

## Isolation and Molecular Identification of Mycobacteria Spp from Human in Iraq

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### Abstract

*Total of 110 sputum samples were collected included 70 sample from Chest and Respiratory Diseases Center in Baghdad city and 40 sample from birds owners, The cultural results, acid fast stain and molecular identification of 110 human samples revealed that all human isolates were Nine isolates from 110 (8.18 %) which isolated only from T.B center patients from female and male respectively. All the isolates were from elderly patients and one of them under go from diabetes. were belong to one isolate was *M. persicum* (11.11) some results were the first isolated of these NTM spp, one isolate was *M. fortuitum* (11.11%), three isolate were belong to *M. simiae* ( 33.33%) one isolated from female live in rural area, One isolate was *M. abscessus* (11.11), *M. tuberculosis* group which represent the high percent (33.33%)*

*mycobacterium persicum ID:MN936127.1 has been identified and first recorded on a molecular basis in Iraq. Mycolicibacterium fortuitum ID:MN559537 was recorded on molecular basis in Iraq for humans.*

**Keywords:** *M. persicum, M. fortuitum, M. simiae, M. abscessus and M. tuberculosis*

### Introduction

The genus *Mycobacterium* consists of more than 180 species with validly published names, Most of them are saprophytic species widely distributed in the environment, some of which are pathogenic to animals and humans (Tan *et al.*, 2016).

*Mycobacteria* are non-motile, aerobic, acid-fast bacteria from the family *Mycobacteriaceae*. *Mycobacteria* infections have been documented a zoonotic disease, its spread from birds to humans, causing epidemics in nature, at the same times most of them

have been identified as humans pathogens (Shukla *et al.*, 2013; Das *et al.*, 2016).

The most pathogenic *mycobacteria spp* in human are, *M. smiae*, *M. fortuitum* and *M. abscessus* ( Dvorska *et al.*, 2004, Gopinath and Singh, 2010, Kankya *et al.*, 2011).

Humans exposed to infected birds may acquire a zoonotic infection, particularly immunocompromised people such as those with human immunodeficiency virus (HIV), in addition to antibiotic treatment with the infected individuals (Saggese *et al.*, 2008).

Non-tuberculous mycobacteria NTM is fewer virulent than *M. tuberculosis* complex, but recently years have been increasingly recognized as causative agents of opportunistic infections both immunocompetent and immunocompromised persons (Tortoli, 2006; van Ingen, 2015).

NTM- occur in humans with either an abnormal lungs structure or immunosuppressed, such as in cystic fibrosis, chronic obstructive pulmonary disease (COPD), or bronchiectasis (Prevots *et al.*, 2010; Prevots and Marras, 2015; Adjemian *et al.*, 2017).

*M. fortuitum* mainly causes skin and joint / bone infections in both immunocompromised and immunocompetent patients (Griffith *et al.*, 2007),

*M. fortuitum* it has been found in environments, including fresh vegetables, drinking water, ghost frogs (Brown-Elliott *et al.*, 2011; Lorencova *et al.*, 2013; Perez-Martinez *et al.*, 2013; Cerna-Cortes *et al.*, 2015; Gcebe *et al.*, 2018).

*Mycobacterium simiae* is a Photochromogens NTM colonies are produce orange or yellow pigment when exposure to light after being grown in the dark and colonies take morethan 7 days to maturing and appear on solid media (Wayne, 1984; Bercovier and Vincent, 2001).

*Mycobacterium tuberculosis*. slowly growing mycobacteria , (Frieden *et al.*,2003; Alexander *et al.*, 2010; Lawn *et al.*, 2011).

*M. persicum* Rapidly growing on L-J media of cultivation, on LJ medium pigmented (Yellow pigmentation). developed only after light exposure Rough colonies grew in about 10 days at 37°C, no growth was achieved either at on MacConkey agar without crystal violet or 42°C gram-positive bacilli acid-fast rods ( Shahraki *et al.*, 2017).

*Mycobacterium abscessus* found in, water, soil, and dust, and causing serious infections in skin and soft tissue, disseminated disease lung infections ( CDC 2010. Aitken *et al.*, 2012; Brown *et al.*, 2015; Tortoli *et al.*, 2016).

## Materials and Methods

During the study period (december2018 to April 2019) human sputum samples were collected, including 70 (40 male ,30 female) samples (patient's negative acid fast for pulmonary tuberculosis) obtained from Chest and Respiratory Diseases Center in Baghdad city and 14 samples from birds sellers (14 male)

Sputum samples about 3-5 ml were collected from patients in (TB center) , birds sellers and birds workers in sterile screw plastic container and labeling them with name, identifying number, age, gender and transported to laboratory with sterile cold box.

Each sample of human and birds were digested and decontaminated. About 5 ml. of sputum was transferred to a sterile test tube and equal amount of sterile 4% NaOH was added. The tube was incubated at 37°C for 15 minutes with vortex shaking every 5 minutes. The mixture was centrifuged at 3,000 rpm for 15 minutes and a part supernatant poured off. The deposit was neutralized by 2N HCL using a drop of phenol red as indicator. LJ was inoculated by 0.1ml. of deposit and incubated at 28- 37°C (Petroff, 2015).

## Molecular Identification of Mycobacteria

According to the WizardGenomic DNA Purification DNA was isolated from bacterial growth, Promega. PCR Kit, protocol was performed for identify *Mycobacteria spp.*

on (MYCGEN-F 5`-AGA GTT TGATCC TGG CTCAG-3`),

(MYCGEN-R 5`-TGC ACACAG GCC ACA AGGGA-3)

1ml of culture for 2min at 13000 rpm then solution added to pellet and vortex. Incubated at 37 °C in water bath for 30min. After incubation samples centrifuge at 13000 rpm for 2min. From Lysis Solution, 600µl. added and mixing were incubated at 80°C, for 5 min, then lysis cooled., 3µl of RNase Solution. Mixed, incubated for 15 mins at 37°C., 200µl of Precipitation Protein add to cell lysate. Vortexing incubated at (-30)centrifuge for 5 min at 13,000 rpm. Diluted DNA transfer to tube with 600µl isopropanol. Mixing then centrifuge as in "Pellet Cells". 70% ethanol, 600µl were added centrifuge at 13,000 rpm for 2 min aspirate Ethanol and air-dried the pellet. rehydrated in 100µl of Solution for 1 hour at 65°C.

Table 1 : PCR program for detection of mycobacteria

Steps	°C	m:s	Cycle
Initial Denaturation 95	95	05:00	1
Denaturation	95	00:30	30
Annealing	62	01:00	30
Extension	72	01:00	30
Final extension	72	07:00	1
Hold	10	10:00	1

agarose gel used to detect the presence of amplification PCR. Solutions

DNA ladder marker 1 X TAE buffer, Ethidium bromide loading dye (10mg / ml). Agarose preparation 100 ml of 1X TAE was taken. and 1.1 gm of agarose

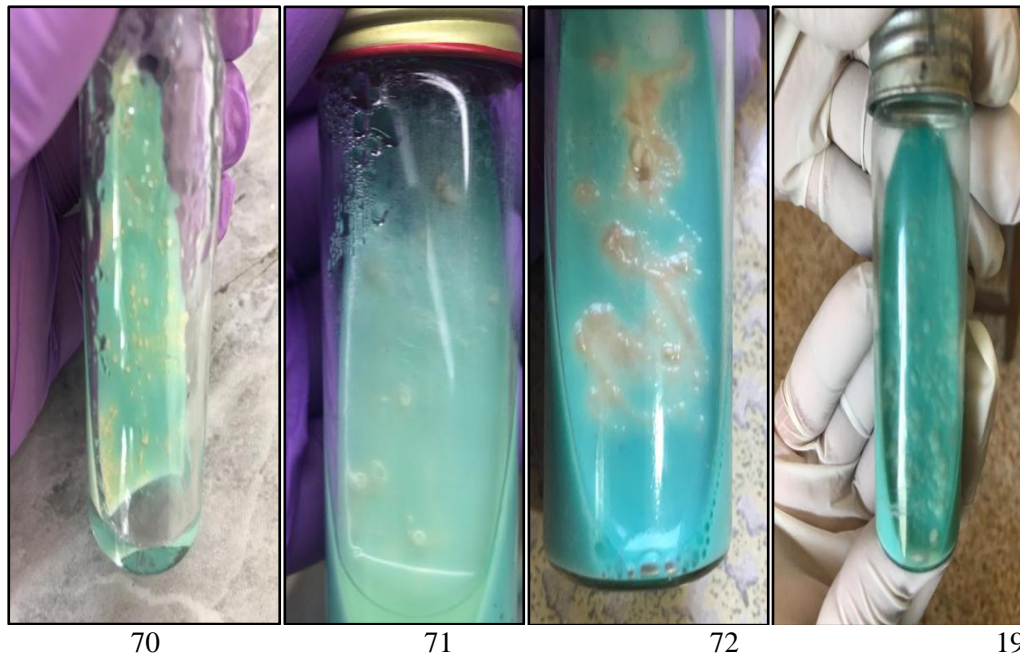
added to the buffer. Then heated to boiling until dissolved. Then add 1µl of Ethidium Bromide to the agarose. to get mixed The agarose was stirred, The cooled at 50-60°C. The solution poured into the gel tray and sealed the edges with cellophane tapes and solidified for 30 min. The comb removed. The tray was filled with 1X TAE-electrophoresis buffer 3-5mm over the

surface of the gel. DNA loading 5 µl of PCR products was loaded to well. At 100v/50mA for 75min. Electrical power was turned on. DNA moves from Cathode to plus Anode poles. The Ethidium bromide stained the bands in gel by using Gel Imaging.

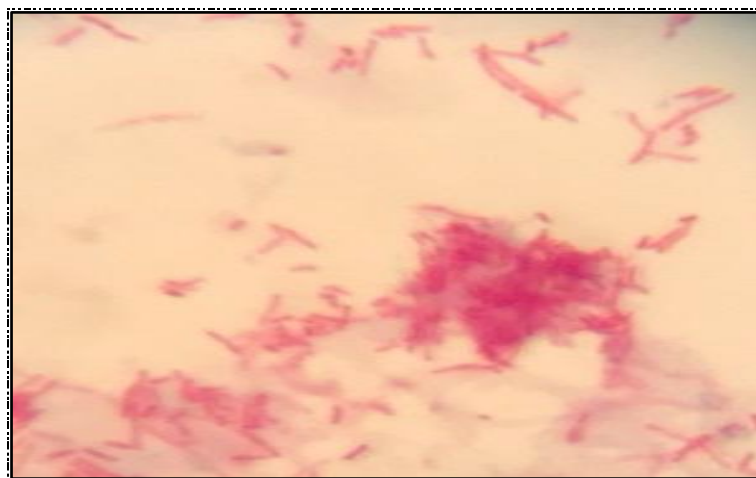
## Results

Nine isolates (8.18 %) were identified as MTC & NTM and diagnosis based on their ability to grow in LJ medium, colonies of the isolated appeared within (3 days-8 weeks). acid-fast rods presence in Ziehl-Neelsen (ZN). The isolates

grown at 28-30, 37°C. Colonies on LJ were pigmented and typical nonpigmented (Figure 1). On acid-fast stain long rods or short, branching were observed (Figure 2) on the MacConkey agar pink color colonies (Figure 3). mycobacterium spp. Based on *16S rRNA* gene analysis, Results indicated that nine spp. Of mycobacterium were identified, one isolate was *M. persicum* (11.11%), one isolate was *M. fortuitum* (11.11%), three isolate were belong to *M. simiae* (33.33%) one of isolated from female live in rural area, One isolate was *M. abscessus* (11.11%), three isolate were belong to *M. tuberculosis* group which represent the high percent (33.33%) including one of them isolated from male working for long periods in farm animals show Figure (4).

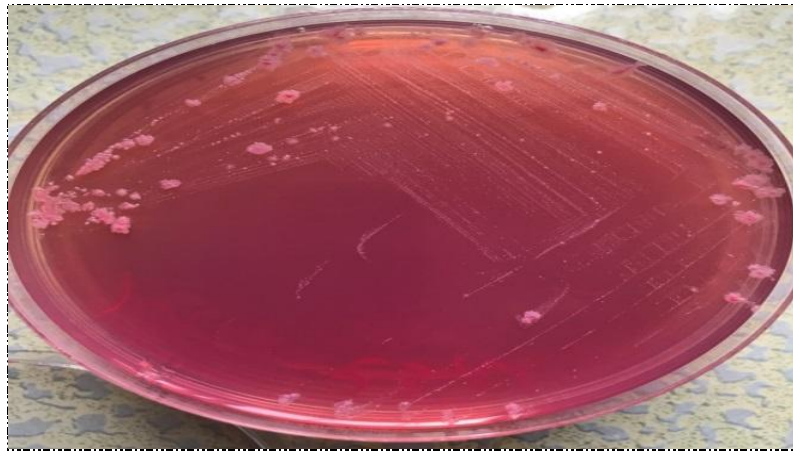


**Figure(1):**sample70 :*M. persicum*on LJ medium pigmented (Yellow pigmentation) after 7-10 days of cultivation, 37°C, Sample 71:*M.fortuitum* on LJ medium. after 8-10 days of cultivation, 35°C form typical nonpigmented white, round, rough, Sample 72: *M.simiae*. on LJ medium after 20-22days of cultivation, 37°C form typical pigmented, rust color after exposure to light, Sample 19H: *M.tuberculosis*.on LJ medium after 24-28days of cultivation, typical nonpigmented, rough, dry colonies.



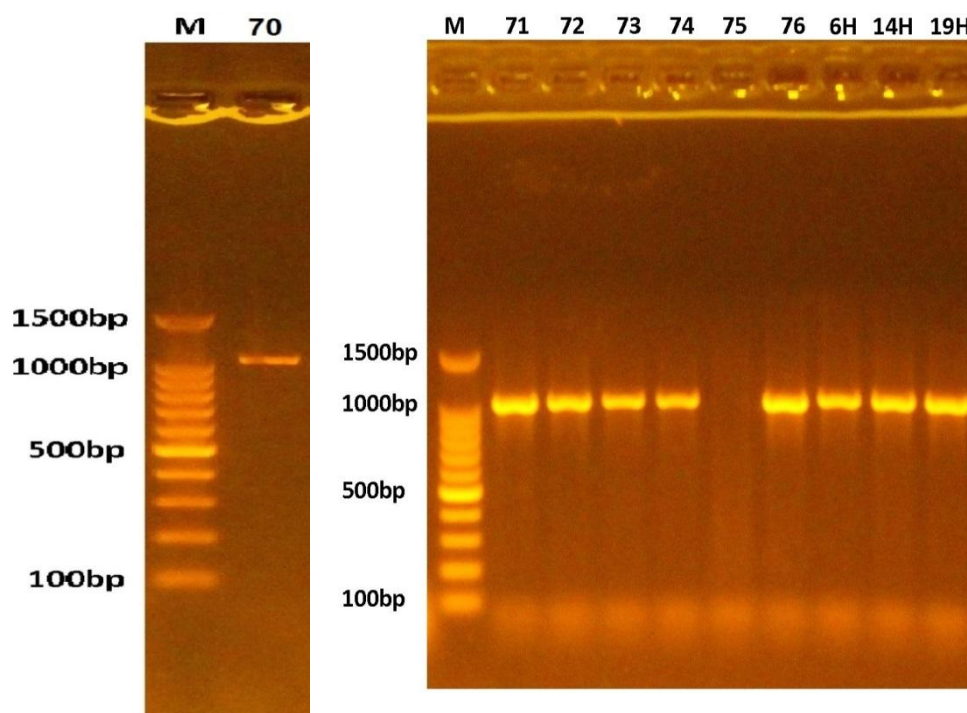
**Figure(2) : Acid fast bacilli.** short or long rods and no cross-barring or cord formation. Non-capsulated, non-branching and non-spore forming





**Figure(3):**NTM grow on macConkey agar without crystal violat from human isolate 76:

*M.abscessus* on macConkey agar (light pinke colonies).



**Figure (4 ):**Results of the presence of 16s RNA gene of the humans MTC& NTM Isolate fractionated on 1% agarose gel electrophoresis stained with Eth.Br. Lane1:100bp DNA marker all isolate appeared (1030 bp); 70:*M persicum*.

71:*M.fortuitum*.72,73and74:*M.semiae*.76:*M.abscessus*.6H,14Hand19H: *M.tuberculosis*.

### Discussion

There are little study on molecular identifications of mycobacteria from human in

Iraq. In this study the mycobacteria of human were only 9 isolates out of 110 sample 8.1% from pulmonary infections in patients suspected T.B which are belong to mycobacteria spp. include: *M. persicum*, (11.11%), *M. fortuitum*(11.11%), *M. siamae*(33.33%),*M. abscessus* (11.11% ) and *M. tuberculosis*(33.33%)which were isolated from patients as the first reported,

This result agrees with in study in Iraq Al-jebory *et al*( 2017) when isolated 40 Mycobacterium isolates from One hundred sputum samples from patients diagnosed as 35 (87.5%) *Mycobacterium tuberculosis*. Also In Iraq, NTM (2.6%) were positive from 114 sputum sample from T.B. patients in Baghdad were examined microscopically using Ziehl-Nelsen stain and culturing, (Saleh and Hammed, 2013).

And agrees with study of Al-Mashhadani and Al-Gburi (2018) isolated *M. porcinum* 25% (1/4) was isolated, *M. fortuitum* 50% (2/4) was and *M. cosmeticum* 25% (1/4).also A Mokaddas and Ahmad, 2008 in Kuwait isolates *M. fortuitum* followed by , *M. chelonae*, *M. abscessus*, *M. kansasii*,of from sputum.,and agree with the result of study In Saudi Arabia, The major species isolated were *M. avium*, *M.fortuitum* and *M.abscessus* (Varghese *et al.*, 2013; Varghese *et al.*, 2017).

Al-Harbi 2016 noted from another study in Saudi Arabia. when examined respiratory specimens of 142 patients; 28% of them were diagnosed with definitive pulmonary NTM infections caused mainly by MAC and *M. abscessus*. And Al-Ghafli and Al-Hajoj (2017) isolated *M. abscessus* (17%) and *M. fortuitum* (29%) with respiratory diseases. AS well as Velayati *et al.*, (2015) isolated *Mycobacterium fortuitum* from both environmental (46.7%) and clinical (60.1%) samples. *Mycobacterium avium* complex (MAC) was the most common slowgrowing mycobacteria(SGM) isolated from clinical samples (21.9%). And Nasiri *et al.*, ( 2018) identified *Mycobacterium simiae* (38.7%), followed by *Mycobacterium kansasii* (17.7%), *M. fortuitum* (19.3%)and *M. avium* complex (8.0%).



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