

# Isolation of Nontuberculous Mycobacteria (NTM) from Air Conditioner Dust

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## 에어컨 먼지내 비결핵 항산균의 동정

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Nontuberculous mycobacteria (NTM) contains *M. avium*-intracellular complex (MAC), *M. fortuitum*, *M. chelonae*, *M. abscessus*, *M. kansasii*, etc., which causes infections in swine, poultry, and other animals. These bacteria are opportunistic pathogens that can contaminate people, and in doing so, cause various social economic problems. This study proved that NTM are also present in air conditioners of multiuse facilities in frequently visited populated areas. The results of this study are meaningful because they showed that pathogenic microbes can live in the surroundings and cause diseases to people with impaired immunity. This study used the AFB stain, L-J medium culture, and PCR method for the detection of MTB and NTM in air conditioner dust. MTB was not detected in any of the collected samples, while NTM was detected in 2 out of 40 samples (5%). Most people living in the modern environment cannot avoid being exposed to air conditioners so special attention is needed for sanitary inspections and the management of air conditioners. Moreover, it is important to study NTM through various individuals, accumulate the related data and establish the methods and standards of hygiene management system to reduce NTM infections in public areas. Moreover, it would be necessary to identify the precise species of NTM through DNA sequencing and evaluate the effects of NTM on the pulmonary tuberculosis in immunodeficiency patients.

**Key words:** Nontuberculous mycobacteria, Polymerase chain reaction, Air conditioner

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## INTRODUCTION

Nontuberculous mycobacteria (NTM) are also called atypical mycobacteria and contaminants present in various natural environments such as soil and stream around us. In the past, NTM were known to live only in the natural environment. However, recent studies investigated whether NTM live in the artificial environment and be contagious to humans or not [1-4].

Many studies found that NTM exists even in an artificial environment, different from the natural habitat condition. These studies showed that NTM could live in the multiuse facility, frequently used by people, and NTM could cause various diseases to humans as contaminants. Consequently, it became very important and necessary to study the risk of NTM to human beings and the habitat range of NTM. Previous studies reported that immunocompromised patients such as patients with AIDS were more prone to tuberculous infections

in the United States, Europe, and Japan since the 1980s and more adults in normal immune status also had more lung diseases induced by NTM [5-7]. There were many follow-up studies to identify the causes of it. Someone reported that NTM were found in the hospital tap water as well. Moreover, it was found that some bacterial species of NTM live in drinking water, in the vicinity of human habitats, and in the natural environment [8,9]. Additionally, it was emphasized to pay a caution to the induction of tuberculosis when in contact with immunodeficiency elderly people [8,9]. Hillebrand-Haverkort et al [9] recently proved that *M. avium* was a bacterium species causing tuberculosis-related diseases to immunodeficient patients. In other words, it was reported that the NTM induced disease in the hospital was originated from NTM living in the contaminated environment [10,11]. Moreover, some bacterial species such as *M. avium* and *M. szulgai* were identified on the surface of water and water in the spray. Furthermore, Schulze-Robbeke et al [12] showed that *M. fortuitum* could be found in the nearby sewage and dust and argued that it would be needed to investigate the surrounding environment and media since contaminated tap water could cause the disease to people. As NTM were found in the living environment of human beings such as tap water, it was assumed that NTM would exist in the multiuse facility where people frequently used. Consequently, researchers demanded to study the multiuse facility. If the presence of NTM in the in the multiuse facility is proven, the multiuse facility can be considered as a pathway to cause various diseases such as lung disease to people with impaired immunity or even healthy adults.

Many recent studies have reported that NTM were found in multiuse facilities. If NTM are detected in various multiuse facilities, it can be considered as a serious social health problem affecting the people's health condition directly and indirectly.

Therefore, this study was conducted to precisely evaluate the danger of the situation. Samples were collected from 40 air-conditioning facilities in the capital area to test the presence of NTM in multiuse facilities. Acid-fast staining and Löwenstein-Jensen (L-J) medium inoculation were used to detect NTM. Moreover, the degrees of MTB and NTM contamination were investigated by conducting PCR with the

cultured bacteria.

## MATERIALS AND METHODS

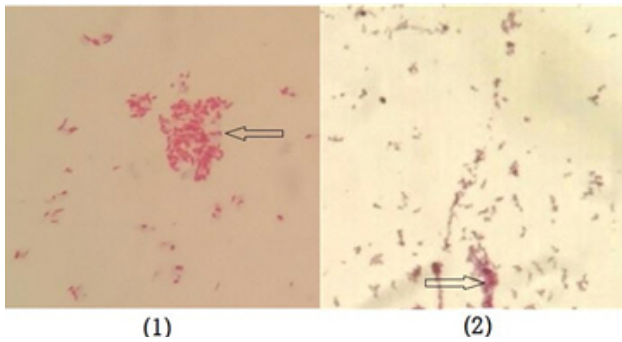
### 1. Sample collection and direct AFB stain, direct PCR

Samples were collected from air-conditioners by using sterilized cotton swabs. The sample was inserted into a Falcon tube with sterilized distilled water and centrifuged at 2,000 rpm for 30 minutes. Afterward, the supernatant was discarded and 4% NaOH was added to the precipitate. Then, it sat for 20 minutes. Acid-fast staining was performed (Direct AFB stain) and it was inoculated on L-J medium (Difco, USA), which is the Kinyoun method. PCR (Direct PCR) analysis was carried out [13].

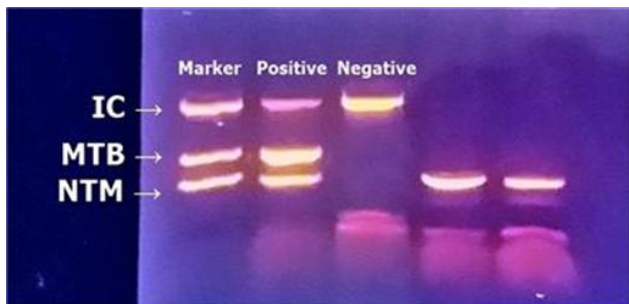
### 2. Indirect AFB stained and indirect PCR using cultured bacteria

It was inoculated on L-J medium with sterilized cotton swab and loop and the medium was cultured at 37°C for 8 weeks. Acid-fast staining was performed with the cultured bacteria and indirect PCR (2nd PCR) was conducted. Indirect PCR was performed by adding sterilized distilled water to the sample and centrifuging it at 2,000 rpm for 30 minutes. Then, the supernatant was discarded and the precipitate was suspended in 1 mL of TE buffer [14]. Indirect PCR was performed by adding TE-Buffer 1 mL into a colony bag cultured in an L-J medium with using sterilized loop into and centrifuging it at 13,000 rpm for 5 minutes. Then, the supernatant was discarded and 200 µL of DNA extract was added to it. It was whirled for 5 minutes and heated at 100°C for 20 minutes to extract DNA. After heating, DNA was isolated by centrifugation at 13,000 rpm for 5 minutes and 3 µL of the upper layer was used for PCR.

The final 20 µL of PCR reaction was made by adding 3 µL of extracted DNA, 4 µL of 5×MTB/NTM ACE PM, 3 µL of 8-MOP solution, and 10 µL of 2×Multiplex Master to PCR premix (Bioneer, Korea). The reaction conditions of the thermal cycler were an initial reaction at 94°C for 15 minutes, followed by denaturation (94°C for 30 seconds), binding (62°C for 30 seconds), and elongation (72°C for 30 seconds) reactions. Total 42 cycles were performed. For checking the



**Figure 1.** Result of indirect acid-fast stain ( $\times 1,000$ ). The Figure 1 (1, 2), showed the result of indirect AFB stain. Indirect AFB stain means AFB stain using cultured colony from L-J media. No 1,2 is number indicating cultured colony from L-J media. All of No 1,2 showed positive result from AFB stain (arrow).



**Figure 2.** Result of indirect PCR. The Figure 2, showed the result for NFB and MTB to Indirect PCR assay. Indirect PCR assay means PCR assay method using cultured colony from L-J media. All of No 1,2 congruenced with marker of NTM control band. Abbreviations: PCR, polymerase chain reaction; IC, Internal control; MTB, Mycobacterium tuberculosis; NTM, nontuberculous mycobacteria.

PCR results, DNA was stained with 10  $\mu$ L of gel red in 2% agarose gel. The 3  $\mu$ L of the DNA size marker and PCR reaction product was electrophoresed at 100 volts for 20 minutes to confirm it at a UV transilluminator [15,16].

## RESULTS

The study was conducted to prove the presence of NTM in samples collected from air conditioners of 40 multiuse facilities in the capital area. The AFB staining of samples (hereafter "direct AFB staining") and direct PCR of samples were negative. The two of L-J medium tests showed colonies. They were collected by a sterilized loop and cultured to confirm the presence of NTM and acid-fast (hereafter "indirect AFB staining") using a cultured colony and indirect PCR were conducted. The results of indirect AFB staining

**Table 1.** Result of AFB Stain and PCR

Test	NTM Positive No (%)	MTB Positive No (%)
Total	40 (100)	40 (100)
Direct AFB staining	0 (0)	0 (0)
Direct PCR	0 (0)	0 (0)
L-J medium culture	2 (5)	0 (0)
Indirect PCR	2 (5)	0 (0)
Indirect AFB	2 (5)	0 (0)

Abbreviations: NTM, nontuberculous mycobacteria; MTB, Mycobacterium tuberculosis; AFB, acid-fast bacilli; L-J, Löwenstein-Jensen; PCR, polymerase chain reaction.

showed that two of 40 samples were positive (5%) and the indirect PCR also confirmed 2 NTM positive samples (Figure 1, 2 and Table 1).

## DISCUSSION

The study was conducted to confirm the presence of MTB and NTM in the air conditioners of multiuse facilities. The results of the study showed that the detection rate of NTM in these air conditioners was 5%. Previous studies reported 20.4% detection rate from tap water [17], 54% from ice samples, and 35% from drinking water in the market [18]. Moreover, Argueta et al [19] found that 25 of 121 food samples had NTM (20.6% detection rate).

The results of this air conditioner study showed a lower detection rate than the detection rates of previous studies. However, the samples of the study were collected from air conditioners of multiuse facilities, which are within the close range of our lives. Therefore, the results of this study are very meaningful. The results of this study indicated that NTM were factors threatening the health of citizens because NTM were present in the public place. The results were remarkable, even though there are differences in the environment between countries. Consequently, based on the results of this study, MTB and NTM tests should be conducted for more various subjects continuously. Moreover, more studies should be conducted to identify the risk of the disease exposure. The results of this study clearly showed that NTM inhabited in the multiuse facilities in addition to the natural environments, which agreed with previous studies [20].

## 요약

비결핵 항상균(Nontuberculous Mycobacteria, NTM) *M. avium*-intracellular complex (MAC), *M. fortuitum*, *M. chelonae*, *M. abscessus*, *M. kansasii* 등을 함유한다.

비결핵 항상균은 자연 환경속에 서식하며 다양한 가축등에 감염한다. 그리고 사람한테는 기회감염을 유발하여 사회적 경제적 문제를 야기한다. 본 연구는 면역결핍 사람한테 기회감염을 유발하는 비결핵 항상균이 사람들이 흔히 이용하는 다중이용시설내 존재 유무를 확인하기위해 40개 에어컨 먼지를 채취하여 직접 AFB 염색, 직접 PCR, 간접 AFB 염색, 간접 PCR 등을 실시했다. 그 결과 채취한 샘플의 직접 AFB 염색, 직접 PCR 에서는 인형결핵균(MTB), 비결핵 항상균 모두 발견되지 않았다. 배양된 집락을 이용한 간접 AFB 염색, 간접 PCR 결과 인형결핵균 (MTB)은 모두 음성반응을 나타냈으며, 비결핵 항상균은 40개 샘플 중 2개(5%)에서 양성반응을 나타냈다. 본 실험결과는 자연 내 비결핵 항상균이 존재한다는 사실을 증명했으며 비결핵 항상균이 기회감염의 원인임을 감안했을 때 다중이용시설의 청결한 위생처리가 중요함을 암시한다. 다만 비결핵 항상균의 종(species) 감별과 기회감염 유발 유무는 좀 더 연구가 필요하다고 사료된다.

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