Transactions of the Royal Society of Tropical Medicine and Hygiene 105 (2011) 148–152

Contents lists available at ScienceDirect

Transactions of the Royal Society of Tropical Medicine and Hygiene

journal homepage: http://www.elsevier.com/locate/trstmh

# Trends of mycobacterial clinical isolates in Taiwan

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ARTICLE INFO

*Article history:*
Received 24 March 2010
Received in revised form 9 November 2010
Accepted 9 November 2010
Available online 15 January 2011

*Keywords:*
*Mycobacterium tuberculosis* complex
non-tuberculous mycobacteria
pulmonary infection

ABSTRACT

Non-tuberculous mycobacteria (NTM) can cause chronic pulmonary infection, however, NTM infection is generally overlooked. This retrospective study analyzed the frequencies of *Mycobacterium tuberculosis* complex (MTBC) and NTM clinical isolates from 99 200 specimens of patients suspected with pulmonary mycobacterial infection in Taiwan from 2002–2007. A total of 8024 mycobacterial isolates, including 5349 MTBC and 2675 NTM, were obtained from the 99 200 specimens in the study period. The overall mycobacterial isolation rate was 8.09% (8024/99 200), and the overall MTBC and NTM isolation rate was 5.39% (5349/99 200) and 2.7% (2675/99 200), respectively. Notably, the prevalence of NTM isolates among the identified mycobacteria strains was increased 2.6 fold from 2002 (17.54%, 147/838) to 2007 (45.80%, 659/1439). The frequencies of MTBC and NTM isolates showed a reciprocal trend: the NTM isolation rates were steadily increasing while the overall mycobacterial isolation rates remained stable over the study period. Our results suggest that the diagnosis, identification and susceptibility tests for NTM should be standardized and integrated in clinical routines, for providing the information of NTM infection and prescribing clinical treatment in a more precise and efficient way to reduce the increasing NTM in the studied area.



## 1. Introduction

Tuberculosis (TB) is a serious threat to human health.[1,2] Approximately 2 billion people, which corresponds to one third of the global population, suffer from this disease.[3] Southeast Asia is the area with the highest TB prevalence where TB patients account for 60% of the global TB burden.[4] Nine million new TB cases and three million deaths are reported annually.[5] In developing countries, the number of patients who die from TB corresponds to about 99% of the annual TB death toll worldwide.[6]

Tuberculosis is one of the most dangerous communicable diseases in Taiwan. Although Taiwan's GDP (Gross Domestic Product) has reached US$13 000, about 14 000 new cases of tuberculosis are reported each year, with a mortality rate of 3.4 per 100 000 people in 2007.[6] The mortality rate of tuberculosis has plunged 98.8% and 50.9%, respectively, from 1947–2007 and in the decade from 1997–2007.[6] Factors such as health care resources, population density and environmental hygiene conditions

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doi:10.1016/j.trstmh.2010.11.005

**Table 1**
Categories and numbers of specimens for mycobacterial culture 2002–2007

| Year<br>Specimens | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | Total (%)<br>*n* = 99 200 |
|---|---|---|---|---|---|---|---|
| Sputum | 10 349 | 12 759 | 19 602 | 17 179 | 15 068 | 16 764 | 91 721 (92.46) |
| Pleural effusion | 454 | 593 | 758 | 691 | 752 | 865 | 4113 (4.15) |
| BAL | 167 | 180 | 184 | 180 | 176 | 246 | 1133 (1.14) |
| CSF | 98 | 91 | 128 | 79 | 88 | 107 | 591 (0.60) |
| Urine | 89 | 97 | 63 | 95 | 59 | 58 | 461 (0.46) |
| Ascites | 38 | 91 | 100 | 79 | 89 | 68 | 465 (0.47) |
| Pus | 44 | 25 | 47 | 41 | 45 | 49 | 251 (0.25) |
| Endotracheal aspirate | 100 | 20 | 0 | 0 | 0 | 0 | 120 (0.12) |
| Synovial fluid | 3 | 14 | 19 | 15 | 14 | 27 | 92 (0.09) |
| Pericardial effusion | 5 | 16 | 11 | 15 | 8 | 13 | 68 (0.07) |
| Blood | 10 | 5 | 11 | 4 | 12 | 5 | 47 (0.05) |
| Tissue | 19 | 1 | 5 | 5 | 1 | 12 | 43 (0.04) |
| Abscess | 2 | 0 | 1 | 0 | 5 | 11 | 19 (0.02) |
| Discharge | 12 | 0 | 0 | 1 | 0 | 1 | 14 (0.01) |
| Body fluid | 7 | 0 | 0 | 0 | 0 | 6 | 13 (0.01) |
| Gastric juice | 2 | 0 | 0 | 0 | 0 | 5 | 7 (<0.01) |
| Throat swab | 6 | 0 | 0 | 0 | 0 | 0 | 6 (<0.01) |
| Other[a] | 9 | 1 | 0 | 1 | 1 | 24 | 36 (0.04) |
| Total | 11 414 | 13 893 | 20 929 | 18 385 | 16 318 | 18 261 | 99 200 (100) |

[a] Other: including stool, bone marrow, subcutaneous wash, tumor, bioscopy, aspirate.
BAL: bronchial alveolar lavage; CSF: cerebrospinal fluid.

contribute significantly to the decreased TB incidence, mortality and drug resistance rate in Taiwan.

Non-tuberculous mycobacteria (NTM) are opportunistic pathogens that cause skin infection, lymphadenitis, and chronic pulmonary infection. Nevertheless, NTM infection does not cause much attention and is generally overlooked. Al Jarad et al. reported that the NTM infection rate among mycobacterial infections is about 24.4%.[7] Clinical pulmonary syndrome and the corresponding X-ray manifestations of NTM infection are easily confounded with that caused by *Mycobacterium tuberculosis* (MTB). In addition, studies regarding the clinical isolates, incidence and antibiotic resistance of TB mainly focus on investigating the major TB pathogen MTB. In this context, drug susceptibility testing methods for NTM are not standardized and although some NTM-infected patients may present with unique characteristics, these are often overlooked as there has been limited documentation and study of NTM-related disease.[8]

Accordingly, it is intriguing to investigate the distribution of *M. tuberculosis* complex (MTBC) and NTM clinical isolates, which would provide valuable clues to clinicians for controlling the chronic infection. The present study analyzed the frequencies of MTBC and NTM clinical isolates in central Taiwan from 2002–2007 to determine the trends of mycobacterial infection. Our results reveal that MTBC and NTM isolate rates showed a reciprocal trend during the study period. The data described in this study provide important information for the development of optimal intervention and strategy to control mycobacterial infection.

## 2. Materials and methods

### 2.1. Sample collection

This study retrospectively analyzed MTBC and NTM isolation rates from 2002–2007. Samples were collected from patients admitted to hospitals or clinics (including medical centers, regional hospitals, clinics, medical laboratories and health centers) in central Taiwan (including Taichung City, Taichung County, Miaoli County, Nantou County, and Changhua County) suspected with mycobacterial infection. All the 99 200 samples (91 721 sputum [92.46%], 4113 pleural effusion [4.15%], 1133 bronchial-alveolar lavage [BAL; 1.14%] and others) were submitted to the TB center of Chung Shan Medical University Hospital for further processing, bacterial culture and identification. Detailed information regarding numbers and categories of the specimens used in this study are listed in Table 1. Sample collection procedures conformed with the guidelines from Department of Health, Taiwan.[9] In brief, expectorated sputum samples were obtained shortly after the patients awoke in the morning. Pleural effusions were collected from patients with thoracocentasis. BAL fluids were collected from patients with bronchoscopy. Sediments from sterile specimens (including pleural effusions, cerebrospinal fluids [CSF], and other body fluids) after centrifugation were submitted to acid-fast staining and bacterial culture. Stool specimens were collected in clean containers with tightly fitting lids.

### 2.2. Sample processing and mycobacterial isolation

All the laboratory procedures of specimen processing were performed in the TB Center, Chung Shan Medical University Hospital. The samples were decontaminated and digested using fresh prepared NALC (N-acetyl-L-cystein)-NaOH solution,[9] microscopically examined after Kinyoun staining,[9] and cultured into both Lowenstein-Jensen medium and BACTEC™ MGIT™ 960 Mycobacterial Detection System (Becton, Dickinson and Company, New Jersey, USA). Identification of MTBC and NTM was performed using BD ProbeTec system (Becton, Dickinson and Company) according to the manufacturer's instructions.