EVALUATION OF GENEXPERT MTB/RIF ASSAY FOR RAPID DIAGNOSIS OF TUBERCULOSIS AND DETECTION OF RIFAMPICIN RESISTANCE IN PULMONARY AND EXTRAPULMONARY SPECIMENS AT MWANANYAMALA REGIONAL REFERRAL HOSPITAL, DAR-ES-SALAAM, TANZANIA.

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ABSTRACT
This study was conducted to evaluate the use of GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampicin resistance in pulmonary and extra pulmonary specimen among the patients at Mwananyamala Regional Referral Hospital from October 2015 to May 2016, Dar-es-salaam, Tanzania. The study was prospective and retrospective study, hospital based. The study consists of 505 individuals in which male were more 48.3% and females 51.7%. All age groups were included from 1 to 90 years. There are pulmonary and extra pulmonary specimens 94.5% and 5.5% respectively. Out of 505 specimens 19.6% of samples positive for M. tuberculosis and reaming 80.4% were negative. Out of 99 samples of positive M. tuberculosis from that 85 of them were sensitive that is 85.9% and 14.1% were resistance to rifampicin.

KEYWORDS: GeneXpert MTB/RIF.

INTRODUCTION
Tuberculosis (TB) is a disease caused by infection from the bacteria Mycobacterium tuberculosis. If not treated properly, TB can be fatal (CDCP, 2009). Currently, the World Health Organization estimates that over 13 million people have TB and about 1.5 million die each year from the disease. Tuberculosis most commonly affects the lungs (pulmonary TB). Patients with active pulmonary TB usually have a cough, an abnormal chest x-ray, and are infectious. TB can also occur outside of the lungs (extrapulmonary), most commonly in the central nervous, lymphatic, or genitourinary systems, or in the bones and joints (CDCP, 2009). Tuberculosis which occurs scattered throughout the body is referred to as miliary TB. Extrapulmonary TB is more common in immunosuppressed persons and in young children (ALA, 2009).

In Tanzania Tuberculosis continues to be among the major public health problems in the country. The number of tuberculosis cases has steadily increased from 11,753 in 1983 to about 65,665 in the year 2004, almost six-fold. The majority of cases appear in young adult population groups aged 15-45 years, the same age group affected by HIV/AIDS. The rapid increase of tuberculosis in Tanzania is mainly attributed to the HIV epidemic, but factors like population growth and urban overcrowding have also contributed (Manual of Tanzania, 2006).

M. tuberculosis is a very slow-growing, intracellular organism. Consequently, treatment requires the use of multiple drugs for several months (Goodman and Lipman, 2008). With appropriate antibiotic treatment, TB can be cured in most people. Treatment usually combines several different antibiotic drugs that are given for at least 6 months, sometimes for as long as 12 months. However, many M. tuberculosis strains are resistant to one or more of the standard TB drugs, which complicates treatment greatly (NIAID, 2009). Currently, there are 10 drugs approved by the U.S. Food and Drug Administration for the treatment of TB. Of the approved drugs, isoniazid (INH), rifampin (RIF), ethambutol (EMB), and pyrazinamide (PZA) are considered first-line antituberculosis agents. These four drugs form the foundation of initial courses of therapy.

Drug-resistant TB is major problem for the treatment of the disease. Multidrug-resistant TB (MDR-TB) is defined as disease caused by TB bacilli resistant to at least isoniazid and rifampicin, the two most powerful
anti-TB drugs (WHO, 2009). MDR-TB is intrinsically resistant to drugs but its resistance can be exacerbated by inconsistent or partial treatment. When patients do not take all their medicines regularly for the required period because they start to feel better, drug-resistant bacteria can arise. While drug-resistant TB is generally treatable, it requires extensive chemotherapy (up to two years of treatment) with second-line anti-TB drugs. These second line drugs produce more severe adverse drug reactions more frequently than the preferred first line drugs.

GeneXpert MTB/RIF is an automated, cartridge-based nucleic amplification assay for the simultaneous detection of TB and rifampicin resistance directly from sputum in less than two hours. The technology is based on the GeneXpert platform and was developed as a partnership between the Foundation for Innovative New Diagnostics (FIND), Cepheid Inc. and the University of Medicine and Dentistry of New Jersey, with support from the US National Institutes of Health. WHO recommended use of the technology in December 2010 and is monitoring the global roll-out of the technology to promote coordination (WHO, 2011). Xpert is more sensitive than sputum smear microscopy in detecting TB, and has similar accuracy as culture (Boehme et al., 2010).

In many parts of the world as well as Tanzania TB was recognized out of control including multi drug resistance. The study aimed to investigate the evaluation of the Gene Xpert MTB/RIF assay for rapid diagnosis of tuberculosis in pulmonary and extra pulmonary specimen. Also, it evaluates the detection of rifampicillin resistance in pulmonary and extra pulmonary specimen. Furthermore, this study added more knowledge to the diagnosis of tuberculosis by using GeneXpert method.

**METHODOLOGY**
The data were collected from pulmonary and extra pulmonary specimens from TB department in the hospital. In the study the *Mycobacterium tuberculosis* and rifampicillin resistance detected by GeneXpert and data analysis in the study was the goal of study.

**Study Design**
The study was prospective and retrospective study, hospital based.

**Study Area**
The study area was at Mwananyamala Regional Referral Hospital, Dar es salaam, Tanzania.

<table>
<thead>
<tr>
<th>DATE</th>
<th>S.NO</th>
<th>PATIENT ID</th>
<th>AGE</th>
<th>SEX</th>
<th>Mycobacterium tuberculosis detected</th>
<th>Rifampicillin resistance detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Study Population**
People suspected with tuberculosis and those with tuberculosis attending TB clinic at MRRH from October 2015 to May 2016.

**Inclusion and exclusion criteria**

**Inclusion**
All detected *Mycobacterium tuberculosis* and rifampicillin resistance that has been performed from October 2015 to May 2016.

**Exclusion**
All detected specimens outside the specified study period.

**Sampling and Sample Estimation**

**Sampling method**
Systematic sampling method was used and data recorded from the patients.

**Sampling estimation**
Sample size based on the minimum sample size required to have 90% of positive results, 5% level of significance and confidence interval of 95%.

This was calculated as the formula below

\[ N = \frac{Z^2 \times p (1-p)}{E^2} \]

Where,

- \( N \) = minimum sample size
- \( Z \) = standard normal deviation
- \( p \) = the proportion from literature review
- \( E \) = maximum likely error tolerance

**Data Collection**

**Method**
Data were analyzed from the files of patients attending MRRH who suspected to have TB and those who already had the disease.

This will be observation and study of recorded patients results of specimens received.

**Data collection tool**
All the data were collected from then patient’s results from the TB department in the laboratory, and the following table was used.
Data analysis
Computer was used to analyze data where by SPSS program. Differences in prevalence, antibiotic susceptibility and antibiotic resistance were calculated using chi($\chi^2$) square method.

Ethical Consideration
- Permission to check the files was obtained from the department at MRRH.
- No patient’s names are used; instead patents ID code numbers are used.
- All information obtained will be treated as confidential.

The research proposal was submitted to the ethical committee for approval according to guidelines of the ethical committee.

RESULTS

Demographic Characteristics
Table 1 shows distribution table according to gender/sex of respondent. The findings shows that in 505 collected data of people attended tuberculosis section there were more females than males, 261 (51.7%) female and 244 (48.3%) male.

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>261</td>
<td>244</td>
<td>505</td>
</tr>
<tr>
<td>Percentage</td>
<td>51.7</td>
<td>48.3</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2 shows the age frequency from one to ninety years. From the study of 505 cases, 208 were from age group 1 to 10 equals to 41.2%, from age group 11 to 20 there were 57 (11.3%) patients, 51(10.1%) were aged from 21 to 30 years, 91 individuals out of 505 were aged from 31 to 40 that is 18%.9.9% among 100% which equals to 50 aged from 41 to 50,35 attendants (6.9%) aged from 51 to 60,6 attendants(1.2%),4 equals to 0.8% and 0.6% 3 attendants ranged from age group 61 to 70,81 to 90 respectively.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>1-10</th>
<th>11-20</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>51-60</th>
<th>61-70</th>
<th>71-80</th>
<th>81-90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>208</td>
<td>57</td>
<td>51</td>
<td>91</td>
<td>50</td>
<td>35</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Percentage</td>
<td>41.2</td>
<td>11.3</td>
<td>10.1</td>
<td>18</td>
<td>9.9</td>
<td>6.9</td>
<td>1.2</td>
<td>0.8</td>
<td>0.6</td>
</tr>
</tbody>
</table>

As shown in the table 3, Out of 505(100%) specimens 477(94.5%) were pulmonary while 28(5.5%) were extrapulmonary.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pulmonary</th>
<th>Extra pulmonary</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>477</td>
<td>28</td>
<td>505</td>
</tr>
<tr>
<td>Percentage</td>
<td>94.5</td>
<td>5.5</td>
<td>100</td>
</tr>
</tbody>
</table>

In 505 samples of the attendants 99 of them were positive that is 19.6% and 406 were negative 80.4%, as shown in table 4.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>406</td>
<td>99</td>
<td>505</td>
</tr>
<tr>
<td>Percentage</td>
<td>80.4</td>
<td>19.6</td>
<td>100</td>
</tr>
</tbody>
</table>

Table shows the Frequency and percentage of sensitive and resistance. Out of 505 samples 99 samples were positive 85 of them were sensitive that is 85.9% and 14 (14.1%) were resistance.

<table>
<thead>
<tr>
<th>Sensitive</th>
<th>Resistance</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>85</td>
<td>14</td>
</tr>
<tr>
<td>Percentage</td>
<td>85.9</td>
<td>14.1</td>
</tr>
</tbody>
</table>

DISCUSSION
The study shows there were more females than males who attended the tuberculosis section 51.7% female and male were 48.3%. All ages were included from 0 to 90 years, age group 1 to 10 has 41.2% compared to other age groups because in MRRH samples that are processed...
in geneXpert are children samples mostly the elders are processed by staining technique because of the shortage of geneXpert machine. The elders samples are processed for follow up and immunocompromised patients (HIV/AIDS) patients.

GeneXpert process both pulmonary and extra pulmonary specimens but pulmonary specimens are many compared to extra pulmonary specimens in which 94.5% are pulmonary and extra pulmonary specimens are 5.5%. Pulmonary tuberculosis affects the lungs and is the commonest form of the tuberculosis and this is the infectious form of the disease.

Among 505 samples (100%) 406(80.4%) were negative 99(19.6%) were positive. The study shows that only 19.6% of the study have been affected by tuberculosis awareness of the disease on the transmission of the disease taking care of affected individuals, availability of treatment to the victims government support on free treatment to the affected and free supply of medicine and education that is given to the society have contributed to the decrease of the rate of disease. 14.1% of the positive samples were rifampicin resistance while 85.9% were sensitive.

This study has shown that the rate of tuberculosis in MRRH for 8 months is less than 20 percent but this is only for risk population that is for children, immune compromised people and for follow up because only these groups have been given priority of using gene Xpert machine. The study shows that Tuberculosis infects all gender male and female 48.3% and 51.7% respectively and all age groups.

The geneXpert has short turnaround time and detect the resistance of rifampicin resistance unlike other technique and from the study 5.5% of positive sample were resistance to rifampicin hence early treatment to the patients. GeneXpert detects mycobacterium tuberculosis, from the study 19.6% was detected in both pulmonary and extrapulmonary specimen.

In conclusion, Xpert is not a panacea. Its implementation presents major challenges, particularly related to cost and infrastructure, which call for a thoughtfully phased and careful introduction.

REFERENCES