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# Circulation of Multidrug-Resistant (MDR) and Extensively Drug-Resistant (XDR) *Mycobacterium tuberculosis* Strains in Mexico

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**Abstract:** Emergence of Extensively Drug-Resistant (XDR) Tuberculosis (TB) is an important public health problem worldwide. In Mexico, the extent of circulation of XDR-TB strains is unknown. Here, the drug-resistance patterns of TB isolates collected in Mexico between 2006 and 2008 were analyzed. Among 137 TB isolates, (58%) were classified as MDR exclusively, (33%) were Pre-XDR and (9%) were assigned as XDR. The results confirmed the circulation of Pre-XDR and XDR-TB in Mexico. Surveillance and drug-susceptibility testing should be improved in Mexico to monitor the circulation of XDR strains.

**Keywords:** Tuberculosis, MDR, Pre-XDR, XDR, Mexico

## Introduction

Tuberculosis (TB) is caused by the infection of *Mycobacterium tuberculosis*. This is an airborne infectious disease and a major public health problem worldwide (Ahmad, 2014) World Health Organization (WHO) estimated 8.6 million people infected with *M. tuberculosis* and 1.3 million deaths in 2012 (WHO, 2013). In 2013, WHO declared Multidrug-Resistant TB (MDR-TB) as a public health crisis and estimated ~450,000 people affected by MDR-TB with ~170,000 MDR-TB deaths in 2012 (WHO, 2014). The emergence of MDR and Extensively Drug-Resistant (XDR) TB brings several concerns and jeopardize the efficacy of strategies aimed to control disease spread. MDR is a form of TB which is characterized by resistance at least to isoniazid (H) and Rifampicin (R). Besides resistance to H and R, XDR strains are also resistant to any of the fluoroquinolones (such as Ofloxacin [Ofx] or Ciprofloxacin [Cip]) and to at least one of three injectable second-line drugs (amikacin [Amk], Kanamycin [Km] or Capreomycin [Cm]) (WHO, 2011).

The development of drug resistance is commonly due to misuse and mismanage of anti-TB drugs (Shin *et al*., 2010). Alternatively, XDR-TB can also be transmitted from individuals carrying drug resistant strains. XDR-TB therapy is challenging, expensive and the likelihood of success is much lower than in patients with ordinary TB or even MDR-TB (Haydel, 2010). Treatment success rate for MDR-TB is about 60%; however, the success of therapy is reduced to half for XDR-TB (Kim *et al*., 2008). Moreover, MDR and XDR-TB require longer treatment and are usually accompanied by more severe side effects (Caminero *et al*., 2010).

Different studies conducted in Latin-American countries have shown the increase of MDR-TB (Pelly *et al*., 2004). The rising of drug resistance in Latin-America and other parts of the world (Migliori *et al*., 2010), highlights the need of national efforts and international cooperation to implement strategies for TB control. Interestingly, rates of latent TB along the Mexico-USA border have been found to be 21.8% in Latino versus 5.6% for non-Latino individuals (Sipan *et al*., 2003). Likewise, young Latinos living near the border are at higher risk for undiagnosed infection and inadequate treatment. Incompleteness of treatment among Latino individuals has been associated with idiosyncrasy and cultural barriers that

play role in adherence and ultimately in treatment success (Kandula *et al*., 2004). Interestingly, Hispanic patients born outside the US or Mexico were more likely to have MDR-TB than were those born within these countries (Bojorquez *et al*., 2013).

In this study, we assessed the circulation of XDR-TB isolates in Mexico. The presence of Pre-XDR strains in different geographical regions of the country highlights the importance of surveillance for the appropriate monitoring of drug resistant strains. within parentheses.

## Materials and Methods

Here, we analyzed 137 epidemiologically non-related *M. tuberculosis* isolates associated with drug resistance collected via the State Laboratory Network in Mexico during 2006 and 2008. Colony morphology and acid-fast bacilli identification on smears by Ziehl-Neelsen technique and culture in Löwenstein-Jensen solid slants were performed. Biochemical analyses for differentiation included nitrate reduction, niacin accumulation test and catalase test both 37 and 68°C. All isolates were subjected to routine culture-based drug susceptibility testing to determine the drug resistance patterns. Susceptibility profiles to first line drugs were determined by the fluorometric method MGIT 960 (Becton Dickinson, Sparks, MD). Susceptibility testing to second line drugs (Amk, Km, Cm, Ofx and Cip), was evaluated by the Proportions Method. Mycobacterial strains were classified into three groups according to their resistance profile. MDR, Pre-XDR (MDR strains also resistant to either a fluoroquinolone or a second-line injectable agent but not both) and XDR-TB. Additionally, resistance to Streptomycin (S), pyrazinamide (Z), Ethambutol (E) and Ethionamide (Eto) was assessed.

## Results

The clinical samples were originally obtained from 26 different states in Mexico (Fig. 1 and Table 1). The patterns of drug-resistance showed that 80 (58%) isolates were exclusively MDR, while 45 (33%) and 12 (9%) were classified as Pre-XDR and XDR, respectively. Overall, resistance to fluoroquinolones (25.5, 21.2% for Ofx and Cip, respectively) was more frequent than resistance to Amk (11%), Km (19%) and Cm (8.7%). This pattern was also observed among Pre-XDR strains where resistance to fluoroquinolones was also more common (Table 1).

In general, resistance among Pre-XDR strains was evenly distributed between strain resistant to fluoroquinolones (22) and injectable drugs (23). Twenty Pre-XDR isolates exhibited resistance to only one fluoroquinolone (3) or one injectable drug (17). Twenty strains were resistant to both fluoroquinolones and only one isolate was resistant to Amk and Km. Additionally, four isolates displayed resistance to all injectable drugs. All twelve XDR strains were resistant to Ofx and only three were susceptible to Cip. Most XDR strains were also resistant to Km and only half to Amk. Resistance to Cm was observed among 5 isolates.

Overall resistance to other first-line drugs ranged between 35-39% (37.2, 38.7 and 35.8% for S, Z and E, respectively). Resistance to Eto was slightly higher (39.4%). Resistance to first-line drugs was slightly higher among Pre-XDR isolates than MDR strains. The small number of XDR samples did not allow establishing resistance patterns (Table 2). However, resistance to Eto was observed in almost all XDR strains, while ~30% of the MDR and Pre-XDR isolates showed resistance to Eto (Table 2).

## Discussion

In this study, we have shown the characteristic drug resistance patterns of *M. tuberculosis* strains circulating in Mexico. The results showed the increasing problem with Pre-XDR and XDR-TB. In Mexico, different studies have reported the presence of MDR strains (Zazueta-Beltran *et al*., 2011, Bojorquez-Chapela *et al*., 2013). Circulation of XDR strains in the country has also been suggested (Banerjee *et al*., 2008). Here, a significant number of Pre-XDR strains were observed. The circulation of such resistant strains is a major concern considering that acquisition of resistance to a second-line anti-TB agent is highly possible in an environment where inappropriate treatment management is frequently observed (Laniado-Laborín and Cabrales-Vargas, 2000).

Table 1. Strain distribution per state

| State | N (%) |
|---|---|
| AGU | 3 (2.2) |
| BCN | 2 (1.5) |
| BCS | 5 (3.6) |
| CAM | 3 (2.2) |
| CHH | 7 (5.1) |
| CHP | 16 (11.7) |
| COA | 2 (1.5) |
| COL | 3 (2.2) |
| GRO | 2 (1.5) |
| GTO | 7 (5.1) |
| HGO | 11 (8) |
| JAL | 8 (5.8) |
| MIC | 2 (1.5) |
| MOR | 7 (5.1) |
| NAY | 1 (0.7) |
| NLE | 7 (5.1) |
| OAX | 6 (4.4) |
| PUE | 6 (4.4) |
| ROO | 3 (2.2) |
| SIN | 5 (3.6) |
| SON | 4 (2.9) |
| TAB | 2 (1.5) |
| TAM | 4 (2.9) |
| VER | 17 (12.4) |
| YUC | 3 (2.2) |
| ZAC | 1 (0.7) |

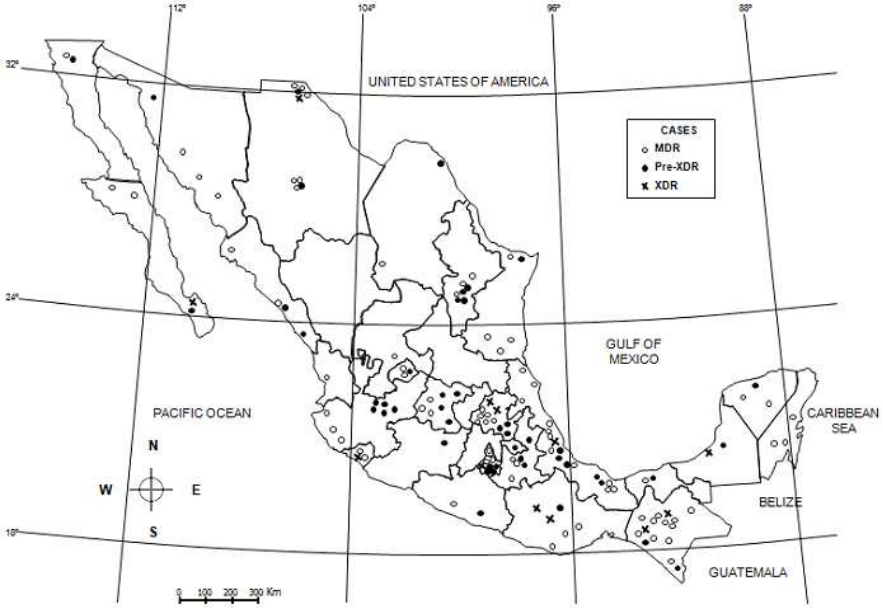


Fig. 1. States from which resistant *Mycobacterium tuberculosis* isolates were recovered are indicated on the map. MDR, Pre-XDR and XDR-TB isolates are depicted on the map

Table 2. TB strains resistance patterns

| | N% | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | First-line | | | | Second-line | | | | | |
| | N | S | Z | E | Amk | Km | Cm | Ofx | Cip | Eto |
| MDR | 80 | 29(36.2) | 28(35) | 25(31.2) | | | | | | 26(32.5) |
| Pre-XDR | 45 | 20(44.4) | 22(48.8) | 21(46.6) | 9(20) | 15(33.3) | 7(15.5) | 23(51.1) | 20(44.4) | 17(37.7) |
| XDR | 12 | 2(16.6) | 3(25) | 3(25) | 6(50) | 11(91.6) | 5(41.6) | 12(100) | 9(75) | 11(91.6) |

Pre-XDR-TB strains have been reported to circulate in the USA, China, South Africa and Korea (Banerjee *et al*., 2008; Kim *et al*., 2010; Qi *et al*., 2012; Van Der Plas *et al*., 2011). The presence of these Pre-XDR-TB strains in different parts of the world highlights the importance of timely diagnosis aimed to prevent the development of XDR-TB.

While in most bacteria drug resistance is usually acquired by horizontal transfer of mobile genetic elements (plasmids, transposons or integrons), in the case of *M. tuberculosis* acquisition of resistance is mediated primarily by spontaneous mutations in chromosomal genes, which are selected under sub-optimal therapy regimes (Kochi *et al*., 1993). Thus, drug resistance has been associated with several mycobacterial genes (Kozhamkulov *et al*., 2011). It is noteworthy that classical mutations associated with resistance to a given drug could initiate resistance towards other drugs (Safi *et al*., 2008), as in the case of resistance to H which is also related to resistance to Eto (Banerjee *et al*., 1994). Therefore, it is not surprising the high degree of resistance to Eto observed among all these MDR-TB strains. Likewise, the presence of Pre-XDR strains is a latent risk for development of XDR strains.

Cross-resistance to fluoroquinolones is commonly assumed; however, the presence of mutations conferring resistance to particular fluoroquinolones has also been reported (Von Groll *et al*., 2009). Here, four isolates with resistance to Ofx but sensitive to Cip were identified. However, the molecular bases responsible for this resistant phenotype were not assessed. Cross-resistance between second-line injectable drugs has also been shown (Alangaden *et al*., 1998). Conversely, other studies have suggested that Km and Amk cross-resistance is not absolute, showing variable patterns and levels of resistance (Kruuner *et al*., 2003). Our results showed a high correlation between resistance to Km and both Amk and Cm. Importantly, universal cross-resistance to second-line injectable agents was not observed.

The main limitation of this study is the likelihood of underestimating the actual number of XDR-TB since complete drug susceptibility testing (other fluoroquinolones such as moxifloxacin) was not performed. Therefore, we cannot rule out resistance to other fluoroquinolones. Also, underreporting is a major concern because most likely not all TB cases are identified by the current surveillance system. Finally, the characterization of the mutations

responsible for drug resistance among these specimens further limits our understanding on the molecular basis controlling TB drug resistance.

## Conclusion

In conclusion, it is of the utmost importance to evaluate the drug resistance profile, as a compulsive component in the therapeutic monitoring of TB patients. Identification of resistance patterns against the first and second-line of anti-TB drugs in Mexico will contribute to develop better strategies for disease control. Surveillance and drug susceptibility testing should be improved in Mexico to monitor the circulation of MDR, Pre-XDR and XDR-TB strains.

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## Author's Contributions

Carlos A. Vazquez-Chacon and Armando Martinez-Guarneros contributed equally in this study.

## Ethics

The authors of this manuscript declare no conflict of interest.

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