Environmental mycobacteria in areas of high and low tuberculosis prevalence in the Islamic Republic of Iran

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المتفطّرات المتواجدة في بيئة المناطق ذات الانتشار المرتفع أو المنخفض لمرض السل في جمهورية إيران الإسلامية

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الخلاصـة: أُجريت في إطار هذا البحث مقارنة بين الأنماط المختلفة لأنواع المتفطّرات وأعدادها في عينات من التربة أُخذت من منطقتَيْن من مقاطعة كولستان، في جمهورية إيران الإسلامية، وتعاني إحداهما من ارتفاع معدلات انتشار السل، بينما تشهد المنطقة الأخرى معدلات طفيفة. فمن 220 عينة، أظهرت 91 عينة (41.4٪) نتائج إيجابية لمزرعة السل، شُخِّصت فيها 161 ذرِّية مختلفة. وكانت المتفطرة التصادفية *M. fortuitum*، والمتفطرة المصفرة *M. flavescens*، والمتفطرة الخراجية *M. chelonae* أكثر الأنواع التي تم استفرادها شيوعاً. وقد كان تواتر المتفطّرات المتواجدة في بيئة المناطق ذات معدلات الانتشار المنخفضة أعلى من تلك الموجودة في بيئة المناطق ذات معدلات الانتشار المرتفعة، وربّما كان مردّ ذلك إلى اختلاف العوامل البيئية.

ABSTRACT This research compared the numbers and types of different *Mycobacterium* species in soil samples taken from 2 areas of Golestan province, Islamic Republic of Iran, 1 with a high prevalence of tuberculosis and 1 with a low prevalence. From 220 samples, 91 grew positive cultures (41.4%) and 161 different strains were diagnosed. The most common species isolated were *Mycobacterium fortuitum*, *M. flavescens* and *M. chelonae*. The frequencies of environmental *Mycobacterium* in the low-prevalence area were much higher than in the high-prevalence area, perhaps due to different environmental factors.

Mycobactéries environnementales dans des zones à forte et faible prévalence tuberculeuse en République islamique d'Iran

RÉSUMÉ La présente recherche a comparé le nombre et les types des différentes espèces de *Mycobacterium* dans des échantillons de sol prélevés dans deux zones de la province de Golestan (République islamique d'Iran), l'une ayant une forte prévalence tuberculeuse et l'autre une faible prévalence. Sur les 220 échantillons, 91 ont produit des cultures positives (41,4 %) et 161 souches différentes ont été diagnostiquées. Les espèces les plus couramment isolées étaient *Mycobacterium fortuitum, M. flavescens* et *M. chelonae*. La fréquence des mycobactéries environnementales dans la zone à faible prévalence était beaucoup plus élevée que dans la zone à forte prévalence, ce qui tient sans doute à différents facteurs environnementaux.

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Introduction

There are currently 71 recognized or proposed species of *Mycobacterium* [*1*], all of which, except *M. tuberculosis* complex and *M. leprae*, are considered as environmental mycobacteria and can usually be isolated from environmental samples including water, soil and dust [*2*]. The different environmental mycobacteria are very similar and for many years were mistakenly assumed to be *M. tuberculosis* in patients [*1,2*]. Nowadays these bacteria are believed to have an important role in infections, allergies, immunity to other pulmonary infections and the efficacy of bacille Calmette-Guerin (BCG) vaccination [*3*].

These facultative pathogens can cause infection, especially among immunosuppressed or immunodeficient patients [*4*]. Four types of opportunistic mycobacterial disease of humans have been described: skin lesions (following traumatic inoculation of bacteria), localized lymphadenitis, tuberculosis (TB)-like pulmonary lesions and disseminated disease [*5*]. However, the relative incidence of the environmental *Mycobacterium* spp. involved in human disease varies from region to region and is related to the occurrence of the species in the environment [*6,7*]. These bacteria are ubiquitous, so the nature, route and dose of exposure to environmental saprophytes are variables that depend on where and how an individual lives.

Another reason for the medical importance of environmental *Mycobacterium* is the potential impact on the immune response. Exposure to environmental mycobacteria has been suggested to influence immune responsiveness to the pathogenic mycobacteria and to sensitize individuals to skin test reagents; it can also affect the efficacy of BCG vaccination [*3,7–9*]. Contact with different species of environmental *Mycobacterium* can cause acquired immunity to *M. tuberculosis* or increase the efficacy of BCG vaccine protection (*M. vaccae*, *M. microti*), although some species of these bacteria reduce the efficacy of BCG vaccine (*M. scrofulaceum*) [*8,10–13*].

Golestan province, which is located in north of the Islamic Republic of Iran, near the Caspian Sea, has one of the highest rates of incidence of TB in the country (45.5 per 100 000). However, the distribution of TB is not homogenous and the incidence is higher in the western than the eastern part of the province: mean incidences are 54.6 to 28.1 per 100 000 respectively (provincial health centre, personal communication). Our objective in this research was to compare the number and types of different species of *Mycobacterium* in the soil from these 2 areas of the province.

Methods

The study took place in 2001–02. During the summer of 2001, a total of 220 soil samples were taken from humid and wet mud from a depth of 3–5 cm; 120 samples from 20 locations in an area of high TB prevalence in the western part of Golestan province and the remainder from 24 locations in an area of low TB prevalence in the eastern part of the province. The locations, mostly agricultural areas, were chosen randomly and 5 samples were taken from each.

Samples were taken from upper layers of soil, at a depth of 5 cm, and placed in separate sterile glass tubes. Samples were transferred directly to the Microbiology Laboratory of Golestan University of Medical Sciences. A suspension was made using 1 g of soil in 10 mL of sterile distilled water containing 2–3 drops of Tween 80 [*14*]. The prepared suspensions were shaken for 30 min, left for undisturbed for 30 min, then 3 mL of supernatant was transferred to another test tube and 3 mL of solution (11.7 g/L NaOH and 5% NaHCl, ratio 6:1) added to decontaminate the samples. The test tubes were centrifuged for 20 min at 2000 rpm, then the supernatant was decanted and a few drops of sterile distilled water were added to the sediment to neutralize the sample, 200 µL of sample were inoculated in Lowenstein–Jensen media and growths were assessed every 2–3 days for a period of 1 month. The isolated colonies were assessed by growth rate, morphology of colony, pigmentation in dark and light, and the results of a battery of standard biochemical tests: catalase production test at 25 ºC and 68 ºC, growth on MacConkey agar plate, NaCL tolerance, niacin production test, tellurite and nitrate reduction test, urease test and Tween 80 hydrolysis [*6,15*].

Results

From 220 soil samples, environmental mycobacteria were isolated from 91 samples (41.4%). Overall 161 strains of *Mycobacterium* belonging to 12 species were isolated. The isolation rate of environmental mycobacteria in the high-prevalence area was 20.8% (25 samples) but in the low-prevalence area was 66.0% (66 samples). This difference was statistically significant ($P < 0.05$) (Table 1).

In the high-prevalence area, 47 strains of environmental mycobacteria were isolated from 25 positive samples and *M. fortuitum*, *M. flavescens* and *M. chelonae* were the most common species. In 9 samples 2–5 strains were isolated. All the isolated *Mycobacterium* from this area belonged to 8 species (Table 2).

In the low-prevalence area, 114 strains belonging to 11 species were isolated from 66 positive soil cultures, and *M. flavescens, M. chelonae* and *M. fortuitum* were more prevalent than other strains (23, 21 and 19 cases, respectively).

M. phlei were isolated from 14 soil samples in the low-prevalence area but were not isolated from the high-prevalence area. *M. terrae, M. gordonae, M. kansasii* were also isolated in limited numbers from the low-prevalence but not from the high-prevalence area. *M. gastri* was only isolated from the high-prevalence area (Table 2). In 35 samples 2–6 strains were isolated.

Discussion

In this study in Golestan province 41.4% of soil samples contained environmental mycobacteria. This figure is similar to the results from other provinces in the north of the Islamic Republic of Iran bordering the Caspian Sea [*16–18*], presumably due to the

Table 1 **Partial distribution of positive soil culture of environmental *Mycobacterium* spp. in a Caspian Sea province in areas of high and low tuberculosis prevalence**

Variable	High-prevalence area		Low-prevalence area		Total	
	No.	%	No.	%	No.	%
Samples taken	120		100		220	
Positive cultures	25	20.8	66	66.0	91	41.4
Isolated species	8		11		12	

Table 2 **Partial distribution of different environmental *Mycobacterium* spp. isolated from soil in a Caspian Sea province in areas of high and low tuberculosis prevalence**

Species	High-prevalence area		Low-prevalence area		Total	
	No.	%	No.	%	No.	%
M. fortuitum	16	34.0	19	16.6	35	21.8
M. flavescens	10	21.2	23	20.1	33	20.5
M. chelonae	6	12.8	21	18.4	27	16.8
M. thermoresistibile	5	10.6	15	13.1	20	12.4
M. phlei	–	–	14	12.3	14	8.7
M. triviale	4	8.5	6	5.3	10	6.2
M. terrae	–	–	8	7.1	8	4.9
M. gordonae	–	–	4	3.5	4	2.5
M. fallax	2	4.3	2	1.8	4	2.8
M. gastri	3	6.4	–	–	2	1.9
M. marinum	1	2.1	1	0.9	2	1.2
M. kansasii	–	–	1	0.9	1	0.6
Total	47	100.0	114	100.0	161	100.0

similarity in climate (Mediterranean-type), humidity and plant flora in these areas.

In the present study, the isolation rate of *Mycobacterium* spp. in the area of high TB prevalence (20.8%) was significantly lower than in the area of low TB prevalence (66.0%). Environmental factors are the most likely explanation for this difference (high-prevalence areas are warmer, dryer, further from the sea with less herbal coverage than low-prevalence areas). It suggests, however, that people living in low-prevalence areas have more contact with environmental *Mycobacterium* strains than residents of high-prevalence areas.

The most prevalent species isolated from the soil in this study was *M. fortuitum* complex. This is similar to Kampala's study from south India [*19*]. Some of the isolated environmental *Mycobacterium*, such as *M. marinum*, *M. kansasii* and some rapid-growing species such as *M. chelonae* and *M. fortuitum*, can be pathogenic for humans [*1,6*]. They are almost always acquired directly from the environment. A major factor determining the occurrence of infections caused by the various species is their distribution in the environment, especially in water pipes [*6*]. As for the high prevalence of 2 species of *M. fortuitum* and *M. chelonae* in this region, physicians should be aware of opportunistic *Mycobacterium* when encountering patients with pulmonary and soft tissue diseases [*5,6*].

Geographical variation in the efficacy of *Mycobacterium bovis* BCG vaccination against TB is well recognized [*12*]. Different studies in human populations and experimental studies with animals show that infection with some environmental mycobacteria can offer a level of protection against TB similar to that offered by BCG and that, depending on the nature of the environmental mycobacteria and the timing, this exposure can also enhance, mask or interfere with the effect of subsequent BCG vaccination. Thus, regional differences in environmental mycobacteria flora along with several other mechanisms could be responsible for the widely varying results of BCG trials or could influence the course of subsequent infection with virulent TB bacilli [*6,11,13,19*]. A study in Karonga district in Northern Malawi (a region in which BCG vaccination has no effect against pulmonary TB but provides over 50% protection against leprosy) demonstrated that prior sensitization with environmental mycobacteria can inhibit BCG multiplication and thereby prevent the induction of an efficient BCG-mediated immune response and protection against TB challenge [*8*]. Geographic gradients of leprosy prevalence are reported in Karonga District, with the disease more common in the north than in the south, a pattern that may be related to protective mycobacterial exposure in the low-incidence area [*9*].

The data presented in the above studies provide strong evidences that exposure to various environmental mycobacteria can influence the immune response to BCG vaccination and subsequent prevalence of TB [*9*]. However, there have been few studies on the effect of repeated environmental mycobacteria contact on those individuals whom received earlier BCG vaccine (in infancy) and in its efficacy on inhibition of TB.

BCG vaccination is given to all infants at the time of birth in Golestan province, and the prevalence of environmental mycobacteria is higher in locations of high TB incidence than locations of low TB incidence. This raises the question whether these mycobacteria can act as booster dose of BCG vaccine to continually stimulate the immune system and keep it as active form in case of contact with *M. tuberculosis*. Further studies are required to answer this question.

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