the infectiousness can rapidly decline, even after a few days. It's estimated that person with smear positive TB undiagnosed and untreated, transmit the bacilli to 10 to 20 people per year (this varies according to lifestyle and environment).

2-2-1 Clinical Presentation of TB:

Pulmonary TB:

The main symptoms of pulmonary tuberculosis are:

- Persistent cough for 2 weeks or more.
- Fever for more than 2 weeks or more.
- Night sweats.
- Unexplained weight loss.

This symptom may be accompanied by:

- Symptoms: shortness of breath, chest pains, haemoptysis.
- Constitutional symptoms: loss of appetite and fatigue.

PTB should be considered in practice in all patients who have experience respiratory symptoms for 2 weeks or more.

Advanced course include:

- Respiratory insufficiency due to extensive lesion and destroyed lung.
- Massive haemoptysis due to large cavities with hypervascularisation and erosion of vessels
- Pneumothorax due to rupture of cavity in the pleural space. ⁽¹⁾

Physical signs:

Physical signs do not often help much, but do examine the patient carefully. You may find useful signs:

General condition:

Sometimes this may be good, in spite of advanced disease.

But the patient may be obviously ill, very thin, with obvious loss of weight and pale.

Fever:

This can be of any type. There may be only a slight rise of temperature in the evening, the temperature may be high or irregular, often there is no fever.

Pulse:

Is usually raised in proportion to fever.

Finger clubbing:

Finger clubbing may occur, particularly in a patient with extensive disease. Remember that clubbing is more common with lung cancer, lung abscess or bronchiectasis.

Chest:

Often there are no abnormal signs. The commonest sign is:

Fine crepitations (crackles):

In the upper part of one or both lungs. These are heard particularly on taking a deep breath after coughing.

Dullness to percussion

Later there may be dullness to percussion or even bronchial breathing in the upper part of both lungs.

Localized wheeze:

Occasionally there is a wheeze due to local tuberculous bronchitis or pressure by a lymph node on a bronchus. In chronic tuberculosis with much fibrosis (scarring), the

scarring may pull the trachea or the heart over to one side. At any stage the physical signs of pleural effusion may be present. Often, however, you will find nothing abnormal in the chest. ⁽³⁾

Extra-pulmonary TB:

- TB meningitis.
- Disseminated / miliary tuberculosis.
- TB of the bones and joints.
- TB lymphadenitis.
- Tuberculous effusion and empyema.
- Tuberculous pericardial effusion.
- Intestinal/peritoneal/abdominal tuberculosis.
- Other forms of EPTB.

EPTB forms can be developed at any age .young children and HIV infected adult are more susceptible. ⁽¹⁾

Disseminated tuberculosis and tuberculosis meningitis are acute, severe forms of TB, often occurring soon after primary infection.

They occur most commonly in children and young adults.

These acute forms of TB are often fatal. When this form of disease is suspected, treatment should be commenced immediately without waiting for bacteriological proof of diagnosis. ⁽²⁾

2-2-2Diagnosis of Tuberculosis:

Sputum smear microscopy:

The highest priority for TB control is the identification and cure of infectious cases (patients with sputum smear-positive PTB). ⁽⁴⁾

Sputum smear microscopy allows rapid and reliable identification of patients with pulmonary tuberculosis where are there more than 5000bacilli/ml of sputum.

The reliability of sputum microscopy depends on:

1. sputum collection
2. proper preparation and interpretation of the slide
3. training of lab technician
4. Functional EQA system with blind rechecking.

It's recommended that all patients suspected of PTB should submit two sputum specimens the first sample should be collected when the patient is identified as a presumptive TB case, the second sample is collected in the early morning the day after consultation.

2-Molecular techniques

Molecular (genotypic) test can be used

- To diagnose TB through the amplification of nucleic acids.
- To detect MDR through identifying the genetic mutations.

a- Automated real time PCR (Xpert MTB/RIF)

This test can diagnose TB and resistance to Rifampicin. The test is based on real time PCR ,targeting specific nucleic acid sequences in the M.tuberculosis complex genome ,at the same time it provide information about the most common mutations related to Rifampicin resistance. its highly automated ,which runs in closed systems with one cartridge per sample .each instrument can process 4 samples at one time with a processing time of just under 2 hours.

Sensitivities in smear positive is 98% and 72 in smear negative sample, the Xpert MTB/RIF has a good sensitivity (80%) and excellent specificity(>98%)when performed on cerebrospinal fluid ,lymph node material or gastric fluid. ⁽¹⁾

b- Line Prop Assay (LPA)

- This molecular method have giving fast result within a few hours for smear positive patients it detect resistance to Rifampicin and Isoniazid .
- for smear negative patients ,primary culture is needed prior to testing.

Disadvantage:

1. High cost.
2. High infrastructure requirement.
3. High level of technical training.
4. Risk of cross contamination.

3-Culture:

- Culture allows diagnostic confirmation of TB and is more sensitive than microscopy. 10-100 bacilli/ml are required to obtain a positive result. Only specialized laboratories with regular quality assurance procedure in place can be relied upon for culture.
- After decontamination of the sputum specimen to eliminate other organisms, the sample is centrifuged in special medium, in an incubator at 37C
- Culture of sputum slightly improves the number of positives, but it may take 4-8 weeks before you get the result. With milder disease and fewer TB, the smears may be negative but culture positive.

5-Phenotypic drug susceptibility tests (DST)

Phenotypic drug susceptibility test determines if a strain is resistance to an anti-TB drug by evaluating the growth in the presence of the drug. The laboratory performing phenotypic DST should be specialized in mycobacterial cultures, reliable and subject to external quality assessment (e.g. supranational, national lab) ⁽¹⁾

6-X-ray (radiological) examination:

Chest X-ray is a non-specific investigation for TB. It's recommended in smear negative cases and when TB is suspected in children.

Chest X-ray is essential in the diagnosis of Miliary TB, pleural and pericardial TB.

2-2-3 Treatment of tuberculosis patients

Remember the following important and simple rules.

1. Only use recommended drug combinations.
2. It is vital to convince the patient (and his or her family) that he or she must complete the full course of treatment (6 or 8 months) to avoid relapse. Explanatory leaflets are useful and should be. Even with illiterate patients, someone in the family or village can read the leaflet.
3. It is essential to be kind and sympathetic to the patient. Patients are much more likely to come back and to complete treatment if they believe you are their friend and that you want to help them personally.
4. Remember that new a patient and previously treated cases receive first line treatment with different duration and regimen
5. Never use a fluoroquinolone in the treatment of pneumonia without excluding the possibility of tuberculosis (e.g ciprofloxacin for typhoid fever in patients with respiratory symptoms)
6. **The aims of the treatment are:**
 1. to cure patients with minimal toxic drugs and without interruption of usual life patterns
 2. to prevent death of seriously ill patients
 3. to prevent extensive damage to the lungs
 4. to avoid recurrence of the disease
 5. to prevent the emergence of resistant TB (acquired resistance).
 6. to protect the family and community of the patient from infection.

Standardized regimens

The standardized regimens for anti-TB treatment include five essential medicines designated as “first line”:

1. isoniazid (H),

2. rifampicin (R),
3. pyrazinamide (Z),
4. ethambutol (E)
5. streptomycin (S).

Recommended Daily Doses of First-Line Antituberculosis for Adult ⁽⁵⁾

Drug	Recommended dose	
	Daily	
	Dose and range (mg/kg body weight)	Maximum (mg)
Isoniazid	5 (4–6)	300
Rifampicin	10 (8–12)	600
Pyrazinamide	25 (20–30)	–
Ethambutol	15 (15–20)	–
Streptomycin ^a	15 (12–18)	

New patients:

New patients have never had treatment for TB, or have taken anti-TB drugs for less than 1 month. New patients may have positive or negative bacteriology and may have disease at any anatomical site. ⁽⁵⁾

All new patients with strain susceptible to the first line drugs received the same treatment for six month or 12 month depending on the site involved. ⁽¹⁾

For treatment of new cases of pulmonary or extrapulmonary TB, the standardized regimen consisting of two phases.

The initial (intensive) phase uses four drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) administered for two months. This is followed by a Continuation phase with two drugs (rifampicin and isoniazid) for four months.

Reason for giving four drugs in the intensive phase

Combined treatment is so effective because for each drug there are a very small number of resistant 'mutant' TB. If the drug is given alone, these can survive and multiply to replace the sensitive TB that have been killed by the drug. But the mutants that are resistant to each drug are killed by the other drugs. Even if the patient has been infected by TB resistant to one of the drugs (primary resistance), the other drugs will kill those resistant bacilli. ⁽³⁾

The use of rifampicin requires measures to support patients to adhere to treatment and to prevent the development of rifampicin resistance. Daily treatment may be appropriate if the patient is hospitalized, or if the treatment supporter (health worker, neighbor, community or family member) is able to provide care close to the patient's home.

Previously treated cases

Previously treated patients have received 1 month or more of anti-TB drugs in the past, may have positive or negative bacteriology and may have disease at any anatomical site. ⁽⁴⁾

At the global level, 15% of previously treated patients have MDR, which is five times higher than the global average of 3% in new patients. Specimens (sputum or EPTB site) should be obtained from all previously treated TB patients for rapid molecular-based methods (GeneXpert) or culture and drug susceptibility testing (DST) at or before the start of treatment. DST should be performed for at least isoniazid and rifampicin.

Drug resistance is more likely to develop in previously treated patients who continued to be or who became sputum smear (or culture) positive.

The standard regimen for previously treated consists of:

The initial phase: Five drugs in (rifampicin, isoniazid, pyrazinamide, ethambutol and streptomycin). The initial phase is administered for three months, with all five drugs administered for the first two months. Streptomycin is discontinued after two months, and the four remaining drugs are given in the third month.

Continuation phase: Three drugs in the (rifampicin, isoniazid and ethambutol). The continuation phase is administered for five months, daily.

***Maximum dose of Streptomycin 1gram Monitoring the patient ⁽¹⁾**

- All patients should be monitored to assess their response to therapy .Regular monitoring of patients also facilitates treatment completion and allows the identification and management of adverse drug reactions.
- All patients, their treatment supporters and health workers should be instructed to report the persistence or reappearance of symptoms of TB (including weight loss), symptoms of adverse drug reactions, or treatment interruptions.
- Patient weight should be monitored each month, and dosages should be adjusted if weight changes.

A written record of all medications given, bacteriological response and adverse reactions should be maintained for every patient on the TB Treatment Card

2-2-4 Management of treatment interruption:

If a patient misses an arranged appointment to receive treatment, the NTP should ensure that the patient is contacted within a day after missing treatment during the initial phase, and within a week during the continuation phase. The patient can be traced using the locating information previously obtained, It is important to find out the cause of the patient's absence so that appropriate action can be taken and treatment can continue.

The management of patients who have interrupted treatment takes into consideration several factors, each of which, if present, will necessitate further caution and probably additional treatment:

- The patient is found to be smear- or culture-positive upon returning from default.
- Interruption occurs in the intensive, rather than the continuation, phase.
- Interruption occurs early (rather than later) in the continuation phase.
- The interruption is of long duration.
- The patient is immunocompromised (living with HIV or another condition).
- The patient had poor response to treatment before the interruption.
- Drug-resistant disease is known or suspected.

Anti- tuberculosis drugs: ⁽¹⁾

DESCRIPTION	DRUG
First-line oral anti-TB drugs	Isoniazid Rifampicin Ethambutol Pyrazinamide Rifabutin Rifapentine
Injectable anti-TB drugs (injectable agents or parenteral agents)	Streptomycin Kanamycin Amikacin Capreomycin
Fluoroquinolones (FQs)	Levofloxacin Moxifloxacin Gatifloxacin Ofloxacin

<p>Oral bacteriostatic second-line anti-TB drugs</p>	<p>Ethionamide Prothionamide Cycloserine Terizidone <i>p</i>-aminosalicylic acid <i>p</i>-aminosalicylate sodium</p>
<p>Anti-TB drugs with limited data on efficacy and/or longterm safety in the treatment of drug-resistant TB (This group includes new anti-TB agents).</p>	<p>Bedaquiline Delamanid Linezolid Clofazimine Amoxicillin/Clavulanate Imipenem/Cilastatin Meropenem High-dose isoniazid Thioacetazone Clarithromycin</p>

2-2-5 Treatment regimens in special situations:

Pregnancy and breastfeeding:

Women of childbearing age should be asked about current or planned pregnancy before starting TB treatment. A pregnant woman should be advised that successful treatment of TB with the standard regimen is important for successful outcome of pregnancy. With the exception of streptomycin, the first line anti-TB drugs are safe for use in pregnancy streptomycin is ototoxic to the fetus and should not be used during pregnancy.

A breastfeeding woman who has TB should receive a full course of TB treatment. Timely and properly applied chemotherapy is the best way to prevent transmission of tubercle bacilli to the baby. Mother and baby should stay together and the baby should continue to breastfeed. After active TB in the baby is ruled out, the baby should be given 6 months of isoniazid preventive therapy, followed by BCG vaccination. Pyridoxine supplementation is recommended for all pregnant or breastfeeding women taking isoniazid. ⁽⁴⁾

Liver disorders:

In patients with pre-existing liver disease:

Patients with the following conditions can receive the usual TB regimens provided that there is no clinical evidence of chronic liver disease: hepatitis virus carriage, a past history of acute hepatitis, current excessive alcohol consumption. However, hepatotoxic reactions to anti-TB drugs may be more common among these patients and should therefore be anticipated and closely monitored.

- In patients with unstable or advanced liver disease, liver function tests should be done at the start of treatment, if possible. If the serum alanine aminotransferase level is more than 3 times normal before the initiation of treatment.
- Expert consultation is advisable in treating patients with advanced or unstable liver disease.
- Clinical monitoring (and liver function tests, if possible) of all patients with pre-existing liver disease should be performed during treatment.
- The following regimens should be considered, the more unstable or severe the liver disease is, the fewer hepatotoxic drugs should be used.

*In some cases of concurrent acute (i.e. viral) hepatitis not related to TB or TB treatment, it may be possible to defer TB treatment until the acute hepatitis has resolved.

Renal failure and severe renal insufficiency:

The recommended initial TB treatment regimen for patients with renal failure or severe renal insufficiency is :

- 2HRZE/HR, with adjustment of the dose. Ethambutol, pyrazinamide, should be given three times per week at the following doses: pyrazinamide (25mg/kg), and ethambutol (15 mg/kg). While the HR are on daily basis.
- Because of an increased risk of nephrotoxicity and ototoxicity, streptomycin should be avoided in patients with renal failure. If streptomycin must be used, the dosage is 15 mg/kg, two or three times per week, to a maximum of 1 gram per dose, and serum levels of the drug should be monitored.
- No change in dosing is necessary for Isoniazid and rifampicin because they are eliminated by biliary excretion.
- There is significant renal excretion of ethambutol and metabolites of pyrazinamide, and doses should therefore the dose has been adjusted.
- While receiving isoniazid, patients with severe renal insufficiency or failure should also be given pyridoxine in order to prevent peripheral neuropathy. ⁽⁴⁾

2-3 Infection control

Implementation of TB infection control strategies:

There is a trio of infection control levels, which include :

- 1- Administrative controls.
- 2- Environmental measures.
- 3- Personal protective control.

Administrative controls have the potential to have the greatest impact on preventing transmission of TB in health facilities and should be prioritized in all facilities as these are considered the most effective. These measures prevent

droplet nuclei containing *M. tuberculosis* from being spread in the facility thus reducing exposure of staff and patients to TB infection.

Environmental measures are required to reduce the concentration of droplet nuclei in the air. Unfortunately even the combination of administrative and environmental controls can never provide 100% safety, Respiratory protection is therefore needed in specific areas and during the performance of specific tasks to create the desired level of safety. It is important to note that environmental and personal respiratory controls will not work in the absence of solid administrative control measures.

Package of interventions for TB-IC in health-care settings

Administrative control responsibility:

- 1- Identify and strengthen coordinating bodies for planning, development of national guideline and implementation plan.
- 2- Conduct surveillance system for TB at all levels of health system.
- 3- Engage civil society and address advocacy communication and social mobilization.
- 4- Conduct monitoring and evaluation.
- 5- Develop strategies to promptly identify potentially infectious cases (triage).
- 6- Isolation procedure.
- 7- Minimize time spent by the patient in health care settings.

Environmental controls

- 1- Natural ventilation.
- 2- Mechanical ventilation.
- 3- Ultraviolet germicidal irradiation (UVGI) units.
- 4- Health facility design and renovation.

Personal protective control:

1. Respirators
2. training of HCWs and patients on cough etiquette

Administrative controls:

Administrative control measures serve as the first line of defense against spread of TB in health facilities. These measures include practices and procedures to promptly identify potential and known infectious cases of TB, and separate and treat them with the minimal delay. Administrative controls aimed at reducing TB transmission in health care and congregate settings include triaging, physical separation or isolation of patients or TB suspects, cough etiquette and minimizing time spent in health care settings. The work practice and administrative control measures comprise of:

- Facility infection control plan for the different sections and activities in the health facility;
- Administrative support for procedures in the plan, including quality assurance and local training of staff.
- Education of patients.
- Monitoring and enforcement of adherence to standard operating procedures.

Infection Control Plan:

Every health facility and setting should have a written infection control plan that outlines a protocol for the prompt recognition, separation, investigation and referral of patients with suspected or confirmed TB disease. Areas which should be prioritized in the health facilities include where diagnosed or undiagnosed TB patients are found; namely out-patient screening areas, waiting areas in medical outpatient departments, HIV care clinics, medical wards, TB clinics and TB wards. While the latter have generally been regarded as relatively safe since the patients are on treatment and therefore not infectious, the rising problem of drug resistant

TB demands that high levels of airborne precautions continue to be taken even for patients on treatment.

Early recognition of patients with suspected or confirmed TB disease is the first step in the protocol. A staff member should be assigned to screen for patients with cough of more than 2 weeks duration immediately after they arrive at the facility. These patients should be allowed to enter and register without standing in line with other patients and must be given advice on respiratory hygiene/cough etiquette, and provided with a surgical mask or tissues to cover their mouths and noses. They should then be separated from other patients and requested to wait in a separate well-ventilated waiting area. Their investigation should be expedited in order to minimize their stay in the health facility as well as the need to come back for investigations. After ensuring cough hygiene, identified TB suspects who may have attended the clinic for another reason should preferably promptly receive the services they were originally accessing (e.g. VCT, medication refills) before being investigated for TB.

Sputum collection should always be done in a well ventilated area outdoors and away from other people, not in toilets or other enclosed areas.

The facility IC plan should include the following measures:

- Prompt screening of all patients after arrival at the facility to identify persons with symptoms of TB or those who are being investigated or treated for TB disease;
- Instructing the TB suspects and patients in respiratory hygiene/cough etiquette. This includes instructing them to cover their nose and mouth when coughing or sneezing, and providing face masks or tissues to assist them in covering their mouths. Face masks help prevent the spread of *M. tuberculosis* from the patient to others. Paper tissues are less likely to be used effectively but are less costly and less likely to identify people as TB

suspects with the attendant risk of stigma. Tissues and face masks should be disposed of in waste receptacles. Clients and staff should be encouraged to wash their hands after contact with respiratory secretions. *M. tuberculosis* cannot be spread from the hands, but other serious lung infections such as the flu virus can;

- Placing TB suspects and cases in a separate well-ventilated waiting area such as a sheltered open-air space is ideal in warm climates;
- Speeding up management of these persons so that they spend as little time as possible at the facility;
- Ensuring rapid diagnostic investigation of TB suspects and ensuring that persons reporting TB treatment are adhering with their treatment;
- Using and providing regular maintenance of appropriate environmental control measures;
- Training and educating all staff on TB and the TB-IC plan (should include special risks for TB for HIV positive HCWs and patients, and need for diagnostic investigation for those with signs or symptoms of TB).
- Providing voluntary, confidential HIV counseling and testing for staff with adequate access to treatment;
- Monitoring the TB-IC plan's implementation and correcting any inappropriate practices and enforcing adherence to institutional policies. ⁽⁶⁾

Five steps to prevent transmission of TB in health care settings. ⁽⁶⁾

STEP	ACTION	DESCRIPTION
1	Screen	Early recognition of patients with suspected or confirmed TB disease is the first step in the plan. A staff member should be assigned to screen for patients with cough of more than 2 weeks or who are under investigation or on treatment for TB. These patients should be attended to without delay.
2	Educate	Education on cough hygiene ,covering their mouth and noses on coughing, sneezing and where possible be provided with face masks or tissues for use in this regard. Signs and symptoms of TB , TB/HIV
3	Separate	Separate patients identified other patients promptly. They should wait in a separate, well ventilated waiting area, and instructed in cough hygiene
4	Provide HIV Services	Symptomatic patients should be triaged to the front of the line while seeking services (e.g. HIV counseling and testing, medication refills etc) for prompt attention and reduce time to expose other persons to <i>M. tuberculosis</i> .
5	Investigate for TB	Rapid diagnosis of TB as sputum smear , Gene Xpert

2-4 Tuberculosis and HIV infection

Untreated HIV infection leads to progressive immunodeficiency and increased susceptibility to infections, including TB. HIV is driving the TB epidemic in many countries, especially in sub-Saharan Africa and, increasingly, in Asia and South

America. TB in populations with high HIV prevalence is a leading cause of morbidity and mortality. TB programmes and HIV/AIDS programmes therefore share mutual concerns. Prevention of HIV should be a priority for TB control; TB care and prevention should be priority concerns of HIV/AIDS programmes. TB and HIV programmes provide support to general health service providers. Previously TB programmes and HIV/AIDS programmes have largely pursued separate courses. However, a new approach to TB control in populations with high HIV prevalence requires collaboration between these programmes HIV infection increases the demands on TB programmes, which are struggling to cope with the increased TB case load. The impact of HIV exposes any weaknesses in TB control programmes. The rise in TB suspects is putting a strain on diagnostic services. Extrapulmonary and smear-negative pulmonary TB cases, which are more difficult to diagnose, account for an increased proportion of total cases. There are more adverse drug reactions. There is a higher morbidity and mortality, partly due to other, curable, HIV-related infections. The risk of TB recurrence is higher. The diagnosis of TB in young children has always been difficult and is even more so with HIV. ⁽⁵⁾

Infection with HIV increases the risk of progression of recent M. tuberculosis infection and of reactivation of latent M. tuberculosis infection by 5–15% annually. It also increases the rate of relapse and re-infection. ⁽³⁾

Clinical presentation in HIV –infected patients:

TB is a leading cause of HIV related morbidity and mortality, and it is one of the main opportunistic diseases. According to the WHO clinical staging of HIV/AIDS, HIV patients with pulmonary TB are in clinical stage III and HIV patients with extrapulmonary TB are in clinical stage IV. ⁽¹⁾

The following are differences from the usual way tuberculosis appears in patients without HIV infection.

- Fever and weight loss are more common in HIV-positive than in HIV-negative tuberculosis, On the other hand, cough and blood spitting are less common.
- Sputum smears may be negative despite considerable changes in the chest X-ray.
- Extra-pulmonary disease, especially in the lymph nodes, is more common.

*There is often general lymph node enlargement, which is rare in other forms of tuberculosis.

- The tuberculin test is often negative.
- Disseminated disease is common. TB may be isolated from blood culture (which rarely occurs in ordinary tuberculosis).⁽³⁾
- Tuberculosis may occur at unusual sites (e.g. tuberculomas of the brain, abscesses of the chest wall or elsewhere).

People living with HIV should be screened for TB with a clinical algorithm and those who do report any one of the symptoms of: current cough, fever, weight loss and night sweats should be evaluated for active TB⁽¹⁾

People living with HIV who have an unknown or positive TST status and are unlikely to have active TB should receive at least six months of IPT as part of a comprehensive package of HIV care.

2-4-1 Diagnosis of HIV in TB patients:

The definitive diagnosis of HIV infection rests on a positive HIV test. All confirmed TB patients must be offered HIV counseling and testing. In children under 12 years of age, parents or the legal guardian of the child should be counseled and asked to provide consent for the test. Ideally, the offer of an HIV test should take place during the diagnostic work-up for TB or soon after the initiation of TB treatment.

The benefits of knowing the HIV status include:

- Early diagnosis and management of other HIV-related illnesses.
- Opportunities for prevention of other infections (e.g. using cotrimoxazole).
- Access to ART
- Access to HIV care (psychosocial, nutritional, medical)
- Decreased HIV transmission and re-infection through condom use. ⁽²⁾

2-4-2 TB treatment in people living with HIV: ⁽²⁾

Case	Regimen	
	Intensive phase	Continuation phase
New TB patients ¹ living with HIV	2 HRZE	4 HR
Retreatment TB patients ¹ living with HIV	2 HRZES + 1 HRZE	5 HR

2-5 GeneXpert system

GeneXpert systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence using Real-time reverse transcriptase PCR (RT-PCR) and real-time PCR assay.

2-5-1 Principles of Operation

Polymerase chain reaction is an amplification method that increases quantities of specific copies of DNA or cDNA sequences. Real-time polymerase chain reaction uses fluorescence to detect the specific sequences and includes a mechanism to determine the cycle at which the DNA or cDNA of interest first appears at appreciable copies (called the cycle threshold).

Polymerase chain reaction consists of a series of cycles during which the DNA or cDNA is heated and cooled at specific temperatures for a certain duration.

After **Initial Denaturation** (when the polymerase used to amplify the DNA or cDNA is activated) a cycle occurs, which is usually a three-step process, consisting of:

1. The **Denaturation** step which divides the DNA strands.
2. An **Annealing** step in which a primer is needed by the polymerase to amplify the DNA. The primer will bind to the DNA or cDNA sequence if complementary.
3. The **Extension** step, where the DNA strands will be extended.

2-5-2 GeneXpert Module

The PCR cycle indicates 40 cycles performed by the I-CORE module. The denaturation temperature is 95 °C; the annealing temperature is 60 °C; and, the extension temperature is 72 °C. Each of these temperatures must be held by the module for a specific duration, The initial denaturation takes place for 120 seconds for one cycle. The denaturation (5 seconds), annealing (30 seconds) and extension (10 seconds) steps cycle consecutively forty times before the polymerase chain reaction is finally completed.

Each instrument module contains the following components that enable automated sample processing in the cartridge and filling of the tube with the sample-reagent mixture for PCR:

- **Valve Drive** – Rotates the cartridge valve body to address the different cartridge chambers.
- **Plunger Rod** – Dispenses fluids into the different cartridge chambers.
- **Ultrasonic Horn** – Lyses the sample (if applicable).
- **I-CORE Module** – Performs PCR amplification and detection.

A cartridge loading and unloading mechanism assures the proper movement of the cartridge in the instrument. In addition, the system is designed to perform a self-test before each test starts to verify that the system is functioning properly.

2-5-3 GeneXpert Cartridge

The disposable, single-use GeneXpert cartridge holds the samples and reagents that are to be processed in the GeneXpert Dx system. Each cartridge consists of the following components.

- **Processing Chambers** – Hold the samples, reagents, processed sample, and waste solutions. One chamber is designated as an air chamber to equilibrate pressures within the cartridge.
- **Valve Body** – Rotates and allows fluid to move to different cartridge chambers and to the reaction tube. Within the valve body, the specimen is isolated, PCR inhibitors are removed, and specimens are ultrasonically lysed (if applicable). After the sample is processed, it is mixed with PCR reagents and moved into the integrated reaction tube.
- **Reaction Tube** – Enables rapid thermal cycling and optical excitation and detection of the tube contents. The reaction tube is automatically inserted into the I-CORE module when the cartridge is loaded into the instrument. The cartridge is designed to keep the reagent contained within the cartridge. It is a closed-system vessel.

The Gene Xpert cartridges are not supplied with the system. To order the assay-specific cartridges, contact Cepheid.

2-5-4 I-CORE Module

The I-CORE (Intelligent Cooling/Heating Optical Reaction) module is the hardware component within each instrument module that performs PCR amplification and fluorescence detection. As part of the cartridge load process, the reactor tube is inserted into the I-CORE module. The sample and reagent mixture are pushed from the cartridge into the reaction tube. During the amplification process, the I-CORE heater heats up and the fan cools down the reaction tube

contents. The optical blocks excite the dye molecules and detect the fluorescence emitted.

2-5-5 Heating and Cooling Mechanisms

Within the I-CORE, the heater consists of two ceramic plates that have high thermal conductivity to assure temperature uniformity and rapid heat transfer .

Resistive heater elements are deposited on the ceramic plates using thick film technologies and a thermistor attached directly to each plate monitors its temperature. A high-efficiency fan cools the reaction tube contents by moving ambient air across the heater plates. During thermo cycling, the instrument firmware controls the temperature inside the instrument module. The firmware incorporates a control loop to ensure rapid heating of the plates while minimizing the temperature overshoot around the desired target temperature.

2-5-6 Explanation of Experimental Methods

The GeneXpert system uses real-time polymerase chain reaction (real-time PCR) to detect the organism's DNA of interest.

Real-time polymerase chain reaction is a variant of polymerase chain reaction and uses the same method of PCR with denaturation, annealing and extension at specified time durations to amplify DNA. Real-time PCR uses fluorescence in the form of either intercalating dyes or probes to detect amplified copies of the DNA of interest and to visualize and monitor the amplified product in real time.

In real-time PCR, primers specifically designed to be complementary to the organism's DNA bind to the DNA and extend it. For example in 5'-nuclease technology, a probe which has a reporter dye and quencher attached to it is also complementary to the organism's DNA and binds to the DNA downstream to the primer. The primer and probe together add a higher level of specificity to identify a sequence specific to the organism. As the DNA strand gets extended, the probe is destroyed and the reporter and quencher are dissociated and become free in

solution. The fluorescent signal becomes detected and increases with each amplification.

The cycle at which the fluorescence becomes detected after appreciable copies of the DNA are made is the cycle threshold (Ct). The most basic definition of a cycle threshold is the first cycle in which there is significant increase in fluorescence above the background fluorescence. The real-time PCR generates a growth curve with number of cycles on the x-axis and fluorescence on the y-axis. The increase in fluorescence is proportional to the amount of amplicon generated and can be used to define cycle threshold. As the growth curve plateaus, it will reach a fluorescent end-point at which other factors are rate-limiting. If the organism's DNA is not detected by the real-time PCR reaction, the growth curve will be flat.

Note:-

*Genexpert can be use all fluid of the body, such as cysctic fluid, lymph node fluid, sliva...etc ,except urine and stool.

* Genexpert need at least 131 bacilli of Mycobacterium tuberculosis for detection. ⁽⁷⁾



GeneXpert Dx System



GeneXpert Cartridge

2-5-7 previous study

In study done by Mekonnen F.2015 .

Back ground:

Multi drug resistant tuberculosis (MDR-TB) is an emerging challenge for TB control programs globally . according to WHO ,2012 report Ethiopia stands 15(th) out of the 27 high priority countries in the world and 3(rd) in Africa . the aim of this study was to assess the prevalence of MDR-TB and associated risk factors in west Armachiho and Metema districts of Northwest Ethiopia .

Method:

Across sectional study was conducted in Armachiho and Metema districts between February 01 and June 25, 2014. A total of 124 pulmonary tuberculosis patients were included in the study.

Socio-demographic and possible risk factor data were collected using questionnaire . test performed by using Genexpert MTB/RIF. Data were analyzed using statistical package SPSS version 20 . P- value <0.05 were considered as statistically significant .

RESULTS: 124 samples of pulmonary TB patients , 117 (94.4%) were susceptible to Rifampicin , while 7 (5.7%) were confirmed to be resistant to Rifampicin and Isoniazid .

Conclusion:

The overall prevalence of MDR-TB was 5.7% among cases at five health centers and a history of previous treatment was found to be a risk factor for being infected by an MDR-TB strain . there fore , maximizing early case detection and treatment , strengthening TB infection control activities and proper implementation of DOTS are recommended to reduce the burden of MDR-TB.

In study done by Ikuabe PO,2016

Introduction :

The diagnosis of tuberculosis and its treatment is challenging in resource-limited settings . The growth and speed of MDR-TB in high burden countries like Nigeria is a growing concern .This study is aimed at determining the prevalence of rifampicin resistance in sputum specimens of patients with pulmonary tuberculosis in Yenagoa ,Nigeria .

Method:

A descriptive survey of all consecutive sputum specimens of adults greater than 15 years of age that presented to the Tuberculosis Referral Hospital laboratory were subjected to the automated Genexpert test between January and December 2016 .

Results:

All 446 specimens were tested using Genexpert automated system . 102 (22.9%) of the sputum specimens were positive for Mycobacterium tuberculosis , with 15(14.7%) showing rifampicin resistance .

Conclusion :

There was significantly high prevalence of MDR-TB much higher than the World Health Organization (WHO) prediction of 3.2-5.4% for Nigeria.

Chapter Three

Materials and Methodology

3-Material and Methodology

3:1: Study Design:

This Study was prospective laboratory base descriptive study , conducted in Atbara town during the period between January-2018 to June -2018.

3:2: Study area:

The study was conducted in Atbara central diagnostic lab receive specimens for diagnosis and identification of resistant bacilli collected from different centers distributed in different locality of the state.

3:3: Study Population Sampling:

150 sputum samples were obtained during the period between January-2018 to June-2018. The patients referred to central lab either suspected new cases of pulmonary tuberculosis or suspected multidrug resistance patients .

The average age were ranged from (2-90) years. The mean age was 46 year. The PCR screen was revealed 66.6% (100/150) positive pulmonary tuberculosis.

3:4: Material and instrument:

- Container.
- NaoH solution.
- Centrifuge.
- Pasteur pipettes.
- Cartridge barcode.
- GeneXpert machine.

3:5: Principle of method:

GeneXpert systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence using Real-time reverse transcriptase PCR (RT-PCR) and real -time PCR assay.

Each GeneXpert module processes one sample. The sample and applicable reagents are inserted in to a GeneXpert cartridge and a test is created on the GeneXpert systems to run the test. The cartridge is then loaded in to an available instrument module and then is started. ⁽⁷⁾

3:6: Steps of GeneXpert:

1. Sputum sample collected in vacutainer (if delaying keep sample in refrigerator).
2. Add NaoH solvent in sputum sample in percent 2:1, and shake well.
3. Sample Incubated 15 minutes, after that we took not less than 2 ml from the sample in cartridge, then enter cartridge in GeneXpert machine.
4. Moves the sample and reagents into different chambers in the cartridge for sample preparation.
5. Hydrates the reagent beads.
6. Performs probe checks to ensure that the sample preparation is successful (only if the assay definition requires this step).
7. Moves the sample and the reagent mixture which contains reverse transcription (if applicable) and real-time PCR specific components into the reaction tube.
8. Starts the RT-PCR (if applicable) and PCR cycles and real-time detection .

The GeneXpert Dx System uses the I-CORE module heating and fan cooling system to perform the real-time polymerase chain reaction used to exponentially amplify and detect the organism's DNA or cDNA sequence of interest.

3:7: Data collection tools:

Data was collected by using questionnaires.

3: 8: Data analysis:

The gathered data was analyzed with SPSS soft ware program in computer.

3:9: Data presentation:

Data was presented in the form of tables.

Chapter Four

Results

4-Results

This Study was prospective laboratory base descriptive study, the study was conducted in Central Diagnostic Laboratory in Atbara , received specimens for diagnosis and identification of suspected cases of pulmonary tuberculosis and resistant bacilli collected from different centers distributed in different locality of the state .

150 sputum samples were obtained during the period between January to June-2018. The patients referred to central lab either suspected new cases of pulmonary tuberculosis or suspected multidrug resistance patients . The average ages were ranged from 2-90 years. The mean age was 46 year. The PCR screening test was revealed 66.6% (100/150) positive for Mycobacterium tuberculosis.

Table (4-1) Shows distribution of tuberculous patients according to the gender:

Sex	Frequency	Percentage
Male	75	75
Female	25	25
Total	100	100

Table (4-2) Shows distribution of tuberculosis according to the age:

Age group	Frequency	Percentage
1-16 years	8	8
17-35 years	49	49
Above35 years	43	43
Total	100	100

Table (4-3) Shows distribution of tuberculosis according to the social status:

Social status	Frequency	Percentage
Single	38	38
Married	54	54
Divorced	8	8
Total	100	100

Table (4-4) Shows distribution of tuberculosis according to the geographical area:

Geographical area	Frequency	Percentage
Rural	67	67
Urban	33	33
Total	100	100

Table (4-5) Shows distribution of tuberculosis according to the educational levels:

Educational levels	Frequency	Percentage
Illiterate	27	27
Primary	41	41
Secondary	22	22
Collegiate	10	10
Above collegiate	0	0
Total	100	100

Table (4-6) Shows distribution of tuberculosis according to the occupation:

Occupation	Frequency	Percentage
No job	30	30
Student	8	8
Free business	46	46
Employee	16	16
Total	100	100

Table (4-7) Shows distribution of clinical complain in patients in rolled in the study:

Clinical complain	Frequency	Percentage
Night sweats	1	1
Cough for 2weeks or more	55	55
Bloody sputum	29	29
Weight loss	15	15
Total	100	100

Table (4-8) shows duration of patients clinical complain before management:

Duration time to start treatment	Frequency	Percentage
Days	1	1
Weeks	56	56
Months	43	43
Total	100	100

Table (4-9) Shows the relation between tuberculous patients with HIV co-infection:

HIV	Frequency	Percentage
Positive	1	1
Negative	2	2
Unknown	97	97
Total	100	100

Note:

.1% of TB patients positive for HIV.

.2% of TB patient negative for HIV.

.97% not screened before .

Table (4-10) Shows distribution of chronic diseases such as (diabetes-hypertension.....) in tuberculous patients:

Other diseases	Frequency	Percentage
Yes	28	28
No	72	72
Total	100	100

Table (4-11) Shows distribution rate of recurrence:

Recurrence	Frequency	Percentage
Yes	59	59
No	40	40
Unknown	1	1
Total	100	100

Table (4-12) Shows distribution of recurrence cases of tuberculosis in relation to the suspected cause (59 patients):

Causes of recurrence	Frequency	Percentage
Failure of treatment	6	10.2
Relapse	30	50.8
Lost of follow up	23	39
Total	59	100

Table (4-13) Shows distribution of MDR patients among TB patients:

Patients	Frequency	Percentage
TB	90	90
MDR	10	10
Total	100	100

Table (4-14) Relation between Cases s and Previous treatment:

Cases	Previous treatment		NO (percent)	P.value
	Yes	No		
MDR	5(5%)	5(5%)	10 (10%)	0.000
TB	49(49%)	41(41%)	90 (90%)	
Total	54(54%)	46(46%)	100 (100%)	

P value=(0.000) is Significant.

Chapter Five

Discussion

Conclusion

Recommendations

5-1 Discussion

This Study was prospective laboratory base descriptive study, conducted in Atbara town during the period between January to June 2018. 150 sputum samples were obtained during the period between January to June-2018. The patients referred to central lab either suspected new cases of pulmonary tuberculosis or suspected multidrug resistance patients .

The average age were ranged from 2-90 years, Mean age was 46 year. The PCR screening test was revealed 66.6% (100/150) positive for Mycobacterium tuberculosis.

Tuberculosis was higher in males 75% (75) than females 25% (25) , male /female ratio was (3:1), most of the cases encountered in the age group from 17 to 35 years 49% (49/100), the frequency of tuberculosis was more in married individual 54% (54) than singles, patients from rural area were 67% (67) while 33%(33) from urban area, the frequency of patients symptoms in the study were cough for 2 weeks or more 55% , haemoptysis 29% , weigh loss 15% , and night sweat 1%. The patients were diagnosed after appearance of symptoms 56% within weeks, 43% within months ,and 1% within days, 97% of patients unscreened for HIV, the reminder patients 1% was HIV seropostive ,and 2% were negative, superimposed diseases such as diabetes and hypertension were encountered in 28% of the patients included in the study , the study revealed that recurrent rate of infection was 59% (59/100) due to treatment failure or lost of follow up . MDR for tuberculosis was 10%(10/100). The study revealed there was a significant difference between new and recurrent cases of tuberculosis patients, p.value = 0.000. In this study MDR was 10% (10), which was higher with the study conducted by Mekonnen F.2015 in west Armachiho and Metema districts of northwest Ethiopia, revealed 124 samples of pulmonary TB patients , 5.7% (7)

were confirmed to be resistant to Rifampicin and Isoniazid . the study revealed lower frequency of MDR than study conducted by Ikuabe Po, during 2016 in Yenagoa-Nigeria, revealed 446 samples were tested by Genexpert automated system ,22.9%(102) s of the sputum specimens were positive for Mycobacterium tuberculosis ,with14.7% (15) Rifampicin resistant .

5-2 Conclusion

We concluded that :

.Gene Xpert MTB/RIF is useful tool for detection of TB and RIF resistance on primary respiratory specimens.

.This study clear the possible risk factors such as (sex, ages, irregular treatment...).

.we found that MDR relatively high (10% of patients in this study are MDR, and 90% are MTB).

5-3 Recommendations

. Health education program about tuberculosis especially in rural area.

.MTB patients follow up by using Genex pert technique, and MDR patients through culture.

.We recommend to use Genex pert test in all towns for diagnosis and follow up of tuberculosis in the state.

بسم الله الرحمن الرحيم

استبيان

هذا الاستبيان تم اعداده لمرضى الدرن

Questionnaire

This questionnaire related to patients of tuberculosis.

رقم المريض:.....

Patient No:.....

1/ النوع:

أ.ذكر () ب.أنثى ()

2/ العمر:

أ.1-14 سنة () ب.15-35 سنة () ج.أكثر من 35 سنة ()

3/ الحالة الاجتماعية:

أ. أعذب () ب.متزوج () ج. مطلق ()

4/ المنطقة الجغرافية:

أ.ريف () ب. حضر ()

5/ المستوى التعليمي:

أ.أمي () ب. ابتدائي () ج. ثانوي () د. جامعي () هـ.فوق جامعي ()

6/ المهنة:

أ.غير عامل () ب.طالب () ج.أعمال حرة () د.موظف ()

7/ الأعراض المشتكى منها:

أ.ألم بالصدر () ب.تعرق ليلي () ج. كحه لمدة أكثر من أسبوعين ()

د.بلغم مصحوب بدم () هـ.فقدان وزن () و.حمى ()

ز.تعب وإرهاق () ر.أخرى ()

8/ المدة التي أستغرقتها منذ طلبك الرعاية من المؤسسة الصحية إلي أن تم تشخيصك بالدرن :

أ.أيام () ب.أسابيع () ج.أشهر ()

9/فحص الايدز:

أ.إيجابي () ب.سلبى () ج.غير معروف ()

10/ هل تعاني من أمراض أخرى(ضغط - سكر.....)؟

أ.نعم () ب.لا ()

11/ هل سبق علاج المريض من الدرن ؟

أ.نعم () ب.لا () ج.غير معروف ()

12/ إذا كان الجواب (نعم) وضح نوع الحالة ؟ وهل المريض من المخالطين؟

أ.فشل () ب.انتكاسة () ج.فقد متابعه () د.مخالطين ()

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