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Molecular and phenotypic characterisation of *Mycobacterium tuberculosis* resistant to anti-tuberculosis drugs

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SUMMARY

SETTING: Dr Cetrángolo Hospital, Buenos Aires, Argentina.

OBJECTIVES: To characterise drug-resistant (DR), multidrug-resistant (MDR-) and extensively drug-resistant (XDR-) *Mycobacterium tuberculosis* isolates, and identify their genetic profiles, drug resistance levels and resistance-conferring mutations.

DESIGN: Phenotypic drug susceptibility testing methods were used to determine drug resistance profiles. Minimal inhibitory concentrations (MICs) of isoniazid (INH), rifampicin (RMP) and levofloxacin (LVX) from 169 DR tuberculosis (TB) isolates, 78 of them mono-resistant to INH, 13 to RMP, 7 to LVX, and 71 MDR-TB, were determined. Multiplex allele-specific polymerase chain reaction and DNA sequencing were used to detect mutations in *kat*G, *rpo*B and *gyr*A/B genes. Genotyping was performed using spoligotyping and insertion sequence *6110* restriction fragment length polymorphism.

RESULTS: In total, 38.9% of the INH-resistant (INH^R) isolates had an MIC ⩾ 32 μg/ml; 61.3% of the RMP-resistant (RMP^R) isolates had an MIC ⩾ 64 μg/ml and 55.6% of the LVX-resistant (LVX^R) isolates had an MIC 4–⩾16 μg/ml. The main mutations found in INH^R isolates were *kat*G315 (53.7%) and *inh*AP-15 (25.5%), whereas in RMP^R isolates the main mutations were *rpo*B531 (61.9%), followed by *rpo*B526 (16.7%). LVX^R isolates showed mutations in *gyr*A94/90. Haarlem, LAM and T were the main spoligotyping families found. *kat*G315 was mainly associated with Haarlem and LAM, whereas *inh*AP-15 was associated with T.

CONCLUSIONS: Several isolates showed an association between high INH^R levels and *kat*G mutation; others from the Haarlem family were prone to becoming MDR-TB and continue to circulate in the community.

KEY WORDS: *M. tuberculosis*; genotypes; drug resistance levels; genetic mutations

TUBERCULOSIS (TB) remains a major global health problem and is the second leading cause of death from infectious diseases worldwide after the human immunodeficiency virus (HIV) infection. The global burden of TB remains enormous, and the World Health Organization (WHO) estimates that around one third of the world population is infected with *Mycobacterium tuberculosis,* of whom 10% will develop active TB. In 2011, there were almost 9 million new TB cases (13% HIV co-infected) and 1.4 million TB deaths.[1,2]

Drug-resistant (DR, defined as resistance to at least one anti-tuberculosis drug), multidrug-resistant (MDR-TB, resistance to isoniazid [INH] plus rifampicin [RMP]) and extensively drug-resistant TB (XDR-TB, defined as MDR-TB that is also resistant to one injectable agent [amikacin, kanamycin or capreomycin] plus one fluoroquinolone [FQ]), show increasing trends and are of major public health concern in several countries.[2,3] All of these forms of TB pose a threat to TB control efforts, mainly in countries where HIV and TB prevalence are highest. Globally, 3.7% of new TB cases and 20% of previously treated cases are estimated to be MDR-TB. The WHO estimates that around 500 000 new MDR-TB cases occur each year, and around 5–9% of these will evolve to XDR-TB, which has so far been identified in 84 countries.[2–4] Argentina reported a total of 10 336 TB cases in 2011, 2.2% of which were patients with MDR-TB who had not previously received treatment for TB (NSM, personal communication, XLIV Annual Argentinean Meeting, 2012).

The purpose of the present study was to obtain an overview of MDR-TB transmission in the North Buenos Aires City area (NBA), which contributes 30% of TB and XDR/MDR-TB cases to the country burden. The study was aimed at describing the relationship among genetic profiles, drug resistance levels and resistance-conferring mutations in MDR-/XDR-TB organisms.

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MATERIALS AND METHODS

M. tuberculosis clinical isolates from patients receiving medical attention at the Dr Antonio Cetrángolo Hospital were included from 2006 to 2010.

Mycobacterial isolates

A total of 20 959 clinical specimens were processed and decontaminated using sodium hydroxide/*N*-acetyl-L-cystein or the hypertonic solution of sodium chloride (since 2008), and then concentrated and inoculated onto Löwenstein-Jensen (LJ), Stonebrink and the BACTEC™ MGIT™ 960 system (BD, Vicente Lopez, Argentina).[5] One drop of this solution was used to prepare the smears.

Identification

Mycobacterial isolates were identified following an algorithm previously described based on biochemical and molecular tests.[6] Isolates belonging to the *M. tuberculosis* complex were confirmed using BD MGIT™ TBc (TBc ID) and spoligotyping.[7,8]

Drug susceptibility testing

The reference strain H37Rv was used as the control for drug susceptibility testing (DST). Table 1 shows the drug concentrations used in each of the methods.

Drug resistance profile

The indirect proportion method on LJ (ILJ) and the MGIT 960® SIRE kit were used to determine the drug resistance profile of isolates against first-line anti-tuberculosis drugs and levofloxacin (LVX). LVX was only tested using ILJ.[9,10]

Drug resistance levels

Drug resistance levels were explored by determining the minimal inhibitory concentrations (MICs) of isoniazid (INH), rifampicin (RMP) and LVX using the microplate colorimetric method with resazurin (resazurin microtiter assay [REMA]) as redox indicator as per a previously described protocol.[10,11]

Molecular detection of drug-resistant mutations

DNA extraction from clinical isolates was carried out by boiling a loopful of colonies in 400 μl of sterile water for 30 min. The supernatant was used for the polymerase chain reaction (PCR).[12]

Table 1 Drug concentrations used in drug susceptibility testing methods

Drug	MGIT 960 μg/ml	Indirect LJ proportion method μg/ml	Colorimetric micromethod Range μg/ml	Colorimetric micromethod Cut-off μg/ml
Isoniazid	0.1	0.2	0.03–32.0	⩾0.25
Rifampicin	1.0	40.0	0.06–64.0	⩾0.50
Levofloxacin	—	2.0	0.13–16.0	⩾0.50

MGIT = Mycobacteria Growth Indicator Tube; LJ = Löwenstein-Jensen.

Isoniazid and rifampicin resistance

A previously assessed multiplex allele-specific PCR (MAS-PCR) and DNA sequencing were used to detect mutations conferring resistance.[12] MAS-PCR detected INH and RMP resistance and mutations in codon 315 of the *kat*G gene in site −15 of the *inh*A gene promoter region (*inh*AP-15) and in codons 516, 526 and 531 of the *rpo*B gene. The following forward and reverse primers (5′–3′) were used: *kat*GR ATA CGACCTCGATGCCGC, *kat*GF GCAGATGGGGCT GATCTACG, *inh*APR CACCCCGACAACCTATCG, *inh*APF GCGCGGTCAGTTCCACA, *rpo*BR TTGA CCCGCGCGTACAC, *rpo*B-516 CAGCTGAGCCAA TTCATGGA, *rpo*B-526 CTGTCGGGGTTGACCCA and *rpo*B-531 CACAAGCGCCGACTGTC.[12]

Sequencing of only a fragment of the *inh*AP (648 base pairs [bp], containing site −15) and *katG* genes (435 bp, including codon 315) was carried out to detect INH resistance. Sequencing primers (5′–3′) were as follows: *kat*GF GCAGATGGGGCTGATCTACG and *kat*GsR AACGGGTCCGGGATGGTG. Sequencing of the whole *inh*A gene (open reading frame [ORF]) was also performed using forward and reverse primers (5′–3′): *inh*AF GTATGGGCCACTGA CAACAC and *inh*AR CCGCCGAACGACAGCA GCAGGA. To detect RMP resistance, 250 bp of the *rpo*B gene (including the hot-spot region) were sequenced using forward and reverse primers (5′–3′): ROF GTCGCCGCGATCAAGGA and RIR TGA CCCGCGCGTACAC.[12] H37Rv was used as wild-type (WT) control for MAS-PCR and sequencing.

Levofloxacin resistance

Sequencing of the quinolone resistance-determining region (QRDR) of *gyr*A (320 bp) and *gyr*B (375 bp) was performed on LVX-resistant (LVX^R) isolates. Sequencing primers (5′–3′) used were as follows: *gyr*AF CAGCTACATCGACTATGCGA and *gyr*AR GGG CTTCGGTGTACCTCAT; *gyr*BF CCACCGACATCG GTGGATT and *gyr*BR CTGCCACTTGAGTTTGT ACA.[13] Sequencing was performed at the Biotechnology Institute, National Institute of Agricultural Technology using the 3130xl Genetic Analyzer (Applied Biosystems, Buenos Aires, Argentina).

Genotyping

Spoligotyping

Spoligotyping was used as a screening tool on DR *M. tuberculosis* isolates as per the protocol described by van Embden et al.[14] SPOLDB4 (http://www.pasteur-guadeloupe.fr/tb/bd_myco.html) was used to group genetically related isolates in different clades and previously reported spoligotyping families (SPOF).

IS6110 restriction fragment length polymorphism

IS*6110* RFLP was performed on DR isolates grouped using spoligotyping to differentiate whether or not the isolates were identical. The protocol used was a modification of that used by van Embden et al.[15]

Ethics

Informed consent was obtained at the moment of the TB diagnosis. The study did not affect treatment decisions or medical behaviour; approval was obtained from the Ethics Committee of Dr Cetrángolo Hospital, Buenos Aires, Argentina.

RESULTS

A total of 2777 (13.2%) mycobacterial isolates were recovered: 2607 (93.9%) were identified as *M. tuberculosis*, while 6.1% were non-tuberculous mycobacteria.

Drug susceptibility testing

Drug resistance profiles

DST results from 1305 isolates were obtained and, based on their drug resistance profile, 169 DR *M. tuberculosis* isolates were finally included (Table 2).

Drug resistance levels

Table 3 shows the MIC results for INH, RMP and LVX obtained using REMA. MIC (in μg/ml) results were obtained in 96.6% (144/149) of the INH-resistant (INH^R) isolates, in 89.3% (75/84) of the RMP-resistant (RMP^R) isolates and in 81.8% (9/11) of the LVX^R isolates. Isolates with invalid results failed to grow in REMA.

Molecular detection of drug resistance

Isoniazid and rifampicin

MAS-PCR. MAS-PCR detected 53.7% (80/149) of INH^R isolates and strains (MDR-TB + INH-monoresistant) with mutation in *kat*G315, and 25.5% (38/149) in *inh*AP-15. In addition, four isolates (2.7%) had both mutations, while no mutations were found in 27 (18.1%) INH^R isolates. Of 71 MDR-TB isolates, 43 (60.5%) had *kat*G315 mutations, while 16 (22.5%) had *inh*AP-15 mutations ($P = 0.0213$); 47.4% (37/78) of INH-monoresistant isolates had a *kat*G315 mutation, while 28.2% (22/78) had an *inh*AP-15 mutation ($P = 0.2381$).

Of 84 RMP^R isolates, 52 (61.9%) had mutations in codon 531, 16.7% (14/84) in codon 526 and 4.8% (4/84) in codon 516 of the *rpo*B gene. No mutations were found in 14 (16.7%) RMP^R isolates.

Table 2 Isolates included in the study according to drug resistance profile

Resistance	*n* (%)
Isoniazid*	78 (46.2)
Rifampicin	13 (7.7)
Multidrug-resistant tuberculosis†	71 (42)
Levofloxacin	7 (4.1)
Total	169 (100)

*1 isolate plus levofloxacin resistance.
†3 isolates plus levofloxacin resistance.

Table 3 Drug resistance levels expressed as MIC ranges using the colorimetric micromethod obtained from phenotypically drug-resistant *M. tuberculosis* isolates*

Indirect LJ proportion method	Colorimetric micromethod MIC ranges, μg/ml	Isolates†	
		n (%)	Total *n* (%)
INH^R ($n = 149$)	⩾32	56 (38.9)	144 (96.6)
	2–16	64 (44.4)	
	0.25–1	24 (16.7)	
RMP^R ($n = 84$)	⩾64	46 (61.3)	75 (89.3)
	16	1 (1.3)	
	0.5–8	25 (33.3)	
	0.06–⩽0.25	3 (4.0)	
LVX^R ($n = 11$)	4–⩾16	5 (55.6)	9 (81.8)
	0.5–2	1 (11.1)	
	0.06–⩽0.25	3 (33.3)	

*Cut-off (μg/ml): INH ⩾ 0.25; RMP ⩾ 0.50; LVX ⩾ 0.50.
†Isolates with MIC results.
MIC = minimal inhibitory concentration; LJ = Löwenstein-Jensen; INH^R = isoniazid-resistant; RMP^R = rifampicin-resistant; LVX^R = levofloxacin-resistant.

DNA sequencing

Global agreement in detecting mutations was observed between both molecular methods. *kat*G and *inh*A (promoter region and ORF) were sequenced in those 27 INH^R isolates that showed no mutations using MAS-PCR. One isolate (INH MIC 2 μg/ml) had *kat*G TGG321TGC (W>C) mutation, and two (INH MIC 1 μg/ml; INH MIC 8 μg/ml) had a GGG83AGG (G>R) *inh*A mutation. DNA sequencing increased by 2% the detection of gene mutation with respect to MAS-PCR. Mutations in 84% of INH^R isolates were detected with both methods.

A 250-bp fragment of *rpo*B was sequenced in 14 RMP^R isolates; no mutations were detected using MAS-PCR. Only two showed double mutations: CAA513CCA (Q>P) and GAG565CAG (E>Q). CAA 513CCA (Q>P), TCG522TTG (S>L) and ATC572 TTC (I>F) mutations were also found in three different monoresistant isolates. Nine isolates showed no mutations in the sequence studied. Sequencing of the whole *rpo*B gene was performed in five of these isolates, and no mutations were found.

Three of the 11 LVX^R isolates showed mutations in *gyr*A94 (1 GAC94CAC: D>H, 2 GAC94TAC: D>Y) and 1 in *gyr*A90 (GCG90GTG: A>V), while 7 had a WT sequence. In addition, two isolates had the AGC95ACC (S>T) polymorphism in *gyr*A and all of the LVXR isolates showed WT sequence for *gyr*B. H37Rv was correctly detected as WT for all the genes studied. 100% concordance between phenotypic DST (ILJ/MGIT 960) and molecular methods was found.

Drug resistance levels and mutations

Most INH^R isolates with the *kat*G315 mutation showed high (INH MIC ⩾ 32 μg/ml) to intermediate (INH MIC 4–16 μg/ml) resistance levels, while more than 50% of those with the *inh*AP-15 mutation presented low resistance levels ($P = 0.0352$). Most

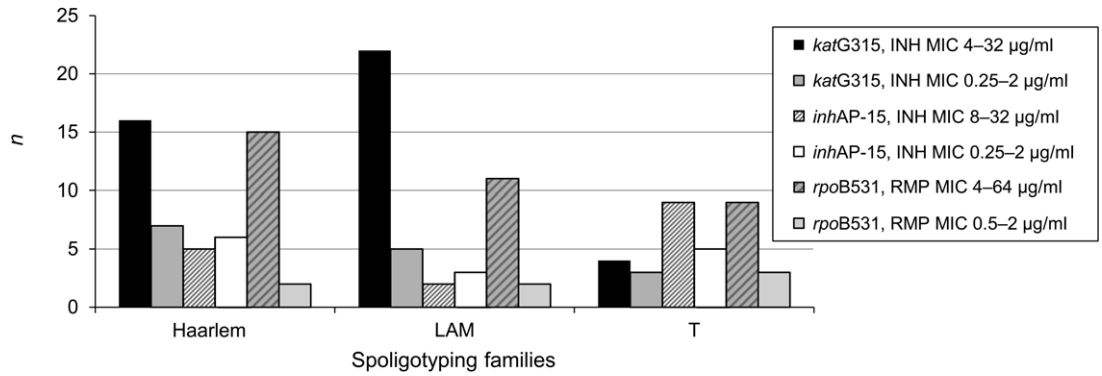

Figure Number of INH^R and RMP^R isolates mutated in different genes according to the spoligotyping families and MIC values. LAM = Latin-American and Mediterranean family; T = ill-defined T Super Family; INH = isoniazid; MIC = minimum inhibitory concentration; RMP = rifampicin; ^{R} = resistant.

RMP^R isolates (42.9%) had an *rpo*B531 mutation and high RMP resistance levels (RMP MIC ⩾ 64 μg/ml); half of the *rpo*B526 mutated strains had high RMP resistance levels and 3 of 4 *rpo*B516 strains showed high drug resistance levels (Appendix Table A.1).*

Three *gyr*A94 mutated isolates had high drug resistance levels (LVX MIC ⩾ 16 μg/ml), while those mutated in *gyr*A90 had low LVX resistance levels (LVX MIC ⩾ 1 μg/ml). Three LVX^R isolates detected using ILJ and with no mutations in either *gyr*A or *gyr*B showed lower MIC values (0.25 μg/ml; Appendix Table A.1).

Genotyping

Spoligotyping

DR isolates were grouped in nine SPOFs with different sub-types. The SPOFs contained 140 (82.8%) isolates, another 16 isolates (9.5%) belonged to different clades and only 13 (7.7%) had orphan spoligotyping patterns (OP). Most DR isolates belonged to the Haarlem (H; 51/169, 30.2%), Latin-American and Mediterranean (LAM; 43/169, 25.4%) and the ill-defined T-Super families (T; 35/169, 20.7%; $P = 0.04633$). Appendix Table A.2 shows the SPOFs found in DR isolates, DST patterns and resistance-conferring mutations. Appendix Table A.3 shows the percentages of mutated strains in *rpo*B, *kat*G and *inh*A according to their spoligotyping patterns.

Relationship between minimal inhibitory concentration, spoligotyping and mutations

INH^R isolates belonging to the Haarlem and LAM SPOF with the *kat*G315 mutation were more frequently associated with high than with low INH resistance levels ($P_{\text{Haarlem}} = 0.0173$, $P_{\text{LAM}} < 0.0001$). For those T SPOF isolates with *kat*G315 mutations, there were no significant differences between the two levels ($P = 0.5077$); the same occurred among the number of isolates that mutated in *inh*AP-15 with high or low INH resistance levels in any of the three main SPOFs ($P = 0.4300$). RMP^R isolates were grouped mostly at high RMP resistance levels (RMP MIC 4–64 μg/ml), independently of the SPOF and resistance-conferring mutation (Figure).

Insertion sequence 6110*-restriction fragment length polymorphism*

Valid results were obtained from 113 DR isolates grouped in SPOF/clades showing 90 different RFLP patterns. As the spoligotyping analysis detected 13 OP, a total of 103 (81.7%) different genetic patterns were represented in DR *M. tuberculosis* isolates. RFLP analysis detected 66.7% of polymorphisms in the LAM (subtypes 3, 5 and 9), 67.5% in the H (H2 and H3) and 79.2% in the T families.

DISCUSSION

Most *kat*G315 mutated isolates had high to intermediate INH resistance levels, while more than half of the *inh*AP-15 mutated isolates had low drug resistance levels. These results are in concordance with previous reports.[16–18] Sequencing showed that the *kat*G AGC315ACC (S>L) mutation is the most frequent in this gene, which is in line with previous reports.[12,19–21] Of the 149 INH^R isolates, 24 (16%) could not be confirmed molecularly, which suggests that other mechanisms or mutated genes may be responsible for this drug resistance phenotype.[15,22,23]

Most RMP^R isolates had high drug resistance levels, as reported previously.[24,25] Using sequencing, the TCG531TTG (S>L) mutation was mainly found in codon 531 of the *rpo*B gene, as previously reported in NBA and elsewhere.[12,21,26,27] When sequencing the whole gene, 6.3% (5/80) of the RMP^R isolates showed no mutation. According to the literature, approximately 5% of the RMP^R isolates cannot be mutated in the *rpoB* gene.[28]

*The Appendix is available in the online version of this article at http://www.ingentaconnect.com/content/iuatld/ijtld/2013/00000017/00000008/art00017

Using both MAS-PCR and sequencing, drug resistance was detected in more than 80% of INH^R/RMP^R isolates. As reported previously, mutations in *gyr*A94 and *gyr*A90 were most frequently found in LVX^R isolates.[29,30] In this study sample, 11 fluoroquinolone-resistant (FQ^R) isolates were included. LVX is used in the treatment of acute respiratory and urine infections. Prescription of these drugs for patients with active TB before diagnosis could generate a selection of FQ^R micro-organisms. As FQ use is associated with MDR-TB treatment, the assumption of MDR-TB without microbiological confirmation could generate FQ^R cases if an inappropriate regimen is used.

In NBA, FQ^R may be mainly due to the global use of these drugs for the treatment of common infections acquired in the community. Mutations outside of the QRDR region of the *gyr*A/B gene may have been responsible for phenotypic resistance in isolates where gene mutations were not observed. Furthermore, efflux pumps have been proposed as alternative mechanisms of drug resistance.[22,23] Alterations in these pumps could increase drug (INH, RMP, FQ) flux outside the micro-organism.[22,23,31]

As reported previously, worldwide and in Argentina in particular, drug resistance is related to the mutated genes described above (*kat*G315, *inh*AP-15 and *rpo*B); also, DR isolates were distributed mainly among the H SPOF, followed by the LAM and T (reported mainly in America, Europe and Africa) families.[32,33] The Beijing, X, F36, U and S SPOF were also found in this and previous reports.[32,33]

In our study, *kat*G315 mutations were mainly associated with MDR-TB and *inh*AP-15 with monoresistance to INH, in line with other reports.[34,35] Moreover, *kat*G315 was mainly associated with H and LAM, while T was most often associated with *inh*AP-15. In addition, mutations in *rpo*B531 and 516 were represented among the Haarlem, LAM and T families, while the *rpo*B526 mutation was absent from the LAM family.[32,33] Interestingly, in our setting, genetic diversity of more than 80% was found among DR *M. tuberculosis* isolates.

CONCLUSIONS

Phenotypic and molecular characterisation of DR *M. tuberculosis* isolates provided further information on mutations, drug resistance levels and genotypes present in NBA. The absence of mutations in isolates phenotypically resistant to INH, RMP and LVX opens the possibility for further studies on other genes related to drug targets or different mechanisms of drug resistance.

Mutations and genetic patterns prevalent in NBA are common to other geographic areas.[33,35] Information generated by this study may thus be of interest for TB control both globally and locally, as it might help to prevent the development of MDR-TB from an initial INH^R isolate profile. In NBA, the prevalence of INH resistance is >20% (new and relapsed cases; NSM, personal communication). Information on low resistance levels associated with *inh*AP-15 mutations would allow clinicians to adjust treatment regimens, retaining INH in potentially useful doses, particularly in settings where rapid molecular testing is available.[12,21]

Furthermore, the genotyping analysis of DR isolates would contribute to surveillance activities on the clonal dispersion of the community transmission of the main lineages, and to estimate the proportions of endogenous reactivation and active transmission. The fact that most DR/MDR-TB isolates were not grouped in RFLP clusters suggests that these cases may mostly have occurred due to endogenous reactivation; approximately 20% may have been due to active transmission.

Clinical reasons for the study findings were not completely elucidated. When a possible explanation was found, it was taken into consideration; however, further investigation into these issues is required.

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Conflict of interest: none declared.

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APPENDIX

Table A.1 Relationships between drug resistance levels and mutations found in drug-resistant *M. tuberculosis**

Indirect LJ proportion method	Drug range, μg/ml	Gene mutation					Total *n* (%)
		*kat*G 315	*inh*A (−15)	*kat*G-*inh*A (315, −15)	*inh*A ORF	None detected	
INH^R (n = 149)	INH						
	4–⩾32	57	15	2	2	11	87
	0.25–2	18	20	2	1	13	54
	⩽0.03–0.13	0	1	0	0	2	3
	Total						144 (96.6)
		*rpo*B 516	*rpo*B 526	*rpo*B 531	Other codons	None detected	
RMP^R (n = 84)	RMP						
	⩾64	3	7	36	0	0	46
	16–32	1	0	0	0	0	1
	4–8	0	3	8	2	1	14
	0.5–2	0	2	3	2	4	11
	0.06–⩽0.25	0	1	2	0	0	3
	Total						75 (89.3)
		*gyr*A 90	*gyr*A 94	*gyr*B 515	Other codons	None detected	
LVX^R (n = 11)	LVX						
	4–⩾16	0	3	0	0	2	5
	0.5–2	1	0	0	0	0	1
	0.06–⩽0.25	0	0	0	0	3	3
	Total						9 (81.8)

*Cut-off (μg/ml): INH ⩾ 0.25; RMP ⩾ 0.50; LVX ⩾ 0.50.
LJ = Löwenstein-Jensen; INH = isoniazid; ^{R} = resistant; ORF = open reading frame; RMP = rifampicin; LVX = levofloxacin.

Table A.2 Spoligotyping patterns and mutations in drug-resistant clinical isolates

Spoligotyping family/clade		Spoligotyping pattern	*n*	INH[R]	RMP[R]	MDR-TB	Mutation					
							*kat*G 315	*inh*AP –15	*kat*G/*inh*AP 315/–15	*rpo*B 516	*rpo*B 526	*rpo*B 531
Haarlem (*n* = 47)	H1	777777774020771	1	0	1	0	0	0	0	0	0	1
	H2	000000004020771	23	5	0	18	22	0	1	1	3	14
	H3	000000004220771	2	0	0	2	1	0	0	0	0	1
		617777777720771	1	1	0	0	0	0	0	0	0	0
		777777777720771	19	13	3	3	2	11	0	0	3	1
	H4	777777777420771	1	0	0	1	1	0	0	0	0	0
Beijing (*n* = 2)		000000000003771	2	1	1	0	0	0	0	0	0	1
LAM (*n* = 43)	3	776137607760771	2	0	0	2	0	2	0	0	0	2
		776177607760731	2	1	1	0	1	0	0	0	0	1
		776177607760771	6	4	0	2	4	1	0	0	0	0
		776177607700171	1	1	0	0	0	0	0	0	0	0
	5	777737607760770	18	10	0	8	17	0	1	0	0	8
		777737607760771	1	1	0	0	1	0	0	0	0	0
	9	777777607760771	11	8	0	3	3	1	1	1	0	1
		777677607760771	1	0	0	1	1	0	0	0	0	1
		377777607760771	1	1	0	0	0	0	0	0	0	0
T (*n* = 35)	1	777777777760771	12	4	2	6	2	4	0	0	5	3
		677777777760771	1	1	0	0	1	0	0	0	0	0
		777775777760771	1	1	0	0	1	0	0	0	0	0
		777777777760700	1	1	0	0	0	0	0	0	0	0
		777741017760771	3	0	0	3	0	3	0	0	0	3
		777774077560771	2	2	0	0	0	2	0	0	0	0
		777777663760771	2	2	0	0	2	0	0	0	0	0
		777740777760771	1	0	0	1	1	0	0	0	0	1
	2	777777407560731	3	2	0	1	3	0	0	1	0	0
	3	777737777760771	2	1	0	1	0	1	0	0	0	1
	5	775777757760771	1	0	0	1	0	1	0	0	0	1
	TV	777740017760771	3	0	1	2	0	2	0	0	0	3
	T5_MAD2	777777557760771	3	0	1	2	0	0	0	0	0	0
U (*n* = 2)		777777600000771	2	1	1	0	0	0	0	0	1	0
U (likely H; *n* = 1)		777777770000000	1	0	0	1	0	1	0	0	0	1
X (*n* = 4)	1	777776777760771	2	0	1	1	0	0	0	0	0	0
	3	700036777760771	2	1	0	1	1	0	1	0	0	1
S (*n* = 1)		776377777760771	1	1	0	0	1	0	0	0	0	0
F36 (*n* = 1)		000000007760771	1	0	0	1	1	0	0	1	0	0
C79		777777663360771	7	5	0	2	7	0	0	0	1	0
C86		757777777660771	2	0	0	2	0	0	0	0	1	0
C59		777777777730771	2	0	0	2	0	2	0	0	0	2
C91		776357777760771	1	1	0	0	0	1	0	0	0	0
C106		777741000760771	1	1	0	0	0	1	0	0	0	0
C69		777740000760771	1	0	0	1	1	0	0	0	0	1
OP			13	8	1	4	6	3	0	0	0	4

INH = isoniazid; [R] = resistant; RMP = rifampicin; MDR-TB = multidrug-resistant tuberculosis; H = Haarlem; LAM = Latin-American and Mediterranean family; T = ill-defined T Super Family; C = previously found spoligotyping clade; OP = orphan spoligotyping pattern.

Table A.3 Gene mutations in drug-resistant *M. tuberculosis* belonging to different spoligotyping families

INH					RMP				
						*rpo*B			
SPOF	*kat*G315* *n* (%)	*inh*AP-15† *n* (%)	*kat*G/*inh*AP (315/–15) *n* (%)	No mutations detected *n* (%)	SPOF	516 *n* (%)	526 *n* (%)	531 *n* (%)	No mutations detected *n* (%)
H (*n* = 43)	26 (60.5)	11 (25.6)	1 (2.3)	5 (11.6)	H (*n* = 28)	1 (3.6)	6 (21.4)	17 (60.7)	4 (14.2)
LAM (*n* = 42)	27 (64.3)	4 (9.5)	2 (4.7)	9 (21.4)	LAM (*n* = 17)	1 (5.9)	0	13 (76.4)	3 (17.6)
T (*n* = 31)	10 (32.3)	13 (41.9)	0	8 (25.8)	T (*n* = 21)	1 (4.8)	5 (23.8)	12 (57.1)	3 (14.3)

*Significant difference between H and LAM with T ($P < 0.020052$).
†Significant difference between H with T ($P < 0.05$).
INH = isoniazid; RMP = rifampicin; SPOF = spoligotyping family; H = Haarlem; LAM = Latin-American and Mediterranean family; T = ill-defined T Super Family.

RÉSUMÉ

CONTEXTE : Hôpital Dr Cetrángolo, Buenos-Aires, Argentine.

OBJECTIFS : Caractériser les isolats de *Mycobacterium tuberculosis* résistants aux médicaments (DR), multirésistants (MDR) et ultrarésistants (XDR) en identifiant leurs profils génétiques, les niveaux de résistance aux médicaments et les mutations responsables de la résistance.

SCHÉMA : On a utilisé des méthodes phénotypiques pour tester la sensibilité aux médicaments afin de déterminer les profils de résistance. On a déterminé les concentrations minimales inhibitrices (MIC) pour l'isoniazide (INH), la rifampicine (RMP) et la lévofloxacine (LVX) dans 169 isolats DR dont 78 monorésistants à INH, 13 à RMP, 7 à LVX et 71 TB-MDR. On a utilisé une réaction polymérase en chaîne (PCR) multiplex-allele spécifique ainsi que le séquençage de l'ADN pour détecter des mutations dans les gènes *kat*G, *rpo*B et *Gyr* A/B. Pour le génotypage, on a utilisé le spoligotypage ainsi que le polymorphisme de la taille des fragments de restriction de séquence d'insertion *6110*.

RÉSULTATS : Au total, une MIC ⩾ 32 μg/ml a été observée dans 38,9% des isolats résistants à INH (INH^R); une MIC ⩾ 64 μg/ml dans 61,3% des isolats résistants à RMP (RMP^R) et une MIC 4–⩾16 μg/ml dans 55,6% des isolats résistants à LVX (LVX^R). Les mutations principales observées dans les isolats INH^R ont été *kat*G315 (53,7%) et *inh*AP-15 (25,5%), et dans les isolats RMP^R, *rpo*B531 (61,9%) suivi par *rpo*B526 (16,7%). Dans les isolats LVX^R, on a trouvé des mutations de *gyr*A94/90. Les principales familles de spoligotypes observées ont été Haarlem, LAM et T. *kat*G315 est principalement en association avec Harlem et LAM, alors qu'*inh*AP-15 l'est avec T.

CONCLUSIONS : On a trouvé une association entre un niveau élevé de résistance à l'INH et la mutation de *kat*G dans plusieurs isolats ; d'autres isolats de la famille Haarlem tendaient à devenir TB-MDR et ont continué à circuler dans la collectivité.

RESUMEN

MARCO DE REFERENCIA: Hospital Dr Cetrángolo, Buenos Aires, Argentina.

OBJETIVOS: Caracterizar aislamientos de *Mycobacterium tuberculosis* drogorresistentes (DR), multidrogorresistentes (-MDR) y extensivamente resistentes (-XDR), identificando su patrón genético, los niveles de drogorresistencia y las mutaciones causantes de dicha resistencia.

DISEÑO: Fue investigado el perfil de drogorresistencia de los aislamientos. Se determino la concentración inhibitoria mínima (MIC) a isoniacida (INH), rifampicina (RMP) y levofloxacina (LVX) en 169 aislamientos DR: 78 monorresistentes a INH, 13 a RMP, 7 a LVX y 71 TB-MDR. Mutaciones en *kat*G, *inh*A, *rpo*B y *gyr*A/B fueron investigadas mediante reacción en cadena de la polimerasa y secuenciación. La genotipificación incluyó spoligotyping y secuencia de inserción *6110*-polimorfismo de longitud de fragmentos de restricción.

RESULTADOS: Un total de 38,9% aislamientos resistents a INH (INH^R) tuvieron MIC ⩾ 32 μg/ml; 61,3% aislamientos resistents a RMP (RMP^R) MIC ⩾ 64 μg/ml y 55,6% resistents a LVX (LVX^R) MIC 4–⩾16 μg/ml. Las principales mutaciones en aislamientos INH^R fueron *kat*G315 (53,7%) y *inh*AP-15 (25,5%), en aislamientos RMP^R fueron *rpo*B531 (61,9%) y *rpo*B526 (16,7%). Los aislamientos LVX^R mostraron mutaciones en *gyr*A94/90. Haarlem, LAM y T fueron las familias de spoligotyping mayoritariamente encontradas, *kat*G315 fue asociada con Haarlem y LAM y *inh*AP-15 con T.

CONCLUSIONES: Varios aislamientos mostraron asociación entre altos niveles de INH^R y mutación en *kat*G, otros pertenecientes a Haarlem fueron propensos a desarrollar TB-MDR y a continuar circulando en la comunidad.