International Journal of Mycobacteriology xxx (2016) xxx–xxx

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/IJMYCO

Full Length Article

Adequacy of examining one sputum specimen in tuberculosis drug resistance surveys

S. Balaji, Vanaja Kumar, Prabu Seenivasan, R. Radhakrishnan, Gomathi Sekar, T. Kannan, V. Chandrasekaran, Soumya Swaminathan, N. Selvakumar *

National Institute for Research in Tuberculosis, Chetpet, Chennai, India

ARTICLE INFO

Article history:
Received 11 April 2016
Accepted 20 April 2016
Available online xxxx

Keywords:
Drug resistance survey
Löwenstein–Jensen media
Mycobacterium tuberculosis
Sputum

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.

* Corresponding author at: Department of Bacteriology, National Institute for Research in Tuberculosis, Chetpet, Chennai 600 031, India.
E-mail address: Selvakumar.nagamiah@gmail.com (N. Selvakumar).

Peer review under responsibility of Asian African Society for Mycobacteriology.
http://dx.doi.org/10.1016/j.ijmyco.2016.04.002

Please cite this article in press as: S Balaji et al. Adequacy of examining one sputum specimen in tuberculosis drug resistance surveys. Int. J. Mycobacteriol. (2016), http://dx.doi.org/10.1016/j.ijmyco.2016.04.002

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 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 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.

Please cite this article in press as: S Balaji et al. Adequacy of examining one sputum specimen in tuberculosis drug resistance surveys. Int. J. Mycobacteriol. (2016), http://dx.doi.org/10.1016/j.ijmyco.2016.04.002