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Timely diagnosis of MDR-TB under program conditions: is rapid drug susceptibility testing sufficient?

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SUMMARY

Timely diagnosis and effective, safe treatment are essential to reduce transmission and improve outcomes for patients with tuberculosis. Aside from laboratory methods, many programmatic factors influence the overall turnaround time (TAT) in diagnosing multidrug-resistant tuberculosis (MDR-TB). We measured each step in the overall TAT required for MDR-TB in two of five health districts of Lima, Peru. The total TAT, from initial sputum specimen to diagnosis and appropriate treatment, was 5 months, almost twice as long as the bacteriological procedures per se. Expensive investments in laboratory technology may yield low returns unless the programmatic aspects of the diagnostic process are streamlined at the same time.

RÉSUMÉ

Le diagnostic en temps opportun de la tuberculose et son traitement efficace et sûr sont essentiels pour diminuer la transmission cette maladie et améliorer les résultats du traitement des patients tuberculeux. Outre les méthodes de laboratoire, de nombreux facteurs programmatiques ont une influence sur le temps nécessaire au diagnostic de la tuberculose à germes multirésistants (TB-MR). Nous avons mesuré le temps nécessaire pour diagnostiquer la TB-MR dans deux des cinq districts de Lima, au Pérou. Le temps nécessaire total (de la collecte d'un spécimen de crachat au diagnostic et à la décision de traitement) était de 5 mois, presque deux fois plus long que le temps requis par les procédures de laboratoire elles-mêmes. Investir dans des technologies de laboratoire coûteuses pourrait ne produire que des avantages limités si cette démarche ne s'accompagne pas d'une rationalisation des aspects programmatiques du processus de diagnostic.

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RESUMEN

El tratamiento seguro, efectivo y el diagnóstico oportuno son esenciales para disminuir la transmisión y mejorar los resultados para los pacientes con tuberculosis. Además de los métodos laboratoriales, muchos factores programáticos influencian sobre los tiempos de demora en el diagnóstico de la tuberculosis multidrogorresistente (TB-MDR). Medimos cada paso en todos los tiempos de demora requeridos para el diagnóstico de la TB-MDR en dos de las cinco Direcciones de salud de Lima, Perú. El tiempo de demora total, desde la solicitud de la muestra inicial de esputo hasta el diagnóstico y tratamiento apropiado, fue de 5 meses, casi dos veces tan prolongado como lo que toma los procedimientos bacteriológicos. Inversiones costosas en tecnología de laboratorio podrían generar una baja recuperación de lo invertido a menos que los aspectos programáticos del proceso diagnóstico estén fuertemente ligados al mismo tiempo.

Keywords

tuberculosis; diagnosis; antimicrobial drug resistance; multidrug resistance; pulmonary diseases

THE PRINCIPAL STRATEGY for tuberculosis (TB) control continues to be timely diagnosis and rapid, effective treatment of pulmonary tuberculosis (PTB). While PTB can be diagnosed in hours to days by sputum microscopy in over 60% of cases, the diagnosis of multidrug-resistant TB (MDR-TB)—defined as tuberculosis strains resistant to at least isoniazid (INH) and rifampin (RMP)—can take up to 3 months considering the maximum incubation time of cultures and drug susceptibility testing (DST). Timely identification of drug resistance is especially important to avoid subjecting patients with resistance to one or two drugs to standard first-line treatment for months while awaiting DST results.[1] Such exposure to inadequate therapy would result in amplified drug resistance and ongoing transmission. It is known that in MDR-TB patients, initiation of proper therapy is associated with sputum culture conversion to negative in half of patients within 3 months and with improved outcomes.[2] For this reason, the quest for affordable, rapid DST is fueled by the need to curtail transmission of drug-resistant TB and to improve patient outcomes in MDR-TB treatment programs in resource-poor settings.[3] Implementation of such methods may prove cost-effective for TB treatment programs.[4] Several rapid diagnostic assays provide accurate identification of MDR-TB strains compared with conventional DST.[5–10] To date, the impact of implementing a rapid DST method under program conditions has not been described.

Despite the potential to significantly reduce the turnaround time (TAT) using rapid DST methods, other programmatic and clinical factors may heavily influence the impact of such an implementation. The infrastructure through which rapid testing for TB and drug resistance can be deployed is particularly important in determining how much benefit a rapid DST method could possibly achieve. In addition to laboratory capacity and methods, selecting patients for culture and DST, timely transportation of specimens and communication of results and prompt follow-up all likely contribute greatly to the potential impact on MDR-TB of using rapid DST in a programmatic setting.

In Peru, the implementation of rapid DST is planned as part of nationwide efforts to scale up services for detection and treatment of MDR-TB. DST for this program was initially performed at the Massachusetts State Laboratory Institute (MSLI) as part of an international collaboration to implement DOTS-Plus.[11] Subsequently, the MSLI has supervised the Peruvian National Reference Laboratory (NRL) in the validation and local implementation of DST against first- and second-line drugs. A final component of the transfer of laboratory capacity to Peru is the plan to decentralize DST to seven regional laboratories which currently provide coverage to 90% of the national TB burden. Several DST methods have been chosen for decentralized implementation, including conventional indirect DST on Löwenstein-Jensen (LJ) medium using the proportion method,[12] indirect DST on Middlebrook 7H10 agar using the proportion method, and the direct nitrate reductase method on modified LJ medium (the 'Griess' method).[13,14] The Griess method is both simple and inexpensive. Used as a direct susceptibility test, the Griess method works well for smear-positive specimens and has already been validated at the NRL and two district laboratories under the supervision of the MSLI.[15]

Given these plans and recognizing the need to optimize other programmatic factors influencing the impact of decentralized rapid DST, we aimed to measure each of the steps contributing to the overall TAT in two of the regions of Lima.

MATERIALS AND METHODS

These data were derived from evaluating TB health services in two health regions, or DISAs (Dirección de Salud, comprised of health districts), of Lima, Peru: Lima Ciudad and Lima Este. Lima Ciudad includes 45 health establishments (24 health centers, nine health posts, and 12 hospitals) serving a population of 1 577 090 in an area of approximately 100 km^2. Lima Este includes 134 health establishments (42 health centers, 87 health posts, and 5 hospitals) serving a population of 1 088 515 in an area of approximately 6340 km^2. Smear microscopy is used to diagnose active TB, while culture and DST are reserved for individuals with confirmed TB and a risk factor for MDR-TB according to National Tuberculosis Program (NTP) norms (e.g., household contact with MDR-TB, persistent or recurrent smear-positive status after 4 months of first-line therapy). Smear microscopy is performed in Level I laboratories in health centers and hospitals. Health posts send sputum samples to their closest health center for smear microscopy. For patients with MDR-TB risk factors, positive sputum samples are sent to the DISA Level II laboratory for culture. Positive cultures are sent to the NRL, located in the Peruvian National Institute of Health, for DST. Results on paper are signed by the laboratory director then sent from the NRL back for registration to the DISA laboratory which, in turn, transmits the results to the health establishment. The patient is then routinely seen by a pulmonologist at the local hospital to review the DST results and if necessary modify the TB regimen. In patients with drug-resistant isolates, an expert committee reviews the case to approve enrollment into MDR-TB therapy.

The two DISA laboratories, the NRL, and 92 health establishments were included in the study (21 health centers and one health post in Lima Ciudad; 49 health centers, 20 health posts, and one hospital in Lima Este). Data were collected from August to October 2004 and

were considered from July 2003 to the date of collection. At each health establishment, medical records were reviewed to identify 10 active patients with smear, culture and DST results. If 10 such patients could not be identified, then patients who had smear and culture data were included. In small health centers without 10 such patients, patients who had a smear result (not necessarily MDR-TB suspects) were also included in the study.

For each sample, data were recorded by trained data collectors using a previously validated data collection form. Sources of data included patient medical records in health establishments and laboratory registers for smear microscopy, culture, and DST results. The data consisted of a series of dates pertaining to an individual specimen from the moment smear microscopy was ordered to the time the patient was re-evaluated by a pulmonologist with DST results in hand:

1. Date of smear microscopy, culture and/or DST requested
2. Date of sputum sample collection
3. Date sputum sample received in the local laboratory
4. Date smear microscopy performed in the local laboratory
5. Date smear microscopy result obtained in the local laboratory
6. Date sputum sample sent for *Mycobacterium tuberculosis* culture
7. Date sputum sample received for culture in the intermediate laboratory
8. Date culture performed in the intermediate laboratory
9. Date of first culture reading in the intermediate laboratory
10. Date culture result sent from the intermediate laboratory
11. Date of receipt of the culture result at the health establishment
12. Date culture sent for DST to the NRL
13. Date DST performed at the NRL
14. Date DST result obtained at the NRL
15. Date of receipt of DST result in the intermediate laboratory
16. Date of receipt of the DST result in the health establishment
17. Date of re-evaluation of patient treatment with new DST result.

Data were entered into Microsoft Excel 2000 (Microsoft Excel, Palisade Corp, Newfield, NY, USA) and analyzed using SAS 9.1 (SAS Institute, Cary, NC, USA). Intervals in days were calculated between adjacent dates. Differences in time intervals between the two health districts were compared using a *t*-test for normally distributed data and a Wilcoxon rank sum test for non-normal data.

Approval was obtained from the Institutional Review Board (IRB) of Brigham and Women's Hospital in Boston, MA, USA.

RESULTS

From the 92 health establishments, data were collected on 719 patients from whom a total of 924 samples were processed for smear microscopy, culture and/or DST. Of the 17 dates, one (No 9: 'Date of first culture reading in the intermediate laboratory') was not routinely registered. For this reason, of the 16 anticipated time intervals to be assessed, we were only able to assess 14.

Table 1 summarizes the number of observations obtained for each time interval. Of note, the information on 'Date culture sent for DST to the NRL' was not available in Lima Este, so the corresponding time interval could not be calculated for Lima Este.

Table 2 presents the average number of days for each time interval. Smear microscopy required less than one day on average, but for culture and DST many days elapsed that were not related to the slow growth rate of mycobacteria. For instance, more than 6 days on average passed from the date of the smear microscopy result to the date of processing for culture. From there, approximately 50 days passed before culture results were available, and 6 more days passed until the health establishment received the culture result. For positive cultures, 7 days elapsed from the date the DISA laboratory sent it to the date the NRL processed it for DST. DST results took approximately 80 days on average, and 12 days passed from date of DST results until this result reached the health establishment. Finally, once the health establishment received the DST results, an average of 49 days passed until the patient's treatment was changed based on those results.

Predictably, the districts differed. Certain intervals were shorter in Lima Ciudad and others in Lima Este. The data display the width of the distribution of time intervals as well as the center.

Table 3 summarizes the cumulative time intervals associated with the processing of smear microscopy, culture and DST. Overall, the total TAT from the time of microscopy request to the time of clinical re-evaluation was approximately 4.9 months, with no significant difference between the two DISAs.

DISCUSSION

There are important limitations to these data. The number of health centers surveyed and the numbers of patients per health center were small, especially within certain time periods measured; thus, the variance around these measurements is wide. Furthermore, the proportion of missing data was large for some of the TAT measurements. This was generally due to incomplete reporting and documentation by providers when filling out forms and/or recording results in patient charts. We found that the proportion of missing data was higher among patients who were hospitalized, given the fact that hard copies of test results were less likely to be included in the patient's health center chart. Otherwise, data appeared to be missing randomly. We therefore speculate that the missing data would have resulted in an underestimation of the TAT measures, as the extra step required to transmit laboratory results from the hospital to the local health center after a patient had returned home would likely contribute further to delays in diagnosis and treatment. It may not be possible to

generalize the results of these two districts to other districts in Lima, to Peru in general, or outside of Peru. Nonetheless, in our experience, these types of TAT are common in middle- and low-income countries where the infrastructure supporting TB laboratories—diagnostic services for TB as a whole—is underdeveloped. These data do not provide information about the reasons for the amount of time taken at each step. Additional information and considerable judgment will be necessary to develop interventions and evaluate their impact.

These data nonetheless highlight significant delays in the process of performing and acting upon DST. The process of collecting and analyzing these data illustrates how to identify key delays in a programmatic process, with the aim of then addressing those amenable to intervention. Efforts in operational research such as this will be important if the scale up of complex interventions in resource-poor settings is to be successful.

As TB treatment programs become more complex, the need for efficient coordination becomes greater. These data demonstrate that management of specimens and results doubles the overall time required to diagnose and treat drug-resistant TB. Microbiologists, medical providers and public health systems may lament the slow growth rate of mycobacteria as the cause of the long time required but, in practical terms, this is far from true. In reality, the rate at which people act (i.e., transfer specimens and results) appears to contribute at least as much time. The impact of implementing new technologies for rapid culture and DST in settings with weak infrastructures may be limited by other factors influencing the overall time required to obtain test results that affect the patient's treatment. These additional factors must also be addressed; otherwise, substantial investments in technology, training, and logistics may yield poor returns.

Among the strategies needed to address these delays, increased coordination between the different levels of the health services (e.g., communication of DST results from the laboratory to the health establishment and then to the treating physician) is crucial. Additional resources are required to establish the infrastructure, management systems, and procedures in the laboratory and in the clinical setting. Training, opportunity, and adequate compensation are needed for personnel. Regulatory measures may be necessary to set standards for consistent timely diagnostic services. For example, in the US, microscopy results must be reported within 24 h, culture within 21 days, and DST results within 1 month. Regulatory measures may be required to set biosafety standards.

Another important factor in the delay in processing DST is due to the increasing demand for this test. Since the DOTS-Plus program began in Peru in 1996, the number of patients receiving individualized treatment for MDR-TB and thus requiring DST has increased from 14 patients enrolled in the first year to 562 patients enrolled in 2003, and 730 patients enrolled through November 2004. Managing MDR-TB requires these specialized laboratory procedures and clinical expertise. For this reason, scaling up this program has necessitated expansion of laboratory capacity to decentralize DST screening services, as previously described. Similarly, individualized treatment will be determined by a district-level committee of specialists instead of relying on a sole central committee, which has been the case until now. Central resources such as the NRL and the national committee of MDR-TB pulmonologists will be responsible for monitoring and evaluation of these decentralized

services, including quality of laboratory procedures and clinical management, as well as Level III laboratory services for selected isolates according to systematic criteria.

More in-depth exploration of these delays has led to strategies to reduce them, taking into account differences between districts. For example, Lima Este is larger in area but has a smaller population, more geographically dispersed, including some rural areas. Efficient transportation of specimens, patients, and results on paper does not occur automatically, but requires resources. Similarly, rapid reporting of results via information systems requires infrastructure, technology, and training. To reduce delays, a multifaceted approach to strengthen diagnostic services as a whole is being implemented in these two districts in Lima, as summarized in Table 4. After these measures are integrated into the program, the measurement of elapsed time at each step of the diagnostic process will be repeated. Future reports will present these results.

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Table 1

Number of observations for each time interval

No.	Time interval	No. observations		
		Total	Lima Este	Lima Ciudad
1	From DST request to sputum sample collection	638	444	194
2	From sputum sample collection to receipt of sputum sample in the local laboratory	643	449	194
3	From receipt of sputum sample to time smear microscopy processed in the local laboratory	732	535	197
4	From time smear microscopy processed to smear microscopy result in the local laboratory	737	536	201
5	From smear microscopy result to time sputum sample sent for *M. tuberculosis* culture	484	265	219
6	From time sputum sample sent for *M. tuberculosis* culture to receipt of sample in intermediate laboratory	547	318	229
7	From receipt of sample to time culture processed in intermediate laboratory	506	277	229
8	From time culture processed to time culture result sent from intermediate laboratory	564	329	235
9	From time culture result sent from intermediate laboratory to receipt at health establishment	125	23	102
10	From time culture sent for DST to time DST processed at NRL	108	0	108
11	From time DST processed to DST result at NRL	233	125	108
12	From DST result to receipt of DST result at intermediate laboratory	149	42	107
13	From receipt of DST result at intermediate laboratory to receipt of DST result at health establishment	76	28	48
14	From receipt of DST result at health establishment to patient re-evaluation with DST result	60	30	30

DST = drug susceptibility testing; NRL = National Reference Laboratory.

Table 2

Time intervals for processing of smear microscopy, culture and DST

No.	Time interval	Median days (range)	25th percentile	75th percentile
1	From DST request to sputum sample collection *	0 (0–4)	0	0
2	From sputum sample collection to receipt of sputum sample in the local laboratory *	0 (0–1)	0	0
3	From receipt of sputum sample to time smear microscopy processed in the local laboratory	0 (0–1)	0	0
4	From time smear microscopy processed to smear microscopy result in the local laboratory *	0 (0–8)	0	1
5	From smear microscopy result to time sputum sample sent for *M. tuberculosis* culture	2 (0–23)	0	4
6	From time sputum sample sent for *M. tuberculosis* culture to receipt of sample in intermediate laboratory *	0 (0–5)	0	0
7	From receipt of sample to time culture processed in intermediate laboratory *	2 (0–58)	1	5
8	From time culture processed to time culture result sent from intermediate laboratory *	48 (11–95)	36	64
9	From time culture result sent from intermediate laboratory to receipt at health establishment	3 (0–99)	0	8
10	From time culture sent for DST to time DST processed at NRL	5 (0–43)	3	11
11	From time DST processed to DST result at NRL	81 (6–166)	71	92
12	From DST result to receipt of DST result at intermediate laboratory *	8 (0–84)	5	10
13	From receipt of DST result at intermediate laboratory to receipt of DST result at health establishment *	6 (0–70)	3	16
14	From receipt of DST result at health establishment to patient re-evaluation with DST result	33 (0–225)	7	69

* Significant difference between time interval measured in Lima Ciudad versus Lima Este, $P < 0.001$.

DST = drug susceptibility testing; NRL = National Reference Laboratory.

Table 3

Cumulative time intervals for smear microscopy, culture and DST

No.	Time interval	Median days (range)	25th percentile	75th percentile
1	Smear microscopy (from smear microscopy request to microscopy result) *	0 (0–8)	0	1
2	*M. tuberculosis* culture (from time of smear microscopy result to time culture result received at health establishment) *	54 (0–151)	39	70
3	DST (from time DST processed to time of patient re-evaluation)	99 (0–320)	86	117
4	Total turnaround time	147 (20–353)	127	177

* Significant difference between time interval measured in Lima Ciudad vs. Lima Este, $P < 0.001$.

DST = drug susceptibility testing.

Table 4

Measures currently implemented in Lima, Peru, to reduce delays in DST processing TAT

Algorithm-based selection of high-risk patients
Reliable, regular transportation of specimens
Expanded, biosafe laboratory facilities and equipment
Improved procedures for culture for primary isolation at district and central laboratories
Introduction of novel rapid screening test for INH and RMP resistance at district laboratories
Bactec 460 culture at NRL for paucibacillary cases, children and HIV-positive patients and for DST of isolates that screen resistant to INH or RMP at district laboratory
Full spectrum DST by agar plate proportion method at the NRL
Electronic reporting of results by internet-based information system
Integrated internal quality control and external quality assurance process

DST = drug susceptibility testing; TAT = turn-around time; INH = isoniazid; RMP = rifampin; NRL = National Reference Laboratory; HIV = human immunodeficiency virus.