Short Communication

Identification of mycobacteria from unhealthy and apparently healthy aquarium fish using both conventional and PCR analyses of *hsp65* gene

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Abstract

Seventy-eight unhealthy and forty-two apparently healthy looking freshwater aquarium fish belonging to twenty-six different species were collected from local aquarium stores in different cities and were investigated for the presence of mycobacteria in north-western Iran by culture and molecular methods. Using the culture method, *Mycobacterium* sp. was isolated from 31.6% of the fish (38 cases), while it was obtained in 30.8% (37 cases) by polymerase chain reaction (PCR) assay for a 439 bp fragment of the heat shock protein 65kD gene (*hsp65*). Acid-fast bacilli (AFB) were seen on the smears of 27 samples (22.5%) by direct microscopic examination method of Ziehl-Neelsen (ZN, for staining *Mycobacterium* spp. isolates). Based on all three methods, 38 purified bacterial isolates, which are well-known pathogens in humans as well as in fish, were identified: nine isolates of *M. fortuitum*, nine *M. marinum*, seven *M. smegmatis*, four *M. terrea*, four *M. flavescens*, three *M. gordonae* and two isolates of *M. asiaticum*. *Mycobacterium marinum*, *M. fortuitum*, *M. smegmatis* and *M. flavescens* were common in both diseased and healthy fish groups. In conclusion, considering diagnostic challenge for both culture and molecular methods of bacterial identification and their importance, especially in apparently healthy looking freshwater aquarium fish, the results of this study demonstrates that, in terms of reliable sensitivity, conventional microbial identification is superior to molecular techniques.

Keywords: mycobacteria, aquarium fish, PCR, zoonosis

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Introduction

Mycobacterium, an aquatic bacterium, causes tuberculosis, an important disease of humans and mammals, while fish do not get tuberculosis but can be long-term carriers of the bacterium. Fish mycobacterial diseases are one of the common infections of freshwater aquarium fish that have been reported in more than 150 species (Pate et al., 2005). Some of the most common fish pathogens that cause mycobacteriosis with a chronic progressive character are *Mycobacterium fortuitum*, *M. chelonae* and *M. marinum*, and many other species have been found in relation to this disease (Pourahmad et al., 2009). The lack of effective treatment and short courses of therapy against these opportunistic fish pathogens and the causative diseases in patients (Daiet al., 2011) plus the economic losses of these infections include decreased marketability and losses in the aquaculture industry due to effects of subclinical infection such as decreased feed efficiency, decreased growth rates and mortality (Zanoni et al., 2008), highlighting the need for exact and rapid identification of mycobacteria.

Fish with mycobacteriosis or tuberculosis normally does not behave differently from uninfected fish until its movements become sluggish and fish colouration fades; additional clinical signs such as skin inflammation, open or epithelial lesions and ulcerations, exophthalmia, and signs of emaciation may also occur (Toranzo et al., 2005, Shukla et al., 2013; Salawudeen et al., 2017). Granulomas or tubercles may appear in all internal organs and commonly on the kidney, liver and spleen (Gauthier and Rhodes, 2009).

Several species of mycobacteria can also cause disseminated and localized infections in man under certain circumstances. It can be transmitted from swimming pools or aquaria, becoming pathogenic (transmissible dermal infections); this condition became known as 'fish tank granuloma' or 'swimming-pool granuloma' in human, which is hard to diagnose and treat (Jernigan and Farr, 2000; Enzensberger et al., 2002; Lewis et al., 2003, Beran et al., 2006; LeBlanc et al., 2012).

Several cases of *M. marinum* infection in human have been reported in Iran (Alaeen et al., 2011; Hosseini et al., 2011). *Mycobacterium marinum* is an opportunistic-cutaneous infection in human hands. It presents itself as a red-to-violaceous plaque or as abscesses or inflammatory nodules with an overlying crust or verrucous surface, and usually in a skin sporotrichotic type of distribution (Jernigan and Farr, 2000; Rallis and Koumantaki-Mathioudaki, 2007). *Mycobacterium chelonae* and *M. fortuitum* are the rapid-growing mycobacterial (RGM) species, widely distributed in the environment (soil and water) and common as agents of nosocomial infection that usually cause superficial lesions and probably lung disease and initial lymphadenopathy, but patients with cervical lymph node disorders and pulmonary disease may also be asymptomatic (Woods et al., 2000; Mainous and Smith, 2005; Watral and Kent Michael, 2007; Kent Michael et al., 2016).

The detection of mycobacterial species in ornamental fish is conventionally based on culture and histopathological methods including growth characteristics, pigmentation and photo reactivity, colonial morphology and bio-chemical reactions, etc. Because there is a demand for rapid and facile detection and identification of mycobacterial species and non-Tuberculous mycobacteria, molecular-based methods such as PCR of the 439 bp of heat shock protein 65kD gene (*hsp65* is present in all mycobacterial species) (Schinnick et al., 1987) or PCR of the 441 bp of *hsp65* followed by restriction fragment pattern analysis by BstEII and HaeIII enzymes proposed by Telenti et al. (1993) have also been developed (Moghim et al., 2012; Shukla et al., 2013).

Mycobacteriosis as a common systemic disease was reported by many studies (Kent Michael, 2007; Marzouk et al., 2009; Novotny et al., 2010; Peterson et al., 2013; Watral and Kent Michael et al., 2016) on different aquarium fish species such as Carassius sp., Danio sp., Colisa sp., and Trichogaster sp., etc. However, few studies have been conducted of the incidence of mycobacteria in apparently healthy fish populations (Beran et al., 2006; Mrlik et al., 2012; Shukla et al., 2013).

The purpose of the present study was, therefore, to identify the species of mycobacteria isolated from 120 (26 species) apparently healthy and unhealthy aquarium fish in Iran by molecular method compared to conventional (culture) identification test in order to check the reliability of the detection method for routine clinical use.

Materials and Methods

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.= 120416/ November 03, 2015).

Fish samples: One hundred and twenty aquarium fish of 26 species were collected from local ornamental fish stores of Maragheh, Bonab, Malekan, Ajabshir and Tabriz cities in Iran from March through September 2016. The number of fish and the species studied were: 5 black molly (*Poecilia sphenop*), 3 clown loach (*Botia macracantha*), 8 goldfish (*Carassius auratus auratus*), 6 freshwater angel fish (*Pterophyllum scalare*), 4 three-spot gourami (*Trichogaster trichopterus*), 7 guppy (*Poecilia reticulata*), 4 dwarf gourami (*Colisa lalia*), 4 southern platy fish (*Xiphophorus maculatus*), 10 rosy barb (*Puntius conchonius*), 4 siamese fighting fish (*Betta splendens*), 3 fire mouth cichlid (*Thorichthys meeki*), 8 oscar (*Astronatus ocellatus*), 4 variable platy (*Xiphophorus variatus*), 12 sailfin molly (*Poecilia latipinna*), 2 green neon tetra (*Paracheirodon simulans*), 3 green flower (*Flower Horn Fish*), 2 neon tetra jumbo (*Paracheirodon innesi*), 4 cichlid fish (*Microgeophagus altispinosus*), 4 severum (*Heros severus*), 3 black widow tetra (*Gymnocorymbus ternetzi*), 3 catfish (*Corydoras* spp.), 3 green terror (*Andinoacara rivulatus*), 3 royal red discus (*Symphysodon* spp.), 5 swordtail (*Xiphophorus helleri*), 3 zebra danio (*Danio rerio*) and 3 bala sharks (*Balantiocheilus melanopterus*).

Of the 120 freshwater aquarium fish samples investigated, 42 samples did not show any symptoms

of infection while 78 cases were unhealthy with symptoms of listlessness and anorexia, epithelial lesions, blindness, malformation of gills and distended abdomen. To avoid any epidemiological consideration, only one live ornamental fish from each aquarium was considered and transported to the laboratory where they were then immersed in clove lube (50 µL/L) and examined immediately.

Biochemical and physical examination of specimens for mycobacteria: The fish samples were dismembered with sterile gadgets. Internal organs including kidney, liver, spleen, intestine, heart, brain, eye and ovary plus whole fish except the organs were placed separately in sterile bags and homogenized with a vortex mixer. Then, smears made from the homogenized specimens were stained by Ziehl-Neelsen (Z-N) in order to identify the acid fast rods.

For culture examination, the homogenized samples of fish organs were centrifuged and decontaminated by the Petroff method 1 (Petroff, 1915). First, the suspension (1 ml) was treated with sodium hydroxide (NaOH at 0.5 N concentration) for 20 min at room temperature with intermittent agitation and then neutralized with 1 N hydrochloric acid (HCl). The suspension was then centrifuged at 2,500 × **g** for 20 min to pellet the bacilli and then resuspended in sterile saline at a concentration of 10^7 bacilli/ml. From the sediment, Löwenstein-Jensen medium was inoculated and the culture slants were incubated at 30°C and 37°C at room temperature (Kane et al., 2007). The obtained isolates were subjected to biochemical and physical examination of specimens for mycobacteria via evaluation of the rate of growth at different temperatures, pigmentation and colony morphology. Biochemical tests included growth on agar and the following enzymatic activities: arylsulphatase activity (3 days), niacin and urease production, nitrate reductase, pyrazinamidase, semi quantitative and heat stable catalase 68°C and tween 80 hydrolysis.

PCR-based detection of mycobacteria: The procedure was divided into 3 steps: 1) DNA isolation from cultured material, 2) amplification by PCR and 3) detection of PCR product by gel electrophoresis.

DNA isolation from cultured material: DNA was extracted from the homogenized and decontaminated samples. According to the protocol of genomic DNA extraction kit, 200 µL of each sample was placed in 1.5-mL microcentrifuge tubes and purified with 20 µL of proteinase K treatment. Then, 200 µL of binding buffer was added and the mixture was incubated at 60°C for 10 min, followed by addition of 100 µL isopropanol and centrifugation for 1 min at 6000 × *g*. Binding columns were moved into new 2-mL microcentrifuge tubes and washed twice with 500 µL of washing buffer by centrifugation for 1 min at 6000 × *g*. Ethanol was removed by centrifugation for 1 min at 13400 × *g*. Thereafter, the binding columns were transferred into new 1.5-mL microcentrifuge tubes, followed by addition of elution buffer. After 5 min at room temperature centrifugation was carried out for 1 min at 6000 × *g*.

Amplification by PCR: The gene encoding the 65-kDa surface antigen of mycobacteria (heat shock protein) was chosen as the target for the diagnostic PCR. For the amplification of a 439 bp mycobacterial DNA fragment of the *hsp 65* gene area, 5 µL of the template DNA was added to each reaction tube (Figure 1). The PCR blend consisted of 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 10% glycerol, 200 µM (each) deoxynucleotide, 0.5 µM of each primers and 1.25 U Taq DNA polymerase. The amplification reaction was subjected to 45 cycles (1 min at 94°C, 1 min at 60°C, 1 min at 72°C), followed by 10 min of extension at 72°C. Primers (Tb11 5´-ACCAACGATGGTGTGTCCAT and Tb12 5´-CTTGTCGAACCGCATACCCT) amplified a 439 bp segment between positions 398 and 836 of the published gene sequence (Schinnick et al., 1987).

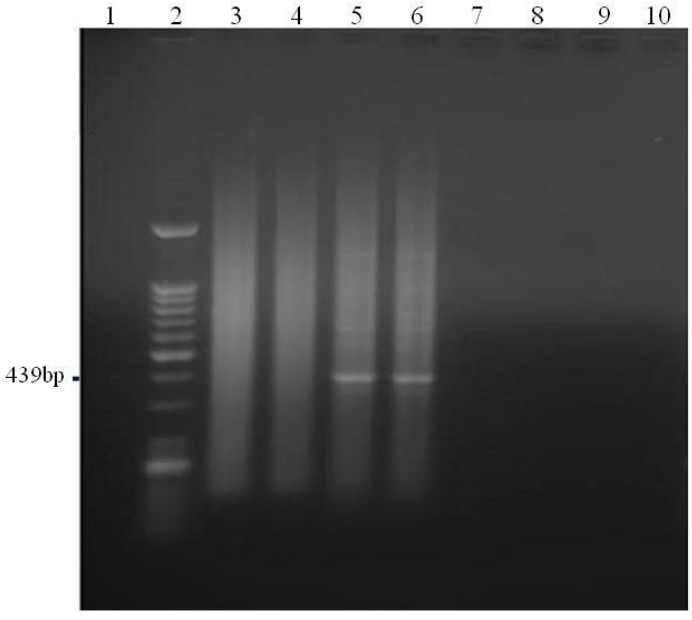

Figure 1 Detection of mycobacterial PCR products. Column 2: 439 bp of DNA molecular weight marker; Column 3: Negative control; Column 6: Positive controls consisting of DNA extracted from BCG vaccine.

Detection of PCR product by gel electrophoresis: To detect the amplified products, 2.5 µl of the reaction mixture was separated electrophoretically on agarose gels (2%). After gel electrophoresis and staining with ethidium bromide, DNA bands were visualized under ultraviolet light. Each PCR run included negative and positive controls. The negative controls contained the reaction mixture without DNA, which was replaced with equivalent volume of sterile bi-distilled water. The positive controls consisted of total DNA extracted from the BCG vaccine.

Results and Discussion

Using the culture method, 38 (31.6%) mycobacterial isolates were obtained from the 120 investigated samples (78 unhealthy and 42 apparently healthy fish) as shown in Tables 1 and 2. However, *Mycobacterium* spp. was isolated from 37 cases (30.8%) by the PCR assay (Table 3). According to these comparison results, 26 out of 78 unhealthy cases and 12 and 11 out of 42 apparently healthy samples were positive in both culture and molecular methods, respectively. In addition, 19 smears out of 78 ZN-stained specimens of the unhealthy group and 8 smears out of 42 ZN-stained specimens of the healthy group gave positive microscopic results for mycobacteria.

Identification of the species on the basis of physical (rate of growth) and biochemical examination was successfully accomplished for 38 (31.6%) isolates: 9 were identified as *Mycobacterium fortuitum*, 9 as *M. marinum*, 7 as *M. smegmatis*, 4 as *M. terrea*, 4 as *M. flavescens*, 3 as *M. gordonae* and 2 as *M. asiaticum* (Table 1). A mixed infection of more species of mycobacteria was also found in tow fish. *M fortuitum*, *M smegmatis*, *M marinum* and *M. flavescens* were identified from the apparently healthy samples.

Table 1 *Mycobacterium* spp. isolated from freshwater aquarium fish based on culture method

Fish species	No. fish	No. pos	Apparently healthy samples							Unhealthy samples						
			Mfl	Ma	Mm	Mfo	Ms	Mt	Mg	Mfl	Ma	Mm	Mfo	Ms	Mt	Mg
Poecilia sphenop	5	3	-	-	-	+	-	-	-	-	-	+	-	+	-	-
Botia macracantha	3	1	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Carassius auratus auratus	8	3	-	-	+	-	+	-	-	-	-	-	+	-	-	-
Pterophyllum scalare	6	2	-	+	-	-	-	+	-	-	-	-	-	-	-	-
Trichogaster trichopterus	4	1	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Poecilia reticulata	7	4	-	-	-	+	-	-	+	-	-	-	+	+	-	-
Colisa lalia	4	2	-	-	+	-	-	-	-	-	-	+	-	-	-	-
Xiphophorus maculatus	4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Puntius conchonius	10	4	-	-	+	+	+	-	-	-	-	-	+	-	-	-
Betta splendens	4	1	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Thorichthys meeki	3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Astronatus ocellatus	8	3	-	-	-	+	-	+	-	+	-	-	-	-	-	-
Xiphophorus variatus	4	2	-	-	+	-	-	-	+	-	-	-	-	-	-	-
Poecilia latipinna	12	5	-	+	-	+	+	-	-	-	-	+	+	-	-	-
Paracheirodon simulans	2	1	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Heros severus	3	1	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Paracheirodon innesi	2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Microgeophagus altispinosus	4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Heros severus	4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gymnocorymbus ternetzi	3	2	-	-	-	-	-	-	+	-	-	-	-	+	-	-
Corydoras spp.	3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Andinoacara rivulatus	3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Symphysodon spp.	3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xiphophorus helleri	5	2	-	-	+	-	-	-	-	-	-	+	-	-	-	-
Danio rerio	3	1	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Balantiocheilus melanopterus	3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	120	38	3	2	5	5	4	4	3	1	0	4	4	3	0	0

Mfo = *M. fortuitum*; Ms = *M. smegmatis*; Mm = *M. marinum*; Mt = *M. terrae*; Ma = *M. asiaticum*; Mg = *M. gordonae*; Mfl = *M. flavescens*

Table 2 Biochemical profiles of 38 mycobacterial isolates from apparently healthy and unhealthy freshwater aquarium fish in Iran from March 2016 to September 2016

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

+: The isolate was positive for the test; -: A negative reaction for the test. P = Photochromogenic; Sc = Scotochromogenic; N = Non-chromogenic; R = Rough; S = Smooth

Table 3 Comparison results

Fish populations	Apparently healthy samples (N=42)		Unhealthy samples (N=78)	
Detection method	Positive cases	Negative cases	Positive cases	Negative cases
Ziehl-Neelsen staining	8	34	11	67
Culture	12	30	26	52
PCR	11	31	26	52

Due to the importance of this zoonotic disease in terms of public health, in our study the identification of mycobacteria from 26 species of healthy and unhealthy fish was performed based on three detection techniques: 1) Ziehl-Neelsen staining (traditional), 2) culture, and 3) PCR.

According to the results, Ziehl-Neelsen staining method showed low acid-fast bacilli (AFB) detection (27 specimens, 22.5%) compared to the culture examination results (38 isolates, 31.6%). Our results are in agreement with those reported by Ang et al. (2000) and Pate et al. (2005).

The AFB smear stain and culture are two separate tests always performed together in public health laboratories. The Ziehl-Neelsen stain, known as AFB stain, is an old detection method of mycobacteria in tissues. Generally, the causes of failure to identify AFB through microscopic examination may include destruction of mycobacteria or lack of AFB through direct microscopy even though granulomatous lesions are present. Also, mixed mycobacterial cultures can be contaminated with other bacteria, and this can make it difficult to interpret the biochemical profile (Moghim et al., 2012; Shukla et al., 2013). Therefore, microscopic examination is less sensitive than culture examination. However, as an auxiliary diagnostic method, it may provide valuable information, particularly when the bacterial culture is negative probably due to the low number of viable *Mycobacterium* in tissues or in cases where *Mycobacterium* is probably killed by the host defense system (Lescenko et al., 2005).

Overall, molecular methods are being used increasingly in mycobacterial identification, replacing the time-consuming cultural and biochemical testing, although some studies indicated that the sensitivity of molecular method was less than that of the culture method (Pate et al., 2005). Our study failed to detect *Mycobacterium* in one case by molecular methods and mycobacterial infection in 37 out of 120 samples was detected by PCR, while it was obtained in 38 cases (31.6%) using the culture method.

Although conventional method is slow and limits reliable information for identification of mycobacteria (Ong et al., 2010; Shukla et al., 2013), this method is still considered as the gold standard technique for detection of mycobacterial infections because it is simple, well-established, accurate, relatively inexpensive, and highly sensitive in mycobacterial lineages and treatment outcomes,.

On the other hand, the interpretation of results by biochemical tests is often difficult and variations between strains may occur. There are very small differences in the biochemical profile of *Mycobacterium* species causing infection in fish (Pate et al., 2005; Moghim et al., 2012; Shukla et al., 2013). Some species, such as *M. chelonae* and *M. fortuitum* or *M. gordonae* and *M. Marinum*, have most physical properties similar, causing difference in one or two tests. *Mycobacterium smegmatis*, *M. flavescens*, *M. gordonae*, *M. terrea*, *M. asiaticum*, *M. marinum* and *M. fortuitum* were the species isolated from the unhealthy fish samples, and *M. marinum* and *M. fortuitum* were the species most identified (15 out of 33). These findings suggest that the mentioned species commonly cause mycobacteriosis in ornamental fish. Many studies revealed that common isolates that infect ornamental fish were either of *M. marinum* or *M. fortuitum* species (Lewis et al., 2003; Mainous and Smith, 2005; Zanoni et al., 2008; Marzouk et al., 2009).

In our study, *Mycobacterium fortuitum*, *M. smegmatis*, *M. marinum* and *M. flavescens* were identified from the apparently healthy samples. Few studies examined the presence of *Mycobacterium* spp. in clinically healthy ornamental fish. Three species, namely *M. fortuitum*, *M. gordonae* and *M. terrae*, were reported as the causative agent of fish mycobacteriosis in Czech Republic (Beran et al., 2006).

In conclusion, fish mycobacteriosis has a global distribution and the organism has widely occupied aquatic environments. Non-tuberculous mycobacteria such as *Mycobacterium marinum* are transmitted from infected ornamental fish or contaminated water when cleaning or handling the fish.

As a serious matter of public health, opportunistic infections via non-tuberculous mycobacteria in the elderly or immunocompromised patients are on the rise especially in Iran because the infections are so difficult to treat and require long courses of therapy with or without adjunctive surgical intervention. Most common among these clinical syndromes are soft tissue and osteoarticular infections, superficial lymphadenitis, chronic pulmonary infections, and disseminated disease.

In this study, some bacterial isolates such as *Mycobacterium marinum*, *M. fortuitum*, *M. smegmatis* and *M. flavescens*, which are well-known pathogens in fish and humans, were common in both unhealthy and healthy fish. Hence, considering the importance of exact bacterial identification, especially in apparently healthy looking aquarium fish, the results of this study demonstrates that, in terms of reliable sensitivity, conventional microbial identification is superior to molecular techniques, also relatively cheap, easy to perform and quick to detect different mycobacterial species.

The present study detected *M. marinum* and *M. fortuitum*, the most important and most frequently found pathogens, in apparently healthy fish. Therefore, more attention should be paid to the presence of mycobacteria in aquarium fish in order to reduce the rate of bacterial transmission to human using rapid and exact detection tools and techniques of bacterial identification.

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บทคัดย่อ

การตรวจพิสูจน์เชื้อ mycobacteria จากปลาป่วยและปลาปกติ โดยวิธีเพาะเลี้ยงเชื้อและวิธี PCR สำหรับยีน *hsp65*

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การศึกษาเพื่อตรวจหาเชื้อ Mycobacteria โดยเก็บตัวอย่างปลา 26 ชนิด จำแนกเป็นปลาป่วยจำนวน 78 ตัวอย่างและปลาปกติจำนวน 42 ตัวอย่าง จากสัตว์น้ำในหลายเมืองในเขตตะวันตกเฉียงเหนือ ของประเทศอิหร่าน ผลการศึกษาพบว่า วิธีการเพาะเลี้ยงเชื้อ สามารถเพาะเลี้ยงเชื้อ *Mycobacterium* sp. ได้ 31.6% (38 ตัวอย่าง) และวิธี PCR ด้วยการตรวจหายีน heat shock protein 65kD ให้ผลบวกคิดเป็น 30.8% (37 ตัวอย่าง) ผลการตรวจด้วยวิธีย้อมสี Ziehl-Neelsen เพื่อหาเชื้อ Acid-fast (AFB) ให้ผลบวก 22.5% (27 ตัวอย่าง) จากผลการตรวจหาเชื้อทั้ง 3 วิธี สามารถแยกเชื้อได้ 38 ตัวอย่าง ซึ่งเป็นเชื้อ *Mycobacteria* ที่สามารถก่อโรคในคนและปลา ได้แก่ *M. fortuitum* (9 ตัวอย่าง) *M. marinum* (9 ตัวอย่าง) *M. smegmatis* (7 ตัวอย่าง) *M. terrea* (4 ตัวอย่าง) *M. flavescens* (4 ตัวอย่าง) *M. gordonae* (3 ตัวอย่าง) และ *M. asiaticum* (2 ตัวอย่าง) โดยเชื้อ *Mycobacterium marinum, M. fortuitum, M. smegmatis* และ *M. flavescens* เป็นเชื้อที่พบได้บ่อยในปลาปกติและปลาป่วย โดยสรุปการวินิจฉัยเชื้อในปลาด้วยวิธีการเพาะเลี้ยงเชื้อและวิธีตรวจหาเชื้อในระดับโมเลกุลบ่งชี้ว่าความไวและความน่าเชื่อถือ ของวิธีการเพาะเลี้ยงเชื้อสูงกว่าวิธีตรวจหาเชื้อในระดับโมเลกุล

คำสำคัญ: mycobacteria ตู้ปลา PCR โรคสัตว์สู่คน

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