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# MISDIAGNOSIS AND CLINICAL SIGNIFICANCE OF NON-TUBERCULOUS MYCOBACTERIA IN WESTERN KENYA IN THE ERA OF HUMAN IMMUNODEFICIENCY VIRUS EPIDEMIC

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## ABSTRACT

***Objectives*: To determine and document the role of non-tuberculous mycobacteria (NTM) in TB-like disease morbidity and demonstrate the confusion they cause in the diagnosis of TB in Western Kenya.**
***Design*: A cross-sectional study.**
***Setting*: One provincial and nine District hospitals in Western Kenya.**
***Subjects*: Tuberculosis suspects.**
***Interventions*: Sputa from 872 tuberculosis suspects underwent microscopy and culture on solid and liquid media. The growth was identified using the Hain's GenoType® *Mycobacterium* CM and GenoType® *Mycobacterium* AS kits. Consenting clients were screened for HIV infection using Trinity Biotech Uni-Gold™ test and positive cases were confirmed with the enzyme linked immunosorbent assay. A questionnaire was used to obtain demographic data.**
***Main outcome measures*: ZN smear positivity / negativity; Culture positivity or negativity; *Mycobacterium* species isolates (tuberculous or non-tuberculous); HIV status.**
***Results*: Sputa from 39.1% (341/872) of the participants were ZN smear positive, of these 53.1% (181/341) were culture positive. Only 3.8% (20/531) of the ZN smear negatives were culture positive. In total 41.4% (361/872) participants were infected with mycobacteria, of which 44.3% (160/361) were culture negative and 55.7% (201/361) were culture positive. The culture positives yielded 92.5% *M. tuberculosis* complex and 7.5% NTM. The overall prevalence of the NTM disease was 1.72% (15/872).**
***Conclusion*: A low prevalence of NTM pulmonary disease in western Kenya is reported in this study, but some the NTM disease cases could have been misdiagnosed as TB cases.**

## INTRODUCTION

The genus *Mycobacterium* causes more morbidity and mortality worldwide than all other bacterial infections combined. The most notorious has been the *M. tuberculosis* complex also known as tubercle bacillus, followed by the lepra bacillus, *M. leprae*, the aetiological agent of leprosy (1). However, the emergence of non-tuberculous mycobacteria (NTM) as opportunistic pathogens in HIV / AIDS patients is gaining clinical significance. The NTM are *Mycobacterium* species different from those belonging to *M. tuberculosis* complex (2), most of them being saprobes (3). However, some are opportunistic pathogens, which may cause severe and fatal TB-like syndromes (4). Skin test data suggest that a high proportion of people have been exposed to one or more NTM species. The predominant NTM species may vary from country to country and between different areas of a country (3, 5, 6).

*Mycobacterium avium* complex (MAC), also referred to as *M. avium- intracellure* (MAI) complex, is the most common cause of NTM disease. The MAC consists of 28 serovars of two distinct species,

*M. avium*, and *M. intracellulare* (7), and is responsible for progressive and usually fatal disease if untreated, especially in immunocompromised patients (1, 8). *Mycobacterium kansasii* is second to MAC in the causation of NTM lung disease (1). The American Thoracic Society (ATS) (9) reports MAI complex, *M. kansasii M. fortuitum* and *M. chelonae* as the most common NTM causing chronic respiratory disease, with *M. kansasii* causing chronic pulmonary disease similar to reactivation TB. *Mycobacterium kansasii* infection occurs worldwide but is most common in the USA and UK (10). In Kenya, *M. fortuitum* / *M. chelonae*, *M. szulgai*, *M. kansasii,* and *M. terrae* are among the NTM species that have been isolated from patients who present with acute radiologically confirmed pneumonia (11).

Lately however, new NTM species have emerged as opportunistic pathogens in HIV / AIDS patients. *Mycobacterium genasense* was first isolated in 1990 from a Swiss patient, and is now being reported in other European countries, USA, and Australia. *Mycobacterium celatum*, which seems biochemically indistinguishable from *M. avium*, but shows mycolic acid patterns closely related to that of *M. xenopi*, is also being reported to cause disease (12). The other NTM species which have been associated with lung disease in HIV / AIDS patients include *M. malmoense*, *M. xenopi* (13), *M. abscessus*, *M. chelonae*, *M. fortuitum* (14), *M. asiaticum* (15), *M. haemophilum* (16), *M. triviale*, *M. szulgai* and *M. smegmatis* (17). Death rates from NTM disease are high even with treatment (13). Due the wide spread of HIV in developing countries, the role of NTM in mycobacterioses (TB-like syndromes) may be underestimated particularly in sub-Saharan Africa (4). This study was carried out to determine the role of NTM in TB-like disease morbidity and demonstrate the confusion they cause in the diagnosis of TB in western Kenya.

## MATERIALS AND METHODS

*Study Design*: A cross-sectional study was conducted between September 2007 and September 2009. It was carried out to provide a snapshot (one point in time measurements) description of the significance of the NTM in causing TB-like disease in Western Kenya.

*Study site and population:* The study was done at one provincial and nine district hospitals in western Kenya. These were Busia, Bungoma, Kisumu, Migori, Kisii, Narok, Kericho, Uasin Gishu and Lodwar district hospitals, and Nakuru Provincial General Hospital. Western Kenya includes the expansive former Rift Valley, Nyanza and Western Provinces, with a cumulative population of about 19.8 million people. This constitutes about 52.1% of the Kenyan population, according to the Kenya Census of 2009.

*Sampling frame and patient characteristics*: The participants suspected of having pulmonary TB were enrolled into the study between September 2007 and September 2009 as they sought healthcare services at the chest and paediatric clinics. Cases who had prior treatment before attending to the clinics were carefully screened and those already on anti-TB were excluded. Participants were suspected of having TB if they had a cough of more than two weeks not responding to antibiotic treatment (NLTP, 2003)

*Collection of demographic data*: A questionnaire was used to obtain participant demographic data. Data collected included age, gender, previous anti-TB treatment, HIV status and anti-retroviral therapy (ART).

*Collection of samples:* At least two millimetres of three sputum specimens (spot, early morning, spot) (18) were collected from 872 participants with suspected TB under the supervision of trained and competent medical staff. The patient were requested to cough so that expectoration will come from deep down the chest as possible, and spit into sterile 50 ml blue cap tubes. The samples were refrigerated at 4$^{o}$C awaiting transportation in cool boxes to the Mycobacteria Reference Laboratory, Moi University School of Medicine (MRL, MUSOM) weekly for analysis. At the MRL, MUSOM, the samples were refrigerated at 4$^{o}$C till processing. However, the samples were processed within seven days of collection in order to minimize loss of viability of the mycobacteria. Consenting 695 participants also underwent phlebotomy for HIV testing. The blood was delivered into Vacutainer Brand STERILE interior EDTA (K3) tubes and stored at –20$^{o}$C awaiting processing. The samples were transported in cool boxes to MRL, MUSOM, Eldoret, and processed within two weeks. The safety for research assistants and healthcare workers during collection and handling of sputum specimens was ensured by observing the WHO guidelines (19).

*HIV testing*: Screening for HIV infection was done by screening serum by the Trinity Biotech Uni-Gold™ test (20) and positives confirmed with the enzyme linked immunosorbent assay (21).

*Microscopic examination of specimens*: Diagnosis for mycobacterial disease was done after staining specimens with carbol-fuchsin using the ZN method (18).

*Isolation of mycobacteria and identification of mycobacteria:* Sputum specimens were processed for isolation of mycobacteria following standard protocols (22). 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 (23).

The suspects with ZN smear positive but culture negative sputa were treated as smear negative pulmonary TB cases.

*Data analysis:* Data were entered in MS Excel 8.0 and analysed using Epi Info version 3.5.1 to calculate proportions. Descriptive statistics were used to summarise data.

*Ethical issues:* The proposal for this study was approved by ITROMID / KEMRI's Scientific Steering Committee (SSC) and Ethical Review Committee (ERC) [SSC No. 837] and by Moi University School of Medicine (MUSOM) / Moi Teaching and Referral Hospital (MTRH) Institutional Research and Ethics Committee (IREC) [FAN No.00092]. The study was conducted in accordance with the Declaration of Helsinki (24). Results on TB, NTM disease and HIV infection were availed to respective healthcare givers for appropriate patient care. The HIV positive cases were referred for post-test counselling and enrolment to HIV / AIDS Programme.

## RESULTS

*Study participants*: A total of 872 TB suspects were enrolled into the study, 54.9% (477) males and 45.1% (393) females. Their median age was 32 years. The majority (33.1%) were in the 25-34 age-group, followed by those in the 35-44 (21.8%) and 15-24 (18.7%) age brackets respectively. Paediatric cases (0-14 age-group) were the lowest with 4.6%, with children below five years accounting for only 0.6% (Table 1).

*Smear microscopy and culture:* Sputum specimens from 39.1% (341/872) cases were ZN smear positive, of which 53.1% (181/341) were culture positive. Of the ZN smear negative, 3.8% (20/531) were culture positive. Hence, of the 41.4% (361/872) cases with mycobacterial disease, 44.3% (160/361) were culture negative and 55.7% (201/361) were culture positive. Among the culture positives, 92.5% of the isolates were *M. tuberculosis* complex and 7.5% were NTM. The 42.6% (160/341) cases that were ZN smear positive but culture negative were regarded and treated as TB. No cultures yielded tuberculous and non-tuberculous mycobacteria co-infection. Five of the NTM isolates were identified as *M. intracellulare* (3 isolates), and *M. fortuitum* and *M. peregrinum* one isolate each. The remaining ten NMT isolates could not be identified to species level.

Of the 15 NTM disease cases, ten were males and five were females. The majority (40%) of the NTM infection cases were in the 25-34 year age-group, followed by the 15-24 year age-group with 20% (Table 2). Four of the NTM disease cases (three males and one female) had been previously treated for TB. Six (40%) of cases were co-infected with HIV, five (33.3%) were HIV negative, and four (26.7%) were of unknown HIV status. Three of the NTM-HIV co-infection cases were on antiretroviral therapy (ART).

**Table 1**
*Distribution of study participants by gender-age*

| Age-group | N (%) | Males (%) | Females (%) |
|---|---|---|---|
| 0-14 | 39(4.5) | 22(2.5) | 18(2.1) |
| 15-24 | 163(18.7) | 80(9.2) | 83(9.5) |
| 25-34 | 288(33.1) | 162(18.6) | 126(14.4) |
| 35-44 | 190(21.8) | 108(12.4) | 82(9.4) |
| 45-54 | 89(10.2) | 108(12.4) | 36(4.1) |
| 55-64 | 54(6.2) | 29(3.3) | 25(2.9) |
| > 64 | 48(5.5) | 25(2.9) | 23(2.6) |
| Total | 872(100) | 479(54.9) | 393(45.1) |

**Table 2**
*Gender-age distribution of NTM disease cases*

| Age-group | N (%) | Males (%) | Females (%) |
|---|---|---|---|
| 0-14 | 1(6.7) | 0 | 1(6.7) |
| 15-24 | 3(20.0) | 2(13.3) | 1(6.7) |
| 25-34 | 6(40.0) | 4(26.7) | 2(13.3) |
| 35-44 | 2(13.3) | 2(13.3) | 0 |
| 45-54 | 1(6.7) | 0 | 1(6.7) |
| 55-64 | 1(6.7) | 1(6.7) | 0 |
| > 64 | 1(6.7) | 1(6.7) | 0 |
| Total | 15(100) | 10(66.7) | 5(33.3) |

## DISCUSSION

The NTM disease is being associated with HIV/AIDS and encountered with increasing frequency in non-aids patients (9). However, most of the data reporting high rates of NTM disease come from developed countries (4). In Africa, the contribution of NTM to the clinical problem of TB has so far only been examined at a very low scale. In South Africa for instance, two studies have reported prevalence rates of NTM colonisation / infection of 1, 400 and 6,700 per 100,000 respectively (25). In Zambia, Buijtels [4] has reported NTM colonisation rate of 14/154 (9%) in the patient population with a disease rate of 3/154 (2%), and a colonisation rate of 61/383 (16%). In present study, 7.5% (15/201) of the mycobacterial disease cases were NTM TB-like syndromes, giving an overall prevalence of 1.72% (15/872). However, since NTM diseases are frequent in HIV infected patients in low income countries (26), some of the 160 ZN smear positive but culture negative cases treated as TB in the current study could be NTM disease cases. This could imply underestimations of the prevalence rates of non-tuberculous mycobacterioses in high HIV prevalence countries.

While tremendous progress has been made in tuberculous and non-tuberculous mycobacterioses diagnostics in developed countries, techniques for the diagnosis of these diseases have remained relatively unchanged (invariably based on ZN smear microscopy) in Africa and other resource-poor settings, albeit supplemented by chest X-ray in some settings. The level of sophistication and cost associated with the new and more sensitive techniques have made their general applicability unfeasible in developing countries (4), where the basis for TB diagnosis has continued to be ZN smear microscopy of specimens to visualise acid-fast bacteria (AFB), which may capture 50-69% of the cases when carried out by experienced technicians. However, in most low-income countries, much lower rates of case detection are achieved due to poor quality microscopes, heavy workload, and shortage of trained staff. Moreover, ZN smear microscopy is usually negative in less advanced disease especially among HIV/AIDS co-infected patients and extra-pulmonary TB cases. The proportion of cases detected by microscopy in some low-income countries is often as low as 20-30% of all cases (4, 27).

The diagnosis of smear-negative pulmonary TB (PTB) may even be more complicated when reliance is solely placed on clinical and radiological features, which may not distinguish NTM TB-like respiratory syndrome from PTB. However, to guide the diagnosis of the NTM pulmonary mycobacterioses, the bacteriological diagnostic criteria established by the American Thoracic Society (ATS) (9) can provide support; a single NTM culture from bronchial washing fluid or two positive sputum cultures, in a symptomatic patient with nodular or cavitary opacities in the chest radiograph (28). In the present study, however, NTM disease was diagnosed based on at least two positive cultures from two separate expectorated sputa from symptomatic patients with chest pain, with or without ZN smear positivity. However, lack of patient chest X-ray data to augment clinical, microscopic and culture findings could be a significant limitation of the current study.

The highly discouraging finding in the current study is the high rate (42.6%) of ZN smear positive but culture negative cases treated as TB observed in the present study. Not all acid fast bacilli represent mycobacteria, let alone *M. tuberculosis* complex. The NTM (26) and some other bacterial species including *Nocardia* and *Rhodococcus* species which are widely spread in the environment yield positive results in ZN smear detection of acid-fast bacilli (AFB) and may present with similar radiological features (29). Moreover, a number of NTM including *M. haemophilum*, *M. genavense*, *M. avium* subsp. *paratuberculosis* (formerly *M. paratuberculosis*), and *M. ulcerans* are fastidious and require special nutrient supplementation for optimal recovery on cultures. For instance, *M. haemophilum* grows only on media supplemented with iron-containing compounds such as ferric ammonium citrate, hemin, or haemoglobin, while *M. genavense* and *M. avium* subsp. *paratuberculosis* require mycobactin J incorporated into the medium. Similarly, *M. ulcerans* may be optimally recovered with egg yolk supplementation (9). Worthy noting also is that a significant proportion of patients, especially HIV positive may give negative ZN smear results.

From the foregoing and the results of current study, it is evident that ZN microscopy as a diagnostic tool for TB is imprecise and causes a significant over-diagnosis of TB among HIV/AIDS patients. Some of NTM disease cases could be misdiagnosed as TB and put on anti-TB chemotherapy, even though the treatment of NTM disease is generally not directly analogous to TB treatment (9,28). Multi-drug regimes are used for NTM TB-like disease treatment, the cornerstone agents being a newer macrolide (azithromycin, clarithromycin) (28), ethambutol, and rifamycin, and require prolonged durations of therapy aimed to facilitate clearance of the mycobacteria and minimise the emergence of drug resistance (9, 28). However, cure of NTM disease is not the goal of therapy in all patients. Palliation of symptoms or minimisation of disease progression may be the desired result for some patients. Symptomatic, radiographic, and microbiologic improvement (conversion of sputum cultures) may be the desired treatment outcome (9). Patients frequently show clinical improvements within four to six months of beginning therapy, with negative sputum cultures

typically occurring within six to twelve months on multiple-drug regimens. The requirement that treatment be continued for up to 12 months of documented negative sputum cultures translates to treatment duration of 18 to 24 months, but it may be longer for some patients. However, treatment failure is not uncommon (no clinical improvements after six months or positive sputum culture after 12 months of appropriate therapy), which may be related to treatment non-compliance or intolerance, anatomic defects (cavitation or bronchiectasis), or drug resistance (especially to macrolides). Relapses and re-infections are also common and may not be related to drug susceptibility (30). Additionally, the treatment regimens are expensive, and often poorly tolerated because of frequent side effects (toxicity), with patients often describing the treatment to be worse than the disease itself (9).

In conclusion, the prevalence of NTM disease in Western Kenya may be considered low, but a comprehensive national survey on NTM TB-like morbidity is necessary. Some of the NTM pulmonary disease cases could have been misdiagnosed as TB, considering that a high number of ZN smear positive but culture negative cases were treated as TB cases.

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