

**ISOLATION AND DIAGNOSIS OF MYCOBACTERIA SPECIES IN LOCAL
WATER FISHES IN NINEVAH PROVINCE**

H H Ali and M A Hamad

Department of Microbiology, College of Veterinary Medicine University of Mosul,
Mosul, Iraq

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ABSTRACT

This study was conducted to isolate and diagnose mycobacteria species from local freshwater fish samples collected from different markets in Nineveh province. Seventy samples were examined by staining and study of cultural characteristic of colonial growth, two mycobacteria species were isolated from 10 (14.3%) samples, seven (10%) of them were *M. fortuitum*, while 3 (4.3%) were *M. marinum* from different species of fishes belongs to family Cyprinidae which includes the genus of Barbus, *B. sharpeyi* (2%), *B. grypus* (3%), *B. xanthopterus* (2%) and family siluridae includes the genus *Silurus glanis* (1%), and the genus of carp, *Cyprinus carpio* (2%).

عزل وتشخيص العصيات الفطرية من اسماك المياه العذبة المحلية في
محافظة نينوى

هيفاء حسين علي و محمد علي حمد

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

أجريت هذه الدراسة لعزل وتشخيص جراثيم العصيات الفطرية من عينات اسماك المياه العذبة التي تم الحصول عليها من الاسواق المحلية لمحافظة نينوى وشملت الدراسة فحص 70 نموذج من الانواع المختلفة للاسماك المحلية بالاعتماد على الصفات المجهرية ودراسة الصفات المزرعية فيها حيث اعطت عشرة عينات (14.3%) نتيجة موجبة للعصيات الفطرية سبعة (7%) منها كانت للنوع *M. fortuitum* و 3 (4.3%) كانت للنوع *M. marinum* والتي عزلت من انواع مختلفة للاسماك المحلية والتي تعود إلى عائلة الشبوطيات Cypiridae وتشمل اجناس كل من البني Barbus فتمثله بـ *B. sharpeyi* (2%) والشبوط الاعتيادي *B. grypus* (3%) والكطان *B. xanthopterus* (2%) أما جنس الجري الاوربي *Silurus glanis* (1%) الذي يعود لعائلة siluridae و جنس الكارب carp بـ النوع *Cyprinus carpio* (2%).

INTRODUCTION

Mycobacteria found in soil and surface water have been isolated from tissues of animals. Mycobacteriosis in fish is a disease caused by certain bacterial species within genus *Mycobacterium*. Many species have been isolated from 151 different species of fish from 40 different families (1, 2). There are many different species of mycobacteria which can infect human and animals, however those species which cause mycobacteriosis in fish are referred to as nontuberculous mycobacteria and do not cause major disease in normal healthy people. This infection was first described from carp in France by Battalio, Dubard, and Terre 1397 and identified as *M. piscium* in 1902, *M. salmoniphilii* by Ross and Brancato 1959 which reclassified as *M. fortuitum* by Thoen, Schliesser (3). The genus mycobacterium includes many species that cause disease in mammals, birds and reptiles as well as fish. Most incidence of mycobacterial disease in fish were caused *M. marinum*, *M. fortuitum*, *M. chelonae* and because of the slow progression of disease, younger fish infected with mycobacteriosis show no external signs, a chronic progressive disease develops that may be characterized by emaciation, inflammation of the skin, exophthalmia "pop-eye", open lesions or ulceration. Fish may become sluggish, bloated refuse to eat and develop fin, tail as well as scale loss, internally gray white granulomas (4, 5, 6). Mycobacterium infected fish do have the potential to cause disease in humans such as granulomas associated with *M. marinum* acquired from fish tanks and swimming pools have been reported (7, 8, 9) *M. fortuitum* produce branching filaments in smears of pus or may produce filamentous colonial form (10, 11).

MATERIALS AND METHODS

A total of 70 freshwater fish (all fishes were alive and died while it taken out from aquarium) were collected during spring and summer seasons- 2002 from local fish market in Mosul city and transferred to the laboratory for study of the microscopical and cultural characteristics of the mycobacterium. Samples examined including viscera (spleen, liver, kidney, intestine, ovary) taken from different types of genus under cyprinidae family. There were *Barbus sharpeyi*, *B. grypus*, *B. xanthopterus*, *silurus glanis* and genus of carp fish which were classified according to (12). Tissues were homogenized for

decontamination with 2% HCl 4% NaOH to be neutralize the acid. The samples were left at room temperature for about 15 minutes, then centrifuged for about 10 minutes at 1650 xg, the supernatant fluid was discarded, sediments washed and centrifuged then inoculated on to each of two tubes of Lowenstein- Jensen medium with glycerin (Merck), incubated at (27-37 °C) for about 4 weeks (13), then acid fast stain was applied on the growing colonies, which appeared within 72 hours after incubation period, that characteristic of a cream color smooth, non chromogenic and rapid growing then biochemical tests applied which were included nitrate reduction test, urease hydrolysis test also tolerance to 5% NaCl ; growth on MacConkey agar (table 1). (14, 15, 16).

RESULTS AND DISCUSSION

This study revealed that 10 (14.3%) from 70 samples which were taken from freshwater fish were positive for acid fast staining and depending on the cultural characteristics and biochemical tests two species of the genus Mycobacterium, *M. fortuitum* 7(10%), *M. marinum* 3(4.3%) were identified. *M. fortuitum* isolates identification based on colonial appearance, cream color, large size, rough, non chromogenic, rapid growing and biochemical tests and biological properties of positive result for nitrate reduction tests and tolerance of 5% NaCl, urease test, growth on MacConkey agar but negative for tween 80 hydrolysis test and the growth was very well at (27), and (37), while *M. marinum* isolates were yellow color, smooth, slow growing, negative for nitrate reduction, tolerance to 5% NaCl, growth on MacConkey agar and positive urease test and tween hydrolysis test, growth in (27), and (37) also very well (Table 1). The results of this study were nearly close to other studies (17, 18). *M. fortuitum* was isolated from (10%) and these results were similar to other observation for isolation of this species from fish (4, 12). While *M. marinum* were isolated from (4.3%) and these results are in accordance with results found on the incidence of infection by *M. marinum*(19, 20). Mycobacteriosis is a disease of concern in fish for several reasons, the incidence of mycobacteriosis in wild fish can be as high as 8% in fish from a river in U.S.A., (20, 21). The severity of Mycobacteriosis in fish may be related to the age of the fish, its nutritional state, oxygen tension and stocking densities. Skin lesions caused by handling or parasitic infestation may facilitate infection such as water fleas; snails may act as reservoirs of the organisms (4). Feeding on uncooked fish or viscera may also be responsible for a number of outbreaks of Mycobacteriosis in fish (1), although the transmission of organism from infected fish to humans is rare but it is important to take care about the potential occurrence (4, 22).

Mycobacterium species which cause disease in fish occasionally cause a skin infection in people which referred to a fish handler disease or fish tank granuloma. The most common method of transmission is assumed to be ingestion of infected material, this can occur in fish which fed on fish products that have not been thoroughly cooked or if an infected fish dies and is consumed by other fish in the population (23). Vertical transmission (transmission from parent to offspring) may occur through the egg or sperm products (23, 24). Trace amounts of certain minerals, particularly zinc and iron have also been correlated with increased number of environmental mycobacteria. Therefore it is best to destroy infected stocks and thoroughly disinfect facilities prior to restocking for decontaminated water and equipment. People who may be exposed to infected material should wear gloves and wash thoroughly with 70% isopropyl alcohol and an antibacterial soap. Immunocompromised individuals should be prevented from having direct contact with mycobacteria infected materials (5, 23).

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