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Determining First Line Anti-Tuberculosis Drug Resistance among New and Re-treatment Tuberculosis/ Human Immunodeficiency Virus Infected Patients, Nairobi Kenya

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Abstract

Drug resistant tuberculosis (T.B) is a state when *Mycobacterium tuberculosis* (MTB) organisms are resistant to antimicrobial agents at the levels attainable in blood and tissue. Scarce data exists on the prevalence of resistance to first line anti-tuberculosis drugs in populations with high rates of tuberculosis and human immunodeficiency virus (H.I.V). Strains of MTB complex from MGIT were subjected to drug susceptibility testing for isoniazid (INH), Rifampicin (R), Streptomycin(S), and Ethambutol (E) using the proportional method on (MGIT). A total of 145 TB patients were enrolled for study. Of the 138 patients who had valid results for analysis, 79(57.2%) were male and 59(42.8%) were female.

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Most of the patients (20.3%) were aged between 35-39 years with the lowest proportion (3.6%) being in the younger category <20 years. Among the pulmonary tuberculosis patients 34% were new cases while 66% were retreatment cases. A total of 43(31.2%) strains showed resistance to at least one drug tested, while 112(81.2%) were susceptible. The isolates showed different resistance patterns with mono-resistance in 15(11%) isolates, total multi- drug resistance (MDR) in 6(4.3%) isolates with new and retreatment cases being 0(0.0%) and 6(6.6%) respectively. Mono-resistance was recorded in all four drugs tested. The isolates were resistant to the antibiotics as follows; 16(17.6% and 0(0.0%) were resistant to INH; 9(9.9%) and 0(0.0%) were resistant to R; 10(11.0%) and INH (2.1%) were resistant to E; 7(7.7%) and 0(0.0%) were resistant to S; 6(6.6%) and 0(0.0%) were multi drug resistant among retreatment and new cases respectively. Our study concluded that there were high levels of drug resistance among those previously treated for TB.

Keywords: Multi drug resistant tuberculosis; New and Retreatment; HIV; Resistance

1. Introduction

Tuberculosis is a serious public health problem, a third of the world population that is two billion people are infected with TB, 9 million develop TB disease and close to 2 million die annually, 650,000 develop MDR-TB while 8% are co-infected with HIV [14]. 95% of these TB cases occur in developing countries, where 1 in 14 new cases occur in individuals who are infected with HIV and 85 % of these cases occur in Africa [10]. According to the WHO global report of 2008, about 9.2 million new cases and 1.7 million deaths from TB occurred in 2006 and of these around 709,000 (7.7%) new cases and 200,000 deaths were estimated to have occurred in HIV positive individuals [12]. Highest rates of TB are reported in the countries of Eastern Europe, where weakened economies and public health efforts are the main causes of its resurgence, and where internationally recommended control strategies need further expansion and strengthening. In Western Europe, there are pockets of increasing incidence, particularly in major cities with socially marginalized immigrants from high burden TB countries [13-4]. Studies on drug resistance in various countries in the 1960's showed that developing countries had a much higher incidence of drug resistance than developed countries[3]. Resistance to (INH) and (S) was more than resistance to (R) and (E) and the rate of primary drug resistance to (INH) as a single agent ranged from 0% to 16.9% among HIV infected individuals [6]. (INH) forms the core of anti-tuberculosis drugs, and its use in TB preventive therapy has been known to reduce incidence in high risk individuals for more than 40 years [7]. Despite the confirmed efficacy of preventive therapy, concerns about drug resistance, have limited its uptake [7].

HIV infection by impairing the cell mediated immunity is the most potent known risk factor for the reactivation of latent TB infection and rapid progression to active disease [8]. Overall an estimated 8% of new cases are attributable to HIV co- infection [14]. An estimated 13% of the 1.5 million TB deaths in 2010 were attributed to HIV infection, but in the African region this proportion has been much higher because of the high HIV prevalence [2]. The risk of death in co-infection is twice that of HIV infected individuals without TB, even when CD4 cell count and ART therapy are taken into account [2-16]. Some groups of people are at higher risk for TB disease because they are more likely to be exposed to or infected. Risk of infection is; poor housing and crowding, large pool of untreated persons. Risk of developing disease after infection is increased by low

immunity (extreme of ages, HIV, diabetes). This includes close contacts of people with infectious TB disease, people in areas where TB is common, elderly, drug users, and people with certain medical conditions especially HIV infections. For people with HIV and TB, the risk of developing TB disease is about 10% each year, in contrast, people infected only with TB; the risk of developing TB disease is 10% over a lifetime [13]. Control of drug resistance involves; identifying and treating drug resistant cases, treating all cases, treating latent TB, improving cure rate, active case finding, and reducing development of secondary drug resistance by improving adherence [5].

Introduction of the first anti-tuberculosis drugs, (INH), (S), (PAS), was slowly followed by resistance, which was observed in clinical isolates of *MTB* [3]. Over 60% of new cases of pulmonary TB in most developing countries are now co-infected with HIV [9-1]. The WHO approach (identifying of TB bacilli microscopically) to TB diagnosis is failing in a number of HIV infected patients, as smear negative TB has been linked to poor treatment outcomes, including death [9]. According to the national MDR-TB surveillance data 2011, approximately 150 HIV positive individuals were diagnosed with MDR-TB and two cases confirmed with extremely drug resistance tuberculosis (XDR-TB) in Kenya [18]. These figures have been on the rise because of inadequate and insensitive diagnostic methods, leading to increased mortality and morbidity among those infected [5]. This study seeks to determine first line anti-TB drug resistance among HIV infected patients attending Maryland comprehensive care centre.

This study was undertaken to determine *M. tuberculosis* resistance patterns against first-line drugs used for treatment in patients diagnosed with pulmonary TB and living with HIV.

2. Materials and Methods

2.1 Setting

The study was conducted in Mathare 4A, Nairobi the capital city of Kenya. The population of Mathare is nearly 150,000 and is steadily growing due to rural /urban migration. This poses a lot of challenges. A significant proportion of the residents of Mathare belong to low economic social class, with high population densities. Mathare valley is approximately 6km to the north east of Nairobi's central business district. It is bordered by Thika road to the north and Juja road to the south. The study was cross sectional, eligible patients (new and retreatment) randomly sampled during the intake period, who gave consent were enrolled for the study. The study consisted of 79(57.2%) male and 59(42.8%) female patients. The intake period was between April and November 2013.

2.2 Specimen Collection and Transport

One early morning sputum and a spot sample were collected on screw capped bottles. Genexpert was done on the first sample to confirm T.B diagnosis of suspected patients. Second samples from the *MTB* positive patients were then transported weekly by smith-line courier service, to central reference laboratory (CRL) for culture and drug susceptibility testing (DST). The CRL is located within the centre for respiratory diseases research, Kenya Medical Research Institute (CRDR-KEMRI) at Kenyatta National Hospital.

2.3 Culture of M. Tuberculosis and Drug Susceptibility Testing

Sputum culture and DST for *M. tuberculosis* was conducted in the central reference laboratory. Primary culture of *M. tuberculosis* was performed using non radiometric method Mycobacterium growth indicator tube (MGIT) 960. The sputa were decontaminated with NAOH solution (40%w/v) combined with 2.9% sodium citrate solution and N-acetyl -L-cystein (NALC) powder. Sterile phosphate buffer was added and the organisms concentrated by centrifugation at 3,000rpm for 15 minutes. The supernatant was decanted and the sediment suspended with phosphate buffer and inoculated in liquid MGIT media and incubated along with a growth control and an external control H37Rv at 37^0C in BACTEC 960 systems (BD Diagnostic Systems, Sparks, MD, USA). The MGIT tubes were incubated until the instrument flagged them positive. After a maximum of six weeks, the instrument flagged the tubes negative if there was no growth at 37^0C. A positive culture of *M. tuberculosis* confirmed the diagnosis of active disease.

2.4 Sensitivity Testing of M. tuberculosis

All culture positive tubes were tested for contamination before sensitivity tests using the standard method used in Kenya for drug susceptibility BACTEC MGIT 960 liquid culture system (Becton Dickson Company Sparks, MD, USA. A total of four first line drugs collectively referred to as SIRE (Streptomycin (S)-1.00ug/ml; Isoniazid (I)-0.10ug/ml; Rifampicin (R)-1.00ug/ml and Ethambutol (E)-5.00ug/ml were tested for sensitivity. A control tube was matched with all the isolates tested. An external control of H37Rv was also set in all culturing and sensitivity testing processes. All readings were performed inside the machine and results were printed as susceptible, resistant or indeterminate.

3. Ethical Approval

The research proposal was approved and ethically cleared by the national ethical review committee (ERC) and Scientific Steering Committee (SSC) at the Kenya Medical Research Institute (KEMRI). Each patient who consented to enroll was required to complete an informed consent form.

4. Results

A total of 145 patients were enrolled for study in the Maryland comprehensive care centre (Figure 3). Of the 138 patients for whom data was available, 47(34.1%) were new and 91(65.9%) previously treated for TB. A total of 138 sensitivity tests were performed from pulmonary tuberculosis patients of whom 79(57.2%) were male and 59(42.8%) were female (Figure 2). Most of the patients (20.3%) were aged between 35-39 years with the lowest proportion (3.6%) being in the younger category <20 years (Figure 1).

A total of 26(18.8%) strains showed resistance to at least one drug tested, while 112(81.2%) were susceptible. The isolates showed different resistance patterns with mono-resistance in 15(11%) isolates, total multi- drug resistance in 6(4.3%) isolates with new and retreatment cases being 0(0.0%) and 6(6.6%) respectively. Mono-resistance was recorded in all four drugs tested.

The isolates were resistant to the antibiotics as follows; 16(17.6% and 0(0.0%) were resistant to INH; 9(9.9%) and 0(0.0%) were resistant to R; 10(11.0%) and 1NH (2.1%) were resistant to E; 7(7.7%) and 0(0.0%) were resistant to S; 6(6.6%) and 0(0.0%) were multi drug resistant among retreatment and new cases respectively. Seventeen (12.3%) males and 9(6.5%) female patients showed resistance to at least one drug tested (Table 1). The proportion of patients that was sensitive to all drugs among the retreatment cases (72.5%) was significantly low compared to the new cases (97.9%), (OR=0.06 [95% CI=0.01 – 0.44]; p<0.001). Implying that, a retreatment case was 94.0% less likely to be sensitive to all drugs compared to a new case. Resistance to Isoniazid and Rifampicin was significantly associated with TB retreatment (P=0.001, and P=0.028 respectively) (Table 1).

Table 1: Patterns of resistance to first line anti-tuberculosis drugs in relation to treatment status

	Total (n=138)		**RT (n=91)**		**New (n=47)**		**95% CI**			
Antibiotic	**N**	**%**	**N**	**%**	**N**	**%**	**OR**	**Lower**	**Upper**	**p value**
Sensitivity to all	112	81.2%	66	72.5%	46	97.9%	0.06	0.01	0.44	**<0.001**
Any resistance pattern										
Isoniazid (H)	16	11.6%	16	17.6%	0	0.0%	UD	UD	UD	**0.001**
Rifampicin (R)	9	6.5%	9	9.9%	0	0.0%	UD	UD	UD	**0.028**
Ethambutol (E)	11	8.0%	10	11.0%	1	2.1%	5.68	0.70	45.79	0.098
Streptomycin (S)	7	5.1%	7	7.7%	0	0.0%	UD	UD	UD	0.095
Monoresistance TB										
Isoniazid (H)	6	4.3%	6	6.6%	0	0.0%	UD	UD	UD	0.095
Rifampicin (R)	2	1.4%	2	2.2%	0	0.0%	UD	UD	UD	0.548
Ethambutol (E)	4	2.9%	3	3.3%	1	2.1%	1.57	0.16	15.50	1.000
Streptomycin (S)	3	2.2%	3	3.3%	0	0.0%	UD	UD	UD	0.551
Multi drug resistance TB (MDR TB)										
H+R	2	1.4%	2	2.2%	0	0.0%	UD	UD	UD	0.548
H+R+E	2	1.4%	2	2.2%	0	0.0%	UD	UD	UD	0.548
H+R+S	1	0.7%	1	1.1%	0	0.0%	UD	UD	UD	1.000
H+R+E+S	1	0.7%	1	1.1%	0	0.0%	UD	UD	UD	1.000
Total MDR TB	6	4.3%	6	6.6%	0	0.0%	UD	UD	UD	0.095
Other resistant Patterns										
H+E	2	1.4%	2	2.2%	0	0.0%	UD	UD	UD	0.548
H+S	1	0.7%	1	1.1%	0	0.0%	UD	UD	UD	1.000
H+E+S	1	0.7%	1	1.1%	0	0.0%	UD	UD	UD	1.000
R+E	1	0.7%	1	1.1%	0	0.0%	UD	UD	UD	1.000
E+S	0	0.0%	0	0.0%	0	0.0%	-	-	-	-
R+S	0	0.0%	0	0.0%	0	0.0%	-	-	-	-
R+E+S	0	0.0%	0	0.0%	0	0.0%	-	-	-	-

Table 1; presents patterns of resistance to first line anti-tuberculosis drugs in relation to treatment status.

Age distribution among the patients was normally distributed (Figure 1) with a mean age of 35.0 (±10.0 SD), and a median age of 35.0, ranging between 16 and 61 years. Figure 1 presents the distribution of study patients by age categories. Most of the Clients (20.3%) were aged 35-39 years with the lowest proportion (3.6%) being in the younger category (Age<20 years).

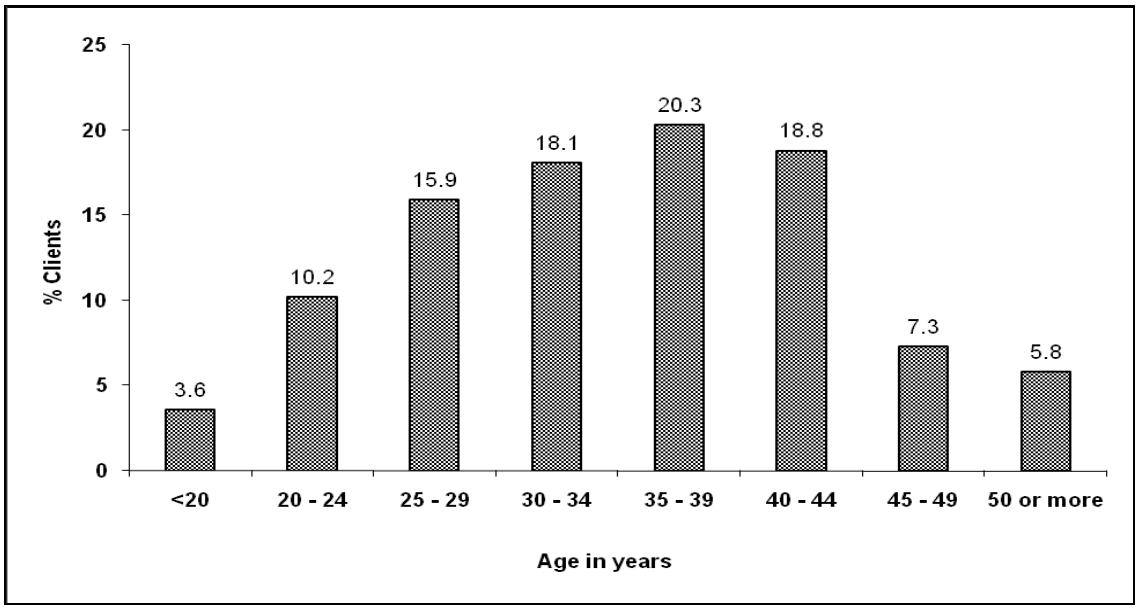

Figure 1: Age distribution among the study patients

Figure 2, presents distribution of gender among the study patients with tuberculosis. 79 (57.2%) and 59 (42.8%) of the patients were male and female respectively. The majority of the cases were male.

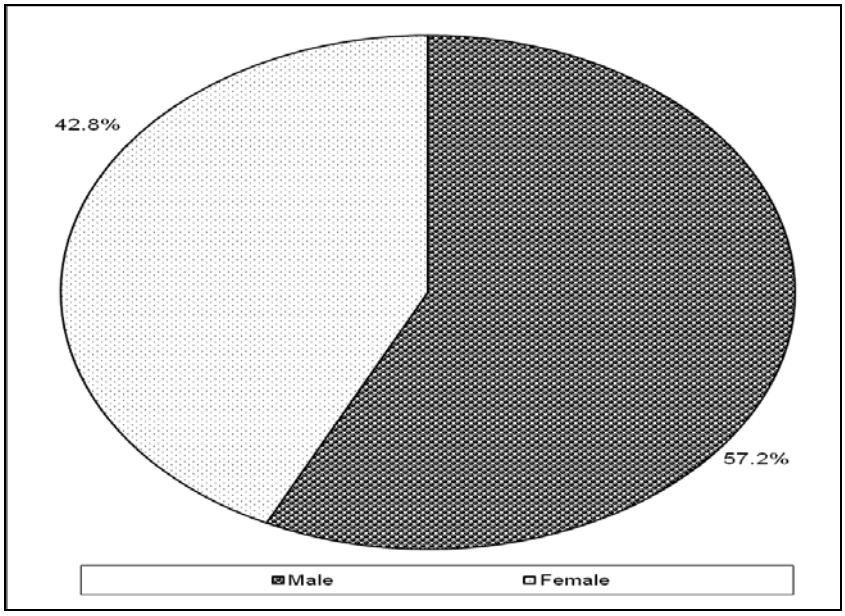

Figure 2: Gender distribution among the study patients

Figure 3, presents a flow diagram on patient recruitment. Seven patients were removed from the study due to various reasons namely death, withdrawal, and culture contamination.

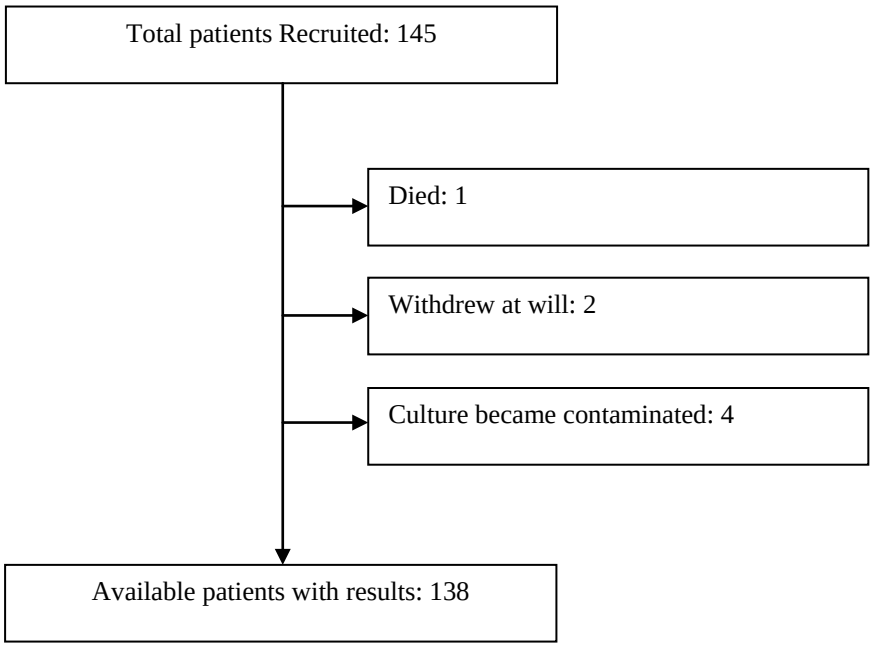

Figure 3: Flow diagram on Patient recruitment

5. Discussion

T.B drug resistance is a serious public health, and worldwide problem and a major challenge in T.B care and control. H.I.V infection is the major risk factor for the reactivation of latent TB infection and rapid progression to active disease [15].

5.1 Resistance Patterns

The overall resistance to all the drugs tested 18.8% was significantly lower to that reported in earlier studies in Kenya, where 30.1% isolates were resistant to at least one drug [19]. The results of this study differ with that conducted in Central Asia where resistance was 30.5% [24]. Resistance rates in the present study were slightly higher than rates observed in studies in Tanzania where only 14 out of 280 (5.83%) isolates were resistant to at least one drug [20], while in South Africa and Korea total resistance to the drugs tested was 7.4% [21] and 18.7% [22] respectively.

The results of the present study show a high rate of resistance among retreatment cases, with decreased resistance among new cases (46.5% and 2.1% respectively, while MDR-TB prevalence was 0.0% and 6.6% among new and previously treated patients respectively). This is comparable with studies conducted in Uganda where prevalence of resistance to any of the first-line anti-TB drugs was 8.3% and 25.9%; with an MDR-TB prevalence of 1.4% and 12.1% among new and retreatment cases respectively [31], in the Republic of Tanzania any resistance was 8.3% and 20% among new and retreatment cases respectively [23]. While in Kenya any

resistance was 21% and 45% among new and retreatment cases respectively [34]. All these were one-time studies performed in single facilities in the respective countries, similarly the present investigation was a one-time study performed in a single facility.

Resistance to (INH) in this study was 11.6% which was slightly lower than results obtained in earlier studies in Ethiopia, where one isolate was resistant to (INH) [25], with Bangladesh at 5.4% [26] and Sri-lanka at 12.2% [28]. According to WHO (INH) resistance rates higher than 10% can predict the development of MDR-TB. This high resistance may be caused by both its wide use in the treatment of TB as a first-line drug or poor compliance by patients. In this study resistance to (RIF) was 6.5% which is higher than that observed in earlier studies in Kenya, where resistance was 1.3 % [19]. This rate is also higher than reports from studies in Bangladesh where resistance was 0.5% [28] and Ethiopia where resistance to (RIF) ranged from 0% to 1.8% [20-22]. Rif has several adverse effects such as nausea, vomiting, rashes, hepatitis, GIT upset, flulike symptoms, fever, and jaundice, which could result in patients non adherence and hence may lead to the selection of resistant strains.

Resistance to (S) in this study was 5.1% which was comparable to the resistance of 5.2% [19] reported in another study in Kenya but lower than that reported in Ethiopia 26% [25] and Sri-Lanka 9.9% [27]. Resistance to (E) in this study was 8.0% which was higher than rates in Ethiopia 2.7% [25]. It was however; lower than studies conducted in Sri-lanka where 14.5% resistance was reported [27]. Ethambutol enhances the effect of many drugs including beta lactams to different Mycobacteria species and can be used to develop a regimen for MDR-TB [29]. In this study a high number of patients with TB showed IHN resistance but significantly susceptible to Rifampicin. It is therefore possible for these patients to recover fully if WHO guidelines for re-treatment are followed under strict supervision to prevent them from developing MDR-TB. However, the high rate of INH resistance is significant since it is a first line drug which is used throughout the course of treatment. This indicates a high probability for developing MDR-TB in the future since it has been observed that MDR often develops from initial INH mono-resistant strains. INH is also the drug of choice for chemoprophylaxis of TB and is used in developed countries for treating latent TB. The high level of INH resistance among the study population also is an indicator that this drug will be completely useless for both these purposes in Kenya.

In the present study MDR-TB prevalence was 0(0.0%) and 6(6.6%) among new and previously treated patients respectively. The result of this study compares with that of study conducted in Uganda where MDR-TB prevalence was 1.4% and 12.1% among new and previously treated patients respectively [32-18]. Higher levels of MDR-TB prevalence among retreatment's cases raises concerns about the quality of directly observed therapy and adherence to treatment. The existence of very low primary resistance to INH, R, E, and S implies no ongoing transmission of drug resistant strains in the community. This would imply strengthened infection control measures which should therefore be further strengthened through dissemination of TB infection control guidelines by the National Leprosy and Tuberculosis Program (NLTP). Whereby priority should be accorded to TB infection control training for health care workers, in the TB diagnostic and treatment centre's especially those which offer comprehensive TB/HIV care.

The scale up of Xpert MTB/RIF screening in Kenya will allow for a more expedient diagnosis of rifampicin resistant TB and may improve TB outcomes by shortening diagnostic delays and ineffective initial therapy [30-

33]. The results of this study indicate the need for strict enforcement of the DOTS method and better epidemiological surveillance of Tb cases.

5.2 Comparison of Pulmonary TB and Resistance on basis of gender

There was a significantly high number of male diagnosed with TB than female (57.2% and 42.8% respectively). This compares with earlier studies in Kenya where more males were associated with pulmonary TB than female (60.4% and 39.5% respectively; $p<0.05$. Globally a 70% predominance of males over female patients was reported [20-16]. The world health organization reported that 67.2% of the global male population was diagnosed with TB as compared to the female population [35-15]. The great number of males compared to females could be attributed to behavioral factors such as smoking, which is a predisposing factor to TB with more males being smokers than females [36-2]. Alcohol consumption, malnutrition, and the delay to seek treatment, especially by men [36-37] are other factors that have been associated with higher numbers of males than females with TB, with 57% of the patients in the current study being male. In the present study more males were associated with drug resistance than females (17(12.3%) and 9(6.5%) respectively. This is consistent with earlier studies in Kenya, where more males were associated with drug resistance than females 52(18.2%) and 34(11.9%).

6. Study Limitation

This study was conducted over a short period of seven months and it was limited to Mathare, similar studies should be conducted in other regions.

7. Conclusion

Presence of MDR-TB should be awake up call to continue monitoring its course in addition to promoting the improvement and expansion of control activities. There is need for patients to access rapid diagnosis and rapid drug susceptibility tests and treatment with more effective drugs and also regimens shorter than the current two year period for MDR-TB. Introduction of rapid molecular diagnostic tests like Xpert MTB/RIF; makes diagnosis of MTB and identification of rifampicin resistance quicker, this makes patient management easier. DST of the other first line TB drugs E, S, H should be made available and accessible. There is a high prevalence of drug resistance among retreatment TB cases compared to new cases. This raises concerns about the quality of directly observed therapy and adherence to treatment. The fact that these patients are previously treated for TB could be a possible risk factor for the development of resistance due to incomplete and irregular treatment of tuberculosis in them.

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