A population-based study of first and second-line drug-resistant tuberculosis in a high-burden area of the Mexico/United States border

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The resistance of 139 Mycobacterium tuberculosis *(MTB) isolates from the city of Monterrey, Northeast Mexico, to first and second-line anti-TB drugs was analysed. A total of 73 isolates were susceptible and 66 were resistant to anti-TB drugs. Monoresistance to streptomycin, isoniazid (INH) and ethambutol was observed in 29 cases. Resistance to INH was found in 52 cases and in 29 cases INH resistance was combined with resistance to two or three drugs. A total of 24 isolates were multidrug-resistant (MDR) resistant to at least INH and rifampicin and 11 MDR cases were resistant to five drugs. The proportion of MDR-TB among new TB cases in our target population was 0.72% (1/139 cases). The proportion of MDR-TB among previously treated cases was 25.18% (35/139 cases). The 13 polyresistant and 24 MDR isolates were assayed against the following seven second-line drugs: amikacin (AMK), kanamycin (KAN), capreomycin (CAP), clofazimine (CLF), ethionamide (ETH), ofloxacin (OFL) and cycloserine (CLS). Resistance to CLF, OFL or CLS was not observed. Resistance was detected to ETH (10.80%) and to AMK (2.70%), KAN (2.70%) and CAP (2.70%). One isolate of MDR with primary resistance was also resistant to three second-line drugs. Monterrey has a high prevalence of MDR-TB among previously treated cases and extensively drug-resistant-MTB strains may soon appear.*

Key words: tuberculosis - *Mycobacterium tuberculosis* - first and second-line drug resistance

In 2010, 8.8 million new tuberculosis (TB) cases and 1.1 million deaths from TB were reported (WHO 2011).

Although this disease has been the subject of control programs since the early XX century, TB is still an endemic disease in Mexico and remains a serious challenge to public health (SSM 2008).

Mexico has reported 15,932 new cases of pulmonary TB (PTB), with an incidence of 13.7/100,000 inhabitants. The states of Baja California, Sonora, Chihuahua, Coahuila, Nuevo León (NL) and Tamaulipas form part of the border with the United States of America (USA) and constitute the greatest development zone in Mexico. Mexico reported in 2010, there were 4,627 PTB, equivalent to 32% of the estimated total number of cases (SSM 2011). The spread of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) threatens global efforts to control TB (WHO 2010). According to the World Health Organization (WHO 2008), MDR-TB is a form of TB that does not respond to the standard six-month regimen using first-line drugs [i.e., resistant to isoniazid (INH) and rifampicin (RIF)]. MDR-TB can require two years of treatment with drugs that are more toxic and 100 times more expensive. If the drugs used to treat MDR-TB are inappropriately administered, further resistance can occur. XDR-TB, an even more dangerous form of TB, is caused by *Mycobacterium tuberculosis* (MTB) strains that are resistant to INH, RIF, fluoroquinolones and any of the injectable second-line anti-TB drugs, including amikacin (AMK), kanamycin (KAN) and capreomycin (CAP) (WHO 2010).

During the last two years, MDR-TB has been reported among 7.8%, 13.6% and 17.9% of TB isolates in México by Ferrer et al. (2010), Sánchez-Pérez et al. (2010) and Zazueta-Beltran et al. (2011), respectively. The presence of XDR-TB (WHO 2010) has also been described. Nevertheless, little is known regarding the prevalence of circulating MTB strains that are resistant to both first (Garcia-Garcia et al. 2001) and second-line anti-TB agents (Ferrer et al. 2010). This information is essential for identifying XDR-MTB.

Our research centre is located in Monterrey, NL, México. The incidence of PTB in NL is 20.7 per 100,000 inhabitants (SSM 2011). Most of the TB cases that have been reported by the government of NL have occurred in the metropolitan area of Monterrey (MAM), where 75% of the population of NL lives (INEGI 2010). The Mexican Institute of Social Security (IMSS) provides

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health care to 95.72% of workers and their families living in NL (imss.gob.mx/delegaciones/nuevoleon/Pages/estadisticas.aspx). Northeast and northwest MAM have the heaviest burden of PTB. The aim of this study was to analyse the patterns of resistance of MTB strains, isolated from patients attending an IMSS health facility in northwest MAM, to first and second-line anti-TB drugs and the possible emergence of XDR-PTB.

PATIENTS, MATERIALS AND METHODS

Patients - The patients attended a Family Medicine Clinic 28 of the IMSS. This clinic provides health care services to 118,000 individuals, including workers and their families. The incidences of PTB and mortality rates at this facility are 78.9 and 6.4 per 100,000 individuals, respectively.

For a period of four years, all patients with a clinical diagnosis of PTB (290), made by their family physician, were referred to the epidemiology service of their clinic for registration and treatment. Clinical PTB was diagnosed by taking into account respiratory symptoms and radiological data for each patient. All 290 patients were included in the present study after they provided informed consent. Every participant answered a questionnaire to collect socio-demographic (Table I) and clinical (Table II) information. Participants belonged to three clearly distinguishable groups: previously treated cases who received anti-MTB therapy for one month or longer, including relapsed cases (3-8 per year), new MTB cases who had not received anti-MTB medications at the time of their enrolment and patients with an unknown treatment history.

Bacteriological methods - Sputum samples were processed by smear microscopy with Ziehl Neelsen staining (Ellis & Zabrowarny 1993) and cultured in L**ö**wenstein-Jensen (LJ) or Middlebrook BACTEC 12B medium (Becton Dickinson, Sparks, MD, USA). Middlebrook BACTEC 12B medium was supplemented with 0.1 mL of antimicrobial PANTA (Becton Dickinson), which contains polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin. Cultures with typical mycobacterial colonies were sub-cultured and typed according to the methods of Kent and Kubica (1985) and Balandrano-Campos et al. (1996). MTB cultures grown in 12B medium were preliminarily identified using the p-nitro-alpha-acetyl-amino-beta-hydroxypropiophenone test (BACTEC 460 TB System, Becton Dickinson Microbiology Systems, Sparks, MD, USA), resuspended in 7H9 medium supplemented with 10% glycerol and stored at -70ºC until use.

Isolate recovery - Frozen MTB isolates were thawed at -20ºC overnight and at 4ºC for 3-4 h. Mycobacteria were inoculated on LJ slants and incubated at 37ºC until growth was evident.

Drug susceptibility testing (DST) for first-line anti-TB drugs - DST was performed using a Bactec 460 radiometric system (Becton Dickinson Microbiology

TABLE I

Frequencies of sociodemographic descriptors of the target population having positive *Mycobacterium tuberculosis* cultures (n = 139)

Sex n (%)		Age n (%)		Schooling n (%)				Occupation n (%)				
									Workers			
Male	Female	15-54	> 55	Unknown	Elementary school	High school	Bachelor	Housewife	White-collar	Blue-collar	Inactive	Unknown
82 (59)	57 (41)	82 (59.6)	43 (30.9)	14 (10.1)	45 (32.4)	85 (61.2)	9 (6.5)	26 (18.7)	11 (7.9)	46 (33.1)	23 (16.6)	33 (23.7)

TABLE II

Frequencies of risk factors and clinical status of participants having positive *Mycobacterium tuberculosis* cultures (n = 139)

Risk factors n (%)				Status of pulmonary tuberculosis n (%)		Comorbidity n (%)		
Alcoholism	Drug addiction	Tabagism	None	Not cavitated	Cavitated	Diabetes	COPD or neumoconiosis	None
49 (35.25)	7 (5.03)	55 (39.57)	28 (20.14)	73 (52.52)	66 (47.48)	41 (29.49)	20 (14.39)	78 (56.11)

COPD: chronic obstructive pulmonary disease.

Systems, Sparks, MD, USA). The assayed drugs were 0.1 µg/mL INH, 2.0 µg/mL streptomycin (SM), 2.0 µg/mL RIF, 2.5 µg/mL ethambutol (EMB) and 100 µg/mL pyrazinamide (PZA).

DST for second-line anti-TB drugs - DST was performed using the microplate Alamar Blue assay (MABA) according to the method of Luna-Herrera et al. (2003). The drugs AMK, CAP, clofazimine (CLF), ethionamide (ETH), KAN, ofloxacin (OFL) and cycloserine (CLS) (Sigma Chemical Company, St. Louis, MO, USA) were tested. Stock solutions (1 mg/mL) were prepared, filter-sterilised and stored in aliquots at -20ºC. Microplates were prepared as described elsewhere (Luna-Herrera et al. 2003).

The DST inoculum was prepared from a seven-21-day culture in Middlebrook 7H9 medium plus 0.2% (v/v) glycerol, 10% (v/v) oleic acid, albumin, dextrose, catalase (Becton Dickinson Co Microbiological Media BBL, Sparks, MD, USA) and 0.05% (v/v) Tween 80 (Sigma). The turbidity of bacterial suspensions was adjusted to the McFarland 1 standard and diluted 1:50 with supplemented 7H9 medium. The drug concentrations in the microplates were as following: OFL 0.5-16 µg/mL, KAN 0.5-16 µg/mL, AMK 0.5-16 µg/mL, CAP 1.0-32.0 µg/mL, ETH 0.5-16 µg/mL, CLF 0.125-4 µg/mL and CLS 2.0-64 µg/mL. One 1:100-diluted and four undiluted growth controls were included in each plate. The microplates were incubated at 37ºC in a 5% CO_2 atmosphere. After a five-day incubation period, 20 µL of MABA solution (Trek Diagnostics, Westlake, OH, USA) and 12 µL of 20% Tween 80 (Sigma) were added to one growth control and the microplates were incubated overnight. If the MABA turned pink in the positive controls, the entire microplate was developed and incubated overnight. If the MABA did not turn pink, the preparations were incubated for 12-24 h longer until a colour change was observed. The concentration of each drug in the first well where the colour remained blue was recorded as the minimal inhibitory concentration (MIC). The absence of a colour change denoted an absence of mycobacterial growth, which was verified microscopically.

Ethical considerations - This project was approved by the institutional scientific, biosecurity and ethical review board and registered under protocol 1908. Signed informed consent, socio-demographic data and one to three sputum samples were collected from participants. Therefore, this study presented a low risk to participants.

RESULTS

General findings - Fig. 1 shows that among the 290 sputum samples, 57.59% were positive for acid-fast bacteria (AFB) or growing of colonies of mycobacteria, 52.42% for growing colonies of mycobacteria and 48.97% for AFB and mycobacteria cultures. Conversely, 42.41% of samples were negative for either both AFB and/or mycobacterial cultures.

Mycobacteria isolates - Fig. 2 summarises the findings related to mycobacteria identification and drug sensitivity. MTB was identified in 91.45% of the 152 isolates, 88.82% were pure MTB cultures, 2.63% were MTB cultures mixed with nontuberculous mycobacteria (NTM) and 8.55% were NTM cultures. Therefore, MTB were isolated from 47.93% of the 290 sputum samples. Of the 139 MTB cultures, 52.52% were sensitive to SM, INH, RIF, EMB and PZA and 47.48% were resistant to one or more first-line anti-TB drugs.

Sociodemographic and clinical description of a target population with positive MTB cultures - Table I shows that the frequency of PTB was 30.49% higher in men than women. The majority of patients had a high school education and almost one-third had a low education level.

Table II shows the frequency of risk factors and clinical status of our target population. The majority of patients (80%) were addicted to alcohol and tobacco and a minority were addicted to other drugs. Diabetes and chronic obstructive pulmonary disease (COPD) were the predominant co-morbidities, diabetes being the most frequent. In contrast, more than half of the participants did not report associated diseases and none were human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS)-positive according to the survey responses. In addition, none of the patients' medical records indicated an HIV/AIDS-positive

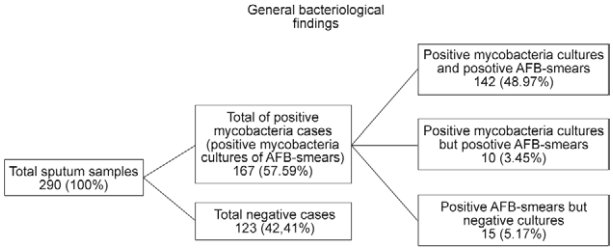

Fig. 1: diagrammatic representation of general bacteriological findings. Sputum samples corresponded to every patient. Positive cultures formed not identified mycobacteria colonies in Lowenstein-Jensen medium. Acid-fast bacteria (AFB) were observed with a microscope in Zhiel-Neelsen-stained sputum smears.

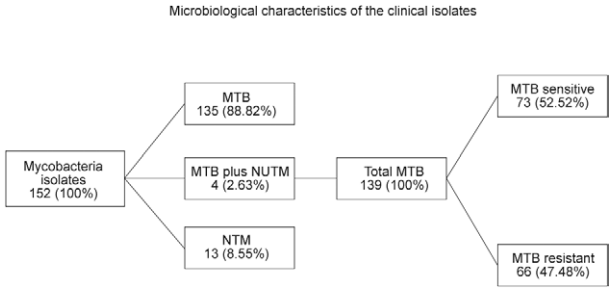

Fig. 2: schematic representation of the main characteristics of the *Mycobacterium tuberculosis* (MTB) clinical isolates. MTB plus non-tuberculosis mycobacteria (NTM) were cultures of mixed *M. tuberculosis* and non-tuberculosis mycobacteria. MTB sensitive and MTB resistant were MTB cultures inhibited or not by streptomycin, isoniazid, rifampin, ethambutol or pirazinamid.

status. Almost half of the patients had advanced PTB. Approximately one-third of patients had been previously treated for PTB, whereas two-thirds of the target group had not been previously treated and a minority of patients (8%) had an unknown history of anti-PTB treatment. The majority of patients [58% (25-64 years old)] were of working age and 16.5% were of school age (15-24 years old). Most patients were blue-collar workers, followed by homemakers, unemployed or retired workers and white-collar workers.

Resistance patterns - Table III describes the distribution of the 139 MTB isolates according to resistance patterns. We identified three groups of MTB isolates: (i) those with resistance to one drug (monoresistance), (ii) those with resistance to two, three or four drugs, but not to INH (polyresistance), and (iii) those with MDR, with resistance to INH and RIF and one or more additional first-line drugs. Furthermore, three groups of patients were formed according to their history of anti-PTB treatment. Table III also shows that our MTB isolates

TABLE III

Patterns of drug-resistance to first-line drugs

	Total number of cases (% of resistance) (n = 139)			
	Previously treated n = 45	New n = 83	Unknown n = 11	
	Cases per category[a] (%)			
	77.78	30.12	18.18	
Patterns	Isolates by category and profile of resistance n (%)			Total n (%)
Monoresistant				
SM	2 (1.44)	7 (5.04)	1 (0.72)	10 (7.19)
INH	5 (3.60)	7 (5.04)	3 (2.16)	15 (10.79)
RIF	0 (0)	0 (0)	0 (0)	0 (0)
EMB	0 (0)	4 (2.88)	0 (0)	4 (2.88)
PZA	0 (0)	0 (0)	0 (0)	0 (0)
Subtotal	7 (5.04)	18 (12.95)	4 (2.88)	29 (20.86)
Polyresistant				
INH + SM	0 (0)	5 (3.60)	0 (0)	5 (3.60)
INH + EMB	1 (0.72)	1 (0.72)	0 (0)	2 (1.44)
INH + PZA	1 (0.72)	0 (0)	0 (0)	1 (0.72)
INH + SM + PZA	1 (0.72)	0 (0)	0 (0)	1 (0.72)
INH + EMB + PZA	2 (1.44)	0 (0)	0 (0)	2 (1.44)
INH + SM + EMB + PZA	2 (1.44)	0 (0)	0 (0)	2 (1.44)
Subtotal	7 (5.04)	6 (4.32)	0 (0)	13 (9.35)
Multidrug-resistant				
INH + RPM + SM	1 (0.72)	0 (0)	0 (0)	1 (0.72)
INH + RPM + PZA	4 (2.88)	0 (0)	0 (0)	4 (2.88)
INH + RPM + PZA + SM	2 (1.44)	1(0.72)	0 (0)	3 (2.16)
INH + RPM + SM + EMB	3 (2.16)	0 (0)	0 (0)	3 (2.16)
INH + RPM + PZA + EMB	2 (1.44)	0 (0)	0 (0)	2 (1.44)
INH + RPM + SM + PZA + EMB	9 (6.47)	0 (0)	2 (1.44)	11 (7.91)
Subtotal	21 (15.11)	1 (0.72)	2 (1.44)	24 (17.27)
Total	35 (25.18)	25 (17.99)	6 (4.32)	66 (47.48)

a: with respect to the number of previously treated, new and unknown-cases; EMB: ethambutol; INH: isoniazid; PZA; pyrazinamide; RIF: rifampicin; SM: streptomycin.

were resistant to at least one of the five first-line anti-TB drugs included in this study. All polyresistant MTB isolates were resistant to INH. Combining the number of monoresistant, polyresistant and MDR-MTB isolates and calculating their percentages with respect to the 139 MTB isolates, the rates of general resistance to each of the drugs were as follows: SM (36/139, 25.90%), EMB (26/139, 18.71%), PZA (26/139, 18.71%), RIF (24/139, 17.27%) and INH (52/139, 37.41%). With respect to the historical treatment category, the highest percentage of resistance among the total number of cases was observed in the group of previously treated cases and was 2.6 and 4.3 times higher than the resistance percentage in new and unknown cases, respectively.

Monoresistance - The percentage of monoresistance was 20.86% (29/139 MTB isolates). Within this group, resistance was only observed against three first-line drugs (SM, INH and EMB), with the highest frequency found against SM and INH [25 of 29 MTB isolates (86.21%)]. The highest percentage of resistance to SM and INH was found in the group of formerly untreated patients [14/29 MTB isolates (48.27%)]. Furthermore, all four EMB-monoresistant MTB isolates were identified in the previously untreated group. Overall, monoresistance to INH was the most frequent phenotype, followed by resistance to SM.

Polyresistance - The overall polyresistance rate was 9.35% (13/139 MTB isolates).The previously treated group of PTB patients exhibited a variety of drug resistance profiles in which two, three or four drugs were involved. Resistance to INH was found in all of these profiles. Combined resistance to INH and SM was not found in the isolates from treated patients, but was found among those who were untreated (5 cases). Moreover, we isolated an MTB strain from untreated participants that was resistant to INH and EMB. Specifically, MTB resistance to INH, SM and EMB was found in both monoresistant and polyresistant isolates, although the latter already demonstrated combined resistance. In patients with an unknown treatment history, we did not find polyresistant MTB.

Multidrug-resistance - MDR was the second most frequent type of resistance. The previously treated group exhibited the highest frequency of MDR among the MDR isolates; additionally, one MDR case was found in the group of patients who had not been previously treated and two cases were found in the group with an unknown treatment history. The prevalence of MDR isolates with respect to all MTB isolates was 17.27% (24/139). In the previously treated group, MDR isolates resistant to three (5/21; 23.81%), four (7/21; 33.33%) and all five (9/21; 42.86%) first-line drugs were found. Two isolates from patients with an unknown history of previous treatment were also resistant to all five drugs.

Susceptibility and resistance to second-line anti-TB drugs - Table IV shows that all of the 37 polyresistant or MDR-MTB isolates were sensitive to CLF, OFL, AMK and CLS. The rates of resistance against the remaining second-line agents (ETH 10.80%, KAN 2.70%, AMK 2.70% and CAP 2.70%) were remarkably low. None of the 24 MDR strains tested in this study exhibited an XDR profile. Nevertheless, a particular strain isolated from a patient who had not been treated previously attracted our attention; in addition to being MDR, this strain was resistant to PZA and SM and all of the second-line aminoglycoside drugs (AMK, KAN and CAP).

DISCUSSION

In this paper, we have presented a socio-demographic and clinical description of a population served by the IMSS. The IMSS is the foremost institution providing social health care to Mexican workers and their families. Within this framework, we have described for the first time the prevalence of resistance to second-line anti-TB drugs in a population living in NL, which is one of the 10 states forming the Mexico/USA border. Moreover, we have contributed to the extension of knowledge regarding the prevalence of resistance to first-line treatments for PTB.

The socio-demographic data collected in this study are consistent with the national data reported in 2011 by the Mexican authorities and the state-wide data reported for NL. The proportion of males with PTB was higher than that of females; we found a prevalence of 30.48% and the rates for the nation and NL were 34.32 and 39.11%, respectively. The majority of our target group was of working age (15-65 years). This situation is also occurring throughout the country (CENAVECE 2012).

Sick people of working age and the high proportion of homemakers observed in this study have a devastating impact on our society because most are workers and fathers and mothers who must provide economic support and care to their families.

In contrast, we noted the following differences between our target population and the national population. First, a significant percentage of diabetics (29.5%) and

TABLE IV

Resistance and sensitivity to seven second-line antitubercular drugs of polyresistant and multidrug-resistant (MDR) *Mycobacterium tuberculosis* (MTB)-isolate

Drug	Concentration range (μg/mL)	Cut-off (μg/mL)	MTB-isolates n (%)	
			Resistant	Sensitive
CLF	0.065-4.0	2.0	0 (0)	37 (100)
ETH	1.0-16.0	4.0	4 (10.80)	33 (9.17)
OFL	0.5-16.0	4.0	0 (0)	37 (100)
KAN	0.5-16.05	4.0	1 (2.70)	36 (97.29)
CAP	1.0-32.0	4.0	1 (2.70)	36 (97.29)
AMK	0.5-64.0	16.0	1 (2.70)	36 (97.29)
CLS	5.0-80.0	40	0 (0)	37 (100)

estimated as minimum inhibitory concentration. AMK: amikacin; CAP: capreomycin; CLF: clofazimine; CLS: cycloserine; ETH: ethionamide; KAN: kanamycin; OFL: ofloxacin.

individuals with respiratory diseases [COPD or pneumoconiosis (14.4%)] and PTB. Alcoholism and smoking were relevant risk factors in our patients (35.25% and 39.57%, respectively), whereas throughout the country, the incidence of PTB among alcoholics was only 7% and the incidence of PTB among smokers was not reported. The incidences of PTB among diabetics and PTB among COPD patients in our target population were 0.32 and 14.4 times the corresponding national rates, respectively. Interestingly, we did not identify patients with PTB and HIV/AIDS, although we intentionally sought these patients. The absence of patients with PTB and HIV/AIDS was confirmed by the absence of this diagnosis in the medical records of the patients. Conversely, at the national level, the rates of PTB among HIV/AIDS patients were reported as 5% and 12% by Castellanos-Joya and García Avilés (2008), respectively. The above differences between our target population and the national population could be due, at least in part, to the fact that the workers and their families affiliated with the IMSS belong to a special population that, in accordance with the law, has paid employment and health insurance. In contrast, the national statistics encompass all Mexicans, including those living in conditions of poverty or extreme poverty [52 million (46.43% of the total population)] (CONEVAL 2011). Most of these individuals have no education or have barely completed the first two or three years of elementary school and lack adequate housing, health care and nutrition. Therefore, these people are significantly more vulnerable than the workers and their families described above.

We collected one to three sputum samples in succession from each participant to determine the presence of AFB and mycobacteria grown in LJ medium until positive results were obtained or all three samples were negative for AFB or mycobacteria. The percentages of positive AFB (57.59%) and mycobacteria isolates (52.41%) found in this study were similar to those found by other researchers (Abe et al. 1992). Therefore, we consider our bacteriological findings acceptable. In addition to MTB, we obtained several NTM isolates, the clinical importance of which needs to be investigated.

The prevalence of MDR strains in our target population (24/139, 17.27%) is within the third quartile of the MDR-TB global prevalence (0.0-28.3%). Mexico has reported a prevalence of 12-33%. Zazueta-Beltran et al. (2011) found a prevalence of MDR-TB of 17.90% in the Mexican state of Sinaloa and Yang et al. (2001) found a rate of 18% in a third-tier hospital in Monterrey. Therefore, the data for our group of patients are consistent with national values and those of the MAM.

The percentage of MDR-TB among new TB cases in our target population was 0.72% (1/139 cases). The range of global rates of MDR-TB among new cases from 1994-2009 was 0-28.3% (WHO 2010). Therefore, the percentage of MDR-TB among new TB cases was 39 times lower in our target population than that in countries with the highest prevalence of MDR-TB among resistant isolates, i.e., the Russian Federation. In contrast, during 1994-2009, Canada, the United States, most countries in the Caribbean and South America, the nations in East, North and Northeast Africa, the Scandinavian peninsula and part of Western Europe, Western India and some Asian countries reported percentages of MDR-TB among new cases similar to those in the MAM (WHO 2010). With respect to MDR in new TB cases in Mexico, only four states (Baja California Norte, Sinaloa, Oaxaca and Chiapas) reported MDR-TB in new TB cases. All of these Mexican states reported rates of 0% to < 3% from 1994-2009 (WHO 2010). At the same time, Sánchez-Pérez et al. (2010) reported that in Chiapas, the proportion of MDR-TB among new cases was 4.6% (2/43). Therefore, our data are comparable to those at the national level. Our data rank among the first quartile of the national prevalence range and were 6.4 times lower than the percentage in the state of Chiapas.

The proportion of MDR among previously treated TB cases worldwide (from 1994-2009) ranged from 0-61.6% (WHO 2010). In the present study, we found a prevalence of 25.18% (35/139 cases). These results place the prevalence of MDR among previously treated TB cases in the second tier on the stratified epidemiological map published by the WHO. The first tier on this map corresponds to a prevalence of 50% or higher and the prevalence range corresponding to our data is 30% to < 50% (WHO 2010), which is comparable to that reported by Yang et al. (2001). The countries with rates similar to ours are Lithuania, Egypt and the Sinai Peninsula, countries of the Malay Peninsula, Israel, Iran and Thailand (WHO 2010). Mexico reported a MDR prevalence of 12% to < 30% among previously treated cases. Sánchez-Pérez et al. (2010) reported that in the state of Chiapas, the percentage of patients with MDR-TB among previously treated patients was 7.95%. Therefore, the prevalence found in our study group was situated in the second tier of the WHO global loads and near the upper limit of the percentages of MDR-TB in Mexico. Furthermore, the MDR-TB prevalence found in this study is three times higher than the MDR-TB prevalence in the state of Chiapas.

The analysis of second-line anti-TB DST is now a widespread practice (Hazbon et al. 2006) due to the emergence of XDR-TB. To our knowledge, this is the first study conducted in North Mexico that was designed to estimate the prevalence of resistance to second-line drugs and was performed with polyresistant MTB and MDR clinical isolates in ambulant PTB patients. Among the cost-effective options for evaluating drug resistance, we chose the MABA (Luna-Herrera et al. 2003), which is a simple and useful method for determining drug resistance to second-line anti-TB drugs. Nevertheless, there is currently no consensus regarding the drug concentration cut-off in the microplate DST that determines whether a strain is resistant or susceptible to a particular drug. Therefore, we utilised the values suggested by Martin et al. (2003) and Morcillo et al. (2004) because Martin's group used resazurin, which is the same redox indicator contained in MABA, and Morcillo et al. (2004) used 3-(4,5-dimethylthiazol-2-yl)2-5-dephenyltetrazolium as the indicator. We chose the AMK cut-off used in the radiometric method because similar MICs were found in the MTB H37Rv strain using MABA and Bactec 460 (Collins et al. 1998). Based on the above criteria, our

results strongly suggest that the clinical isolates analysed in the present study had not developed resistance to CLF, CLS or OFL and that XDR-TB has not emerged. However, the aforementioned presence of one isolate resistant to AMK, KAN and CAP demonstrates a real risk that some circulating MTB strains could acquire additional resistance and become XDR-MTB. Furthermore, in other zones of the MAM, XDR-MTB may already be circulating.

In conclusion, the MDR prevalence among new cases is still low and is equivalent to the national prevalence and to the lowest global burden. The resistance of MDR-TB to second-line drugs is also low, although some MDR-MTB strains that are resistant to all first and second-line anti-TB drugs have already appeared. Therefore, drastic measures are urgently needed to stop the spread of these strains. Otherwise, it is just a matter of time until XDR-TB appears in our population, along with all of the well-known undesirable consequences.

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