# Bacteriological Studies On The Effect Of Some Disinfectants And Antibacterial Agents On Mycobacterium tuberculosis Complex Isolated From Human And Animal Samples

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## ABSTRACT

This study was carried out to evaluate the effect of some disinfectants and antibacterial agents on Mycobacterium tuberculosis complex isolated from animal and human samples. The animal samples were collected from suspected animals from El-Sharkia, El- Menofya and El-Gharbiya governorate included lymph nodes (57 samples), raw milk samples (1250 sample) and 143 human sputum samples collected from the workers (attendance and cow men) at infected farms. The bacteriological findings of the examined infected lymph nodes samples revealed that 31 sample out of 57 were harbored mycobacterium species with a percent of 54.4%, while 30 isolates of mycobacterium spp. were recovered on Lowenstein-Jenseen media with a percentage of 2.4% from the milk samples. On the other hand, the results of Bacteriological examination of human sputum samples (143) proved that 6 samples (4.2%) were harbored the acid fast bacilli by microscopical examination using Ziehl- Neelsen stain. The efficacy of both first and second line antituberculous drugs on the isolated strains were examined using method of proportion and showed that Rifampicin and Ethambutol had relative potent activity against the isolated mycobacteria also; Ciprofloxacin and Kanamycin had relative potent activity against the isolated mycobacteria. Evaluating the efficacy of disinfectants (phenol 5%- 10% and ethyl alcohol 70%) using qualitative suspension test revealed that phenol 5% was effective at 20m' exposure, phenol 10% was effective at 15m exposure and ethyl alcohol 70% was effective at 20m.

## INTRODUCTION

Mycobacteria are widely spread in nature and they remain an important source of worldwide infections in human and animals (1). Tuberculosis is caused by members of the Mycobacterium tuberculosis complex (MTC), which includes Mycobacterium tuberculosis, the main pathogen in human, Mycobacterium bovis, the main pathogen in cattle (2). Bovine tuberculosis is a zoonotic disease caused by Mycobacterium bovis with potential public health and socio-economic significance. While Human tuberculosis is a

contagious-infectious disease mainly caused Mycobacterium tuberculosis establishes its infection usually in the lungs. (3). Mycobacteria are widely spread in nature and they remain as important cause of worldwide infections in human and animals (3).Eradication programs of tuberculosis among cattle lead to decrease in its incidence among human (4). Prompt detection, isolation, identification susceptibility testing of mycobacteria from clinical specimens are essential for control and prevention of disease (5). Culture procedures are generally believed to be more

sensitive than the microscopic examination for the detection of mycobacteria in clinical specimens. So its advantage is obvious in cases where specimens contained only small amounts of tubercle bacilli and for isolation of tubercle bacilli for further identification of the organisms and for the performance of drug susceptibility tests (6,7).

Mycobacteria, with a very thick cell wall, impermeability being responsible for resistance to disinfectants that limits uptake of biocide into the cells. The components of the cell wall that are responsible for this high levels of resistance are unknown, but both mycolic acid and arbinoglactan appear to be involved.

Additionally, efflux system may remove toxic drug and biocide molecule from the cell, although a key issue related to the concentration at which the compounds are used. Thus, with drugs it is usually necessary to equate with blood serum levels. By contrast, biocides are essentially used for external purpose at concentrations are likely to be efflux from bacterial cells. Nevertheless, the possibility remains that low 'residual' concentrations could act as a focus for the survival of organisms containing efflux genes or for the gradual or rapid development of biocide-resistance bacteria (8, 9).

Adequate sterilization or disinfection of reusable medical devices is essential to prevent cross contamination and possible transmission of infection (10). Tuberculosis is usually treated with first-line drugs; rifampin isoniazid, pyrazinamide and ethambutol while second line drugs are aminoglycosides as Kanamycin and Amikacin & new classes of fluoroquinolones Ciprofloxacin as Gatifloxacin. So, this work is planned to evaluate the effect of first line antituberculous drugs (Streptomycin, Isoniazid, Ethambutol and Rifampicin), second line antituberculous drugs (Ciprofloxacin ,Amikacin, Kanamycin and Clarithromycin) and disinfectants (Phenol 5%, phenol 10% and Ethyl alcohol 70%) on Mycobacterium tuberculosis complex through the isolation of different strains from different sources.

## **MATERIALS AND METHODS**

Samples

Animal Samples: A total of 57 lymph nodes from tuberculin positive cows from El-Sharkia, El - Menofya and El-Gharbia abattoirs were collected at postmortem examination under complete aseptic conditions. After collection of samples they were transported to the lab and kept for bacteriological examination, Isolation and Identification of Mycobacterium tuberculosis complex were carried out according to Mark's technique (11) .On the other hand, 1250 raw milk cows' were collected from dairy cows at El-Sharkia governorate in sterile containers. After cleaning and washing the teats and udder, the last strip of milk was collected. Samples were transferred in ice box laboratory for bacteriological examination according to Quinn (12).

Human samples: Three morning sputum samples were collected from the workers at infected farms on 3 consecutive days in clean tightly closed plastic disposable containers. Samples were clearly labeled with identification number and then transported to the laboratory as soon as possible to be processed according to Petroff's technique (13).

Conventional method: After preparation and processing of samples, they were cultured on Lowenstein –Jensen media and kept under observation for 6-8 weeks at microaerophilic conditions (14) .Colonies were analysed for identification by microscopic examination with Ziehl-Nelseen stain.

Evaluating the effect of some antibiotics and disinfectants

To evaluate the effect of Isoniazid  $0.2\mu g$  / ml, Streptomycin 4  $\mu g$  / ml, Ethambutol 2  $\mu g$  / ml and Rifampicin 40  $\mu g$ /ml; (from Sigma) they were prepared and tested using drug incorporation method (15). The tested disinfectants were phenol 5&10% and ethyl alcohol 70% which evaluated by the qualitative suspension test (16).

#### RESULTS

The bacteriological finding of animal samples

The bacteriological findings of examining the infected lymph nodes of slaughtered tuberculin positive animals, showed that 31 out of 57 examined animals were harbored the acid fast bacilli of

mycobacterium species with a percentage of 54.4%. As regards to raw milk samples (1250) revealed that 30 isolation of Mycobacterium spp. were recovered on Lowenstein- Jensen media with a percentage of isolation reached 2.4%.as shown in table (1).

Table 1. Prevalence of M.bovis in tuberculin positive animals cows

SOURCE	N. C 1	Bacteriological findings			
	No.of samples	No. of isolates	%		
Lymph nodes	57	31	54.4		
Raw milk	1250	۳.	2.4		
Total	1307	61	4.66		

Results of microscopic examination and culture of human samples

The Bacteriological examination of 143 sputum samples showed that 6 samples (4.2%) harbored the acid fast bacilli by microscopical examination and using Ziehl-Neelsen stain. The use of culture method revealed the isolation of (8) isolates of Mycobacterium spp. (5.6%) on Lowenstein-Jensen media.

Results of susceptibility testing of mycobacterial isolates on the first line of antituberculous drugs using the Method of proportion (MOP) Table (2) showed that the isolates were highly resistant to Streptomycin and Isoniazid while showed susceptibility to Ethambutol and Rifampicin.

Results of susceptibility testing of mycobacterial isolates to second line antituberculous drugs using the MOP The

obtained isolates were sensitive to Ciprofloxacin and Amikacin while these strains were resistant to Clarithromycin and Kanamycin as shown in table (3).

Results of bactericidal action of disinfectants on different mycobacterial after varying period of exposure using qualitative suspension test: Table (4) illustrates the results of susceptibility of 50 mycobacterium isolated from different sources (human and animal) to phenol 5% using qualitative suspension test. Phenol 5% showed efficacy towards mycobacterial strains after 20m' of with a susceptibility percent exposure 88%, while the bactericidal action of phenol 10% on different mycobacterial isolates appeared after 15m' contact with susceptibility percent 82%. Ethyl alcohol showed its bactericidal action on different mycobacterial spp. after 20m' with a percent

Table 2. Results of susceptibility testing of mycobacterial isolates to the first line antituberculous drugs using MOP

Types of species	No.of -								
M.bovis	species -	Streptomycin		Ison	iazid	Etha	mbutol	Rifampicin	
		No.	%	No.	%	No.	%	No.	%
Human	8	4	50	2	25	4	50	6	75
Animal	42	20	47.6	26	77	37	88.09	30	71.4
Total	50	24	48	28	56	41	82	36	72

Table 3. Results of susceptibility testing of mycobacterial isolates to the second line antituberculous drugs using MOP

Types of species	No.of	Antituberculous drugs										
M.bovis	species	Kanamycin		Ami	ikacin	Ciprofloxacin Clari			thromycin			
WI.DOVIS		No.	%	No.	%	No.	%	No.	%			
Human	8	0	0	4	50	1	12.5	1	12.5			
Animal	42	11	26.19	9	21.42	31	73.8	6	14.3			
Total	50	11	22	13	26	32	64	7	14			

Table 4. Bactericidal action of different disinfectants on different Mycobacterium tuberculosis types with different periods of exposure using Ost

	Type of	
Disinfectant	tubercle	No.
Disiniectant	bacilli	INO.

Culture after minutes of contact with disinfectant

	M.bovis		1	l		5	1	0	]	15	2	0	3	30
Phenol 5%			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I Henor 5 /	Human	8	0	0	0	0	1	12.5	8	100	8	100	8	100
	Animal	42	0	0	2	4.8	7	16.7	35	83.3	36	85.7	42	100
To	tal	50	0	0	2	4	8	16	43	86	44	88	50	100
Phenol	Human	8	4	50	4	50	5	62.5	6	75	6	75	8	100
10%	Animal	42	10	23.8	12	28.57	16	38.1	35	83.3	35	83.3	42	100
To	tal	50	14	28	16	32	21	42	41	82	41	82	50	100
Ethyl	Human	8	0	0	0	0	0	0	3	38.5	6	75	8	100
alcohol . 70%	Animal	42	0	0	0	0	5	12	30	7.1	35	83.3	35	83.3
То	tal	50	0	0	0	0	5	10	33	66	41	82	43	86

## DISCUSSION

Mycobacteria are widely spread in nature and they remain an important cause of worldwide infections in human and animals Eradication programs of bovine tuberculosis among cattle lead to decrease in its incidence among human (4). Prompt isolation, identification detection, susceptibility testing of mycobacteria from clinical specimens are essential for control and prevention of disease (5). Adequate sterilization or disinfection of reusable medical devices is essential to prevent cross contamination and possible transmission of infection (10).

In the present study direct microscopic examination of 57 infected lymph nodes of slaughtered tuberculin positive animals using Ziehl-Nelseen stain revealed 31 positive cases (54.4%) (17, 18). The cause of variation in the results comparing with others may be due

to the difference in the number of samples included in other studies that might affect the percentage of results.

1250 raw milk samples were examined for the presence of mycobacteria by Ziehl Neelsen staining method and culture in Löwenstein- Jensen medium, 30 samples were positive by culture, revealed the acid fast bacilli of Mycobacterium species with a percentage of 2.4%. This result is lower than that obtained in Tunisia in 2011 which was attributed to the small quantity of produced milk that may not be sold to dairy industry for pasteurization, but sold at retail and may be consumed raw or used for producing fermented dairy products (19).

While examining of 143 sputum samples using Ziehl-Nelseen stain gave 6 positive isolates (2.4%), while culturing on Lowenstein –Jensen media gave 8 positive isolates (5.6%). The differences between our

results and that obtained by (17,18,20) may be attributed to the discarded contaminated cultures and low centrifugal force used in the present study.

Treatment of infections caused by mycobacteria have been complicated by their resistant to antimicrobial agents, so the in vitro susceptibility test results have been used to predict the therapeutic efficacy of various drug regimens (21).

In the present study, the percentage of resistance was high for Streptomycin and Isoniazid 52% and 44% respectively while the percentage of resistance to Ethambutol Rifampicin was 18% and respectively (22,23). Ethambutol was the powerful antituberculous drug and increasingly used in cases of drug resistant tuberculosis (24), these results agree with the present work. The high resistance percentage may be attributed to the availability of Isoniazid in the private market and their wide spread use as antibacterial agent has been related to the emergence of resistant strains.

It was apparent table (3) that the resistance percentage of isolated mycobacteria was high with the first line antituberculous drugs, for Clarithromycin, Kanamycin 86%,78% respectively; while the percentage of resistnace for Ciprofloxacin Amikacin and was 32% and 74% respectively.

Our results were in agreement with that recorded by other investigators who recorded that the Ciprofloxacin was found to be the most active, their activity against the different strains of M.tuberculosis unrelated to their susceptibility or resistance to the antituberculous drugs Moreover, (24).Ciprofloxacin was active against all tested strains in vitro (25,26), while it disagreeed with that recorded by other investigators who reported that Amikacin was significantly active drug when tested against M.avium complex (27). In addition, the growth of M.fortuitum was inhibited by Amikacin and Neomycin which

inhibitory against *M.tuberculosis* and *M. scrofulaceum* (28).

Resistance rate of Kanamycin was 12.6%, which was higher than that reported in other study (18).

Several laboratories showed that drug resistant strains were less viable in vitro and less likely to cause disease in experimental animals (29).

Chemical disinfectants are often the only practical means of rapid disinfection, the efficacy of the disinfectants against potential pathogen, and surface compatibility of the product should tested. be Testing disinfectants against mycobacteria, presents a number of practical problems. There is no standardization, well recognized test that is universally accepted partly M.tuberculosis are slow growers and highly pathogenic (30).

The obtained results from the present work, revealed that 5%phenol was much more effective at 20 m' exposure and it gave susceptibility percentage 88% as shown in table (4), but 10%phenol was more effective 15 m' exposure with susceptibility percentage 82% as shown in table (4) (30). These results are in agreement with other investigators who reported that phenol and derivatives are known tuberculocidal even when organic matter is present (31), also phenol 5% was effective under all test conditions, producing at least a 4log10 reduction in cfu (32).

Phenol and phenol derivatives are used in the disinfection of environmental surfaces however, the strong odour and toxicity associated with phenolic derivatives make it unsuitable for use in food preparation areas and places of housing infants and children (33).

Ethyl alcohol 70% was more effective mycobactericidal at 20 m' exposure to produce susceptibility 82% by using qualitative suspension test as shown in table (4). These results are in agreement with others who found that ethanol 70% was most

effective disinfectant even after only 15 minutes exposure (34), while these results disagree with that proved ethanol 70% is effective against *M.tuberculosis* at one minute contact time (32).

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## الملخص العربي

بعض الدراسات البكتريولوجية عن تأثير بعض المطهرات والمضادات البكتيرية على مجموعة ميكروبات السل من مصادر مختلفة

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يعتبر مرض السل من أهم الأمراض المشتركة بين الانسان والحيوان و هو من الامراض الواسعة الانتشار في العالم ويكتسب هذا المرض اهميته لما يسببه من خسائر اقتصادية هائلة وكذلك مقاومته لادوية علاج السل.

وقد تمت هذه الدراسة على عدد ٥٧ غدة ليمفاوية تم أخذها من حيوانات مذبوحة بعد ان أظهرت ايجابية لاختبار التيوبركلين في المزرعة وقد تم عزل الميكروب من ٣١ عينة بنسبة ٤,٤٥% بالطرق التقليدية على أوساط السل البيئية (ميديا لوفنتشتين) في حين تم فحص ١٢٥٠ عينة لبن من الحيوانات المصابة بالمزارع واظهرت النتائج عزل الميكروب من ٣٠ عينة بنسبة ٤,٢% وتم فحص عينات من الأشخاص العاملين بالمزارع والمخالطين للحيوانات المصابة بها عددها ١٤٣ عينة بصاق واظهرت نتائج الفحص المجهري باستخدام صبغة الزيل نيلسن ان عدد العينات الايجابية ٦ بنسبة ((8,7,7)) في حين ان ٨ عينات بنسبة (8,7,7) كانت ايجابية بعد زرعها على بيئة لوفنيشتين جنسن.

وباجراء اختبار الحساسية للعترات المعزولة على المضادات الحيوية للميكروب تبين ان أعلى نسبة كانت لكل من الايثامبيوتول والسيبروفلوكساسين بنسب 4% و 4% على الترتيب كما اوضحت نتائج فاعلية المطهرات تجاه العترات المعزولة من ميكروب السل أن فاعلية الفينول 4% عند 4% دقيقة كانت بنسبة 4% فيما اظهر الفينول 4% فاعلية بعد 4% دقيقة بنسبة 4% اما الكحول الايثيلى 4% فقد اظهر فاعلية على العترات المعزولة بعد 4% دقيقة بنسبة 4%

وبالتالى فان هذه الدراسة تظهر فاعلية كل من الايثامبيوتول والسيبر وفلوكساسين تجاه معزولات ميكروب السل كما ان مركبات الفينول والكحول الايثيلى ذات فاعلية كمطهرات قوية بالمعامل والمزارع تجاه ميكروب السل.