

# Detection Rats Technology for Diagnosis of Tuberculosis in High-Risk Populations

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**Abstract:** Prevalence of tuberculosis (TB) in prisoners in Tanzania and other sub-Saharan African countries is considered to be higher than in other populations thus prisons are important source of TB transmission. Control of TB in prisons through appropriate screening and diagnosis is challenging in most low-income countries such as Tanzania that is among world's 22 countries with high burden of TB. Commonly used TB diagnostic test (smear microscopy) have low sensitivity, and most advanced GeneXpert method is rather expensive for developing countries. SUA-APOPO TB detection rats' technology is most promising and increases TB case detection by over 40% in hospitals in Dar es Salaam Tanzania and Maputo Mozambique. This paper reports on improved TB detection in a selected prison in Tanzania using TB detection rats. Sputum samples (n = 11,424) were collected from 5,840 patients whom 3,491 were men, 2,349 were women. Of these, 386 patients were children altogether seeking diagnosis of TB at Ukonga prison dispensary from January 2013 to October 2015) and Keko prison dispensary from February to October 2015). Samples were routinely examined by Ziehl Neelsen (ZN) staining and later tested by rats APOPO TB laboratory, Sokoine University of Agriculture, Morogoro. Rats' positive samples were concentrated and confirmed by fluorescent microscopy (LED-FM) or ZN microscopy. A total of 709 individuals (12%) were diagnosed as smear-positive TB by the prison hospital, whereas rats detected an additional 302 TB patients. This increased the case detection in the prison population by 43%. The use of rats' technology increased the prevalence of smear-positive TB in prisons from 12% to 17.3% (n = 1,011) that is higher than prevalence reported in prisons elsewhere using microscopy. This finding shows that detection rats' technology can help reduce the burden of TB in developing countries. There is need to expand application of this technology to other risk populations including miners. This technology can improve workforce, livelihood and socio-economy by reducing TB related expenses.

**Keywords:** smear-negative TB, prisons population, trained rats, source of transmission.

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## INTRODUCTION

The prevalence of tuberculosis (TB) in prisoners in sub-Saharan Africa is considered higher than in the general population (Nyangulu *et al.* 1997; O'Grady *et al.* 2011). Prisons are also generally considered as reservoirs of TB transmission. Control of TB in prisons through appropriate screening and diagnosis is challenging in most low-income countries such as Tanzania that is also among world's 22 countries with high burden of TB (WHO 2015). The most commonly used TB diagnostic test in these countries is the direct smear microscopy which its sensitivity is low ranging between 20-60% (Urbanczik 1985; Mfinanga *et al.* 2007). Thus around 40% of TB cases remain undetected by this method. The sensitivity of smear microscopy drops further in populations with high prevalence of TB and human immunodeficiency virus (HIV) co-infections (Elliott *et al.* 1993, Johnson *et al.* 1998; Perkins & Cunningham 2007). Sub Saharan Africa has the largest proportion of TB/HIV co-infections hence many TB cases are not diagnosed (WHO 2013). There are new and more sensitivity

diagnostic tools such the GeneXpert MTB/RIF which can give results in two hours and detect drug resistance (Boehme *et al.* 2011). However, Xpert MTB/RIF test is rather expensive for most developing countries (Pantoja *et al.* 2013) and the roll-out to-date is limited to few hospitals. SUA-APOPO TB detection rats' technology is the most promising low cost diagnostic technology which can increase TB case together with smear microscopy to over 40% (Poling *et al.* 2010; Mahoney *et al.* 2011). The use of these rats as second-line screening tool after smear microscopy in hospitals in Dar es Salaam, Coast Region and Morogoro Tanzania and Maputo in Mozambique shows similar findings indicating the potential use of this technology to reduce TB problems in high risk populations. This has not yet been determined therefore the aim of this study was to determine the impact of TB detection rats in a prisons population in Dar es Salaam over a 3-year intervention period. The proportion of TB in prisons hospital was also compared to that in general hospital to determine the magnitude of differences.

### Materials and methods

Sputum samples were collected from prison inmates, prison staff and family members and other presumptive TB patients seeking TB diagnosis at Ukonga and Keko prisons dispensaries in Dar es Salaam.

Samples were first evaluated by smear microscopy at prisons DOTS centres and thereafter were securely transported in sealed containers packed inside cool boxes to APOPO TB detection laboratory at Sokoine University of Agriculture, for rat re-evaluation. Samples were heat inactivated at 100°C for 30 minutes to kill microorganisms before evaluating by rats as biosafety precaution. Samples were randomly arranged in bars (Fig. 1) and presented to rats in special training cage. Demonstration of rat TB detection rat evaluation can be viewed on this website link ([http://www.youtube.com/watch?v\\_KoRvdyuHxdE](http://www.youtube.com/watch?v_KoRvdyuHxdE)). Rats were rewarded when they correctly identified a TB positive control sputum sample. All other indications of unknown samples was recorded and those samples were pre-concentrated by centrifuging to obtain sediment for concentrated smear microscopy which is more sensitive than the direct smear microscope (Uddin *et al.* 2013). Samples indicated as TB positive by rats and confirmed by concentrated smear examined by LED fluorescence microscopy or ZN were reported to hospitals for tracking the patients and initiating treatment.



Figure 1. Heat-inactivated sputum samples arranged in bars ready for presentation to rats

## DATA ANALYSIS

The proportion of smear-positive TB detected by rats in prisons hospitals (17.3%, N =5840) was compared with previous proportion (23.1%, N = 6500, *unpublished*) obtained from a general hospital population also in Dar es Salaam. Comparison was done using MedCalc utilizing the "N-1" Chi-squared test according to Campbell (2007) and Richardson (2011) to determine whether the difference is statistically significant at  $P < 0.05$ .

## RESULTS

Sputum samples (n = 11,424) were collected from 5,840 patients whom 3,491 were men, 2,349 were women. Of these patients, 386 were children.

A total of 709 individuals (12%) were diagnosed as smear-positive TB by the prison hospitals whereas rats detected an additional 302 TB patients yielding a total of 1,011 smear positive TB patients. This increased the case detection in the prison population by an average of 43% (Fig.2). The increase in case detection decreased from 66% recorded in 2013 to 23% in 2015 (Fig. 2).

The use of detection rats' technology increased the prevalence of smear-positive TB in prisons from 12% to 17.3% (n = 1,011/5,840). Rats evaluated 140 sputum samples for 20 minutes.

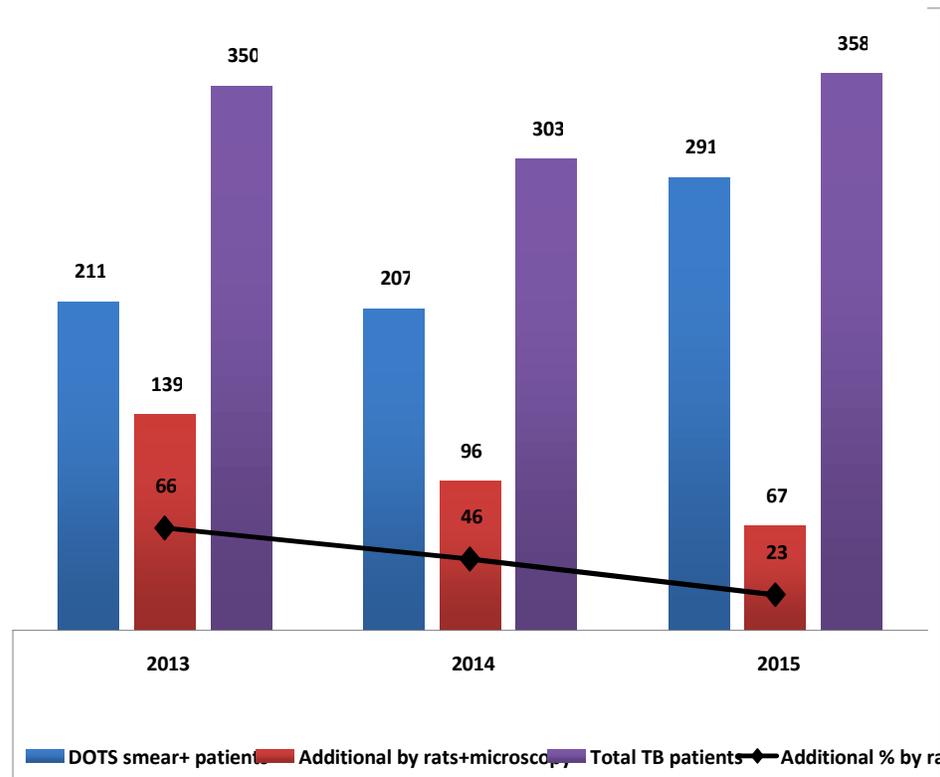


Figure 2. Tuberculosis screening in prison hospitals by microscopy and detection rats. Significant increase in case detection obtained by using rats

### Comparisons of proportions of TB

The observed proportion of smear-positive TB in prisons hospitals (17.3%) was not statistically significant from that previously obtained from general hospital (23.1%) (unpublished) whereby the difference was 5.8, 95% CI -11.5300 to 23.1315 Chi-squared = 0.095, DF 1,  $P = 0.7584$ .

## DISCUSSION

Detection rats improved TB case detection in prison populations by 43%. The rats detected more TB cases than smear microscopy and raised the proportion of smear-positive TB from 12% detected by microscopy to 17% in two prisons hospitals. This is based on confirmation by concentrated smear microscopy which has a higher sensitivity than the direct smear microscopy (Uddin *et al.* 2013) used in hospitals. Confirmation by more sensitive diagnostic methods such as the Xpert MTB/RIF could provide different findings as this methods detects nucleic acids of *Mycobacterium tuberculosis* pathogens which may be present in sputum containing very few bacterium below the detection limits of microscopy. Such scenario may reveal more TB positive patients as rats are targeting specific volatile odour compounds produced only by *Mycobacterium tuberculosis* (Mgode *et al.* 2012 *a, b, c*). These trained rats have also high ability of distinguishing clinical sputum with *M. tuberculosis* from those with other mycobacteria and related pathogens (Reither *et al.* 2015). There was a decrease in the percentage of case detection from 66% recorded in 2013 to 23% in 2015 (Fig. 2) although the average percent increase in TB case detection is 43% that corroborates previous reports (Poling *et al.* 2010; Mahoney *et al.* 2011). The two prisons hospitals started using Xpert MTB/RIF hence many cases were detected by this method in the hospitals. Comparative studies of rats and this technology are much needed as well as determining other causes of the observed decrease which may also be associated with improved performance in the hospitals since rats are kind of re-check up tool after smear microscopy.

Findings of this study show that use of detection rats can reveal more TB patients and enhance treatment of a significant number of patients missed by hospital thus help prevent deaths due to TB infection and prevent transmission of TB to other people living or in contact with misdiagnosed patients. In this case, the 302 patients detected by rats could have transmitted TB to 3,020 up to 4,520 individuals in the prison surrounding given than 1 undetected TB patient can transmit the disease to 10 up to 15 persons per year if remains undetected.

The high sample throughput whereby rats can analyze an average of 140 sputum samples in 20 minutes that is faster than available conventional methods such as microscopy with which a technician can examine an average of 20 sputum samples per day (WHO 2005); reasonable accuracy, and low operational costs makes the detection rat technology suitable for scaling up and mass screening of high-risk populations in countries with high TB burden.

On the other hand, the observed 17% smear-positive TB by rats indicates that prisons may not be the major source of TB transmission as reported elsewhere (Nyangulu *et al.* 1997; O'Grady *et al.* 2011). Previous evaluation of smear-positive TB in a selected DOTS clinic in Dar es Salaam that serves the general population showed a prevalence of 23.1% which is not statistically significant ( $P= 0.7584$ ). Further studies utilizing detection rats, Xpert MTB/RIF and culture methods are needed to explore the actual proportions of TB in prisons and general population. Multi-spacer sequence typing of the *M. tuberculosis* isolated from such study may provide insights on actual TB sources by assessing genotypes and transmission patterns. Recent study reported 10 genotypes of *M. tuberculosis* in Dar es Salaam (Mgode *et al.* 2015) where the present study sites are located. These can be explored in prisons hospitals to further unravel questions of actual TB burden in prisons population.

With these findings we can conclude that there is high potential of reducing TB problems by using detection rats technology that improve TB detection yield rapidly.

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