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Quality indicators in a mycobacteriology laboratory supporting clinical trials for pulmonary tuberculosis

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ABSTRACT

Background: Documentation of structured quality indicators for mycobacteriology laboratories supporting exclusively controlled clinical trials in pulmonary tuberculosis (PTB) is lacking.
Objective: To document laboratory indicators for a solid (Lowenstein–Jensen medium) culture system in a mycobacteriology laboratory for a period of 4 years (2007–2010).
Methods: The sputum samples, collected from PTB suspects/patients enrolled in clinical trials, were subjected to fluorescence microscopy, culture and drug sensitivity testing (DST). Data was retrospectively collected from TB laboratory registers and computed using pre-formulated Microsoft Office Excel. Laboratory indicators were calculated and analyzed.
Results: The number of samples processed in a calendar year varied from 6261 to 10,710. Of the samples processed in a calendar year, specimen contamination (4.8–6.9%), culture positives (78.4–85.1%) among smear positives, smear positives (71.8–79.0%) among culture positive samples, smear negatives among culture negative samples (95.2–96.7%), and average time to report DST results (76–97 days) varied as shown in parentheses.
Conclusion: Values of quality indicators in mycobacteriology laboratories supporting exclusively clinical trials of PTB have to be defined and used for meaningful monitoring of laboratories.

Introduction

The World Health Organization (WHO) declared Tuberculosis (TB) a global emergency in 1993. Although all efforts are being made to control the disease in high burden countries, the control of drug-resistant TB remains a big challenge [1]. The diagnosis of drug-resistant TB is achieved by the services rendered by the solid culture and drug susceptibility testing (DST) laboratories that are being scaled up throughout the world, apart from other rapid diagnostic methods [2]. The quality of these laboratory services has to be ensured in order to give maximum benefits to the patients. The quality indicators for diagnostic mycobacteriology laboratories have been recently suggested by McCarthy et al. [3], and the TB laboratory registers have been designed to collect such performance indicators.

Efficacies of various treatment regimens for PTB are being assessed in controlled clinical trials. These controlled clinical trials are ably supported by mycobacteriology laboratories. The TB laboratory registers in these mycobacteriology laboratories supporting exclusively controlled clinical trials are presumably not standardized, and each and every laboratory may have designed their own TB laboratory registers to record

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the observations made in the laboratory. The quality of mycobacteriology laboratory indicators, although monitored by the investigating teams, is not well documented.

The National Institute for Research in Tuberculosis (NIRT), Chennai, which is a National Reference Laboratory under the Revised National Tuberculosis Control Program (RNTCP), India, as well as the Supra-national Reference Laboratory for the South East Asian region under the World Health Organization (WHO) is supporting controlled clinical trials for PTB and extra-PTB since 1956, and the TB laboratory registers designed then are being used continuously [4]. In this study, an attempt is made to compile the information available in TB laboratory registers in the mycobacteriology laboratory at NIRT supporting exclusively controlled clinical trials for 4 years to get some insight into the quality indicators for sputum AFB microscopy and culture of *Mycobacterium tuberculosis*.

Materials and methods

Study subjects

The NIRT conducts controlled clinical trials for pulmonary tuberculosis (PTB) [5,6]. It investigates new smear-positive and previously treated PTB patients referred from hospitals in and around Chennai city for inclusion in clinical trials. It obtains informed consent from all patients prior to enrollment into the studies. Studies conducted by the NIRT are approved by the Institutional Ethics Committee.

Sample collection and processing

Sputum specimens, both spot (S) and early morning home collection (H) in about 5 ml, were collected from all registered patients. Four sputum samples viz. two "S" and two "H", were collected from all patients before initiating treatment; one "S" and two "H" samples were collected from patients during the treatment period and one "H" and one "S" were collected once in 3 months from patients during the follow-up period ranging from 2 to 5 years depending on the study. The sputum samples, with occasional exceptions, were processed on the same day or on the next working day. Their direct smears were subjected to fluorescence microscopy and were processed by modified Petroff's method for culture by standard operating procedures followed at the NIRT [7]. Processed deposits were inoculated onto two of Lowenstein–Jensen (LJ) medium and cultures were incubated at 37 °C. A weekly growth reading was taken for 8 weeks and culture grading was recorded. Biochemical tests, such as niacin and catalase tests, and growth on para-nitrobenzoic acid were performed to confirm *M. tuberculosis* was isolated. The date on which DST was set up got noted against the culture selected for testing.

Table 1 – Distribution of smear and culture results.

Year	Smear results[a]	Culture results[b]								
		3+	2+	1+	Cols	Any positive	NEG	Cont	NTM	TOTAL
2007	3+	69	43	6	0	118	2	2	0	122
	2+	213	188	36	8	445	16	5	9	475
	1+	170	529	234	94	1027	183	11	50	1271
	Any positive	452	760	276	102	1590	201	18	59	1868
	NEG	13	163	270	180	626	7551	191	474	8842
	Total	465	923	546	282	2216	7752	209	533	10710
2008	3+	69	34	1	0	104	5	0	0	109
	2+	214	146	31	15	406	21	10	5	442
	1+	179	429	313	103	1024	258	20	29	1331
	Any positive	462	609	345	118	1534	284	30	34	1882
	NEG	10	86	177	143	416	6445	143	310	7314
	Total	472	695	522	261	1950	6729	173	344	9196
2009	3+	44	34	3	0	81	2	0	0	83
	2+	121	155	44	13	333	13	3	1	350
	1+	86	299	196	91	672	206	12	20	910
	Any positive	251	488	243	104	1086	221	15	21	1343
	NEG	3	50	196	102	351	5017	108	305	5781
	Total	254	538	439	206	1437	5238	123	326	8467
2010	3+	29	12	4	1	46	4	2	0	52
	2+	144	119	40	15	318	39	0	3	360
	1+	77	216	196	78	567	187	2	19	775
	Any positive	250	347	240	94	931	230	4	22	1187
	NEG	10	25	117	95	247	4555	42	230	5074
	Total	260	372	357	189	1178	4785	46	252	6261

[a] 1+ = 4 AFB in at least 50 fields or <5 AFB/field in at least 50 fields; 2+ = 5–100 AFB per field: 3+ = >100 AFB per field.

[b] Cols = 1–19 colonies; 1+ = 20–100 colonies; 2+ = innumerable colonies; 3+ = confluent growth.

Table 2 – Performance of quality indicators in the mycobacteriology laboratory.

Indicator	Year			
	2007	2008	2009	2010
Total no. of specimens	10710	9196	8467	6261
M. tuberculosis positive	2216	1950	1437	1178
M. tuberculosis negative	7752	6729	6581	3368
Contaminated samples among total samples	742/10710 (6.9%)	517/9196 (5.6%)	449/8467 (5.3%)	298/6261 (4.7%)
Bacterial contamination among total contaminated samples	209/742 (28.1%)	173/517 (33.5%)	123/449 (27.4%)	46/298 (15.4%)
Non Tuberculosis Mycobacteria (NTM) among total contaminated samples	533/742 (71.9%)	344/517 (66.5%)	326/449 (72.6%)	252/298 (84.6%)
Smear positives among total contaminated samples	77/742 (10.4%)	64/517 (12.4%)	36/449 (8.0%)	26/298 (8.7%)
Smear positives among samples contaminated with bacteria	18/209 (8.6%)	30/173 (17.3%)	15/123 (12.2%)	4/46 (8.7%)
Smear positives among samples contaminated with NTM	59/533 (11.1%)	34/344 (9.89%)	21/326 (6.4%)	22/252 (8.7%)
M. tuberculosis positives among smear positive samples (Recovery rate)	1590/1868 (85.1%)	1534/1882 (81.5%)	1086/1343 (80.8%)	931/1187 (78.4%)
M. tuberculosis among smear negative samples (Recovery rate)	626/8842 (7.1%)	416/7314 (5.7%)	351/5781 (6.1%)	247/5074 (6.8%)
Smear positives among *M. tuberculosis* positive samples (Sensitivity)	1590/2216 (71.7%)	1534/1950 (78.7%)	1086/1437 (75.6%)	931/1178 (79.0%)
Smear negative among *M. tuberculosis* negative samples (Specificity)	7551/7752 (97.4%)	6445/6729 (95.8%)	6360/6581 (96.6%)	4555/4785 (95.2%)
Average turnaround time (days) for DST	97	88	80	76

Documentation and analysis

Once the culture results (*M. tuberculosis*, contamination with bacteria, and contamination with NTM) were recorded by the laboratory personnel in registers, all the details of the specimens in the registers were transferred immediately, again with occasional exceptions, to the patients' case sheets for clinical management.

The following information for each specimen was collected from the registers: laboratory number, smear and culture results, and the dates on which DST was set up and reported. The data was entered in pre-formulated Microsoft Office Excel program. The laboratory performance indicators, such as percentages of specimen contaminated with bacteria and NTM among samples processed in a calendar year, culture positivity among smear positive samples, smear negativity among culture-negative samples and average turnaround time (from the date of collection of the sample until the DST results were reported) were calculated and analyzed as suggested by McCarthy et al. [3].

Results

Table 1 shows the details of the samples received and the distribution of smear results against their corresponding culture results. A total number of 34,634 samples were processed. The maximum number received was in the year 2007 and there was a gradual decline in the number of samples received in the subsequent years.

The performance of quality indicators for a period of 4 years (2007–2010) in the mycobacteriology laboratory are given in Table 2. The overall contaminated samples among the total samples processed in a year varied from 4.7% to 6.9%. Among the total contaminated, the specimen contaminated with bacteria (15.4–33.5%) and the specimen contaminated with NTM (66.5–84.6%) varied as shown in parenthesis. Culture-positives among smear-positive samples (rate of recovery) ranged between 78.4% and 85.1%. Smear-positives among culture-positive samples (sensitivity of microscopy) varied from 71.7% to 79.0% and smear-negatives among culture-negative samples (specificity of microscopy) varied from 95.2% to 97.4%. Average turnaround time for doing DST ranged from 76 to 97 days.

Discussion

During the study period, there was a decline in the number of samples processed during a calendar year. This was due to the decline in the number of patients referred from health posts in and around Chennai city for assessment to enroll in clinical trials: 2007 – 342; 2008 – 279; 2009 – 254; and 2010 – 83.

In the study, the recovery of *M. tuberculosis* from smear-positive samples varied from 78.4% to 85.1%. The reduced recovery from smear-positive samples, as observed in the present data analysis, could be attributed to the inclusion of diagnostic and follow-up samples. In the TB laboratory register, the information concerning whether the sample is for diagnostic or for follow-up examination is not recorded in order to avoid bias in investigations. Moreover, the diagnostic

samples in the study include those from patients who were assessed and excluded for several reasons, including the history of previous treatment for more than a month. It is to be pointed out that the proportion of smear-positive samples yielding culture-positive results is known to be reduced in patients currently being treated [8,9]. It is also known that, in patients receiving a rifampicin-containing regimen, around 20–25% of smear-positive samples can yield culture-negative results in samples collected during the follow-up period [10].

The sensitivity of microscopy varied from 71.7% to 79.0%. The reduced sensitivity of microscopy was again as a result of the inclusion of diagnostic and follow-up sputum samples from clinical trials. In a separate study conducted at the NIRT, the sensitivity of microscopy for diagnostic and follow-up samples for the same cohort of patients in a clinical trial respectively was found to be 95.0% and 60.0% [11]. However, this information was obtained from specially designed culture cards for individual patients.

The specimen contamination rate among the total samples processed ranged between 4.7% and 6.9% during the study period. The rate of contamination with NTM among the total contaminated samples varied from 66.5% to 84.6%. NTM could be environmental or laboratory contaminants, although their probable recovery from sputum samples cannot be ruled out. Unless NTM is recovered from the same patient in multiple samples and with heavy growth, it cannot be considered as atypical mycobacteriosis, and also this status cannot be ascertained from the TB laboratory registers unless efforts are taken to sort the data based on each patient [12]. The contamination with NTM among the total samples varied from 3.7% to 5.0%. It was reported from this laboratory that as much as 8–10% of sputum samples, collected from patients in TB surveys in epidemiological studies carried out in the Thiruvallur area, which is 40 kilometers away from the present study area, yielded NTM [13]. The low recovery of NTM in the study suggests that NTM is not common in the controlled clinical study area, or it could be due to selective populations studied.

Turnaround time (TAT) for MIC method is 70 days (6 weeks for culture isolation and 4 weeks for DST). The average TAT for DST varied between 76 and 97 days. The delay in TAT in 2007 and 2008 could be owing to a large number of cultures selected and batched for DST in the laboratory. It improved over the years when the sample load decreased. It should be mentioned that cultures from diagnostic samples and from relapses were subjected to DST on priority. The cultures from follow-up samples were batched and DST was set up.

In the present analysis, performance indicators reveal the quality of laboratory processes. McCarthy et al. (2008) [3] earlier collected the data from four regional and national TB reference laboratories in Bangkok, Thailand, and analyzed the data using the set of indicators mentioned above. McCarthy's mycobacteriology laboratory performance indicator scheme is used, for the first time, to know its relevance for the data collected from standard smear and culture registers maintained in a mycobacteriology laboratory supporting controlled clinical trials of PTB. The indicator values provide an insight, for the first time into the performance of a mycobacteriology laboratory supporting exclusively clinical trials for PTB.

Conclusion

The findings of this study provide meaningful mycobacteriology laboratory performance indicators that can be defined and used to monitor the quality of laboratories supporting exclusively the controlled clinical trials in pulmonary tuberculosis.

Conflict of interest

None declared.

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