

Original article

Rapid isolation and identification of *Mycobacterium tuberculosis* from pulmonary tuberculosis patients

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Abstract

Objective: The Study aimed to develop a rapid inexpensive and simple method for preliminary isolation and detection of *M. tuberculosis* from clinical specimens. Also to probe the impact of deteriorating health measures on the pulmonary tuberculosis control program in Basra city. **Methods:** A simple monophasic-diphasic setup (MDCS) was used for the isolation and preliminary identification of *M. tuberculosis*. This setup consists of a slanted Lowenstein-Jensen medium, the bottom of it is covered with 1 mL of tuberculosis broth thus establishing a diphasic solid liquid environment at the bottom of the screw capped test tube and above them a monophasic one. **Results:** During 7 months period from the beginning of December 2003 to the end of June 2004, 1 295 cases were managed in Tuberculosis and Chest Diseases Clinic in Basra city. Only 348 cases were diagnosed as active pulmonary tuberculosis. They consist of 232 new cases and 116 previously registered ones. The MDCS method showed higher recovery of isolation rate of *M. tuberculosis* (92.3%) than Lowenstein-Jensen, and pyruvate methods with 78.1% and 51.9% respectively. Also this method revealed more rapid appearance of results of 12 days than Lowenstein-Jensen, and pyruvate media with 22 days and 23 days respectively by the traditional culturing methods. Concurrently the study revealed that drug resistance against one or more anti tuberculosis drugs was 23.1% for new cases and 70.8% for the old ones. Multi drug resistance accounts for 20% of total isolates from old cases. This was based on using the critical concentration and 1% proportional procedures. Tuberculosis was more common among males than females with 69.4% and 30.6% respectively. A significant correlation ($P < 0.05$) was found between the productive age group (25-54) and incidence of tuberculosis. Tuberculosis was higher among married (83.8%) than unmarried peoples (22%). Children, husbands and wives showed higher infection rates than others indicating kinship influence. Failure of treatment was highly affected by improper use of anti tuberculosis drugs. **Conclusion:** The MDCS method exhibited several advantages over other culturing techniques exemplified by elimination of transport medium, establishment of simultaneous but two separate environments to accomplish both the detection and preliminary identification of *M. tuberculosis*, providing flexibility in the kinds of media used in the liquid and solid phases and it is inexpensive.

Keywords: *Mycobacterium tuberculosis*; Pulmonary tuberculosis; Detection; Isolation

INTRODUCTION

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* complex. The disease usual-

ly affects the lungs although in up to one third of cases other organs are involved^[1].

Beginning in the mid-1980s in many industrialized countries, the number of tuberculosis cases notifications which has been falling steadily stabilized or even began to increase. Approximately 3.8 million new cases of tuberculosis 90% of them from developing countries were reported annually to the world health organization in the 2001^[2].

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Tuberculosis is usually classified as pulmonary or extra pulmonary. Pulmonary tuberculosis can be categorized as primary or post primary (secondary)^[3]. It can be diagnosed by clinical manifestation and by taking history of contact; a chest radiograph may show the typical picture of upper lobe infiltration with cavitations^[3]. This can be documented by Acid fast microscopy and microbial culture^[4] and by one of the most promising diagnostic techniques such as complete sequence of *Mycobacterium tuberculosis* genome and new genetic and physiology methods to identify targets in *Mycobacterium tuberculosis* or RNA nucleotide sequence by probe hybridization^[5,6]. Most of these methods are very expensive or not available in Iraq.

The aim of the study is to attempt a simple, easy and inexpensive method for rapid isolation and diagnosis of *Mycobacterium tuberculosis* in samples from Tuberculosis and Chest Diseases Clinic in Basra city.

MATERIALS AND METHODS

Studied cases

A total of 1 295 cases attending Tuberculosis and Chest Disease Clinic in Basra city complaining of chest problems were studied. All cases were submitted to full medical examination and sent for chest radiographs, 348 cases of them were diagnosed as pulmonary tuberculosis and these cases were divided into two groups, 232 cases visiting the Tuberculosis and Chest Diseases Clinic for the first time as new cases and the previously registered 116 old cases. Samples of smear negative cases were subjected to digestion decontamination and concentration.

Cultures

A total of 115 smear positive sputum samples from the new cases and 68 old ones were cultured using Lowenstein-Jensen and pyruvate media according to Collee, J et al^[7]. Monophasic-diphasic culture setup (MDCS) was prepared as described by Al-Sulami AA, et al^[8] and used for the isolation and identification

of *Mycobacterium tuberculosis*.

Identification and confirmation of 136 isolates as *Mycobacterium tuberculosis* was carried out by acid fast staining and biochemical tests^[9].

Anti tuberculosis drugs susceptibility test was carried out on 104 isolates from new cases and 65 isolates from old cases according to Collee J^[7] relying on critical concentration and the 1% proportional method.

RESULTS

Acid fast staining of sputum samples from the 232 new cases revealed 85 (36.6%) were smear positive and 147 (63.4%) smear negative. Additional 51 smear positive cases were obtained after digestion and decontamination and concentration of smear negative cases, rising up the ratio of smear positive cases to 58.6%.

The MDCS method showed 92.3% recovery rate of isolation and identification of *Mycobacterium tuberculosis* as to Lowenstein-Jensen, and pyruvate with 78.1% and 51.9% respectively (Table 1). Also this method revealed more rapid appearance of results of 12 days than Lowenstein-Jensen and pyruvate media with 22 and 23 days respectively by the traditional culturing methods (Table 2).

All isolates were acid fast non pigmented, positive for niacin and negative for catalase at 68°C and have been identified as *Mycobacterium tuberculosis*.

The drug resistance against one or more anti tuberculosis drugs was observed in 23.1% for new cases and for the acquired drug resistance 70.8%. The multi drug (Rifampicin-Isoniazid) resistance (MDR) accounts for 20% of total isolates from old cases (Table 3).

Tuberculosis was more common among males than females with 69.4% and 30.6% respectively. A significant correlation ($P < 0.05$) was found between the productive age group (25-54) and incidence of tuberculosis (Table 4). Failure of treatment was highly affected by improper use of anti tuberculosis drug (Table 5).

Table 1 Percent and number of smear positive isolates cultured on the traditional LJ & pyruvate methods and MDCS method.

Medium	Method	No. of cultured samples	No. of positive samples	%
LJ + TB broth	MDCS	183	169	92.3
LJ	Traditional	183	143	78.1
Pyruvate	Traditional	183	95	51.9

Table 2 Rate of isolation by the traditional methods & MDCS method.

Method	Medium	Rate of isolation in days
MDCS	LJ + TB broth	12
Traditional	LJ	22
Traditional	Pyruvate	23

Table 3 No. and percent of anti tuberculosis drug resistant isolates among 65 old cases.

No. of cases	Sensitive		Resistant		MDR		Type of the case
	No.	%	No.	%	No.	%	
11	3	27.3	6	55	2	18.2	Treatment failure
14	4	28.6	8	56.1	2	14.3	Relapse
30	9	30.0	16	53.3	5	16.7	Defaulters
10	3	30.0	3	30.0	4	40.0	Chronic cases
65	19	29.2	35	50.8	13	20.0	Total

Table 4 Relation of age with TB infection among smear positive & smear negative patients.

No.	Age group (year)	Smear positive		Smear negative		Total (%)
		Male	Female	Male	Female	
1	0-14	2	1	1	0	4 (1.7)
2	15-24	13	4	10	2	29 (12.5)
3	25-34	27	13	20	13	73 (31.5)
1	0-14	2	1	1	0	4 (1.7)
2	15-24	13	4	10	2	29 (12.5)
3	25-34	27	13	20	13	73 (31.5)
4	35-44	21	9	16	10	56 (24.1)
5	45-54	18	6	8	5	37 (16.0)
6	55-64	10	3	4	2	19 (8.2)
7	65-	7	2	4	1	14 (6)
	Total	98	38	63	33	232

Table 5 Type of cases of improper treatment among 116 old cases.

Type of the case	Total No. of cases	Irregularly treated cases	
		No. of cases	%
Treatment failure	24	16	66.7
Relapse	31	26	83.9
Defaulters	48	48	100
Chronic cases	13	10	77

DISCUSSION

The aim of this study was arrived at by comparing the performance of MDCS^[8] with current methods using Lowenstein Jensen and pyruvate media with an increase in percentage of recovery of *Mycobacterium tuberculosis* from 78.1% and 51.7% in Lowenstein

Jensen and pyruvate media receptively to 92.3% in MDCS system. It Shows rapid growth rate from 22 days and 23days in Lowenstein-Jensen and pyruvate receptively to 12 days in MDCS system. So the Patient can get effective treatment in less than 3 weeks instead of 8 weeks a difference which is statistically highly significant.



The higher performance of MDCS is due to the manner in which the solid and liquid media are set-up, in solid and liquid phases of MDCS system the *Mycobacterium tuberculosis* cells cohere to form growth attached to the solid slant of the solid media instead of the wall of the culture vessel^[8], easier and more rapid than the liquid medium only, providing rapid growth, diagnosis, and faster susceptibility testing and treatment.

The diagnostic value of the MDCS lies in its versatility, as there is a liquid phase in direct contact with a solid one and above of them there is a solid phases, thus phase with different composition can be prepared.

MDCS is economic because of small quantities of liquid and solid media consumed, the omission of transport media and the short incubation time within one test tube.

The emergence of resistance of *M. tuberculosis* to drugs, whether primary or acquired ones is a problem of great concern to health authorities worldwide^[10]. Again this is clearly apparent from results in Tables 3.

Regarding the profile of the age influence on TB percentage it has been noticed that productive age range of 15-54 years has the highest one (84.5) which is compatible with the WHO report^[2]. Age group 0-15 showed the lowest percentage (1.17) and which is in agreement with data available in Iraq^[11]. This has been ascribed to the usage of BCG vaccine and the difficulties associated in taking sputum samples from children and the disease being of the primary kind.

The more frequent occurrence of TB among males than women could be attributed to environmental and social causes. In our society, men are more stressed than women because of succession of wars and instability. Also women generally experience less contact social wise. The Pulmonary Tuberculosis Control Program is based on the Directly Observed Therapy Strategy (DOTS). However shortage of resources, lack of proper supervision and suitably trained personnel made its impact limited. In this context, defaulting from antituberculosis treatment is reflected

in high rates of relapse, failure of treatment and occurrence of chronic disease (Table 5). Similar situation has been observed in Chiapas, Mexico^[12].

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