

Causative Agents of Mycobacterial Diseases in Aquarium Fish and their Importance for Human Health

MAHDI SEYFAHMADI¹, SEYYED REZA MOADDAB², AZAR SABOKBAR³

ABSTRACT

Introduction: The isolation of Non-Tuberculous Mycobacteria (NTM) from human and fish mycobacteriosis specimens increasingly has turned to be a major concern. NTM can cause opportunistic infections in elderly and immunocompromised patients; it is not only difficult to treat but also requires long course of therapy.

Aim: To isolate and identify the species of non-tuberculous mycobacteria in aquarium fish and to study the importance of their opportunistic infections to the general health of society.

Materials and Methods: Fifty-three common freshwater aquarium fish were included in the study for the diagnosing of mycobacteria by culture and Ziehl-Neelsen staining methods. The Mycobacteria species were identified by morphological characteristics of colonies and biochemical tests. Drug susceptibility tests were also performed using the proportional method on Lowenstein-Jensen media.

Results: Among 53 ZN-stained specimens, 13.2% gave positive direct microscopic results for mycobacteria. Using the bacterial

culture method, *Mycobacterium* spp. colonies was observed in 28.3% Lowenstein-Jensen agar tubes. Identification of different species of *mycobacterium* from 15 isolates obtained by culture based on phenotypic characteristics and biochemical tests was as follows: *M. fortuitum*, 6 cases; *M. marinum*, 3 cases; *M. smegmatis*, 3 cases; *M. terrea*, 1 case; *M. flavescens*, 1 case; and *M. asiaticum*, 1 case. The resistance rates of isolates were 93.33%, 80%, 80%, 66.66%, 60%, 40% and 20% against streptomycin, isoniazid, rifampin, ethambutol, kanamycin, amikacin and ciprofloxacin, respectively.

Conclusion: In this study, some isolated mycobacteria which have been considered as potential pathogens in fish and humans were identified, such as *Mycobacterium marinum*, *M. fortuitum*, *M. smegmatis* and *M. flavescens*. All of them were found to be resistant to the most of tested drugs. It's important to be aware of the contamination of some aquarium fishes with mycobacteria species to reduce the risk of bacterial transmission to humans.

Keywords: Fish mycobacteriosis, Non-tuberculous mycobacteria, Public health

INTRODUCTION

The isolation of NTM from clinical specimens is increasing among both immunocompromised and immunocompetent and also in the elderly patients in the last years [1-3]. NTM are typically environmental microorganisms and certain species of them may cause opportunistic diseases in human with various predisposing conditions of host-related factors. Pulmonary infections related to NTM are the most frequent disease type of these bacteria, although involving other parts of the body has also been reported. Among immunocompromised patients; the rate of reported infections like in the lymph nodes, soft tissues and cutis, catheter and bone-joints have been increased [4]. The genus *Mycobacterium* contains more than 150 species. Detection of mycobacteria at species level is necessary and itself provides useful information on epidemiology and successful options for treatment [5].

Mycobacteriosis in fish is a chronic progressive disease. Some mycobacterial species that normally cause mycobacteriosis in ornamental fish are *Mycobacterium marinum* (*M. marinum*), *Mycobacterium chelonii* (*M. chelonii*), and *Mycobacterium fortuitum* (*M. fortuitum*), and several other species of mycobacteria have also been identified to be associated with this disease [6]. Diagnosis of this disease in aquarium fish is usually done by histopathology, culture, determination of bacterial growth characteristics, pigment type, colony morphology, and biochemical tests. *M. marinum* is an opportunistic pathogen causing several human infections

reported worldwide including Iran [7,8]. Non-tuberculosis mycobacteria such as *M. marinum* can be transmitted to human from contaminated ornamental fish or contaminated environment (contaminated water) during contact with the aquarium while cleaning or touching the fish through cuts or scratches on the skin [9-12]. *M. chelonii* and *M. fortuitum* are aggressive species growing fast and distributed widely in the environment (soil and water), which are the common cause of nosocomial infections that usually cause superficial lesions, possibly pulmonary disease, and primary lymphadenopathy [13]. Isolation of rapidly growing mycobacteria were frequently reported in pulmonary infections from Taiwan, China, and Singapore [14,15].

Due to the zoonotic character of fish mycobacteriosis, increasing opportunistic infections via NTM in the elderly and immunocompromised patients and on the other hand existence of difficulties in long-term treatment of NTM infections, the aim of this study was to isolate and identify the species of non-tuberculous mycobacteria from aquarium fish samples. Detection of drug resistance of isolates was done to isoniazid, ethambutol, rifampin, Streptomycin, kanamycin, ciprofloxacin, and amikacin.

MATERIALS AND METHODS

Fish samples: Fifty-three aquarium fish of eight different species including eight *Carassius auratus*, four *Xiphophorus maculatus*, four *Microgeophagus altispinosus*, four *Andinoacara rivulatus*, three

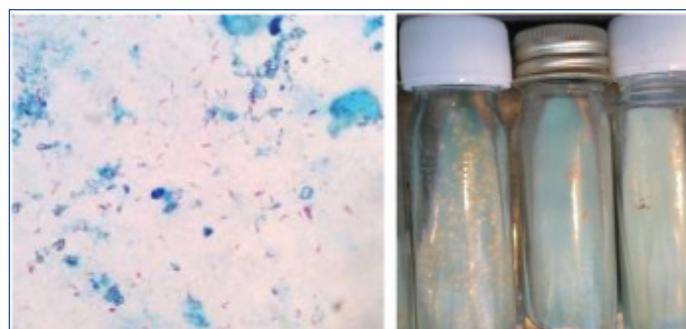
Heros severus, 10 *Puntius conchonius*, 12 *Poecilia latipinna*, and eight *Astronatus ocellatus* were randomly selected from different aquarium stores in Tabriz. In some aquarium fish, there were signs of illness such as lethargy, scale loss and skin lesions, and some of the samples had no clinical signs and appeared to be healthy. The samples were immediately transferred to the Tuberculosis and Lung Disease Research Center of Tabriz University of Medical Sciences for laboratory work.

Laboratory examination: The internal organs including liver, spleen, kidney, intestines, and different organs of the fish were separated by sterile scissors, scraped off in small sizes, and homogenized in a vortex machine for three minutes in a sterile falcon tube containing distilled water and sterile glass pearl. Then, the sample was filtered by transferring them from the current containers into the new falcon tubes by inserting the sterilized gas into the tubes, and used them for Ziehl-Neelsen staining (ZN staining) to see AFB. In each of the slides prepared, one hundred fields of vision were examined with optical microscope with magnification of 1000.

For culture examination, the homogenized samples of fish organs were also centrifuged and decontaminated by the Petroff method [16]. One volume of the sample was poured into two volumes of Sodium Hydroxide 1N solution in a falcon tube and soda 1N was added. After the samples passed through digestion and decontamination, they were placed at a room temperature for half an hour in a shaking machine to homogenize and then centrifuged at a speed of 3000 rpm for half an hour. In the stage after centrifugation of the samples, the supernatants were discarded under a ventilation hood and by adding a drop of phenolphthalein 0.01% as a colour reagent and with the help of 1N hydrochloric acid neutralizing the pH of the samples was performed (pH 6/8). Then, under the hood, 20 µl of the sediment was inoculated in Lowenstein-Jensen Culture media (each sample in two culture media) and incubated for eight weeks at 30°C and 37°C [17]. Culture media were examined daily for four weeks and then once a week for three months to observe growth and determine the growth rate of mycobacteria. Samples that were positive for development but their cultivation remained negative until the end of this period were incubated for another four weeks (for a total of 12 weeks) [17,18].

Identification of isolated mycobacterium species: Identification of isolated *mycobacterium* species was done using phenotypic characteristics (growth rate, growth at different temperatures, colony morphology, and pigment production test) and biochemical tests {niacin and urease production, heat resistant catalase test (68°C), hydrolysis between 80, 3-day arylsulfatase test, and nitrate reduction test} [18].

Evaluation of drug resistance of isolates: Drug resistance of isolated strains was studied proportionally. For this purpose, first, a dilution equal to half McFarland was prepared from suspension of isolated mycobacterium species and then a concentration of 0.01 was prepared from that, and 0.2 mL of diluted suspension was inoculated to Lowenstein-Jensen Culture media containing isoniazid 0.2 µg/mL, ethambutol 2 µg/mL, rifampin 40 µg/mL, streptomycin 4 µg/mL, kanamycin 20 µg/mL, ciprofloxacin 4 µg/mL, and amikacin 20 µg/mL antibiotics, and Lowenstein-Jensen Culture media without antibiotics (as control) and incubated for six weeks at 37°C. If the bacterial growth rate (the number of colonies) in antibiotic-containing media was less than 1% in comparison with the control culture medium, the strain was considered susceptible; and if the growth rate was equal to or greater than 1% relative to the control medium, it was considered as a resistant strain. The standard *Mycobacterium tuberculosis* strains H37Rv, which was sensitive to all the drugs used in this test, was used as control strain [19-21].



[Table/Fig-1]: ZN-stained specimen. **[Table/Fig-2]:** *Mycobacterium* sp. colonies on Lowenstein-Jensen agar. (Images from left to right)

Ethical Approval

The experimental protocol was reviewed and approved by Ethical Review Committee, Islamic Azad University of Karaj and conducted according to Institutional Animal Care and Use Committee of Iran (IACUC no.= 140426/ February 12, 2016).

RESULTS

Among fifty-three ZN-stained specimens, seven (13.2%) gave positive direct microscopic results for mycobacteria [Table/Fig-1].

Using the bacterial culture method, out of 53 samples, *Mycobacterium* spp.colonies was observed in 15 (28.3%) Lowenstein-Jensen agar tubes [Table/Fig-2].

Identification of different species of mycobacterium from 15 isolates obtained by culture based on phenotypic characteristics and biochemical tests was as follows [Table/Fig-3,4]: *M. fortuitum*, 6 cases; *M. marinum*, 3 cases; *M. smegmatis*, 3 cases; *M. terrea*, 1 case; *M. flavescens*, 1 case; and *M. asiaticum*, 1 case.

In drug sensitivity study, from among the strains isolated (14 cases) 93.33% were resistant to streptomycin (12 cases) 80% to isoniazid, (12 cases) 80% to rifampin, (10 cases) 66.66% to etambutol, (9 cases) 60% to kanamycin, (6 cases) 40% to amikacin, and (3 cases) 20% to ciprofloxacin. The results indicated that streptomycin had the highest drug resistance and the lowest drug resistance was related to ciprofloxacin.

DISCUSSION

In direct microscopic examination using ZN staining method, 13.2% of AFB were identified, and in the bacterial culture (28.3%) AFB were isolated. Although direct microscopic sensitivity is less than culture experiment, it may provide valuable information, especially when bacterial culture fails probably due to the destruction of mycobacterium species by the host defense system or very low levels of living bacteria in the tissue [6].

Mycobacteria have a global distribution and widely swarm in aquatic environments [13,22,23]. Mycobacteriosis is one of the common infections in ornamental fish reported in more than 150 species [24]. When the fish is infected with mycobacteria, it usually does not behave differently from non-infectious fish. We only suspected their being infected when their mobility weakens, the colour of the skin of the fish becomes faint, and other clinical symptoms such as the inflammation of the skin, skin lesions or wounds, exophthalmos and so on occur [25]. As various species of non-TB mycobacterium can potentially be transmitted to humans from fish, water, and their living environmental, such as aquariums and reservoirs and fish ponds and cause skin infections [9,11,13,26,27], the people working in aquarium stores and anyone who is somehow associated with ornamental fish should take precautionary and preventive steps, even when in contact with seemingly healthy fish. Drug therapy is not recommended for fish and has a limited value for the disease as treatment does not

Growth at temperatures 30°C-37°C		Colony morphology	Pigment production	Growth rate	Biochemical testes						Mycobacterium spp.
					Niacin	Nitrate Reduction	Catalase heat stable 68°C	Tween 80 Hydrolysis	Arylsulfatase (3days)	Urease	
+	+	S	N	Growth < 7 days	-	+	+	-	+	+	<i>M. fortuitum</i>
+	-	S	P	Growth > 7 days	-	-	-	+	-	+	<i>M. marinum</i>
+	+	S	N	Growth < 7 days		+	+	+	-		<i>M. smegmatis</i>
+	+	S	N	Growth > 7 days	-	+	+	+	-	-	<i>M. terrae</i>
+	+	S	Sc	Growth < 7 days	-	+	+	+	-	+	<i>M. flavescens</i>
+	+	S	P	Growth > 7 days	-	-	+	+	-	-	<i>M. asiaticum</i>

[Table/Fig-3]: Phenotype characteristics and biochemical tests

+: the isolate was positive for the test; -: a negative reaction for the test.

P= Photochromogenic; Sc= Scotochromogenic; N= Non-chromogenic; R= Rough; S= smooth

Aquarium fish species	Samples	Contaminated cases	Isolated mycobacterial species					
			Mfl	Ma	Mm	Mfo	Ms	Mt
<i>Poecilia latipinna</i>	12	5	-	+	+	++	+	-
<i>Xiphophorus maculatus</i>	4	0	-	-	-	-	-	-
<i>Carassius auratus</i>	8	3	-	-	+	+	+	-
<i>Microgeophagus altispinosus</i>	4	0	-	-	-	-	-	-
<i>Andinoacara rivulatus</i>	3	0	-	-	-	-	-	-
<i>Puntius conchoniis</i>	10	4	-	-	+	++	+	-
<i>Heros severus</i>	4	0	-	-	-	-	-	-
<i>Astronatus ocellatus</i>	8	3	+	-	-	+	-	+
Total	53	15	1	1	3	6	3	1

[Table/Fig-4]: Mycobacterial species isolated from samples

Mfl = *M. flavescens*; Ma = *M. asiaticum*; Mm = *M. marinum*;

Mfo = *M. fortuitum*; Ms = *M. smegmatis*; Mt = *M. terrae*

remove mycobacteria from the populations of infected fish [24].

Considering the development of the aquaculture industry and the increase in the importance of domestic ornamental fish, it is necessary to provide fast and accurate tools for the identification of pathogens and their treatment. Moreover, it is important for veterinarians and aquaculture specialists to be aware of clinical signs of the disease transmissible between fish and humans and ways to minimize the risk of exposure to these organisms. Among important feature of many of the mycobacterial species causing this disease are their opportunistic character and the disease being often associated with people who have a weakened immune system. Most infections occur among people having aquariums at home, or breeders and fishermen and may be a business risk for specific people at risk for this infection [24,28]. When washing of an aquarium by immunosuppressed patients, safety precautions such as wearing gloves, shaving hands and even wearing a mask and the adequate chlorination of tanks should be carefully considered [24].

Increase in reports of infections caused by opportunistic pathogens, including non-TB mycobacteria in the elderly or immunocompromised patients on the one hand, and very difficult treatment and the need for long-term treatment for these infections on the other have made this a serious issue in relation to the general health of society [3,23,29].

Mycobacterium flavescens has been isolated from pulmonary diseases, osteomyelitis, keratitis, disseminated post-injection infection and gluteal abscess [13]. *Mycobacterium smegmatis* has been known as the causative agent of skin and soft tissue

infection, pulmonary disease, endocarditis, osteomyelitis, lymphadenitis, arthritis and disseminated infections [30,31]. *M. asiaticum* has been isolated from progressive pulmonary disease and extrapulmonary disease in human such as flexor tenosynovitis, keratitis and olecranon bursitis [32].

In humans, *M. marinum* could be cause of nodular and granulomatous inflammation of the tendon sheaths of hands and fingers, subcutaneous tissues, skin and is referred to as 'fish tank granuloma', 'swimming pool granuloma', 'fish fanciers disease', 'fish handlers', or 'fish TB' [24,33,34]. *Mycobacterium fortuitum* has been diagnosed in post-injection abscess disease, sternal wound infection, traumatic osteomyelitis, mastitis, cellulitis and peritonitis [13].

The results of studying the samples showed that *M. marinum* and *M. fortuitum* were isolated more than other species (9 out of 15). Many studies have shown that these species commonly cause mycobacteriosis in ornamental fish. Three species of *M. fortuitum*, *Mycobacterium gordonii*, and *M. terrae* have been reported in relation to fish mycobacteriosis in the Republic of Kazakhstan [35]. Many of the infections caused by *M. marinum* in human have a slow and spontaneous recovery in 1 to 6 years. Small lesions may heal themselves, but in other cases, multiple antibiotic treatments are required for several months [29,36]. Antibiotic treatment may prevent the progression of deep-seated infection, but the bacteria are highly resistant to the treatment [24,27]. Antibiotic therapy for humans include isoniazid, rifampin, perazinamide, ethambutol, streptomycin, kanamycin, ciprofloxacin, azithromycin and some quinolones. Infection responds to appropriate antibiotic therapy

slowly and depending on the extent and severity of infection, the course of antibiotic treatment may vary from 2 weeks to more than 18 months [13,21,29].

Normally, the infection is not very aggressive and responds to treatment, although months of treatment may be necessary. Sometimes, the infection can spread to deeper tissues such as the bone that hardly responds to treatment [4,21]. Deep infections usually require both antibiotic and surgical treatments. Removal of necrotic tissue including joint synovial, joint tenosynovial or joint removal is essential. Moreover, amputation to control the infection despite proper antibiotic treatment and multiple removal of necrotic tissue may be required [21]. Considering the use of the drugs mentioned in the treatment and the results of investigating the susceptibility and drug resistance of isolated mycobacterium species, streptomycin had the highest drug resistance with 93.33% and the lowest drug resistance was related to amikacin (40%) and ciprofloxacin (20%). Thus, ciprofloxacin and amikacin can be used as selective therapies for treatment, but this is a serious challenge to the treatment of diseases caused by NTM species. Probably one of the factors contributing to high resistance to streptomycin is the use of this drug in the treatment of other infectious diseases [21,37].

LIMITATION

The limitation was low number of viable *Mycobacteria* in the tissue. It may be caused by killing of *Mycobacteria* through host defense mechanisms or destruction of the *Mycobacteria* during the preparation of the samples. This could lead to negative cultivation results. Additionally, biochemical examination is a time consuming method, the results are often difficult to interpret and variations among strains may occur. The reason for the difficult interpretation of the biochemical profile may lie in mixed mycobacterial cultures due to contamination with other bacteria among some samples.

CONCLUSION

In recent years, mycobacterial infections in aquarium fish have received much attention, which is due to the increasing importance of aquariums in the world and more attention to public health. This study isolated some bacteria from aquarium fish such as *M. marinum*, *M. fortuitum*, *M. smegmatis*, and *M. flavescens* known as pathogens from fish and humans. It was also indicated that isolated strains were resistant to most of the drugs used in the treatment. Given the zoonosis of the disease, the difficulty of treatment, and the need for a long-term treatment period, those who are involved with aquariums and aquaculture equipment, or even those who keep ornamental fish at home for entertainment, especially the people with immunodeficiency. More considerations are needed to prevent or reduce the transmission of disease to humans.

ACKNOWLEDGEMENTS

Hereby, the authors of the study shows their gratitude to all the colleagues in the Tuberculosis and Lung Disease Research Center of Tabriz University of Medical Sciences who immensely helped in the study.

REFERENCES

- [1] Ryu YJ, Koh W-J, Daley CL. Diagnosis and treatment of nontuberculous mycobacterial lung disease: clinicians' perspectives. *Tuberc Respir Dis (Seoul)*. 2016;79(2):74-84.
- [2] Gunaydin M, Yanik K, Eroglu C, Sanic A, Ceyhan I, Erturan Z, et al. Distribution of nontuberculous mycobacteria strains. *Ann Clin Microbiol Antimicrob*. 2013;12(1):33.
- [3] Gundavda MK, Patil HG, Agashe VM, Soman R, Rodrigues C, Deshpande RB. Nontuberculous mycobacterial infection of the musculoskeletal system in immunocompetent hosts. *Indian J Orthop*. 2017;51(2):205-12.
- [4] Tortoli E. Clinical manifestations of nontuberculous mycobacteria infections. *Clin Microbiol Infect*. 2009;15(10):906-10.
- [5] Nour-Neamatollahie A EN, Siadat SD, Vaziri F, Eslami M, Sepahi AA, Masoumi M, et al. Distribution of non-tuberculosis mycobacteria strains from suspected tuberculosis patients by heat shock protein 65 PCR-RFLP. *Saudi J Biol Sci*. 2017;24(6):1380-86.
- [6] Pate M, Jencic V, Zolnir-Dovc M, Ocepek M. Detection of mycobacteria in aquarium fish in Slovenia by culture and molecular methods. *Dis Aquat Organ*. 2005;64(1):29-35.
- [7] Alaeen A, Alaeen A, Alaeen H. A case report of fish tank granuloma in Khomein. *HBL_Journals*. 2011;14(4):113-17.
- [8] Fard SMH, Yossefi MR, Esfandiari B, Sefidgar SAA. *Mycobacterium marinum* as a cause of skin chronic granulomatous in the hand. *Babol-Caspjimm*. 2011;2(1):198-200.
- [9] Enzensberger R, Hunfeld K-P, Elshorst-Schmidt T, Böer A, Brade V. Disseminated cutaneous *Mycobacterium marinum* infection in a patient with Non-Hodgkin's Lymphoma. *Infection*. 2002;30(6):393-95.
- [10] Jernigan JA, Farr BM. Incubation period and sources of exposure for cutaneous *Mycobacterium marinum* infection: case report and review of the literature. *Clin Infect Dis*. 2000;31(2):439-43.
- [11] Lewis FM, Marsh BJ, von Reyn CF. Fish tank exposure and cutaneous infections due to *Mycobacterium marinum*: tuberculin skin testing, treatment, and prevention. *Clin Infect Dis*. 2003;37(3):390-97.
- [12] Sette CS, Wachholz PA, Masuda PY, da Costa Figueira RBF, de Oliveira Mattar FR, Ura DG. *Mycobacterium marinum* infection: a case report. *J Venom Anim Toxins Incl Trop Dis*. 2015;21(7):1678-9199.
- [13] Garcia-Agudo L, Garcia-Martos P. Clinical significance and antimicrobial susceptibility of rapidly growing *Mycobacteria*. *Science against Microbial Pathogens*. 2011;77:363-77.
- [14] Simons S, Van Ingen J, Hsueh P-R, Van Hung N, Dekhuijzen PR, Boeree MJ, et al. Nontuberculous mycobacteria in respiratory tract infections, eastern Asia. *Emerg Infect Dis*. 2011;17(3):343-49.
- [15] Lim JM, Kim JH, Yang HJ. Management of infections with rapidly growing *Mycobacteria* after unexpected complications of skin and subcutaneous surgical procedures. *Archives of Plastic Surgery*. 2012;39(1):18-24.
- [16] Petroff S. A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. *J Exp Med*. 1915;21(1):38-42.
- [17] Kane AS, Stine CB, Hungerford L, Matsche M, Driscoll C, Baya AM. *Mycobacteria* as environmental portent in Chesapeake Bay fish species. *Emerg Infect Dis*. 2007;13(2):329-31.
- [18] Moghim S, Sarikhani E, Esfahani BN, Faghri J. Identification of nontuberculous mycobacteria species isolated from water samples using phenotypic and molecular methods and determination of their antibiotic resistance patterns by E-test method, in Isfahan, Iran. *Iran J Basic Med Sci*. 2012;15(5):1076.
- [19] Chen CC, Chen SY, Chen YS, Lo CY, Cheng PW. *Mycobacterium fortuitum*-induced persistent parotitis: Successful therapy with clarithromycin and ciprofloxacin. *Head Neck*. 2007;29(11):1061-64.
- [20] Gayathri R, Therese KL, Deepa P, Mangai S, Madhavan H. Antibiotic susceptibility pattern of rapidly growing mycobacteria. *J Postgrad Med*. 2010;56(2):76-78.
- [21] Rallis E, Koumantaki-Mathiodaki E. Treatment of *Mycobacterium marinum* cutaneous infections. *Expert Opin Pharmacoth*. 2007;8(17):2965-78.
- [22] Bi S, Hu F-S, Yu H-Y, Xu K-J, Zheng B-W, Ji Z-K, et al. Nontuberculous mycobacterial osteomyelitis. *Infect Dis (Lond)*. 2015;47(10):673-85.
- [23] Xu D, Han C, Wang M-S, Wang J-L. Increasing prevalence of non-tuberculous mycobacterial infection from 2004-2009 to 2012-2017: a laboratory-based surveillance in China. *Journal of Infection*. 2017;75(6):473-598.
- [24] Haenen O, Evans J, Berthe F. Bacterial infections from aquatic species: potential for and prevention of contact zoonoses. *Rev Sci Tech*. 2013;32(2):497-507.
- [25] Shukla S, Sharma R, Shukla SK. Detection and identification of globally distributed mycobacterial fish pathogens in some ornamental fish in India. *Folia Microbiol (Praha)*. 2013;58(5):429-36.
- [26] Lowry T, Smith SA. Aquatic zoonoses associated with food, bait, ornamental, and tropical fish. *J Am Vet Med Assoc*. 2007;231(6):876-80.
- [27] Pandian T, Deziel P, Otlej C, Eid A, Razonable RR. *Mycobacterium marinum* infections in transplant recipients: case report and review of the literature. *Transpl Infect Dis*. 2008;10(5):358-63.
- [28] Aubry A, Chosidow O, Caumes E, Robert J, Cambau E. Sixty-three cases of *Mycobacterium marinum* infection: clinical features, treatment, and antibiotic susceptibility of causative isolates. *Arch Intern Med*. 2002;162(15):1746-52.
- [29] Kankya C, Muwonge A, Djonne B, Munyeme M, Opuda-Asibo J, Skjerve E, et al. Isolations of non-tuberculous mycobacteria from pastoral ecosystems of Uganda: public health significance. *BMC Public Health*. 2011;11(1):320.
- [30] Bohsali A, Abdalla H, Velmurugan K, Briken V. The non-pathogenic mycobacteria *M. smegmatis* and *M. fortuitum* induce rapid host cell apoptosis via a caspase-3 and TNF dependent pathway. *BMC Microbiol*. 2010;10(1):237.
- [31] Best CA, Best TJ. *Mycobacterium smegmatis* infection of the hand. *Hand (N Y)*. 2009;4(2):165-66.

- [32] Grech M, Carter R, Thomson R. Clinical significance of *Mycobacterium asiaticum* isolates in Queensland, Australia. *J Clin Microbiol*. 2010;48(1):162-67.
- [33] Atkins BL, Gottlieb T. Skin and soft tissue infections caused by nontuberculous mycobacteria. *Curr Opin Infect Dis*. 2014;27(2):137-45.
- [34] Afzal A, Nadeem M, Aman S, Kazmi AH. *Mycobacterium marinum* infection: A case report. *Journal of Pakistan Association of Dermatology*. 2016;19(1):48-51.
- [35] Řehulka J, Kaustová J, Řehulková E. Causal agents of mycobacterial diseases in freshwater ornamental fish and their importance for human health in the Czech Republic. *Acta Veterinaria Brno*. 2006;75(2):251-58.
- [36] Velu PP, Fernandes SE, Laurenson IF, Noble DD. Pulmonary *Mycobacterium marinum* infection: 'fish tank granuloma' of the lung. *Scott Med J*. 2016;61(4):203-06.
- [37] Wang H-x, Yue J, Han M, Yang J-h, Gao R-l, Jing L-j, et al. Nontuberculous mycobacteria: susceptibility pattern and prevalence rate in Shanghai from 2005 to 2008. *Chin Med J (Engl)*. 2010;123(2):184-87.

PARTICULARS OF CONTRIBUTORS:

1. Student, Department of Microbiology, College of Basic Sciences, Karaj Branch, Islamic Azad University, Karaj, Alborz, Iran.
2. Associate Professor, Department of Laboratory Sciences, Paramedical Faculty and Tuberculosis and Lung Disease Research Center, Tabriz University of Medical, Tabriz, East Azerbaijan, Iran.
3. Associate Professor, Department of Microbiology, College of Basic Sciences, Karaj Branch, Islamic Azad University, Karaj, Alborz, Iran.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Seyyed Reza Moaddab,
Associate Professor, Department of Laboratory Sciences, Paramedical Faculty and Tuberculosis and Lung Disease Research Center, Tabriz University of Medical, Tabriz, East Azerbaijan, Iran.
E-mail: seyedreza_moaddab@yahoo.com

Date of Submission: **Mar 28, 2018**Date of Peer Review: **Apr 25, 2018**Date of Acceptance: **May 18, 2018**Date of Publishing: **Sep 01, 2018****FINANCIAL OR OTHER COMPETING INTERESTS:** None.