

## Special Article - Mycobacterium Tuberculosis

# Prevalence of Multidrug Resistant Mycobacterium tuberculosis and Risk Factors among Youth Attending MDR-TB Unit in Mulago Hospital

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## Abstract

**Background:** Multidrug resistant tuberculosis is the TB bacilli revealing resistance to at least isoniazid and/or rifampicin drugs. Tuberculosis is one of the world's leading causes of adult morbidity and mortality with an estimated 8.8 million incident cases and 1.4 million deaths in 2010 mainly in Sub-Saharan Africa. The etiologic agents are *Mycobacterium tuberculosis* in human and *Mycobacterium bovis* in cattle and mainly attack the lungs. Tuberculosis is primarily an air borne disease, the bacteria are transmitted from person to person through tiny microscopic infective droplets when a TB infected patient coughs, sneezes, speaks, sings or laughs.

**Methods:** Sputum samples were collected from suspected TB patients who visited Mulago National Referral Hospital (ward 5 and 6) in Kampala, Uganda. Ziehl-Nelsen technique was performed on the sputum samples and then after subjected to the gene-Xpert machine which simultaneously detects *Mycobacterium tuberculosis* complex and resistance to Rifampicin in less than 2 hours using the real time polymerase chain reaction (real time-PCR) technique for the detection and amplification of the resistant *Mycobacterium tuberculosis* gene.

**Results:** From a total of 177 sputum samples analyzed for MDR-TB, 1.69% (3/177) was positive for multidrug resistant tuberculosis revealing a prevalence rate of 1.69%. The risk factors ranged from age, sex, patients with TB re-infections or relapse cases, HIV, TB, alcohol, religion, tribe and the occupation of the patients. This study however, revealed that patients with TB re-infections or relapse cases and smokers were comparatively at higher risk of developing MDR-TB as compared to the other factors.

**Conclusion:** Although the prevalence rate is low, MDR- *M. tuberculosis* is still a problem in Uganda. Smokers and relapse cases have higher chances of contracting MDR-*M. tuberculosis*.

**Keywords:** Prevalence; MDR-*Mycobacterium tuberculosis*; Ziehl Nielsen; RT-PCR

## Abbreviations

AIDS: Acquired Immune Deficiency Syndrome; HIV: Human Immunodeficiency Virus; MDR-TB: Multidrug Resistant Tuberculosis; PCR: Polymerase Chain Reaction; PLHIV: People Living with Human Immunodeficiency Virus; RIFAM: Rifampicin Drug; ZN: Ziehl Neelsen

## Background

Multidrug resistant *M. tuberculosis* is defined as TB bacilli revealing resistance to at least isoniazid and/or rifampicin whereas extensively drug resistant tuberculosis is the TB bacilli that develops resistance to at least isoniazid and rifampicin and at least one of the second line anti TB injectable drugs like Kanamycin, Amikacin or Capreomycin [1].

The current worldwide burden of Multidrug resistant Tuberculosis is approximately 500,000 cases, this means that almost a third of

the world's population is infected and 9 million new cases of active *Mycobacterium tuberculosis* with about 2-3 million deaths occurring annually (95%) in developing countries [2]. This estimates to about 4000-5000 deaths every day mainly because of drug resistance due to inappropriate use of anti-TB drugs by patients with drug susceptible strains [3], therefore globally, Multidrug resistant TB is at 3.4% and 19.8% for all new and previously treated TB cases respectively, with a general increase in Botswana, Peru, the Republic of Korea and a decline in Estonia, Latvia and the United States of America [4].

However it remains uncommon in most parts of Africa especially in sub Saharan Africa due to inadequate laboratory services that makes it difficult to estimate the actual burden of MDR-TB even if surveillance showed a prevalence of 1-4% among new cases and 4-17% among previously treated TB cases in Cote d'Ivoire, Ethiopia, Madagascar, Rwanda, and Senegal [5]. In Uganda, MDR-TB prevalence was at 0.5% and 4% among new and previously treated TB cases respectively with about 12.7% prevalence in Kampala and a low

resistance to second line drugs [6].

The discovery of effective anti-TB drugs in the 1940s made TB curable. However, worldwide surveys documents drug resistant TB as a rapidly increasing problem hence requiring a rather complicated second line drugs for the treatment of the multidrug resistant TB some of which are only injectable, less efficacious, more toxic and more expensive than the first line agents. Development of anti-TB drugs resistance can occur due to mycobacterium genetic factor, previous anti-TB treatment-related factor and also migration, age, sex, HIV infection, and socio-economic factors are associated with the increased prevalence of MDR-TB. Not only does HIV increase the risk of reactivating latent *M. tuberculosis* infection, but it also increases the risk of rapid TB progression soon after infection or re-infection with the bacteria.

The mortality rates in MDR/XDR-TB patients co-infected with HIV/AIDS was at 80% for XDR-TB patients and 63% for MDR-TB following diagnosis and because of HIV spreads into Asia and eastern Europe where drug resistant is more firmly established, the number of persons infected with both HIV and MDR-TB is likely to increase. Generally, individuals who are infected with *Mycobacterium tuberculosis* have approximately 5-10% lifetime risk of developing TB disease, however in individuals with HIV-infection/AIDS, the risk is at 5-10% per year and this contributes HIV-infection/AIDS to the outbreak of MDR-TB and XDR-TB. Therefore the burden of TB increases generally with increase in HIV infection [7]. This study aimed at determining the prevalence and risk factors predisposing the youth to multidrug resistant *M. tuberculosis* in Kampala, Uganda.

## Methods

### Study site and design

A cross sectional study was carried out at Mulago national referral hospital (wards 5 and 6) in Kampala, Uganda.

This study involved sample collection from suspected TB patients who visited the hospital and questionnaires were administered to them which helped to obtain pertinent information about their life style and environment that may have had any influence to their contraction of MDR-TB. The study was carried out in MDR-TB wards in Mulago national referral hospital. All participants recruited in this study were referral cases between the ages of 18 and 35 years. Informed consent was obtained from the patients before enrolment into the study.

### Sample and data collection

The sample size was determined to be 177 subjects. It was calculated using an acceptable error of 5% and the estimated prevalence of the disease at 12.7% [8] and was based on a standard sample size formula for cross-sectional studies (Martin, 1998). Questionnaires were administered to the study subjects and these included age, sex, tribe and religion, whether they had been infected previously with TB, TB treatment history, HIV status and previous contact with MDR-TB patients. History of smoking, level of education, alcoholism, occupation and financial status before and after contracting the disease were also recorded.

Standard sputum sample collection procedures were strictly followed during sample collection to avoid contamination of samples.

Records of their symptoms and physical examination were taken. A single sample of about 5mls was collected using a wide mouth, sterile, leak proof sputum sample collection container from the patients in the area designated for sample collection in the vicinity of the ward by following the instruction given strictly early morning before brushing their teeth. After collection the samples were brought to the laboratory immediately for labeling with the laboratory number and processing under a bio-safety cabinet level II then later examined.

### Macroscopic sputum examination

The sputum sample appearance was reported as salivary, mucoid, muco-purulent or blood stained. The samples that appeared white/yellow meant that they had large numbers of white cells/neutrophils and eosinophils, green/yellow samples indicated presence of pus, bacteria and cellular debris and this were described as purulent or muco-purulent and those streaked with blood were called blood stain samples [9].

### Microscopic sputum examination

Ziehl-Neelsen staining technique was used to demonstrate the presence of the acid fast bacilli. A new scratch free microscope slide was cleaned using gauze or dry cotton wool and labeled with the district code, unit code and patients laboratory number using a diamond pencil or lead pencil on the frosted side of the slide. The portion of the sputum that appeared abnormal was picked up using a sterile wire loop or an applicator stick and spread in the middle of the slide to a size of about 1 by 2 cm, allowed to air dry and the wire loop rinsed in 5% Lysol solution in a beaker which removed traces of sputum from it and later heated in a Bunsen burner flame to sterilize the loop. All this processes was carried out from the bio-safety cabinet which prevented contamination of the environment with the aerosols coming from the sputum during processing.

The dried smear was then fixed using 70% alcohol for about 2-3 minutes and later stained by flooding the slide with strong carbol fuchsin and heated using a cotton wool socked in alcohol and wrapped around a pair of tongs or an applicator stick until vapor arose from the stain and kept warm for 15 minutes. The slides was then picked using a pair of forceps, rinsed in a thin stream of water, decolorized with 20% Sulphuric acid for 2 minutes and later counter stained using 0.1% Methylene blue for 1 minute. The slide was then washed in a thin stream of water, dried at room temperature and examined with immersion oil using  $\times 100$  objectives of the microscope.

### Determination of *M. tuberculosis* resistance to rifampicin and/or isoniazid

Sputum sediments were prepared by centrifugation and re-suspended in 67mM Phosphate/H<sub>2</sub>O buffer for testing using Xpert MTB/RIF Assay. After re-suspension, at least 0.5 mL of the resuspended sediment was used for the Xpert MTB/RIF Assay. The cartridge was labeled on the sides with the sample ID ensuring that the existing 2D barcode on the cartridge was not covered. Specimens with obvious food particles or other solid particulates were rejected.

GeneXpert was used to detect *M. tuberculosis* complex and resistance to Rifampicin using Real-time Polymerase Chain Reaction (Real-time PCR) technique for the amplification of the resistant *Mycobacterium tuberculosis* gene. At least 0.5 mL of the total re-suspended pellet was transferred to a conical, screw-capped tube

**Table 1:** Analysis of risk factors for multi-drug resistant *M. tuberculosis*.

RISK FACTOR	NUMBER OF CASES (out of 177(%))	ODDS RATIO, AND 95% CONFIDENCE INTERVAL	P VALUE
<b>AGE</b>			
18-25	99(55.9%)	1	
26-35	78(44.1%)	1.00(0.97- 1.03)	0.956
<b>SEX</b>			
Female	91(51.4%)	1	
Male	86(48.6%)	0.96(0.93- 1.00)	0.023
<b>SUSPECT</b>			
Relapse	5(2.8%)	1	
New	172(97.2%)	0.55(0.50- 0.60)	0
<b>HIV STATUS</b>			
Neg	41(23.2%)	1	
Pos	136(76.9%)	1.01(0.97- 1.05)	0.515
<b>TUBERCLOSIS</b>			
Neg	152(85.9%)	1	
Pos	25(14.1%)	0.97(0.92-1.02)	0.232
<b>ALCOHOL</b>			
No	165(93.2%)	1	
Yes	12(6.8%)	0.99(0.92-1.06)	0.732
<b>SMOKING</b>			
No	174(98.3%)	1	
Yes	3(1.7%)	1.20(1.07-1.34)	0.002
<b>RELIGION</b>			
Islam	31(17.5%)	1	
Christians	146(82.5%)	0.97(0.93-1.00)	0.082
<b>OCCUPATION</b>			
Causal job	132(74.6%)	1	
Professional	9(5.1%)	1.08(1.00-1.08)	0.681
<b>TRIBE</b>			
Non bantu	14(7.9%)	1	
Bantu	152(85.9%)	1.08(1.02-1.14)	0.011

of the Xpert MTB/RIF using a transfer pipette. Alternatively, the entire sample was processed in the original tube. 2mL of Xpert MTB/RIF Sample Reagent (SR) was transferred to 0.5mL of resuspended sediment using a transfer pipette and then vigorously shaken 10 to 20 times or vortex for at least 10 seconds. The sample was incubated for 10 minutes at room temperature, and then shaken vigorously 10 to 20 times or vortex for at least 10 seconds plus an additional 5 minutes incubation at room temperature.

Each Xpert MTB/RIF cartridge was labeled with the sample ID. In a leak-proof sputum collection container, the sample was diluted in the ratio 2:1 using SR diluents and shaken vigorously 10 to 20 times or vortex for at least 10 seconds, incubated for 10 minutes at room temperature and shaken again vigorously 10 to 20 times or vortex for at least 10 seconds before finally incubating it at room temperature for an additional 5 minutes. The cartridge lid and the sample containers were opened and the liquefied sample transferred

into the cartridge using the transfer pipette slowly before the cartridge lid closed firmly. The test was started within 4 hours of adding the sample to the cartridge. The GX 4.0 software or higher was loaded and Xpert MTB/RIF assay definition file was imported into the software. Laboratory work sheet was developed specifically for this study and used to record patients' laboratory results.

The GeneXpert generated the results from measured fluorescent signals and embedded calculation algorithms. A positive test result did not necessarily indicate the presence of viable organisms. It was however, presumptive for the presence of MTB and RIF's resistance. If the results showed "Rif Resistance DETECTED" this meant that MTB target was present within each sample and a mutation in the rpoB gene was detected that fell within the valid delta Ct setting hence reported as positive. "Rif Resistance NOT DETECTED" indicated that MTB target was present within the sample but no mutation in the rpoB gene had been detected and hence reported as negative.

## Data analysis

Data was analyzed using a computer software program (SPSS, Microsoft Excel) and the prevalence of MDR-TB determined. The different risk factors were analyzed and their measure of association with MDR-TB was determined and hence the results were considered significant at  $p < 0.05$  (95% confidence interval).

## Ethical clearance

Permission to collect samples from patients attending Mulago National Referral Hospital was sought from the administration. Informed consent was obtained from all study subjects. In addition ethical clearance was obtained from the Institutional Review Board of School of Biomedical Sciences of Makerere University College of Health Sciences.

## Results and Discussion

Multidrug resistance *M. tuberculosis* prevalence was 1.69% in the 177 samples analyzed. A number of factors were assessed in relation to MDR-TB prevalence; this included among others age, sex, suspects, HIV, TB, alcohol, smoking, religion, tribe and the occupation of the patients. The results however revealed that patients with TB re-infections or relapse cases, sex, smokers and religion had significantly ( $p < 0.05$ , Chi square) higher risk of developing MDR-TB in relations to the other factors at 95% confidence interval. The females are more likely to develop MDR-TB as compared to the males. Similarly the Bantu had higher prevalence compared to the non Bantu as shown in (Table 1).

The results of this study indicate that MDR-TB prevalence is slightly below the national MDR-TB prevalence of 2.3% and the prevalence of 12.7% in Kampala city [6]. This difference could be the study being restricted to the youth and only concentrated in Mulago Hospital.

Among the predisposing factors assessed, patients with TB re-infections or relapse cases, smokers, gender or sex and tribe were statistically significant ( $p < 0.05$ ) as compared to other risk factors like age, HIV, alcohol, religion and occupation which confirms with previous studies done in Latvia, this study revealed that new TB cases are 0.55 times less likely to develop MDR-TB as compared to relapse cases. This is comparable with a similar study in Latvia showing that relapses are 5.7 times more likely to develop MDR-TB as compared to new cases, this is mainly because relapse cases have been previously infected and treated for tuberculosis and the *M. tuberculosis* strain might have mutated to the first line anti tuberculosis treatment and hence leading to resistance to these drugs [10].

Cigarette smokers are about 1.195 times more likely to develop MDR-TB than non-smokers. This is because smoking damages the lungs and causes lung cancer, this makes the lungs very susceptible to the *M. tuberculosis* infection and hence increasing the risk of multidrug resistant tuberculosis infection. Studies conducted in five countries: the US, Spain, South Africa, Pakistan, and Vietnam showed that smokers were 2.08 more likely to develop MDR-TB as compared to non-smokers although the timing of smoking in relation to the study varied. Tobacco smoking, passive smoking, and indoor air pollution from biomass fuels have been implicated as the main cause [11]. This is in conformity with the results of the present study.

According to the results obtained from this study, the Bantu are at a higher risk of developing MDR-TB as compared to the non Bantu tribes. This is because the city is mainly congested by the Bantu tribes whose majority of them are business oriented and since most of the samples were collected from within Kampala, this could probably explain these results.

MDR-TB prevalence is higher in females than in males which could be due to the female population in Uganda being higher than their male counterparts [12]. The professional workers are 1.076 times more at risk of developing MDR-TB as compare to the causal laborers. This could be because the professionals like health workers are always in close contact with TB patients than the causal laborers hence exposing them to the disease agent. Miners also are in constant inhalation of toxic gases and dust particles that damages the lungs and hence making it more susceptible to infection by the MDR-TB strain [13,14], which confirms that most professionals are highly susceptible to the disease.

HIV patients are at a higher risk of developing MDR-TB due to their low immunity which makes them more susceptible to infections including MDR-TB. This results confirms to earlier studies which showed that not only does HIV increase the risk of reactivating latent *M. tuberculosis* infection but also increases the risk of rapid TB progression soon after infection or re-infection with the bacteria to MDR-TB [15].

The mechanism of drug resistance by modification of drug targets where Pathogenic bacteria are able to avoid antibacterials agents through structural modifications of their targets; this reduces their antibiotic binding affinity. Studies have shown that exposure of the bacterial cells to sub-lethal levels of bactericidal antibiotics promotes cellular mutagenesis hence leading to increased mutations in other drug resistance genes. *Mycobacterium* is also able to inactivate antibiotics via direct chemical modifications through deletion of the RND-4 system (BCAL2820 to BCAL2822) leading to decreased sensitivities to different antimicrobial compounds and hence suggesting its involvement in intrinsic drug resistance [16-38].

## Conclusion

Although the prevalence rate is low, MDR-*Mycobacterium tuberculosis* still a problem in Uganda. Smokers and relapse cases have higher chances of contracting MDR-*Mycobacterium tuberculosis*. The community should be sensitized properly on the predisposing factors so that the burden of MDR-TB is lessened.

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## Authors' Contributions

JNK, DO and EW conceptualized the project, performed most of the laboratory experiments and wrote the manuscript. ST assisted in the laboratory analysis of the samples while CA did the statistical data

analysis. JGN assisted in drafting and finalizing the manuscript. All authors read and approved the final manuscript.

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