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Methods of phenotypic identification of non-tuberculous mycobacteria

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ABSTRACT

Non-tuberculous mycobacteria (NTM) are composed of mycobacterial species other than the *Mycobacterium tuberculosis* complex. Initially thought to be mere contaminants when isolated from clinical specimens, literature is increasing by the day showing NTM as proven pathogens. Due to the difference in antimicrobial susceptibility of different species, it becomes imperative for the microbiology laboratory to identify them to the species level. Molecular methods are available for rapid and accurate identification, but in a resource limited nation, phenotypic methods, albeit time consuming, are of paramount importance. By means of this article, the authors intend provide a concise summary of the basic biochemical reactions which can be done to identify most commonly isolated NTM.

1. Introduction

Non-tuberculous mycobacteria (NTM) are composed of mycobacterial species other than the *Mycobacterium tuberculosis* complex. They are widely distributed in nature and were initially thought to be mere contaminants when isolated. However, in the last few years they have been reported to cause a varied spectrum of diseases ranging from hospital acquired infections to infections following environmental exposure like hurricanes and tsunamis [1,2], in both immunocompromised and immunocompetent individuals.

Epidemiological data from the Infectious Diseases Society of American Emerging Infections Network and information from referral centers suggest that NTM infections have been consistently on the rise [3]. Almost all diseases caused by rapidly growing mycobacteria in humans are due to *M. fortuitum, M. chelonae*, and *M. abscessus* [4]. They can affect respiratory tract, skin and soft tissue, causing diseases that include pulmonary infections [5–8], and extra-pulmonary infections [6,9–11], including traumatic and surgical wound infections [5,12–17], skin and soft tissue infections [2,18–28], implant-associated [29,30], and catheter-associated infections [4,31]. NTM have even been isolated from hospital cockroaches [32]. Sheer number of literature on NTM as pathogens highlight the importance of their identification which is still a dark zone, not many workers wanting to venture-into.

Numerous articles have been published on their classification [33,34], ecology and physiology, virulence factors [35], risk factors [18,36,37], diseases and clinical features [11,19]; and molecular characterization [38–40]. However, on review of literature very scanty

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data on phenotypic methods of identification of NTM is found, that too is scattered and difficult to comprehend for routine use by the students and laboratory staff. Hence, there is a necessity of information at one place for the benefit of research community. In a resource limited nation of South-East Asia, where laboratories have less than basic infrastructure, shortage of trained staff and molecular detection methods are available only in reference laboratories; there is a need to know phenotypic methods of speciation of NTM. This knowledge is required to prevent irrational use of antimicrobial agents as the susceptibility of *Mycobacterium* varies with species [41–43]. It should be remembered that all *Mycobacterium* isolates from patients are not *Mycobacterium tuberculosis* [44].

With the availability of commercial media and ready to use antibiotic susceptibility testing plates, reproducible and reliable results are obtained. This article is therefore, an attempt to describe the various simple yet cost effective methods of identification of the NTM species in a resource constrained setting.

2. Procedures

Described below are the procedures to perform various biochemical tests along with their principles. It is reiterated to follow universal safety precautions and all tests should be carried out in a bio-safety cabinet. Various biochemical tests with their principle and procedure are as follows:

2.1. Niacin accumulation test

- Principle: All *Mycobacterium* species produce niacin ribonucleotide; but only *M. tuberculosis, M. simiae* and some strains of *M. chelonae* lack the enzymes to convert it further into nicotinamide adenine dinuceotide (NAD). Thus, niacin accumulates in the culture medium from which it can be extracted with sterile water or physiologic saline. The test demonstrates the presence of cyanogen chloride formed through the reaction of chloramine T and potassium thiocyanate in the presence of citric acid. The cyanogen chloride breaks the pyridine ring of niacin, forming the aldehyde gamma-carboxyglutamate that binds with the aromatic amine producing a yellow color.
- Materials: Niacin Detection Kit, Modified for Mycobacteria (K048; HiMedia Laboratories, Mumbai) which contains:
  - ➢ Part A
  - ➢ Part B
  - ➢ R055
- Procedure:

➢ Use only more than three week old mycobacterial culture grown on Lowenstein-Jensen (LJ) Medium Slants showing heavy growth. Cultures grown on other types of media do not produce enough niacin to yield a positive result by this method. (False negative test results, from the use of cultures, with too few organisms or from cultures grown on media other than LJ). Do not use this test on mixed cultures.
➢ Add 2 ml of sterile distilled water or saline to the slant.
➢ Cut or stab the slant with a spade or needle.
➢ Incubate the slant upright at 37 °C for two hours to allow extraction of niacin into the distilled water.
➢ Retain the slant in an upright position for five minutes.
➢ Use 1 ml of this solution as a test sample.
➢ Test isolate: Transfer content of Part A (1 ml) to Part B (1 ml). Use this as a reagent solution for further test.
➢ Transfer test sample (1 ml) to reagent solution (Part A+Part B) using a syringe.
➢ **Positive** reaction: Development of yellow color within five minutes (Fig. 1).
➢ **Negative** reaction: No development of yellow color within five minutes. Reagent solution remains colorless.

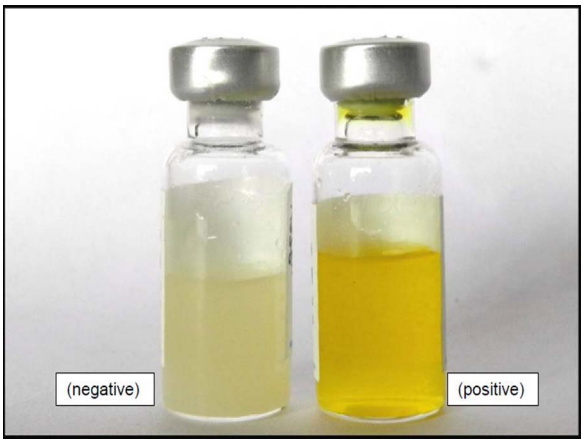

Fig. 1. Niacin accumulation test.