



## Evaluation of Non-Tuberculous Mycobacterioses and multidrug resistant Tuberculosis in western Kenya

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### SUMMARY

**Background:** The diagnosis of tuberculosis (TB) in Africa is mostly based on the microscopy. However, both *Mycobacterium tuberculosis* and non-tuberculous mycobacterioses (NTM) yield positive results in the microscopic detection of acid-fast bacilli (AFB). The conventional anti-mycobacterial chemotherapy is not analogous to the treatment of tuberculosis-like syndromes caused by the NTM. This cross-sectional study was conducted in western Kenya in 2008 – 2009 to determine the role of NTM in the aetiology of TB-like disease, and demonstrate the confusion they present in the treatment of TB.

**Methods:** The sputa from 128 smear positive patients were cultured and growth was identified using the Hain's GenoType<sup>®</sup> *Mycobacterium* CM and GenoType<sup>®</sup> *Mycobacterium* AS kits. The *M. tuberculosis* complex isolates underwent anti-TB drug susceptibility testing to determine their susceptibility to rifampicin and isoniazid.

**Results:** No case of multi-drug resistant tuberculosis was isolated. The NTM constituted 7.5% of the isolates.

**Conclusion and recommendations:** Tuberculosis classically defined on the basis of ZN-smear positive of sputum may be over-diagnosed in western Kenya because some of the infections are caused by NTM. Conventional anti-TB drugs are ineffective against many of the NTM which may be confused with anti-TB multidrug resistance

**Key words:** Non-tuberculous mycobacteria; anti-TB drug resistance

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### Introduction

Tuberculosis is a chronic infectious disease caused by a bacillus called *Mycobacterium tuberculosis* (Mtb) [1]. Multi-drug resistant tuberculosis is defined as resistance to isoniazid and rifampicin whether there is resistance to other drugs or not [1, 2]. NTM refers to

all the species in the family of mycobacteria that may cause human disease, but do not cause TB or leprosy [3].

Tuberculosis (TB) is a global emergency due to its high morbidity and mortality. The disease is out of control in many parts of the world. The advent of HIV



pandemic has led to the TB–HIV/AIDS lethal combination with global prevalence, devastating morbidity and massive mortality [4, 5, 6]. The resurgence of multi–drug resistant TB (MDR–TB) and its transmission in the general community further complicates this problem and the emergence of non–tuberculous mycobacteria (NTM) as opportunistic infections in HIV/AIDS patients has worsened matters [7,8].

In poor resource settings, microscopy is still the only tool available for diagnosis of tuberculosis. All mycobacteria share the characteristic of acid fastness i.e. after staining with carbol–fuchsin, they do not decolorize with acidified alcohol [9]. Therefore it is not possible to differentiate tuberculosis and NTM infections using the ZN staining. To avoid over diagnosis of tuberculosis on the basis of positive ZN staining there is need to improve the diagnosis of TB and NTM disease by use of culture and molecular detection. This cross–sectional study was conducted in Western Kenya to determine the risk factors and the role of NTM in the aetiology of TB–like disease, and demonstrate the confusion that they can cause in diagnosis and treatment of TB.

Treatment of NTM infections is not analogous to the treatment of tuberculosis. Treatment of NTM requires not less than 24 months as opposed to eight months treatment of tuberculosis [10]. Multi–drug regimens which include a newer macrolide (azithromycin, clarithromycin) is used [10]. Failure to respond to treatment among patients with NTM infections could be attributed to multi–drug resistance.

## **Materials and methods**

**Study Design:** A cross–sectional study was conducted between December 2008 and June 2009.

**Study site and population:** The study was done at chest clinics Lodwar, Narok, Bungoma, Busia, and Uasin Gishu district hospitals. The target population comprised of the ZN smear positive clients.

**Sampling frame and patient characteristics:** The ZN smear positive patients were included if they sought healthcare services at the chest clinic between December 2008 and June 2009.

**Collection of demographic data:** Counseling and collection of demographic data were done by clinicians / nurses running the chest clinics. An interviewer administered questionnaire was used to obtain the data that included gender, age and past medical history.

**Collection of sputum samples:** Early morning and second spot sputum samples were collected from 128 ZN smear positive clients. The patient were requested to cough so that expectoration would come from as deep down the chest as possible, and spit into a sterile 50 ml blue cap tubes. The samples were refrigerated at 4°C awaiting transportation in cool boxes to the Mycobacteria Reference Laboratory, Moi University School of Medicine (MRL, MUSOM) Eldoret weekly for analysis. At the MRL, MUSOM, the samples were maintained at 4°C and were processed within 7 days of collection in order to minimize loss of viability of the mycobacteria.

**Microscopic examination of specimens:** Diagnosis for mycobacterial infection was carried by staining sputum specimens with carbol–fuchsin using the Ziehl–Neelsen (ZN) method [11].

**Isolation of mycobacteria:** Sputum specimens were processed (by digestion, decontamination and concentration) using the N–acetyl–L–cysteine sodium hydroxide (NaOH–NALC) method. The processed specimens were cultured for isolation of mycobacteria



using the Mycobacteria Growth Index Tube 7 mL (BBL™ MGIT™) supplemented with BACTEC™ MGIT™ Growth Supplement, BBL™ MGIT™ PANTA™ Antibiotic Mixture [12]. In addition to culture in the BACTEC MGIT 960, 0.2 to 0.3 ml (2–3 drops) of the concentrated specimens was inoculated onto LJ slants for maximum recovery of the mycobacteria. The bottles were incubated at 36–37 °C and examined on a weekly basis until growth was detected. Cultures were reported as negative if no growth occurred after 8 weeks of incubation [12]. Positive tubes (both MGIT and LJ) were processed for identification of mycobacteria.

**Identification of mycobacteria:** The mycobacterial isolates were identified as *M. tuberculosis* complex or species of non-tuberculous mycobacteria (NTM) using Hain's GenoType® Mycobacterium CM and GenoType® Mycobacterium AS Molecular Genetic Assays, following manufacturer's instructions [13].

**Anti -TB drug susceptibility testing:** The isolates underwent drug susceptibility testing (DST) using the Hain's GenoType® MTBDR<sub>plus</sub> Molecular Genetic Assay for Identification of Resistance to rifampicin and isoniazid, following manufacturer's instructions [13].

**Data analysis:** The data was entered into SPSS and analyzed using frequencies and cross tabulations.

**Ethical issues:** The proposal for this study was approved by Moi University School of Medicine (MU-SOM) / Moi Teaching and Referral Hospital (MTRH) Institutional Research and Ethics Committee (IREC) [FAN No: 000364]. The study was conducted in accordance with the Declaration of Helsinki [14]. Results on TB, NTM disease and HIV infection were availed to respective healthcare givers for appropriate patient care.

## Results

**Study population:** A total of 128 respondents were recruited in the study, 65% males and 35 % females. The age of the respondents ranged from 18 to 75 years with a mean of 30.7 years  $\pm$  10.8 SD. The age group of 28–37 years accounted for the majority (43%) of the respondents.

**Past medical history:** Past medical history indicated that 28.9 % of the patients had been diagnosed and treated for TB before, and 50% of the respondents had been diagnosed with HIV/AIDS before of whom 5% were on ARVS. Only 17.9% of the respondents had been diagnosed with both TB and HIV AIDS.

**Culture results:** A total of 115 specimens had growth on the liquid medium while 110 had growth on the solid medium. Part of the specimen had growth on both media, therefore some specimen made part of the 115 as well as part of 110 (Table 1).

**Table 1:** Cultures

Culture	Growth	No growth	contaminated	Total
MGIT	115	10	3	128
LJ	110	14	4	128

A total of 111(92.5%) isolates were identified as *M. tuberculosis* complex and 9 (7.5%) as NTM. Six of the NTM isolates were identified as *M. intracellulare* (2), *M. fortuitum* (2), and *M. perigrinum* (2). Three NTM isolates could not be identified due to species level.

**Anti-TB drug resistance:** Three 3 (2.7 %) isolates were resistant to rifampicin and 6 (5.4%) were resistant to isoniazid. No isolate was resistant to both



rifampicin and isoniazid. Therefore no case of multi-drug resistant strains was isolated (Table 2).

**Table 2:** Anti-TB drug resistance

Drug	Susceptible	Resistant	Total
Rifampicin	108	3	111
Isoniazid	105	6	111

## Discussion

Tuberculosis and associated HIV/AIDS are robbing many countries of resources and capacities on which human security and development depend. Dozens of resource poor countries including Kenya are already in the grip of serious epidemics [15]. The resurgence of MDR-TB is adversely affecting patient care further worsening the situation [16]. While studies in developed countries have shown that NTM play a significant role in the aetiology of TB-like syndromes (non-tuberculous mycobacterioses) in HIV-positive patients, their contribution to the TB problem in Africa and other poor-resource has hardly been examined [10].

In this study both liquid and solid media were used to culture each of the sputum specimen for isolation of mycobacterium. The rate of growth was higher for the liquid medium (89.8%) as compared to the rate of growth on the solid medium (85.9%). Some of the specimens which had no growth on the solid medium were able to grow on the liquid medium.

The rate of contamination from both media was comparable where 2.4% of the specimens cultured on the liquid medium were contaminated as compared to 3.1% contaminated specimens cultured on solid medium. Although sputum culture is still the gold standard test for the diagnosis of pulmonary

tuberculosis, the application of this diagnostic tool in most hospitals in low-income countries is not standard practice. To investigate the occurrence of NTM by culture, the use of MGIT tubes in combination with a solid medium is recommended. This would provide the number of colonies grown on solid medium and would help to determine the clinical relevance of a positive culture [17].

Solid culture for mycobacteria requires 3–8 weeks, which limits the usefulness of culture as a first line diagnostic test. In the last decade, liquid culture methods have become available in the Western world, with a shorter turn-round time of 1–3 weeks [18]. However, liquid culture systems have hardly been applied in Africa because of the costs and maintenance of the equipment involved [18]. In addition, culture of mycobacteria requires highly trained personnel and a dependable supply of water and electricity.

The current study found out that 92.5% of the isolates were Mtb while 7.5% isolates were NTM. Other studies in South Africa indicated a prevalence rate of NTM disease of 6.7 % [19]. In Africa the contribution of NTM to the clinical problem of diagnosis of tuberculosis has been examined at a very small scale [17]. The only available population-based studies on epidemiology of NTM in Africa have been conducted in South Africa and these were generally limited to selected populations.

In this study NTM were isolated from 9 of the 120 positive mycobacterial cultures. These sputum were tested by molecular method for the possible presence of *M. tuberculosis* i.e. Geno Type MTBDRplus of which they were negative. The specific NTM species were then identified using the combination of Genotype



mycobacterium CM and Geno Type Mycobacterium AS. Six isolates were identified as 2 *M. Intracellulare*, 2 *M. fortuitum*, 2 *M.perigrinum*. Three NTM isolates were not suitable for identification due to failure to re-culture. Worldwide, the most common NTM species causing human disease are the slow growing mycobacteria of the *M.avium* complex (*M.intracellulare* and *M.avium*) and *M.Kansasii* [10]. In a study done in Zambia the most commonly isolated NTM in patients and controls was *M. intracellulare*. Other species isolated included *M. lentiflavum*, *M. avium*, *M .gordanae*, *M. fortuitum*, *M. chelonae* ,and *M.peregrinum* [17]

In Africa despite the fact that environmental exposure to NTM is very high, infection by NTM is rare, even among patients with AIDs [20]. However, it is not clear whether this is due to a true low prevalence of the NTM infection or that the magnitude of this problem in terms of prevalence and distribution has not yet been established. A study done in Nigeria among people with persistent lower respiratory tract infections indicated a prevalence rate of NTM infections of 11%. Among these NTM, six were classified as *M. avium*, four *M. kansasii*, and one *M. fortuitum* [21]. In Kenya a study done among rural and urban population of adults who presented with acute pneumonia indicates a prevalence rate of NTM infections of 3–6 % [22]. The NTM species identified included three *M. fortuitum*, two *M. szulgai*, two *M. kansasii* one *M. terrae* and three other NTM species.

In general NTM infections in Africa are not treated currently and indications have been found that at least a part of the NTM isolated in African patients is clinically relevant [10]. Patients diagnosed with NTM disease should be evaluated for initiation of therapy and outcome should be strictly followed for extended

time periods. The presentation of NTM infections mimic TB, confounding the diagnosis of TB especially MDR–TB. In this study 7.5 % of the respondent had NTM infections, but on the basis of ZN–smear positive of sputum, these patients had been diagnosed with tuberculosis and started on Anti TBs in the respective district hospitals. The treatment of NTM is not similar to the treatment of tuberculosis. Multi–drug regimens which include a newer macrolide (azithromycin, clarithromycin) is used [10]. The duration of therapy for NTM pathogens is at least one year until sputum cultures are consecutively negative while on therapy as opposed to eight months treatment of tuberculosis [17]. Failure to respond to treatment in this group of patients could have been attributed to multidrug resistance while the real problem was in the diagnosis and hence the type of treatment the patients were put on. In the light of this, methods for distinguishing *M. tuberculosis* and NTM in clinical materials should be implemented on a broader scale in western Kenya and Africa in general.

The current study showed that more *M. tuberculosis* isolates resistant to isoniazid (5.4%) as compared to rifampicin (2.7%). No isolate was resistant to both drugs. These results were in agreement with the 1993–1994 WHO anti TB drug resistance survey, in which Kenya reported no multi–drug resistant TB. However, isoniazid mono–resistance was reported to be 5 % and 10% for primary and combined resistance respectively. No resistance to rifampicin was reported [2]. However, in 2008 [23], the DLTLTD through the Central Reference Laboratory (CRL) reported 102 *M. Tuberculosis* complex isolates out of 5,604 specimens examined resistant to both isoniazid and rifampicin. In 2009 [24] , the DLTLTD identified and notified to the WHO a total of 150 MDR–TB cases out of 6,569



specimens received at the CRL with one XDR-TB case isolated and initiated on treatment at Moi teaching and Referral Hospital, Eldoret. This is as result of existing policy supporting MDR-TB diagnosis and treatment and the USAID support of routine MDR-TB surveillance in Kenya.

A plausible explanation for the much lower resistance levels observed in the present study compared to the DLTLD report is that the CRL deals with retreatment cases (relapses and treatment failures) country wide. However, recurrences can be either relapses (endogenous reactivation) or exogenous re-infections. A relapse (R) is defined as a smear-positive TB patient who has previously been treated and declared cured. Treatment failure (TF) is a patient with a positive smear at the end of five months despite being on anti-TB treatment, hence failing to respond to treatment. The probability of anti TB drug resistance is higher in relapses and treatment failures.

Tuberculosis classically defined on the basis of ZN-smear positive of sputum may be over-diagnosed in western Kenya because 7.5% of the tuberculosis-like syndromes in the region is caused by NTM. The treatment of such infections is different from that of classical tuberculosis and therefore failure to respond to the first line anti-tuberculosis drugs may erroneously be attributed to multidrug resistant tuberculosis.

### **Conclusion and Recommendations**

The study showed a high prevalence of non-tuberculosis mycobacterioses of 7.5% as compared to other studies done in Kenya. No MDR-TB cases were reported in the present study, and the first-line anti-TB drugs can still be used for effective treatment of TB cases in western Kenya. Tuberculosis classically defined on the basis of ZN-smear positive of sputum

may be over-diagnosed in western Kenya because some of the infections are caused by NTM. There is need to incorporate the diagnosis of NTM in pulmonary disorders in order to reduce misdiagnosis of TB. Clinicians should be empowered with knowledge on how to treat NTM infections through workshops and training.

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