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INCIDENCE AND CHARACTERIZATION OF ATYPICAL MYCOBACTERIA IN SOIL

(With 2 Tables)

By

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مدى تواجد وتصنيف الميكوبكتريا الغير قياسية فى التربه

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تم جمع خمسة وثلاثون عينه تربه من أماكن ايواء ورعاية الحيوانات . عشرون عينه من محافظه سوهاج وخمسة عشر عينه من محافظة أسيوط وقد تم عزل الميكوبكتريا الغير قياسيه سريعة النمو من ١١ عينه بنسبة ٤٣ ر ٣١٪ ووجد ان معاملة التربه ب ٤٪ هيدوكسيد الصوديوم و٥٪ حامض أوكساليك كانت طريقة ناجحه لعزل هذه الميكروبات بصورة نقيه من التربه وقد تم عزل وتصنيف ١٥ عترة صنفت كما يلى :

٧ من الميكوبكتريم فليای ، ٤ عترات من الميكوبكتريم فوريتوتم ، ٢ عترة من الميكوبكتريم فلافسنس وعدد اثنين عتره من الميكوبكتريم كيلونى هذا وقد تمت دراسة الاهميه الصحيه والاحتياطات الواجبه لتقليل تلوث التربه بهذه الميكروبات .

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SUMMARY

Thirty-Five samples of soil were collected from animal dwellings and careing centers in Sohag (20) and Assiut (15) Governorates. Atypical mycobacteria were recovered from 11 out of 35 soil samples with an incidence of 31.43% Fifteen strains were isolated from 11 positive samples and were identified as follows: *Mycobacterium phlei* (7), *Mycobacterium fortuitum* (4) *Mycobacterium flavescens* (2) and *Mycobacterium* chelonei (2). The public health significance as well as the suggestive measures to prevent contamination of soil by these organisms are discussed.

INTRODUCTION

Atypical mycobacteria are a heterogenous group of acid-fast organisms. Recently this group of bacteria attracted the attention of many research workers due to the increasing reports of its role as etiological agents of diseases in man and animals. Besides their pathogenicity and disease problems as well as their role in the appearence of false positive reaction in tuberculin tested human and animals can not be overlooked *(ACHA and SZYFRES, 1989).*

The ability of soil to harbour atypical mycobacteria has assumed greater significance as many authors reported that soil is the main probable source of atypical mycobacteria and the reservoirs for many human infections with atypical mycobacteria *(KUBICA et al., 1961; WOLINSKY and RYNEARSON, 1968 and DONAHUE & SISK, 1979).*

In Egypt, the available literature dealing with the incidence and significance of atypical mycobacteria in soil is lacking. Therefore, this work has been undertaken to determine the incidence of atypical mycobacteria and identification of isolated organisms.

MATERIAL and METHODS

Sampling:

Thirty-five soil samples were collected from animal dwelling of different farms and animal careing center in Sohag (20) and Assiut (15) Governorates. 50 gms collected by scraping a superficial layer of the soil with a sterile spatula and transferred to a sterile covered containers. Collected samples were submitted as rapidly as possible to the laboratory where they were subjected to mycobacteriological examination.

Methodology

- Isolation of Mycobacteria

Treatment of soil samples by using combination of 4% Sodium hydroxide solution and 5% Oxalic acid solution *(BEERWERTH, 1971)*. One gram of each soil sample was placed in a sterile centrifuge tube. The tubes were then filled with 4% sodium hydroxide solution with intensive sterring and left for 20 minutes. Then the supernatent fluid was poured off and the sediment was treated with 15 ml. Oxalic acid 5% solution and mixed well. After 20 minutes centrifugation at 3000 r.p.m was done, the supernatent fluid was discarded and acid sediment was used as inoculum. Four slants of Lowenstein Jensen medium: (two glycerinated and two non-glycerinated) were inoculated and incubated at 37°C. The incubated slant media were examined daily for 7 days and periodically once a week thereafter upto 8 weeks. The type and rate of growth was recorded. Direct smears were made from isolated colonies, fixed by gentle heating, stained by Ziehl-Nelsen method and examined microscopically for acid-fast organisms. Suspected colonies were purified and streaked onto glycerinated and non-glycerinated slants and incubated at 37°C for further identification.

- Further identification of isolates:

A) Culture screening test:

Strains that could be confirmed as acid-fast by the Ziehl-Nelsen method were tested as follows: Pigment production in the light (Photoactive) and in the dark; mature growth in < 7 days; Optimum growth temperature *(KENT and KUBICA, 1985)* and growth on Lowenstein-Jensen medium containing 5% (W/V) sodium chloride *(JARNAGIN and PAYEUR, 1988)*.

B) Cytochemical reactions:

1) Niacin test: as described by *JARNAGIN and PAYEUR (1988)*.
2) Catalase test: as mentioned by *WAYNE and DOUBEK, 1968* was applied.
3) Arylsulfatase test: as described by *CRUICKSHANK et al. (1975)*.
4) Tween 80 hydrolysis test *(JARNAGIN and PAYEUR, 1988)*.
5) Nitrate reduction test: as described by *KENT and KUBICA (1985)*.
6) Utilization of organic acid. Sodium citrate and sodium succinate were used in the utilization of organic acids as carbon source and the technique was described by *CRUICKSHANK et al. (1975)*.

RESULTS

All results are shown in tables 1 and 2.

DISCUSSION

The summerized results reported in (Table,1) show that atypical mycobacteria could be isolated from 11 out of 35 soil samples with an incidence percentage of 31.43%.

Comparing the results presented in this work with those reported by other investigators, one can easily conclude that our results were significantly low as comnpared with that reported by *KUBICA et al. (1963); JONES and JENKINS (1965); BEERWARTH (1971) and HAMMAM (1981);* who detected atypical mycobacteria in 50%, 83%, 84% and 47.5% respectively.

The lower yield of atypical mycobacteria from soil in the present study may be due to naturally low incidence of these organims in upper Egyptian soil or due to the high contamination of soil from animal dwellings which interferred much with the isolation.

Information derived from our aforementioned results declares that the islation of atypical mycobacteria from soil has been difficult since they are present in small numbers and, ordinarily, are quickly overgrown by a large variety of other microoiganisms therefore, several methods of isolation of atypical mycobacteria from soil have been recommended by *FRAY and HAGAN (1931); GORDON and HAGAN (1937) JONES and JENKINS (1965); TSUKAMURA (1967); WOLINSKY and RYNEARSON (1968) and BEERWERTH (1971).* Information derived from the results of HAMMAM (1981) revealed that Beerwereth method of decontamination of soil (combination of 4% NaoH and 5% oxalic acid solution) was successful and resulted in high isolation rate.

In the present study, 15 atypical mycobacterial isolates were recovered, by using Beewereth method, from 11 positive soil samples (Table 1). Nine cultures were scotochromogenic and six were non chromogenic. All isolates grown on Lowenstein - Jensen medium showed acid-fastness on staining by the Ziehl-Nelsen method. The cells differ morphologically from cocco-bacilli, short rods to long and clynder nonfilamentous ones, some of the cells showed dark stained parts called beads. All isolates are rapid grower (2-7 days) and grow well at 37°C and some at 28°C. Incubation at 45°C is destructive to some organisms while seven cultures grow at 52°C. The pigment production among coloured cultures (Chromogenic) was not influenced by light (Scotochromogenic).

The results of biochemical and physical tests are present in Table 2. It is shown from the table that a number of isolates differed in the typical characteristics of the species that most closely resembled. However the isolated strains of atypical mycobacteria, in the sequence of frequency were *Mycobacterium phlei, Mycobacterium fortuitum, Mycobacterium flavescens* and *Mycobacterium chelonei.* These findings agree to a certain extent with those reported by *AKULOV et al. (1967); HAMMAM (1981) and ACHA & SZYFRES (1989).* A contradictory results were reported by *TSUKAMURA (1967) and DONAHUE & SISK (1979).*

In view of the limitted numbers of soil samples studied in this work, the failure of isolating slow growers strains should not indicate that soil is not a source of these organisms. *TSUKAMURA (1967)* isolated two new species of slow grower non-chromogenic mycobacteria from soil.

From the results achieved, it can be concluded that the soil of animal dwellings and animal careing centers harbour some atypical mycobacteria and under certain circumstances the soil may be the source of atypical mycobacterial infection to man and animals. *Mycobacterium fortuitum* and *Mycobacterium chelonei* were reported by many investigators as one of the etiological agents in human chronic progressive pulmonary and extra pulmonary infection; as well as in some chronic inflammatory disease of soft tissues, bones and joints *(CHAPMAN, 1982 and BAILY, 1983).* As cattle are also readily sensitized, it is possible that these strains of mycobacteria could produce a subsequent reaction in this species to the comparative tuberculin test. The floors should therefore be made of concrete and kept dry and clean as much as possible. Frequent disinfection with an efficient disinfectant must be carried out.

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Table 1: Incidence of atypical mycobacteria in soil.

No. of soil samples examined	No of samples positive for atypical mycobacteria	%	No. of Isolates
35	11	31.43	15

Table 2: Differential characteristics of atypical mycobacteria isolated from soil.

	Mycobacterium Phlei	Mycobacterium fortuitum	Mycobacterium flavescens	Mycobacterium chelonei
Number of isolates	7	4	2	2
Rate of growth	rapid	rapid	rapid	rapid
pigment production	Scoto-chromogen	Non-chromogen	Scato chromogen	Non-chromogen
Photochromogenicity	-ve	-ve	-ve	-ve
Growth at 28°C	-	+	+	±
37°C	+	+	±	+
45°C	+	-	-	+
52°C	+	-	-	+
Catalase test(>45 mm*)	+	+	+	±
Nitrate reduction test	+	+	±	+
Tween 80 hydrolysis test	+	+	v	+
Growth on 5% Nacl	+	-	+	+
Arylsulfatse test in:				
3 days	-	+	-	+
14	+	-	-	+
Niacin test	-	-	-	-
Utilization of sodium citrate	+	+	-	+
Utilization of sodium succinate	+	+	-	+

+ percentage of strains positive.
= > 84%. ± = 60 - 84%. v= 40 - 59%.
∓ = 16 - 39%. - = < 16%. * amount of foam (mm).