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Adequacy of examining one sputum specimen in tuberculosis drug resistance surveys

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ABSTRACT

Objective/background: Collection of one spot and one morning sputum specimen is recommended for tuberculosis (TB) drug resistance surveys. This was a retrospective analysis of *Mycobacterium tuberculosis* cultures isolated from two spot sputum specimens collected from smear positive TB patients in a TB drug resistance survey. It was conducted to understand the value of a second specimen.

Methods: A TB drug resistance survey was conducted in the state of Tamil Nadu, India, to estimate the prevalence of drug resistance among new sputum smear-positive (NSP) and previously treated (PT) patients diagnosed in Revised National Tuberculosis Control Program microscopy centers. A total of 2425 patients (1524 NSP and 901 PT cases) were enrolled in the study. From these patients, two spot sputum specimens (*C* and *D*) were collected within a period of 2 h. No preservative was added to sputum. The samples were transported at ambient conditions without cold storage to the central laboratory for culture of *M. tuberculosis*. Culture yield from each sample was computed and analyzed.

Results: The proportion of cultures retrieved from *C* and *D* specimens among NSP cases (89.3% and 89.7%) and PT cases (90.8% and 90.3%) were similar. The culture grades of *C* and *D* samples were comparable (chi-square test, 3560.135; $p < .001$) and the agreement was moderate (kappa test, 0.454).

Conclusion: The findings of the study reveal the adequacy of single spot sputum specimen from smear positive pulmonary TB patients for bacteriological examination in a quality-assured TB laboratory to determine precisely the level of drug resistance in a province of India.

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Introduction

Bacteriological examination of sputum specimens is considered the most appropriate method for the definitive diagnosis of pulmonary tuberculosis (PTB) and detection of drug resistance caused by *Mycobacterium tuberculosis*. Its components—microscopy, culture methods, and drug-susceptibility testing (DST) for anti-TB drugs—are established and are being scaled up in many of the national TB control programs all over the world for case detection, monitoring, and treatment, and for drug-resistance surveillance. However, the number of sputum specimens to be collected from PTB patients, the logistics, including the biosafety, involved in ensuring their transportation to the central laboratories, and the cost of laboratory investigations constitute a huge financial burden for TB program managers trying to accomplish their goals of adequate health care services to TB patients. The World Health Organization (WHO) and the International Union against Tuberculosis and Lung Diseases initially recommended three sputum specimens in an algorithm of Spot–Morning–Spot for the diagnosis of PTB by sputum smear microscopy [1]. They revised their decision and recommended the use of two specimens in an algorithm of Spot–Morning for diagnosis of TB after reviewing extensive data on the contribution of a third specimen for case detection [2]. Other laboratory investigations have already shown the adequacy of a single sputum specimen for diagnosis of PTB by Ziehl–Neelsen smear microscopy [1,3,4]. Recently, the findings of a study in India have informed the policy makers of the utility of using a single specimen in bacteriological profiling of multidrug-resistant TB patients during follow up investigations [5]. However, for TB drug resistance surveys (DRSs)/surveillance, WHO has until now recommended two sputum specimens for culture and drug susceptibility of *Mycobacterium tuberculosis*, which has been followed in several surveys [6–8]. In this retrospective analysis of the data, collected in a DRS conducted in Tamil Nadu, India, following the WHO recommended guidelines, the inference of adequacy of a single sputum specimen for the bacteriological investigations in DRSs is presented and discussed.

Materials and methods

A TB DRS, based on cross-sectional cluster survey as recommended by World Health Organization [6], was conducted in the state of Tamil Nadu, India, in 2011 to estimate the prevalence of drug resistance among new sputum smear-positive (NSP) and previously treated (PT) cases diagnosed in Revised National Tuberculosis Control Program microscopy centers [8]. Its sample size was calculated based on the TB DRS data available in one of the provinces in India, Gujarat (prevalence of multidrug resistant-TB, 2% among NSP and 12% among PT cases) [7], and it was estimated to be 1,680 for NSP and 992 for PT cases, with 50% precision, 10% loss, and a design effect of 2.

Prior to the survey, training was given to all staff in the periphery on all aspects of the survey, including sputum collection, safe package and transportation of specimens, and documentation of information in the clinical information form. From each sputum smear-positive PTB patient, diagnosed under the programmatic conditions, two additional spot sputum specimens, collected within a period of 2 and 3 h (Specimens *C* and *D*), were transported, using existing courier services, to the central laboratory (NIRT, Chennai, India). The sputum specimens were not preserved with Cetyl Pyridinium Chloride (CPC) and were transported under ambient conditions. The specimens were processed by modified Petroff's method and cultured on solid Löwenstein–Jensen (LJ) media. The culture isolates were subjected to DST by 1% proportion method (economic variant) on LJ medium as per the WHO guidelines. NIRT is being continuously monitored externally by the coordinating supranational TB reference laboratory at Antwerp, Belgium, to ensure the quality of mycobacterial culture and DST. The quality and performance indicators in the laboratory were monitored continuously on a routine basis as described before [9].

The data were entered into a Microsoft Excel (Version 11.0 developed by Microsoft for Windows) spreadsheet and cross verified by a statistician. The number of *C* and *D* specimens received was enumerated and, the proportion of cultures retrieved, contaminated and nontuberculous mycobacteria isolated from NSP and PT cases, were calculated. A *Z*-proportion test was done to determine the significance of observed differences between the proportions. A two-way table, comparing culture grades of paired specimens from 1518 NSP and 894 PT patients, was created after excluding patients who produced a single specimen. Kappa statistics was performed to find the agreement between *C* and *D* specimens. Chi-square and *Z* proportion tests were performed to determine the significance of differences between *C* and *D* specimens.

Ethical statement

Informed patient consent was obtained from all the study participants. The permission for the retrospective analysis of the data was obtained from the institutional ethical committee.

Results

A total of 2425 patients (1524 NSP and 901 PT cases) were enrolled in the study. Of these, six NSP and seven PT cases, were excluded as they produced single sputum specimen. As the numbers excluded were very small, the pairs of samples from the remaining 1518 NSP and 894 PT cases (2412 cases in total) were included for the present comparison.

The proportion of cultures retrieved from *C* and *D* specimens among NSP cases (89.3% and 89.7%) and PT cases (90.8% and 90.3%) were very high. The numbers of *M. tuberculosis* isolated, contaminated cultures and nontuberculous mycobacteria isolated from *C* and *D* specimens were not significantly different among NSP and PT (Table 1).

The quantitative comparison of culture results between *C* and *D* specimens, from NSP and PT cases, is given in Table 2.

Table 1 – Details of specimens received from patients in the survey.

	New smear positive cases		Z	Previously treated cases		Z
	C	D		C	D	
Specimens received	1524	1518	—	901	894	—
After exclusion of single specimens	1518	1518	—	894	894	—
Culture positive	1355 (89.3%)	1361 (89.7%)	.764	812 (90.8%)	807 (90.3%)	.746
Culture negative	105 (6.9%)	103 (6.8%)	.944	45 (5%)	42 (4.7%)	.830
Culture contaminated	53 (3.5%)	48 (3.2%)	.685	22 (2.5%)	35 (3.9%)	.107
NTM	5 (0.3%)	6 (0.4%)	.985	15 (1.7%)	10 (1.1%)	.420

Note. Z = proportion statistic test at 5% level of significance.

There was a very high degree of agreement between the C and D samples. The chi-square test showed a significant association (3560.135; $p < .001$) and kappa test showed a moderate agreement between C and D specimens (0.454; $p < .001$).

Discussion

The guidelines for drug resistance surveillance and testing in laboratories were developed as early as 1969 [10]. Later, to have the comparable global data on drug resistance, WHO framed guidelines to carry out a global drug resistance surveillance program through its collaborating centers for bacteriology of TB [6,11]. The guidance is to collect two sputum samples from smear-positive patients enrolled under national TB control programs and to perform DST in quality assured reference laboratories. Accordingly, in Tamil Nadu survey, two additional sputum specimens were collected from Ziehl–Neelsen-positive patients and transported to the central laboratory to carry out culture and susceptibility testing.

This retrospective analysis of DRS was done to determine the adequacy of single sputum specimen over the current recommendation of two (one spot and one morning) sputum specimens in the TB DRSs. It showed an insignificant contribution of second specimen (<2% additional yield) in isolation of *M. tuberculosis*. It is evident that it is redundant to collect and process as many as 2412 specimens to get additional yield of 2% isolates. Evidently, when adopting a single specimen, the workload will be decreased considerably in TB reference laboratories of the province, which have limited resources and capacity. The entire logistics involved in sputum package and transportation will also be minimized. The cost of processing specimens in the laboratory can be considerably reduced. The patient noncompliance and nonadherence to the study may also be reduced by resorting to single spot specimen. Nonetheless, the real value of a spot sputum specimen from smear-positive PTB patients for isolation and DST of *M. tuberculosis* is yet to be ascertained in an exclusive study. The guidance for two specimens was to avert the unexpected contamination/loss of specimens/loss of viability of cultures in the very complex TB survey procedures. This can be negated once the quality and performance indicators in the reference laboratory, where the DRS is to be performed, are ensured. Even if there is any shortfall due to loss of cultures and eventual loss of DST results, additional patients can be recruited to compensate the loss as is being practiced in DRS.

In a study in a remote setting, a successful recovery (94.4%) of *M. tuberculosis* was achieved with the BACTEC MGIT 960 system using a single sputum specimen, even though the specimens were stored for a long time in the field and sent to the reference laboratory without any chemical preservative or decontamination prior to transport [12]. The authors recommended studies in other resource-limiting settings to confirm their finding. They further suggested inclusion of solid culture media to augment recovery of *M. tuberculosis*. The present analysis revealed adequate recovery of (97%) of *M. tuberculosis* utilizing solid LJ media and using one sputum specimen.

Table 2 – Quantitative comparison of culture grades between C and D sputum specimens.

		C specimen							Total
		1+	2+	3+	Col	Neg	Cont	NTM	
D specimen	1+	183	81	29	31	13	8	1	346
	2+	92	287	197	9	6	17	0	608
	3+	43	172	878	6	8	16	1	1,124
	Col	33	11	6	19	18	3	0	90
	Neg	12	6	5	13	99	6	4	145
	Cont	14	8	29	3	4	23	2	83
	NTM	0	0	0	0	2	2	12	16
Total		377	565	1,144	81	150	75	20	2,412

Note. 1+ = growth of 20–100 colonies of mycobacteria on Löwenstein–Jensen medium (LJ); 2+ = growth of >100 colonies of mycobacteria; 3+ = confluent or innumerable growth of mycobacteria on LJ; Cont = contamination; Col = growth of 1–19 colonies of mycobacteria on LJ; Neg = negative; NTM = nontuberculous mycobacteria.
Chi-square statistics, 3560.135; $p < .001$; kappa agreement, 0.454; $p < .001$.

The following are the limitations of the analysis and should be considered with caution for careful interpretation: (1) the current survey utilized two spot samples; (2) the findings cannot be extrapolated to liquid culture (MGIT 960 system); and (3) the specimens must be transported to the laboratory with in 72 h from the time of collection. Delay in transportation lead to contamination which further leads to loss of culture positivity. In this survey, majority of specimens were transported within 48 h using the well-organized network of courier services in this province.

Despite all the merits and demerits described above, the outcome of this retrospective analysis of the data may inform the decision makers to review the data collected in similar surveys/surveillance and draw conclusions about the adequacy of single specimen for future DRs/surveillances.

Conclusion

The findings of the study reveal the adequacy of a single spot sputum specimen, without any preservative, from smear-positive PTB patients enrolled in a TB control program, for bacteriological examination in a quality assured TB laboratory to determine precisely the level of drug resistance in a province.

Conflicts of interest

All authors have no conflicts of interest to declare.

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