

## Multidrug-Resistant Human *Mycobacterium tuberculosis* Strain Resisted Inactivation by 10% Formalin

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### Abstract

Multidrug-resistant strain of *Mycobacterium tuberculosis* (MDR-Tb) and Multidrug-sensitive *Mycobacterium tuberculosis* (MDS-Tb) isolated from humans were injected subcutaneously into the guinea pig. One set of infected lungs of animals was fixed in 10% formalin pH 7.2, while the other set was treated with 75% ethanol, 2 hours prior to fixation in 10% formalin.

After six months of fixation, MDS-Tb strains were completely inactivated by both methods of fixation. MDR-Tb strains resisted inactivation by 10% formalin, but were inactivated by the treatment with 75% ethanol 2 hours prior to 10% formalin fixation.

**Keywords:** Formalin; Ethanol; Inactivation; *Mycobacterium tuberculosis*

### Introduction

Formalin is widely used to fix histological preparations, and as preservatives, in embalming solutions. It is an age-long practice in medical laboratories [1-3]. It is generally accepted that the risk of contracting tuberculosis is relatively high among Medical Laboratory workers and Pathologists. It is an assumption that once tissue is fixed in formalin, the risk for transmission and subsequent infection of *Mycobacteria* is greatly reduced, if not altogether eliminated [4-6]. Cadavers remain a practical teaching tool for Anatomists and Medical educators teaching gross anatomy. Infectious pathogens in cadavers that present particular risks include *Mycobacterium tuberculosis*, Hepatitis B and C, the AIDS virus HIV, and Prions that cause transmissible spongiform encephalopathies [7].

Recent studies have however, suggested that formalin does not effectively inactivate *Mycobacterium tuberculosis* in formalin-fixed tissue [4-13]. In this study we tried to test the inactivation ability of (a) 10% formalin fixation and (b) treatment of tissue with 75% ethanol, 2 hours prior to 10% formalin fixation. Multiple Drug Resistant (MDR) and Multiple Drugs Susceptible (MDS) strains of *Mycobacterium tuberculosis* were employed.

### Methods

Official consent was obtained from the Head of Department, Chest Clinic, Baptist Medical Centre Ogbomoso. All subjects included in this study were volunteers.

Sputa were collected from subjects at the Chest clinic of Baptist Medical Centre Ogbomoso. Samples were stained by Ziehl-Neelsen's (ZN) technique for Acid Fast Bacilli (AFB). Positive AFB samples were cultured on Lowenstein-Jensen (L-J) medium before the commencement of multiple drug therapy [14,15]. The different drugs used at the clinic include; rifampicillin, isoniazid (INH), pyrazinamide and ethambutol.

Primary growth on L-J was kept. Another round of sputa was collected from the same sets of subjects after the second and fifth months of directly observed multidrug therapy (DOT). The samples were stained. The positive AFB smears at the second and fifth month

of DOT were considered to be MDR-Tb [16,17]. The negative AFB smears at the second and fifth months of DOT were considered MDS-Tb, and the primary growth from such samples were used as MDS-Tb.

MDR-Tb sputa samples were cultured on L-J, and incubated. One set of animals was injected subcutaneously with the primary MDS-Tb growth, while another set was injected with the MDR-Tb growth [14]. Control animals were injected with sterile normal physiological saline. Animals were kept, fed on pellets and sterile water. They were watched for symptoms of disease, after which they were scarified and lungs excised.

Parts of the lungs were processed for culture on L-J media to confirm infection in the animals. Parts of infected lung tissue were fixed in 75% ethanol for 2 hours and then in 10% formalin. Other parts of the lungs were fixed in 10% formalin pH 7.2. The fixed tissues were kept for 6 months. Lung tissues were processed for culture on L-J media, and incubated aerobically at 36°C.

### Results

Fifty AFB-positive subjects were encountered in this study. Four (8%) were carrying MDR-Tb.

The animals injected with *mycobacteria* cultures developed local swellings and weight-loss after one month, and *mycobacteria* were isolated from their lungs when cultured on L-J medium. There was no growth from the lung tissue of the control animals. There was no growth from the lungs of MDS-Tb infected animals in both fixation methods (Figures 1 and 2). In the MDR-Tb infected lungs, there was no growth in the 75% ethanol, 10% formalin fixation, but there was growth in the 10% formalin fixation (Figures 3 and 4).

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Figure 1: MDS-Tb fixed in 10% formalin – No growth.

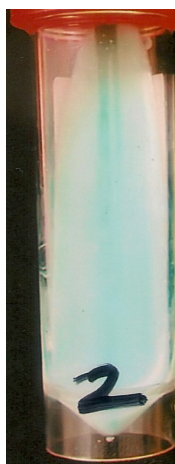


Figure 2: MDS-Tb fixed in 75% ethanol/10% formalin – No growth.



Figure 3: MDR-Tb fixed in 75% ethanol/10% formalin – No growth.

This shows that both methods of fixation were able to inactivate MDS-Tb in lung tissue. MDR-Tb was not inactivated by 10% formalin fixation, but was inactivated by 75% ethanol/formalin fixation.



Figure 4: MDR-Tb fixed in 10% formalin-showing growth of *Mycobacterium*.

## Discussion

The results of our study show that treatment of lung tissue with alcohol before fixation in 10% formalin was able to inactivate *Mycobacterium tuberculosis* in tissue including MDR-strain that resisted 10% formalin.

Environmental persistence of *M. tuberculosis* is subject of speculation. However, the reality that infected post mortem tissue can be a danger to Pathologists and embalmers has worrisome implications. A few experimental studies have demonstrated the ability of *Mycobacterium tuberculosis* to withstand exposure to embalming fluid and formalin. Gerston et al. succeeded in isolating live tubercle bacilli from 3 of 25 formalin fixed tissues. In a following report using a larger sample of 138 formalin-fixed lungs, they were able to obtain growth of *Mycobacteria* in three cases [5]. Treatment of tuberculosis is far from easy and frequently results in failure or even death [16-19]. The world is recording a high rate of death due to multiple-drug resistant tuberculosis. The results of workers suggested that there is a risk of contracting tuberculosis from tissue that has been fixed in formalin if aerosolization or accidental inoculation should occur [5]. In view of these, more effective fixative method should be employed to prevent occupational hazards to Medical Laboratory Scientists, Pathologists, Anatomists and Medical educators.

It has been reported that alcohols, especially 70% ethanol, have rapid mycobactericidal activity [1], and a tuberculocidal fixation may be prepared with 10% formalin in 50% ethanol [10]. Treatment of tissues with 95% ethanol prior to fixation in 10% formalin has been suggested [20]. Multiple toxic effects of alcohols on structure and metabolism are suspected, primarily due to protein denaturation and coagulation. Alcohol had been reported to disrupt the amino acid-amino acid bond to form an amino acid-alcohol hydrogen bond [1].

The result of this investigations are in agreement with the suggestions of other workers for the use of alcohol in addition to the conventional 10% formalin fixation of tissues [10,20,21]. This is pertinent, more so now that more people die due to MDR-TB infections.

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