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IN VITRO EFFECTS OF SOME AVAILABLE DRUGS AND DISINFECTANTS ON ATYPICAL MYCOBACTERIA

(With 2 Tables)

By

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(Received at 30/12/1993)

دراسات على كفاءة بعض العقاقير والمطهرات المتاحه على الميكوبكتيريا الغير قياسيه

عادل الياس ، الفونس فخرى ، دانيال ميخائيل
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استهدف البحث دراسة كفاءة أربعة عشر عقار وستة مطهرات على عدد ٦٢ عتره من عترات الميكوبكتيريا الغير قياسيه ولقد أظهرت الدراسه ان الميكوبكتيريا الغير قياسيه شديدة التغير من ناحية نظام حساسيتها للعقاقير المختبره وفى نفس الوقت ، أوضحت النتائج ان فنيك عابدين بتركيز ٥٪ ، هيبوكلوريت الكالسيوم بتركيز ٥٪ والجير المطفى بتركيز ٢٠٪ تقتل عتيرات الميكروب المعنى فى خلال وقت قصير .

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SUMMARY

The study dealed with the effects of Fourteen drugs and six disinfectants against sixty-two strains of atypical mycobacteria. It was been found that atypical mycobacteria were highly heterogenous in their drug sensitivity patterns. In the sametime, experimental results revealed that Phenique Abdin 5%, Calcium hypochlorate 5% as well as 20% Slaked lime were effective in Killing atypical mycobacteria within a short time.

INTRODUCTION

It is well known that many of the atypical mycobacteria are pathogenic *(EFTHIMIOU, 1984; HANSON* et al., *1987 and PANG, 1991)*. Knowledge concerning the sensitivity of these organisms to drugs is, however, scattered. Some species are fairly well studied *(HEJNY, 1982)*, whereas information about most of these organisms is sparse or non existent.

During the last few years the interest in the control of atypical mycobacteria has ben increased through the measures used in improving the field of hygiene. One of these successful methods used is the disinfectant *(ANDRUSHCHENKO, 1975)*. In this respect, few trials has been carried out by certain workers *(SAVOV, 1973; VIALLIER* et al., *1978 and KOLYCHEV; 1985)*.

In view of the above considerations, this work was planned to study the effects of some available drugs and disinfectants on atypical mycobacteria.

MATERIAL and METHODS

Part one: In vitro effects of drugs :

(I) **Materials :**

(a) Strains for investigation: Sixty-two atypical mycobacterium strains including *M. Kansassi, M. intracellulare, M. chelonei, M. fortuitum, M. smegmatis, M. flavescens, M. phlei, M. gordonae and M. scrofulaceum* were isolated from milk (40), dairy products (6), soil (13) and lymph nodes of clinically healthy slaughtered buffaloes (3). All the strains with the exception of *M.kansasii* were collected in a previous study by one of the authors *(BASTAWROUS, 1992 and 1993)*; those of *M.kansasii* were obtained by another author *(MIKHAIL, 1985)*. All these strains were completely identified for its pourity according to *BONICKE (1962)*.

(b) Media : The tests were performed on Middlebrook 7H10 with OADC enrichment media containing drugs and others as control.

(II) **Methods :**

(a) Inoculum : One loopful (the inner diameter of the loop was 4 mm) of bacterial mass from Löwenstein-Jensen Cultures was suspended in 2 ml phosphate buffer solution and 0.1 ml of this suspension was used as inoculum, according to *RIDELL (1983).*

(b) Drugs: Data concerning the drugs used are presented in table one (Critical testing concentrations given in parentheses). The first five drugs may be classified as antituberculous agents *in vitro* [Isoniazid (0.2 ug/ml), Ethambutol (6.0 ug/ml) and Streptomycin (10.0 ug/ml); the next eight as anti-microbial drugs active primarily against organisms other than mycobacteria [Amikacin (12.5 ug/ml), Chloramphenicol (20.0 ug/ml), Kanamycin (20 ug/ml), Pencillin G (80 IU/ml), Gentamycin (3 ug/ml), Cephradine (16 ug/ml) Oxytetracyclin (20 ug/ml) and Erythromycin (10 ug/ml), and the last one as Sulphonamide (Sulphamethoxazol (50 ug/ml).

(c) Methodology : The technical performance of the test was described by *KENT and KUBICA (1985).*

Part two: In vitro effects of disinfectants :

(a) Disinfectants :

1- Phenique Abdin (70% medium oil and 3% Sodium hydroxide) 5% in water.
2- Compound Cresol Solution 5% in water "Savlon".
3- Ethyl alcohol 70%.
4- Formalin (40% Formaldhyde Solution) 3% in water.
5- Calcium hypochlorite (30% available chlorine) 5% in water.
6- SLaked lime suspension 20% in water.

(b) Strains for investigation :

One strain from each species of atypical mycobacteria as recorded in part one.

(c) Bacterial suspension inoculum :

As described in part one but 5 drops of the suspension was used as inoculum.

(d) Methodology :

The plan of experiment was recorded by *LOTFY and GUINDI (1963) and ISMAIL and KAMEL (1972).*

RESULTS

All results are shown in tables (1) and (2).

DISCUSSION

Since with atypical mycobacteria the disc method of drug sensitivity testing is not adequate or precise enough *(HEJNY, 1982)*, and therefore, we devised the indirect method on solid medium with a single concentration of drug and a standardized inoculum as described by many authors *(WOLINSKY et al., 1957; RIDELL, 1983 and KENT and KUBICA, 1985).*

A standard loopful was used as a standard measure to overcome the difficulty of obtaining a homogenous suspension since some species of the atypical mycobacteria are autoagglutinable.

Informations derived from the results reported in table (1) reveal that the majority of the atypical mycobacterial strains were resistant to the standard antituberculous group of drugs except in case of ethambutol and streptomycin. On the other hand, no much difference was noticed between the behaviour of the atypical mycobacterial strains, whether sensitive or resistant, towards the antibacterial agents active primarily against organisms other than mycobacteria. Nearly similar results were reported by *WOLINSKY et al. (1957); GUY and CHAPMAN (1961); OLITZKI et al. (1967); DALOVISIO and PANKEY (1977), SCHNEIDER et al. (1978); YATES and COLLINS (1981), JACKSON et al. (1981), ATEF et al. (1982); HEJNY (1982) and KANTOR et al. (1985).*

From the same table, it is evident that the 9 species of the atypical mycobacteria were heterogeneous in their susceptibility to anti-tuberculosis drugs as well as to other antibiotics active primarily against organisms other than mycobacteria. This heterogenecity may be attributed hypothetically to the variety of their biotypes. Their drug sensitivity heterogenecity not only interspecific but also intraspecific. Practically each strain of atypical mycobacteria would represents a distinct biological unit in respect of drug sensitivity.

From the findings of *HANSON et al. (1987)* and from the present results atypical mycobacteria resistant to anti-tuberculosis drugs *in vitro* may respond to the same drugs *in Vivo* and the treatment requires testing of the causal agent for its sensitivity pattern. As a result of this examination the effecient treatment include anti-tuberculosis drugs, antibiotics and sulphonamides.

Disinfection is an important part of controlling disease agents, to obtainhigh standards of sanitation and to protect the quality of environment.

As shown from table (2), it will be noticed that the most effective bactericidal action of the disinfectant within the shortest exposure time was obtained by alcohol 70%, Freshly prepared slaked lime 20% suspension and Phenique Abdin 5% in water.

These results substantiate what was reported by *DYKSTRA (1961) and viallier (1978).* A contradictory results were reported by *JARNAGIN and PAYEUR (1988).*

On the basis of the experimental data of this study *Mycobacterium phlei, Mycobacterium chelonei and Mycobacterium smegmatis* have much higher resistance to the action of disinfectants rather than the other species of atypical mycobacteria.

Comparing these results with those of by *LOTFY and GUINDI (1963) and ISMAIL and KAMEL (1972)* who reported the differential resistance *in vitro* of the human, bovine and avian tubercle bacilli to a certain concentration of disinfectants, one may conclude that atypical mycobacterial species were resistant to disinfectants that were capable of killing *Mycobacterium tuberculosis, Mycobacterium bovis.*

To the best of our knowledge no available literatures could be traced dealing with this subject, and therefore, it was hard to discuss the aforementioned results but generally these findings agree with those reported by *SAVOV (1973).*

Results obtained from table (2) revealed that *Mycobacterium phlei* exhibited higher resistance to the action of all the disinfectants tested, whereas *Mycobacterium kansasii* was the least resistant one.

Accordingly, *Mycobacterium phlei* may be recommended as a test organism for mycobactericidal effeciency of any disinfectant used in veterinary and public health practices.

From the achieved results, it may be concluded that from the economical point of view, Phenique Abdin 5%, Calcium hypochlorate 5% and 20% Slaked lime freshly prepared are practically recommended for disinfecting premises and yards contaminated with atypical mycobacteria.

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Table (1) In Vitro Drug Susceptibilites of 52 strains of atypical mycobacteria
(Figures Represent Number of strains)

Atypical mycobacteria tested		(0.2 ug/ml) Isoniazid			(6.0 ug/ml) Ethambutol			(1.0 ug/ml) Rifampicin			(25.0 ug/ml) Pyrazinamide			(10.0 ug/ml) Streptomycin			(12.5 ug/ml) Amikacin			(20.0 ug/ml) Chloramphenicol			(20/ ug/ml) Kanamycin			(30 I.U/ml) Penicillin G			(3 ug/ml) Gentamycin			(15 ug/ml) Cephradine			(20 ug/ml) Oxytetracycline			(10 ug/ml) Erythromycin			(50 g/ml) Sulphamethoxazol		
		S	Si	R	S	Si	Int	R	S	Si	R	S	Si	R	S	Si	R	S	Si	R	S	Si	R	S	Si	R	S	Si	R	S	Si	R	S	Si	R	S	Si	R	S	Si	R
M.kansasii	3	0	1	2	3	0	0	0	1		2	0	0	3	0	1	2	2	1	0																								
M.scrofulaceum	2	0	0	2	0	2	0	0	0	2		0	0	2	0	1	1	1	1	0																								
M.terrae	6	0	0	6	0	1	1	5	0	0	6	0	0	6	0	0	6	1	1	4																								
M.flavescens	10	0	0	10	0	2	0	8	0	0	10	0	0	10	2	0	8	6	1	3																								
M.phlei	17	0	0	17	0	5	0	12	0	1	15	0	0	17	6	4	7	5	4	8																								
M.gordonae	6	0	0	6	4	2	1	0	0	0	6	0	0	6	3	2	1	3	1	2																								
M.scrofulaceum	5	0	0	5	1	1	1	3	0	0	5	0	0	5	0	1	4	3	1	1																								
M.gastri	6	0	0	6	1	1	1	4	0	0	6	0	0	6	0	0	6	3	2	1																								
M.intracellulare	8	0	0	7	3	2	0	5	0	3	4	0	0	7	0	1	5	0	3	4																								

Figures given in parentheses represent critical testing concentration of drug

List of Abbreviation: S = Susceptible (No colonies).

Si = Slightly resistant (1 - 20 colonies (Discrete colonies)

R = Resistant (confluent growth).

Table (2) Time needed for the bactericidal action of different disinfectans on atypical mycobacteria.

Disinfectant under test	Time of death								
	M. phlei	M. Fortuitum	M. smegmatis	M. flavescens	M. gordonae	M. scrofulaceum	M. intracellular	M. chelonei	M. kansasii
Formalin 3%	12 hrs	5 hrs	8 hrs	5 hrs	6 hrs	5 hrs	5 hrs	8 hrs	4 hrs
Comp.Cresol-solution 5% "Savlon"	3 hrs	30 min	60 min	20 min	30 min	20 min	20 min	2 hrs	30 min
Calcium hypochlorate 5%	72 hrs	20 min	48 hrs	10 min	20 min	10 min	20 min	48 hrs	10 min
Slaked lime solution 20%	5 hrs	6 hrs	2 hrs	60 min	2 hrs	2 hrs	60 min	60 min	30 min
Phenique Abdin 5%	60 min	20 min	30 min	20 min	30 min	20 min	20 min	30 min	10 min
Alcohol 70%	30 min	10 min	20 min	10 min	10 min	20 min	10 min	20 min	10 min