

The role of nontuberculous mycobacteria in the diagnosis, management and quantifying risks of tuberculosis in Tanga, Tanzania

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Abstract

Background: The role of nontuberculous mycobacteria (NTM) in tuberculosis (TB) diagnosis is well documented in many developing settings. However, this has not been the case in many resource poor settings like Tanzania. This study aimed at understanding the role of NTM in the diagnosis and management of TB in resource poor settings of Tanzania.

Methods: A cross-sectional study was conducted in Tanga, Tanzania. Patients with symptoms suggestive of TB self-referred to health care facilities were recruited. Two sputum samples were collected for standard direct smear microscopy. Culture was performed using BacT/Alert 3D system, Löwenstein-Jensen and Gottsacker slopes. Identification of *Mycobacterium tuberculosis* and NTM was done by using GenoType[®]MTBC and GenoType[®]CM/AS, respectively.

Results: A total of 372 patients were involved in the study. Eighty-one (21.8%) patients were diagnosed as having *M. tuberculosis* by the isolation of the organism from cultures of sputum. Further analysis of culture showed that 8.1% (30/372) were NTM with 7/372 (1.9%) cases of NTM classified as pulmonary tuberculosis (PTB) patients. Ziehl Neelsen stain had a sensitivity of 68.8% and produced 10 false negative results. On the other hand, Fluorescence stain had a sensitivity of 85.7% and gave seven false negative samples when compared with culture results. Weight loss ($p = 0.0001$), fatigue ($p = 0.003$), fever ($p = 0.038$) and night sweats ($p = 0.004$), young population (18-40 years) ($p = 0.0352$), males ($p = 0.0025$) were important risk factors for TB. Four out of 30 NTM diagnosed by culture received first line anti-TB treatment suggesting that a good proportion of patients (4/65, 6.2%) were mistreated as TB patients.

Conclusion: Inefficient screening of TB patients in resource poor settings and prevalent increase of NTM may contribute to over diagnosis of TB cases. The need to integrate NTM diagnosis in the routine management of TB is urgently needed for designing effective tuberculosis prevention and control strategies in the country.

Keywords: tuberculosis, nontuberculous mycobacteria, management, Tanzania

Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) complex remains a major public health challenge worldwide and a leading cause of morbidity and mortality throughout sub-Saharan Africa (WHO, 2009). The emergency of multidrug-resistant (MDR) TB and co-infection with HIV have continued to threaten global TB control, especially in resource poor settings of sub-Saharan Africa (Cobelens *et al.*, 2008; Migliori *et al.*, 2008a,b; WHO, 2010a). The problem in many resource poor settings like Tanzania is further compounded by weak health care systems, and inadequate laboratory infrastructure.

The incidence of all forms of TB in Tanzania is estimated at 295 cases per 100,000 persons per year (NTLP, 2011). The country has achieved commendable landmarks in fighting

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against TB, and listed among 13 countries from the high-burden countries (HBCs) that achieved treatment success rate target of 85% set by the World Health Organization for new sputum smear-positive cases of pulmonary TB (WHO, 2010a). Case detection rate for new smear positive TB cases remains low at 51%, far below the WHO's target of 70%. The WHO report further shows an alarming trend of increased active TB cases in Tanzania due to HIV/AIDS epidemic, which is associated with 60 percent increase in active TB (WHO, 2010a).

In spite of these achievements, a number of challenges face the TB control programme. The current surveillance system still relies on passive case finding among individuals who self-referred to health facilities, followed by diagnosis based on the presumptive clinical TB symptoms and laboratory diagnosis using insensitive direct sputum smear microscopy (Mfinanga *et al.*, 2007; Matee *et al.*, 2009). Typically, this method has been shown to be poor in performance, with low detection rate of 56 to 68% resulting in missing of half of the cases (WHO, 2009). The sensitivity is reported to be further reduced to 43-51% among TB/HIV co-infected patients (Sonnenberg *et al.*, 2001).

Several studies have demonstrated that serious infections with non-tuberculosis mycobacteria (NTM) remain a major public health concern in many TB-endemic countries (Crump *et al.*, 2009; Buijtelts *et al.*, 2010; Maiga *et al.*, 2012). NTM can present with clinical and radiologic manifestations comparable to *M. tuberculosis*. Most often, it is not feasible to differentiate between NTM and MTB microscopically in situations where sputum culture and other diagnostic tests are not done. Increased prevalence of nontuberculous mycobacteria (NTM) infections among TB patients which are difficult to differentiate from MTBC further jeopardises the control efforts to contain the disease (Migliori *et al.*, 2008).

It is apparently therefore, that the need for a quick and accurate diagnosis of TB is critically important. Accurate diagnosis of MTB will not only ensure accurate and timely management of TB patients, but also will reduce morbidity, mortality, economic loss, further spreading of infection and emergence of MDR strains (Kox *et al.*, 1994; Moulding *et al.*, 2004). This study therefore aimed at determining the role of nontuberculous mycobacteria (NTM) in diagnosis, treatment and quantifying risks for TB transmission in Tanga, Tanzania.

Materials and Methods

Study area, design and population

This cross-sectional study was conducted in Tanga, Tanzania from November 2012 to January 2013. The study involved four health facilities, namely Makorora and Ngamiani health centres and Bombo Regional Referral hospital in Tanga City and the Designated District Hospital in the nearby Muheza district. All patients with clinical signs and symptoms suggestive of TB self-referred to four primary health care facilities were eligible for the study. Demographic information and data were collected only after provision of informed consent. A careful cross examination of patient history for previous anti-TB treatment using a structured questionnaire was used to classify patients as 'new' or 'previous treated' TB cases. A patient was considered as "new case" if had no prior anti-TB treatment for more than 1 month and was considered 'previous treated' if had received anti-TB treatment lasting at least 1 month (Abdel-Aziz, 2003). There were no restrictions on inclusion criteria regarding clinical symptoms and age of the patients.

Sputum and data collection

Demographic data were obtained using structured questionnaire administered to the patients attending the health care facilities who provided informed consent to the study. Two sputum samples (one spot during the initial visit to the clinic and one early morning) were collected into small autoclavable wide mouth glass bottles. The specimens were examined using the standard direct smear microscopy at the respective facilities using either Ziehl Neelsen (ZN) stain (Makorora and Ngamiani health centres) or fluorescence stain (Bombo and Muheza). Diagnosis of smear-

positive TB was performed based on the National Tuberculosis and Leprosy Programme Guidelines (NTLP, 2006). All morning sputum samples kept at -20°C at the respective clinics. No preservatives were added until shipped to the mycobacteriology laboratory at the Institute of Medical Microbiology and Epidemiology of Infectious Diseases, University Hospital, Leipzig, Germany for culture and molecular analysis. HIV status of the patients was determined by rapid HIV screening method at the treatment and care centres (CTC) of the respective clinics.

Sputum culture and identification of mycobacterial isolates

Sputum specimens were digested and decontaminated using N-acetyl-L-cysteine-sodium hydroxide method (Kent & Kubica, 1985) and were re-examined for the presence of acid-fast bacilli by fluorescence stain in Leipzig. The isolates were cultured in BacT/Alert 3D liquid culture system (bioMérieux, Marcy-l'Étoile, France) and on Löwenstein-Jensen and Gottsacker slants (Artelt-ENCLIT GmbH, Wyhra, Germany). Gottsacker slopes contains sodium pyruvate for isolation of *M. bovis*. Cultures were incubated at 37°C for up to 8 weeks. Confirmation of *M. tuberculosis* and NTM was done based on presumptive phenotypic appearance of colonies and by line probe assay GenoType®MTBC for identification of *M. tuberculosis* and GenoType®CM/AS for the identification of common and additional NTMs. The assay kits were obtained from Hain Lifescience, Nehren, Germany.

Data analysis

Descriptive statistics such as frequencies, means and proportions for different study attributes were computed by using Open Epi software version 3. A 2x2 contingency table for comparison of direct smear microscopy and the gold standard cultural method was built and prevalence, sensitivity, specificity and kappa statistic of agreement were calculated by using SAS software version 9. The Chi-square test of association between TB status of study participants and clinical signs were computed at significant level of 5%. The risk factors associated with positive TB status were quantified by logistic regression using SAS software version 9. The model building process followed a backward stepwise strategy whereby univariable analysis of all variables preceded the multivariable analysis. The variables included age, gender, occupation, previous TB history, smoking, diabetes and HIV/AIDS status. A variable qualified for multivariable analysis when it had a p-value of less than 0.25. A multivariable model was run and an iterative process of removing a variable with highest p-value was done until all variables in the model had p-value of less than 0.05. A variable was regarded as a confounder and retained in the final model when its removal from the multivariable model caused a more than 25% or between -0.4 and 0.4 changes in coefficient of any other variable. Model fitness test was assessed by Hosmer-Lemeshow test.

Ethical considerations

The protocol for this study was approved by the Medical Research Coordinating Committee of National Institute for Medical Research, Tanzania (Certificate No. NIMR/HQ/R.8a/Vol. IX/1401). Written informed consents were obtained from all participants of this study. For illiterate participants, there was an independent witness present during the consenting process, who then signed the relevant witness section of the consent form.

Results

Comparison of results of staining techniques and culture

A total of 372 TB suspects including 312 new and 60 previously treated cases were recruited. Eighty-one of the 372 (21.8%) patients were diagnosed as having *M. tuberculosis* by the isolation of the organism from cultures of sputum. A total of 176 samples were analysed by ZN staining while 196 samples were analysed by fluorescence staining. ZN stain was able to detect 22 out of 32 positive samples by culture technique. This means ZN method had a sensitivity of (68.8%) and produced 10

false negative results. On the other hand, Fluorescence staining was able to detect 42 out of 49 positive samples by culture technique (sensitivity = 85.7%) and seven (7) samples were false negative (Table 1). Moreover, culture results showed that 8.1% (30/372) of the sputum was positive for NTM, with 7/372 (1.9%) of culture confirmed NTM recorded as TB positive case by the health facilities. The remaining 68.3% (254/372) were culture negative for *M. tuberculosis* at 8 weeks.

Table 1: Relationship of results between ZN stain, Fluorescence stain and culture (n=372)

			Culture		
			positive	negative	Total
Stain	ZN	Positive	22	0	22
		Negative	10	144	154
	Fluorescence	Positive	42	0	42
		Negative	07	147	154
		Total	81	291	372

Assessment of criteria for suspecting TB cases in Tanga

Currently, one of the defining criteria for suspecting TB is cough for over 2 weeks. In attempt to establish how correctly the clinics were able to assess the patients presented with TB cardinal symptoms and the actual TB cases, we analysed the association of TB status with the clinical manifestations presented during patients visit to the TB facilities. Results show that weight loss ($p = 0.0001$), fatigue ($p=0.003$), fever ($p=0.038$) and night sweats ($p=0.004$) were statistically significantly associated with TB positive status of patient in Tanga (Table 2).

Table 2: Assessment of TB status and clinical signs presented by patients in Tanga (n=372)

Variable	Category	n	TB +	TB -	Df	X ² value	p-value
Cough \geq 2 weeks	Yes	327	75	252	1	2.14	0.14
	No	45	6	39			
Bloody sputum	Yes	44	11	33	1	0.3	0.58
	No	328	70	258			
Night sweats	Yes	249	65	184	1	8.29	0.004
	No	123	16	107			
Fatigue	Yes	208	57	151	1	8.78	0.003
	No	164	24	140			
Fever	Yes	254	63	191	1	4.31	0.038
	No	118	18	100			
Dyspnoea	Yes	176	46	130	1	4.04	0.045
	No	194	34	160			
Weight loss	Yes	138	49	89	1	24.16	0.0001
	No	228	31	197			

Quantification of risk factors for occurrence Tuberculosis in patients in Tanga

Univariable analysis of TB status (dependent variable) against age, gender, occupation, TB history, smoking, diabetes, HIV/AIDS (independent variables) was run. Results of this analysis revealed that age, sex and occupation were significantly associated with positive TB status in patients in study area (Table 3).

Table 3. Single and modelled multiple risk factors for occurrence of TB

Risk factor	Response	n (%)	TB positive n (%)	OR	95% CI	P value
Age (years)	5-17	16	4 (4.9)	0.211	0.046 – 0.962	0.3637
	18-40	184	49 (60.5)	0.193	0.067 – 0.561	0.0352
	41- 60	111	24 (29.6)	0.254	0.084 – 0.772	0.3896
	> 60	61	4 (4.9)			
Sex	Male	192 (51.6)	54 (66.7)	2.217	1.323 – 3.715	0.0025
	Female	180 (48.4)	27 (33.3)			
Occupation	High contact	147 (39.5)	46 (56.8)	2.472	1.497 – 4.083	0.0004
	Low contact	225 (60.5)	35 (43.2)			
Previous TB history	Yes	61	11 (13.6)	0.757	0.374 – 1.533	0.4399
	No	311	70 (86.4)			
Smoking	Yes	79	22 (27.5)	1.558	0.881 – 2.753	0.1273
	No	293	58 (72.5)			
Diabetes	Yes	2	1 (1.2)	3.613	0.223 – 58.396	0.3657
	No	370	80 (98.8)			
HIV/AIDS status	Positive	57	7 (8.6)	2.235	0.954 – 5.235	0.0682
	Negative	100	23 (28.4)	1.047	0.597 – 1.837	0.2691
	Unknown	215	51 (63)			
Final Model Multiple risk†						
Sex	Male	192 (51.6)	54 (28.13)	1.909	1.124-3.244	0.0167
	Female	180 (48.4)	27 (15)			
Occupation	High contact	147 (39.5)	46 (31.3)	2.188	1.31- 3.654	0.0028
	Low contact	225 (60.5)	35 (15.6)			

†The multivariable analysis resulted into a final model with two variables, namely gender and occupation. Male patients had 1.9 times higher risk of suffering TB while people involved in high contact activities had 2.188 times odds of getting TB. This model well fitted the variables as shown by Hosmer-Lemeshow test with $X^2 = 0.0092$, $df = 2$ and p-value of 0.99.

Comparison of diagnostic results and treatment

We evaluated the treatment of different subjects based on smear microscopy results and culture results. Among the 65 subjects receiving first line anti-TB drugs, 25 (38.5%) were diagnosed by ZN staining and 40 (61.5%) by Fluorescence staining. Moreover, 64 subjects whose samples were processed by ZN staining and 47 subjects by fluorescence staining were empirically treated with broad spectrum antibiotics. Out of 196 subjects who did not receive any medication, 87 and 109 subjects had their samples processed by ZN and fluorescence staining, respectively (Table 4).

Table 4. Distribution of treatment type by staining technique (n=372)

		Smear technique		
		ZN	Fluorescence	Total
Treatment	1 st line anti-TB drugs	25	40	65
	Broad spectrum antibiotics	64	47	111
	No treatment	87	109	196
	Total	176	196	372

Of the 81 patients diagnosed as TB positive by culture, 54 were already on first line anti-TB drugs, while 11 were empirically treated with broad spectrum antibiotics and 16 were not given any medication following smear microscopy results. Out of 291 TB negative subjects, 11 were given first line anti-TB drugs, 100 were given broad spectrum antibiotics and 180 received no therapy (Table 5).

Table 5. Comparison of diagnostic results and treatment for the study subjects (n=372)

		Culture results		
		Positive	Negative	Total
Treatment	First line anti-TB	54	11	65
	Broad spectrum antibiotics	11	100	111
	No treatment	16	180	196
Total		81	291	372

Further analysis indicated that subjects diagnosed as NTM positive by culture had already received different medication following smear microscopy results (Table 6). Four subjects received first line anti-TB drugs, 12 received broad spectrum antibiotics and 14 received no medication.

Table 6. Association of NTM diagnosis and treatment for the study participants (n=372)

		NTM diagnosis		
		Positive	Negative	Total
Treatment	1 st line anti-TB	4	61	65
	Broad spectrum antibiotics	12	99	111
	No medication	14	196	196
Total		30	342	372

Discussion

Sputum smear microscopy is the mainstay for pulmonary tuberculosis screening in high-burden countries (WHO, 2011). The method has proven to be rapid and easily affordable means of diagnosing PTB in many resource poor settings, where TB is still a major public health burden. Findings from this study showed that the sensitivity of ZN method was 68.8% and that of Fluorescence staining was 85.7% when compared to culture results. This finding is not surprising since ZN stain is known to have a low level of sensitivity compared to the Fluorescence method (Ulukanligil *et al.*, 2000). Finding that about one-fifth of the patients were diagnosed with *M. tuberculosis* by the isolation of the organism from cultures of sputum suggest that a significant proportion of the patients had sputum culture positive for *M. tuberculosis*. These findings reaffirms the consolidate the findings that the performance characteristics of smear microscopy is poor in the diagnosis of PTB (WHO, 2009). In patients co-infected with HIV the performance has shown to be further lower (Sonnenberg *et al.*, 2001).

Generally, smear-negative PTB accounts for 30% of PTB cases per annum in developing countries (Scherer *et al.*, 2007). The findings suggest that, while from the public health point of view smear-positive cases are considered more significant in diagnosing PTB, smear-negative patients on the other hand may be actual cases of TB as proved at some cases by culture (Tadesse *et al.*, 2011). Since smear microscopy does not only play a pivotal role in the initial diagnosis of PTB but also in monitoring of anti-TB treatment, false negatives can continue to be infection source while false positives may be subjected to unnecessary anti-TB medication. Our results show that over half of the patients diagnosed by Fluorescence method received anti-TB treatment compared to about a third when ZN method was used. In general, passive case finding strategy combined with sub-optimal diagnostic procedures at diagnostic clinics may pose a serious problem in underestimating the burden of the disease.

The diagnostic value of smear microscopy in general is reportedly being eroded not only by increased cases of HIV-related smear negative PTB, but also due to the influence of NTM (Lalit, 2001; Valadas *et al.*, 2003). The finding that a few of the patients had culture confirmed NTM elucidates an important public health and diagnostic challenge of continuing relying on smear microscopy for diagnosis of PTB in settings where dual infections are on the increase. Furthermore, classification of a few of culture confirmed NTM as PTB patients based on the smear microscopy results means that, such patients were managed as individuals with PTB.

NTM can present with clinical and radiologic manifestations comparable to *M. tuberculosis*. Since it is not feasible to differentiate between NTM and MTB microscopically in situations where sputum culture and other identification tests are not done, it could imply therefore, that NTM cases may have been treated as PTB for long periods of time. Essentially, NTM treatment differ from PTB treatment. Thus, such patients may show unfavourable response to anti-TB therapy. Additionally, since NTM isolates are often resistant to first-line anti-TB drugs, it may result into classifying and treating these individuals as MDR-TB cases (Tabarsi *et al.*, 2009), that may require the use of second-line drugs which are more toxic and expensive, consequently adding up to waste of resources due to mismanagement of such patients. Several studies have demonstrated that serious infections with NTM remain a major public health concern in many TB-endemic countries (Crump *et al.*, 2009; Buijtelts *et al.*, 2010; Maiga *et al.*, 2012).

The presumptive diagnosis of PTB has in practice been based on an algorithm of clinical and radiological (where available) criteria coupled with smear microscopy. Based on this fact, we assessed the defining criteria in predicting PTB positivity at the primary care facilities. Our results showed that, weight loss, fatigue, fever and night sweats were the most likely criteria for TB positivity among patients in Tanga. Since one of the defining criteria for suspecting TB is cough for ≥ 2 weeks, it can be argued that, this generalization might be contributing to missing a reasonable number of TB cases. This is because patients reporting at the primary diagnostic facilities with cough of less than two weeks are likely to have not been screened for TB under routine programme (Ngadaya *et al.*, 2009). Conversely, experience from the study shows that, a tendency of generalizing all complaints of cough for ≥ 2 weeks as a gold standard for PTB suspect leads not only to unnecessary increase of workload to already overwhelmed clinics, but also to unrealistic case detection. Inclusion of all patients with cough of more than two weeks may include patients with simple chest infections that are sometimes complicated with cough (Ngadaya *et al.*, 2009; Swai *et al.*, 2011). Furthermore, it may also contribute to increased negative results, delayed reporting and ultimately eroding patient's faith in the services of the laboratory and of the general health system (Wandwalo & Mørkve, 2000).

Results of risk factors quantification indicated that age, sex and occupation were the important risk factors associated with TB positivity. Young population at the age between 18 to 40 years and males were at most risk of TB infection. Similar trend was also reported during the 2012 prevalence survey (NTLP, 2011). These findings suggest that the patient population in this setting is marked with active transmission; hence, TB is largely seen in younger population. Furthermore, "high contact" subjects like students, community gatherings and other crowd related human activities (including public commute transports) remain at high risk of contracting TB.

In conclusion, unrealistic TB case detection may create a cluster of infectious cases in the community that maintain active TB transmission. Furthermore, classification of 1.9 % of culture confirmed NTM as TB patients at the primary health care facilities suggest that, a substantial number of patients may have been wrongly managed as PTB cases. An inefficient screening plus sub-optimal diagnostic procedures at the primary health care facilities may lead to unrealistic detection of TB cases. There is an urgent need to integrate NTM diagnosis in management of TB for designing effective tuberculosis prevention and control strategies in the country. Promoting of favourable health seeking behaviours and education especially among males, younger population and those at high risk such as crowded human activities need to be strengthened.

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Competing interests

All authors declare that they have no competing interests.

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