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Rapid detection of multidrug-resistant *Mycobacterium tuberculosis* by multiplex allele-specific polymerase chain reaction

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SUMMARY

SETTING: Dr Cetrangolo Hospital, Buenos Aires Province, Argentina.

OBJECTIVE: To evaluate a multiplex allele-specific polymerase chain reaction (MAS-PCR) to detect multidrug-resistant tuberculosis (MDR-TB) clinical isolates and to describe the main mutations conferring resistance to isoniazid (INH) and rifampicin (RMP).

DESIGN: Drug-resistant *Mycobacterium tuberculosis* clinical isolates were tested to detect mutations using MAS-PCR. The genes involved were *kat*G, *inh*A promoter and *rpo*B.

RESULTS: Among 193 clinical isolates included in the study, 52.6% of the INH-resistant isolates presented a mutation in the *kat*G (315) gene, 28.1% in the *inh*AP (−15) and 3.0% in both. For the *rpo*B gene, 60% of the RMP-resistant isolates showed a mutation in codon 531, 17.5% in 526 and 2.5% in 516. Results were compared with those obtained by sequencing, and 100% concordance was obtained for the detection of the mutation in *kat*G (315), 94.1% for *inh*AP (−15), and 97.8% for *rpo*B. The global concordance between both methods was 98%.

CONCLUSIONS: The MAS-PCR system allowed the simultaneous and rapid detection of approximately 80.0% of the drug-resistant clinical isolates. This method could be used as a rapid and simple screening tool to detect drug-resistant TB in clinical practice.

KEY WORDS: tuberculosis; multidrug-resistant; MAS-PCR

ISONIAZID (INH) and rifampicin (RMP), the two first-line anti-tuberculosis drugs that are most effective against *Mycobacterium tuberculosis*, are used in the treatment of fully drug-susceptible tuberculosis (TB). Resistance to both drugs is defined as multidrug-resistant tuberculosis (MDR-TB). Extensively drug-resistant TB (XDR-TB), caused by MDR-TB with additional resistance to a fluoroquinolone and one other injectable second-line drug (capreomycin, amikacin and/or kanamycin), was defined in 2006.[1,2]

Point mutations in DNA sequences of certain genes are the primary genetic basis of the development of *M. tuberculosis* drug resistance (DR). Mutations in specific genes correlate well with particular phenotypic DR.[3]

As resistance to INH is generally the first step in the development of MDR-TB, the study of its molecular basis in clinical isolates is very important. Several mechanisms are involved in the development of INH resistance: mutations in codon 315 of the *kat*G gene (*kat*G315) are responsible for 50–80% of INH-resistant (INH-R) isolates.[4–6] Second, the point mutation in the promoter region (−15) of the *inh*A gene (*inh*AP-15) was found to be responsible for 8–20% of INH-R. No more than 5% of INH-R isolates contain mutations in the *inh*A gene.[7–9]

It was previously demonstrated that RMP resistance is mainly caused by point mutations in the 'hot spot region' (81 base pairs [bp]) of the *rpo*B gene of *M. tuberculosis*.[10] Nearly 95–97% of RMP-resistant (RMP-R) isolates contain mutations in this region, which comprises codons 507 to 533.[11,12] The main mutations of the *rpo*B gene correspond to codons 516, 526 and 531.[12,13]

In clinical practice, and in low- and middle-income settings, the detection of DR-TB is based mainly on phenotypic drug susceptibility testing (DST) using the time-consuming indirect proportion method on Löwenstein-Jensen (ILJ). Faster methods are therefore needed for prompt and accurate detection of drug resistance to avoid incorrect treatment and to prevent transmission of resistant forms of the disease in the community.

Technology that is affordable for reference TB diagnostic and surveillance laboratories should be a priority in those areas of the world where TB remains

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a menace, including medium-incidence countries such as Argentina.[14] Molecular methods based on the detection of mutations in genes involved in *M. tuberculosis* DR have been developed as a rapid alternative for the detection of MDR-TB cases.[15,16]

The aims of the present study were to evaluate a simple molecular method to detect DR and MDR-TB clinical isolates and to describe the main mutations conferring resistance to INH and RMP. To accomplish these aims, a multiplex allele-specific polymerase chain reaction (MAS-PCR) for the simultaneous detection of resistance to INH and RMP was assessed.

MATERIALS AND METHODS

M. tuberculosis *clinical isolates*

From 2004 to 2009, a total of 2767 TB cases were diagnosed in Northern Buenos Aires (NBA), Argentina. DST and identification results were available for 1847 clinical isolates identified as *M. tuberculosis* complex. A total of 198 isolates (each belonging to a single patient), 151 DR and 47 fully drug susceptible (DS), were included in the study. To obtain isolates, specimens from both non-sterile and sterile body sites were processed as previously described.[17] Cultures were performed on Löwenstein-Jensen (LJ) and Stonebrink media and the BACTEC™ Mycobacterial Growth Indicator Tube [MGIT]™ 960 system (BD, Buenos Aires, Argentina).[17–19] *M. tuberculosis* complex identification and speciation were performed by spoligotyping and biochemical methods.[20–22]

Drug susceptibility testing

ILJ and the BACTEC MGIT 960 SIRE kit (BD) were used to determine the DR profile of the isolates against first-line anti-tuberculosis drugs.[23,24] The reference strain H37Rv ATCC 27294 was used as DST control.

DNA extraction

After DST, the isolates were frozen in Middlebrook 7H9 medium until the time of study, when they were thawed and subcultured onto LJ slants. To obtain DNA, a loopful of colonies was boiled for 30 min in 400 μl sterile water; 10 μl of the supernatant was then used for MAS-PCR.[25,26]

MAS-PCR

MAS-PCR was designed to detect the main point mutations previously described that are involved in INH and RMP resistance.[26–29] To detect INH-R strains with mutations in *kat*G315 and *inh*AP-15, primers katGF, katGR, inhAPF and inhAPR were synthesised. Primers rpoB-516, rpoB-526, rpoB-531 and rpoBR were included in the MAS-PCR to detect mutations in codons 516, 526 and 531 of the *rpo*B gene (Table 1). Primers were adopted from Mokrousov et al. and Yang et al.[13,28]

For practical purposes, MAS-PCR was performed using two separate tubes. Both mixes were prepared as follows: 10 μl DNA, 5 μl buffer 10×, 6 μl Cl_2Mg (magnesium chloride) 25 mM, 4 μl 2.5 mM dNTPs (deoxynucleoside triphosphate) mix, 2.5 U Taq DNA polymerase (Fermentas, Foster City, CA, USA); mix 1: 5 pmol rpoB-516, 25 pmol rpoB-531 and 30 pmol primer rpoBR; mix 2: 4 pmol katGR and katGF, 25 pmol of inhAPF and inhAPR, and 25 pmol rpoB-526 and rpoBR. Both mixes were completed up to 50 μl final volume with nuclease-free water (Figure).

The MyClycler™ thermal cycler was used (Bio-Rad, Richmond, CA, USA), with a touchdown protocol including a denaturation step at 96°C for 3 min, followed by 9 cycles at 95°C for 50 s, at 72°C for 40 s and at 72°C for 1 min. The initial annealing temperature (72°C) decreases by 0.5°C per cycle during the 9 cycles, followed by 30 cycles, with denaturation at 95°C for 50 s, annealing at 68°C for 40 s and extension at 72°C for 1 min. The PCR was ended with a final extension at 72°C for 7 min.[30]

The amplification results were analysed using electrophoresis of 10 μl PCR products on 2.5% UltraPure™ Agarose-1000 (Invitrogen, Carlsbad, CA, USA) gel in 1× tris-acetic acid-ethylenediaminetetraacetic acid (TAE) buffer. The 20 bp DNA ladder O'RangeRuler (Fermentas) was used to estimate the molecular weight of the PCR bands. PCR products of 292 bp and 270 bp indicate the presence of wild type (wt) *kat*G315 and *inh*AP-15 sequence, respectively, and products of 218 bp, 185 bp and 170 bp indicate a wt sequence for codons 516, 526 and 531 of the *rpo*B gene. The absence of amplification indicates mutation at the targeted codon of the gene studied.

DNA sequencing

Sequencing was performed to confirm mutations found by MAS-PCR. A 435 bp fragment of the *kat*G gene (containing codon 315), 648 bp of the *inh*A promoter

Table 1 Primers used in multiplex allele-specific polymerase chain reaction to detect isoniazid and rifampicin resistance

Target	Reverse primers (5′-3′)		Forward primers (5′-3′)	
*inh*AP-15	inhAPR	CACCCCGACAACCTATCG	inhAPF	GCGCGGTCAGTTCCACA
*kat*G315	katGR	ATACGACCTCGATGCCGC	katGF	GCAGATGGGGCTGATCTACG
*rpo*B 516	rpoBR	TTGACCCGCGCGTACAC	rpoB-516	CAGCTGAGCCAATTCATGGA
526			rpoB-526	CTGTCGGGGTTGACCCA
531			rpoB-531	CACAAGCGCCGACTGTC

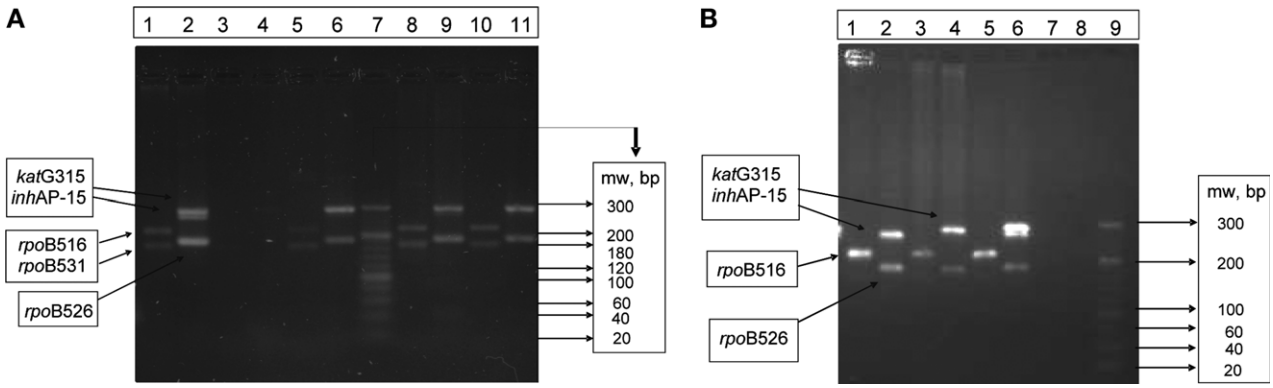

Figure MAS-PCR assay examples. **A.** Band 1: H37Rv mix 1; Band 2: H37Rv mix 2; Band 3: negative control mix 1; Band 4: negative control mix 2; Bands 5, 8 and 10: INH-R strains with mutation in *inh*AP-15 mix 1; Bands 6, 9 and 11: INH-R strains with mutation in *inh*AP-15 mix 2; Band 7: 20 bp mw; mix 1 *rpo*B 516, *rpo*B531; mix 2 *kat*G315, *inh*AP-15, *rpo*B526. **B.** Band 1: MDR-TB strain with mutation in *kat*G315 and *rpo*B531 mix 1; Band 2: MDR-TB strain with mutation in *kat*G315 and *rpo*B531 mix 2; Band 3: MDR-TB strain with mutation in *inh*AP-15 and *rpo*B531 mix 1; Band 4: MDR-TB strain with mutation in *inh*AP-15 and *rpo*B531 mix 2; Band 5: RMP-R isolate with mutation in *rpo*B531 mix 1; Band 6: RMP-R isolate with mutation in *rpo*B531 mix 2; Band 7: negative control mix 1; Band 8: negative control mix 2; Band 9: 20 bp mw; mix 1 *rpo*B 516, *rpo*B531. mw = molecular weight; bp = base pair; MAS-PCR = multiplex allele-specific polymerase chain reaction; MDR-TB = multidrug-resistant tuberculosis; INH-R = isoniazid-resistant; RMP-R = rifampicin-resistant.

(including −15), and 250 bp of the *rpo*B gene (including the 'hot spot region') were sequenced.

The complete *inh*A gene was also sequenced in INH-R isolates with no mutations detected in either the *kat*G gene or the *inhA* promoter using MAS-PCR.[26,28,31] H37Rv and 10 DS isolates were used as wt controls for sequencing. Table 2 shows the primers used for PCR and sequencing: 20 pmol of each primer were used for PCR reactions. To amplify *kat*G, the *inh*A promoter and the *inh*A gene, the cycler protocol included denaturation at 96°C for 3 min, 25 cycles at 95°C for 3 min, at 68°C for 40 s and at 72°C for 1 min and a final extension at 72°C for 10 min. The *rpo*B conditions were 96°C for 3 min, 25 cycles at 95°C for 60 s, at 65°C for 40 s and at 72°C for 1 min, and a final extension at 72°C for 10 min.[28]

The PCR products were purified using the QIAquick PCR purification kit (QIAGEN GmbH, Hilden, Germany) and quantified on 0.8% agarose gel using QuantiMarker (Biodynamics, Buenos Aires, Argentina). The purified products were later sequenced using a DNA sequencer 3130*xl* Genetic Analyzer (Applied Biosystems, Buenos Aires, Argentina). Double-stranded DNA was sequenced for each isolate. The results were analysed using the Basic Local Alignment Search Tool (National Center for Biotechnology Information, Bethesda, MD, USA).

Determination of MAS-PCR sensitivity

To determine the sensitivity of the system from pure cultures, 10-fold serial dilutions of mycobacterial suspensions were prepared from one with turbidity comparable to 1 McFarland and up to 10^{-8}. DNA was extracted by boiling 500 μl bacterial suspensions. MAS-PCR was performed following the protocol previously described. A total of 200 μl of the above-mentioned suspensions were cultured in duplicate on LJ to calculate the number of colony forming units per ml (cfu/ml) and the average number per suspension. The sensitivity of MAS-PCR was determined using a cycler protocol with and without touchdown, and was related to the average number of cfu/ml recovered.

RESULTS

Drug susceptibility testing

Based on the phenotypic DST on ILJ and/or MGIT960 SIRE, 47 isolates were DS, 66 were INH-R, 13 RMP-R and 72 MDR-TB. All isolates were identified as *M. tuberculosis* using biochemical tests and spoligotyping.

Detection of DR and MDR-TB isolates using MAS-PCR

Valid results for 193 of the 198 (97.5%) clinical isolates were obtained using MAS-PCR. Five isolates showed doubtful results and were excluded from the

Table 2 Primers used for polymerase chain reaction and DNA sequencing of *M. tuberculosis* drug resistance genes

Gene	Forward primers (5′-3′)		Reverse primers (5′-3′)	
*kat*G	katGF	GCAGATGGGGCTGATCTACG	katGsR	AACGGGTCCGGGATGGTG
*inh*A (ORF)	inhAF	GTATGGGCCACTGACAACAC	inhAR	CCGCCGAACGACAGCAGCAGGA
*inh*AP	inhAPsF	AATTGCGCGGTCAGTTCCACAC	inhAPsR	CTGCGCGATGCCCGTTGAGC
*rpo*B	ROF	GTCGCCGCGATCAAGGA	RIR	TGACCCGCGCGTACAC

Table 3 Multiplex allele-specific polymerase chain reaction results among drug-resistant clinical isolates

		Mutated genes					
					*rpo*B (hot spot region)		
Drug resistance	Clinical isolates *n*	*kat*G 315 *n* (%)	*inh*AP −15 *n* (%)	*kat*G-*inh*A 315, −15 *n* (%)	516 *n* (%)	526 *n* (%)	531 *n* (%)
INH	66	32	21	0	0	0	0
RMP	11	0	0	0	0	4	4
MDR-TB	69	39	17	4	2	10	44
Total	146	71 (52.6)	38 (28.1)	4 (3.0)	2 (2.5)	14 (17.5)	48 (60.0)
Global detection		113 (83.7)			64 (80.0)		

INH = isoniazid; RMP = rifampicin; MDR-TB = multidrug-resistant tuberculosis.

study. Of the INH-R clinical isolates, 52.6% presented a mutation in the *kat*G315 gene, whereas 28.1% presented a mutation in *inh*AP-15. Four (3.0%) isolates had both mutations, while no mutation was detected in 22 (16.3%) INH-R isolates. For the *rpo*B gene, 60% of RMP-R isolates showed a mutation in codon 531, 17.5% in codon 526 and 2.5% in codon 516. No mutations were found using MAS-PCR in 16 (20%) of the RMP-R isolates (Table 3).

INH-R isolates were correctly identified as wt for the *rpo*B fragment included in MAS-PCR. The same occurred with RMP-R isolates when they were tested for *kat*G and *inh*AP. Neither the DS isolates nor H37Rv presented mutations for the genes studied using MAS-PCR. No false DR results were found with MAS-PCR, as full agreement was observed between the isolates detected as DR using both MAS-PCR (absence of amplified genes) and phenotypic DST.

Sequencing results

We found 100% concordance between MAS-PCR and sequencing when the mutation was present in *kat*G315 (Table 4). Of the 75 INH-R isolates, 68 (91.0%) with a mutation in *kat*G315 detected by MAS-PCR were sequenced; the results show that 95.6% of the isolates had the AGC315ACC mutation. Mutations AGC315ACA, AGC315AAC and AGC315AGA each occurred in 1.5% of the INH-R clinical isolates, with a mutation in *kat*G315 (Table 5).

A concordance of 94.1% was found for *inh*AP-15 (Table 4). To confirm the mutations found in *inh*AP-15 using MAS-PCR, 81% (34/42) of the INH-R isolates with this mutation were sequenced: 94.1% (32/34) had the mutation C-15T and two strains (5.8%) had T-8A and/or G-17T mutations (Table 5).

A concordance of 97.8% was found for the *rpo*B gene, as one strain showed a mutation in 526 by MAS-PCR and in 522 by sequencing. Seventy per cent (45/64) of the RMP-R isolates with a *rpo*B mutation using MAS-PCR were sequenced: the main mutation in *rpo*B was TCG531TTG (77.8%), followed by CAC526TAC (8.9%), CAC526CTC (6.7%), CAC526GAC (2.2%) and CAC526CGC (2.2%). One strain had a TCG522TTG (2.2%) mutation and one MDR-TB isolate with a mutation at *rpo*B 531 also showed several silent mutations along the fragment sequenced (Table 5). The global concordance between both methods was 98% (Table 4). Several DR isolates with no mutations found using MAS-PCR were also sequenced to explore regions that were not included in the system.

The *inh*AP, *kat*G and *inh*A genes were also sequenced in 22 INH-R isolates detected as wt by MAS-PCR. No mutation was found in *inh*AP; the *kat*G TGG321TGC (W→C) mutation was found in one isolate, two showed GGG83AGG (G→R) and one the ACG241ACC (S→T) mutation in the *inh*A gene. It was therefore not possible to detect mutations in the remaining 18 INH-R isolates.

Sixteen RMP-R isolates with no mutation found by MAS-PCR were sequenced flanking the 'hot spot region' of the *rpo*B gene. A double mutation [CAA 513CCA (Q→P) and GAG565CAG (E→Q)] was observed in two isolates. CAA513CCA (Q→P), TCG 522TTG (S→L) and ATC572TTC (I→F) mutations were found in three different isolates. Eight isolates still showed a wt sequence, and no valid results were obtained in three resistant isolates. The 47 DS isolates were correctly identified using the wt sequences of the genes studied.

MAS-PCR sensitivity

The sensitivity of MAS-PCR using the touchdown protocol in the thermal cycler corresponded to the 10^{-7} McFarland suspension (~10 microorganisms per millilitre). When a cycler protocol without touchdown was used, the sensitivity was 10^{-1} McFarland suspension

Table 4 Concordance between MAS-PCR and sequencing in drug-resistant clinical isolates

Gene mutation	MAS-PCR/sequencing concordance *n*/*N* (%)
*kat*G 315	68/68 (100)
*inh*AP-15	32/34 (94.1)*
*rpo*B	44/45 (97.8)†
Total	144/147 (98.0)

*Two strains showed a mutation in −8 and −17 position of the *inh*A promoter.
†One strain showed a mutation in codon 522 instead of 526 of the *rpo*B gene.
MAS-PCR = multiplex allele-specific polymerase chain reaction.

Table 5 Sequencing results in drug-resistant clinical isolates with mutation detected by multiplex allele-specific polymerase chain reaction

Sequencing, mutation	Aminoacid change	Isolates *n* (%)
INH-R (*n* = 68) *kat*G, 435 bp		
AGC315ACC	S→T	65 (95.6)
AGC315ACA	S→T	1 (1.5)
AGC315AAC	S→N	1 (1.5)
AGC315AGA	S→R	1 (1.5)
INH-R (*n* = 34) P.*inh*A, 648 bp		
C-15T	—	32 (94.1)
T-8A	—	1 (2.9)
G-17T	—	1 (2.9)
RMP-R (*n* = 45) *rpo*B, 250 bp		
TCG522TTG	S→L	1 (2.2)
CAC526CTC	H→L	3 (6.7)
CAC526TAC	H→Y	4 (8.9)
CAC526GAC	H→D	1 (2.2)
CAC526CGC	H→R	1 (2.2)
TCG531TTG	S→L	34 (75.6)
TCG522TCC, CGA529CGC, TCG531TTG,* GGG534GGC, TCA539TCC, CGT542CGC, GGG544GGC, CGC548CGT, GTG550GTC, CCG552CCC, GAA562GAG, GGG566GGT	S→S, R→R, S→L, G→G, S→S, R→R, G→G, R→R V→V, P→P, E→E, G→G	1 (2.2)

*Mutation responsible for RMP resistance.
INH-R = isoniazid-resistant; bp = base pair; RMP-R = rifampicin-resistant.

(data not shown). For this reason, a protocol with touchdown was chosen to perform MAS-PCR.

DISCUSSION

Due to the increase in MDR-TB worldwide and the recent identification of XDR-TB as a public health problem, the need for rapid and efficient diagnostic tools that are affordable in low- and middle-income countries is becoming more pressing each year. The development of simple, rapid, easy to implement methods is a priority in microbiological research in TB.[32] This study explored a PCR-based system and a simple electrophoresis detection method to demonstrate the presence of molecular resistance related products of *M. tuberculosis* genes.

The MAS-PCR system used in this study led to the detection of INH and RMP resistance in 80% of DR isolates in one working day. We detected point mutations associated with INH-R in other DNA bases of the *inh*A promoter: −8 and −17.[33] One isolate with a mutation in *rpo*B 522 was detected as mutated in *rpo*B 526 by MAS-PCR. The detection of mutations in other positions close to the target of the MAS-PCR is an advantage of the system in determining DR but not for describing specific mutations.

Our results correlated well with previously published studies, which reported *kat*G315 (AGC→ACC, S→T) as the main mutation associated with INH-R clinical isolates, followed by −15 C→T mutation of the *inh*A promoter.[10,17,29,32] In the analysis of RMP-R isolates, the main mutation conferring resistance corresponded to *rpo*B TCG531TTG. These results are also in line with previous reports from the same geographic area.[12]

Three different mutations that, to our knowledge, have not been previously reported were found in INH-R isolates and corresponded to TGG321TGC (W→C) of the *kat*G gene, and to GGG83AGG (G→R) and ACG241ACC (S→T) of the *inh*A gene. These mutations could be responsible for the INH-R phenotype.

This method may be a low-cost alternative for implementation in clinical reference laboratories. The estimated cost of the MAS-PCR system per individual diagnosis of MDR-TB, taking into account the items listed by Acuña-Villaorduna et al., such as staff, capital, overheads and medical supplies, was calculated to be approximately US$14.[34] The same authors reported that RMP-R molecular diagnosis alone using the commercially available INNO-LiPA Rif TB™ (Innogenetics, Ghent, Belgium) costs US$94.[34] Furthermore, the cost of MDR-TB diagnosis using ILJ, the colorimetric microplate-based assay and the BACTEC MGIT 960 automated system were also calculated at respectively US$16.05, US$16.74 and US$31.[34]

As it is based on DNA amplification, MAS-PCR has the clear advantage of producing results faster than indirect culture-based methods. The time saved in using MAS-PCR as against ILJ is approximately 28–41 days. Compared against the MGIT 960 and colorimetric-based methods, the time saved is respectively 7–15 and 8–12 days.[35]

CONCLUSION

The MAS-PCR assay is a rapid, low-cost molecular method that is easy to implement in clinical reference laboratories and could be used as screening tool in cases suspected of drug-resistant TB or MDR-TB, especially in low- or middle-income countries.

Further studies are needed on isolates without mutations that exhibit a phenotypic DR pattern. These studies should include sequencing of the entire *kat*G, *inh*A promoter and other genes previously reported to be involved in some mechanisms of INH resistance (*ahp*C, *oxy*R, *fur*A).[33,36]

For RMP-R isolates with no mutations in the *rpo*B gene, sequencing of the complete *rpo*B gene should be performed to establish the cause of DR. The distribution of mutations in the genes studied was in line with previous reports. This system could therefore be useful in diagnosing MDR-TB and/or DR in clinical isolates in our settings.

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RÉSUMÉ

CONTEXTE : Hôpital Dr Cetrangolo, Province de Buenos Aires, Argentine.

OBJECTIF : Evaluer une réaction de polymérase en chaîne spécifique pour des allèles multiplex (MAS-PCR) en vue de la détection d'isolats cliniques à germes multirésistants (MDR), et décrire les mutations principales conférant la résistance à l'isoniazide (INH) et à la rifampicine (RMP).

SCHEMA : Des isolats cliniques de *Mycobacterium tuberculosis* résistant aux médicaments ont été testés pour y détecter les mutations au moyen de MAS-PCR. Les gènes impliqués ont été *kat*G, le promoteur d'*inh*A et *rpo*B.

RESULTATS : On a inclus dans l'étude 193 isolats cliniques. Il existait une mutation au niveau du gène *kat*G (315) chez 52,6% des isolats cliniques résistants à l'INH, chez 28,1% dans le *inh*AP (−15) et chez 3,0% dans les deux. Pour le gène *rpo*B, il y avait une mutation dans 60% des isolats résistants à la RMP au niveau du codon 531, dans 17,5% au niveau du codon 526 et dans 2,5% dans le codon 516. Ces résultats ont été comparés avec ceux obtenus par le séquençage ; on a observé une concordance de 100% en ce qui concerne la détection de la mutation dans *kat*G (315), de 94,1% pour *inh*AP (−15) et de 97,8% pour le gène *rpo*B. La concordance générale entre les deux méthodes a été de 98%.

CONCLUSIONS : Le système MAS-PCR a permis une détection simultanée et rapide d'environ 80% des isolats cliniques résistants aux médicaments. La méthode pourrait être utilisée dans la pratique clinique comme outil de dépistage rapide et simple pour la détection d'une tuberculose résistante aux médicaments.

RESUMEN

MARCA DE REFERENCIA: Hospital Dr Antonio A Cetrángolo, Provincia de Buenos Aires, Argentina.

OBJETIVO: Evaluar una reacción en cadena de la polimerasa múltiple alelo específica (MAS-PCR) para detectar aislamientos clínicos multirresistentes y describir las principales mutaciones que confieren resistencia a isoniazida (INH) y rifampicina (RMP).

DISEÑO: Aislamientos clínicos de *Mycobacterium tuberculosis* fueron analizados por MAS-PCR para detectar mutaciones. Los genes estudiados fueron *kat*G, la región promotora del *inh*A y el *rpo*B.

RESULTADOS: Se incluyó un total de 193 aislamientos clínicos. Alrededor del 52,6% de los resistentes a INH presentaron mutación en *kat*G (315), 28,1% en *inh*A (−15) y un 3,0% en ambos genes. Para el gen *rpo*B, 60,0% de los aislamientos resistentes a RMP mostraron mutación en el codón 531, 17,5% en 526 y 2,5% en 516. Los resultados fueron comparados con los obtenidos por secuenciación; se obtuvo 100% de concordancia para detectar mutaciones en *kat*G (315), 94,1% para *inh*A (−15) y 97,0% para el gen *rpo*B. La concordancia global entre ambos métodos fue del 98,0%.

CONCLUSIONES: El sistema de MAS-PCR permitió la detección rápida y simultanea de aproximadamente 80,0% de los aislamientos clínicos drogo-resistentes. Este método podría ser usado como una herramienta de tamiz rápida y sencilla para detectar tuberculosis drogo-resistente en la práctica clínica.