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Full Length Research Paper

Isolation of rapid growing mycobacteria from soil and water in Iran

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A total of 350 soil samples were collected from different part of Uremia city and suburbs. We used 3% sodium lauryl sulfate and 1% NaOH for decontamination of soil samples. Of 350 samples, mycobacteria were isolated from 65 (18.3%) specimens. *Mycobacterium fortuitum* with 18(5.14) strains yielded the highest frequency of isolation. The other isolates were: *Mycobacterium peregrinum* 11(3.14%), *Mycobacterium flvescens* 10 (2.85%), *Mycobacterium chelonae* 6 (1.71%), *Mycobacterium mucogenicum* 6(1.71%), *Mycobacterium thermoresistable* 4(1.14%), *Mycobacterium abscessus* 3 (0.85%), *Mycobacterium neoaurum* 2(0.57%), *Mycobacterium smegmatis* 2 (0.57%) and *M. fortuitum* third biovalant complex 3 (0.85%). The mean pH of soil was 7.89 ± 0.379 (max 8.5, min 7.5). Our data showed an abundant occurrence of mycobacteria in low pH (P value = 0001). We also collected 120 water samples from rivers, brooks and drinking water. Water samples decontaminated were by adding cetyl pyridinium chloride (CPC) to give final concentration of 0.05%. Mycobacteria isolated from 12 water samples. The predominant isolated species were *M. fortuitum* and *Mycobacterium cheloni*. The majority isolates were from brooks and surface waters.

Key words: Rapid growing mycobateria, soil, water.

INTRODUCTION

Soil bacterial populations are large and diverse and are influenced by biotic factors, such as climate and soil type, as well as by local vegetation and other biotic factors. Like many other groups of bacteria, some mycobacterium species are common, soil and water inhabitants. These organisms are often referred to as environmental, non-tuberculosis mycobacteria (NTM) or mycobacteria other than tuberculosis (Parashar et al., 2004; Brook et al., 1984; Wang et al., 2004). Several species of environmental mycobacteria have been known to be important human pathogen especially in immuno-compromised patients. Further exposure to these micro-organisms is believed to alter immunity to the vaccines like *Mycobacterium bovis* BCG ((Primm et al., 2004; Livanainen et al., 1993; Chilima et al., 2006).

Investigation of environmental mycobacteria in soil is limited by the lack of appropriate methods. Culturing also can be used to screen samples from raw and treated water systems, which may harbor opportunistic patho-gens' species of mycobacteria. However, laboratory protocols that are commonly used to investigate the presence of mycobacteria in clinical specimens are insufficient for isolation of these organisms form soil and natural water samples (Chilima et al., 2006).

In spite of large –scale BCG trial conducted in our country during past decades, BCG did not offer protection against pulmonary tuberculosis. One of several hypotheses put forward to explain the results of these trials suggest that exposure to NTM, which is present in the environ-ment of this area, could have played a role in modulating the immune response to subsequent BCG vaccination.
In the present study, an attempt has been made for the first time in this area to isolate and identify NTM species present in the soil and water.

MATERIALS AND METHODS

This study took place during spring and summer of 2004. A total of

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Abbreviations: NTM, Non-tuberculosis mycobacteria; **CPC,** cetylpyridinium chloride; **BCG,** bacillus Calmette-Guérin.

350 soil samples were collected from different sites of the city and suburbs located in North West of Iran. Soil samples were taken from depth of 3 - 5 cm with a sterile trowel and were collected in a one sterile Mc-Carteny bottle. Samples were transferred directly to the microbiology laboratory of Urmia University of Medical Sciences. Soil pH was determined after suspending 5 g of soil to distilled water in a 50-ml Erlenmeyer flask. Reading was made with a Fisher Accument meter (Model 310, Fisher scientific, Pittsburg PA) equipped with a Sensorex combination pH electrode (S300 Sesorex, Weatmister, CA). The probe was held in the sample and the sample was swirled intermittently. For culture, 5 g of soil were suspended in 20 ml of sterile 0.1% nutrient broth (Diagnostic group UK) and shacked manually for 60 s and centrifuged at 1000 rpm at room temperature for 10 min.

The supernatant were processed by Engbaek's methods (3% sodium lauryl sulfate and 1% NaOH (Kamala et al., 1994a). Each treated sample was inoculated in Lowenstein - Jensen (LJ) medium and incubated at 37°C for 12 weeks (Portals et al., 1988). For detection of mycobacteria in water, a total of 120 water samples were taken from drinkable rivers, brooks and ponds' water. For decontamination of water, cetylpyridinium chloride (CPC) method was carried out (Neumann et al., 1997; Peters et al., 1995). Briefly, CPC was added to the sample to give a final concentration of 0.05%. The mixture was shaken for 30 s. After an exposure for 30 min, the samples were immediately filtered through cellulose acetate membrane filter (No .11106-50-CAN: Satorius, Gottingen, Germany: diameter, 50 mm, pore size 0.45 µm) and rinsed in 100 ml of sterile water to remove residual CPC. A strip 10-mm wide was then aseptically cut out from the center of the filter and placed on the medium. After inoculation, the media were incubated at 37°C for 12 weeks and checked for growth every 2 - 3 days. At the end of the 12 weeks incubation period, a representative number of colonies grown was selected and characterized. The selection was done by picking at least one colony of each colony type grown in each tubes. Different colony types were distinguished by their pigmentation, size, growth rate and surface structure. The colonies were checked for acid and alcohol fastness by Ziehl-Neelsen staining method. Further identification was performed on single colonies derived from subculture of original isolates.

All isolates were identified by combination of their growth rate, pigmentation, colonial morphology and biochemical reactions such as arysulfatase activity (3 and 14 days), heat stable and semi-quantitive catalyse, iron uptake, nitrate and tellurite reduction, urease activity, niacin reaction growth on MacConkey agar and tolerance of 5% NaCl. Also the capacity to use compounds such as citrate, inositol, manitol and sorbitol as a sole carbon source were assessed (Mahon et al., 2007).

RESULTS

A total of 350 soil samples were collected from different region of city and suburb of Urmia city were examined. Mycobacteria were isolated from 64 soil sample (18.2%). All isolates were rapidly growing species. Traditionally, mycobacteria whose colonies appear on solid media in 7 days or less are named as fast or rapidly growing mycobacteria and mycobateria whose colonies appear on solid media later than 7 days are referred to as slow growing mycobacteria (Mahon et al., 2000). *M. fortuitum* was the predominant isolated species (18 strains). Other isolates included: *M. peregrinum* (11 strains), *M. flavescence* (10 strains), *M. chellonae* (6 strains), *M. mucogenicum* (6 strains), *M. thermoresistible* (4 strains), *M. abscessus* (3 strains), *M. neoaurum* (2 strains), *M. smagmatis* (2 strains) and *M. fortuitum* third biova (3 strains). We could not isolate slow growing mycobacteria in this study. The mean pH of examined soil was 7.89 (SD ±0.378, min 7.5 max 8.5). There was a correlation between pH and frequency of isolated mycobacteria ($P < 0.001$) (Table 1). The frequency of isolated mycobacteria from low pH was high.

For isolation of mycobacteria from water in total of 120 samples were examined. The mean pH was 7.63 ± 0.42) temperature of water samples were 5-25°C (mean temperature 15.1 ± 4.5°C). Of 120 water samples only 10 samples (8.34%) yielded NTM. The frequency of isolated mycobacteria included: *M. fortuitum* 3 strains (2.5*%) M. cheloni* 3 strains (2.5%) and *M. pergrinum, M absessus*, *M. smegmatis* each one strains (0.84%) isolated.

DISCUSSION

Previous studies have revealed that most of the myco-bacteria isolated from soil are "fast-growing" species, although some slow growers, including *M. avium* have been reported (Chilima et al., 2006). In this study, we tried to isolate and identify mycobacteria from soil and water. In our study, all isolated mycobacteria were rapid grower and *M. fortuitum* with 18 (5.14%) strains yielded the highest frequency of isolation followed by *M. chelonae* 6 (1.71%). Rate of isolation of NTM from water in comparison with soil was low and we isolated only 12 strains of NTM from water and *M. chellonae* each with 3 isolates were the predominant isolates.

Studies from other regions of Islamic Republic of Iran have reported isolation of NTM from environmental sources such as soil. In a study by Ghazi-Saidi (1992) and his co workers in Ahar region of East Azerbaijan, the frequency of isolated NTM was 18%. In a study by Roayei et al. (1996) in the Ahwaz region in South of Iran, the isolation rate of NMT from soil was 27.75%. In our study, the rate of isolation was 18.2% and all isolated strains were rapid growing NMT. In our study, the highest number of strains belongs to *M. fortuitum* while in study of Ghazi-saidi et al. (1992) and Roayaei et al. (1996), the highest number of strains belonged to *M. avium* and *M. szulgai*, respectively. In a recent study by Ghaemi et al. (2006) in Golestan provinces in North of Iran, the most prevalent species isolated from the soil was *M. fortuitum* followed by *M. flavescense*, which were similar to those obtained in our study. Differences in frequency of isolation of NTM from soil in present study and other studies may be due to several factors. Moisture of soil is an important ecological factor or determinant for distribution of NTM. Another important factor for isolation of NTM from environmental sources is decontamination methods. There are many decontamination methods for isolation of mycobacteria from soil and water. In a study by Kamala and his co-workers (1994a, 1994b), six methods were compared for isolation of Mott from soil and water.

On the basis of the results obtained, the method of

Table 1. Number and frequency of various species of mycobacteria isolated from Soil in different studies in Iran.

Species	Present study	Gazi saedi Gazisaedi	Roayaei
M. avium	0 (0)	11 (16.92)	0 (0)
M. intracellular	0 (0)	4 (6.15)	0 (0)
M. gastri	0 (0)	5 (7.62)	3 (3.84)
M. gordonae	(0)	8 (12.3)	11 (14.1)
M. kansasii	0 (0)	7 (10.76)	5 (6.4)
M. triviale	0 (0)	7 (10.76)	0 (0)
M. szulgai	0 (0)	1 (1.53)	12 (15.38)
M. xenopi	0 (0)	8 (12.38)	8 (10.25)
M. terrae complex	0 (0)	3 (4.61)	1 (1.28)
M. phlei	0 (0)	0 (0)	3 (3.84)
M. diernhoferi	0 (0)	2 (3.07)	0 (0)
M. flavescens	10 (15.62)	0 (0)	10 (12.82)
M. fortuitum	18 (28.12)	0 (0)	6 (7.69)
M. neoaurum	2 (3.12)	0 (0)	1 (1.28)
M. rodesiae	0 (0)	0 (0)	1 (1.28)
M. smegmatis	2 (3.12)	1 (1.53)	0 (0)
M. vaccae	0 (0)	8 (12.27)	1 (1.28)
M. agri	(0)	(0)	2 (2.56)
M. abscessus	3 (4.68)	0 (0)	0 (0)
M. mucogenicum	6 (9.37)	0 (0)	(0)
M. peregrinum	11 (17.18)	0 (0)	0 (0)
M. chelonae	6 (9.27)	0 (0)	0 (0)
M. thermo resistable	4 (6.25)	0 (0)	0 (0)
M. nonchromogenicum	0 (0)	0 (0)	1 (1.28)
M. aichenis	0 (0)	0 (0)	1 (1.28)
M. fortuium 3rd biova	2 (3.12)	0 (0)	0 (0)

using 3% sodium lauryl sulfate in combination with 1% NaOH (Engbaek method) yielded more positive culture than other methods. The method of choice in our study was Engbaek's method, while other investigators in other parts of Iran used other methods such as Perttrof's method. The Petrrof's methods usually used form isolation of mycobateria from clinical specimens. One reason for difference in frequency of isolation of NTM in our country may be due to using of different decontamination methods.

There are only a few reports available on mycobacterial ecology in soils. These studies have shown a relation between large numbers of mycobacterial and high soil acidity as mentioned earlier (Livanainen et al., 1996). In our study, soils with neutral pH yielded high frequency of NMT. Other factors more than pH are such as temperature and contents of Ca and K ions in soil are important (Livanainen et al., 1997).

In our study, mycobacteria were isolated from 12 water samples. The predominant isolated species were *M. fortuitum* and *M. cheloni*. There is only one documented study for isolation of mycobactreria from water in our country. In a study by Ghazi Saiidi et al. (1998), 307 samples were taken from sediments of different fish breeding pools in northern Iran. In their study, a total of 107 cases of mycobacteria were isolated. The most common species were *M. fortuitum* (13.97%) followed by *M. gordonae*. In our study, *M. fortuitum* and *M. chelonea* accounted more than half isolates from water. The prevalence of mycobacteria isolated from water in other studies in other countries is different. In a study by Torvinen et al. (2004) in Finland, drinking water distribution system was analyzed for presence of mycobacteria. In their study, over 90% of mycobacteria isolated from water and deposits belonged to *M. lentiflavum*, *M. tusciae* and *M. gordonae*, while in a study by Dantec et al. (2002) in USA, a total of 139 water samples were examined. In their study, NMT were isolated from the 139 samples analyzed and *M. gordonae* was the only NMT species recovered from drinking water samples from distribution systems that use ground water as the source water. *M. mucogenicum* was the most frequently isolated organism from drinking water samples from distribution system that use surface water as the source water. *M. fortuitum* was the isolate obtained most frequently from ice samples. The prevalence and rate of isolation NMT form waters in different studies varied. There are many factors that affect recovery of mycobacteria from water. These factors including decontamination methods and climate conditions

(Chilima et al., 2004; Ghaemi et al., 2006).

Prevalence of isolation NTM from clinical specimens is also different in various studies. In a multi-country retrospective study survey by Martín-Casabona et al. (2004), in which data were collected on isolation of NTM from clinical laboratories, *M. ovum* complex, *M. gordonae*, *M. xenopi*, *M. kansasii* and *M. fortuitum* were five species most frequently isolated NTM from patients. In their study, *M. fortuitum* was most frequent in Iran and Turkey. There may be a relationship between high frequency of isolation of *M. fotuitum* from environmental source and patients in our country.

Conclusion

The data obtained in our study revealed that, rapid growing mycobacteria such as *M. fortuitum* are the predominant isolated NTM from soil and water. Extensive works are needed to asset the features that allow and contribute the proliferation of mycobacteria in soils and water.

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