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Original Article

Identification of Non-Tuberculosis Mycobacteria by Line Probe Assay and Determination of Drug Resistance Patterns of Isolates in Iranian Patients

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ABSTRACT

The potentially pathogenic Non-Tuberculosis Mycobacteria (NTM) are emerging nowadays which result in pulmonary and non-pulmonary infections in human. This group of bacteria consists of at least 200 different species. While the pulmonary disease is the most common form of NTM infections, NTM can cause diffused infections as well as extrapulmonary infections in every organ, such as bone marrow, skin, eye, and brain. The NTM cause tuberculosis-like infections, therefore, correct identification of these Mycobacteria is necessary to avoid faulty treatment. Different species of NTM isolates were identified from clinical specimens using phenotypic methods and Line Probe Assay. Minimum Inhibitory Concentration for selected antibiotics was obtained by the broth micro-dilution method. Totally, 42 NTM isolates were identified in this study. Moreover, the frequency of NTM between all positive mycobacterium cultures was estimated at 12%. The most common Rapidly Growing Mycobacteria included *Mycolicibacterium fortuitum* (30.9%), *Mycobacterium abscessus* (7.1%), and *Mycobacterium chelonae* (2.3%), whereas *Mycobacterium simiae* (40.4%), *Mycobacterium kansasii* (16.6%), and *Mycobacterium avium* complex (2.3%) were the most recurring among the Slowly Growing Mycobacteria. Amikacin, clarithromycin, and ciprofloxacin were the most effective antibiotics against isolated NTM. The NTM isolates are frequently being separated from Iranian patients, and are mostly resistant to the wide spectrum of antibiotics. Correct identification and determination of antibiotic susceptibility can be helpful in the healing process of the patients who suffer from non-tuberculosis mycobacterial infections.

Keywords: Drug Resistance Patterns, Line Probe Assay, Non-Tuberculosis Mycobacteria

Identification des Mycobactéries non Tuberculeuses en Utilisant le Test de Sonde de Ligne et la Détermination des Modèles de Résistance aux Médicaments des Isolats chez les Patients en Iran

Résumé: Les mycobactéries non tuberculeuses potentiellement pathogènes (MNT) sont en train d'émerger de nos jours, ce qui entraîne des infections pulmonaires et non pulmonaires chez l'homme. Ce groupe de bactéries comprend au moins 200 espèces différentes. Bien que la maladie pulmonaire soit la forme la plus courante d'infections à MNT, la MNT peut provoquer des infections diffusées ainsi que des infections extrapulmonaires

dans tous les organes, tels que la moelle osseuse, la peau, les yeux et le cerveau. La MNT provoque des infections de type tuberculose, par conséquent, une identification correcte de ces mycobactéries est nécessaire pour éviter un traitement défectueux. Différentes espèces d'isolats de MNT ont été identifiées à partir d'échantillons cliniques en utilisant des méthodes phénotypiques et un test de sonde de ligne. La concentration minimale inhibitrice pour les antibiotiques sélectionnés a été obtenue par la méthode de micro-dilution du bouillon. Au total, 42 isolats de MNT ont été identifiés dans cette étude. De plus, la fréquence de MNT entre toutes les cultures de mycobactéries positives a été estimée à 12%. Les mycobactéries à croissance rapide les plus courantes comprenaient *Mycolicibacterium fortuitum* (30,9%), *Mycobacterium abscessus* (7,1%) et *Mycobacterium chelonae* (2,3%), tandis que *Mycobacterium simiae* (40,4%), *Mycobacterium kansasii* (16,6%) et le complexe *Mycobacterium avium* (2,3%) étaient les plus récurrents parmi les mycobactéries à croissance lente. L'amikacine, la clarithromycine et la ciprofloxacine étaient les antibiotiques les plus efficaces contre la MNT isolée. Les isolats MNT sont fréquemment séparés des patients iraniens et sont pour la plupart résistants au large spectre des antibiotiques. L'identification et la détermination correctes de la sensibilité aux antibiotiques peuvent être utiles dans le processus de guérison des patients qui souffrent d'infections mycobactériennes non tuberculeuses.

Mots-clés: Modèles de résistance aux médicaments, Test de sonde de ligne, Mycobactéries non tuberculeuses

INTRODUCTION

Non-Tuberculosis Mycobacteria (NTM) consist of more than 200 species (Shahraki et al., 2015). Most of the NTM species are environmental and non-pathogenic. Except for *Mycobacterium tuberculosis (MTB)* complex, *M. leprae* and *M. ulcerans* species are real pathogens (Chavarro-Portillo et al., 2019). Other species are opportunistic pathogens in transplant patients as well as impaired immunity patients, such as those who are affected with human immunodeficiency virus/acquired immune deficiency syndrome HIV/AIDS, malignancies, and chronic obstructive pulmonary disease (COPD) (Swenson et al., 2018). While the pulmonary disease is the most common form of the NTM infections, NTM can cause diffused infections as well as extrapulmonary infections in every organ, such as bone marrow, skin, and brain (Swenson et al., 2018). The NTM are capable to survive in the environment and are resistant to disinfectants (Waak et al., 2019). Therefore, they can be isolated as pseudo-infectious agents (Waak et al., 2019). In order to presume an NTM species as the real infectious agent, clinical and microbiological features must be considered following the American Thoracic Society (ATS) criteria, (Schiff et al., 2019). The NTM infection rate is increasing in developing and developed countries (Cowman et al., 2018). Iran is among the countries in which mycobacterial infections are endemic, and different species of these organisms need to be identified in medical laboratories (Nasiri et al., 2018). In addition, the NTM species are differently distributed in geographical regions and the epidemiology of the NTM infections has a local pattern (Spaulding et al., 2017). The World Health Organization (2018) estimated the rate of tuberculosis (TB) at about 14 per 100,000 population in Iran. Moreover, the NTM have similar properties the same as *MTB* in terms of clinical signs and phenotypic test results (Schiff et al., 2019). On the other hand, NTM are inherently resistant to the wide spectrum of antibiotics, and each isolated strain should be examined for antibiotic susceptibility pattern in order to select a correct antibiotic regimen (Cowman et al., 2016). Meanwhile, the differentiation of NTM from *MTB* is very confusing and time-consuming by phenotypic methods. However, molecular methods, such as real-time polymerase chain reaction (PCR) and PCR

sequencing could help in the distinction; nonetheless, the simultaneous identification of NTM is not possible by a single PCR test due to the high variation in NTM (Fedrizzi et al., 2017). Furthermore, sequencing is an expensive test, and it is not affordable for developing countries to perform sequencing for each isolated NTM (Fedrizzi et al., 2017). Therefore, Line Probe Assay (LPA), which is an accurate test, can be used for the identification of 21 NTM species in a single test. Additionally, the identification of mycobacteria can be performed directly in clinical samples, as well as colonies using LPA (Mäkinen et al., 2006). Since the correct identification of NTM can be achieved in less than 10 days, it can be very helpful for the timely and correct treatment of NTM infections.

MATERIAL AND METHODS

Samples. In total, 5061 different clinical specimens, including sputum, Bronchoalveolar Lavage (BAL), blood, soft tissue, cerebrospinal fluid (CSF), urine, and stool, were collected at Tehran Regional Reference Laboratory for Tuberculosis, Tehran, Iran, to diagnose TB from December 2017 to June 2019.

Culture and Phenotypic Identification. Non-sterile samples, such as sputum, BAL, and stool were decontaminated by 2% NaOH, and direct smear and culture were performed using decontaminated samples. Sterile samples, such as CSF and blood were directly centrifuged, and the concentrated sample was used for further analysis. Furthermore, direct smears were stained by the Ziehl-Neelsen method. Lowenstein-Jensen (LJ) medium was used to grow the bacteria. Cultures were incubated at 37 °C and 32 °C for a month, and they were checked for growing colonies every day until the growth was detected. Phenotypic characteristics, such as pigment productions, growth rate, and colony morphology were used along with biochemical tests, including niacin production and nitrate test, in order to define *MTB* complex and NTM isolates.

Molecular Identification. Genomic DNA was extracted from colonies grown on LJ medium using the QIAamp DNA Mini Kit (Qiagen, Germantown, MD, USA) according to the manufacturer's instruction. The LPA was performed according to the reverse blot hybridization assay kit instructions (YD Diagnostics, Korea). The PCR and hybridization assays were performed as instructed by the supplier. In order to confirm LPA test results, *16s rRNA* gene was amplified using 16s-F (5`-gagagtttgatcctggctcag-3`) and 16s-R (5`-tgcacacaggccacaaggga-3`) primers, and PCR products were sent for sequencing.

Antibiotic Susceptibility Testing. Minimum Inhibitory Concentration (MIC) was determined using the broth microdilution test according to the Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2011). A loop full of bacterial colonies was suspended in a tube containing glass beads and 5 ml of sterile saline buffer. Considering Rapidly Growing Mycobacteria (RGM), 0.5 MacFarland turbidity of each strain was prepared and diluted to 10^4 cells /mL concentration. About 50 µl of the diluted suspension was added to 96-well round-bottom microtiter plates containing serial concentrations of each antibiotic and incubated at 28-30°C for 3 days. Similarly, regarding the Slowly Growing Mycobacteria, 1 McFarland turbidity was prepared for each isolate and diluted to 10^2 cells/mL, and the plates were incubated at 37°C. Amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, and imipenem were used for rapidly growing NTM (CLSI, 2011). Furthermore, rifampin, isoniazid, and ethambutol were used for *M. kansasii,* and clarithromycin, amikacin, doxycycline, linezolid, and ciprofloxacin were tested for other slowly growing NTM. In addition, clarithromycin was used at pH 6.5 and 7.3 for *M. avium* complex (CLSI, 2011). Table 1 summarizes the concentrations of antibiotics. The MIC was determined after 4 days and 2 weeks for RGM and Slowly Growing Mycobacteria (SGM), respectively, compared to the control sample. *Enterococcus faecalis* ATCC 29212, *M. peregrinum* ATCC 700686,

Pseudomonas aeruginosa ATCC27853, and *Staphylococcus aureus* ATCC 29213 were used as standard control strains.

RESULTS

Samples. In total, 5061 clinical samples were tested for mycobacterium species. Out of this number, 303 (6%) and 350 (6.9%) samples were smear-positive and culture-positive, respectively. Among 350 isolated mycobacteria, 290 (82.8%) isolates were *MTB* complex species and 60 (17.1%) isolates were NTM. In addition, 42 isolates of the isolated NTM met the ATS criteria for being a real pathogen agent, and 17 NTM isolates bypassed from the study. Subsequently, the true rate of NTM was 12% among the positive cultures for mycobacteria in this study. The mean age of the patients infected by NTM was 58.3±18.3 years (age range: 20-90 years). Out of 60 patients, 40 (66.6%) cases were male. The NTMs were isolated from sputum (n=38), BAL (n=12), CSF (n=2), blood (n=2), soft tissue (n=2), and abscess (n=1) samples. Among 42 isolated NTM strains, 55% of them had a positive direct smear. Cough, hemoptysis, dyspnea, fever, and weight loss were the most common clinical symptoms, and all the respiratory NTM were isolated from patients with at least two of the above-mentioned symptoms.

Phenotypic Identification / Nontuberculous Mycobacterial Species. According to phenotypic tests, isolated NTM were categorized into two groups of RGM and SGM. Totally, 17 RGM and 25 SGM were identified by culture. In addition, 20 and 22 isolates were photochromogens and non-chromogens, respectively.

Molecular diagnosis. According to the LPA test, *M. simiae* (n=17) was the most common NTM species, and the other frequent species included *M. fortuitum* complex (n=13), *M. kansasii* (n=7), *M. abscessus* (n=3), *M. avium* (n=1), and *M. chelonae* (n=1) (Table 1). A sample of LPA tests has been presented in Figure 1. Moreover, for 9 isolates [i.e., *M. simiae* (n=4), *M. chelonae* (n=1), *M. kansasii* (n=1), *M. fortuitum* (n=1), *M. abscessus* (n=1), and *M. avium* (n=1)], *16s rRNA* gene was sequenced and the LPA results were approved (Table 2).

Drug Susceptibility Pattern. Drug Susceptibility Pattern for Rapidly Growing Mycobacteria: The MIC was determined for each RGM species according to the CLSI (2011) criteria. Amikacin was the most effective antibiotic against *M. fortuitum* and just one *M. fortuitum* isolate was resistant to amikacin. Ciprofloxacin was in the second rank, and the majority of RGM were susceptible, except for two *M. fortuitum* isolates.

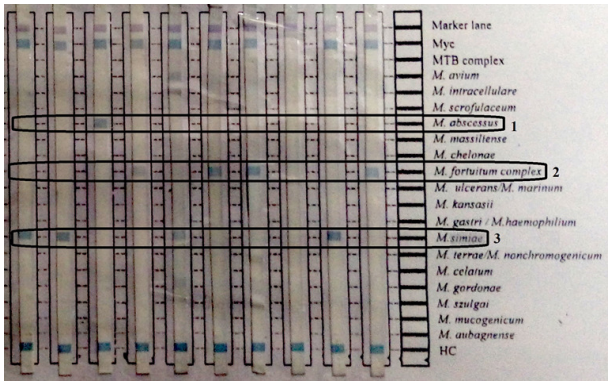

Figure 1. Line Probe Assay results for Non-Tuberculosis Mycobacteria and Mycobacterium Tuberculosis complex. Myc and HC showing the Mycobacterium genus and Hybridization control, respectively. Rectangles 1, 2, and 3 showing positive lines for *M. abscessus, M. fortuitum complex, and M. simiae*, respectively.

Totally, three *M. abscessus* isolates were separated from patients with the mean age of 60 years while suffering from pulmonary signs, such as cough and decreased respiratory capacity. According to the drug susceptibility pattern (DSP) of *M. abscessus* isolates, amikacin and linezolid had just moderate activity, and high rates of resistance were observed for other selected antibiotics. One of the *M. chelonae* species was susceptible to amikacin, imipenem, and ciprofloxacin. Among three *M. abscessus,* just one isolate was susceptible to ciprofloxacin and amikacin, and the other two isolates were completely resistant to all tested antibiotics (Table 3). Drug Susceptibility Pattern for Slowly Growing Mycobacteria: Considering *M. kansasii,* the MIC was calculated for

Table 1. Non-Tuberculosis Mycobacteria species isolated from clinical samples and identified by Line Probe Assay

Non-Tuberculosis Mycobacteria species	Sample						Total (%)
	Sputum	Bronchoalveolar Lavage	Cerebrospinal fluid	Abscess	Soft tissue	Blood	
M. simiae	9	6	1	-	1	-	17(40.47)
M. fortuitum	8	4	-	-	1	-	13 (30.95)
M. kansasii	4	2	1	-	-	-	7 (16.6)
M. abscessus	2	-	-	1	-	-	3 (7.14)
M. avium	-	-	-	-	-	1	1 (2.38)
M. chelonae	-	-	-	-	-	1	1 (2.38)
Total	23	12	2	1	2	2	42 (100)

Table 2. Sequenced *16s rRNA* gene results

	Species	Accession Numbers
1	*M. simiae*	MN197670
2	*M. simiae*	MN238716
3	*M. simiae*	MN238717
4	*M. simiae*	MN238718
5	*M. chelonae*	MN238719
6	*M. kansasii*	MN238720
7	*M. fortuitum*	MN251913
8	*M. abscessus*	MN238721
9	*M. avium*	MN251914

Table 3. Antibiotic susceptibility pattern for isolated rapidly growing mycobacteria

Non-Tuberculosis Mycobacteria species (Isolates)	Range	Minimum Inhibitory Concentration (µg/ml)		No. of Isolates		
		50%	90%	Susceptible	Intermediate	Resistant
M. fortuitum (n=13)						
Amikacin	1-128	2	8	12	-	1
Linezolid	0.5-32	8	32	5	-	8
Imipenem	1-32	>32	>32	-	-	13
Ciprofloxacin	0.125-16	0.5	1	10	1	2
Cefoxitin	2-256	>256	>256	-	-	13
Clarithromycin	0.06-64	>64	>64	1	-	12
M. chelonae (n=1)						
Amikacin	1-128	4	4	1	-	-
Linezolid	0.5-32	32	32	-	-	1
Imipenem	1-64	4	4	1	-	-
Ciprofloxacin	0.125-16	0.125	0.125	1	-	-
Cefoxitin	2-256	256	256	-	-	1
Clarithromycin	0.06-64	64	64	-	-	1
M. abscessus (n=3)						
Amikacin	1-128	64	128	-	1	2
Linezolid	0.5-32	16	32	1	-	2
Imipenem	1-64	32	64	-	-	3
Ciprofloxacin	0.125-16	8	16	-	-	3
Cefoxitin	2-256	128	256	-	-	3
Clarithromycin	0.06-64	32	64	-	-	3

ethambutol, isoniazid, rifampin, clarithromycin, and linezolid. All isolates were resistant to isoniazid, and two isolates were separately susceptible to rifampin and ethambutol. Clarithromycin had the highest efficiency against *M. kansasii,* and all isolates were susceptible to clarithromycin. In addition, ciprofloxacin, linezolid, and amikacin had a moderate effect on *M. kansasii* isolates, and 3, 3, and 4 of these isolates were susceptible to these antibiotics, respectively. With respect to *M. simiae*, antibiotic susceptibility was performed against isoniazid, rifampin, ethambutol, amikacin, linezolid, prothionamide, imipenem,

clarithromycin, ciprofloxacin, and ceftriaxone. The highest susceptibility was observed against amikacin and ciprofloxacin. Furthermore, all the *M. simiae* isolates were resistant to isoniazid, rifampin, imipenem, and prothionamide. In the same line, *M. avium* isolate was susceptible to amikacin, azithromycin, ciprofloxacin, linezolid, and clarithromycin at pH 7.3 and 6.8. This isolate was very resistant to first-line anti-TB antibiotics (Table 4).

DISCUSSION

During recent years, the incidence of TB has been decreased in Iran, whereas the NTM has been isolated more frequently, compared to that in the past decades (Khosravi et al., 2018; Nasiri et al., 2018). This may be due to the increasing trend of transplant patients as well as immunodeficiency syndromes, such as malignancies, COPDs, and HIV/AIDS (Velayati et al., 2014). Moreover, based on several reports on NTM infections, it seems that medical laboratories are paying more attention to better identification of NTM species. However, most of the TB laboratories still do not perform NTM identification tests due to the lack of proper equipment and trained experts. Since phenotypic tests are very confusing and time-consuming, routine medical laboratories are unable to diagnose the exact and correct species of NTM by these methods (Azadi et al., 2018). New methods, such as LPA can result in better identification of NTM species as well as the identification of mycobacteria to the species level in a shorter time and at a high confidence level. Despite the time-consuming nature of the phenotypic tests along with imprecise results, these tests were performed for the identification of species (Moghim et al., 2012). The

Table 4. Antibiotic susceptibility pattern for isolated slowly growing mycobacteria

Non-Tuberculosis Mycobacteria species (Isolates)	Range (µg/ml)	Minimum Inhibitory Concentration (µg/ml)		No. of Isolates		
		50%	90%	Susceptible	Intermediate	Resistant
M. simiae (n=17)						
Isoniazid	16-128	>128	>128	-	-	17
Rifampin	0.5-128	32	32	1	-	16
Ethambutol	4-64	32	32	-	-	17
Amikacin	1-128	4	32	9	-	8
Linezolid	0.5-32	16	32	1	-	16
Imipenem	1-64	32	64	-	-	17
Ciprofloxacin	0.125-16	2	8	9	-	8
Cefoxitin	2-256	128	256	-	-	17
Clarithromycin	0.06-64	8	32	5	2	10
M. kansasii (n=7)						
Isoniazid	0.25-16	8	16	-	-	7
Rifampin	0.125-16	8	16	2	-	5
Ethambutol	4-64	16	32	1	-	6
Amikacin	1-128	16	32	4	-	3
Linezolid	0.5-32	8	32	3	1	3
Imipenem	1-64	32	64	-	-	7
Ciprofloxacin	0.125-16	4	8	6	-	1
Ceftriaxone	2-256	64	128	-	-	7
Clarithromycin	0.06-64	4	16	5	1	1
M. avium (n=1)						
Amikacin	1-128	2	2	1	-	-
Linezolid	0.5-32	16	16	-	-	1
Imipenem	1-64	32	32	-	-	1
Ciprofloxacin	0.125-16	1	1	1	-	-
Cefoxitin	2-256	4	4	1	-	-
Clarithromycin pH 7.3	16-64	<16	<16	1	-	-
Clarithromycin pH 6.8	4-32	8	8	1	-	-
Azithromycin	128-512	<128	<128	1	-	-

RGM and SGM were identified in this study in less than a week and two weeks, respectively, after culturing the sample using the LPA method. On the other hand, the identification of RGM and SGM took at least two weeks and one month, respectively, using phenotypic tests (Moghim et al., 2012). While the frequency of NTM has been reported between 73% and 92% in the United Kingdom, it was estimated at 4% and 15.1% in Iranian patients (Cowman et al., 2018; Khosravi et al., 2018; Nasiri et al., 2018). Moreover, the frequency of the isolated NTM in this study was 12%. It should be noted that the results obtained from this study were consistent with the findings of other studies in Iran. It seems that during the last decade, the rate of NTM infection was stable among Iranian patients. Moreover, *M. simiae*, *M. fortuitum*, and *M. kansasii* were the most common NTM species in the current study. Unlike developed countries in which *M. avium* complex has been reported as the most frequent NTM species (Cowman et al., 2018), *M. simiae* has become the most common NTM species in Iran (Shafipour et al., 2013; Nasiri et al., 2018). This species can mimic the TB disease, and 9% of *M. simiae* variants are also non-pigment forming (Mahon et al., 2011). On the other hand, the niacin accumulation test was positive for the majority of the *M. simiae* isolates, and TB laboratories could misdiagnose *M. simiae* with Multidrug-Resistant (MDR)-TB in phenotypic methods (Mahon et al., 2011). To the best of our knowledge, niacin accumulation tests and pigment production are the main tests for the differentiation of *M. simiae* and *MTB* in Iranian TB laboratories. The correct identification of NTM and DSP tests plays a critical role in choosing the suitable antibiotic regimen for the treatment of NTM infections. In Iran, the first treatment for any mycobacterial infection is the first line anti-tuberculosis (anti-TB) antibiotics (Shahraki et al., 2015). However, it can be seen in this study that the majority of the NTM isolates are completely resistant to first-line anti-TB antibiotics; in addition, these infections are very prone to misdiagnosis with MDR-TB infections. *M. fortuitum* complex was the most frequent RGM in this study. Approximately, all *M. fortuitum* isolates were resistant to clarithromycin, imipenem, cefoxitin, and linezolid. According to a study conducted by Heidarieh et al. (2016) in Iran, 14% and 16% of *M. fortuitum* isolates were resistant and intermediate to clarithromycin, respectively. The studied NTM isolates in the current study were from Tehran, Iran. However, the NTM species were isolated from five different cities in Iran in the aforementioned study (2016). According to the variable origin of NTM from different geographical regions, this discrepancy could result from different sources of NTM isolates (Spaulding et al., 2017). In this study, the highest susceptibility of *M. fortuitum* species was observed against amikacin and ciprofloxacin. Amikacin had high activity against RGM in previous studies. It seems that amikacin is a good treatment choice independent of the geographic source of the isolate. Linezolid was mentioned as a perfect antibiotic regimen for *M. fortuitum* isolates in previous studies, and 66% of the isolates were susceptible according to the results of the study carried out by Heidarieh et al. (2016). However, 61.5% of our isolated *M. fortuitum* strains were highly resistant to this antibiotic. This shows that linezolid therapeutic value is decreasing during recent years against *M. fortuitum* complex species. Although *M. abscessus* is naturally susceptible to cefoxitin, amikacin, and imipenem, *M. abscessus* isolates in our study were completely resistant to imipenem, ciprofloxacin, cefoxitin, and clarithromycin; moreover, one isolate was moderately sensitive to amikacin. A high rate of antibiotic resistance has been reported from *M. abscessus,* and our results confirm that in our region, *M. abscessus* strains have the same pattern of DSP as other parts of Iran and the world (Cowman et al., 2016; Heidarieh et al., 2016). In a study conducted by Van Ingen et al. (2010), *M. abscessus* isolates were completely resistant to amikacin; however, it has been mentioned that *M. abscessus* strains were somehow susceptible to linezolid and amikacin (Cowman et al.,

2016). The results obtained from this study confirm those in previous studies. In line with previous reports (Van Ingen et al., 2010; Cowman et al., 2016), *M. chelonae* isolate was resistant to linezolid and clarithromycin; however, due to the limited rate of *M. chelonae* infections in our study, DSP of *M. chelonae* could not be assessed in Iran. Regarding the SGM, *M. simiae* was the most common species. In previous studies conducted in Iran, *M. simiae* was one of the frequent isolated NTM, and our results were consistent with the findings in previous studies (Khosravi et al., 2018; Nasiri et al., 2018). The majority of the *M. simiae* isolates were cultured from respiratory samples and approximately all the patients had TB like symptoms. This shows that *M. simiae* is mostly considered as an infective agent when patients are suspicious of TB. Unlike *MTB* isolates, *M. simiae* isolates were resistant to first-line anti-TB antibiotics. Mistakes in the identification of *M. simiae* and *MTB* complex can result in the misdiagnosis of infection as MDR-TB infection (Shahraki et al., 2015). In our study, the majority of *M. simiae* isolates were resistant to rifampin, isoniazid, and Ethambutol. In the same line, in the study performed by Heidarieh et al. (2016), a high rate of resistance to rifampin was reported from *M. simiae*, and most of the isolates were susceptible to ethambutol. In other studies, *M. simiae* was also found resistant to rifampin, isoniazid, and ethambutol (van Ingen et al., 2010; Cowman et al., 2016). All of *M. simiae* isolates in our study were resistant to these antibiotics. This may be because of genetic changes in Iranian *M. simiae* isolates during past years. Furthermore, our results are in line with the findings obtained from a comprehensive study carried out by Cowman et al. (2016). They estimated that 0.0%, 5.5%, and 20% of *M. simiae* isolates were susceptible to isoniazid, rifampin, and ethambutol, respectively (Cowman et al., 2016). Amikacin, ciprofloxacin, and clarithromycin had moderate efficacies on *M. simiae* isolates, and 50% of the isolates were susceptible. Additionally, Cowman et al. (2016) revealed that *M. simiae* isolates had 89% and 64% susceptibility rates to amikacin and ciprofloxacin, respectively. The combination of these antibiotics with other treatment options could have a better response (Cowman et al., 2016). *M. kansasii* was the second most common SGM among the isolated NTM in the current study. Among the *M. kansasii* isolates in this study, 85.7%, 71.4%, 57.1%, and 42.8% of them were susceptible to clarithromycin, ciprofloxacin, linezolid, and amikacin, respectively. These rates have been reported 99% and 94% for amikacin and ciprofloxacin, respectively (Cowman et al., 2016). Although the rate of susceptibility in our study is lower than the reported values in other studies across the world, clarithromycin, ciprofloxacin, linezolid, and amikacin have still better efficacy than the first-line anti-TB antibiotics against *M. kansasii* isolates. In addition, a high rate of susceptibility has been reported for rifampin in the treatment of *M. kansasii* (Bakula et al., 2018). Nonetheless, *M. kansasii* isolates had high rates of resistance against rifampicin in this study. Single point mutations have a critical role in resistance against rifampin, and a high rate of rifampin resistance in the current study could result from the point mutations.

M. avium complex isolate was isolated from an end-stage patient with AIDS who had CD4+lymphocytes counts less than 200 per deciliter and the onset of MAC infection during the last 12 months (Welch and Morse, 2002). This strain resulted in disseminated infections in the patient. Different specimens, including blood, stool, bone marrow, and sputum of the patient were positive in smear and culture. The incidence rate of disseminated infections in patients with HIV/AIDS is 110-200 per 100,000 population/year in the United States, and few frequencies of disseminated MAC infections in this study are in line with those in previous studies (Collins et al., 2017). According to some studies, HIV/AIDS is one of the most common risk factors for susceptibility to *M. avium* complex infections (Collins et al., 2017). Despite susceptibility of this isolate to amikacin, ciprofloxacin, cefoxitin, clarithromycin, and azithromycin, the therapy outcome was not good and the patient passed away. Moreover, a

delay in the treatment with suitable anti-MAC antibiotics could result in a weak treatment outcome (Busatto et al., 2019). Although the frequency of *M. avium* complex isolates was low in this study, due to the unsuccessful treatment outcome in this study which led to patient mortality, it could be possible to detect high rates of virulence of *M. avium* complex in patients with HIV/AIDS.

The NTM infection rates are considerable in Iran, and most of the NTM isolates are MDR. The treatment of MDR-NTM requires precise identification and drug susceptibility testing. In addition, inefficient phenotypic methods for the identification of NTM resulted in the misdiagnosis of MDR-TB and NTM infections. The LPA is a rapid and reliable test for the identification of NTM which has the potential to differentiate NTM infection from MDR-TB. The implementation of this method can be very helpful for the treatment of Iranian patients in Iran.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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