Culture and drug sensitivity testing among patients with pulmonary tuberculosis in Mexico: national data for 2009–2013

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ABSTRACT *This study documented the number and results of mycobacterial culture and drug sensitivity testing (CDST) in Mexico from 2009–2013 and assessed whether states with a higher risk of multidrug-resistant tuberculosis (MDR-TB) performed more CDST and had more cultures showing MDR-TB. Data for this longitudinal, descriptive, operational research study came from the electronic records of 31 state public health laboratories in Mexico. The total number of CDSTs was 6 470, increasing from 2 143 in the first 2 years to 4 327 in the latter 3 years. There was a significant increase in the proportion of cultures showing sensitivity to all drugs, from 53.1% to 60.9% in 2011–2013 (P < 0.001) and a significant decrease in the proportion showing MDR-TB, from 28.2% in 2009 to 19.8% in 2013 (P < 0.001). Cases of extensively drug resistant tuberculosis were < 1% per year. In the 12 states with higher risk for MDR-TB, significantly more CDSTs (2 382 test) were done in 2011–2013 than in the other 19 states (1 945 tests). Also, for each year the proportion of cultures showing MDR-TB was significantly higher in high risk MDR-TB states than in lower risk ones (P < 0.001). During the 5-year study period, CDST was scaled up in Mexico, particularly in high-risk MDR-TB states where a higher proportion of cultures showed MDR-TB. Scale up and wider coverage of CDST should continue.*

Keywords Tuberculosis; culture techniques; microbial sensitivity tests; tuberculosis, multidrug-resistant; extensively drug-resistant tuberculosis; operations research; Mexico.

In 1996, the National Tuberculosis Program (NTP) of Mexico introduced the Stop TB Strategy developed by World Health Organization (WHO). The strategy was implemented for nationwide control of tuberculosis (TB) and its WHO international guidelines have been followed since then.

During 2009–2013, there were approximately 20 000 cases of TB of all types and categories notified annually, of which 1 500 (7.5%) had been previously treated (1, 2).

In 2008, Mexico conducted a National Survey on Drug Resistance in patients with TB (3). According to the survey, the prevalence of multidrug-resistant TB (resistant to both isoniazid and rifampicin; MDR-TB) nationwide was 2.8% and the risk of MDR-TB among those previously treated was 3 times higher at 7.2%. Further analyses at the national level found that MDR-TB was associated with other co-morbidities or risk factors, such as diabetes mellitus, malnutrition, alcoholism, drug abuse, HIV / AIDS, and smoking (in descending order).

To diagnose drug-resistant TB or MDR-TB, culture and drug sensitivity testing (CDST) is required for at-risk patients, including contacts of patients with MDR-TB and patients with previously treated TB. Poor case detection of drug resistance can be due to insufficient coverage of CDST in at-risk populations. For example, in Mexico only 148 (9%) of 1 644 patients requiring TB retreatment in 2012 were assessed for MDR-TB (1). Furthermore, it was also estimated in that year that Mexico had

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480 cases of MDR-TB, and yet only 114 (24%) were actually notified to the WHO (3). Other countries, such as China, Malawi, and Nepal, have experienced similar problems with CDST coverage, resulting in low numbers of diagnosed MDR-TB cases compared to estimated numbers (4–7).

Since the 2008 National Drug Resistance Survey, the NTP in Mexico has endeavored to increase the number of patients given CDST by public health laboratories, especially in states where the risk of MDR-TB has been higher in the last 10 years. Testing and results are recorded in electronic registers at each state laboratory. The aim of this study was to document the number and results of CDST and mycobacterial cultures performed in 2009–2013, and to assess whether states at high risk of MDR-TB also performed more CDST and had more results showing MDR-TB.

MATERIALS AND METHODS

This was a longitudinal, descriptive, operational research study of patients with TB who had mycobacterial culture and drug sensitivity testing performed in 2009–2013, based on data from the electronic records of 31 state public health laboratories in Mexico.

Setting

In 2013, Mexico had an estimated population of 118 million (5). It is a democratic republic made up of 31 states and one Federal District. The country's gross domestic product (GDP) is approximately US$ 11 000 per capita, which according to the World Bank is the highest in Latin America (5).

The NTP of Mexico is located at the Secretary of Health. TB case finding, diagnosis, and treatment are standardized throughout the country and follow national and WHO guidelines (8, 9). In brief, patients who present to health services with chronic cough are evaluated with sputum smear microscopy using Ziehl-Neelsen stains and chest radiography when smears showing no acid-fast bacilli. Extrapulmonary TB is diagnosed according to clinical and guideline criteria (7, 8). Once diagnosed, patients are registered and treated with standardized regimens. All TB microscopy services and treatment are provided free of charge in Mexico.

CDST is performed in symptomatic contacts of index patients with MDR-TB, in those with previously treated TB, and in new patients from 12 states where

prevalence of MDR-TB is thought to be particularly high. Sputum specimens can be sent to any one of 31 state laboratories, including the National Reference Laboratory, for mycobacterial culture using Löwenstein-Jensen media and Mycobacterium Growth Indicator Tube (MGIT). Drug sensitivity testing is performed in only 14 laboratories, including the National Reference Laboratory. Laboratories without this capability send cultures to one of the 14 for CDST. The proportion method in agar Middlebrook 7H10 in plaque is used for CDST, which includes sensitivity to first-line anti-tuberculosis drugs (isoniazid, rifampicin, ethambutol, streptomycin, pyrazinamide) and, for previously treated patients, second-line drugs (ciprofloxacin, kanamycin, capreomycin, cycloserine and p-amino salicylic acid). External quality control of CDST in Mexico is provided by the Centers for Disease Control and Prevention (Atlanta, Georgia, United States).

Individual CDST results are recorded on individual laboratory forms. In the 14 CDST performing laboratories, the data are aggregated each month and entered into their electronic registers with no linkage of clinical and laboratory data. In the 17 non-CDST performing laboratories, individual results are received and entered to their electronic registers, again with no linkage of clinical and laboratory data. The information on CDST and drug resistance is transmitted to state program managers. Patients diagnosed with MDR-TB are referred for treatment with regimens individualized according to drug resistance patterns.

Data collection and variables

Data were obtained in February–June 2015 from each of the state laboratories and the National Reference Laboratory using an electronic structured questionnaire. Variables included state, year, number of CDSTs performed annually, and results. Sources of data were state laboratory electronic registers. Data were further stratified into 12 states with high risk of MDR-TB (Baja California, Coahuila, Chiapas, Chihuahua, Guerrero, Hidalgo, Nuevo León, Oaxaca, Sinaloa, Sonora, Tamaulipas, and Veracruz) and the others.

Analysis and statistics

Data from the questionnaires were double-entered into Microsoft Excel™ (Microsoft Corporation, Redmond, Washington, United States) and EpiData

(EpiData Association, Odense, Denmark). States were stratified into those regarded as high-risk for MDR-TB, based on MDR-TB notifications over a 10-year period, and the remainder. Frequencies and proportions were calculated for categorical variables, and comparisons were done using the chi-square test or chi-square test for trend with levels of significance set at $P < 0.05$.

Ethics

Local ethics approval was not needed as this was deemed to be a programmatic activity of the NTP. However, ethics approval for data analysis and publication was obtained from the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease (Paris, France). Patient names and identifiers were not collected.

RESULTS

The total number of CDSTs performed in 2009–2013 was 6 470. Number of CDSTs performed annually and their results are shown in Table 1. In the first 2 years (2009 and 2010), the number of tests was similar, totalling 2 143. For the latter 3 years (2011, 2012, and 2013), the annual numbers increased, totalling 4 327. There was no difference in the proportion of cultures showing monoresistance or polyresistance in 2009–2013. However, compared to 2009, the period from 2011–2013 saw a significant increase in the proportion of cultures showing sensitivity to all drugs ($P < 0.001$), and conversely, a significant decrease in the proportion of cultures showing MDR-TB ($P < 0.001$). There were very few cases of extensively drug resistant tuberculosis (XDR-TB), less than 1% per year.

The numbers of CDSTs performed, along with MDR-TB and XDR-TB results, for the 12 high-risk MDR-TB states and the other states in 2009–2013 are shown in Table 2. In 2009 and 2010, there were no significant differences in the numbers of CDSTs performed in high-risk MDR-TB states versus other states, but in 2011–2013, there were significantly more ($P < 0.05$). In the high-risk MDR-TB states, there was no significant trend in the proportion of cultures showing MDR-TB over the 5-year study period, but in the other states there was a significant decrease (chi-square test for trend = 20.36; $P < 0.001$). For each of the years, the proportion of cultures showing MDR-TB was significantly higher in high-risk MDR-TB

TABLE 1. Number of culture and drug sensitivity tests (CDSTs) performed and results, Mexico, 2009–2013

Characteristic	2009 No.	2009 %	2010 No.	2010 %	2011 No.	2011 %	2012 No.	2012 %	2013 No.	2013 %
Total CDST tests	1 061		1 082		1 444		1 263		1 620	
Fully sensitive to all drugs	563	53.1	578	53.4	904	62.6	805	63.7	987	60.9
Monoresistant[a]	115	10.8	115	10.6	166	11.5	136	10.8	194	12.0
Polyresistant[b]	80	7.5	80	7.4	94	6.5	101	8.0	110	6.8
MDR-TB[c]	299	28.2	301	27.8	279	19.3	212	16.8	320	19.8
XDR-TB[d]	4	0.4	8	0.7	1	0.1	9	0.7	9	0.6

Source: prepared by the authors from the study data.
[a] Resistance to one first-line anti-tuberculosis drug (e.g., isoniazid, streptomycin, ethambutol), including monoresistance to rifampicin.
[b] Resistance to two or more first-line antituberculosis drugs (e.g., isoniazid, streptomycin, ethambutol), but not to both isoniazid and rifampicin.
[c] Multidrug resistant tuberculosis (isoniazid and rifampicin).
[d] Extensively drug resistant (MDR-TB plus resistance to one of the second-line fluoroquinolone drugs and one of the injectable second-line drugs).

TABLE 2. Number of culture and drug sensitivity tests (CDST) performed and results for multidrug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) in high-risk MDR-TB states and other states in Mexico, 2009–2013

Characteristic	2009 No.	2009 %	2010 No.	2010 %	2011 No.	2011 %	2012 No.	2012 %	2013 No.	2013 %
States with a high risk of MDR-TB[a]: (*n* = 12)										
CDST tests	593		531		829		643		910	
MDR-TB	219	37.0	215	40.5	212	25.6	163	56.5	244	26.8
XDR-TB[b]	0		0		0		6	0.9	3	0.3
Other states: (*n* = 20)										
CDST tests	468		551		615		620		710	
MDR-TB	80	17.1	86	15.6	67	10.9	49	7.9	76	10.7
XDR-TB	4	0.9	8	1.5	1	0.2	3	0.5	3	0.4

Source: prepared by the authors from the study data.
[a] Multidrug resistant tuberculosis (resistance to isoniazid and rifampicin).
[b] MDR-TB plus resistance to one of the second-line fluoroquinolone drugs and one of the injectable second-line drugs.

states than in other states ($P < 0.001$). There were small numbers with XDR-TB in the different groups of states.

DISCUSSION

During the last 3 years of the study period, there was a gradual increase in the number of CDSTs performed in the country, with an increase in the proportion of cultures showing drug sensitivity and a decrease in the proportion showing MDR-TB. There has been an increase in CDST in high risk MDR-TB states with, not surprisingly, higher rates of MDR-TB cultures compared to other states. The proportion of cultures with XDR-TB has been low.

A strength of the study is the full national sample of CDST over a 5-year period. Also the study procedure and reporting followed the Strengthening the Reporting of Observational studies in Epidemiology guidelines (10). These study results have several important programmatic implications.

First, the yield of MDR-TB appears to be higher in the high-risk MDR-TB states, and it therefore makes sense to prioritize the scale up of CDST in these areas to ensure that all previously treated TB patients and MDR-TB contacts are appropriately screened. Furthermore, nine patients with

XDR-TB were identified in these high-risk states in 2012 and 2013, further justifying focused vigilance, a prioritized approach, and the addition of sentinel surveillance where appropriate.

Second, the CDST monitoring and evaluation system needs to be improved. Details of type and category of TB linked with CDST results were only recorded on paper-based forms with the state laboratory electronic registers recording aggregate data. Electronic registers need to capture individualized clinical and CDST data so that it can be matched and analyzed for a better understanding of the epidemiology of drug resistance in Mexico. A new system of this kind was initiated at the beginning of 2015.

Third, Mexico needs to consider alternative and more rapid methods of identifying drug resistance. Xpert® MTB/RIF (Cepheid Incorporated, Sunnyvale, California, United States) is a sensitive and specific, fully automated and commercially available nucleic acid amplification test for use with sputum and other body specimens with results in less than 2 hours. Sensitivity and specificity for the diagnosis of TB and of rifampicin resistance are high (11), and this is a useful approach to rapidly diagnosing MDR-TB. Xpert® MTB/RIF machines have already been placed in

eight state laboratories with a view to countrywide scale up.

Limitations

The main study limitation was the inability to cross-link CDST results with TB type and category, nor with contacts of MDR-TB, and so it was not possible to determine what proportion of patients with previously treated TB or contacts of MDR-TB had undergone CDST.

Conclusions

This study shows that in 2009–2013, CDST was effectively scaled up in Mexico, particularly in high-risk MDR-TB states where a higher proportion of cultures show MDR-TB. Methods to address the data linkage deficiencies are in place, and future operational research will be able to provide more in-depth analysis of CDST performance.

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RESUMEN

Cultivo y antibiograma en pacientes con diagnóstico de tuberculosis pulmonar en México: datos nacionales del 2009 al 2013

Se verificó el número y los resultados de los cultivos de micobacterias y antibiogramas realizados en México del 2009 al 2013 y se investigó si los estados con un mayor riesgo de tuberculosis multirresistente (MR) realizaban más pruebas de ambos estudios y obtenían más cultivos diagnósticos de tuberculosis MR. Los datos para este estudio longitudinal descriptivo de investigación operativa se tomaron de los registros electrónicos de 31 laboratorios estatales de salud pública en México. Se realizaron 6 470 pruebas; esta cifra aumentó de 2 143 en los dos primeros años a 4 327 en los últimos tres años estudiados. Se observó un incremento significativo de la proporción de cultivos con sensibilidad a todos los medicamentos, de 53,1% en el 2011 a 60,9% en el 2013 (p < 0,001) y una disminución significativa en la proporción de casos de tuberculosis MR, de 28,2% en el 2009 a 19,8% en el 2013 (p < 0,001). Los casos de tuberculosis extremadamente farmacorresistente fueron inferiores a 1% por año. En los 12 estados con mayor riesgo de tuberculosis MR, el número de cultivos y antibiogramas realizados del 2011 al 2013 (2 382 pruebas) fue significativamente más alto que en los otros 19 estados (1 945 pruebas). Asimismo, en cada año la proporción de cultivos que revelaban tuberculosis MR fue significativamente mayor en los estados con mayor riesgo de tuberculosis de este tipo, que en los estados con menor riesgo (p < 0,001). Durante el período de 5 años del estudio se amplió la aplicación de las pruebas de cultivo y antibiograma en México, sobre todo en los estados con un alto riesgo de tuberculosis MR, donde la proporción de cultivos que revelan multirresistencia es más alta. La aplicación y la cobertura de las pruebas de cultivo y antibiograma deben ampliarse.

Palabras clave

Tuberculosis; técnicas de cultivo; pruebas de sensibilidad microbiana; tuberculosis resistente a múltiples medicamentos; tuberculosis extensivamente resistente a drogas; investigación operativa; México.