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Research Article

Prevalence of Acid Fast Bacilli in Laboratory Waste Sputum Samples Collected from Government and Private Sector Laboratories in Hooghly and Burdwan District, West Bengal, India

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Abstract: Laboratory staffs become prone to infection when they are exposed to infectious biological agents in the laboratory. The mode of transmission of any pathogen like mycobacterium in the laboratory can be secondary besides primary infection. In our country, particularly in West Bengal the systematic study about safe disposal of laboratory wastes and measures taken for prevention of laboratory acquired microbiologic infection to working staff in government and private run laboratories is scanty. A cross sectional study was conducted to explore the risks of laboratory waste related environmental contamination after getting ethical clearance certificates. Five disposed sputum (remain untreated and kept for disposal) samples were collected randomly from each of the 21 laboratories selected arbitrarily of 'Hooghly' district and 20 laboratories of 'Burdwan' district (both government and private laboratories) situated within five kilometer radius surrounding the district/sub-divisional/rural hospitals with prior consent of the laboratory authorities. Study included microbiological examination of sputum for microscopy for AFB by Ziehl Neelsen staining and for culture of AFB using LJ media. The Z.N. smear test positivity for 24% cases and AFB culture test positivity for 24% revealed the existence of acid fast bacilli in laboratory waste samples out of 25 samples from government laboratories, whereas out of total 80 samples, collected from private laboratories, 8.75% samples were found positive for Z.N. smear test and 7.5% samples were found positive for AFB culture for 'Hooghly' district. The Z.N. smear test confirmed 6.67% positivity for acid fast bacilli which was established by 10% AFB culture positive test for samples collected from the government sector laboratories of 'Burdwan' districts. In private sector laboratories, out of total collected samples 5.71% samples showed positivity for Z.N. smear test and 4.29% samples showed positivity for AFB culture test. Mycobacterial findings of LW-sputum for AFB by Z.N. staining was higher in 'Hooghly' district than of 'Burdwan' district in both government and private sector, which reflected the same pattern in case of AFB culture positive reports in the said district. In 'Burdwan' district incident of AFB culture positivity raised a higher position than AFB smear positive cases in government and private sector of both districts. It may be a result of higher attendance of the labour class tubercular patients of industrial areas, who belonged to 'Hooghly' district with a poor socio economic life style. Better result in 'Burdwan' district may be the outcome of good health awareness, knowledge and environment disseminated by the medical colleges of that district. Training in this respect is essential for the staff exposed professionally to clinical specimens and laboratory wastes. Risks of laboratory acquired infection (LAI) can be minimized among technical staff by practicing the standard precautions. Proper laboratory waste management is only possible through stringent follow of its standard procedures and rules.

Keywords: Laboratory waste management, Sputum Samples, Mycobacterium -Acid Fast Bacilli, transmission of pathogen, Hooghly and Burdwan district

INTRODUCTION

Mycobacterial diseases in India persists in such a magnitude that the national level RNTCP has been launched since 1997 with its activities in several wings [1] in public health sector. Huge amount of money and manpower are incorporated for controlling and prevention of the disease. Rising trend in tubercular infection with multi drug resistant (MDR) strains is alarming, especially for the workers involved in laboratory services and biomedical waste disposal activities [2, 3]. In the present study it was tried to observe the presence of AFB in used up sputum specimen generated as laboratory waste in the laboratory. At present public is better aware about the running national level prevention and control programmes for tuberculosis by the government's various awareness programmes including advertisements [4]. So, laboratory investigation and treatment for tubercular diseases is mainly dependent on government sector health care system. A small portion of the population is dependent on private sector for this purpose [5]. Patient's load is higher in government sector laboratory than private sector in respect of this regard. Sputum samples are examined either for AFB or other purposes. Unknowingly the specimens are handled without confirmation for presence of AFB by the untrained workers [4, 6]. Sometimes a positive growth reported in AFB culture from a smear negative sputum sample [7]. Hence, the entire sputum specimens are supposed to be potential for mycobacterial contaminations, which were advised for examination to exclude any respiratory tract infection.

Aerosol or droplets inhalation contaminated with AFB is the cause of pulmonary tuberculosis [8]. Proper handling and disposal of sputum specimen collected by the laboratories is essential unless that there is a great chance of spread of the causative agent in the surrounding community and environment. Sputum samples received by the laboratories were used for multipurpose examination by them and were collected by us in our present study before discarding and examined for AFB in two ways -1. Smear examination to obtain any AFB morphology and 2. Mycobacterial culture. Results may help the laboratory authorities, staffs, disposal authorities and the government health authorities related to the programmes for control and prevention of tuberculosis.

MATERIALS AND METHODS

To explore the risks of laboratory waste related environmental contamination (LW– mycobacteriology) sputum samples were collected from the disposed specimens of government and private laboratories of included two districts. The cross-sectional study was followed during the period of 2007 to 2012 after getting ethical clearance certificates issued by 'The Principal and Chairman, Institutional Ethics Committee, Medical College & Hospital', Kolkata, Vide Memo No.- 5105/1(2) dated 08.12.2006 and 'Institutional Ethics Committee, 'Vidyasagar University', Midnapore, Vide Memo No.- IEC/4/2/15 Dt. 19.6.15. It included randomly selected 21 laboratories (5 government sector laboratories and 16 private sector laboratories) from 'Hooghly' district and 20 laboratories (6 government sector laboratories + 14 private sector laboratories) from 'Burdwan' district. The data regarding government and private registered laboratories were taken from the 'Directory of Medical Institutions–West Bengal 2004' and from the records of the office of 'Chief Medical Officer of Health' (CMOH), 'Hooghly' and 'Burdwan'. Five (5) sputum samples were collected randomly from each selected laboratory after explanation of study protocol and taking written informed consent from the authority of the selected laboratories. The samples were provided without following any inactivation technique or treatment for disposal of laboratory waste. The phase included the microbiological examination of sputum for microscopy for AFB by Z.N. staining and for culture of AFB using LJ media.

Sputum for AFB provided by the laboratory was collected in sterile wide mouth screw cap plastic container, which was brought to our laboratory. An ideal smear with the sputum was prepared onto a new clean and grease free glass slide. It was fixed by flame heat and stained by ZN technique and examined under oil immersion lens of microscope to obtain any AFB [9] (**Plate 1**).

Standard 'Petrof's method' for concentration and decontamination was followed for AFB culture of sputum. The centrifuged deposit of treated sputum was inoculated onto egg based LJ media. Incubated up to six weeks with regular observation for any typical or atypical growth of mycobacteria on the surface of the media [9] (**Plate-2**). Smear prepared from that growth and stained by ZN technique followed by microscopical examination for acid fast bacilli. Reports were recorded as AFB culture positive on presence of AFB or in absence of any AFB recorded as negative.

Plate 1: Sputum smear stained by ZN technique and micrpohotography of ZN stained sputum smear showed AFB (⟶).

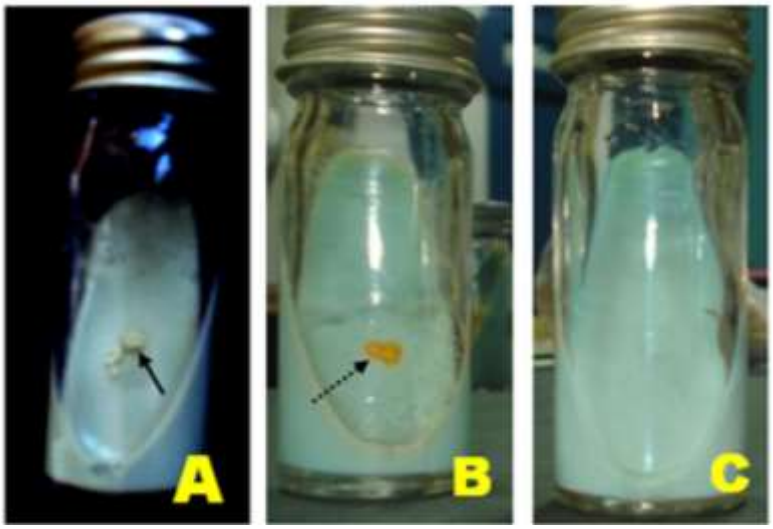

Plate 2: Mycobacterial growth in LJ Media. [A] Typical growth indicated by ➞ [B] Atypical growth indicated by ⇢ [C] No growth

Baseline data were prepared using laboratory analysis of samples, results obtained were analyzed and compared for significant difference between experimental groups following two-tail 't' test using standard statistical software (Statistica, Ver-6.0; Origin Lab, Ver-8.0; MS Excel, Ver-07).

RESULTS

The Z.N. smear test positivity for 24% (6) cases and AFB culture test positivity for 24% (6) revealed the existence of acid fast bacilli in laboratory waste samples (25 samples) from government laboratories of Hooghly district, where as out of total 80 samples, collected from private laboratories of 'Hooghly' district, 8.75% (7) samples were found positive for Z.N. smear test and 7.5% (6) samples were found positive for AFB culture (**Table 1, Figure 1**).

Table 1: Intra-district comparison considering government sector and private sector laboratories of 'Hooghly' district from the view point of LW (Sputum for AFB)

LW - Sputum for AFB (Hooghly) (N=105)						
	Government sector laboratories (5) Sample Collected = 25			Private sector laboratories (16) Sample Collected = 80		
	AFB Positive	AFB Negative	p-value	AFB Positive	AFB Negative	p-value
Z.N. Smear	24% (6 ± 0.18^a)	76% (19 ± 0.84^b)	<0.001	8.75% (7 ± 0.33^a)	91.25% (73 ± 3.26^b)	<0.001
AFB Culture	24% (6 ± 0.18^a)	76% (19 ± 0.84^b)	<0.001	7.5% (6 ± 0.31^a)	92.5% (74 ± 3.29^b)	<0.001

Each horizontal row represents percentage value and mean ± SEM within parenthesis for each group. Comparison was performed in between values of same category for same parameter. Analysis was performed by two tail't' test. Values of rows with different superscripts (a, b) differ from each other significantly at the level of p<0.001.

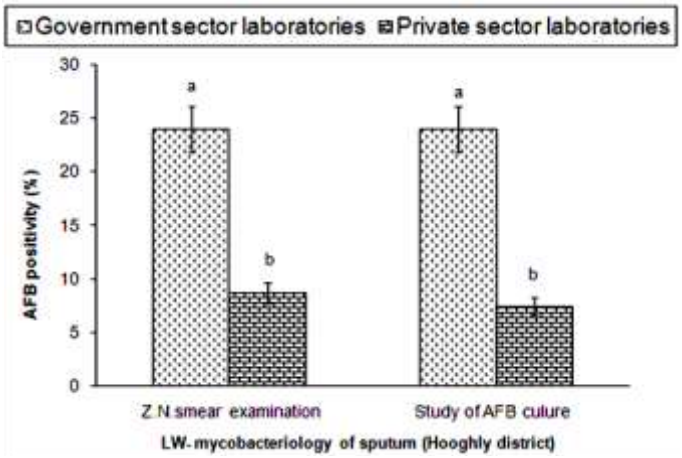

Fig-1: Mycobacteriological examination of LW sputum for AFB positivity and intra-district comparison considering government sector and private sector laboratories of 'Hooghly' district

Each bar represents mean ± SEM. Analysis was performed by two tail 't' test. Bars with different superscripts (a, b) differ from each other significantly at the level of p<0.001.

The Z. N. smear test confirmed 6.67% (2) positivity for acid fast bacilli which was established by 10% (3) AFB culture positive test out of 100% (30) LW sputa samples collected from the government sector laboratories of 'Burdwan' district. In private sector laboratories, out of total 100% (70) LW sputa samples 5.71% (4) samples showed positivity for Z.N. smear test and 4.29% (3) samples showed positivity for AFB culture test (**Table 2, Figure 6.2**).

Table 2: Intra-district comparison considering government sector and private sector laboratories of 'Burdwan' district from the view point of LW (Sputum for AFB)

LW - Sputum for AFB (Burdwan) (N=100)						
	Government sector laboratories (6) Sample Collected = 30			Private sector laboratories (14) Sample Collected = 70		
	AFB Positive	AFB Negative	p-value	AFB Positive	AFB Negative	p-value
Z.N. Smear	6.67% (2 ± 0.09^a)	93.33% (28 ± 1.25^b)	<0.001	5.71% (4 ± 0.18^a)	94.29% (66 ± 2.95^b)	<0.001
AFB Culture	10% (3 ± 0.10^a)	90% (27 ± 1.21^b)	<0.001	4.29% (3 ± 0.14^a)	95.71% (67 ± 2.99^b)	<0.001

Each horizontal row represents percentage value and mean ± SEM within parenthesis for each group. Comparison was performed in between values of same category for same parameter. Analysis was performed by two tail't' test. Values of rows with different superscripts (a, b) differ from each other significantly at the level of p<0.001.

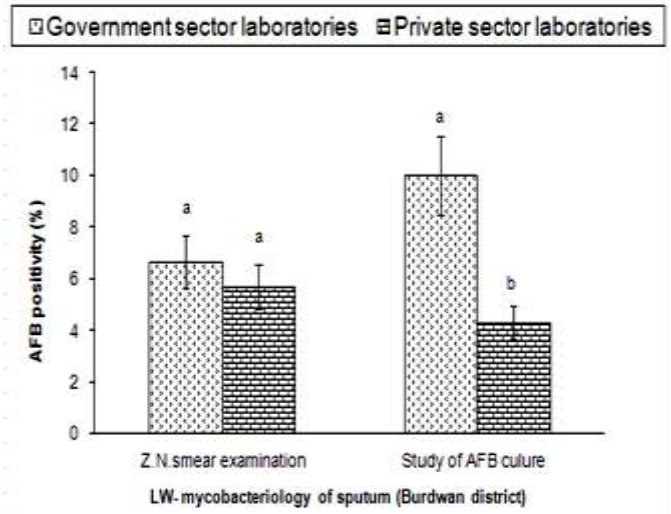

Fig-2: Mycobacteriological examination of LW sputum for AFB positivity and intra-district comparison considering government sector and private sector laboratories of 'Burdwan' district

Each bar represents mean ± SEM. Analysis was performed by two tail 't' test. Bars with different superscripts (a, b) differ from each other significantly at the level of $p<0.001$ and bars having same superscript (a) differ from each other significantly at the level of $p>0.05$

Mycobacterial findings of LW-sputum for AFB by Z.N. staining was higher in 'Hooghly' district than of 'Burdwan' district in both government and private sector , which reflected the same pattern in case of AFB culture positive reports in the said district. In 'Burdwan' district incident of AFB culture positivity raised a higher position than AFB smear positive cases in government sector.

Table 3: Inter district comparison of LW-mycobacteriology in private sector laboratories of 'Hooghly' and 'Burdwan' district

Comparison of LW– mycobacteriology in Government Sector Laboratories (Hooghly Vs Burdwan)						
	Hooghly (Positive)	**Burdwan (Positive)**	**p-value**	**Hooghly (Negative)**	**Burdwan (Negative)**	**p-value**
Z.N. Smear	24% (6 ± 0.18^{a})	6.67% (2 ± 0.09^{b})	<0.001	76% (19 ± 0.84^{a})	93.33% (28 ± 1.25^{b})	<0.001
AFB Culture	24% (6 ± 0.18^{a})	10% (3 ± 0.10^{b})	<0.001	76% (19 ± 0.84^{a})	90% (27 ± 1.21^{b})	<0.001
For 'Hooghly' district, sample collected = 25; For 'Burdwan' district, sample collected = 30						

Each horizontal row represents percentage value and mean ± SEM within parenthesis for each group. Comparison was performed in between values of same category for same parameter. Analysis was performed by two tail 't' test. Values of rows with different superscripts (a, b) differ from each other significantly at the level of $p<0.001$.

Table 4: Inter district comparison of LW-mycobacteriology in private sector laboratories of 'Hooghly' and 'Burdwan' district in respect of prevalence rate

Comparison of LW– mycobacteriology in Private Sector Laboratories (Hooghly Vs Burdwan)						
	Hooghly (Positive)	**Burdwan (Positive)**	**p-value**	**Hooghly (Negative)**	**Burdwan (Negative)**	**p-value**
Z.N. Smear	8.75% (7 ± 0.33^{a})	5.71% (4 ± 0.18^{b})	<0.001	91.25% (73 ± 3.26^{a})	94.29% (66 ± 2.95^{b})	<0.001
AFB Culture	7.5% (6 ± 0.31^{a})	4.29% (3 ± 0.14^{b})	<0.001	92.5% (74 ± 3.29^{a})	95.71% (67 ± 2.99^{b})	<0.001
For 'Hooghly' district, sample collected = 80; For 'Burdwan' district, sample collected = 70						

Each horizontal row represents percentage value and mean ± SEM within parenthesis for each group. Comparison was performed in between values of same category for same parameter. Analysis was performed by two tail 't' test. Values of rows with different superscripts (a, b) differ from each other significantly at the level of $p<0.001$.

LW- mycobacteriology of sputum for Z.N. smear showed highest positive result in 'Hooghly' district (12.35%) followed by 'Burdwan' district (6.0%) and international status (4.84%) [2,10]. LW-sputum for AFB culture result showed almost same picture of LW-sputum Z.N. smear examination except a minimum diminished positive percentage (11.4%) in 'Hooghly' district (**Table 5**).

Table 5: **Comparison of findings in 'Hooghly' and 'Burdwan' district in respect of international status from the view point of LW study (sputum for AFB in smear and in culture) with special emphasis on tuberculosis**

LW-sputum	International status	Hooghly district	Burdwan district
	%		
Z.N. Smear	4.84 ± 1.04^{a}	12.35 ± 2.11^{b}	6.0 ± 1.28^{c}
A.F.B. Culture	4.84 ± 1.04^{a}	11.4 ± 1.98^{b}	6.0 ± 1.27^{c}

Each row represents mean ± SEM for each group. ANOVA followed by multiple comparison two tail 't' test. Values of rows with different superscripts (a, b, c) differ from each other significantly at the level of $p<0.01$.

DISCUSSION

Probability of mycobacterial infection is more amongst the staff occupationally exposed to the contaminated laboratory waste of sputa samples either during analytical procedure or disposal of it. If not properly decontaminated or inactivated prior to disposal of the potentially positive samples may cause environmental pollution with a possibility of spreading of tuberculosis to the surroundings [11] (NCCLS, 1991). Incidence of tuberculosis in laboratory personnel is to be three to nine times than the individuals in other occupations [12, 13].

In our LW-mycobacterial study results revealed the higher incidence of AFB positivity in sputum microscopy in government sector (Table 1). It would be resulted from common people suffering from suspected tubercular diseases preferred the government sector laboratory to avail the diagnostic services as well as for free treatment with medication [14]. During the submission of sputum samples and new TB patient's attendance at DOTs clinic there is also a chance of spreading of infection to other patients and their associates through contaminated aerosol inhalation within the hospital or laboratory premises [15]. Higher positive incidence rate for AFB culture in 'Hooghly' district indicated the constant viability of mycobacteria in sputum specimen submitted to the government laboratories for examination (Table 3 and 4). In private sector the said rate is slightly diminished that may be happened due to one sample did not show positive AFB culture for undergoing treatment with anti-tubercular drugs. Isolation rate was higher for AFB by culture than smear examination in both the district supported by the fact that AFB culture is the gold standard test and superior to smear examination [16]. Lesser sensitivity of smear examination for AFB than culture was significant in this respect. Inadequate effectiveness of the RNTCP could have reflected the higher positivity of AFB in 'Hooghly' district.

Reports obtained from the sputa samples from Burdwan district showed a better result with lower incidence for both the test parameters of AFB microscopy and AFB culture (Table 2). The result indicated a better awareness, prevention and control in respect of tuberculosis which also lowered the chances of environmental pollution from the LW-sputa specimens [17].

Efficacy of RNTCP might have resulted better in Burdwan than of 'Hooghly' district. From this view point strict follow up of proper bio-safety and disposal methods for sputum and other laboratory wastes should be maintained. Recommended inactivation technique for the DOTs clinics might be effective partially [15], which should be maintained to prevent the spread of infection from the LW-sputum. To achieve the goal of eradication of tuberculosis proper disinfection and disposal of laboratory wastes may play a greater role in future [1, 18].

LW-sputum for mycobacteriology indicated the higher incidence for both Z.N. smear and culture positive which was lesser in the developed countries. Low socioeconomic life style and poor health awareness for tuberculosis leads to discontinuation of treatment may be the cause for ours. However, the opposite pictures were sustained in the developed countries which leaded a lower result for AFB (Table 5) [19, 20].

CONCLUSION

Inter district comparison showed though insignificant difference between two districts, still relatively a higher rate of positivity was found in

'Hooghly' district. It may be a result of higher attendance of the labour class tubercular patients of industrial areas, who belonged to 'Hooghly' district with a poor socio economic life style. Better result in 'Burdwan' district may be the outcome of good health awareness, knowledge and environment disseminated by the medical colleges of that district. District health authorities could play an effective role for this better picture in their district. Training in this respect is essential for the staff exposed professionally to clinical specimens and laboratory wastes. Immune status of the working staffs also to be assessed at a regular interval, which may help to control the laboratory waste associated infection. Molecular level detection for presence of mycobacteria in laboratory waste may develop an earlier initiation to prevent the chances of environmental pollutions. Introduction of automation for laboratory waste handling and management may the alternative avenue for controlling of unwanted spreading of infections.

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