Anti-tuberculosis drug resistance in Nairobi, Kenya.

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SUMMARY

Background: **Drug resistant tuberculosis (TB) which is a state when *Mycobaterium tuberculosis (MTB)* organisms are resistant to antimicrobial agents at the levels attainable in blood and tissue pose a serious threat to TB control programs. Limited information exists on the exact prevalence of resistance to anti-tuberculosis drugs in populations with high rates of tuberculosis and HIV co-infection such as those in Nairobi, Kenya.**

Setting: **A cross sectional study was conducted among new and previously treated consecutive sputum smear positive pulmonary tuberculosis (PTB) patients of 14 years and older at 16 diagnostic and treatment facilities in Nairobi, Kenya, between February and August 2010.**

***Objective*:** **To determine the magnitude of drug resistance to first line antituberculosis drugs among *MTB* isolates obtained from a study addressing the diagnosis and epidemiology of drug resistant tuberculosis in Nairobi, Kenya.**

Methods: **Sputum samples from patients with bacteriologically confirmed PTB on microscopy were cultured on Lowenstein Jensen (LJ) media. Participants were offered diagnostic testing and counselling for HIV testing. Strains of *MTB* complex from Lowenstein Jensen (LJ) slopes were subjected to drug susceptibility testing (DST) to isoniazid (H), rifampicin (R), streptomycin (S), and ethambutol (E) using the proportional method on the Mycobacterium Growth Indicator Tube (MGIT) conventional method.**

Results: **A total of 595 TB patients had their *MTB* strains DST done. Of the 568 (95.4%) patients who had valid results for analysis, 369 were new and 199 previously treated. About eighty five percent and seventy seven percent of the strains from new patients and previously treated patients were fully sensitive to all the drugs tested respectively. Any resistance to isoniazid, streptomycin, ethambutol and rifampicin was 10.3%, 4.3%, 5.1% and 0.81% respectively among new patients. Among previously treated patients any resistance to isoniazid, streptomycin, ethambutol and rifampicin was 18.1%, 10.5%, 7.03% and 9.04% respectively. The prevalence of MDR TB defined as resistant to at least both isoniazid and rifampicin was 0.54% and 8.54% among new and previously treated patients respectively.**

Conclusion: **The study found high levels of drug resistant TB in Nairobi compared to other previous studies done in the country. MDR TB in Kenya is now a reality and the situation in Nairobi being the largest cosmopolitant city is worrying. The upword trend of MDR TB in Nairobi is course of concern. This calls for urgent concerted efforts to address the problem especially the strenghthening of the implementation of the comprehensive framework of the DOTS-Plus strategy for appropriate management of MDR-TB.**

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Introduction:

Drug resistance of *Mycobacterium tuberculosis* which is a state when *Mycobaterium tuberculosis* organisms are resistant to antimicrobial agents at the levels attainable in blood and tissue was recognized soon after the introduction of anti-tuberculosis drugs in late 1940s[1,2]. The re-emergence of drug resistant TB especially multidrug resistant TB (MDR TB) poses a serious threat to TB control programs all over the world[3]. Resistance to antituberculosis drugs develop naturally through

mutations, non-adherence to drugs and inadequate tuberculosis treatment. Primary resistance is the presence of drug resistant MTB in a patient with no or less than one month of previous antituberculosis drug therapy. On the other hand acquired resistance the patient has received at least one month of antituberculosis drugs in the past. Primary resistance reflects the transmission of TB over the years while acquired resistance is a good measure of TB programme perfomance since it includes relapse cases, treatment failures and defaulters[4].

Early detection of TB drug resistance allows prompt initiation of the appropriate treatment in patients and surveillance of drug resistance. It is one of the key priorities of the TB control program. However limited information exists on the exact prevalence of resistance to anti-tuberculosis drugs in populations with high rates of tuberculosis and HIV co-infection such as those in Nairobi, Kenya. Data from routine drug sensitivity testing from the National reference laboratory (CRL) shows resistance to rifampicin and isoniazid to be 10% and 20% respectively for previously treated pulmonary tuberculosis patients[5]. In 2007 alone 118 Multidrug resistance TB cases were reported in Kenya from the routine MDR-TB surveillance. This was based on only 1,500 (14% of all retreatment cases) samples analyzed at the central reference laboratory[6]. As of April 2009 one case of XDR-TB had been reported in the Western part of Kenya in Busia[7]. The high rates of drug resistance TB currently being reported in Kenya is alarming. In 2006, WHO estimated a total of 400,000 Multi-drug resistant TB (MDR-TB) to have occurred in the world of which 7,000 MDR-TB cases were reported from African countries[8]. In most areas of the world where TB is common, reliable pretreatment drug susceptability results are not available. However epidemiological studies have shown that most previously treated patients with drug resistance initially had primary drug resistance[9]. *Mycobacterium tuberculosis* strains resistant to anti-TB drugs can be transmitted the same way MTB strains susceptible to anti-TB drugs are transmitted. Evidence that MDR TB strains have the potential for transmission has been reported in a series of outbreaks in hospitals[10, 11] amongst health care workers[12,13] and in prisons[14].

Resistance to any anti-TB drug is predictable if the drug is used alone. A similar resistance is expected if only one drug is in a regimen were effective because of preexisting resistance to other drugs in the regimen[15]. Rifampicin needs special protection because it is the key sterilizing drug in short course treatment of tuberculosis[16].

Among patients with drug resistance TB, additional drug resistance may develop if a regimen with multiple drugs is prescribed that includes drugs against which primary resistance is present. Such a regimen may be equivalent to a single drug therapy. In Vietnam it was shown that primary resistance was a strong factor for failure and relapse and for acquiring further resistance[17]. MDR-TB is a severe form of drug resistance and is on the increase in many countries of the world . Most childhood contacts of adults with MDR-TB are likely to be infected with MDR-TB. Contact tracing is therefore important in identifying children at risk and also know the susceptibility patterns of the index case in order to choose a proper treatment regimen for the contacts[18, 19]. TheWorld Health Organization has proposed in the global plan to stop TB 2006-2015 several components including addressing TB/HIV and MDR-TB[20]. Human Immunodeficiency Virus (HIV) infection curtails the effects of TB control programme by lowering the life expectancy of those receiving TB treatment. The “direct costs” of diagnosis and treatment are significant for poor families, but the greatest economic loss occurs as a result of “indirect” costs, such as loss of employment, travel to health facilities, sale of assets to pay for treatment related costs, and in particular, lost productivity from illness and premature death[21]. The greatest economic benefits of the global plan to stop TB are greatest in African countries especially those with high levels of HIV[22].

Directly Observed treatment short course (DOTs) strategy can rapidly reduce the transmission and incidence of both drug susceptible and drug resistance TB. However, standardized or individualized treatment regimen may be required based on the Drug Sensitivity Testing (DST) to reduce mortality rates due to MDR-TB[23]. Primary drug resistance is a strong risk factor for failure and relapse for acquiring further resistance. In Vietnam it was shown that 17 out of 40 (43%) of the failure patients enrolled for retreatment had primary MDR-TB and 15 out of 25 (65%) of the patients enrolled for retreatment who did not have primary MDR-TB had acquired MDR-TB[24]. The treatment of MDR-TB is expensive and takes long time though most MDR-TB patients can be treated with the use of

appropriate regimen. Second line drug also have many side effects[25]. Aminoquinolones are among the second line anti-TB drugs and the widespread use of aminoquinolones such as ciprofloxacin and ofloxacin for other infections may render them significantly less effective alternative therapy for MDR-TB[26].

Extensively drug resistance TB (XDR-TB) also defined as MDR-TB with further resistance to at least three of the six classes of second line drugs anti-TB drugs is also becoming a threat to public health worldwide. Of the 221 patients who had MDR-TB and were studied in South Africa in September 2006, 53 cases were identified with XDR-TB. This represented almost one-sixth of all the known XDR-TB cases in the whole world[27].

Materials and Methods

Setting: The study was conducted in Nairobi, the capital city of Kenya. The population of Nairobi is steadly growing due to rural-urban migration and immigration from unstable countries e.g Somalia and Sudan. A significant proportion of the residents of Nairobi belong to the middle or low social economic class with high population densities in certain areas. The total area of Nairobi is 696 sq km with about 60% of the people living in less than 5% of this area in overcrowded informal settlements in the form of shelters. Nairobi has the highest TB case load compared to other regions in Kenya. In 2007 there were 18,902 (684/100,000) of all forms of TB cases and 6,634 (242/100,000) of the new smear positive TB cases.

There are 8 health districts in Nairobi divided based on the geographical location and population sizes for operational issues. All the 8 districts were selected for the study. The study was cross sectional and to get the number of patients per district the required sample size was divided proportionately to the 8 districts depending on the number of smear positive TB patients diagnosed by each district in 2007. A list of all public diagnostic centers in each district was compiled and ranked according the number of new smear positive TB cases diagnosed in 2007. The intake period was between February and August 2010 i.e at 6 months maximum duration in order to minimise operational costs. The diagnostic facility in each of the health districts with the highest number of patients diagnosed in a quarter of a year was selected. This approach was used because the facilities selected would capture most of the TB patients in that particular health district and reach the required sample size within the intake period. If the calculated number of patients in the first facility was not adequate for the particular district, then additional patients were recruited from the next nearest dignostic facility within the district. Consecutive eligible patients (both new and previously treated) diagnosed and/or registered for treatment at each of the selected diagnostic sites during the intake period and gave consent were enrolled for the study .

Specimen collection and transport

At the peripheral laboratory, the standard Acid –fast (AFB) direct smear microscopy using Zeihl-Neelsen (ZN) staining was done on the initial sputum to confirm TB diagnosis of suspected patients. A second sputum specimen was then collected before start of treatment and then transported to the Central reference Laboratory (CRL) on the same day for culture. The central reference laboratory is located within the Centre for Respiratory Diseases Research, Kenya Medical Research Institute (CRDR- KEMRI) at Kenyatta National Hospital.

Culture of M. Tuberculosis and drug susceptability testing.

Central reference laboratory (CRL) is the only public laboratory which does sputum culture and drug susceptability testing for *M. tuberculosis.* Primary culture of *M. tuberculosis* was perfomed on egg based solid culture media, Lowenstein-Jensen (LJ). Sputum specimens were first decontaminated using 4% sodium hydroxide to eliminate the associated commensal flora. The decontaminated sputum specimens were then homogenized by vortexing and then centrifuged for 15 minutes at 300 revolutions per minute (rpm). The sediment obtained after centrifuging was inoculated directly to the LJ culture media slopes and incubated at 37^0C. The slopes were examined weekly for any visible growth. A positive culture of *M. tuberculosis* confirmed the diagnosis of active TB disease. Strains of MTB complex from Lowenstein Jensen (LJ) slopes were subjected to drug susceptibility testing (DST) to Isoniazid (INH), rifampicin (RIF), streptomycin (SM), and ethambutol (EMB) using the Mycobacterium Growth Indicator Tube (MGIT) method.

A loopful of the colonies was harvested from the LJ slants, suspended in 1ml sterile saline and vortexes to break the large clumps. The suspension was adjusted to a standard 0.5 McFarland turbidity by visual comparison.

The drug containing and drug-free growth control MGIT tubes were inoculated with the standardized 0.5 McFarland inoculums of the *M. tuberculosis* isolate and entered into the MGIT960 automated machine in a special rack-carrier with a printed barcode; this is read by the machine when entering the tubes to identify the test and apply the adequate algorithm for susceptibility or resistance interpretation. All readings were performed inside the machine and the results were printed as susceptible or resistant for each drug tested.

Results

A total of 691 pulmonary tuberculosis patients were enrolled for study in the 8 health districts of whom 438 were new smear positive PTB patients and 253 were previously treated smear positive patients.

Of the 690 patients for whom data was available, 446

Table 1: Patterns of resistance to first line anti-tuberculosis drug in Nairobi, 2010

	Newly diagnosed patients			Previously treated patients			Total		
Item	**Number**	**%**	**95% CI**	**Number**	**%**	**95% CI**	**N**	**%**	**95%CI**
Number of DST	369	100		199	100		568		
Sensitive to all	312	84.6	80.6-87.9	153	76.9	70.7-82.3	465	81.9	78.6-84.9
Any Resistance									
Isoniazid (H)	38	10.3	7.5-13.7	36	18.1	13.2-23.8	74	13.2	10.4-15.9
Rifampicin (R,)	3	0.81	0.19-2.10	18	9.04	5.59-13.5	21	3.7	3.24-5.57
Ethambutol (E)	19	5.1	3.19-7.72	14	7.02	4.02-11.1	33	5.8	4.08-7.94
Streptomycin (S)	16	4.3	2.56-6.74	21	10.5	6.8-15.3	37	6.5	4.68-8.74
Monoresistance TB									
Isoniazid (H)	24	6.50	4.28-9.33	13	6.5	3.64-10.5	37	6.5	4.68-8.74
Rifampicin (R,)	1	0.27	0.01-1.28	1	0.50	0.02-2.36	2	0.35	0.05-1.12
Ethambutol (E)	10	2.71	1.36-4.71	5	2.51	0.9-5.3	15	2.6	1.52-4.18
Streptomycin (S)	8	2.17	0.99-4.0	4	2.0	0.6-4.66	12	2.1	1.13-3.53
Multidrug resistance TB (MDR TB)									
H+R	0	0	0	3	1.51	0.36-3.92	3	0.5	0.13-1.39
H+R+E	1	0.27	0.01-1.28	2	1.0	0.15-3.17	3	0.5	0.13-1.39
H+R+S	0	0	0	6	3.0	1.19-6.04	6	1.05	0.42-2.14
H+R+E+S	1	0.27	0.01-1.28	6	3.0	1.19-6.04	7	1.2	0.53-2.38
Total MDR TB	2	0.54	0.083-1.72	17	8.54	5.19-12.9	19	3.35	2.06-5.04
Other resistance Patterns									
H+E	5	1.36	0.48-2.91	1	0.5	0.02-2.36	6	1.05	0.42-2.14
H+S	5	1.36	0.48-2.91	5	2.51	0.9-5.3	10	1.76	0.88-307
H+E+S	2	0.54	0.83-1.72	0	0	0	2	0.35	0.054-1.12
R+E	0	0	0		0	0	0		
E+S	0	0	0	0	0	0	0		
R+S	0	0	0	0	0	0	0		
R+E+S	0	0	0	0	0	0	0		

(64.7%) were males and 244 (35.3%) were females with a mean age of 31.3 years, median and mode of 30 years. The minimum age was 14 years and maximum age 84 years. A total of 485 patients (311 males and 174 females) were tested for HIV of whom 155 (32%) tested positive while 330 (68%) tested negative for HIV. and 199 previously treated. Strains of 312 (84.5%) of new patients and 153(76.9%) of previously treated patients were fully sensitive to all antituberculosis drugs tested. Any resitance pattern among new patients was as follows; isoniazid 38 (10.3%). ethambutol 19 (5.1%), streptomycin 16 (4.3%) and rifampicin 3 (0.81%). Among previously treated patients the resistance pattern was isoniazid 34 (18.1%), streptomycin 21 (10.5%) ethambutol 14 isolates (7.02%) and rifampicin 18 (9.04%). Two (0.54%) and 17 (8.54%) strains from new and previosely treated patients respectively were multidrug resistant TB (MDR TB) defined as resistant to at least both isoniazid and rifampicin **(Table 1).**

Discussions:

Drug resistance in tuberculosis is a matter of great concern for TB control programs since resistant *Mycobacterium tuberculosis* strains could spread around the world. The increasing prevalence of TB and HIV infection is also worrying. Resistance to at least one anti-tuberculosis drug in the world ranges from 0% in two Western European countries i.e. United Kingdom and Iceland to 56.3% in Baku and Azerbaijan. Among previously treated patients' resistance to at least one anti-tuberculosis drug ranges from 0% in three European countries to 86% in TashKent and up to 60% for MDR-TB with 4012 MDR-TB cases reported all over the world between 2002-2006, 301 (7%) being XDR-TB cases[28]. As of January 2008 a total of 58 countries worldwide including Kenya had reported to the World Health Organization (WHO) at least one case of XDR-TB. WHO recommends that in order to prevent emergency of XDR-TB, countries should strengthen laboratory diagnostic services to ensure rapid and accurate DST and also increase disease surveillance efforts to asses epidemiological trends[29].

Initial drug resistance surveys performed in Kenya in 1964, 1974 and 1984, indicated that Initial resistance ranged from 7.1% to 8.9% for isoniazid (H), from 0.5% to 1.4% for streptomycin and from 1.3% to 1.4% for both H and S combined while acquired resistance ranged between 16% and 17% for H and between 2% and 16% for both H and S. There was no resistance to Fourty one percent of the female patients tested positive while only 27% of the males tested positive for HIV. Culture was positive in sputum from 595 (86.1%) patients. Of the 595 strains subjected to drug suaceptabilty testing, 568 cultures of *M. tuberculosis* were available for analysis of whom 369 were new cases rifampicin[30]. A countrywide survey carried out by Githui *et al.* in Kenya in 1994, showed that among patients with no previous exposure, 6.3% had a strain resistant to either isoniazid (5.3%) or both isoniazid and streptomycin (1%) while in those with previous exposure 37% of the patients had a strain resistant to either isoniazid (30.4%) or both isoniazid and streptomycin (6.5%). However no resistance was recorded in rifampicin and ethambutol unlike in the present study where resistance was recorded in both new and previously treated patients[31]. In another study carried out in Nairobi between September 1999 and October 2001, MDR TB was documented in 17 (11.4%) out of 209 isolates[32]. Recurrent TB has been identified as the most important risk factor for resistance to isoniazid, rifampicin or both in a study carried out by Zhiyuan et al in New Jersey, USA[33]. Irregular treatment which is also a common problem in big cities like Nairobi due to frequent movements has been associated with MDR-TB[34]. High prevalence of drug resistance in HIV infected TB patients has been reported. Studies in Lativia showed that any resistance and MDR-TB were significantly associated with HIV infection. Taylor *et al.,* found out in a study in Texas that TB patients with HIV were more likely to have rifampicin resistance and less likely to have isoniazid resistance[35]. However a study done in Puna, Maharashltra India on anti-TB drug resistance showed that prevalence of drug resistant isolates among HIV seropositive patients was similar to that of HIV seronegative TB patients indicating that HIV infection may not be associated with drug resistant TB[36]. Tuberculosis infections in high incidence countries have been shown to be recently transmitted and failure to contain MDR TB and XDR TB reflects inability to diagnose the problem early to prevent transmission of the same while continuing to prescribe an ineffective regimen[37]. Once MDR TB has developed, further progression to pre-XDR and XDR is only a question of time and will take place over few months or even years.

The patient remains infectious and transmission of MDR TB and XDR TB continues particularly in areas with high incidence of HIV and overcrowding. The

emergence of MDR TB in Nairobi is therefore a course of concern and calls for urgent attention to address the problem of drug resistant TB. Concerted efforts are therefore required to prevent drug resistance and effectively treat patients with MDR-TB as a crucial protection of public health and TB control. The DOTS strategy developed in 1970s and 1980s can prevent MDR TB from becoming a serious problem in a population and also reduce MDR TB once it has occurred. We call for strenghthened efforts on the implementation of the comprehensive framework of DOTS-Plus strategy that add components for MDR-TB diagnosis, management and treatment integrated within the DOTS programs.

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