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Isolation and identification of nontuberculous mycobacteria from specimens of lower respiratory tract of transplanted patients based on the evaluation of *16SrRNA* gene

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Key words: NTM, Transplantation, Infection, Mycobacterium spp, Bone marrow, 16SrRNA gene
Parole chiave: NTM, Trapianti, Infezioni, Mycobacterium spp, Midollo osseo, gene 16SrRNA

Abstract

Background. *Nontuberculous mycobacteria are pervasive microorganisms and are often present as saprophytes in humans, animals, and the environment. Today, these bacteria are known as the most important environmental opportunists and, in the last decades, infections by nontuberculous mycobacteria have multiplied, due to increased immunodeficiency (cancer, transplant recipients, HIV).*
Study design. *This study aimed to investigate the infections by nontuberculous mycobacteria in transplanted patients.*
Methods. *The study was performed on 57 samples from respiratory secretions of transplant recipients taken by standard methods. Nontuberculous mycobacteria were identified by culture method and molecular identities of clinical isolates were investigated by PCR amplification using 16SrRNA gene and sequence analysis and Blast of the sequences. Demographic data were evaluated by Spss software.*
Results. *The prevalence of nontuberculous mycobacteria in transplant patients was 22.8%, the age of patients was between 23 and 52 years. The most common involvement of nontuberculous mycobacteria in our transplanted individuals were 6 strains of M avium-intracellulare Complex (42.87%), followed by 2 strains of M marinum (14.29%) and 1 strain each (7.14%) of M xenopi, M chelonae, M intracellulare, M kansasii, M simiae. At the conclusion of the tests, one final strain was identified as M tuberculosis (7.14%).*
Conclusion. *The prevalence of nontuberculous mycobacteria indicates their importance in the fate of these patients. The identification of nontuberculous mycobacteria is a neglected part of microbiology laboratories, due to the lack of sufficient facilities and the risk associated with their culture. Therefore developing routine methods for the identification of these infections appears to be critical, especially in hospitals with the transplantation ward.*

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Introduction

Historically, human infections caused by *Mycobacteria* were almost exclusively due to *Mycobacterium tuberculosis (M tuberculosis)*. Recently, other species of *Mycobacteria* have been diagnosed with clinical diseases (1, 2). Tuberculosis caused by *M tuberculosis* complex include *M tuberculosis, M bovis*, *M africanum*, *M microti* and *M canetti*; *M leprae* and *M lepromatosis* cause leprosy (Hansen disease); nontuberculous mycobacteria (NTMs) are also known as "Environmental Mycobacteria" or "Mycobacteria other than tuberculosis", which cannot cause diseases such as tuberculosis or leprosy (3, 4). All Mycobacteria are highly aerobic and are predominantly intracellular pathogens (5, 6). Apart from advanced classification methods, for example *16S rRNA* gene sequencing, most Mycobacteria are classified by their physiological and morphological characteristics, which are dependent on the wax and fat of their cell wall. They are also known as gram-positive bacteria and can be stained by Ziehl-Neelsen (7, 8). NTMs are ubiquitous in nature and can be found in soil, dust, rocks, water and bio-aerosols. These organisms can be found increasingly in unfair environments, characterized by low levels of nutrients, low pH and extreme temperatures. The survival strategy of these organisms in such environments is the formation of biofilms (3), which can cause opportunistic infections (9). NTMs that grow rapidly are mostly found in patients with catheter infections, eye surgery, skin and soft tissue infections, postoperative cosmetic surgery, heart surgery and pulmonary infections (3). NTMs have been traditionally classified by Runyon (10). Rapidly growing Mycobacteria (RGMs) grow in solid media (e.g. Middlebrook 7H11) within 7 to 10 days, as compared to slowly growing Mycobacteria (SGM) species, that usually grow within 10 to 21 days, but could take up to six weeks (11). Members of the RGMs, which are pathogenic but not chromogenic, are *M peregrinum, M chelonae, M fortuitum and M abscessus,* but also *M smegmatis* and *M flavescens,* which are only occasionally pathogenic. SGMs are divided into three Groups, according to the production or non-production of pigments: Group I SGMs ("Photochromogen Mycobacteria") only produce pigment when exposed to light, and include *M intermedium, M marinum M. simiae* and *M kansasii.* Group II SGMs ("Scotochromogen Mycobacteria") become pigmented in darknes, and include *M gordonae, M scrofulaceum, M interjectum, M szulgai, M hiberniae* and *M cooki*. Group III SGMs are classified as "Non-chromogenic" because they never produce pigments and include some opportunistic pathogens known as the "*M avium* complex" (*M avium* + *M intracellulare*), and also *M malmoense, M haemophilum, M genavense, M chimera, M xenopi, M terrae* and *M ulcerans* (12). Since the early 1980s, the number of diseases caused by NTMs has increased. NTM infections are mostly found in people with suppressed immunity, especially those infected with Human Immunodeficiency Virus (HIV) (13). The epidemiological evaluation of this concept has not yet been fully elaborated and the determination of the epidemiology of NTMs is more challenging than that of *M tuberculosis* (14). Humans get infected directly from environmental sources (15). There are no reports of direct or indirect transferring of NTMs to respiratory patients (16), with the exception of the *M abscessus* respiratory disease outbreaks in patients with cystic fibrosis (17). Exposure to NTMs is likely to be very common (18). In addition, there are NTMs isolation from the respiratory tract samples of colonized people, who actually are not patients (15). In many parts of the world, NTMs are not to be notified to public health authorities; therefore, epidemiological information and surveillance are simply unavailable (15).

Despite the barriers to the epidemiological studies of pulmonary diseases due to NTMs, the evidence suggests that the outbreaks of pulmonary diseases dues to NTMs have increased dramatically over the past decades. Several studies reported that NTMs diseases have increased in patients undergoing hematopoietic stem cell transplant (HSCT) and solid organ transplantation (SOT) (16). In several hospitals, transplantation departments are present, and associated risk factors need to be considered. The aim of this study was to investigate the importance and risk of NTMs infections in patients receiving transplants, and to document the presence and types of *Mycobacterium* species isolated from the respiratory tract of transplanted individuals in Imam Reza and Shahid Ghazi Tabatabai Hospitals.

Materials and methods

1. Study population

The present study was conducted on all subjects hospitalized, from December 2018 to February 2019, in the "Shahid Ghazi Tabatabaee" and "Imam Reza" hospitals of Tabriz, Iran. Because NTMs can be responsible for respiratory infections, sputum, bronchoalveolar lavage (BAL) and tracheal aspiration were selected for this study. Sputum was collected voluntarily from patients but BAL or tracheal aspiration were harvested only when collected for diagnostic purposes and after an informed consent form was obtained from the patients: about half of the patients accepted to participate in this study. Sputum specimens were collected by deep coughing from the inferior part of the respiratory tract, in the morning before eating and drinking, in a screwed, sterile container with a wide opening. BALs and tracheal aspirations were obtained by nurses according to the current medical guidelines. The specimens were transferred to the microbiological laboratory of the Center for Applied Drug Research of Tabriz University of Medical Sciences. The samples were cultured in Lowenstein-Jensen medium on the same day or, at most, 24 hours after digestion and decontamination. The contaminating microorganisms had to be removed in order to permit the growth of the non-NTM strains. Digestion and decontamination were performed according to the Petroff method (16, 17). Briefly, a volume of sputum specimen was added to equal amount of 4% NaOH. The container were tightened and shaked to digest, let stand for 15 minutes at room temperature and centrifuged at 3000 rpm for 15 minutes. The supernatants were dissolved and added a drop of the phenolphthalein indicator, to appear purple color. Then, a few drops of HCl were added to the residue to make it colorless. The precipitates were inoculated into a Lowenstein-Jensen medium and were incubated at 37°C for growth. The colonies after 7 days were stained with the Ziehl-Neelsen method and assessed for acid-fast bacilli.

2. DNA extraction

Two loopful of fresh cultured suspected NTMs were suspended in 1X Tris-EDTA (TE) buffer and were incubated at 80°C for 20 min. DNAs were extracted by lysozyme, SDS (Sodium dodecyl sulfate), proteinase K and CTAB (Cetyl trimethylammonium bromide). Extracted DNAs were sedimented with isopropanol and washed with 70% ethanol. After evaporation of ethanol, DNAs were dissolved in 100 µl TE buffer (19).

3. PCR amplification

In this study, the PCR method and sequencing of *16SrRNA* gene were used for the identification of the isolates. *16SrRNA* gene of all isolates were amplified with 16SrRNA-F (5'- ATGCACCACCTGCACACAGG -3') and 16SrRNA-R (5'-GGTGGTT TGTCGCGTTGTTC -3') 473bp fragment (20), and 16SrRNA-F (5'-

Table 1 - General information of isolates from lower respiratory secretions of transplanted patients.

Patient	Age, Sex	Type of samples	Cause of disease	Smoking	Type of blood group	Type of transplantation	Antibiotic treatment	Length of hospitalization	Mycobacterium species
1	31,F	Sputum	CDK[1]	-	O^+	Kidney	Transplant drugs, cyclosporine	27 day	*M. avium complex*
2	23,M	Tracheal tube	Hodgkin lymphoma	+	B^+	Bone marrow	Transplant drugs, cefazolin	14 day	*M. avium complex*
3	43,F	BAL	CRF[2]	-	O^+	Kidney	Transplant drugs, cefazolin, salbutamol	17 day	*M. avium complex*
4	55,M	Tracheal tube	CDK	+	B^+	Kidney	Transplant drugs, prednisolone, fluconazole, ceplalexin	25 day	*M. avium complex*
5	34,F	Tracheal tube	Multiple myeloma	-	A^+	Bone marrow	Transplant drugs, colistin, vancomycin, ciprofloxacin, levofloxacin	1 month	*M. avium complex*
6	50,M	Tracheal tube	PKD[3]	+	O^+	Kidney	Transplant drugs	51 day	*M. avium complex*
7	48,M	Sputum	CKD	-	O^+	Kidney	Transplant drugs, clindamycin, azithromycin	16 day	*M. intracellulare*
8	52,M	Sputum	PKD	+	A^+	Kidney	Transplant drugs	23 day	*M. xenopi*
9	25,M	Tracheal tube	ARF[4]	+	B^+	Kidney	Transplant drugs, ceftriaxone	16 day	*M. marinum*
10	44,F	Sputum	ESRD[5]	-	O^+	Kidney	Transplant drugs	19 day	*M. marinum*
11	49,F	BAL	PKD	-	B^+	Kidney	Transplant drugs	28 day	*M. chlonae*
12	48,M	Sputum	PKD	+	A^+	Kidney	Transplant drugs	27 day	*M. simiae*
13	33,M	Tracheal tube	ESRD	+	O^+	Kidney	Transplant drugs	17 day	*M. kansasii*
14	34,M	Tracheal tube	CRF	+	A^-	Kidney	Transplant drugs	19 day	*M. tuberculosis*

CDK= Chronic kidney disease ; CRF= Chronic renal failure; PKD= Polycystic kidney disease; ARF= Acute renal failure; ESRD= End stage renal disease.

AGAGTTTGATCMTGGCTCAG -3'), 16SrRNA-R (5'-CCGTCAATTCMTTTRAGTTT-3') 921bp fragment (21). The *16SrRNA* gene was amplified as follows: initial denaturation at 95° C for 5 min, 30 cycles followed by 95° C for 30s, 65°C for 30s and 72°C for 45s, and final polymerization at 72° C for 5 min. The PCR products were analyzed by electrophoresis through 1.5% agar and were assessed for 473bp and 921bp fragment. The positive PCR products were sequenced by PRISM 3100 Genetic analyzer (Applied Biosystems) and the sequences were compared with the nucleotide database in GenBank at NCBI (www.ncbi.nlm.nih.gov/blast/).

Results

In this study, 57 specimens were collected, 9 of which were obtained from bone marrow transplanted patients and 48 from kidney transplanted patients. The sources of specimens from patients included 37 tracheal aspirations, 12 sputum, and 8 BAL specimens. The age of the patients varied from 23 to 52 years, with a mean of 39.07 years (Fig. 1). 37 patients (65%) were male and 20 (35%) female. Overall, 14 isolates grew in Lowenstein-Jensen medium. DNA of the isolates was extracted and *16srRNA* of the isolates were amplified by PCR method and were sequenced. Unexpectedly, one of the strains was identified as *M tuberculosis*, coming from the tracheal tube specimen of a kidney transplanted recipient. The isolations of NTMs from kidney transplant recipients (11/47 or 23.4%) was not so different compared to those from bone marrow transplant recipients (2/9 or 22.2%). The sequences obtained indicated that the most frequent isolates were *M avium-intracellulare Complex* (7/13 or 53.8%), followed by *M marinum* (2/13 or 15.4%), and one isolate each was obtained for: *M xenopi*, *M kansasii*, *M simiae*, *M chelonae* (7.14% each). Only one NTM strain belonged to the RGM group (*M chelonae*), while all the other 12 NTM isolates were SGM; more precisely, 4 strains referred to the group 1 (Photochromogens): 2 *M marinum*, 1 each *M kansasii* and *M simiae*; and 8 strains referred to the Group 3 (Non-chromogens): 7 *M avium-intracellulare Complex*, 1 strain *M xenopi*. No strains of Group 2 (Scotochromogens) were present. The results of this study based on the morphology and phenotypic characteristics demonstrated that only *Mycobacterium chelonae* was RGM and all other species were SGM. The results indicated that the specimens contained potentially pathogenic *Mycobacteria* spp. such as *M avium-intracelluare* complex, *M simiae, M kansasii, M chlonae,* as well as environmental saprophytes *Mycobacteria,* such as *M marinum* and *M xenopi*.

Discussion

Nowadays, it is known that the majority of NTM species, except for a few, are opportunistic pathogens for humans and have the ability to produce pulmonary diseases and also some kinds of non-pulmonary diseases (such as skin infections, lymphadenitis, and disseminated diseases) in healthy individuals and in immunodeficient patients (22). These species are transferred to humans through inhalation, ingestion, and skin ulcers (normal wounds or surgical wounds). NTMs are emerging pathogens that affect patients with immunodeficiency (23). The development of molecular techniques has allowed the description of new species and the correct distribution of NTMs into exact species and subspecies. The incidence of NTM pulmonary diseases is increasing worldwide and true outbreaks are often reported of clinical cases of NTM (23). In the present study, NTM isolates from transplant patients were identified using conventional

and molecular methods. Results indicated the importance of NTMs in our transplant patients. *M avium-intracellulare Complex,* togheter with one case of *M tuberculosis,* were the most important pathogens identified in the present study. According to studies on NTM in transplant patients, in Iran and other countries, we can compare the results obtained from northwestern Iran with other studies, considering that few studies have been conducted on the prevalence of NTM in transplant patients in Iran. In a case study from Iran (24), a woman who had a kidney transplant, a week after transplantation, while taking triple drugs (mycophenolate mofetil, prednisolone, and cyclosporine), developed a double infection by *Aspergillus* spp and *Mycobacterium tuberculosis*. In our study, a man who had a kidney transplant and also used immune-suppressive drugs, had a positive culture of *Mycobacterium tuberculosis* from a sputum specimen. Another study in Iran (25) in transplanted patients showed a 10% estimate of patients with *Mycobacterium tuberculosis*. In Saudi Arabia in 2014 (26) NTM infections occurred in HSCT receptors within 115-1055 days after HSCT. Anyway, most NTMs were isolated from HSCT receptors, including *M haemophilum*, *M gordonae*, *M fortuitum*, *M abscessus*, and *M chelonae* (26). In the present study, we had isolates of *M avium-intracellulare Complex* from bone marrow transplants, and *M chelonae* from a patient with kidney transplant. In a study at the South Korean University Hospital, for adult patients undergoing bone marrow transplantation, the most common NTMs were *M avium* complex (n = 15) and *Mycobacterium abstruse* (n=5) (27). In our study, we had two patients with bone marrow transplantation and isolation of *M avium-intracellulare Complex* (15.2%). In addition, age >50 was considered as risk factor for these patients (Fig. 1).

Shak et al. (28) in 2016 at the Texas Transplantation Center reported isolation of *M abscessus* (46%), *M avium-intracellulare Complex* (36%), *M gordonae* (9%), *M chelona* (7%) and *M fortuitum* (2%), with multiple NTM isolates in 3 different cases. In our isolates, *M abscessus*, *M fortuitum* and *M gordonae* were not observed, but *M avium-intracellulare Complex (*53.8%)

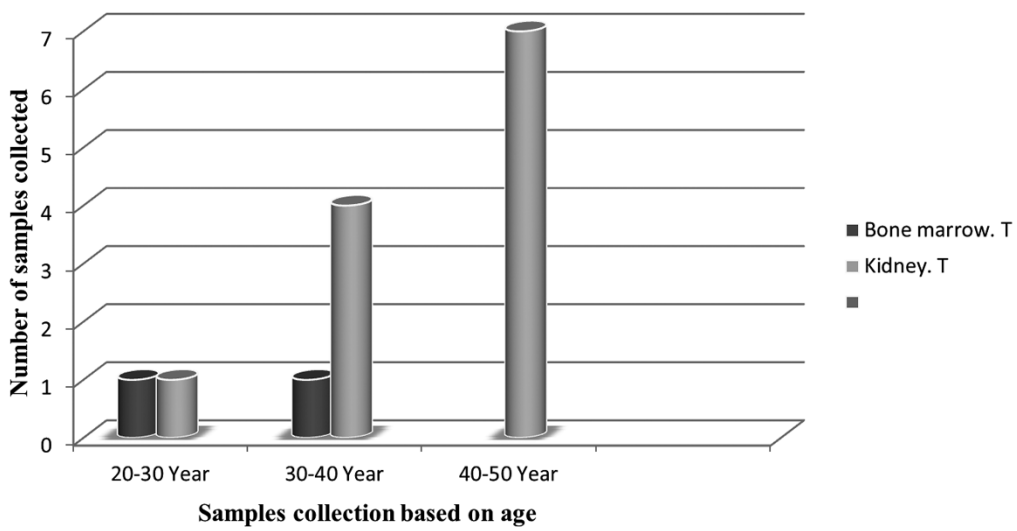

Figure 1 - Frequency of NTM species according to age group.

and *M marinum* (15.4%) had the highest prevalence.

In a similar study by Song et al. (29) in 2018 in a Seoul hospital the most common pathogen (19.1%) was *M chelonae*. In our study, one case of *M chelonae* (7.14%) was found in a kidney transplant recipient.

According to the results and comparison with studies conducted in Iran and abroad, we can conclude that *M chelonae* and *M kansasii* infections were the most important pathogens already seen in studies on transplantation wards worldwide.

According to a study conducted in 2018, the most common NTMs in Europe were: *M mallonus*, *M xenopi*, *M kansasii M. szulgai M simiae* (30). *M simiae* was reported as the most common NTM in clinical specimens in Iran (31). Our general objective in this study was to identify and isolate NTMs from samples of lower respiratory secretions. The prevalence of *M avium-intracellulare Complex* in our transplant recipients was higher than the other species of Mycobacteria. Bone marrow transplanted patients had *M avium-intracellulare Complex,* which should be considered as a risk factor for these patients.

Conclusion

The prevalence of NTMs in hospitalized transplant recipients in Tabriz was 22.8%. This percentage indicates that *Mycobacterium* infections are a serious risk factor in these patients. Although these people, treated with immunosuppressive drugs, also receive preventive medications such as Cotrimoxazole, because of their weakened immune systems they are very prone to NTM infection. NTMs have high adaptive power to settle and multiply in different parts of the body. In the present study, the presence of *Mycobacterium* species in transplant individuals showed no symptoms. Most of *Mycobacteria* species belonged to SGM. Considering the high prevalence of NTM species in transplant patients, timely detection and providing facilities to screen such infections in hospitals with transplantation ward seems critical.

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Conflict of Interest

None to declare

Riassunto

Isolamento ed identificazione con il gene** 16srrna **di micobatteri non tubercolari ottenuti da campioni prelevati dalle basse vie respiratorie di pazienti sottoposti a trapianto

I Micobatteri non tubercolari sono microorganismi capaci di invasività, spesso ospiti come saprofiti nell'uomo, negli animali e presenti anche nell'ambiente. Attualmente essi sono considerati tra i più importanti patogeni opportunisti ambientali, e, nelle ultime decadi, le infezioni da loro prodotte si sono diffuse grazie alla frequenza degli immunodepressi (per tumori, HIV, trapianti).

Disegno dello studio: il nostro studio aveva lo scopo di indagare le infezioni da micobatteri non tubercolari nei soggetti trapiantati.

Materiali e metodi: lo studio è stato effettuato su 57 campioni ottenuti dalle secrezioni delle basse vie respiratorie dei trapiantati con modalità standard. I Micobatteri non tubercolari sono stati identificati con metodi culturali seguiti da identificazione mediante amplificazione PCR con il gene 16SrRNA ed analisi delle sequenze. I dati demografici dei pazienti sono stati elaborati mediante Spss.

Risultati. L'età dei pazienti variava tra i 23 ed i 52 anni. la prevalenza dei suddetti Micobatteri è risultata del 22,8% (13/57), La specie più frequente è stata il

Complesso M avium-intracellulare con 7 ceppi (7/13 or 42,9%), seguita da *M marinum* con 2 (14,3%) e da un ceppo ciascuno di *M xenopi*, *M chelonae*, *M kansasii*, *M simiae* (7,14% ciascuno). Il 14° ceppo è risultato essere un *M tuberculosis* (7,14%).

Conclusioni. La prevalenza di Micobatteri non tubercolari indica la loro importanza per il destino di questi pazienti, ma evidenzia anche che la loro identificazione è negletta in molti laboratori, sia per la obiettiva carenza di adatte tecnologie, sia per il pericolo connesso con la loro coltivazione. Pertanto lo sviluppo di metodi routinari alla portata di ogni laboratorio è un'esigenza critica ed un must negli ospedali che si occupano di trapianti.

References

1. Johnson MM, Odell JA. Nontuberculous mycobacterial pulmonary infections. J Thoracic Dis 2014; **6**(3): 210-20.
2. Asgharzadeh M, Kafil HS, Khakpour M. Comparison of mycobacterial interspersed repetitive unit-variable number tandem repeat and IS6110-RFLP methods in identifying epidemiological links in patients with tuberculosis in Northwest of Iran. Ann Microbiol 2008; **58**(2): 333.
3. Barie PS. Atypical Wound Pathogens. Surg Infect (Larchmt) 2017; **18**(4): 455-60.
4. Leylabadlo HE, Kafil HS, Yousefi M, Aghazadeh M, Asgharzadeh M. Pulmonary Tuberculosis Diagnosis: Where We Are? Tuberc Respir Dis 2016; **79**(3): 134-42.
5. Portaels F. Epidemiology of mycobacterial diseases. Clin Dermatol 1995; **13**(3): 207-22.
6. Asgharzadeh M, Khakpour M, Salehi TZ, Kafil HS. Use of mycobacterial interspersed repetitive unit-variable-number tandem repeat typing to study *Mycobacterium tuberculosis* isolates from East Azarbaijan province of Iran. Pak J Biol Sci 2007; **10**(21): 3769-77.
7. Van Deun A, Hossain MA, Gumusboga M, Rieder HL. Ziehl-Neelsen staining: theory and practice. Int J Tuberc 2008; **12**(1): 108-10.
8. Asgharzadeh M, Shahbabian K, Kafil HS, Rafi A. Use of DNA Fingerprinting in Identifying the Source Case of Tuberculosis in East Azarbaijan Province of Iran. J Med Sci 2007; **7**(3): 418-21.
9. Falkinham JO 3rd. Ecology of nontuberculous mycobacteria—where do human infections come from? Semin Respir Crit Care Med 2013; **34**(1): 95-102.
10. Runyon EH. Anonymous mycobacteria in pulmonary disease. Med Clin North Am 1959; **43**(1): 273-90.
11. Thomson RM. Changing epidemiology of pulmonary nontuberculous mycobacteria infections. Emerg Infect Dis 2010; **16**(10): 1576-83.
12. Koh WJ. Nontuberculous Mycobacteria-Overview. Microbiol Spectr 2017; **5**(1).
13. Chapman J. The atypical mycobacteria and human mycobacteriosis. Springer Science & Business Media, 2012.
14. Kendall BA, Winthrop KL. Update on the epidemiology of pulmonary nontuberculous mycobacterial infections. Semin Respir Crit Care Med 2013; **34**(1): 87-94.
15. Panagiotou M, Papaioannou AI, Kostikas K, et al. The epidemiology of pulmonary nontuberculous mycobacteria: data from a general hospital in Athens, Greece, 2007–2013. Pulm Med 2014; **2014**: 894976.
16. Doucette K, Fishman JA. Nontuberculous mycobacterial infection in hematopoietic stem cell and solid organ transplant recipients. Clin Infect Dis 2004; **38**(10): 1428-39.
17. Tripathi K, Tripathi PC, Nema S, Shrivastava AK, Dwiwedi K, Dhanvijay AK. Modified Petroff's method: an excellent simplified decontamination technique in comparison with Petroff's method. Int J Recent Trends Sci Technol 2014; **10**(3): 461-4.
18. Asgharzadeh M, Samadi Kafil H, Pourostadi M, Asadi Faezi N, Rashedi J, Mahdavipour B. Strain differentiation of Mycobacterium tuberculosis for epidemiology in northwest of Iran. Cell Mol Biol 2016; **62**(8): 15-20.
19. van Soolingen D, de Haas PE, Hermans PW, van Embden JD. DNA Fingerprinting of mycobacterium tuberculosis. Methods Enzymol 1994; **235**: 196-205.
20. Mendum TA, Chilima BZ, Hirsch PR. The PCR amplification of non-tuberculous mycobacterial 16S rRNA sequences from soil. FEMS Microbiol Lett 2000; **185**(2): 189-92.
21. Harmsen D, Dostal S, Roth A, et al. RIDOM: comprehensive and public sequence database for identification of Mycobacterium species. BMC Infect Dis 2003; **3**(1): 26.
22. Dovriki E, Gerogianni I, Petinaki E, Hadjichristodoulou C, Papaioannou A, Gourgoulianis K. Isolation and identification of nontuberculous mycobacteria from hospitalized patients and drinking water samples--examination of their correlation by chemometrics. Environmental

monitoring and assessment. Environ Monit Assess 2016; **188**(4): 247.
23. Koh WJ. Nontuberculous Mycobacteria-Overview. Microbiology Spectr 2017; **5**(1).
24. Petramfar P, Yousefian M, Ashraf MH, Davarpanah MA, Rahmati H. Meningoencephalitis with *Aspergillus* and *Mycobacterium tuberculosis* in a renal transplant recipient. Exp Clin Transplant 2011; **9**(1): 68-71.
25. Ahmadinejad Z, Azmoudeh Ardalan F, Safy S, Nikbakht G. Diagnosis of latent tuberculosis infection in candidates for kidney transplantation (comparison of two tests). Acta Med Iran 2012; **50**(5): 305-10.
26. Al-Anazi KA, Al-Jasser AM, Al-Anazi WKJ Infections caused by non-tuberculous mycobacteria in recipients of hematopoietic stem cell transplantation. Front Oncol 2014; **4**: 311.
27. Kang JY, Ha JH, Kang HS, et al. Clinical significance of nontuberculous mycobacteria from respiratory specimens in stem cell transplantation **4**recipients. Int J Hematol 2015; **101**(5): 505-13.
28. Shah S, McAnally K, Seoane L, et al. Analysis of pulmonary non-tuberculous mycobacterial infections after lung transplantation. Transpl Infect Dis 2016; **18**(4): 585-91.
29. Song Y, Zhang L, Yang H, et al. Nontuberculous mycobacterium infection in renal transplant recipients: a systematic review. Infect Dis (Lond) 2018; **50**(6): 409-16.
30. Basille D, Jounieaux V, Andrejak C. Treatment of Other Nontuberculous Mycobacteria. Semin Respir Crit Care Med 2018; **39**(3): 377-82.
31. Khaledi A, Bahador A, Esmaeili D, Ghazvini K. Prevalence of Nontuberculous Mycobacteria (NTM) in Iranian clinical specimens: systematic review and meta-analysis. J Med Bacteriol 2016; **5**(3): 29-40.

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