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Molecular Identity of Mycobacteria Isolates in New Cases of Pulmonary Tuberculosis Patients in Kisumu County, Western Kenya

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Abstract
Background: Pulmonary tuberculosis (TB) remains one of the most challenging diseases to control in the world today and it has become a major global health problem especially in immunocompromised people such as HIV/AIDS. The problem is compounded by the emergence of non-tuberculous mycobacteria (NTM) of which its treatment is not directly analogous to that of MTB. **Objective**: This study determined the identity of Mycobacteria isolates in new cases of human pulmonary TB patients. **Methods:** It was a cross-sectional study that involved 316 confirmed new cases of pulmonary TB attending JOOTRH and Kisumu County Hospital. Sputa specimen was cultured in MGIT liquid culture medium. The isolates were identified to species level using GenoType® Mycobacterium CM/AS and MTBC Assay from Hain Lifescience Germany. **Results.** Of the 316 culture positive isolates, 91.8% were identified as MTBC and 8.2% were NTM species. Of the 290 MTBC, three different species were identified, 97.6% were *M. tuberculosis*, 1.7% were *M. africanum* and 0.7% were *M. bovis.* The Fisher's exact test was used to assess the associations between patient characteristics and MTBC species identified showed that age category of patients less than 35 years and above 35 years were statistically significant with MTBC species (p=0.020). While sex was not statistically significant with MTBC species (p=0.696). Four different NTM species were identified as 61.5% *M. intracellulare*, 19.2% *M. abscessus*, 11.5% *M. kansasii* and 7.7% *M. fortuitum*. The Fisher's exact test done to assess the associations between patient characteristics and NTM species was identified. Age category (p=0.608) and sex (p=0.182) of patients was not statistically significant to NTM species**.** **Conclusion:** There is a need for routine speciation among members of the MTBC and NTM as it is an important prerequisite for the proper management of patients with mycobacterial infections.
Keywords: *Mycobacterium tuberculosis* complex, Non tuberculous mycobacteria, Tuberculosis

INTRODUCTION

Pulmonary tuberculosis (TB) remains one of the most challenging diseases to control in the world today and it has become a major global health problem especially in immunocompromised people such as HIV/AIDS, diabetic and cancer patients and is the major cause of HIV-related death (WHO, 2013). Due to the powerful interaction between TB and human immunodeficiency virus (HIV) disease, together with the problems of poverty and malnutrition, the incidence of TB is increasing dramatically in sub-Saharan Africa (Raviglione, Snider, & Kochi, 1995). One-third of the estimated 40 million people living with HIV/AIDS are co-infected with TB, Africa harboring 29% of those infected. Sub-Saharan Africa bears the brunt of the dual epidemic, accounting for approximately 78% of the estimated burden in 2013; (WHO, 2013; (Kandwal, Garg, & Garg, 2009). The HIV/AIDS has profound impact on the TB epidemic in Kenya, where up to 60% of TB patients are feared to be HIV co-infected and the mortality rate attributed to TB in this group being above 130 per 100, 000 (Macintyre & Bloss, 2011).

The TB-HIV/AIDS problem is compounded by the emergence of non-tuberculous mycobacteria (NTM) as opportunistic infections in immunocompromised patients, and their treatment is not directly analogous to that of TB (Johnson & Odell, 2014). NTM form a group of organisms diverse in many characteristics, including pathogenicity and clinical disease. Their pathogenic potential is based on the species and they have been isolated from TB-HIV/AIDS co-infected patients in Western Kenya (Nyamogoba *et al*., 2012), Nairobi (Limo *et al.,* 2015). There is uncertainty regarding clinical relevance of many of the species, particularly in settings where facility to isolate and identify them is relatively recent (Van Halsema *et al*., 2015). Conventional identification of mycobacteria is achieved by standard culture and biochemical identification methods, all of which are laborious and time-consuming and is not often performed by diagnostic laboratories. The rise in NTM isolation demands faster methods for their identification and for selection of appropriate therapy. Definitive diagnosis of NTM pulmonary TB is challenging due to: similarity in clinical and microbiologic characteristics to *Mycobacterium*

tuberculosis complex (TB); limited laboratory capacity to isolate and identify NTM species (Herzmann & Lange, 2010); (Perdigão *et al.*, 2014) These NTM causes confusion in the treatment of TB in immunocompromised people and they are presenting as opportunistic mycobacterial pathogens to these patients. The confusion is as a result of misdiagnosis and over concentration in the treatment of TB while neglecting NTM, which acts as opportunistic infections in such patients.

The *Mycobacterium tuberculosis* complex (MTBC) is composed of the closely related species which include: *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, "*M. canetti*", and *M. pinnipedi M. bovis* comprises *M. bovis* subsp. *bovis*, *M. bovis* subsp. *caprae*, and the *M. bovis*-derived BCG vaccine strain; *M. africanum* includes two subtypes, I (West African strain) and II (East African strain); *M. microti*; and "*M. canetti*" may be merely a subspecies of *M. tuberculosis* (Brosch *et al*., 2002); (Niemann, Richter, & Rüsch-Gerdes, 2002). It is important to differentiate MTBC species to distinguish between strict human and zoonotic TB and to initiate an appropriate therapy (Djelouadji, Raoult, Daffé, & Drancourt, 2008). Therefore, rapid differentiation to the species and subspecies levels should be obtained not only for epidemiological purposes but also for adequate treatment of each patient. Therefore, the aim of this study was identification of, and species differentiation within the *M. tuberculosis* complex (MTBC) and non tuberculous mycobacteria (NTM) from confirmed positive cultures from new cases of pulmonary TB patients using the rapid molecular technique a commercially available GenoType Mycobacterium CM/AS and MTBC Assay which provided the first comprehensive insight into the molecular pattern of mycobacterial isolates in the region (Kisumu County, Western Kenya).

METHODOLOGY (MATERIALS AND METHODS)

Study Design: It was a cross-sectional study conducted between February 2016 and August 2016. The study employed experimental method of data collection.

Study population and sample size: The study involved tuberculous and non-tuberculous mycobacteria isolates from new cases of pulmonary TB patients attending Jaramogi Oginga Odinga Teaching & Referral Hospital (JOOTRH) and Kisumu County Hospital. Only new cases of consenting pulmonary TB patients attending the chest clinic of the two facilities and who turned out to be GeneXpert positive and smear positive after spot sputum analysis were recruited. All patients were adults (18 years and above).

Samples Collection (Sputum Specimen)

Once a suspected TB patient visited a chest clinic of JOOTRH and Kisumu County Hospital, sputum was collected under the supervision of a trained and competent medical staff. The patient was instructed to rinse mouth with water before collection. Sputum was collected as previously described by Nyamogoba and colleagues (2012). At least 2 ml of three sputum specimens [(1) first spot sample in the clinic the same day, (2) early morning home-collected sample because the sample would have the highest yield at this time and (3) second spot sample in the clinic the next day] was collected from suspected TB patients. Patients were requested to cough so that expectoration would come from as deep down the chest as possible, and spit into a sterile 50 ml specimen bottles/blue falcon cap tubes (Nyamogoba *et al.*, 2012). Each specimen was labeled with the patient name, hospital number (patient ID), date and time of collection. The samples were refrigerated at $4^{o}C$ before being transported in ice cooler boxes to the Medical Microbiology Laboratory, at Moi University once weekly for analysis. The samples were processed within seven days of collection in order to minimize loss of viability of the mycobacteria. The safety for research assistants and healthcare workers during collection and handling of sputum specimen was ensured by observing the WHO guidelines (Pai *et al*., 2005);(Bock, Jensen, Miller, & Nardell, 2007)

Sample Processing (NaOH-NALC procedure)

All clinical sputum specimens from patients were processed by the digestion-decontamination process as described by Warren *et al*., 2006 using sodium hydroxide-N-acetly-L-cysteine (NaOH-NALC) prepared in the laboratory.

Smear Preparation and Acid-Fast Staining (ZN Method)

Smears were prepared from all processed specimens before inoculation into medium as described by Siddiqi and Rusch-Gerdes (2006) (Siddiqi & Rüsch-Gerdes, 2006). Positive smears were also prepared from positive cultures of *M. tuberculosis* (H37Rv, ATCC #27294 or H37Ra ATCC 21577) and *E.coli* culture was used as negative control which were examined before the slides from clinical specimens.

Inoculation in Mycobacterium Growth Indicator Tube (MGIT) 960 System

Briefly, MGIT tubes were labelled with specimen number, unscrewed the cap and 0.8 ml of MGIT supplement was added. Up to 0.5 ml of well mixed processed specimen was added into the appropriately labelled MGIT tubes of the BACTEC MGIT 960 System (Becton-Dickinson Diagnostic Instrument Systems, Towson, MD, USA) using separate pipette or pipette tip for each specimen. The tubes were immediately recapped tightly, mixed and left at room temperature for 30 minutes before incubation. The tubes were entered into the MGIT 960 instrument, incubated at 370C and monitored automatically every 60 minutes for an increase in fluorescence for a maximum of six weeks until the culture flagged positive (Siddiqi & Rüsch-Gerdes, 2006)

Isolation and Identification of *Mycobacteria tuberculosis* Complex (MTBC) and NTM
The sputum specimens from each patient were processed for isolation of mycobacteria and following standard protocols. A participant with at least one positive MGIT culture was considered as a TB case, while those with three negative culture results were regarded as not having TB. Mycobacterial isolates were identified as *M. tuberculosis* complex (MTBC) or non-tuberculous mycobacteria (NTM) from the positive cultures using three commercially available DNA strip assays Hain's GenoType® Mycobacterium CM, AS and MTBC Molecular Genetic Assays, (Hain Lifescience GmbH, Nehren, Germany) which were executed consecutively. The assays were performed according to the instructions of the manufacturer (Package Insert)

GenoType® Mycobacterium CM/AS and MTBC Assays

DNA Extraction: DNA extraction was performed in accordance with the manufacturer's instructions. In brief, 1 ml of positive bacteria culture grown in MGIT culture media was pelleted by spinning for 15 minutes at approximately 10000 x g. The supernatant was discarded and bacteria resuspended in 100-300 µl of deionized water by vortexing followed by heating or incubation for 20 minutes at 95^{0}C in water bath and further incubation for 15 minutes in an ultrasonic bath, then spinned down for 5 minutes at 13,500 (full speed) and 5µl of the supernatant was directly used for the assay (Hains Lifescience package insert).

DNA Amplification: Amplification mixture (45 µl) was prepared in DNA free room, including 5 µl extracted DNA (20-100 ng DNA) in the reaction mixture contained 35 µl primer nucleotide mix, 5 µl 10 × polymerase incubation buffer for HotStar Taq (QIAGEN, Hilden, Germany), 2 µl 25 mM $MgCl_2$ solution, 0.2 µl HotStar Taq and 3 µl water (biology grade water). Amplification was carried out in a thermal cycler (MJ Research, PTC-100 Thermal Cycler, GMI, Inc, USA), which involved 1 cycles of denaturation solution (DEN) at 95°C for 5 min, annealing of primers at 95°C for 30 s, 2 min at 58°C for 10 cycles, then 20 cycles at 95°C for 25 s, 53°C for 40 s and 70°C for 40 s and final primer extension at 70°C, 8 min for 1 cycle.

Hybridization: Briefly, 20 µl of Denaturation Solution was dispensed in the corner of each of the wells used and then 20 µl of amplified sample was added by pipetting up and down to mix well and incubated at room temperature for 5 minutes. At least 1 ml of pre-warmed Hybridization Buffer was added carefully to each well followed by gently shaking the tray. A strip was placed in each well using tweezers in a manner to make sure complete flooding of solution over strips. Then tray was placed in shaking TwinCubator and was incubated for 30 minutes at 45^{0}C followed by complete aspiration of Hybridization Buffer. Washing was done by 1 ml of Stringent Wash Solution to each strip and incubated for 15 minutes at 45^{0}C in shaking TwinCubator. The work was being done at room temperature from this step forward. Completely remove Stringent Wash Solution. Again each strip was washed once with 1 ml of Rinse Solution for 1 minute on shaking TwinCubator. Then 1 ml of diluted Conjugate was added to each strip and incubated for 30 minutes on shaking TwinCubator. The solution was removed and each strip washed twice for 1 minute with 1 ml of Rinse Solution and once for 1 minute with 1 ml of distilled water on shaking TwinCubator. 1 ml of diluted substrate was added to each strip and incubated protected from light without shaking for 3-20 minutes. As soon as bands were clearly visible brief rinsing was done twice with distilled water to stop the reaction. Strips were removed from the tray and dried between two layers of absorbent paper using tweezers. Evaluation and interpretation of the results were done based on the presence and absence of different bands and compared with reference band provided in the kit.

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]. This study included 290 patients diagnosed with new cases of pulmonary tuberculosis. Permission was also obtained from JOOTRH administration. Informed consent was obtained from patients or their guardians before they were enrolled into the study.

RESULTS

The study comprised of 343 new cases of TB patients out of which 182 (53.1%) were males and 161 (46.9%) were females. The mean age of the patients was 35.07±14.6 (standard deviation) with a range of (18-82) years. Of the 343 samples evaluated, 92.4% (n=317) were positive for GeneXpert, 75.8% (n=290) were positive for smear and 92.1% (n=316) were culture positive. Of the patients who were aged less than 35 years, 93.0% (185/199) were positive for GeneXpert, compared to 91.7% (132/144) who were above 35 years. Among the males 94% (171/182) were positive for GeneXpert compared to 90.7% (146/161) females. 84.4% (168/199) patients aged less than 35 years were smear positive compared to 84.7% (122/144) who were above 35 years. Among the males 82.2% (146/182) were smear positive compared to 89.4% (144/161) females. Prevalence of culture positivity amongst patients less than 35 years was 95.0% (189/199) and 88.2% (127/144) patients above 35 years. Among the patients who were culture positive 94.5% (172/182) were males compared to 89.4% (144/161) females**. (Table I)**. Only the culture positive samples were used. Of the 316 culture positive isolates, 290 (91.8%) were identified as MTBC and 26 (8.2%) were NTM species. **(Table 2)**. Of the 290 MTBC, three different species were identified, 283 (97.6%) were *M. tuberculosis*, 5 (1.7%) were *M. africanum* and 2 (0.7%)

were *M. bovis*. Their distribution is describe in Table 3. The Fisher's exact test was used to assess the associations between patient characteristics and MTBC species identified. The analysis showed that age category of patients less than 35 years and above 35 years were statistically significant with MTBC species identified (p=0.020). While sex was not statistically significant with MTBC species (p=0.696) **(Table 3)**. Out of the 26 (8.2%) NTM identified, four different species were identified which included 16 (61.5%) *M. intracellulare*, 5 (19.2%) *M. abscessus*, 3 (11.5%) *M. kansasii* and 2 (7.7%) *M. fortuitum*. Their distribution is shown in **Table 4**. The Fisher's exact test done to assess the associations between patient characteristics and NTM species was identified. The analysis showed that age category (p=0.608) and sex (p=0.182) of patients was not statistically significant to NTM species **(Table 4).**

DISCUSSION

Treatment and management of patients with MTBC and NTM are entirely different, therefore prompt isolation, detection and differentiation is necessary for effective treatment and suitable management of the disease and for epidemiological purposes (Hasegawa *et al*., 2002); (Park *et al*., 2009). MTBC and NTM may or may not have same clinical presentations, but the treatment regimens are always different (A. K. Singh *et al*., 2013); (Marzouk *et al*., 2011).

In the current study 91.8% MTBC and 8.2% cases of NTM was much lower than previous studies reported in Kenya, where 42.4% of mycobacterial isolates were NTM and 57.6% were MTBC from tuberculosis retreatment cases in National Tuberculosis Reference Laboratory (NTRL), Nairobi (Limo *et al*., 2015**).** Lower NTM prevalence rates was reported elsewhere in Western Kenya 4.2% ((Nyamogoba *et al*., 2012), Belgium 1.4% ((De Keukeleire *et al*., 2017)), India 4.23% ((Narang *et al*., 2005)), and Ghana 8% ((Bjerrum *et al*., 2016) similar with the current study. The results in the current study were almost similar as the study conducted in Serbia where 88.8% were identified as MTBC and 11.2% NTM, however, *M. tuberculosis* was the only MTBC species recognized((Živanović, Vuković, Dakić, & Savić, 2014). Higher rates of NTM have also been isolated from TB patients in India 29% (Umrao *et al*., 2016)

Identification of *Mycobacterium tuberculosis* Complex (MTBC)

Three different MTBC species were identified. The most predominant species was *M. tuberculosis* 97.6% (283/290), being by far the most frequently causative agent of pulmonary tuberculosis worldwide. A study conducted in Serbia to isolate mycobacterial species from respiratory specimens, only *M. tuberculosis* was identified in all samples ((Živanović *et al*., 2014)). *M. tuberculosis* has also been identified as the major cause of pulmonary TB in reports from various previous studies in Uganda, Tanzania and Kenya (Asiimwe *et al*., 2008);(Mbugi *et al*., 2015); (B. Katale *et al*., 2017);(Usagi, Odilla, Maingi, & Kebira, 2016) This suggests its wide distribution in the regions. As it is, most people know that *M. tuberculosis* is the only etiological agent of pulmonary tuberculosis.

M. africanum subtype II was the second predominant MTBC species isolated accounting for 1.7% (5/290) of the isolates. *M. africanum* causes up to half of human pulmonary TB in West Africa. In Kenya and other Sub-Saharan countries, *M. africanum* has been isolated from TB patients. Recent surveys showed highly variable prevalence of *M. africanum* in different regions of Africa; e.g., Ivory Coast 5%; Cameroon 10% , (Niobe-Eyangoh *et al*., 2003) Gambia 39% (de Jong, Antonio, & Gagneux, 2010) , with alarming prevalence being in Guinea-Bissau 60% (Bonard *et al*., 2000) were found to be infected with *M. africanum.* These results from different regions of Africa shows a higher prevalence of pulmonary TB caused by *M. africanum* compared to Kenya more especially in the current study. Previous studies carried out in Kenya have reported a higher prevalence of *M. africanum* 8.9% and *M. bovis* 6.7% (Limo *et al.,* 2015). The commercial GenoType MTBC DNA strip assay (Hain Lifescience GmbH) used in this study does not differentiate *M. canettii* from *M. tuberculosis* and *M. africanum* type I from *M. pinnipedii*, but *M. africanum* type I being a West African strain and therefore there are minimal chances of isolating it from pulmonary TB patients in Kenya. It is an infrequently isolated MTBC species, mostly from patients from, or with connection to Africa (Brosch *et al*., 2002) .

Mycobacterium bovis was the least MTBC species isolated in the current study, accounting for 0.7% which is the causative agent of bovine tuberculosis (bTB) a bacterial zoonosis. It was estimated that about 3.1% of human TB cases worldwide are caused by *M. bovis.* While <1% of human infections are caused by *M. bovis* in Spain, the prevalence in Africa ranges from 3.9% in Nigeria, up to 7% in Uganda, and even 16% in Tanzania (Romero *et al*., 2006); (Malama *et al*., 2014). It was reported that in some developing countries, *M. bovis* is responsible for 5–10% of all human TB cases and 30% of all TB cases in children (Wedlock, Skinner, de Lisle, & Buddle, 2002) *M. bovis* could be isolated from approximately 3.9–5% of human tuberculosis patients in Nigeria (Cadmus *et al*., 2006); (Mawak, Gomwalk, Bello, & Kandakai-Olukemi, 2006) and 0.6–1.85% of patients with pulmonary tuberculosis in Burkina Faso (Sanou *et al*., 2014). *M. bovis* is treated similarly to *M. tuberculosis* but it is usually naturally resistant to pyrazinamide. In fact, healthcare providers might not know that a person has *M. bovis* instead of *M. tuberculosis.*

Identification of NTM

The distribution of NTM species in the current study differs from that found in previous studies in Kenya. In 2015, Limo *et al* (2015) reported seven different NTM species *(M. intracellulare* 52.8%, followed by *M. absessus, M. fortuitum, M. sacrofuloceum, M. kansasii, M. interjectum* and *M. xenopi)*. However, in both studies *M. intracellulare* was the most frequently isolated species. Nyamogoba *et al* (2012) isolated three NTM species with notable absence of *M. kansasii* and *M. absessus, M. peregrinum* being among the isolates. *M intracellulare* has been reported to be predominant from South Africa and Australia (Hoefsloot *et al.*, 2013). These results are in agreement with the current study where *M. intracellulare* was the most predominant species. One study in India the predominated species was *M. abscessus* (31.3%), *M. fortuitum* (22%) and *M. intracellulare* (13.6%) (Umrao *et al.*, 2016) which is in contrary to the current study. The rate of 61.5% isolation of *M. intracellulare* in the present study was much higher compared to the 2.6% isolated in New Delhi (S. Singh *et al.*, 2007), Southern Asia 17.4% , Brazil 15% (Lima *et al.*, 2013) NTM from AIDS patients where 57.8% of the NTM cases was found to be *M. avium*, Turkey 20.8% (Ergin *et al.,* 2004). This results shows that the prevalence rates of NTM is higher than the case in our study. NTM isolation has also been reported in other studies; in the Netherlands (van Ingen *et a*l., 2012) Poland (Safianowska, Walkiewicz, Nejman-Gryz, Chazan, & Grubek-Jaworska, 2010) and Great Britain .(Moore, Kruijshaar, Ormerod, Drobniewski, & Abubakar, 2010) Based on the two studies conducted in Kenya both of which reported high prevalence rates of *M. intracellulare* isolated from pulmonary TB patients suggests its prevalence could be the result of its abundance in the two regions where the studies were done and its feature of drug resistance and pathogenicity promote its persistence in the regions.

M. abscessus was the second most common NTM species isolated in this study which accounted for 19.2%. A previous study in Kenya reported a similar trend, however lower prevalence rates 13.4% (Limo *et al.*, 2015). A study in India reported higher prevalence rates of *M. abscessus* 31.3% (Umrao *et al.*, 2016). *M. abscessus* is being seen with increasing frequency and is particularly di cult to treat medically, because the disease often progresses slowly over years and because older adults are typically a ected (Umrao *et al.*, 2016)

M. kansasii was the third most common NTM in our current study. Comparing with other studies, the isolation rates of *M. kansasii* was higher 11.5% (3/26). Low *M. kansasii* prevalence rates have been reported elsewhere in Kenya; Limo *et al.*, (2015) 3.4% among tuberculosis retreatment cases in Kenya, but a study done in Western Kenya to isolate NTM species of Mycobacteria did not isolate any *M. kansasii* (Nyamogoba *et al.*, 2012); India 1.9% (Umrao *et al.*, 2016). Poland 35% of all NTM (Wassilew, Hoffmann, Andrejak, & Lange, 2016) being the highest, Israel 33.9%, (Shitrit *et al.*, 2007), Europe 5% (Sester *et al.*, 2014). Clinically, *M. kansasii* is a slow-growing acid fast bacilli that causes a chronic, upper-lobe cavitary disease, resembling that from *M. tuberculosis*. The infections are also prevalent in areas where HIV infection is common due to the susceptibility of the hosts. The increasing incidences of pulmonary tuberculosis caused by *M. kansasii* may be due to the broad spectrum of immune deficiencies associated with cancers and their therapies. *M. kansasii* infection is treated differently than *M. tuberculosis* infection, and it does not require contact tracing because it is not transmitted by person-to-person contact. Thus, the clinical identification of *M. kansasii* would be helpful, given the delay before bacteriological confirmation is available.

M. fortuitum was the least predominant NTM species reported in the current study with an isolation rate of 7.7%. This is almost in agreement with the results obtained in Western Kenya 6.7% (Nyamogoba *et al.*, 2012) , Limo *et al.*, (2015) 6.7%. However, slightly higher than that reported in New Delhi-India 5.7% (S. Singh *et al.*, 2007) , however a second study in Lucknow-India reported a 22% rate of *M. fortuitum* which was much higher than the current study (Umrao *et al.*, 2016). *M. fortuitum* has also been isolated from TB patients in other parts of Sub-Saharan Africa; Tanzania (B. Z. Katale *et al.*, 2014)**;** South Africa (Van Halsema *et al.*, 2015); Nigeria (Pokam & Asuquo, 2012).

In Sub-Saharan Africa, especially in East Africa, most of the Mycobacterial species reported in the current study have also been isolated from both new and retreated cases of pulmonary TB. Kenya, Uganda and Tanzania have experienced a gradual rapid increase in the prevalence of pulmonary TB and increase in NTM infections, but it is still unclear if this trend is real or is the result of improvement in the use of new rapid molecular diagnostic techniques. Previous earlier studies conducted in the three countries concluded that Mycobacterial infections caused by NTM were rare in Africa, but recent studies in African countries with the major objective of isolating mycobacteria species responsible for TB using rapid molecular diagnostic methods have reported a high prevalence of NTM and other MTBC species other than *M. tuberculosis*.in immunocompromised patients such as HIV/AIDS. This shows that TB caused by NTM in HIV/AIDS patients is as common as TB caused by *M. tuberculosis*. Correct and sufficient data of human NTM infections in Kenya is yet to be unveiled, but the already conducted studies in specific regions in the country indicates that NTM problem is bigger than previously documented.

CONCLUSION & RECOMMENDATIONS

This results clearly emphasize the need for routine speciation among members of the MTBC and NTM is very

much necessary as it is an important prerequisite for the proper management of patients with mycobacterial infections. Since most NTM are not susceptible to conventional anti-TB drugs, it becomes desirable to identify these isolates up to species level particularly from AIDS and drug-resistant cases. MTBC species have different patterns of anti-TB drug sensitivity. In particular, the distinction between *M. tuberculosis* and *M. bovis* is necessary, as the latter species is naturally resistant to pyrazinamide which is commonly used in the management of pulmonary TB cases in Kenya. GenoType Mycobacterium CM/AS and MTBC Assay Kits if introduced as a required standard into routinely employed laboratory diagnostics in the speciation of mycobacteria infections could be a useful tool for diagnostic and epidemiological purposes in Kenya and more importantly in Kisumu County being a county with the highest prevalence in TB-HIV/AIDS co-infection.

STUDY LIMITATIONS

Most of the slides were AFB-positive because in the absence of culture, the result of AFB was a major criterion for defining diagnosis of TB. It should be noted that only pulmonary cases were included in which *M. bovis* is less frequent. The study only involved the two facilities located in Kisumu urban area, which may reduce the inclusion of patients from rural areas where *M. bovis* may have a higher prevalence.

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Table1.Socio-demographics (n=343)

Socio-demographic variable	GeneXpert(n=343)		Smear Status(n=343)		Culture (n=343)		
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	
Prevalence	317(92.4)	26(7.6)	290(75.8)	53(24.2)	316(92.1)	27(7.9)	Total
Age Category:							
Less than 35	185 (93.0)	14 (7.0)	168 (84.4)	31 (15.6)	189 (95.0)	10 (5.0)	**199**
Above 35	132 (91.7)	12 (8.3)	122 (84.7)	22 (15.3)	127 (88.2)	17 (11.8)	**144**
Sex:							
Male	171 (94.0)	11 (6.0)	146 (82.2)	36 (19.8)	172 (94.5)	10 (5.5)	**182**
Female	146 (90.7)	15 (9.3)	144 (89.4)	17 (10.6)	144 (89.4)	17 (10.6)	**161**

Table 2: The distribution of Mycobacteria species (n=316)

Mycobacteria species	N	%
M. tuberculosis complex (MTBC)	290	91.8
Non-tuberculous Mycobacteria	26	8.2
Total	**316**	**100**

Table 3: Distribution and relationship between patient characteristics and MTBC species (n=290)

	M. africanum n (%)	*M. bovis* n (%)	*M. tuberculosis* n (%)	p-value
Total	5(1.7)	2(0.7)	283(97.6)	
Age Category:				
Less than 35	1 (20.0)	0 (0)	174 (61.5)	0.020
Above 35	4 (80.0)	2 (100.0)	109 (38.5)	
Sex:				
Male	2 (40.0)	0 (0)	127 (44.9)	0.696
Female	3 (60.0)	2 (100.0)	156 (55.1)	

Table 4: Distribution and relationship between patient characteristics and NTM species (n=26)

	M.intracellulare n (%)	*M.abscessus* n (%)	*M.kansasii* n (%)	*M.fortuitum* n (%)	p-value
Total	16 (61.5)	5(19.2)	3(11.5)	2(7.7)	
Age Category:					
Less than 35	9 (56.3)	3 (60.0)	2 (66.7)	0 (0)	0.608
Above 35	7 (43.7)	2 (40.0)	1 (33.3)	2 (100.0)	
Sex:					
Male	11 (68.8)	3 (60.0)	0 (0)	1 (50.0)	0.182
Female	5 (31.2)	2 (40.0)	3 (100.0)	1 (50.0)	