

Isolation of *Mycobacterium Chelonae* in the Sputum and Cervical Lymph Nodes of Patient with Metastatic Breast Cancer

Saeed Zaker Bostanabad^{1,2*}, Pirouz Salehian² and Abdolrazagh Hashemi Shahraki³

¹Department of Medical Microbiology, Islamic Azad University, Parand branch, Tehran, Iran

²Department of Medical Microbiology, Massoud Laboratory, Tehran, Iran

³Department of Medical Microbiology, Ahvaz Joundishapour University of Medical Science, School of Medicine, Ahvaz, Iran

Background

Mycobacterium chelonae is non-tuberculous mycobacteria (NTM), a grouping that encompasses all mycobacteria outside of the *Mycobacterium tuberculosis* complex. *M. chelonae* causes various clinical syndromes mostly in the setting of immuno-suppression, especially AIDS. Herein we report case of infection caused by *M. chelonae* isolated from sputum and lymph biopsy samples of patients with Metastatic Breast Cancer.

Material and method: An unknown strain of mycobacterium was isolated from sputum of the patient suffering from fever, chronic cough and chest pain. The same organism was recovered from biopsy specimen of cervical lymph nodes on right side of neck. Phenotypic and molecular tests carried out for identification of the strain based on standard methods. The susceptibility of the strain to anti-mycobacterial agents was performed by the microbroth dilution method.

Result: Acid fast staining and culture tests has been positive. PCR amplifications tests for detection of *M. tuberculosis* and *M. avium* have been negative.

Phenotypic tests mainly including, positive result for growth at 25°C and 37°C and PNB test, growth on MacConkey agar without crystal violet, arylsulphatase, urease, iron uptake and 68°C and 22°C catalase and negative for tween 80 hydrolysis, tolerance to 5% NaCl, niacin and nitrate reduction test provided evidences that the strain belong to *M. chelonae* species.

Conclusion: Our findings show that *M. chelonae*, however rare its incidence may be, is capable of causing infection in immunocompromised patients.

Introduction

In developing countries like Iran, the high endemicity of tuberculosis has overshadowed the clinical significance of infections caused by mycobacteria other than tuberculosis (MOTT) [1]. They are simply ignored as a contamination or misidentified as tuberculosis which leads to serious problems in patient management.

Here we present an unusual case of infection caused by *M. chelonae* in a female patient who died from metastatic breast cancer.

Case Report

In December 2009, a 48-year-old female patient was admitted to a hospital due to high temperature of 39.6°C and cough. Chest X-ray was normal and tuberculin test was also negative. Other laboratory testing revealed an elevated C-reactive protein of 18 mg/l, an erythrocyte sedimentation rate of 70 mm/h and negative for HIV, HBV and HCV antigens.

The patient was empirically treated with erythromycin and tobramycin for seven days. Even after a course of this treatment, fever and cough continued still, skin lesion of neck associated with mild erythema and induration with serosanguinous discharge was appeared.

Tests for Widal, Weil Felix Dengue, brucella and smear examination for malarial parasites were negative. Discharge taken from lesion by swab for culture examination yielded no growth after 48-72 hours. Blood culture was negative after one week. Ultrasound visualization revealed no abnormalities of the abdomen and pelvis. Local right inguinal fossa and groin examination revealed mesh insitu with no fluid collection. Patient was continued with the same antibiotics along with amoxy-

clavulinic acid for another seven days. She felt better and local pain subsided following this treatment. However, she had febrile episodes off and on with mild chronic cough and subsequently developed severe pain in the right groin, pubic region and right paravertebral region. She was advised to get MRI or CT scan of the right groin. CT scan revealed localized collections.

At this juncture, the patient presented herself at the Department of Microbiology, Masoud laboratory, Tehran. After thorough history taking and examination of the swollen cervical lymph nodes on right side of neck, it was decided to do repeat laboratory diagnostic tests, i.e., chest X-ray, biopsy specimen analysis and sputum examination. Biopsy specimens from both right and left trocar sites and three independent sputum samples were taken for mycobacterial infections. Gram stain on biopsy samples showed a few inflammatory cells and no organisms.

***Corresponding author:** Dr. Saeed Zaker Bostanabad, Department of Medical Microbiology, Islamic Azad University, Parand branch, Tehran-Saveh High away, Parand new town, 3761396361, Iran, Tel: 00982294733070; Fax: 00982294733071; E-mail: saeedzaker20@yahoo.com

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Routine culture for aerobic and anaerobic organisms also yielded no growth. Repeated chest X-ray showed progressive patchy pulmonary infiltrates of the upper zones. After a week, Ziehl Neelsen stain for AFB on two sputum and wound biopsy specimens was found to be positive (Figure 1).

Based on smear microscopy, the patient was put on with first line anti-tubercular treatment (ethambutol 800 mg, isoniazid 300 mg, rifampicin 600 mg and pyrazinamide 450 mg daily). Fever subsided and he was apparently comfortable except for occasional pain at the trocar site with persisting discharge from wound.

Molecular amplification tests were negative for detection of DNA of *M. tuberculosis* and *M. avium*. A part of clinical samples also were cultured on Lowenstein Jensen (LJ) solid medium and grown colonies were identified to the species level using phenotypic tests. Culture of sputum and biopsy specimen on LJ medium showed none pigmented colonies by 6th day (Figure 2), which was presumptively identified as a RGM. The isolated mycobacterium identified as *M. chelonae* based on conventional tests. Therefore, the patient was given amikacin and ciprofloxacin and she made a good recovery and improved remarkably during almost 3 months.

After 2 weeks of recovery of patient from *M. chelonae* infection, pathology report and immunohistochemistry analysis using specific target for breast carcinoma including BRST-1, BRST-2 and Estrogen receptor and progesterone receptor from patient confirmed the diagnosis of metastatic ductal carcinoma of breast to cervical lymph node.

After two months a metastatic tumor was appeared in the brain and the patient is died one week with all the efforts (Figure 3).

Microbiology Testing

Clinical samples including sputum and biopsy specimen from cervical lymph nodes on right side of neck were examined using standard primers for detection of DNA of *M. tuberculosis* (a 750 bp

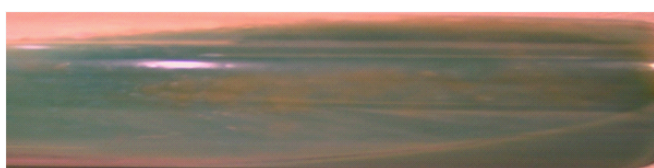


Figure 1: Ziehl Neelsen stain for sputum of patient.



Figure 2: Colony of *M. chelonae* that has been growth in LJ after 6 days.



Figure 3: CT scan showing loculated collections.

segment of the IS6110 gene) and *M. avium* (also a 275 bp segment) by commercial amplification kits (DNA technology kit, Russian company). DNA extraction was done by *DNA technology* kit (Russian company). *M. tuberculosis* H37Rv strain was used as control. PCR amplifications were carried out in 50 µl tube containing 2 µl KCl, 2 µl Tris (pH 8.0), 1.5 µl MgCl₂, 5 µl dNTP, 1 U *Taq* polymerase, 27 µl water (DDW molecular grade), 20 pmol of each primer and 6-10 µl of DNA template. The following thermocycling parameters were applied: initial denaturation at 95°C for 5 min; 36 cycles of denaturation at 94°C for 1 min; primer annealing at 56°C for 1 min; extension at 72°C for 1 min; and a final extension at 72°C for 10 min. PCR amplified products were run onto a 1% agarose gel and stained with ethidium bromide and visualized under ultraviolet (UV) light.

A part of clinical samples also were cultured on Lowenstein Jensen (LJ) solid medium and grown colonies were identified to the species level using phenotypic tests including arylsulfatase activity, catalase production, growth rate, niacin accumulation, nitrate reduction tests, pigment production, tellurite reduction, TCH (2-thiophene carboxylic acid) and urease activity [2]. The susceptibility of the strain to common antimycobacterial agents was performed by the microdilution method according to NCCLS recommendation for RGM [3].

The definite identification of the organism as *M. chelonae* was based on growth at 25°C and 37°C and absence of any growth at 42°C. PNB test, growth on MacConkey agar without crystal violet, arylsulphatase, urease, iron uptake and 68°C and 22°C catalase were positive. Tween 80 hydrolysis, tolerance to 5% NaCl, niacin and nitrate reduction test was negative. The isolate was susceptible to amikacin, doxycycline, imipenem, florenated quinolones, sulphonamides, cefoxitin and clarithromycin.

Discussion

To date, the genus *Mycobacterium* comprises over 150 species and among them several species of mycobacterium other than *Mycobacterium tuberculosis* (MOTB) or non-tubercular mycobacterial (NTM) are becoming increasingly recognized as significant pathogens (<http://www.bacterio.cict.fr/m/mycobacterium.html>). Infections due to NTM are becoming increasingly common [4].

The rapidly growing organisms such as *M. chelonae*, *M. fortuitum* and *M. abscessus* are widespread in nature and in hospital environments [5,6]. They are also highly resistant to antibiotics, antiseptics and disinfectants and hence are important nosocomial pathogens. These organisms are notorious for causing infections of soft tissues, tendons, bones and joints. Surgical procedures, accidental trauma or injections are

also considered as risk factors for infections involving these organisms [6,7]. *M. chelonae* causes various clinical syndromes, including lung disease, local cutaneous disease, osteomyelitis, joint infections and ocular disease [6-8]. With the exception of lung disease, these syndromes commonly develop after trauma. *M. chelonae* is a rare cause of isolated lymphadenitis. Endocarditis has also been documented. Disseminated skin and soft tissue lesions, occurs almost exclusively in the setting of immuno-suppression, especially AIDS [7,8]. Esophageal disorders may place patients at increased risk for pulmonary disease due to rapidly growing mycobacteria. Surgical-site infections due to *M. chelonae* are well documented, especially in association with cardiothoracic surgery and augmentation mammoplasty. *M. chelonae* is an atypical rapidly growing mycobacterium (RGM) which is also known as cold blooded tubercle bacillus originally isolated from a turtle. *M. chelonae*, although being a rare cause of human infection, is often associated with cases of inoculation mycobacterioses, disseminated infections in immuno-compromised patients and rarely involves skin and soft tissues [7,9]. However, here we present an unusual case of this organism isolated from sputum and biopsy specimens of patient who died due to breast cancer. Clinical significance of isolated *M. chelonae* in our patient with breast carcinoma, was assessed using American Thoracic Society (ATS) criteria [10] including pulmonary infiltrates in chest X-ray, presence of mycobacterium in sputum and cervical lymph node biopsy specimens as well as treatment and response to antimycobacterial therapy.

In Conclusion, *mycobacterium* sp. is a *M. chelonae* that has been isolated from patient with metastatic breast cancer and the depilated and immuno-compromised patient was accentuated the virulence of *M. chelonae* in such patients and may be seen including, autoimmune disorders, chronic allergy, chronic osteomyelitis and other disorders disrupting the immuno-surveillance mechanisms.

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