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First-Line Anti-Tuberculosis Drug Susceptibility Patterns of Mycobacterium tuberculosis Complex Strains Responsible for New Cases of Human Pulmonary Tuberculosis in Kisumu County, Western Kenya

Geoffrey Arasa Ouno[1] Rose Kakai[1] Henry N.D. Nyamogoba[2] Biegon R.K.[3] Cornelius K.Magut[4]
1.Maseno University, School of Medicine, Department of Medical Microbiology, Maseno
2.Moi University, School of Medicine, Department of Medical Microbiology & Parasitology
3.Moi University, School of Medicine, Department of Immunology
4.Ampath Reference Laboratory, Moi Teaching & Referral Hospital

Abstract
Background: Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide, drug-resistant tuberculosis being a major public health problem. The emergence and spread of multidrug resistant (MDR) *Mycobacterium tuberculosis* complex (MTBC) strains poses significant challenges to disease control. Continued surveillance of drug susceptibility helps determining proper treatment regimen. The effectiveness of a standard anti-tuberculosis (TB) treatment regimen correlates with *in vitro* drug susceptibility pattern of the infecting tubercle bacilli. The results of the drug susceptibility tests help select a proper treatment regimen or modify treatment regimen for a better management of patients and surveillance and timely control of the spread of the drug resistant TB in the community. Treatment of drug resistant TB is costly, and the outcomes, including survivorship, can be poor. As the result, the drug susceptibility test has become more important than ever. **Objective:** This study aimed to investigate the patterns of first line anti-tuberculosis drug-susceptibility against *Mycobacterium tuberculosis* complex isolates from new cases of pulmonary TB patients in Kisumu County, Western Kenya. **Method**: This was a cross sectional study which included a total of 290 isolates from pulmonary TB patients in JOOTRH and Kisumu County Hospital between February and August 2016. The MTBC isolates identified were *M. tuberculosis, M. africanum*, and *M. bovis*. Drug susceptibility test was performed on the 283 *M. tuberculosis, 5 M. africanum* and *2 M. bovis* isolates by BD BACTEC MGIT 960 SIRE and PZA DST system using five first-line anti-TB drugs: Isoniazid, Rifampicin, Streptomycin, Ethambutol and Pyrazinamide. **Results:** *M. tuberculosis* was highly sensitive to all the anti-TB drugs; Streptomycin(S) 96.8%, Isoniazid (H) 89.8%, Rifampin(R) 98.2%, Ethambutol (E) 94.4%, Pyrazinamide (PZA) 89.8%. *M. bovis* TB species was 100% sensitive to all drug except Pyrazinamide where there was 100% resistance. *M. africanum* varied in its sensitivity to anti-TB drugs; Streptomycin 80%, Isoniazid 60%), Pyrazinamide 4 (80%). Resistance was Streptomycin 20%, Isoniazid 40%, and Pyrazinamide 20%. *M. africanum* was neither resistant to Rifampin(R) nor Ethambutol (E). A total of 20.8% of *M. tuberculosis* strains showed resistance to at least one drug tested, while 79.2% were sensitive. 16.3% were resistant to one drug (mono resistance), 2.1% to two drugs (double resistance), 0.7% to three drugs (triple resistance), 0.4% to four drugs (quadruples) and 1.4% to five drugs (pentagon-resistance). Two isolates of *M. bovis* were resistant to one drug. Two isolates of *M. africanum* were resistance, one case to one drug and another one case to three drugs. **Conclusion:** This study showed high level of resistance in *M. tuberculosis* isolates warranting proper use of anti-TB drugs in Kisumu County.
Keywords: Tuberculosis, *M. tuberculosis* complex, Multi Drug Resistance

INTRODUCTION

Human tuberculosis (TB) remains a major cause of morbidity and mortality worldwide. Despite the worldwide implementation of effective control programs, it has re-emerged at an alarming rate and continues to prevail as one of the deadliest contagious diseases with an estimated one-third of the global population being infected and 1.5 million deaths annually (WHO 2015) not only in the developing world, but also in the developed countries. Its re-emergence indicates failure to control its transmission. The growing number of isolates displaying resistance to the first line conventional anti-TB drugs used in its control causes part of the alarm (Mubarak S Alfaresi, Abdulsalam, & Elkoush, 2006). The TB epidemic is complicated further by the selection and transmission of *Mycobacterium tuberculosis* strains resistant to the most effective first- and second-line conventional anti-TB drugs (WHO 2015). An alarming increase in the global incidence of drug-resistant *M. tuberculosis* infection has created a critical need for methods that can rapidly detect *M. tuberculosis* complex (MTBC) and identify drug-resistant cases. Failure to quickly and effectively identify and treat patients with drug-resistant tuberculosis (TB), particularly multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis, leads to increased mortality, nosocomial outbreaks, and resistance to additional anti-tuberculosis drugs (Blakemore e*t al.*, 2010)

Drug-resistant tuberculosis is a major global public health problem. The emergence and spread of MDR and

XDR *Mycobacterium tuberculosis* complex (MTBC) strains poses significant challenges to disease control (WHO 2010). The prevalence of MDR-TB is increasing throughout the world in both new tuberculosis cases as well as previously-treated tuberculosis cases (Varshney, Shukla, Raza, & Ahmad, 2014). Although previous treatment for tuberculosis is the strongest risk factor for development of MDR-TB, treatment-naive patients are also at risk due to either spontaneous mutations or transmission of resistant strains (Vijay, Balasangameshwara, Jagannatha, & Kumar, 2004).Transmission of resistant strains from close contacts is increasing due to overcrowding and growing burden of MDR-TB patients. Globally, World Health Organization estimated 630 000 cases of MDR-TB among the world's 12 million prevalent cases of tuberculosis worldwide in 2011 (WHO 2012). Worldwide, 3.7% of new cases and 20% of previously treated cases were estimated to have MDR-TB. India, China, the Russian Federation and South Africa have almost 60% of the world's cases of MDR-TB. EXDR-TB has been reported by 84 countries; the average proportion of MDR-TB cases with XDR-TB is 9.0% (Sethi S. *et al*., 2013). India and China accounted for almost 40% of the world's tuberculosis cases (WHO, 2012) In Kenya drug resistant TB still remains a major health concern. There are growing concerns over rising drug-resistant strains of the killer tuberculosis in the country. According to the 2015 TB Drug Resistance Survey, 305 cases of Multi-drug resistant TB had been reported in the country, the highest figure in the last ten years. In 2014, there were 283 cases while in 2013 the number was 285. According to the Ministry of Health latest statistics the number of such cases have been increasing since 2006 when 82 cases were reported. The total number of Kenyans who have so far been reported to have MDR-TB since 2006 is 1790, with many cases believed to go unreported (Ministry of Health; USAID 2009). Majority of the cases were recorded in Dadaab camp owing to the refugee population. Only 2 labs in Nairobi and Kisumu are operative in testing for drug resistant TB (Kamene K 2016). In February 2016, the ministry of health directed that from the July 2016, all TB facilities should adopt the GeneXpert equipment to test individuals suspected to have drug resistant TB or HIV related TB. There is need to frequently monitor resistance patterns of *Mycobacterium tuberculosis* complex to prevent increase of multidrug resistant (MDR) and XDR cases. Drug susceptibility patterns of MTBC isolates is therefore essential for the proper treatment and management of pulmonary tuberculosis caused by *Mycobacterium tuberculosis* complex. In Kenya, like many other developing countries, very few national studies have been conducted to evaluate the current status of MDR-TB. Drug susceptibility test for tuberculosis is not done and if done it is in research institutions such as KEMRI and National Tuberculosis Reference Laboratory, thus little is known on anti TB drug susceptibility patterns in Kenya more especially in Kisumu County. Therefore, the current study was undertaken with the specific objective to determine the in vitro drug susceptibility pattern of 290 *Mycobacteria tuberculosis* complex (*M. tuberculosis, M. africanum* and *M. bovis*) clinical isolates against five conventional first line anti-tuberculosis drugs (Streptomycin, Isoniazid, Rifampicin, Ethambutol and Pyrazinamide) among patients with new cases of pulmonary tuberculosis in Kisumu County, Western Kenya.

MATERIALS AND METHODS

Study Design:-This cross-sectional study was conducted between February 2016 and August 2016 in Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) and Kisumu County Hospital that employed experimental method.

Study population:-The study involved 290 confirmed MTBC isolates from new cases of pulmonary TB patients visiting JOOTRH and Kisumu County Hospital.

Ethical consideration:-The proposal was initially cleared by the School of Graduate Studies, Maseno University. The study proposal was approved by Maseno University Ethical Research Committee (MUERC) [MSU/DRPI/MUERC/00280/16] and Jaramogi Oginga Odinga Teaching and Referral Hospital Ethical Research Committee (JOOTRH ERC) [ACCREDITION NO.01713]. Permission was also obtained from JOOTRH administration. Informed consent and/or assent was obtained from patients or their guardians before they were enrolled into the study.

SIRE DRUG SUSCEPTIBILITY TESITNG (DST)

A total of 290 confirmed positive *Mycobacteria tuberculosis* complex (MTBC) isolates identified using a commercially available DNA strip assay kit GenoType® Mycobacterium MTBC Molecular Genetic Assays, (Hain Lifescience GmbH, Nehren, Germany) were subjected to DST. These isolates were obtained from new cases of pulmonary TB patients attending the two health facilities in Kisumu County (JOOTRH and Kisumu County Hospital). Three MTBC species were identified *M. tuberculosis* (283/290), *M. africanum* (7/290) and *M. bovis* (2/290). The BACTEC MGIT 960 susceptibility test for Streptomycin (S), Isoniazid (I), Rifampin (R) and Ethambutol (E), called SIRE, and the BACTEC MGIT 960 PZA against Pyrazinamide (Becton-Dickson and Company, Sparks, MD, USA) was used.

Reconstitution of SIRE lyophilized drugs: Each critical concentration drug vial was reconstituted with 4 ml of sterile distilled water and mixed thoroughly. At least 0.1 ml (100 µl) of reconstituted drug solution was added

into each of the labelled BACTEC MGIT 960 tubes which resulted in the following critical concentration of drugs in the medium: Streptomycin (S) 1.0 µg/ml of medium; Isoniazid (I) 0.1 µg/ml; Rifampin (I) 1.0 µg/ml; Ethambutol (E) 5.0 µg/ml.

Inoculum Preparation: The inoculum was prepared according to BACTEC MGIT 960 System Manual, 2006. The inoculum was used for both SIRE and PZA Drug Susceptibility Test (DST). The growth was within the recommended timeframe on the day of positivity (Siddiqi & Rusch-Gerdes, 2006).

Inoculation and incubation

It was performed in accordance with the manufacturer's recommendations. For each isolate/test culture, 5 MGIT tubes were labeled. Four of the tubes contained the drugs (BACTEC MGIT SIRE; Becton Dickinson), and one was a drug-free growth control (GC). Aseptically 0.8 ml of BACTEC 960 SIRE Supplement was added to each of the labelled MGIT tubes and 0.1 ml (100 microliter) of reconstituted STR drug in the STR labelled tube was added. Similarly, other drugs in the other labelled tubes were added. No drug was added to the GC tube. The final critical concentrations of each drug in the test tubes was 1.0µg/ml STR, 0.1µg/ml INH, 1.0µg/ml RIF, and 5.0µg/ml EMB. Each of the drug containing tubes was inoculated with 0.5ml of the inoculum (well mixed culture suspension). No inoculum was added to the control. For the control, the test culture suspension was first diluted 1:100 by adding 0.1 ml of the test culture suspension to 10.0 ml of sterile saline, then 0.5ml of this diluted suspension was added into the growth control tube, the caps were tightened and the inoculated broth was mixed well by gently inverting the tube several times. Labelled tubes were placed in the correct sequence in the set carrier (GC, STR, INH, RIF, and EMB) of the MGIT machine. The susceptibility set carrier was entered into the BACTEC MGIT 960 instrument using susceptibility test set entry feature. The order of the tubes in the AST Set Carrier was ensured that it conforms to Set Carrier definitions. For example, GC, STR, INH, RIF, EMB for the SIRE standard testing.

PYRAZINAMIDE SUSCEPTIBILITY TESTING

Drug susceptibility testing was performed on MTBC isolates using the BACTEC MGIT 960 liquid culture system in accordance with the manufacturer's recommendations.

Susceptibility testing against PZA as always was carried out at a lower pH (5.9) of the medium, since PZA is active only at the low pH in vitro.

Reconstitution of lyophilized PZA drug:- Each of the PZA drug vials was reconstituted with 2.5 ml of the sterile distilled/deionized water and mixed well. The reconstitution drug solution now contains 8000 µg/ml of PZA.

Inoculation and incubation

Two MGIT PZA tubes were labeled, one as GC (growth control) and one as PZA (drug containing). Aseptically, 0.8 ml of PZA supplement was added to each of the two tubes followed by addition of 0.1 ml (100 µl) of the reconstituted drug into the PZA tube. This will give you 100 µg PZA per ml of the medium. No drug was added to the GC tube. Inoculation of 0.5 ml of the culture suspension was done to the PZA tube. For growth control inoculation, the inoculum was diluted 1:10 by adding 0.5 ml of the culture suspension (the one used for the drug tube) to 4.5 ml of sterile saline, mixed well followed by addition of 0.5 ml of the diluted suspension into the tube labelled GC and mix well. Note that for PZA susceptibility test, the inoculum of the control is diluted 1:10 and not 1:100 as in SIRE AST. The caps were tightened and both the MGIT tubes were mixed. The tubes were then placed in a two AST Set Carriers with the sequence of first GC and the PZA and the PZA set was entered into the instrument using AST set entry feature. The GC was placed first, and PZA second, in the AST Set Carrier. PZA was selected as the drug in the second tube AST set carrier definition when performing the AST set entry.

RESULTS

The present study included a total of 290 confirmed MTBC isolates from new cases of infected pulmonary TB patients. The MTBC species were *M. tuberculosis* 283/290 (97.6%), *M. africanum* 5/290 (1.7%) and *M. bovis* 2/290 (0.7%). A total of five different first line conventional anti-TB drugs were used in the current study: Streptomycin, Isoniazid, Rifampicin, Ethambutol and Pyrazinamide. All anti-TB drug resistance detected in our study was primary resistance (resistance among newly diagnosed pulmonary TB cases with no previous history of treatment or retreatment with anti-TB drugs or treatment for less than one month). Comparing drug type to MTBC species, *M. tuberculosis* was highly sensitive to all the anti-TB drugs; Streptomycin(S) 274 (96.8%), Isoniazid (H) 254 (89.8%), Rifampin(R) 278 (98.2%), Ethambutol (E) 267 (94.4%), Pyrazinamide (PZA) 254 (89.8%). *M. bovis* TB species was 100% sensitive to all drug except Pyrazinamide where there was 100% resistance. *M. africanum* varied in its sensitivity to anti-TB drugs; Streptomycin 4 (80%), Isoniazid 3 (60%), Pyrazinamide 4 (80%). Resistance was Streptomycin 1 (20%), Isoniazid 2 (40%), and Pyrazinamide 1 (20%). *M. africanum* was neither resistant to Rifampin(R) nor Ethambutol (E). **(Table 1)**

In **table 2**, a total of 59/283 (20.8%) of *M. tuberculosis* strains showed resistance to at least one drug tested, while 224/283 (79.2%) were sensitive/susceptible. 46/283 (16.3%) were resistant to one drug (mono resistance),

6/283 (2.1%) to two drugs (double resistance), 2/283 (0.7%) to three drugs (triple resistance), 1/283 (0.4%) to four drugs (quadruples) and 4/283 (1.4%) to five drugs (pentagon-resistance). Two isolates of *M. bovis* were resistant to one drug. Two isolates of *M. africanum* were resistance, one case to one drug and another one case to three drugs.

DISCUSSION

The current study was conducted to determine the patterns of drug susceptibility of first-line conventional anti-TB drugs against MTBC strains isolated from new cases of pulmonary TB patients in Kisumu County. Unlike the previous earlier studies that only identified and tested *M. tuberculosis*, the current study identified and tested DST for the three members of MTBC (*M. tuberculosis, M. africanum, M. bovis*) isolated from patients. Isoniazid and Pyrazinamide resistance was the most prevalent in our current study both accounting for 10.2%. The overall resistance of *M. tuberculosis* to at least one drug tested was 20.8% which was much lower than a previous study in Kenya where the overall resistance was 30.06% (Ndung'u, Kariuki, & Revathi, 2011), Bangladesh 37.8% (Mottalib, Hossain, Khalil, Islam, & Hossain, 2011), India 44.5% (Varshney et al., 2014), South Africa 30.2% (Green et al., 2010), Ethiopia 23% (Seyoum, Demissie, Worku, Bekele, & Aseffa, 2014), and Abu Dhabi, UAE 23% (Mubarak Saif Alfaresi & Hag-Ali, 2010). However, it was relatively lower than one study in Kenya where resistance to at least one drug was 18.3% (E. S. *et al*., 2000). In Indonesia 18.9% of *M. tuberculosis* isolates were resistant to at least one first-line TB drug (Wiwing, Widysanto, & Lugito, 2015). Lower resistance rates were also revealed in other previous studies compared with our current study. In Tanzania 5.83% of the isolates were resistant to at least one drug (Urassa et al., 2008). A recent study in Cameroon by (Koro Koro et al., 2016) reported a primary resistance rate of 7.2% with the primary mono resistance to isoniazid and streptomycin being the most prevalent (2.58% both) followed by mono resistance to rifampicin (0.52%), but no mono resistance was recorded for ethambutol. Based on the data from different studies, it shows that the rates of drug resistance is higher in Asian countries compared to African countries. All the data in these studies were obtained from studies conducted from new cases of pulmonary TB patients. For example in Bangladesh a study that was conducted among retreated patients, the drug resistance of *M. tuberculosis* to at least one drug was found to be alarming 50% (Mottalib *et a*l., 2011) .

Resistance to individual drug was: Resistance to streptomycin in our current study was 3.2% which was lower than a previous study conducted in Nairobi- Kenya that reported a resistance rate of 5.2% (Ndung'u et al., 2011) , much lower than Bangladesh where resistance was found to be 22% (Mottalib et al., 2011) , Abu Dhabi, UAE 25.6% (Mubarak Saif Alfaresi & Hag-Ali, 2010), Iran 23.1% (Shamaei et al., 2009). India 8.6% (Varshney et al., 2014) and Ethiopia 7% (Seyoum et al., 2014) reported almost a similar trend. However, some studies reported low rates of resistance compared to the current study. In North Eastern Thailand the prevalence rate was 2.1% (Reechaipichitkul, Tubtim, & Chaimanee, 2011). Streptomycin has been in use since the beginning of TB chemotherapy as of 1943 (Amukoye, 2008). Furthermore, being a broad spectrum antibiotic it is widely used in the treatment of other bacterial infections other than TB. Its use is not restricted hence, high rate of resistance to streptomycin is expected.

Mono-resistance to isoniazid in the current study was 10.2%**.** This finding is consistent with previous studies which was slightly lower than earlier studies in Kenya where resistance to isoniazid was reported to be 12.9% (Ndung'u et al., 2011), similar resistance rates to our study was reported in Ethiopia 9.5% (Seyoum *et al.*, 2014). However it was higher than North Eastern Thailand where *M. tuberculosis* resistance to isoniazid was 2.3% (Reechaipichitkul *et al*., 2011), the highest resistance to isoniazid being in India 37.7% (Varshney *et al.*, 2014) followed by Abu Dhabi, UAE 34.8% (Mubarak Saif Alfaresi & Hag-Ali, 2010), Bangladesh 26% (Mottalib *et al*., 2011) and Iran 11.6% (Shamaei *et al*., 2009), which is the most popular anti-TB drug used in the treatment of tuberculosis. WHO 2008 reported a 5.9% resistance rate worldwide (WHO/IUATLD 2008). According to WHO isoniazid resistance rate higher than 10% can predict the development of MDR TB (Githui *et al*., 1998). An alarmingly high rate of resistance to isoniazid (76.03%) was reported from another previous earlier study in Bangladesh (Rahman, Kamal, Mohammed, Alam, & Ahasan, 2009). In the current study, isoniazid resistance rates being slightly above 10% is an indication of slow development of MDR-TB strain in Kisumu County, Western Kenya. This high resistance may be due to the fact that isoniazid is used widely in the treatment of TB as a first-line drug and poor compliance by patients can select for drug resistant mutant strains. Low mono-resistance rates to isoniazid than in the current study were also observed in new cases of TB patients such as in Nepal 7.1% (Thapa, Pant, Khatiwada, & Shrestha, 2016) and 1.4% (Pradhan, Poudyal, Gurung, Acharya, & Bhattacharya, 2014). Rijal *et al*., (2005) reported a mono-resistance rate of 13.33% to isoniazid. Resistance to Isoniazid in this study might be due to poor management or the Isoniazid resistance prior to the current treatment (Nasiri *et al*., 2014).

In the current study, rifampin resistance was 1.8% which is slightly higher than one study done in Nairobi Kenya that reported 1.3% resistance (Ndung'u *et al*., 2011). Higher resistance rates have been reported in various countries Abu Dhabi, UAE 32.5% (Mubarak Saif Alfaresi & Hag-Ali, 2010) reporting the highest resistance, in

South Africa 17.7% (Green *et al*., 2010), India 22.2% (Varshney *et al*., 2014), Bangladesh 12% (Mottalib *et al*., 2011), Nepal 9.5% and 4.2% (Thapa, Pant, Khatiwada, & Shrestha, 2016; Pradhan *et al.,* 2014), Thailand 2.6-2.8% (Jittimanee et al., 2009); WHO/SEARO,2009; Reechaipichitkul *et al*., 2011) , Iran 3.9% (Shamaei *et al*., 2009), Ethiopia reporting low resistance of 1.7% (Seyoum *et al*., 2014). Rifampicin mono-resistance is uncommon but increasing in some areas of the world, more especially in Sub-Saharan Africa, Kenya being among them.

Resistance to ethambutol in this study was 5.6% which was higher than the rates reported in one study conducted in Kenya by Ndung'u *et al*, 2011 and his colleagues that reported a rate of 4.5%, in North Eastern Thailand 3.8% (Reechaipichitkul *et al*., 2011), Iran 3% (Shamaei *et al*., 2009), South Africa ranged from 1.4% (Green *et al*., 2010) and Addis Ababa-Ethiopia the rates were much lower 0.3% (Seyoum *et al*., 2014). It was however much lower than studies carried out in Bangladesh where 20% resistance was reported (Mottalib *et al*., 2011) and India 10% (Varshney *et al*., 2014). Abu Dhabi, UAE 20.9% (Mubarak Saif Alfaresi & Hag-Ali, 2010) reported the highest resistance to Ehambutol.

Resistance to pyrazinamide in this study was also high 10.2%, however various studies conducted to determine the patterns of drug susceptibility of first line conventional anti-TB drugs failed to test for pyrazinamide. From the Netherlands, 0.8% (van Klingeren, Dessens-Kroon, van der Laan, Kremer, & van Soolingen, 2007) resistant rates the results were not in agreement with the findings in the current study. A recent multicenter study has shown a rate of 5.1% PZA resistance among isolates from patients with pulmonary TB in Bangladesh (Zignol *et al*., 2016). Alfaresi and Hag-Ali, (2010) in Abu Dhabi reported a much higher resistance rates to Pyrazinamide 34.8%. Pyrazinamide (PZA) is a frontline anti-tuberculosis drug used in both first- and second-line treatment regimens. Though the prevalence of PZA resistance is higher in MDR-TB cases, due to technical difficulties in the laboratory and the possibility of false susceptibility results, most TB laboratories do not perform or rarely perform PZA susceptibility test (Zhang *et al., 2012)*; (Aono, Hirano, Hamasaki, & Abe, 2002). PZA is active only at low pH, and thus, it requires acidic culture medium for susceptibility testing. The acidic nature of the culture medium is inhibitory to the isolates (Zhang, Permar, & Sun, 2002). As a result, most patients infected with PZA resistant strains fail to get appropriate treatment (Zimic *et al*., 2012). This is also the main reason behind the scarcity of data regarding PZA susceptibility. Though PZA is used in MDR-TB treatment, very few data regarding the prevalence of PZA resistance among MDR-TB patients in Kenya are available. However, further modification (by keeping the pH of the medium around pH 5.7-5.9) and standardization of the medium has significantly improved the performance of the PZA test resulting in reliable and accurate PZA susceptibility data.

Mycobacterium africanum represented 1.7% of the isolates in the current study. Mono resistance was observed in Streptomycin (20%), Isoniazid (40%), and Pyrazinamide (20%). All the five species of *M. africanum* were neither resistant to Rifampicin nor Ethambutol. There was limited data available on the previous studies involving drug susceptibility pattern of *M. africanum* to first line anti-TB drugs. Most studies did a general study on MTBC strains without taking into consideration the different species of MTBC.

Mycobacterium bovis was the least member of MTBC species isolated representing 0.7% of the isolates. Naturally, *M. bovis* isolates are resistant to pyrazinamide because the organism does not produce the enzyme pyrazinamidase which is needed to convert pyrazinamide into pyrazinic acid, the active form of the antimicrobial agent (Barouni, Augusto, Lopes, Zanini, & Salas, 2004). This resistance is one of the basic features which can be used to distinguish isolates of *M. bovis* (universally resistant to pyrazinamide) from *M. tuberculosis* (commonly susceptible). Data regarding anti-TB drug susceptibility patterns for *M. bovis* pulmonary TB in humans is limited.

According to one recent study conducted in Cameroon, none of the *M. africanum* and *M. bovis* strains was responsible of drug resistance (Francioli, K.K. *et al*., 2016). Infection with *M. bovis* followed by pulmonary TB in humans, disease symptoms are indistinguishable from that caused by *M. tuberculosis* and is therefore generally treated in the same way and remedies. In the current study, *M. bovis* MTBC species were 100% sensitive to all the four anti-TB drugs except Pyrazinamide. *M. bovis* is usually susceptible to most antibiotics used to treat human pulmonary TB, caused either by infection with *M. tuberculosis* or *M. africanum*. The susceptibility of *M. bovis* reported in the current study is consistence with the reports from various previous studies: In Brazil none of the isolates of *M. bovis* were resistant to any of the five anti-TB drugs used in the determination of DST, excluding pyrazinamide (Parreiras *et al*., 2004), in Ethiopia (Tadesse, Ameni, & Desta, 2014). In Michigan, the results were also identical to our findings in the current study, where all the isolates were susceptible to isoniazid, streptomycin, rifampin, and ethambutol but resistant to pyrazinamide (100%) (Fitzgerald, Schooley, Berry, & Kaneene, 2011). Reports from one study in Mexico, the results were not in agreement with other previous studies. Mono-resistance was reported among *M. bovis* isolates where 10.9% of the isolates were resistant to Streptomycin. A study from San Diego reported 7% resistance for Isoniazid and 1% for Rifampicin among 167 *M. bovis* TB cases (LoBue & Moser, 2005). The National TB Genotyping Service of the United States reported 17% of Streptomycin resistance among *M. bovis* isolates (Hlavsa *et al*., 2008). The primary resistance to Isoniazid and Rifampicin in *M. bovis* which was not seen in the current study but reported in other studies may indicate human-to-human transmission. It has been suggested that human pulmonary

tuberculosis caused by *M. bovis* (HTBMb) cases may be at a higher risk for developing MDR strains if natural resistance to pyrazinamide is not considered and mono resistance to Isoniazid or Rifampicin is present (Bobadilla-del Valle *et al.*, 2015); (Kurbatova, Cavanaugh, Dalton, S. Click, & Cegielski, 2013). Nevertheless, it should be recognized that TB cases caused by MDR *M. bovis* may result in disease that is harder to treat on a second- line drug regimen. This highlights the need for performing species-level identification and drug susceptibility testing whenever *M. bovis* is suspected.

Identification of mycobacteria to species level is not mandatory in Kenya and therefore pulmonary TB patients infected with NTM fail to respond to conventional first line anti-TB drugs. These patients end up being classified as MDR-TB without knowing the root cause of failure to respond to drugs. The resistance of *M. tuberculosis* to rifampicin and isoniazid as seen in the current study is an alarming marker of MDR-TB. First-line drug susceptibility pattern differences of MTBC in various regions in Kenya and other countries, may be due to the difference in effectiveness of tuberculosis control program in different countries. Mycobacterial species in the *M. tuberculosis* complex undergo low-frequency spontaneous and induced chromosomal mutations which result in genetic resistance to anti-TB drugs. For example, MTBC species undergo a mutation in the β-subunit of their RNA polymerase which is the target site of Rifampicin. However, it is the application of anti-tuberculosis drugs which creates pressure for selection of these strains with mutations. This is generally due to improper therapeutic applications such as insu cient length of drug treatment, poor patient adherence to dosing schedules, using a single anti-tuberculosis drug which does not clear the bacilli instead of the recommended multiple drug therapy, and failure to recognize pre-existing resistance in a tuberculosis case (Jain & Dixit, 2008). These problems then lead to the emergence of either MDR or XDR strains of mycobacteria.

LIMITATIONS OF THE STUDY

Most eligible participants in this study were not ready to give their demographic data such as HIV status, occupation, residential sites, etc. Another limitation was the lack of available enough literature on drug susceptibility to pyrazinamide and drug susceptibility patterns of *M. africanum*.

CONCLUSIONS

Our current study reveals that the overall resistance to first line anti-TB drugs is high. The highest mono drug resistance is detected against isoniazid and pyrazinamide (10.2%). The lowest mono drug resistance is detected against streptomycin. The empiric treatment of pulmonary tuberculosis should be based on the regional drug susceptibility patterns of MTBC). The results of *in vitro* antibiotic susceptibility testing can predict the clinical response to treatment and guide the selection of antibiotics. This study also indicates that it is necessary to determine antibiotic susceptibility patterns in patients with TB to improve treatment outcomes for drug-resistant TB.

RECOMMENDATION

Kisumu County being a high TB prevalent region in Kenya, there is an urgent need of strengthening TB laboratories in health facilities in Kisumu and other counties to counter the MDR-TB strain. This laboratories will help decentralize the services. All TB facilities should adopt the GeneXpert equipment to test individuals suspected to have drug resistant TB or HIV related TB.

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Table 1: **Tabulation of the MTBC species and anti-TB drugs**

Drugs	***M.africanum***		***M.bovis***		***M.tuberculosis***	
	Sensitive n (%)	Resistance n (%)	Sensitive n (%)	Resistance n (%)	Sensitive n (%)	Resistance n (%)
Streptomycin(S)	4 (80.0)	1 (20.0)	2 (100.0)	0	274 (96.8)	9 (3.2)
Isoniazid(H)	3 (60.0)	2 (40.0)	2 (100.0)	0	254 (89.8)	29 (10.2)
Rifampin(R)	5 (100.0)	0	2 (100.0)	0	278 (98.2)	5 (1.8)
Ethambutol(E)	5 (100.0)	0	2 (100.0)	0	267 (94.4)	16 (5.6)
Pyrazinamide(Z)	4 (80.0)	1 (20.0)	0	2 (100.0)	254 (89.8)	29 (10.2)

Table 2: The tabulation of the MTBC species and the anti-TB drug levels

Drugs levels	***M.africanum*** n (%)	***M.bovis*** n (%)	***M.tuberculosis*** n (%)
None	3 (60.0	0	224 (79.2)
Mono	1 (20.0)	2 (100)	46 (16.3)
Double	0	0	6 (2.1)
Triple	1 (20.0)	0	2 (0.7)
Quadruples	0	0	1 (0.3)
Pentagon	0	0	4(1.4)
Total	**5 (100.0)**	**2 (100.0)**	**283 (100.0)**