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Characterization of mycobacterium isolates from pulmomary tuberculosis suspected cases visiting Tuberculosis Reference Laboratory at Ethiopian Health and Nutrition Research Institute, Addis Ababa Ethiopia: a cross sectional study

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ABSTRACT

Objective: To characterize mycobacterium isolates from pulmomary tuberculosis suspected cases visiting National Tuberculosis Reference Laboratory at Ethiopian Health and Nutrition Research Institute, for diagnosis of pulmonary tuberculosis from January 4 to February 22, 2010 with total samples of 263. **Methods:** Sputum specimens were collected and processed; the deposits were cultured. For culturing Lowenstein Jensen medium (LJ) and Mycobacteria Growth Indicator Tube (BACTEC MGIT 960) were used. Capilia Neo was used for detecting NTM isolates from isolates of BACTEC MGIT 960. In Armauer Hansen Research Institute, Addis Ababa Ethiopia, Deletion typing PCR method for species identification (from confirmed *Mycobacterium tuberculosis* complex (MTBC) isolates by Capilia Neo) was done. **Results:** Out of 263 enrolled in the study, 124 and 117 of them were positive for mycobacterium growth by BACTEC MGIT 960 and LJ culture method, respectively. From BACTEC MGIT 960 positive media of 124 isolates, 117 were randomly taken to perform Capilia TB Neo test. From these 7 (6%) of them were found to be NTM and 110 (94%) were MTBC. From these 110 MTBC isolates, 81 of them were randomly taken and run by the deletion typing RD9 PCR method of molecular technique. Out of these 78 (96.3%) were found to be species of *Mycobacterium tuberculosis* and 3 (3.7%) were found to be not in the MTBC. Regarding the types of methods of culture media, Mycobacteria Growth Indicator Tube (BACTEC MGIT 960) method was found to have excellent agreement (with kappa value of 0.78) with the routine method of LJ. **Conclusions:** Pulmonary tuberculosis suspected cases visiting the National Tuberculosis Reference Laboratory at EHNRI that were confirmed to be pulmonary tuberculosis are caused by the species of *Mycobacterium tuberculosis*, hence treatment regimen including pyrazinamide can be applied to the patients as the first choice in the study area in Addis Ababa, Ethiopia. There is indication of the presence of NTM in patients visiting the tuberculosis reference laboratory and this is important because NTM is known to cause pulmonary disease similar with sign and symptom of pulmonary tuberculosis but different in treatment. BACTEC MGIT 960 has excellent agreement with LJ media but it has high tendency of having high contamination rate unless a better decontamination method is designed.

1. Introduction

Mycobacteria are aerobic and nonmotile bacteria (except for the species *Mycobacterium marinum*, which has been shown to be motile within macrophages) that are characteristically acid-alcohol fast. There are different classes of isolates of mycobacterium. These are mycobacterium complex, *Mycobacterium leprae* and non tuberculosis mycobacterium (NTM); *Mycobacterium tuberculosis* (*M. tuberculosis*) complex (MTBC) members are causative agents of human and animal tuberculosis. Species

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in this complex include *M. tuberculosis*, the major cause of human tuberculosis, *Mycobacterium bovis* (*M. bovis*), *M. bovis* BCG, *Mycobacterium africanum* (*M. africanum*), *Mycobacterium canetti*, *Mycobacterium microti* and *Mycobacterium pinnipedii*[1].

NTM are widely distributed in the environment, particularly in wet soil, marshland, streams, rivers and estuaries; different species of NTM prefer different types of environment. Human disease is believed to be acquired from environmental exposures, and unlike tuberculosis and leprosy, there has been no evidence of animal-to-human or human-to-human transmission of NTM, hence the alternative label environmental bacteria is used and NTM are frequently isolated from Oregon residents; more than one-half of all isolates likely represent true disease. Pulmonary NTM is most common among elderly women, and *Mycobacterium avium* causes most disease[2,3].

Most NTM disease cases involve the species *Mycobacterium avium* complex, MAC, *Mycobacterium abscessus*, *Mycobacterium fortuitum* and *Mycobacterium kansasii*. *Mycobacterium abscessus* is being seen with increasing frequency and is particularly difficult to treat; rapidly growing NTMs are implicated in catheter infections, post-lasic, skin and soft tissue (especially post-cosmetic surgery) and pulmonary infections[3]. But there is no much scientific information about the existence of NTM in Ethiopia so far.

As to *M. tuberculosis* complex isolates is concerned, there is an urgent need to evolve and apply techniques that not only rapidly identify but also characterize tubercle bacilli to facilitate epidemiological studies. Investigations on the epidemiology of tuberculosis need strain or species specific markers, which can be used to differentiate *M. tuberculosis* isolates. DNA based technology is now available for molecular characterization of *M. tuberculosis*[4].

It is generally accepted that different strains or species of *M. tubetculosis* complex have distinctive epidemiological and clinical characterstics such as virulence and clinical presentation, and that beahaviour in animal models appear to be strain or species dependent for example some *M. tuberculosis* strain are noted for their dissimination and acquisition of drug resistance while others tend to predominate limited locals. Therefore molecular typing of *M. tuberculosis* isolates is useful in elucidating the natural history of the tuberculosis epidemic and evaluating tuberculosis control efforts[5]. If we review different literature about the proportion of different species of *M. tuberculosis* complexes, 10%-15% of human tuberculosis infection in developing countries is caused by *M. bovis*. However, the contribution of *M. bovis* to the current tuberculosis epidemic is unknown in developing countries. In addition, little is known about the species/strains of mycobacterium that circulate in many developing countries, including Ethiopia[6].

Based on review of the literatures, there is no current study on characteriaton of mycobacterium isolates in Addis Ababa, Ethiopia. Therefore the purpose of this study was to provide preliminary information on the existence and or extent of NTM and type of species of *M. tuberculosis* complex isolates circulating cases in Addis Ababa, Ethiopia, from pulmonary tuberculosis suspected cases, in particular from cases visiting the National Tuberculosis Reference Laboratory at Ethiopian Health and Nutrition Research Institute (EHNRI).

2. Materials and methods

2.1. Study area

The study was conducted at Addis Ababa, Ethiopia. Addis Ababa is the capital city of Ethiopia and it was established in 1889 and is now a city of 2.7 million people. Addis Ababa is a grassland biome, located at 9°1′48″N 38°44′24E″. The city lies at the foot of Mount Entoto. From its lowest point, around Bole International Airport, at 2 326 meters (7 631 ft) above sea level in the southern periphery, the city rises to over 3 000 metres (9 800 ft) in the Entoto Mountains to the north. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), Addis Ababa has a total population of 2 739 551, of whom 1 305 387 are men and 1 434 164 women; all of the populations are urban inhabitants. All Ethiopian ethnic groups are represented in Addis Ababa due to its position as capital of the country[7].

2.2. Study design and target populations

A cross sectional study was conducted from January 4 to February 22 and target populations were pulmomary tuberculosis suspected cases visiting for diagnosis of pulmonary tuberculosis at National Tuberculosis Reference Laboratory of EHNRI.

2.2.1. Sample size and sampling

All pulmomary tuberculosis suspected cases who requested for sputum examination during the study period were included in the study and a total of 263 specimens were collected.

2.2.2. Specimen collection and processing

After getting signed informed consent from each study participant, sputum was collected. The amount of the sputum used was 2-5 mL and then these amounts of the sputum were transferred into centrifuge tube and mixed with equal volume of N-acetyl-L-cysteine-sodium hydroxide (NALC-NAOH) solution. Then vortexing, not more than 20