The Third International Conference for Postgraduate Scientific Research, December 15, 2020
College of Veterinary Medicine, University of Baghdad

THE IRAQI JOURNAL OF
VETERINARY MEDICINE

eISSN:2410-7409
pISSN: 1609-5693

OPEN ACCESS

Isolation and Molecular Identification of Nontuberculous *Mycobacterium* from Different Species of Fish in Karbala Province, Iraq

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Received: 10 March 2020
Accepted: 27 October 2020
Published: 28 December 2020

DOI:
https://doi.org/10.30539/ijvm.v44i(E0).1026

Cite:
Al-Mashhadani AAB, Al-Gburi NM. Isolation and molecular identification of nontuberculous *Mycobacterium* from different species of fish in Karbala province, Iraq. Iraqi J. Vet. Med. 28 Dec. 2020; 44(0):80-87.

A B S T R A C T

This study was designed for isolation and molecular identification of Nontuberculous *Mycobacterium (*NTM) from fish during the period between October and December 2017 from Karbla province, Iraq. This study included 200 fresh fish samples from four different species including *Spondyliosoma cantharus*, *Liza abu*, *Carassius carassius* and *Cyprinuscarpio*. Three samples of each fish were taken including gills, muscles and all internal organs. The samples were processed by decontamination, concentration of 4% sodium hydroxide, and 0.1 ml of sediment was streaking on Löwenstein Johnson (LJ) media; then the bacterial cultures were incubated at 28-30 °C for 3days up to 4 weeks and suspected colonies were stained with acid fast stain to confirm the presence of *Mycobacterium*. Further identification, biochemical tests were carried out to confirm the diagnosis of isolates, PCR was done using *16s RNA* gene for all isolates, *hsp65* gene was used in unidentified NTM spp and to confirm the others. Results revealed that out of 200 fish samples, 19 isolates 9.5% were identified as NTM belonged to Rapid Growth *Mycobacterium* (RGM). of the total isolates, 18.26 % was investigated from Liza *abu* (Kishni, Abu khraiza). NTM (RGM) isolates on spp level identified six spp of these isolates. *M. porcinum* was 26.32% which was followed by *M. fortuitum* of 21.05%, others included *M. neworleansense* and *M. mucogenicum* 10.5% of each, *M. cosmeticum* and *M. pallens* 5.26% of each. The distribution of NTM spp in the fish organs, nine out of 19 (47.37%) NTM isolate were recovered from gills followed by muscles 36.84 %, while 15.79% from internal organs. These results were the first study concerning isolation of these spp of NTM from fish in Iraq, and some spp are not reported in other studies. This study concluded that the fish is an importance source or reservoir for NTM, especially the pathogenic spp.

Keywords: NTM, fish, *Liza abu,* RGM, Iraq

INTRODUCTION

Nontuberculous mycobacteria (NTM) infections (mycobacteriosis) affect many aquatic or marine animals (fish, tropical aquarium fish, amphibians, and reptiles) causing chronic diseases, and are considered as a major cause of morbidity and mortality in free-living fish; and infections may lead to production and economic losses. Different clinical signs of mycobacteriosis in fish may be existed depending on the main sites of the infection and its severity or no clinical signs may associate, after invading the mycobacterial organisms into the body and spreading throughout the body by the circulatory or lymphatic system (1). The average of the incubation period of it is about 3 months and the form of the disease is either acute or chronic. In acute form, the infection is characterized by

heavy growth of the organism causing death of animals within 16 days (2-4). The chronic form, which is most commonly, is characterized by developing of granuloma in internal organs and animals may survive for maximum of 4 to 8 weeks. Signs may be seen with this disease including exophthalmos (bulging eyes), cachexia, pigmentation in fish body, ulcerative dermal necrosis, skeletal changes, swollen and distended abdomen, ulcers and eroded fins and tail rot, heavy mucus coating on the body surface, loss of scales, loss of appetite, weight loss, non-healing open ulcers, skeletal deformities such as curvature spinal or stunting defect pale gills lethargic. They may detach from other fishes and aggregate in corner of the holding facility (5). Nodular lesions on skin, miliary tubercles occurs in the liver, spleen and kidney which is histologically corresponding to granuloma; in addition to the fact that an affected fish may die, the mortality may reach up to 50% with no clinical signs (5, 6).

Some NTM pathogens that infect fish are highly virulent and are pathogenic to human; so, it may be considered as a zoonotic in a public health concern and its spread from fish, birds, and animals to human causing epidemics in nature (7-13). Conventional biochemical tests are not a significant method for NTM identification at the species level in addition, it waste of time (14). The sequencing of the *16S rRNA* gene is universally the gold standard method, especially, for significant and rapid identification of NTM for the correct epidemiological control study and treatments, sequencing of the *hsp65* gene as the second best alternatives (15-17). This study aimed to isolate and identification of NTM spp. from fish.

MATERIALS AND METHODS

Sample Collection

A total of two hundred fresh fish belonged to four fish species were collected from various fish selling markets at Karbala province, Iraq from October to December 2017 from Krabla city. Fish species included were *Spondyliosoma cantharus* (n=35), *Liza abu* (n=104), *Carassius carassius* (n=46), and *Cyprinus carpio* (n=15). Fish were autopsied in a biosafety cabinet; an individual set of sterile instruments was used for each animal, 70% ethanol was applied as anticepsis to the body of the fish. Three samples were taken from each fish included all internal organs (O), Gills (G), and Muscles (M) were removed aseptically, and each part was cut into small pieces then transmitted to sterile porcelain mortar containing sterile normal saline (0.85%) have been mashed, then the supernatant transferred into sterile container (18).

Cultivation of Samples and Identification of NTM

After digestion and decontamination of samples according to (19-21) methods, a loop full of sediment was inoculated to the LJ medium (Hi media, India) and blood agar (Hi media, India) then incubated at 28-30 °C, and the growth observed within 3 days-4 weeks. Ziehl-Neelsen stain (Syrbio, Swiss) was done before and after culturing the samples; and the isolates were identified depending on colonies features, pigmentation production, ability of growth on MacConkey agar without crystal violet (Hi media, India), in addition to, biochemical tests which included catalase production test (room temperature, semiquantitative catalase), Nitrate reductions, Simmons citrate utilizing, NaCl tolerance, urea hydrolysis (urease) and arylsulfatase (at three and fourteen days).

Polymerase chain reaction (PCR) was performed after genomic DNA of the bacteria was extracted; and conventional PCR was done to detect two genes in clinical isolates of NTM including: universal primers*16S rRNA* gene 1400pb for all isolates by using Forward primer-27AGAGTTTGATCTTGGCTCAG, reverse primer -1492 TACGGTTACCTTGTTACGACTT and *hsp65* gene 249pb was used for unidentified *Mycobacterium* spp.; then to confirm the others, Forward primer-GCCAAGAAGACCGAYGACGT and Reverse primer – GGTGATGACGCCCTCGTTGC were used.

The PCR reaction mixture contained 25 µL volume as follows: 12.5 µL of PCR premix, 1 µL of each forwarded and reversed primers, nuclease free water 8.5 µL and 2 µL of the template. Then the mixture was amplified using thermocycler [Master cycler-nexus, Eppendorf, Hamburg. Company???] and the program's conditions were as follows: initial denaturation at 95 °C for 5 min of one cycle; followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 60 °C for 45 sec, and extension at 72 °C for 1 min; final extension at 72 °C for 7 min and hold at 10 °C for10 min (one cycle) (22). While the PCR program for detection of *hsp65* was done according to Akbari et al. (23); initial denaturation at 94 °C for 2 min of one cycle; denaturation at 94 °C for 30 sec, annealing at 62 °C for 30 sec, and extension at 72 °C for 1 min (40 cycle each); final extension at 72 °C for 6 min and hold at 10 °C for 10 min (one cycle). After that, 1% agarose gel electrophoresis was applied to confirm the amplification.

The PCR products were sent to Macrogen Corporation in Korea for Sanger sequencing using an ABI3730XL automated DNA sequencer. The obtained sequences for *16S rRNA* gene were analyzed using SepsTest software and compared with known sequences in the GenBank (http://www. ncbi.nlm.nih.gov), EzTaxon-e, the obtained sequences for *hsp65* were compared with those present in GenBank, and *hsp65*-BLAST database (*hsp65*-BLAST).

RESULTS AND DISCUSSION

Nineteen isolates (9.5%) out of 200 fish samples were identified as NTM; all the isolates belonged to Rapid Growth *Mycobacterium* (RGM) based on the ability of isolates to

grow on LJ medium, acid-fast stain, colony characteristics and biochemical tests. The over all of isolates 18.26 % (19/104) were investigated only from *Liza abu* (Kishni, Abu khraiza) which were isolated in December. Seven spp. of NTM on spp. level were identified. *M. fortuitum* group was the most predominant spp. among fish isolates 15/19 (78.95%). *M. porcinum* reported 5/19 (26.32%) followed by *M. fortuitum* 4/19 (21.05%), other spp. included *M. neworleansense* and *M. mucogenicum* 2/19 (10.5%), *M. cosmeticum* and *M. pallens* 1/19 (5.26%); in addition, three isolates were unidentified on spp. level including (M. *septicum, M. peregrinum, M. porcinum, M. neworleansense, M. boenickei,* and *M. septicum, M. boenickei, M*. *peregrinum*). This result agrees with Abbas (24) who isolated *Mycobacterium* 64%, 54 % both of them were belong to RGM from rectal swabs of different fish species;17 (68%) was from *Liza abu* in Baghdad province. The above high isolates in comparison to the present result may be attributed to that the samples were from rectum (fecal) which are often harbored and more contaminated with microorganism such as *Mycobacterium* and depending only on cultural characteristics compared with present study which was more accurately in diagnosis. The results were also in agreement with a study in Nineveh province when it was found that *M. fortuitum* was the most common isolates (10%), then *M. marinum* 3.4% from different fish species belongs to Cypinindae (25). In a survey in NTM of fish from pet shop fish, the common pathogens isolated were *M. marinum* and *M. fortuitum* (*M. fortuitum* group) (26). Other study NTM found it was about 11.9% in freshwater fish tissue and fish products and the NTM spp. were included *M. porcinum*, *M. peregrinum*, *M. fortuitum*and, *M. phocaicum,* and *M. mucogenicum* (27). As well as *M. porcinum* and *M. septicum, M. peregrinum* were identified from infected goldfish, Guppy, and exotic fish species (13).

The distribution of NTM spp. in fish organs, nine out of 19 (47.37%) NTM isolates were discovered from gills followed by muscles 7 (36.84 %) while 3 (15.79%) was from internal organs. *M. porcinum* was frequently isolated from gills and muscles (66.66%) of each. *M. fortuitum* (75%) was isolated from muscles. *M.neworleansense* was only isolated from gills and only two *M. mucogenicum was* isolated from muscles and internal organs, while other spp. had only one isolate of each organ. *M. porcinum, M. neworleansense, M. boenickei,* and *M. septicum, M. peregrinum* were isolated from gills and *M. septicum*, *M. boenickei*, *M. peregrinum* from internal organs (Table 1). This to some extend came in line with a report of NTM were obtained from 1.7% fish; The species belonged to nine species using sequence analysis of the *16SrRNA* gene, which was isolated more frequently from the skin and gills than from muscles or internal organs (28). This is the first time to isolate *M. neworleansense*, *M. pallens*, *M. cosmeticum* and *M. boenickei* in Iraqi fish. The above spp. habitat in the Iraq environment, including water and the spp. were isolated from gills which might be colonized or transited in the gills although all NTM spp. were isolated in the present study showed to cause infection to man leading to variable clinical signs except for *M. pallens* (29- 32).

Das et al. (11) isolated the NTM from fish associated with granulomatous infection and the most of these pathogens were isolated from skin, gills and other tissues that confirmed by biochemical tests. the above suggested that the infection sound to be affected by the weather temperature, considering that low temperature is favorited for NTM pathogens. The above may agree with other studies in which same NTM spp. were isolated from fish and man in addition to other studies reffering to the possibility of getting infection from fish to man as the fish and its prodects may condiser as an important source of mycobacterial infections. Furthermore, (Kishni, Abu khraiza) reported the harboring of NTM may suggest that fish spp. is most important sources of NTM infections in human (11, 33, 34).

Table 1. Numbers and percentages of nontuberculous *mycobacteria* (NTM) spp. isolated from fish internal organs, Gills, and Muscles

Nontuberculous *mycobacteria* spp.	**Internal organs**		**Gills**		**Muscles**	
	Number	**%**	**Number**	**%**	**Number**	**%**
M. porcinum	1	33.33	2	66.66	2	66.66
M. fortuitum	-	-	1	25	3	75
M. neworleansense	-	-	2	100	-	-
M. boenickei	-	-	1	100	-	-
M. porcinum, M. neworleansense, M. boenickei	-	-	1	100	-	-
M. septicum, M. boenickei, M. peregrinum	-	-	-	-	1	100
M. septicum, M. peregrinum	-	-	1	100	-	-
M. mucogenicum	1	50	-	-	1(50)	50
M. pallens	-	-	1	100	-	-
M. cosmeticum	1	100	-	-	-	-
Total	3	15.79	9	47.37	7	36.84

The cultural characteristics of the NTM isolates of multiplying colony types appeared on the media and the isolates grew at 28-30 °C within 5-10 days as range in the first isolation. In sub culturing grew within 5-6 days. *M. fortuitum* group colonies had a smooth white, creamy some of them appeared as a rough, wrinkled, and irregular edge on LJ. In long incubation, it tended to be white to slightly beige, small in diameter and translucent on blood agar for 3 days at 35 °C. *M. mucogenicum* isolates were mucoid. The colonies of *M. pallens* and *M. cosmeticum* isolates had an orange-yellow color, Scotochromogens and the other isolates had non-produced pigmentation neither in dark nor after exposing to light (Figures 1, and 2). All the isolates grew on MacConkey medium without crystal violet except *M. pallens*; and the reaction on this media were differentiated by lactose ferment (pink) and lactose non ferment (pale yellow).

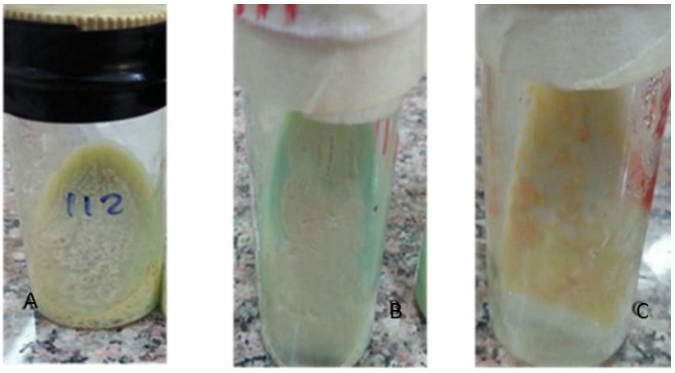

Figure 1. (A) *M. fortuitum*, rough wrinkled, irregular edged; (B) *M. porcinum*, white creamy; (C) *M. pallens*, orange color

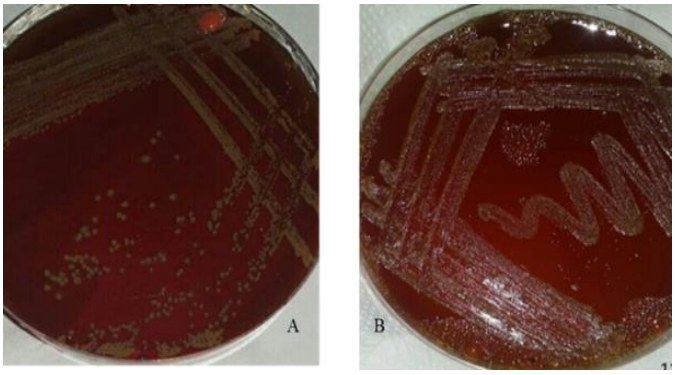

Figure 2. NTM on Blood agar: (A) *M. fortuitum*, slightly beige and small in diameter colonies; (B) *M. bonickei*, white to slightly beige, small and translucent colonies

In addition to the above, all the *M. fortuitum* group isolates had pink color while the two isolates *M. ucogenicum* had a pale yellow; and on acid fast stain, the isolates of NMT were short-long rods and no cord formation, noncapsulated and spore forming (Figures 3 and 4). Biochemical tests revealed these isolates were negative for Simmons' citrate, positive for urease, Catalase test at room temperature and semiquintative catalase <45 mm. Other biochemical showed differences between isolates as it is shown in (Table 2). These results were similar to the phenotypic characteristics described previously (16, 35, 36, 37). Identification of *Mycobacteria* is limited by using classical methods such as morphology, growth characteristics and biochemical tests which may give an inaccurate or limited diagnosis due to unreactive biochemical tests, new or novel *Mycobacterium* spp. discovered causing a confusing to identify at species level (38, 39). Moreover, some *Mycobacterium* spp. are phenotypically similar, causing suspicion in the diagnosis (40, 41).

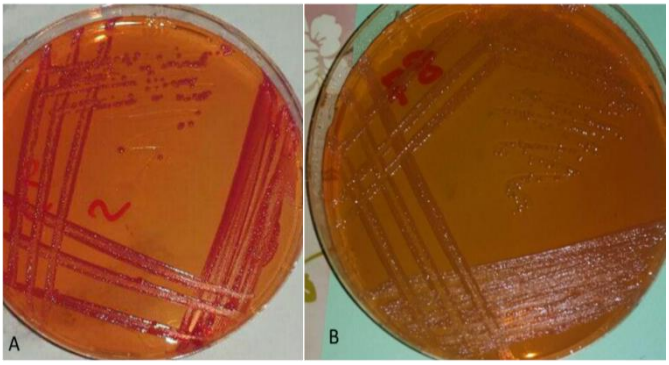

Figure 3. (A) *M. fortuitum* on MacConkey pink colonies, (B) M. mucogenicum pale yellow colonies

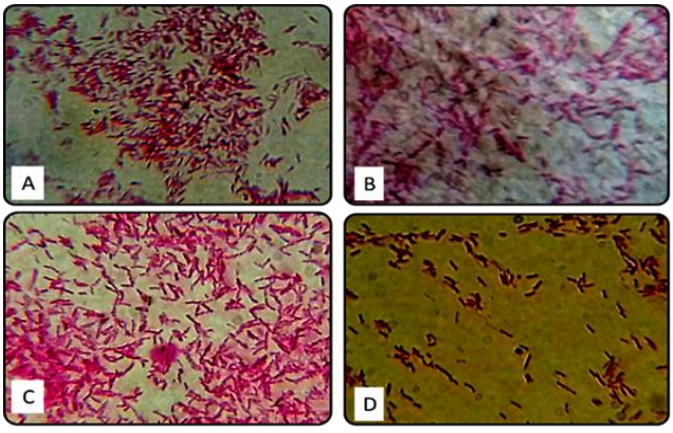

Figure 4. Acid fast bacilli, (A) *M. fortuitum*, (B) *M. pallens*, (C) *M. neworleansense*, (D) *M. mucogenicum* (100×)

In this study, the same results found variable results in one or two biochemical to the same spp. in different isolates; and in many studies there was also differences in some biochemical test for the same spp. Despite that, some isolates could not be identified at the level of spp. because of high closed relation phylogenetically spp. may lead to a new spp. or novel spp. or need more biochemical tests to confirm differentiated. This result agreed with Bahram et al. (42), although biochemical and phenotypic tests were still the most available facilities for identifying species level in most of microbiological laboratories, the present experiment showed that the results of some of the biochemical tests may be variable and using many colonies and changes in amount of bacterial population would affect the biochemical reaction.

Table 2. Biochemical tests result of the nontuberculous *mycobacteria* (NTM) spp

No.	Species (No. of isolate)	Nitrate Reduction	NaCl 5%	Catalase test at 68 °C	Growth on MacConkey	Mannitol sugar agar
1	*M. fortuitum* (4)	+	+	2 -ve, 2 +ve	+ve, pink	-
2	*M. porcinum* (5)	+	+	+	+ve, pink	-
3	*M. neworleansense* (2)	+	+	+	+ve, pink	+
4	*M. boenickei* (1)	-	+	+	+ve, pink	-
5	*M. cosmeticum* (1)	+	+	+	+ve, pink	-
6	*M. mucogenicum* (2)	-	-	-	+ve, pale, yellow	-
7	*M. pallens* (1)	-	-	-	-ve	-

The *16S rRNA* gene was amplified from 19 fish isolates using conventional PCR, which was amplified a product size of approximately 1400 bp (Figure 5). In all cases, the sequence similarity reading for all four public databases ranged between 95.19-100%. SepsiTest blast ranged between 97.2-100%, Genebank 96.63-100%, EzTaxon-e 95.19-10 and 0% Hsp-BLAST 100%. According to this database reading in this study, a similarity of *16S rRNA* or *hsp65* gene and *16S rRNA* were reported and fish isolates were7 isolates give similarity 100%: 3 isolates were *M. fortuitum*, 3 isolates were *M. porcinum,* 1 isolate was *M. pallens,* while 10 isolates give rang of similarity between 95.19-99.9%: 1 isolate were *M. fortuitum,* nine isolates were each isolate was gave vary between two or three species (Table 3). This isolate can be differentiated using biochemical test on isolate even if biochemical test could not be differentiated on level spp. Finally, the other two isolates divided into one had only *M.* spp., and one was unidentified by *16S rRNA*. Therefore, *hsp65* gene to detect 5 spp. was used and this gene gave 100% similarity to these isolates with exception to two isolates in which they varied between two or three spp. These isolates were not differentiated on spp. level as shown in Table 3 even by biochemical test. In study of Joao *et al*., (43) mycobacteria based on two highly conserved genes *16S rRNA* and *hsp65* were used to identify the gen and several public databases to perform the data analysis explaining the cause of using two genes, the *16S rRNA* which is universally used for bacteria identification. Nevertheless, this has several limitations even so it is rare in some cases (*M. terrae* and *M. celatum*complex); and two copies of the *16S rRNA* gene, with different sequences, could be found in the same mycobacteria (44). Williams et al. (45) used *"16S rRNA* sequence analysis for successful performance of 30 NTM isolates giving results of two isolates to fail of show *16S rRNA* PCR product and in this study, the identification was taken as the closest match generated by GenBank blast search analysis, had similarities ranged from 96 to 100% homology over fragment sizes of 448 to 635 bp. They explained the above as there were no standards for the interpretation and analysis of sequence data in a diagnostic setting.

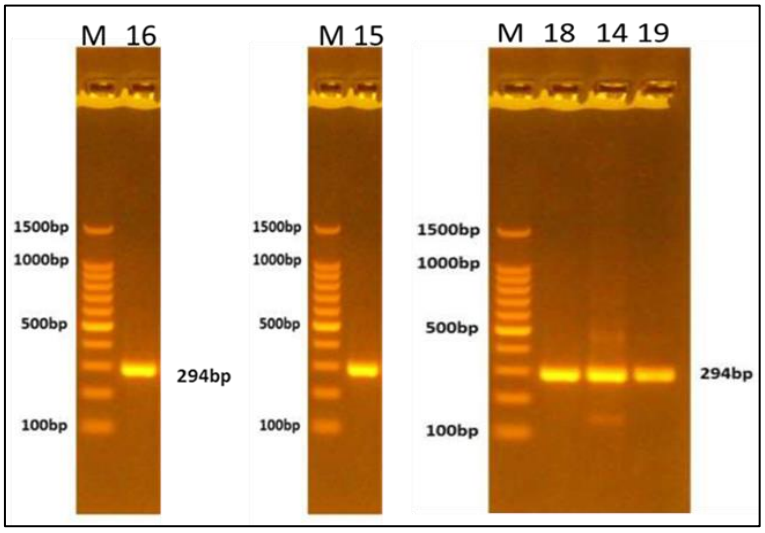

Figure 5. Results of presence of *hsp65* gene with 294 bp, fractionated on1% agarose gel electrophoresisstained withethidium bromide. Lane M=100 bp DNA ladder. Lanes 14, 15, 16, 18, and 19= positive isolates

Therefore, all results should be interpreted on an individual basis in conjunction with the clinical information; and sequence analysis of *16S rRNA* did not differentiate among *M. peregrinum*, *M. septicum* and *M. fortuitum*; *M. kansasii* and *M.gastri*; *M. mucogenicum* and *M. ratisbonense*; *M. chelonae, M. abscessus* group and *M. fuerth* and they suggested that the reason is according to what Han et al. (46) has referred to. The inability to distinguish between *M.*

kansasii and *M. gastri* using *16S rRNA* gene sequence analysis has been noted previously, however, these species could be differentiated by culture characteristics.

The study concluded that fish is a potential source of pathogenic NTM spp. that may increase the risk of public health. In addition to that, using sequence analysis for hsp65 gene with 16S rRNA primer and using multiple public databases are good methods for identifying NTM at the species' level.

Table 3. Results of Sequences Analysis and Identification of nontuberculous *mycobacteria* (NTM)S of Fish isolates by four databases

		16S rRNA (similarity%) 1: sepsiTest blast 2: GenBank, 3: EzTaxon-e			hsp 65 gene sequencing (similarity%) 1: GenBank, 2: Hsp -BLAST		Differentiated by Biochemicals
	Identification	**1**	**2**	**3**	**1**	**2**	
1	*M. porcinum* *M. neworleansense*	100	99.9	99	Not done	Not done	*M. porcinum*
2	*M. fortuitum*	100	100	100	Not done	Not done	*M. fortuitum*
3	*M. porcinum* *M. neworleansense*	99.3	99.3	99.3	Not done	Not done	*M. neworleansense*
4	*M. porcinum* *M. neworleansense* *M. boenickei*	99.7	99.7	99.7	Not done	Not done	*M. porcinum* *M. neworleansense* *M. boenickei*
5	*M. fortuitum*	100	100	99	Not done	Not done	*M. fortuitum*
6	*M. porcinum* *M. neworleansense* *M. boenickei*	99.7	99.7	99.8	Not done	Not done	*M. boenickei*
7	*M. porcinum* *M. neworleansense*	99.8	98.8	99.8	Not done	Not done	*M. neworleansense*
8	*M. porcinum* *M. neworleansense*	100	99.9	99	Not done		*M. porcinum*
9	*M. porcinum*	100	100	100	100		*M. porcinum*
10	*M. fortuitum*	100	100	100	100		*M. fortuitum*
11	*M. fortuitum*	99.5	95.5	99			
12	*M. pallens*	100	100				*M. pallens*
13	*M. porcinum*	100	100	100	100		*M. porcinum*
14	*M.* spp.	=	=		*M. cosmeticum* 100	*M. cosmeticum* 100	*M.cosmeticum*
15	*M. phocaicum* *M. mucogenicum*	99.8	*M. mucogenicum* 99.6	*M. peregrinum* *M. conceptionense* *M. montmartrense* 99.47		*M. mucogenicum* 100	*M. mucogenicum*
16	*M. phocaicum* *M. mucogenicum*	97.2	*M. mucogenicum* 96	*M. mucogenicum* 96.63	*M. mucogenicum* 100	*M. mucogenicum* 100	*M. mucogenicum*
17	*M. porcinum*	100	*M. mucogenicum* 99.6	*M. mucogenicum*	100	100	*M. porcinum*
18	*M. porcinum* *M. neworleansense* *M. boenickei*	98.6	96	*M. porcinum* 95.19	*M. septicum* *M. peregrinum* *M. boenickei* 100	*M. septicum* *M. boenickei* 100	*M. septicum* *M. boenickei*
19	Unidentified by 16S rRNA				*M. septicum* *M. peregrinum* *M. boenickei* 100	*M. septicum* *M. boenickei* 100	*M. septicum* *M. boenickei* *M. peregrinum*

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Anam Kalaf for helping in molecular part in this research.

FUNDING

These authors declare that above-submitted work was not funded by any governmental or private funding source nor supported by any financial projects.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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العزل والتشخيص الجزيئي للمتفطرات اللاسلية من مختلف أصناف الاسماك في محافظة كربلاء، العراق

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الخلاصة

صممت هذه الدراسة لعزل وتشخيص المتفطرات اللاسلية من الاسماك في محافظة كربلاء للفترة خلال شهري أكتوبر وديسمبر 2017. تم جمع 200 سمكة من اربعة انواع مختلفة من الاسماك حيث شمل هذا العدد الانواع التالية: 35سمكة من نوع *Spondyliosoma cantharus* (104 سمكة) من نوع *Liza abu* ، 46 سمكة من نوع *Carassiuscarassius*، 15سمكة من نوع *Cyprinuscarpio* , أخذت ثلاث عينات من كل سمكة تضمنت الغلاصم والعضلات والاعضاء الداخلية.تمت معالجة العينات عن طريق إزالة التلوث ، والتركيز باستخدام هيدروكسيد الصوديوم بنسبة 4٪ ، واخذ 0.1 مل من الراسب وزرع على وسط لونشتاين، ثم حضنت في درجة حرارة تتراوح بين 28-30 درجة مئوية لمدة 3 أيام - 4 أسابيع ، صبغت المستعمرات بصبغة acid fast لتأكيد وجود الميكوبكتيريوم ، تم إجراء اختبارات كيميائية حيوية لتأكيد تشخيص العزلات ، وتم إجراء فحص تسلسل البلمرة باستخدام جين 16sRNA لجميع العزلات ، وتم استخدام جين hsp65 في NTM sppغير مشخصه على مستوى الانواع ولتأكيد االبعض الاخر. ظهرت النتائج أنه من بين 200 عينة سمكية تم عزل 9.5٪ من الأسماك ، حيث إن جميع المتفطرات اللاسلية كانت تنتمي إلى المتفطرات سريعه النمو.وان جميع العزلات بنسبة 18.26٪ كانت من Liza abu (خشني ، أبو خريزة). العزلات شملت *M. porcinum* كانت 26.32٪ ، يليه 21.05 *M. fortuitum*, *M. neworleansens*٪ و *M. mucogenicum* 10.5 ٪ لكل منهما ، *M.cosmeticum* و5.26 *M.Pallens*٪ لكل منهما. المتفطرات اللاسليه كانت 47.37 ٪ من الخياشيم تليها من العظلات % 36.84 بينما 15.75 % من الأعضاء الداخلية. كانت هذه النتائج أول دراسة حول عزل هذه الانواع من الأسماك في العراق ، وكذلك عزل لاول مره للانواع الاخرى. خلصت هذه الدراسة إلى أن الأسماك هي مصدر مهم للبكتيريا الفطرية اللاسليه وخاصة المرضيه منها مما يشكل خطوره على الصحة العامة.

الكلمات المفتاحية: المتفطرات اللاسلية، اسماك، ابو خريزه، خشني، العراق