

Isolation and Identification of *Mycobacterium chelonae* from Human Sputum among Suspected Pulmonary Tuberculosis Infections in Basra-Iraq

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Abstract Objective: The purpose of this study was to estimate the frequency of *Mycobacterium Chelonae* (*M. chelonae*) among tuberculosis suspected patients in Basra governorate and evaluate the antimicrobial susceptibility. **Methods:** Isolation of *M. chelonae* from 150 samples from patients attended to the Advisory Clinic for Chest Diseases and Respiratory (ACCDR) in Basra city, smears were stained with the Ziehl Neelsen technique. Specimens were inoculated on Lowenstein Jensen medium, Identification to species level was achieved on the basis of the growth characteristics. Drug susceptibility were tested to antibiotics (Rifampicin, Isoniazid, Ethambutol, Pyrazinamide and Streptomycin) using proportional method. **Results:** from 150 sputum samples among tuberculosis suspected patients, sixteen samples (10.6%) were Nontuberculosis mycobacterium (NTM), from 16 NTM samples, 4 (2.6%) were *M. chelonae*. Drug susceptibility results showed that all isolates resistance to rifampicin, while one isolate showed intermediate resistance to ethambutol. All isolates of *M. chelonae* were sensitive to pyrazinamide, isoniazid and streptomycin. **Conclusion:** The *M. chelonae* present a high frequency, especially among tuberculosis suspected patients, which requires confirmation on a follow-up, along with the examination of patterns of sensitivity, is an absolute necessity in health centers in Iraq.

Keywords: *mycobacterium chelonae*, Non tuberculosis mycobacterium, Tuberculosis, TB, Antimicrobial Susceptibility Testing

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1. Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous organisms that rarely cause disease in immunocompetent individuals. NTM have been recovered in many parts of the world and from a variety of environmental reservoirs (Al-Sulami *et al.*, 2012). There has been an increase in the incidence of infections caused by nontuberculous mycobacteria species during the last decades, especially in rapidly growing mycobacteria (RGM) (Sethi *et al.*, 2003).

From a clinical viewpoint mainly RGM are opportunistic pathogens. The species most commonly recovered from patients belong to the *Mycobacterium fortuitum* complex, *M. chelonae*, *M. abscessus*, *M. mucogenicum* and *M. smegmatis*, reported almost everywhere worldwide. Occasionally other RGM species can cause disease in humans (García-Agudo and García-Martos, 2011). RGM most commonly complicate lung disease due to previous mycobacterial disease, cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), malignancy, lipid pneumonia, and conditions associated with chronic gastro esophageal reflux, or vomiting (Wali, 2009).

Pulmonary diseases are a common manifestation of RGM infections. and most are due to three species: *M. abscessus*, *M. fortuitum*, and *M. chelonae*. Of these, approximately 80% of chronic pulmonary diseases are caused by *M. abscessus* (Talip *et al.*, 2013). *M. chelonae*, a RGM, also known as cold blooded tubercle bacillus originally isolated from a turtle (Grange, 1981). It is a member of Runyon group IV mycobacteria that are commonly isolated from water and soil. They are generally regarded as commensals in humans (Brown, 1985).

M. chelonae most commonly causes infection in immunocompromised patients (Falsey *et al.*, 2013). It is one of the most common clinical picture is skin disease, sometimes scattered, usually in patients under immunosuppressive therapy for solid organ transplantation, rheumatoid arthritis or other autoimmune process. It can also cause traumatic localized infection (cellulitis, abscess and osteomyelitis), infection of the surgical wound, post-injection disease and disease related to intravascular catheters (Sungkanuparph *et al.*, 2003; Lalitha *et al.*, 2004; Regnier *et al.*, 2009).

It is increasingly recognized as a rare but significant cause of chronic pulmonary infection in immune competent patients but more commonly causes infections

of the skin and soft tissue (Todd *et al.*, 2000; Brown-Elliott and Wallace., 2002).

Furthermore, it has been reported among individuals with chronic lung diseases, such as tuberculosis-old chronic obstructive pulmonary disease and cystic fibrosis to be prone to lung infections *M. chelonae* (Koh *et al.*, 2007).

M. chelonae one of the most pathogenic RGM showing increased resistance to antibiotics (Regnier *et al.*, 2009). The differences in susceptibility patterns of species and resistance to first-line antituberculosis drugs create challenges in the approach to treatment of these organisms (Martín-Casabona *et al.*, 2004).

M. chelonae is resistant to antituberculosis agents but is susceptible to a number of traditional antibacterial agents (Nash *et al.*, 2009). Although amikacin, tobramycin, clarithromycin, linezolid, imipenem, ciprofloxacin, levofloxacin, doxycycline, and azithromycin are considered to be effective (Wallace *et al.*, 2001).

We therefore studied the frequency of *M. chelonae*, identification of these bacteria, obtained from Patients with Suspected Tuberculosis, during a twelve months period. Then testing drug susceptibility to antibiotics.

2. Methodology

2.1. Sample Collection and Processing

This study included the isolation of *M. chelonae* from 150 samples from patients attended to the ACCDR, during nine months period (March to November 2013). Three sputum specimens and a thick-yellow pleural fluid sample were collected. All sputum samples (10 ml) were collected with sterile screw cap containers in the early morning. Expecterated sputum was taken by asking the patient to cough deeply into the container, followed by screwing of the cap immediately. Samples were transported to the laboratory within two hours and processed immediately or refrigerated at 4°C as soon as possible (Sireva, 1998).

2.2. Microbiologic Examination

The specimens were processed on the same day for microscopy and culture using standard procedures (Koneman *et al.*, 1997).

The smears were stained with Ziehl Neelsen (ZN). Samples were inoculated into Lowenstein Jensen (LJ) medium after decontamination procedures and concentration, then incubated at 37°C. The cultures were examined every day for a week, then once a week for eight weeks. The isolates were obtained in a week confirmed as acid-fast bacilli by ZN staining identification to species level was achieved on the basis of the growth characteristics, including growth in less than 7 days, growth at 37°C, growth in presence of NaCl 5%, pigment production, Niacin production, pyrazinamidase, urease, nitrate reduction test, catalase test, heat-stable catalase (pH 7, 68°C), Tween 80 hydrolysis, growth on MacConkey agar, arylsulfatase test, and colony morphology (Miyamoto *et al.*, 2006).

2.3. Susceptibility Tests

The in vitro antimicrobial susceptibility of *M. chelonae* was tested using proportional method (Canetti *et al.*, 1963). It were tested to antibiotics (Rifampicin (1 µg/ml), Ethambutol (2 µg/ml), Pyrazinamide (0.25 µg/ml), Isoniazid (0.2 µg/ml), Streptomycin (2 µg/ml)).

The results must be reading after three weeks of incubation at 35°C. The strain was recorded as resistant when growth on antibiotic containing medium was more than the growth on the medium without antibiotic. When the case was opposite, the strain was considered as susceptible. The resistance percent was calculated according to Vestal 1975.

3. Results

The sputum samples of 150 suspected TB patients (73 male and 77 female) aged from 10 -80 years, the data are shown in Table 1.

Table 1. The clinical features of four patients infected with *M. chelonae*

Case No.	Age(Yrs)	Sex	Case Type	Work Type
26	43	M	Follow up	Earnar
48	60	F	New	House wife
76	51	F	New	House wife
114	25	F	New	House wife

3.1. Microbiologic Examination Results

The direct microbiologic examination results of *M. chelonae* showed acid-fast, gram-positive and acid fast bacilli, pleomorphic rods and non-sporulating and measured 2-7 µm x 0.2 – 0.5 µm (Figure 1).

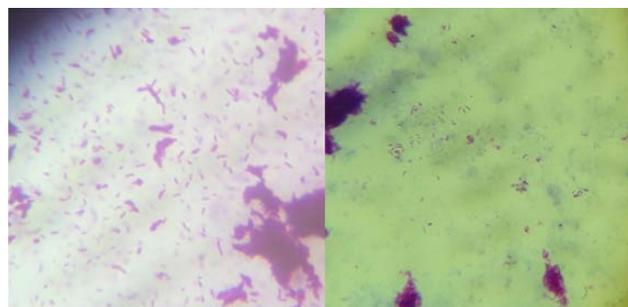


Figure 1. Direct examination of *M. chelonae* on ZN stain

The colonies of *M. chelonae* on LJ medium was circular, smooth, pale-cream colonies (Figure 2). The biochemical and growth characteristics results are summarized in Table 2.



Figure 2. circular, smooth, pale-cream *M. chelonae* colonies on LJ medium

Table 2. Summarized results of biochemical tests *M. chelonae*

Test or property	<i>M. chelonae</i>
Growth at 37°C	+
Growth in less than 7 days	+
Pigment in dark	-
Photoreactive pigment	-
Nitrate reduction	+
Growth on McConkey agar	+
Niacin production	-
Growth in presence of NaCl 5%	+
Arylsulfatase (3 days)	+
Tween 80 hydrolysis	+
Pyrazinamidase	-
Urease	-
Heat stable catalase	-

3.2. Susceptibility Tests Results

Drug susceptibility results showed all isolates *M. chelonae* resistance to rifampicin, while one isolate showed intermediate resistance to ethambutol. All isolates of *M. chelonae* were sensitive to pyrazinamide, isoniazid and streptomycin. The antimicrobial susceptibility result summarized in Table 3.

Table 3. Antimicrobial susceptibility result of four *M. chelonae* isolates from patients

Antimicrobial agent	<i>M. chelonae</i>			
	26	48	76	114
Rifampicin	R(50%)	R(96%)	R(44%)	R(14%)
Ethambutol	S	R(4%)	S	R(2%)
Pyrazinamide	S	S	S	S
Isoniazid	S	S	S	S
Streptomycin	S	S	S	S

4. Discussion

A review of previously reported cases reveals that most patients with pulmonary infections due to *M. chelonae* are non immunosuppressed but have underlying chronic lung disease (Wallace *et al.*, 2014). An unusual increased frequency of isolation of *M. chelonae* was noted (Xiang *et al.*, 2007).

In summary, the results of this study showed that high-frequency of *M. chelonae*, represented 2.6% for all isolates. That indicator of increase of infections by this bacteria, and this agree with previous studies (Hazelton *et al.*, 2000; Todd *et al.*, 2000; Brown-Elliott and Wallace., 2002; Mateo *et al.*, 2006; Tschiedel *et al.*, 2006; David *et al.*, 2007; Metta *et al.*, 2008; Cortesia *et al.*, 2010; Simons *et al.*, 2011; Conaglen *et al.*, 2013). The high frequency of *M. chelonae* due to transmission by inhalation of aerosols or by inoculation, but normally it is not transmitted from person to person (Metta *et al.*, 2008).

Previous study has shown that *M. chelonae* can cause transient colonization as well as pulmonary infection in patients who have undergone bronchoscopy with contaminated equipment (Pappas *et al.*, 1983). Although *M. chelonae* pulmonary disease has not developed in any of the patients, all patients should be followed (Wang *et al.*, 1995).

According to our study of pulmonary *M. chelonae* infection, three of four patients were middle-aged or elderly women (mean 45 years) and one case was male (43 year), and this agree with Hazelton *et al.* 2000.

On the other hand, results of susceptibility of antibiotics, *M. chelonae* isolates showed resistant or partially

susceptible to many antituberculous drugs and this agree with previous studies (Wallace *et al.*, 1992; Brown-Elliott and Wallace., 2002; Regnier *et al.*, 2009; Shaaban *et al.*, 2012).

A treatment strategy for complicated pulmonary diseases has not yet been established. Only one clinical trial on the successful treatment of *M. chelonae* skin infection used clarithromycin (Wallace *et al.*, 1993). Over the last several decades, the bacterial resistant to antibiotics becoming global challenge.

Although the optimal duration of therapy is not well defined, treatment for a minimum of 12 months of sputum- negative patients should be considered (Griffith *et al.*, 2007). However, in Iraq we need to focus on the identification and treatment strategies for *M. chelonae* and other NTM especially in pulmonary diseases, and still needs more prospective studies for reference.

5. Conclusions and Recommendations

This paper has documented the isolation of *M. chelonae* from human sputum. The focus is always on the TB bacteria to their importance and the increasing mortality rates of infection. We find that other types of causing symptoms similar to tuberculosis, such as *M. chelonae* require real pause and reconsider the methods of diagnosis and then a change in the therapeutic regimes depending on the ability of bacteria to resist.

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