

## Original Article

**EVALUATION OF BACTERIOLOGICAL DIAGNOSIS OF SMEAR POSITIVE PULMONARY TUBERCULOSIS UNDER PROGRAMME CONDITIONS IN THREE DISTRICTS IN THE CONTEXT OF DOTS IMPLEMENTATION IN INDIA**C.N. Paramasivan<sup>1</sup>, Pratibha Narang<sup>2</sup>, G. Dakshayani<sup>3</sup>, V. Chandrasekaran<sup>4</sup> and P. Venkataraman<sup>5</sup>*(Original received on 6.3.2006. Revised version received on 27.4.2006. Accepted on 2.5.2006)***Summary**

**Objective:** To study the smear and culture positivity rates in pulmonary tuberculosis patients declared as smear positive in the districts of North Arcot (Tamil Nadu), Raichur (Karnataka) and Wardha (Maharashtra) in India in order to evaluate the diagnosis of pulmonary tuberculosis at the field level under programme conditions.

**Methods:** Two specimens of sputum from each of 320 patients in North Arcot, 314 patients in Raichur and 302 patients from Wardha district, all of whom had been reported as smear-positive at the field level, were examined by smear and culture.

**Findings:** The proportion of specimens found to be smear-negative was 4.7% in North Arcot and 5.7% in Raichur as against 38.7% in Wardha. The proportions of culture negative specimens were 5.7% and 6.3% respectively in North Arcot and Raichur, while it was 35.6% at Wardha. The difference in the smear and culture negativity between Wardha and the other two districts was highly significant.

**Conclusions:** The study revealed an unacceptably high level of false positives in sputum smear microscopy in the Wardha district. This could be attributed to the absence of systematic and intensive training in smear examination consequent to the non-implementation of the DOTS strategy in this district and a high standard of training offered in the RNTCP implemented districts. [*Indian J Tuberc* 2006; 53:196-200]

**Key Words:** Pulmonary Tuberculosis; Smear and Culture Findings; India

**INTRODUCTION**

Bacteriological examination of sputum by smear and culture are the two most important methods in undertaking drug resistance surveillance studies. While direct sputum smear microscopy helped in identifying the infectious cases, culture of sputum provided definite diagnosis of tuberculosis by establishing the viability and identity of the isolates, at the same time yielded viable cultures for drug susceptibility testing.

A recent publication from this Centre reported the drug resistance patterns in two districts of South India, viz., North Arcot in Tamil Nadu and Raichur in Karnataka, using internationally accepted guidelines as well as standardized methods<sup>1</sup>. This centre also undertook drug resistance surveillance (DRS) study in the Wardha district of Maharashtra.

The bacteriological findings on the patients from these three districts are described in this report.

**METHODS****Organization and intake**

The study was undertaken in the composite districts of North Arcot (Comprising Vellore and Tiruvannamalai districts, population 4.5 million), in Tamil Nadu, Raichur, (including the districts of Raichur and Koppal, population 1.8 million) in Karnataka and in the Wardha district (population 1.2 million) in Maharashtra. In all, a total of 23 diagnostic centres in North Arcot, 20 centres in Raichur and 25 centres in Wardha participated in the study. The study in North Arcot district was undertaken during February-April 1999, in July-December 1999 in Raichur and August 2000-May 2001 in Wardha

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district. All newly diagnosed patients (including children under 15 years of age) were included if, during the intake period, they were sputum smear-positive on at least one occasion.

Other procedures such as sample size, training and supervision methodology as well as specimen collection and transportation were undertaken as described earlier<sup>1</sup>. Identical procedures were employed in Wardha district also. In brief, sputum was collected from all eligible patients; smears were made and examined by the Ziehl-Neelsen (Z-N) method. If the smear was positive, two additional specimens of about 5 ml each were collected in universal containers with 5 ml of 1% cetyl pyridinium chloride (CPC) and 2% sodium chloride (NaCl) solution. These specimens were transported to the Tuberculosis Research Centre (TRC), with minimal delay, for bacteriological investigations.

In total, 635 specimens were collected from 320 patients in North Arcot district, 617 from 314 in Raichur district and 587 from 302 in Wardha district.

**Bacteriological investigations**

All the laboratory investigations were carried out at the TRC at Chennai, as per WHO/IUATLD guidelines<sup>2</sup>. Direct smears were made and stained by auramine-phenol and examined by fluorescence

**Table 1:** Smear results of all specimens tested

Smear grade	North Arcot		Raichur		Wardha	
	No.	%	No.	%	No.	%
Negative	30	4.7	35	5.7	227	38.7
1+	520	81.9	457	74.1	314	53.5
2+	83	13.1	106	17.2	45	7.7
3+	2	0.3	19	3.1	1	0.2
<b>Total</b>	<b>635</b>		<b>617</b>		<b>587</b>	

microscopy<sup>3</sup>. Positive smears were graded as 1+, 2+ and 3+. If the direct smear was negative, a second smear was made from the concentrated sputum deposit. This was done since CPC containing sputum specimens being viscous in nature some times did not adhere firmly to the slides when direct smears were made and has a tendency to get washed off while staining. For culture, the specimens were directly centrifuged, the deposit suspended in about 20 ml sterile distilled water, mixed and recentrifuged. The resultant deposit was inoculated onto two slopes of Lowenstein-Jensen (L-J) medium and also one slope of L-J enriched with sodium pyruvate. The slopes were incubated at 37°C and read at weekly intervals for eight weeks. Positive cultures were graded as 1+ (20-100 colonies), 2+ (more than 100 colonies) and 3+ (confluent growth). Actual colony counts were recorded if the growth was less than 20 colonies. Growth of *Mycobacterium tuberculosis* was identified, based on the niacin production test, catalase activity at 68°C/pH 7.0) and susceptibility to p-nitro benzoic acid<sup>4,5</sup>.

**RESULTS**

**Smears**

Of the 635 specimens from North Arcot district, 30 (4.7%) were found to be smear-negative (Table 1). The corresponding figures for Raichur and Wardha district were 35 out of 617 (5.7%) and 227 of 578 (38.7%) respectively. The proportion of smear-negative specimens was significantly higher at Wardha ( $P<0.001$ ) than either of the other two districts. It was also observed that a majority of specimens, 82% from North Arcot, 74% from Raichur and 54% from Wardha, were in the 1+ smear grade. Duplicate smears from the same patient yielded identical grades in 79% of the patients in North Arcot, 69% in Raichur and 71% from Wardha. Concordance in results (within ± 1 grade), was observed in 92% of North Arcot patients, 89% in Raichur and 79% among the patients from Wardha (results not tabulated).

**Cultures**

The proportion of culture negative specimens was 5.7% in North Arcot and 6.3% in

**Table 2:** Culture grades of all specimens tested

Culture grade	North Arcot		Raichur		Wardha	
	No.	%	No.	%	No.	%
Negative	36	5.7	39	6.3	209	35.6
Cols.	42	6.6	40	6.5	65	11.1
1+	63	9.9	103	16.7	101	17.2
2+	281	44.2	165	26.7	77	13.1
3+	166	26.1	245	39.7	114	19.4
Cont.	47	7.4	25	4.0	21	3.6
Total	635		617		587	

Raichur as against 35.6% in Wardha, a highly significant difference ( $P < 0.001$ ) (Table 2). The proportion of cultures lost due to contamination ranged between 3.6-7.4% in the three districts. Of the total number of culture-positive specimens, as many as 81% from North Arcot and 74% from Raichur had yielded moderate to confluent growth (2+/3+ grades) while this proportion was lower (54%) at Wardha. The culture results of duplicate specimens revealed a high level of agreement in all districts (results not tabulated). The number of patients with both cultures negative was 11 (3.4%) in North Arcot, 14 (4.5%) in Raichur as against 79 (26.2%) at Wardha. The proportion of patients lost due to contamination of both specimens was 1.9% at North Arcot and at Raichur while it was 1.0% at Wardha (not tabulated).

#### Smear vs. Culture

A distribution of all specimens according to smear and culture in the three districts revealed that in North Arcot district 10 (1.8%) of the 546 culture-positive specimens were smear-negative. Similar figures were observed in Raichur district also where 14 (2.5%) of the 553 culture-positive

specimens were smear-negative. The corresponding figures for Wardha were 14 (13.2%) of 357 (results not tabulated). The proportion of specimens that were smear-positive and culture-negative was identical (3.6%) in North Arcot and Raichur districts. However, the corresponding proportion for Wardha district was higher, viz., 9.2%.

#### DISCUSSION

The present investigation on the bacteriological findings in DRS studies in three districts of India has yielded valuable information in the context of diagnosis of tuberculosis at the field level, under programme conditions.

The districts, where the DRS studies were undertaken, were at different stages of the implementation of the Revised National Tuberculosis Control Programme (RNTCP) incorporating DOTS strategy. The district of North Arcot was one of the first to implement RNTCP while at Raichur the personnel had undergone training in sputum microscopy although RNTCP was not implemented at the time this investigation was carried out. The district of Wardha has neither implemented the RNTCP nor has the staff been trained intensively but were carrying out routine tuberculosis control programme that has been in existence for more than four decades.

Considering the smear results from the districts in the light of the above, the proportion of specimens reported as smear-negative from North Arcot and Raichur was 4.7% and 5.7% respectively. Amalgamating the two districts, a total of 65 specimens (5.2%) out of 1252 specimens were reported as smear-negative. It should be emphasized that the specimens sent to TRC had not been examined by microscopy at the diagnostic centres as per the global DRS protocol although an earlier specimen from the same patients had been found to be smear-positive. Thus, the discrepancies could have been due to inherent differences between specimens from the same patient. This was substantiated by examining the results of duplicate specimens taken from same patients. Thus, of the 33 patients from whom the 65 specimens had been obtained, only 14

had, both smears, reported as negative. For the remaining, the other specimens were smear-positive. The near 95% agreement in smear positivity between the diagnostic centres and TRC where smears were examined by fluorescence microscopy showed the high level of smear microscopy efficiency at the peripheral laboratories which have only minimal infrastructure and manpower. In contrast, 38.7% of specimens from Wardha district, from reportedly smear-positive patients were found to be smear-negative at TRC. Further, of the 302 patients, as many as 87 (28.8%) had both smears reported as negative. These need to be considered as unacceptably high level of false-positives at the peripheral centres.

The findings with the smear result were also reflected in the culture results, the proportion of culture-negative specimens being 5.7% and 6.3% respectively in the districts of North Arcot and Raichur. Further 36 (55%) of the 65 smear-negative specimens were negative on culture also. The proportion of patients with both cultures-negative was only 3.5% in North Arcot and 4.5% in Raichur. The proportion of patients lost due to contamination of both specimens was less than 2% in either district. In the case of Wardha district, 209 (35.6%) of the specimens were culture-negative. Of these 176 (84%) were also reported as smear-negative. The number of patients with both cultures negative was 79 (25.2%). Only 1% of them were lost due to contamination of both specimens. Such high rates of culture positivity in smear-positive specimens and low contamination rates highlight the utility of the novel inexpensive transportation method used<sup>1</sup>. Out of over 3000 specimens received in such studies to-date at this centre, only one bottle was broken in transit and total leakage of specimens were less than 10 occasions.

However, an aerosol spread was contained with an inbuilt procedure employed in transportation. The majority of specimens from North Arcot were received within 2-3 days while it took 4-6 days from Raichur. The specimens from Wardha, being the farthest from Chennai, required 5-10 days for receipt. However, the viability of the organisms was not affected by the delay due to the use of CPC/NaCl as

a transport agent.

**The findings in the present study, as observed by the differences in smear and culture positivity rates in the RNTCP trained/implemented districts of North Arcot and Raichur on the one hand and the non-RNTCP implemented district of Wardha revealed the extraordinary standard of systematic and intensive training in case selection and diagnosis by sputum smear microscopy, that were undertaken in the DOTS implemented districts in India.** This training which was followed-up by effective monitoring and quality assurance studies by the supervisory personnel sustained high standards at all levels. A rapid scale-up in the implementation of RNTCP in India in recent years yielded better diagnosis and reliable as well as reproducible bacteriological results. As on date, the DOTS programme in India is the second largest and the fastest developed programme in the world, covering almost India's one billion populations<sup>6-8</sup>.

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#### REFERENCES

1. Paramasivan CN, Venkataraman P, Chandrasekaran V, Shripad Bhat, Narayanan PR. Surveillance of drug resistance in tuberculosis in two districts of South India. *International Journal of Tuberculosis and Lung Disease* 2002; **6**:479-484.
2. WHO/IUATLD Global Working Group on Antituberculosis drug resistance surveillance. *Guidelines for surveillance in tuberculosis*. WHO/TB/96.216. Geneva: WHO, 1997.
3. Holst E, Mitchison DA, Radhakrishna S. Examination of smears for tubercle bacilli by fluorescence microscopy.

4. Allen B, Baker RJ. In: *Mycobacteria: isolation, identification and sensitivity testing*. London, UK: Butterworth, 1968: p 17.
5. Kubica GP. Differential identification of mycobacteria. VII: key to features for identification of clinically significant mycobacteria. *American Review of Respiratory Diseases* 1973; **107**: 9-21.
6. Progress towards tuberculosis control, India, 2001. *Weekly Epidemiological Records* 2002; **77(12)**: 89-93.
7. World Health Organization. *Joint tuberculosis programme review, India, February 2000*. WHO SEA.TB.224, 5 May 2000.
8. Khatri GR, Frieden TR. Controlling tuberculosis in India. *New England Journal of Medicine* 2002; **347**: 1420-1430.

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**EXPEDITE DESPATCH OF ABSTRACT/S OF PAPER/S FOR 61<sup>ST</sup>  
NATIONAL CONFERENCE ON TUBERCULOSIS AND CHEST  
DISEASES - UDAIPUR**

All delegates are informed that the 61<sup>st</sup> National Conference on Tuberculosis and Chest Diseases will be held at RNT Medical College, Udaipur, from 23rd to 25th February, 2007. Exact dates of the Conference will be intimated shortly. Delegates who have not submitted their abstract/s of paper/s for presentation at the Conference may kindly do so by 15<sup>th</sup> November, 2006. The registration-cum-brochure forms will be despatched in due course.

Kindly expedite abstract/s of paper/s and help the organizers in making the Conference a grand success.

Dr. M.M. Singh  
Vice Chairman & Editor, IJT