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Fluorescein Diacetate Staining- A Strategy of Laboratories Capacity Building for Efficient Management of Mycobacterium Infections Treatment in Resource Limited Settings

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Abstract

Mycobacterium tuberculosis complex and non-tuberculosis mycobacterium are still caused serious problems of public health around the world, mainly in resource limited settings. The emergence of drug resistant, failure and relapse of mycobacterium infections treatment made the need of efficient and cost-effective tools for treatment monitoring. In resource limited settings with poor facilities, the transfer and implementation by National Tuberculosis Programs of fluorescein diacetate vitality staining could be used as an alternative method of mycobacteria culture for drug therapy monitoring in district level. We described in this review the potential use of fluorescein diacetate staining in the monitoring of infectious disease caused by mycobacterium strains.

Introduction

Rober Koch proved in the 1890s that tuberculosis (TB) was caused by *Mycobacterium tuberculosis* [1,2]. However, recognition of the pathogenic potential of non-tuberculous mycobacteria (NTM) has lagged behind recognition of the pathogenic potential of *M. tuberculosis* [3]. TB is an emerging disease and the number of new cases began to increase since 1980 due to the impoverishment of the population and the emergence of the HIV/AIDS pandemic [1]. At the same time, the NTM began emerged as opportunistic diseases [2,4]. NTM are often the cause of TB treatment failure due to the lack of the appropriate diagnosis tool of mycobacterium species in resource limited settings. So, the emergence of NTM diseases made them a new challenge to health authorities in resource limited settings [2,4]. National Tuberculosis Programs (NTP) would consider them in TB therapy in case of TB treatment failure after confirmed respiratory cases. Some studies have shown that a large number of clinically relevant pulmonary cases show high pathogenic potential of NTM [2]. NTM infections exceed sometimes the number of cases of TB in certain areas. Drug resistant TB has long been a major problem in resource limited settings emphasized by the emergence of multidrug-resistant and extensively drug resistant. In these settings with poor facilities in laboratories, there is a need of simple and easy TB treatment monitoring tools for the districts level [5,6]. The resurgence of TB, resistant TB and NTM has trained many efforts to develop tools for the susceptibility of mycobacterium strains during treatment but these tools are no longer available in resource limited settings due to their cost and the lack of trained technicians for their transfer and implementation. Acid-fast bacilli staining (AFB) is the simplest method used for the detection of mycobacterium, but it can't distinguish viable and non-viable mycobacteria during the monitoring of treatment, especially in cases of therapeutic failure. The interpretation of AFB obtained during the treatment of the disease caused by a mycobacterium infection can be difficult because at the end of the intensive phase, smears were positive in 15% of cases. The distinction between viable and non-viable bacilli would quickly adapt treatment. However, fluorescein diacetate (FDA) and ethidium bromide (EB) equally stain viable and non-viable mycobacterium strains [7-9]. Since the development of fluorescein diacetate vitality staining technique, many applications were tried for the detection of mycobacterium to adapt the method in the routine activity for drug therapy monitoring [10-18].

The purpose of this paper was to describe the applications of fluorescein diacetate staining in mycobacteriology illness treatment management.

Methods

An overview of the most important use of FDA in mycobacteriology diagnosis was done by electronically research in Medline PubMed and HINARI databases. The methods of FDA staining viability of mycobacterium were selected. Strategy of research considered the followings keys words, "Fluorescein diacetate" and "Mycobacterium". We considered mainly papers published in English. The interests' germs were *Mycobacterium tuberculosis* complex, *Mycobacterium leprae*, *Mycobacterium ulcerans* and others NTM.

Findings

Characteristics of Fluorescein diacetate

FDA is cell-permeant esterase substrate that can serve as a viability probe. It could be used in combination with ethidium bromide. Living cells can split FDA to fluoresce green, while dead cells will take the red EB stain. The principle is based on the ability of FDA to pass through the hydrophobic cell membrane of viable mycobacteria and be rapidly hydrolysed to free fluorescein by intrinsic esterase. FDA contains fluorescein, which is detectable by fluorescence microscopy after its

cleavage by esterases in the cell. These esterases are assumed to be present only in living cells. Dead mycobacteria inhibited by anti-mycobacterial agents hydrolyse significantly less FDA [3]. Viable cells are stained green due to their ability to dissociate the non-fluorescent FDA green fluorescein by enzymatic hydrolysis (active esterase) the presence of an intact cell wall allowing the accumulation of fluorescein in the cell. These characteristics have been standardized for mycobacterium viability detection by researchers around the world.

Mycobacterium tuberculosis complex viability screening

M. tuberculosis complex is a group of bacilli responsible for causing TB in humans and animals. Drug resistant TB, treatment failure and relapse have long been a common problem prevailing in resource limited settings. A reliable test to differentiate the living from the dead bacilli could soon be very useful when as decision criteria to change treatment quickly, for example in the case of treatment of multidrug resistance or prolonged intensive phase, and as true indicator of failure therapy. The use of FDA in mycobacteriology laboratories is to do rapid identification of live *M. tuberculosis* among treatment failure cases [19]. Nakamura et al. demonstrated one of the first research results on *M. bovis* BCG for the viability using FDA/EB staining method [7]. Living mycobacterium strains were stained in yellow green in FDA/EB staining while non-viable bacteria were red, but no difference was shown in AFB staining between viable and non-viable mycobacterium strains [7]. In the study of Van Deun et al., positive FDA results late during treatment were a strong predictor of failure to convert to negative culture and of rifampicin resistance [20]. They suggested that a positive FDA test could be considered as sufficient evidence to start second-line treatment, particularly with a well-tolerated regimen and in the context of low prevalence of NTM infections [20]. FDA staining method has been proposed to be appropriate for the rapid diagnosis of drug resistant TB. It can be used directly with suspect TB patients' samples. In 1980, FDA/EB was used to evaluate viability of mycobacterium in colony which was maintained for a long time ago [11]. They confirmed their research by culturing the same material on Lowenstein-Jensen media for growth as a confirmation of the viability of mycobacterium strains [11]. The utility of the FDA/EB staining in the monitoring of therapy was first established with *M. tuberculosis* H37Rv strains [16]. It was proved that FDA could be used for rapid screening of drugs therapy against slow growing mycobacteria [17].

Viability of Mycobacterium leprae

Mycobacterium leprae cannot be cultured, so it's very difficult to determine the viability of the organism in case of leprosy cure management [21]. Drug resistance of *M. leprae* is very important for multidrug therapy. Many efforts were made to develop method of leprosy drug monitoring. Shepart et al. discovered the mouse foot pad (MFP) method in the 1960's and this method was the one that permitting testing of new anti-leprosy drugs, determination of drug-resistant strains of *M. leprae* [21] until FDA-EB was developed. The mouse MFP method was used to determine the viability of *M. leprae* by growing the bacilli in the mouse foot-pad [22] for multidrug therapy monitoring. But MFP was labor-intensive, time-consuming and expensive technique, used for mice [21,23]. It is pointed out that molecular approaches may be more useful tools but this method can't distinguish dead and viable bacilli during monitoring. FDA/EB method has been successful used to assess the viability of *M. leprae* from biopsies of leprosy patients under different periods of treatment

[22]. The feasibility of applying the FDA/EB staining procedure of *M. leprae* has been investigated and the results were encouraging [12]. Loss of viability of *M. leprae* in presence of dapsone and rifampicin was demonstrated with FDA/EB for multidrug therapy monitoring [15]. FDA/EB staining was also used to determine the transmission of viable *M. leprae* by *Aedes aegypti* from lepromatous leprosy patients to the skin of mice through interrupted feeding [24], indicating transfer of bacilli mechanically to the biting spot through intermittent feeding [25].

Viability of Mycobacterium ulcerans

Mycobacterium ulcerans is an environmental NTM and has been detected in stagnant and slowly moving water from many tropical and temperate parts of the world [26]. It was discovered in 1897 by McCallum in Buruli Country at Ouganda. It causes buruli ulcer (BU). BU is the third most important mycobacterium pathogen in immunocompetent individuals after tuberculosis and leprosy and has been recognized by the World Health Organization as an important emerging disease [27]. The disease has been reported in many countries, mostly tropical, in Africa, North America (Mexico), South America, Southeast Asia, and Oceania [27]. The prevalence of BU has been increasing dramatically in West Africa and viability of BU bacilli can be detected by FDA staining [26].

Viability of the other non-tuberculosis mycobacteria

NTM occupy actually an important place in TB diagnosis with pseudo-TB treated as TB and the consequences are the increase of TB treatment failure in many resource limited settings with limited facilities. The most common clinical manifestation of NTM disease is lung disease followed by lymphadenitis in children, skin disease, and extrapulmonary or disseminated infections in immuno-compromised patients [4]. FDA staining can be used for drug susceptibility testing in NTM infections. Carboxyfluorescein diacetate succinimidyl, a derivate of FDA was used in the viability measurement of NTM [28] and could be transferred and implemented in resource limited settings for NTM treatment management.

Conclusion

FDA viability staining technique was used to perform susceptibility testing of non-tuberculosis and tuberculosis mycobacteria as efficient alternative methods which need to be transferred and implemented in resource limited settings by the bias of NTP. The FDA staining method is an alternative method of measuring mycobacterial viability. The decentralized use of FDA staining could decrease the bad effects of the lacks of adequate tools to monitor TB and NTM infectious disease treatments. It showed considerable promise as a rapid method for drug susceptibility test. It could be a valid alternative to the conventional mycobacterium culture, DST and molecular biology that are difficult to be implemented easily in resource limited settings where facilities are rare. With the advent of light-emitting diode fluorescence microscopes, FDA staining could be used in resource limited settings at the districts levels for the capacity buildings of mycobacteriology laboratories.

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