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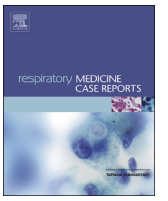

Case Report

Analysis of three cases with false positive PCR results of non tuberculosis mycobacterium

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ABSTRACT

Background: Real-time fluorescent quantitative PCR (RT-PCR) can effectively distinguish between *Mycobacterium tuberculosis* (MTB) and Non-tuberculosis mycobacterium (NTM), but when there are overlapping sequences between other pathogens (such as Nocardia otidiscaviarum, Mycobacterium parantracellulare, Mycolicibacterium fluoranthenivorans) and NTM, abnormal amplification curves may appear.

Case presentation: The clinical manifestations of the three patients were fever and respiratory symptoms. Chest CT showed "multiple lung infections". The acid-fast bacilli were negative by microscopic examination. The results of RT-PCR detection of *Mycobacterium tuberculosis* DNA showed that they are all NTM, while the results of DNA microarray method showed that there were no non-Mycobacterium tuberculosis. Identified by MALDI-TOF mass spectrometry, they are Nocardia otidiscaviarum, Mycobacterium parantracellale, Mycolicibacterium fluoranthenivorans. We found that the sequences of the above three bacteria can be combined with the primers and probes used for NTM PCR detection, resulting in false positive.

Conclusions: In the RT-PCR detection of mycobacteria, if there's abnormal amplification, and the mycobacterial species cannot be identified, the amplified products sequencing or MALDI- TOF mass spectrometry identification will help avoid the omission of rare pathogens.

1. Background

Nocardie is an aerobic gram-positive bacterium, which usually exists in decomposed vegetation, soil and water [1]. Microscopically, nocardiae are filamentous structures of branching hyphae, often defined as opportunistic infections. Knobloch's disease is usually seen in immunocompromised patients [2]. Infections primarily involve the lungs, skin, and central nervous system [3]. The most common Nocardia isolated from specimens of clinically infected patients are Nocardia stellaria and Nocardia brasiliensis, while the Nocardia otitidiscaviarum is rarely reported both at home and abroad [4,5]. It is clinically rare due to its atypical clinical features, and it is easy to confuse patients infected with Nocardia otitidiscaviarum with those with tuberculosis like symptoms. Mycobacterium paraintracellulare was first isolated from Korean sputum. It is an acid fast bacillus with sequence similarity to Mycobacterium intracellulare [6]. Mycolicibacterium fluoranthenivorans was isolated with fluoranthene as the single carbon source from soil of a former coal gas plant, polluted with polycyclic aromatic hydrocarbons [7].

All three of the above were positive curves that we used the RT-PCR method during the detection of DNA from *M. tuberculosis* or nontuberculous mycobacteria. But the experimental results confirmed that these three positive curves were not *Mycobacterium tuber-*

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culosis or Non tuberculosis mycobacteria. After MALDI-TOF mass spectrometry identified as Nocardia otidiscaviarum, Mycobacterium parantracellulare, Mycolicibacterium fluoranthenivorans. These bacteria are extremely rare in the clinical diagnosis and treatment process, and they have been rarely reported in the literature. We will describe the laboratory tests of above three bacteria by case analysis.

2. Case

The first case was a 61-year-old female patient, who developed fever after a cold 20 days ago, the body temperature fluctuated between 38 °C and 39 °C, the body temperature could temporarily decrease after taking antipyretics, the patient had no cough and expectoration, no dyspnea, the patient had a poor effect after anti-infective treatment with " amoxicillin clavulanate ". Nearly half a month later, the patient lost 5 kg of body weight. The patient had suffered from tuberculosis five years earlier and self-complaint was cured. The patient's chest CT results showed multiple infection foci in both lungs, and tuberculosis dissemination was possible. The patient had negative results of acid bacilli smear, sputum bacteriological examination showed no abnormalities, mycological examination showed no abnormalities, tuberculosis infection T cell test was positive. The curve of *Mycobacterium tuberculosis* DNA detection in patients was suggestive of nontuberculous mycobacteria positivity(The amplification curve is shown in Fig. 1)and the patients were continued to be given anti-infective treatment as well as specific treatment against nontuberculous mycobacteria, with unsatisfactory results. The identification of mycobacterial species then suggested that the patient was not a nontuberculous mycobacterial infection. It was identified as Nocardia otitidiscaviarum by MALDI-TOF mass spectrometry.

The second case was a 65-year-old female patient who was admitted to our hospital because of recurrent cough, sputum, wheezing for more than 40 years, aggravation for 10 more days. After the patient's activity, the asthma and distress were obvious, cough, cough white sputum, the amount was small, not easy to expectorate, with chest tightness and shortness of breath, accompanied by fever, body temperature up to 38.5 °C. Admission physical examination electrocardiogram was normal, white blood cells and CRP were increased, pulmonary oncology test showed: squamous cell carcinoma associated antigen 2.6ng/ml, keratin 21-1 10.6ng/ml, acid fast bacilli smear and sputum bacteriology test was negative. The patient CT results were double lung exudative lesions with bilateral pleural effusion. Bilateral multiple bronchiectasis, thickening. The cavity in the posterior region of the upper lobe of the left lung and its inner dense shadow, did not exclude the possibility of Aspergillus. The patient was given etimicin, piperacillin tazobactam anti-infective treatment after admission, giving the patient non-invasive assisted ventilation, hormone anti-inflammatory and other comprehensive treatment, the effect was not ideal, the patient's condition was slightly improved. After tapering of hormonal drugs, gradually white blood cell elevation and fever situation, consider not excluding patients' intrapulmonary lesions as secondary lesions, do not exclude tuberculosis, fungi, Nocardia and other atypical pathogens infection, cause fever and white blood cell elevation may, suggest perfect related examination. So the patient did a series of related examinations and the results showed that, *Mycobacterium tuberculosis* rpoB gene and its mutation, the results were negative, electronic bronchoscopy, bronchial brush Aspergillus examination was negative. Alveolar lavage fluid fungal examination was negative. *Mycobacterium tuberculosis* DNA test curve in the patient suggested non tuberculosis mycobacterium positive(The amplification curve is shown in Fig. 2), then after Mycobacterium species identification suggested that the patient was not non tuberculosis mycobacterium, which was identified by MALDI-TOF mass spectrometry as Mycobacterium paraintracellulare. The patient was discharged from the hospital with intermittent cough, white mucous sputum, chest tightness to suppress asthma did not significantly improve.

The third case was a 27-year-old female patient who presented with chest pain after exertion 4 days previously, chest pain related to breathing, as left anterior chest needle stick like pain on deep inspiration, no cough, expectoration, no blood in sputum and hemoptysis, no dyspnea. The patient self-administered " levofloxacin " treatment, the chest pain symptoms were better than before. The pa-

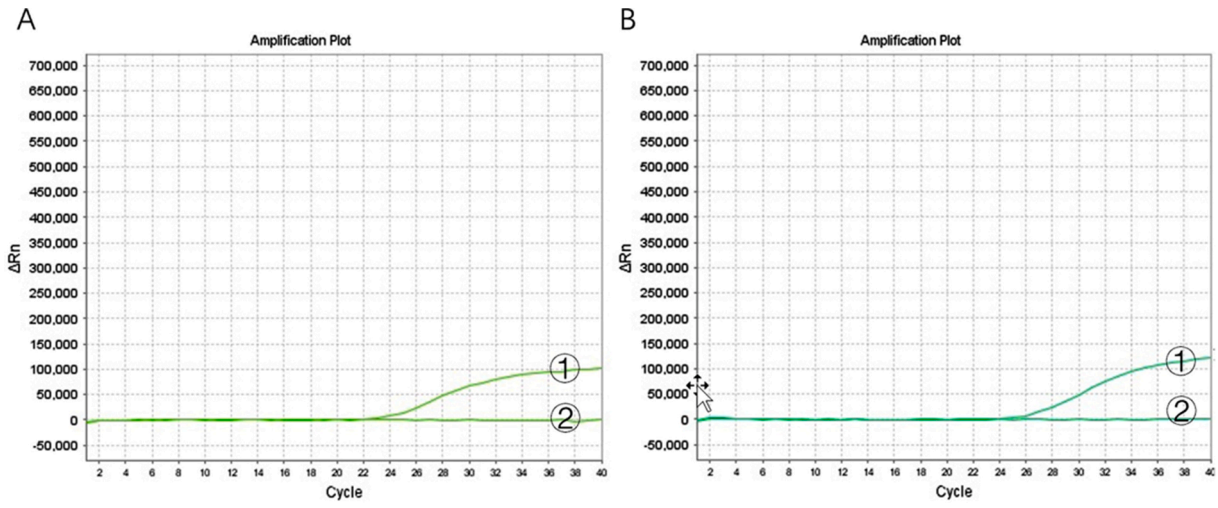

Fig. 1. Amplification curve of Case 1 (①: Amplification curve of non tuberculosis Mycobacterium. ② : Amplification curve of tuberculosis Mycobacterium. Figure A is from sputum sample, and Figure B is from alveolar lavage fluid sample).

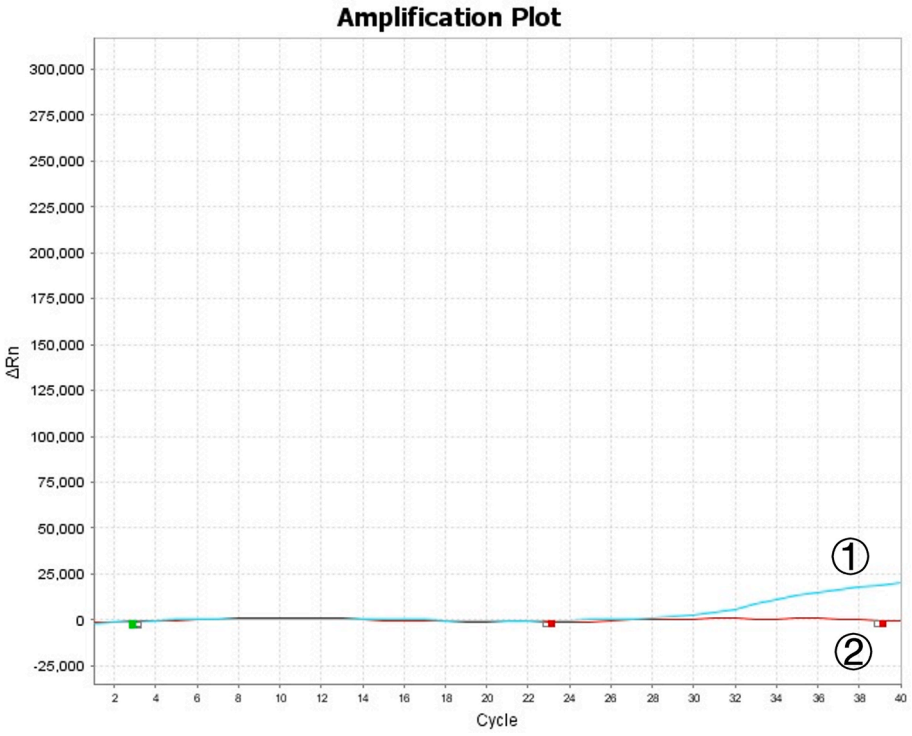

Fig. 2. Amplification curve of Case 2 (①: Amplification curve of non tuberculosis Mycobacterium. ②: Amplification curve of tuberculosis Mycobacterium.).

tient's CT results showed: 1, double lung multiple fibrous cord shadow 2, bilateral pleural thickening, pleural effusion and left lung swelling insufficiency/inflammatory changes. The patient showed elevated white blood cells on blood routine results. The patient had normal ECG results. The patient was admitted to the hospital for examination of sputum acid fast bacilli smear microscopy, sputum bacteriology examination, alveolar lavage fluid bacteriological classification, the results of which were negative. The results of induced sputum cytological classification showed that the neutrophil proportion was 71 %. The patient was given cefazoxime combined with levofloxacin anti-infective treatment, the effect was general, the patient's condition was mildly improved, and the discharged chest CT showed that patient 1. Double lung inflammation, the right lower lung was significant. 2. Multiple small nodules in both lungs, consider benign. 3. Double pulmonary fiber foci; Mild emphysema in middle lobe of right lung. Of note, the PCR amplification curve in the patient's *M. tuberculosis* DNA test was suggestive of nontuberculous mycobacteria positivity, and then after mycobacterial species identification suggested that the patient was not nontuberculous mycobacteria(The amplification curve is shown in Fig. 3), which were identified as Mycolicibacterium fluoranthenivorans by MALDI-TOF mass spectrometry.

3. Discussion and conclusion

In our hospital, a mycobacterial nucleic acid detection kit (PCR fluorescent probe method), which is a combination of duplex PCR and TaqMan probe technology, was used to qualitatively detect mycobacterial nucleic acids extracted from clinical sputum samples. Primers and probes were designed for the *M. tuberculosis* complex and specific sequences of mycobacteria, respectively, and the two probes were labeled with different fluorescent luminescent groups. When nontuberculous mycobacteria test positive, we perform species identification of mycobacteria by DNA microarray, and species identification projects include: *Mycobacterium tuberculosis* (MTB) complex, Mycobacterium intracellulare, *Mycobacterium avium*, Mycobacterium gordonii, Mycobacterium kansas, and Mycobacterium fortuitum, Mycobacterium scrofulaceum infection, Mycobacterium flavum, Mycobacterium soil, Mycobacterium tortoise/abscess, Mycobacterium phlei, Mycobacterium nonchromogenic, Mycobacterium scrofulaceum, Mycobacterium aureus, Threonium Erga/Malmore, Mycobacterium toad, Mycobacterium smegmati [8,9]. When considering tuberculosis or non-tuberculous mycobacterial infection, a mycobacterial DNA test is usually performed. If non-tuberculous mycobacteria have been amplified, further identification of mycobacterial species will be carried out. However, the number of pathogens that can be identified by the microarray method is limited, and there may be some infectious pathogens that are not included in the identification panel. At this time, sequencing the amplified products and comparing them with relevant databases or using MALDI-TOF mass spectrometry for pathogen identification will help to identify the pathogens of the infection [10].

In this case, the primer sequences and probe sequences of the non tuberculous mycobacteria detection reagent were compared with the sequences of the above three bacteria, and it was found that some of the base sequences were identical, which may lead to the non-specific amplification of non-tuberculous fluorescence quantification [11]. This can cause trouble for clinical diagnosis and treatment. The above three cases, when not clearly a rare bacterial infection, were treated poorly following conventional regimens for

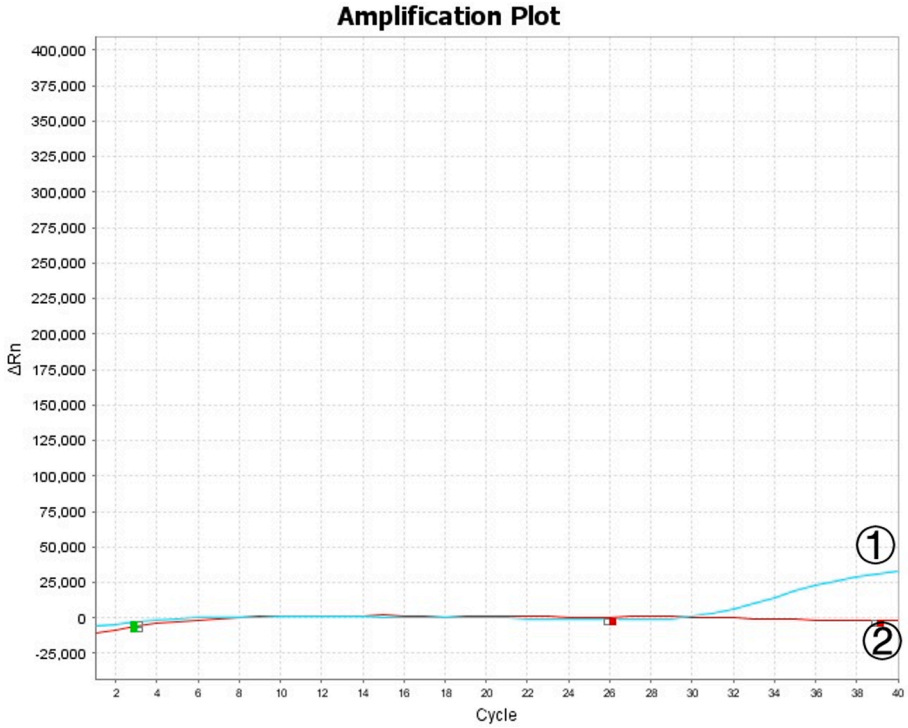

Fig. 3. Amplification curve of Case 3 (①: Amplification curve of non tuberculosis Mycobacterium. ②: Amplification curve of tuberculosis Mycobacterium.).

nontuberculous mycobacteria. In particular, in patients infected with Nocardia otitidiscaviarum, the lung lesions are severe and the prognosis is poor without prompt and effective treatment [12,13]. Selection of antimicrobial agents, which is closely related to the type of species. Therefore, clear identification of the infected species, is essential for the diagnosis and treatment of infection in patients [14].

The RT-PCR method can rapidly detect *Mycobacterium tuberculosis* and nontuberculous mycobacteria, and its specificity and sensitivity are greater than 95 %, which are higher than those of smear and culture methods [15,16]. However, false positives are also possible. Therefore, a positive *M. tuberculosis* DNA test alone cannot confirm the diagnosis of tuberculosis, which needs to be comprehensively evaluated in combination with other items. Therefore, the specificity of their primers should be strengthened during the development of commercial test kits. In addition, DNA detection cannot distinguish between dead or live bacteria, and thus this project cannot be used for the evaluation of the efficacy of anti tuberculosis treatment [17].

If there was amplification in real-time PCR detection of non mycobacteria, but the overall fluorescence value was low, and identification of non mycobacterial species could not identify the pathogen. Clinical examiners encounter this situation and cannot proceed directly to negative or false-positive results. Further measures should be taken to sequence and analyze the amplified products and compare them with relevant databases, or using MALDI-TOF mass spectrometry, which has become a standard routine identification tool for bacteria, which is very helpful for infections of clear pathogens, especially rare [18].

Ethical approval

The authors declare that ethical approval was not required for this study.

Declaration of competing interest

The authors declare no conflicts of interest associated with this manuscript.

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References

[1] P.S. Conville, B.A. Brown-Elliott, T. Smith, A.M. Zelazny, The complexities of Nocardia taxonomy and identification, J. Clin. Microbiol. 56 (1) (2018).
[2] B.A. Brown-Elliott, J.M. Brown, P.S. Conville, R.J. Wallace Jr, Clinical and laboratory features of the Nocardia spp. based on current molecular taxonomy, Clin. Microbiol. Rev. 19 (2) (2006) 259–282.
[3] Q. Luo, S. Hiessl, A. Steinbüchel, Functional diversity of Nocardia in metabolism, Environ. Microbiol. 16 (1) (2014) 29–48.
[4] H.H. Mehta, Y. Shamoo, Pathogenic Nocardia: a diverse genus of emerging pathogens or just poorly recognized? PLoS Pathog. 16 (3) (2020) e1008280.
[5] D. Dhakal, V. Rayamajhi, R. Mishra, J.K. Sohng, Bioactive molecules from Nocardia: diversity, bioactivities and biosynthesis, J. Ind. Microbiol. Biotechnol. 46 (3–4) (2019) 385–407.
[6] S.Y. Lee, B.J. Kim, H. Kim, Y.S. Won, C.O. Jeon, J. Jeong, S.H. Lee, J.H. Lim, S.H. Lee, C.K. Kim, et al., Mycobacterium paraintracellulare sp. nov., for the

genotype INT-1 of Mycobacterium intracellulare, Int. J. Syst. Evol. Microbiol. 66 (8) (2016) 3132–3141.
[7] D. Hormisch, I. Brost, G.W. Kohring, F. Giffhorn, R.M. Kroppenstedt, E. Stackebrandt, P. Färber, W.H. Holzapfel, Mycobacterium fluoranthenivorans sp. nov., a fluoranthene and aflatoxin B1 degrading bacterium from contaminated soil of a former coal gas plant, Syst. Appl. Microbiol. 27 (6) (2004) 653–660.
[8] S. Sankar, M. Ramamurthy, B. Nandagopal, G. Sridharan, An appraisal of PCR-based technology in the detection of Mycobacterium tuberculosis, Mol. Diagn. Ther. 15 (1) (2011) 1–11.
[9] H.B. Xu, R.H. Jiang, W. Sha, L. Li, H.P. Xiao, PCR-single-strand conformational polymorphism method for rapid detection of rifampin-resistant Mycobacterium tuberculosis: systematic review and meta-analysis, J. Clin. Microbiol. 48 (10) (2010) 3635–3640.
[10] T.C. Dingle, S.M. Butler-Wu, Maldi-tof mass spectrometry for microorganism identification, Clin. Lab. Med. 33 (3) (2013) 589–609.
[11] G. Dastgir, M.I. Majeed, H. Nawaz, N. Rashid, A. Raza, M.Z. Ali, M. Shakeel, M. Javed, U. Ehsan, S. Ishtiaq, et al., Surface-enhanced Raman spectroscopy of polymerase chain reaction (PCR) products of Rifampin resistant and susceptible tuberculosis patients, Photodiagnosis Photodyn. Ther. 38 (2022) 102758.
[12] P. Kanchanasin, M. Yuki, T. Kudo, M. Ohkuma, W. Phongsopitanun, S. Tanasupawat, Nocardia terrae sp. nov., an actinomycete isolated from soil in Thailand, Arch. Microbiol. 203 (3) (2021) 1071–1077.
[13] R.J. Wallace, B.A. Brown, J.M. Brown, M. McNeil, Taxonomy of Nocardia species, Clin. Infect. Dis. : Offic. Pub. Infectious Diseases Soci. Am. 18 (3) (1994) 476–477.
[14] S. Tiberi, N. du Plessis, G. Walzl, M.J. Vjecha, M. Rao, F. Ntoumi, S. Mfinanga, N. Kapata, P. Mwaba, T.D. McHugh, et al., Tuberculosis: progress and advances in development of new drugs, treatment regimens, and host-directed therapies, Lancet Infect. Dis. 18 (7) (2018) e183–e198.
[15] D. Machado, I. Couto, M. Viveiros, Advances in the molecular diagnosis of tuberculosis: from probes to genomes, Infect. Genet. Evol. : J. Molecul. Epidemiol. Evolution. Genetic. Infect. Diseases 72 (2019) 93–112.
[16] B. Dahiya, A. Khan, P. Mor, E. Kamra, N. Singh, K.B. Gupta, A. Sheoran, V. Sreenivas, P.K. Mehta, Detection of Mycobacterium tuberculosis lipoarabinomannan and CFP-10 (Rv3874) from urinary extracellular vesicles of tuberculosis patients by immuno-PCR, Pathogens and Disease 77 (5) (2019).
[17] K. Sharma, S. Singh, M. Sharma, N. Batra, V. Kaur, M. Modi, A. Sharma, P. Ray, Multi-targeted loop mediated amplification PCR for diagnosis of extrapulmonary tuberculosis, Indian J. Tubercul. 67 (4) (2020) 479–482.
[18] A. Narang, S.A.E. Marras, N. Kurepina, V. Chauhan, E. Shashkina, B. Kreiswirth, M. Varma-Basil, C. Vinnard, S. Subbian, Ultrasensitive detection of multidrug-resistant Mycobacterium tuberculosis using SuperSelective primer-based real-time PCR assays, Int. J. Mol. Sci. 23 (24) (2022).