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## RESEARCH ARTICLE

### BACTERICIDAL EFFECT OF PHENOL SOLUTION USED IN THE 'DOTS' CLINICS OF HOOGHLY AND BURDWAN DISTRICTS, WEST BENGAL, INDIA

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#### ABSTRACT

Main objective of this study is to observe the success of directly observed treatment, short-course (DOTS) programme in West Bengal in respect of safe disposal of acid-fast bacilli (AFB) positive sputum specimens. The present study focused the mycobacteriocidal effect of supplied phenyl in the DOTS clinics at Hooghly and Burdwan districts, West Bengal, India, where the standard direction of disposal is being followed. Viability of Tubercle bacilli in different concentrations and contact times with that Phenyl has been observed in this study. Sputum samples were examined for (AFB) by Ziehl-Neelson (ZN) staining technique. The existing protocol for using the phenyl with sputum sample is equal volume of sputum and 5.0% Phenol i.e (1:7 dilution of phenyl). The contact time required is about 18 hours approximately. The study focused that concentration of phenyl may be lowered upto 1% without disturbing the mycobacteriocidal effect. So, the dilution which is used in DOTS center is above the threshold dilution of phenyl and so there is no question about the bio-safety in this connection with quality of disposal of sputum of positive patients in this concern.

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#### INTRODUCTION

In 1993 World Health Organization (WHO) declared tuberculosis as a global emergency in recognition of its growing influence as a public health problem (WHO, 2003). Due to the highest number of tuberculosis cases in South East Asia the situation in these countries is described as a "Time Bomb" awaiting to explode (Camilo, 2005). Tuberculosis is a specific infectious disease caused by *Mycobacterium tuberculosis*. The disease primarily affects lungs and causes pulmonary tuberculosis. Tuberculosis can affect intestine, meninges, bones, joints, lymph glands, skin and other tissues of the body. Tuberculosis remains a World-wide public health problem (Sulehri and Ali, 2008). Globally about two million people die with tuberculosis every year (Dye, 1999). Acid-fast bacilli (AFB) are examined under bright light microscope, after preparation, direct smears of sputum samples, stained by Ziehl-Neelsen (ZN). Disposable plastic cups, used to collect sputum samples from pulmonary tuberculosis patients, are generally disinfected using phenolic / hypochlorite solutions and discarded in burial pits (Selvakumar *et al.*, 2007). DOTS clinics are engaged in diagnosis and treatment of the patients under the Revised National Tuberculosis Control Programme (RNTCP), Government of India. Several DOTS clinics are

running in every district of our state, West Bengal, under the supervision of the Directorate of Health Services, West Bengal. There is a common belief in urban and semiurban sectors that the standard direction which is followed during the treatment of the used up sputum samples in the DOTS clinics is not safe properly. The present study has been undertaken to observe the mycobateriocidal effect of the supplied phenolic agent at the stipulated concentration and time as well as to find out the threshold concentration of phynyl in this regard. If Mycobacteria remain viable after treatment of sputam by phenyl at the usable concentration, the chances of environmental pollution would be there and the community's safety will face a large note of interrogation. Regarding the safe disposal of AFB positive sputum specimen, success of the Revised National Tuberculosis Control Programme (RNTCP) will be observed in such type of study.

#### MATERIALS AND METHODS

##### Study area

A total number of twenty DOTS clinics were included in this study, ten from each district of Hooghly and Burdwan in West Bengal. From those DOTS clinics Phenyl samples were collected which were used to treat the discarded sputum

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Table 1. Mycobatericidal effect of phenol solution

Phenol solution (%)	Incubation time (week)											
	1 <sup>st</sup> week		2 <sup>nd</sup> week		3 <sup>rd</sup> week		4 <sup>th</sup> week		5 <sup>th</sup> week		6 <sup>th</sup> week	
	+ ve	- ve	+ ve	- ve	+ve	- ve	+ ve	- ve	+ ve	- ve	+ ve	- ve
Treated with analytical reagent grade phenol												
5.0%	-	-	-	-	-	-	-	-	-	-	-	-
2.0%	-	-	-	-	-	-	-	-	-	-	-	-
1.0%	-	-	-	-	-	-	-	-	-	-	-	*1
Control (Without phenol)	-	-	+ ve	-								
Treated with supplied phenol in DOTS clinic												
5.0%	-	-	-	-	-	-	-	-	-	-	-	-
2.0%	-	-	-	-	-	-	-	-	-	-	-	*3
1.0%	-	-	-	-	-	-	-	*2	-	-	-	-
Control (Without phenol)	-	-	+ ve	-								

\* denotes fungal contamination/growth with number of media. '+ve' = positive, '-ve' = negetive

samples. The phenyl samples were labeled with identification and batch numbers.

### Sample preparation

In our laboratory supplied the phenyl samples were diluted individually with sterile distilled water to get the final concentrations of 1.0%, 2.0% and 5.0%. At the same time a phenolic compound of reputed brand is also diluted and the said three concentrations of it were obtained.

### Microbial sensitivity assay

A bacterial suspension in distilled water was made from a fresh growth of Tubercle bacilli obtained on L.J. Media in a concentration of 0.5 McFarlane's tube (i.e.  $10^7$  CFU/ml.). 10  $\mu$ l of the prepared live bacterial suspension were added to 10 ml. of the diluted Phenyl solution of each three concentrations. The control tubes were also introduced with the same bacterial suspension. All the tubes were kept at room temperature for 18 hours as contact time. On the next day fresh L.J. media were inoculated with 50 $\mu$ l. of each phenyl and bacterial suspensions. A total 66 L.J. media were inoculated out of which 60 were of tests and 6 were for control. A set of 2 L.J. media were inoculated also from the master solution (i.e.  $10^7$  CFU Tubercle bacilli /ml. of distilled water) to see the viability of the given growth. From first 10 tubes of 5%, 2%, 1.0% phenyl solution with 50  $\mu$ l bacterial suspension smears were made and ZN stain was carried out according to standard method (Godkar and Godkar, 2004). AFB was present in good number in all the smears. All the inoculated L.J. media were placed in an incubator at 37°C. At every three days interval media were observed for if any growth is there. Finally ZN stains were made from the suspected growths in different L.J. media to obtain any Acid-fast bacilli.

## RESULTS AND DISCUSSION

There was no growth of AFB in any medium of any concentration of phenol except one fungal growth at 6<sup>th</sup> week in 1.0% phenol treatment. In case of all supplied phenol at graded concentration there was also no growth of AFB in any

culture media which indicates the mycobatericidal effect of that phenol up to a low concentration of 1.0%. There were two fungal contaminated growth at 1.0% concentration on 4<sup>th</sup> week of incubation and three media of 2.0% phenol treated sample became contaminated with fungal growth. Positive control i.e. untreated with phenol proved the viability of the mycobacterial strain used in this study.

Positive control (untreated *Mycobacterium tuberculosis* suspension) showed growth of Tubercle bacilli (AFB) on the 2<sup>nd</sup> week of incubation at 37 C. The concentration of phenol can be minimized up to 1.0% with a contact time of 18 hours which has been observed in both the cases of DOTS supplied and analytical grade phenol. Fungal contamination found only in case of low concentrations and after incubation for long time, usually on 4-6 weeks. Phenols is broad spectrum disinfectants that function by denaturing proteins and inactivating membrane-bound enzymes to alter the cell wall permeability of microorganisms. Phenols is coal-tar derivatives or synthetic formulations and usually have a milky or cloudy appearance when added to water, as well as a strong pine odor (Kennedy, 2000; Shulaw and Bowman, 2011; Grooms, 2003). Phenolic-type antimicrobial agents have been used for their antiseptic disinfectant (Rutala and Weber, 1999, Rutala *et al.*, 2000, Pentella *et al.*, 2000, Bhalla *et al.*, 2004). Phenols is typically formulated in soap solutions to increase their penetrative powers and at 5% concentrations are considered bactericidal, tuberculocidal, fungicidal and virucidal for enveloped viruses (Jeffrey, 1995).

### Conclusion

The existing system of DOTS programmed in respect of disposal of sputum sample is adequately effective where the recommended concentration of phenol is 5.0%. Stringent follow of the standard operation procedure of sputum disposal to be monitored for the safety of the health workers and the chances of community acquired tuberculosis infection. Quality of phenol to be supplied to the DOTS clinic should not be compromised unless which the programmed may become unsuccessful which can increase the national financial burden and suffering of the tuberculosis patients. Further studies

needed to observe the lowest concentration of phenol as antimycobacterial agent.

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