International Journal of Mycobacteriology 1 (2012) 124–130

Available at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/IJMYCO

Drug resistance-related mutations in multidrug-resistant *Mycobacterium tuberculosis* isolates from diverse geographical regions

Senia Rosales-Klintz [a,b,c], *Pontus Jureen* [c,d], *Aksana Zalutskayae* [e], *Alena Skrahina* [e], *Biao Xu* [f], *Yi Hu* [f], *Lelany Pineda-Garcia* [a], *Muayad Aghali Merza* [g,h], *Ionela Muntean* [i], *Freddie Bwanga* [j], *Moses Joloba* [j], *Sven E. Hoffner* [c,d,*]

[a] *Escuela de Microbiologia, Universidad Nacional Autonoma de Honduras, Tegucigalpa, Honduras*
[b] *Division of Global Health (IHCAR), Karolinska Institutet, Stockholm, Sweden*
[c] *Department of Preparedness, Swedish Institute for Communicable Disease Control, Solna, Sweden*
[d] *Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden*
[e] *Republican Scientific and Practical Center for Pulmonology and Phthisiology, Minsk, Belarus*
[f] *School of Public Health, Fudan University, Shanghai, PR China*
[g] *Azadi Teaching Hospital, College of Medicine, University of Dohuk, Dohuk, Iraq*
[h] *National Research Institute of TB and Lung Disease, Tehran, Iran*
[i] *Hospital of Pneumophtisiology, Brasov, Romania*
[j] *Department of Medical Microbiology, School of Medical Sciences, Makerere University, Kampala, Uganda*

ARTICLE INFO

Article history:
Received 8 August 2012
Accepted 11 August 2012
Available online 31 August 2012

Keywords:
MDR-TB
rpoB gene
katG gene
mabA-inhA operon
gyrA gene

ABSTRACT

Background: Drug resistance in *Mycobacterium tuberculosis* is associated with chromosomal mutations in selected genes. These mutations can be screened for an early warning system for drug-resistant tuberculosis. The prevalence of individual mutations differs geographically, which must be considered in developing globally applicable screening tests.

Methods: In order to analyse the geographical distribution and frequency of mutations conferring resistance to rifampicin, isoniazid and fluoroquinolones, the researchers investigated the presence of mutations in the *rpoB* gene, the *katG* gene, the *mabA-inhA* promoter region and the *gyrA* gene in clinical isolates of multidrug-resistant tuberculosis (MDR-TB) from Belarus, China, Iran/Iraq, Honduras, Romania and Uganda. For each study site, the researchers described the distribution of specific mutations in 20 clinical MDR-isolates.

Results: The distribution of resistance-related mutations varied significantly between the study sites. Settings with a high incidence of MDR-TB, such as Belarus, showed a narrower spectrum of mutations related to rifampicin and isoniazid resistance and also a higher prevalence of fluoroquinolone resistance than study sites with a lower MDR-TB prevalence.

Conclusion: This study confirms that there are significant geographical differences in the distribution of resistance-related mutations and suggests that an increased understanding of such differences in the specific distribution of resistance conferring mutations is crucial for development of new, generally applicable, molecular tools for rapid diagnosis of drug-

* Corresponding author. Address: Swedish Institute for Communicable Disease Control, SE 171-82 Solna, Sweden. Tel.: +46 8 457 24 31; fax: +46 8 302566.
E-mail address: sven.hoffner@smi.se (S.E. Hoffner).

2212-5531/$ - see front matter
http://dx.doi.org/10.1016/j.ijmyco.2012.08.001

resistant TB. The fact that a narrower distribution of mutations in high MDR-TB prevalence settings was seen suggests that much of the problems in these settings can be a result of an ongoing transmission of certain MDR-TB strains.

Introduction

Globally, it is estimated that multidrug-resistant tuberculosis (MDR-TB) affects around 650,000 patients [1]. This form of the disease is resistant to at least isoniazid (INH) and rifampicin (RIF), the main first-line drugs used for tuberculosis (TB) treatment [1]. Mismanagement of MDR-TB treatment has caused the development of extensively drug-resistant TB (XDR-TB), defined as MDR-TB also resistant to any of the fluoroquinolones and at least one of the injectable second-line drugs [2]. In some countries, MDR/XDR-TB constitutes a major threat to public health and jeopardizes TB control. Alarming data was recently reported from Belarus where a prevalence of MDR-TB among infectious cases in Minsk was shown to be 49%. Among newly detected patients with infectious (smear positive) pulmonary disease, 35% had MDR-TB and no less than 76% was found in previously treated cases [3]. This is the highest level ever seen of MDR-TB and constitutes a severe challenge for TB control efforts.

Rapid and accurate detection of MDR/XDR-TB is a priority. Timely laboratory diagnosis allows for optimizing therapy and limiting transmission. This is best achieved using molecular screening for resistance-related mutations. Such molecular-based methods have been developed for the timely detection of resistance in *Mycobacterium tuberculosis* based on the knowledge of genetic mechanisms causing drug resistance. Several genetic biomarkers have been identified and shown to be related to phenotypic drug resistance. Among these, an 81 bp region in *rpoB* gene contains more than 95% of the mutations seen in clinical isolates resistant to RIF [4,5]. The *katG* gene [6,7], the promoter region of the *mabA-inhA* operon [8], have been shown to be associated with INH-resistance.

To make sure that the tests are globally applicable, researchers need to know not only which genes and mutations are responsible for resistance, but also their global distribution.

The purpose of this study was to explore the distribution of resistance-related mutations in MDR-TB isolates from seven countries on four continents.

Materials and methods

Mycobacterium tuberculosis isolates and study sites

The molecular characterization was conducted at the Swedish Institute for Communicable Disease Control (SMI), with a convenience sample of 120 clinical MDR *M. tuberculosis* samples collected over somewhat different time periods: Belarus (April–August 2009), China (June 2008–September 2009), Honduras (1994–1995/2004–2009), Iraq (June 2008–June 2009), Iran (May 2008–May 2009), Romania (2007–2008) and Uganda (February 2008–July 2009). Each site contributed with 20 isolates, except Iraq and Iran that contributed ten strains each.

Both the *in vitro* drug susceptibility testing (DST) and the DNA extraction were performed on site in each collaborating center with their respective standard technique. The DST methods used were the absolute concentration method on Löwenstein-Jensen (LJ) medium [9] (Belarus, Romania) and the proportion method on LJ medium [10] (Iran, China, Honduras, Uganda). Bactec MGIT 960 (Becton Dickinson, Sparks, Maryland, USA) [11] was used for ofloxacin (OFX) testing of the Honduran isolates at SMI. The critical concentrations were 0.2/0.1 mg/L for INH, 40.0/1.0 mg/L for RIF and 2.0/2.0 mg/L for OFX for LJ and MGIT respectively. In Belarus a critical concentration of 1 mg/L was used for INH.

PCR amplification and sequencing

DNA isolation was performed as previously described [12,13]. The DNA extracts were amplified and sequenced to identify mutations in the rifampicin resistance-determining region (RRDR) of the *rpoB* gene [4,5]; the *katG* gene [6,7], the *mabA-inhA* promoter region [8] and the quinolone resistance-determining region (QRDR) in the *gyrA* gene [14,15]. Polymerase chain reaction (PCR) mixtures (50 μl) contained 5 μl 10× PCR buffer, 0.2 mM dNTP, 1 U Ampli Taq Gold® polymerase (Applied Biosystems, USA), 0.2 mM of each appropriate pair of primers (Invitrogen, Life Technologies, UK) and 2 μl of DNA extracts. PCR thermal cycling conditions and primers sequences, as well as $MgCl_2$ concentrations used are presented in Table 1.

The PCR products were verified by gel electrophoresis and purified using the GFX™ PCR DNA and gel band purification kit (GE Health Care, Amersham, Little Chalfont, UK).

The purified PCR products were sequenced using the ABI PRISM BigDye® Terminator v3.1 cycle sequencing kit and analyzed in an ABI 3130xl genetic analyzer (Applied Biosystems, CA, USA), following the manufacturer's recommendations. The DNA sequences were aligned using the CLUSTAL W algorithm [16]. All mutations found were compared with the TBDReaMDB database [17].

Results

Of the total of 120 DNA extracts from MDR-TB strains contributed by the different centers, 117 (97.5%) could be genetically characterized.

Rifampicin

A clear majority of 94% (110/117) of the MDR-TB isolates had mutations in the RRDR of *rpoB*. In three of the study sites all 20 isolates had *rpoB* mutations, while only 85% of the isolates from Romania and Iran/Iraq showed such mutations. Of all the 110 mutations detected, 89% showed single, and 5%

Table 1 – Primers and conditions used to amplify and sequence the MDR *M. tuberculosis* isolates.

Gene	Fragment size (bp)	Primers (5′ → 3′)	PCR[a]	Cycle sequencing PCR[b]
rpoB	382	**OPRIF-F** CGG TCG GCG AGC TGA TCC **OPRIF-R** TTG ACC CGC GCG TAC ACC	95 °C/45 s, 53 °C /45 s, 72 °C /45 s	94 °C /10 s (Initial denaturation), 94 °C/10 s, 53 °C/10 s, 60 °C/4 min
katG	704	**F 768** CAT GAA CGA CGT CGA AAC AG **R1458** GCT ACC ACG GAA CGA CGA C	95 °C/30 s, 52 °C /30 s, 72 °C /1 min	96 °C /1 min(Initial denaturation), 96 °C/10 s, 50 °C/5 s, 60 °C/4 min
mabA-inhA Promoter region	1973	**SEP(F1)** CGG AAA TCG CAG CCA CGT TA **SEP(R1)** CCA CGC AGA TGT CGC AAA GA **inhA seq rev 925** CCG GAC CCT GGT GCT CTT CT	95 °C/45 s, 40 °C /30 s, 72 °C /3 min	96 °C /30 s (Initial denaturation), 94 °C/10 s, 50 °C/10 s, 60 °C/4 min
gyrA	602	***gyrA* F180** CCT CGG TTC GTC TGT TGC GTC AAG T ***gyrA* R422** ATC TCC ATC GCC AAC GGG GTC A	95 °C/45 s, 56 °C /45 s, 72 °C /1 min	96 °C /30 s (Initial denaturation), 94 °C/10 s, 50 °C/10 s, 60 °C/4 min

[a] All PCR reactions had an initial denaturation at 95 °C/10 min, 30 cycles, final elongation at 72 °C/7 min.
[b] Cycle sequencing PCR reactions used the same primers, except for *mabA-inhA* (primer inhA seq rec 925) and had 30 cycles.

double mutations (Table 2). The most frequently mutated codons were 531 and 526, found in 61 (52%) and 38 (32%) strains, respectively. The distribution of mutations related to MDR-TB differed significantly between the different settings. For example, the His526Tyr mutation was mainly found in Romanian isolates and the His526Asp mutation mostly in isolates from Belarus. No deletions or insertions were detected. Table 2 summarizes all the *rpoB* mutations detected.

Isoniazid

Most of the MDR-TB isolates (82%) had a mutation in *katG* and/or the *mabA-inhA* promoter region conferring INH-resistance. The most common mutation was *katG* Ser 315Thr found in 83 strains (71%), including 20 strains also showing a *mabA-inhA* (C-15T) mutation.

Also for INH-resistance, a distinct difference was seen in the prevalence of the mutations between the different study settings (Table 3). Most of the strains with mutations both in the *katG* gene and the *mabA-inhA* promoter region were from Romania and Belarus. The two isolates found with *katG* Ser315Thr and *mabA-inhA* T-8 C genotype were both from Belarus. Strains without mutations in *katG* or *mabA-inhA* were mainly from Honduras and Iraq. For one Ugandan isolate, a repeated lack of amplification was observed of the *katG* locus, which might indicate a deletion of this gene. A lack of mutations in *rpoB*, *katG* and *mabA-inhA* promoter regions were found in two isolates from Iran and two from Romania.

Fluoroquinolones

In contrast to the other drugs studied, phenotypic susceptibility data were missing for fluoroquinolones (FQs). Based on genetic characterization, the level of FQ-resistance differed significantly between the MDR-TB strains isolated in different study sites. Most frequently, FQ-related mutations were seen in isolates from Belarus and Romania, with 40% and 35% of the strains having mutations in the QRDR. In isolates from Honduras, no such mutations were seen, and in Uganda and Iran less than 10% of the MDR-isolates carried a QRDR mutation. Altogether, a total of 30 strains (26%) had mutations in the *gyrA* gene. Of those, 22 had FQ-resistance-related mutations in the QRDR. The most common mutation was the substitution Asp94Gly (Table 4).

The single substitution Thr80Ala found in eight Ugandan isolates is not considered to be related to FQ-resistance, but rather seen as an epidemiological marker.

Phenotypic DST results for FQ were only available for 56 of the MDR-TB isolates: from Belarus (20 strains), Honduras (20 strains), Romania (4 strains) and Uganda (12 strains). A full comparison between genetic and phenotypic detection of drug resistance was thus not possible.

Eight out of the nine phenotypically FQ-resistant strains detected in this subset, all from Belarus, had resistance-related mutations in the QRDR. The mutations Ala90Val and Asp94Val were the most common (3 isolates each). Among the 47 phenotypically FQ-susceptible strains, 3 had the single mutation Thr80Ala and 1 had the double mutation Thr80Ala + Asp94Gly. The remaining 43 susceptible isolates had a wild type QRDR.

Thirteen of the 61 strains without phenotypic OFX-DST results had resistance-related mutations in the QRDR. The substitution Asp94Gly was the more frequent (4/13), followed by the Ala90Val mutation (3/13). The majority of these were from Romania (7 isolates) and China (4 isolates).

The overall distribution of mutations found in *gyrA* is shown in Table 4. Taking into account the frequency of FQ-resistance-related mutations only, both Belarus and Romania have a high level of resistance with 40% and 35% of MDR-TB isolates also being resistant to FQ. In contrast, only a minority

Table 2 – Distribution of *rpoB* mutations in 117 MDR-TB isolates.

Amino acid position	Nucleotide change	Amino acid change	No. of isolates	Belarus	China	Honduras	Iran[a]	Romania	Uganda
513	CAA → AAA	Gln → Lys	2	–	–	1	–	–	1
	CAA → CCA	Gln → Pro	2	–	–	–	–	2	–
516	GAC → GTC	Asp → Val	6	–	2	1	1	1	1
526	CAC → TGC	His → Cys	1	–	1	–	–	–	–
	CAC → CTC	His → Leu	1	–	1	–	–	–	–
	CAC → CGC	His → Arg	4	–	–	1	2	–	1
	CAC → TAC	His → Tyr	14	–	3	1	–	10	–
	CAC → GAC	His → Asp	14	8	2	1	–	2	1
531	TCG → CAG	Ser → Gln	1	–	–	–	1	–	–
	TCG → TTG	Ser → Leu	54	9	7	11	11	2	14
	TCG → TTC	Ser → Phe	3	–	–	3	–	–	–
	TCG → TGG	Ser → Trp	2	–	–	–	–	–	2
509 and 526	AGC → ACC and CAC → CTC	Ser → Thr and His → Leu	1	1	–	–	–	–	–
512 and 526	AGC → AAC and CAC → TAC	Ser → Asn and His → Tyr	1	–	–	1	–	–	–
520 and 522	CCG → TCG and TCG → TTG	Pro → Ser and Ser → Leu	1	–	–	–	1	–	–
526 and 531	CAC → CTC and TCG → TGG	His → Leu and Ser → Trp	1	–	–	–	1	–	–
526 and 533	CAC → AAC and CTG → GTG	His → Asn and Leu → Val	2	2	–	–	–	–	–
WT			7	–	1	–	3	3	–
Total			117	20	17	20	20	20	20

[a] Includes isolates from Iraqi Kurdistan. WT = wild type.

(10–20%) of the isolates from Iran/Iraq and China were resistant, whereas in the isolates from Honduras no mutations were seen in the QRDR.

Overall mutation profile

This study also analyzed whether the overall mutation profile for the 4 loci studied related to the geographic origin of the isolates. At least two mutation profiles (same mutations in all the target genes) were shared by more than three isolates from Belarus and Romania. In contrast, the profiles observed in Honduras, Iran and Uganda were more diverse.

Phylogenetic groups

Mutations in the studied genes were also used for classification of *M. tuberculosis* in phylogenetic groups. Based on *katG* 463 and *gyrA* 95 polymorphisms, the MDR-TB isolates were classified into principal genetic groups (PGGs) [18]. One Ugandan isolate could not be categorized, owing to a lack of amplification of *katG* locus. Most isolates belonging to PGG1 ($n = 36$) were from China and Iran, reflecting the high prevalence of ancient *M. tuberculosis* lineages in these countries. Strains from Uganda, Honduras and Belarus belonged to the PGG2 ($n = 62$). The isolates within PGG3 ($n = 12$) were mainly from Honduras and Romania.

Discussion

As far as this study is concerned, this is the first report of mutations related to drug resistance in MDR-TB isolates from Honduras and Romania. The overall results are well in line with earlier reports from other settings on mutations related to RIF, INH and FQ-resistance [19–24]. The mutations earlier reported to be the most common were also most frequently seen in this study. In addition, two "new" mutations were observed in *katG*, Arg385Pro and Ser 446Arg, which have not been reported in the TBDReaMDB database [17].

The most important finding of this study was that the frequency and distribution of mutations detected varied so much between the different study settings. Belarus, having the highest prevalence of MDR-TB of all the study sites, had the narrowest spectrum of RIF and INH-resistance-related mutations, while isolates from Honduras, Iran/Iraq and Uganda, where MDR-TB is much less frequent, generally had a more heterogeneous distribution of mutations. A narrow distribution might indicate an ongoing transmission of MDR.

The fact that Belarus used a higher concentration of INH (1 mg/L) in their DST might have selected for more highly resistant isolates. Mutations in *katG* 315 were seen in all the Belarusian isolates, a finding that has been previously reported [25]. Consequently, it could be questioned if the lower level of INH-resistance caused by a mutation in the *mabA-inhA*

Table 3 – Distribution of *katG* and *mabA-inhA* promoter region mutations in 117 MDR-TB isolates.

Amino acid position	Nucleotide change	Amino acid change	No. of isolates	Belarus	China	Honduras	Iran[a]	Romania	Uganda
300	TGG → GGG	Trp → Gly	1	–	–	–	1	–	–
315	AGC → ACC	Ser → Thr	61	10	10	12	8	6	15
	AGC → AAC	Ser → Asn	2	–	1	–	–	1	–
	AGC → ATC	Ser → Ile	1	–	1	–	–	–	–
	AGC → AGA	Ser → Arg	1	–	–	1	–	–	–
385	CGG → CCG	Arg → Pro	1	–	–	–	1	–	–
446	AGC → CGC	Ser → Arg	1	–	1	–	–	–	–
katG 315 and *inhA*-8	AGC → ACC and T → C	Ser → Thr	2	2	–	–	–	–	–
katG 315 and *inhA*-15	AGC → ACC and C → T	Ser → Thr	20	8	1	–	–	10	1
katG 434 and *inhA*-15	CAG → CCG and C → T	Gln → Pro	1	–	–	–	–	–	1
inhA-15	C → T		5	–	2	2	1	–	–
katG NA and *inhA* WT			1	–	–	–	–	–	1
katG and *inhA* WT			17	–	1	5	7	2	2
katG WT and *inhA* NA			3	–	–	–	2	1	–
Total			117	20	17	20	20	20	20

[a] Includes isolates from Iraqi Kurdistan. WT = wild type. NA = not analyzed.

Table 4 – Distribution of *gyrA* mutations in 117 MDR-TB isolates.

Amino acid position	Nucleotide change	Amino acid change	No. of isolates	Belarus	China	Honduras	Iran[a]	Romania	Uganda
80	ACC → GCC	Thr → Ala	8	–	–	–	–	–	8
90	GCG → GTG	Ala → Val	6	3	2	–	1	–	–
91	TCG → CCG	Ser → Pro	1	1	–	–	–	–	–
94	GAC → AAC	Asp → Asn	3	1	1	–	–	1	–
	GAC → CAC	Asp → His	2	–	–	–	–	2	–
	GAC → GGC	Asp → Gly	7	3	1	–	–	3	–
	GAC → GTC	Asp → Val	1	–	–	–	–	1	–
80 and 94	ACC → GCC and GAC → GGC	Thr → Ala and Asp → Gly	1	–	–	–	–	–	1
91 and 94	TCG → CCG and GAC → GGC	Ser → Pro and Asp → Gly	1	–	–	–	1	–	–
WT			87	12	13	20	18	13	11
Total			117	20	17	20	20	20	20

[a] Includes isolates from Iraqi Kurdistan. WT = wild type.

promoter region would have been detected in the conventional DST, and thus met the criteria for inclusion in this study.

In contrast to the MDR-defining drugs, to which all included isolates were resistant, the studied isolates were included regardless of their resistance to FQ, which in most cases was unknown. Thus the level of FQ-resistance, reflected by the detection of mutations in the QRDR of the *gyrA* gene might give an estimate on the level of FQ-resistance in MDR-TB isolates from the different study sites. The frequency found differed considerably between these, with the highest frequency of FQ-resistance-related mutations seen in Belarus and Romania, from where 40% and 35% of the strains had detectable *gyrA*

mutations. In contrast, such mutations were absent or rare in isolates from Honduras, Iran/Iraq and Uganda.

This finding reflects the more severe problem with FQ-resistance, and thus most likely also with XDR-TB, in East Europe than in the other sites.

An interesting *gyrA* mutation pattern among the Ugandan isolates was observed. The mutation Thr80Ala seen in this study has been previously reported from African clinical isolates [26] and seems to be an allelic variability marker, not conferring resistance to FQ [27]. However, when this mutation occurs simultaneously with a well-known FQ-resistance-related mutation, the isolates are hypersusceptible to FQ [27]. In this study, one Ugandan ofloxacin-susceptible isolate with the genotype Thr80Ala + Asp94Gly was seen. In a recent systematic review, it was postulated that the above-mentioned pattern might be due to a misclassification of the strains or due to the presence of a mixture of two different strains [28]. Further studies on clinical isolates harboring this double mutation might clarify whether or not there are other susceptibility mechanisms involved in the mutation profile, as well as its geographical distribution and possible implications for therapy and on transmission.

The distribution of isolates belonging to the different PGGs may also be used as an indicator of the epidemiological trends in our study settings. The predominance of PGG1 isolates in China and Iran reflects the high prevalence in these countries of ancient *M. tuberculosis* lineages, such as Beijing [29] and Central Asian (CAS) [30]. In Romania and Belarus, mainly PGG 2 and 3 isolates were observed combined with a less diverse mutation profile. This might indicate that modern *M. tuberculosis* lineages, like the Latin American Mediterranean (LAM) and T genotypes, are causing the clonal spread of MDR and pre-XDR isolates. More in-depth, prospective epidemiological studies in these settings are needed to understand the role of non-Beijing genotypes in the occurrence of M/XDR-TB.

A theoretical comparison of these findings with a commercially available rapid molecular test, the line probe assay (LPA) also highlights the importance of knowing the prevalence and distribution of mutations related to resistance before implementing a new test in any specific setting. If the Genotype® MTBDR*plus* (Hain Lifescience, Nehren, Germany) [31] would have been used, 97% of the *katG*/*mabA*-*inhA* mutants and 100% of the *rpoB* mutants would have been correctly detected. Thus, the overall test sensitivity would have been very good, but when site-by-site comparisons are done, only 65% and 75% of the INH-resistant isolates from Iran and Honduras, respectively, would have been identified as resistant. Similarly, the Genotype® MTBDR*sl* (Hain Lifescience, Nehren, Germany) [32] would have been able to detect the fluoroquinolone-resistance-related mutations owing to its high sensitivity for the detection of fluoroquinolone resistance, as it has been reported recently [33].

Conclusion

Knowledge of the prevalence of geographic-specific mutations can allow the development of in-house, PCR-based methods for targeting mutations relevant in a specific setting. Since there are clear geographical differences in the presence and proportion of resistance-related mutations, it is crucial to include enough drug-resistant clinical isolates from various parts of the world when new diagnostic tools aimed at global implementation are developed and evaluated.

Conflict of interest

There are no conflicts of interest.

Acknowledgments

This work had financial support from the Swedish International Development Cooperation Agency (Sida) and the EC projects FP7-HEALTH-2007-A-201690 (FAST-XDR-DETECT) and FP7-HEALTH-2007-B-223681 (TB PAN-NET).

REFERENCES

[1] World Health Organization, Global tuberculosis control: WHO Report 2011, WHO/HTM/2011.16, World Health Organization, Geneva, Switzerland, 2011.
[2] World Health Organization, Report of the meeting of the WHO Global Task force on XDR-TB, WHO/HTM/TB/2007.275, World Health Organization, Geneva, Switzerland, 2007.
[3] A. Skrahina, H. Hurevics, A. Zalutskaya, E. Sahalchyk, A. Astrauko, W. van Gemert, et al, Alarming levels of drug-resistant tuberculosis in Belarus: Results of a survey in Minsk, Eur. Respir. J. 39 (6) (2012) 1425–1431.
[4] S. Ramaswamy, J.M. Musser, Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update, Tuberc. Lung Dis. 79 (1998) (1998) 3–29.
[5] A. Telenti, P. Imboden, F. Marchesi, D. Lowrie, S. Cole, M.J. Colston, et al, Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*, Lancet 341 (1993) 647–650.
[6] B. Heym, B. Saint-Joanis, S.T. Cole, The molecular basis of isoniazid resistance in *Mycobacterium tuberculosis*, Tuberc. Lung Dis. 79 (1999) 267–271.
[7] D.A. Rouse, Z. Li, G.H. Bai, S.L. Morris, Characterization of the *katG* and *inhA* genes of isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis*, Antimicrob. Agents Chemother. 39 (1995) 2472–2477.
[8] A. Banerjee, M. Sugantino, J.C. Sacchettini, W.R. Jacobs Jr., The *mabA* gene from the *inhA* operon of *Mycobacterium tuberculosis* encodes a 3-ketoacyl reductase that fails to confer isoniazid resistance, Microbiology 144 (Pt 10) (1998) 2697–2704.
[9] G. Canetti, W. Fox, A. Khomenko, H.T. Mahler, N.K. Menon, D.A. Mitchison, et al, Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes, Bull. World Health Organ. 41 (1969) 21–43.
[10] G. Canetti, S. Froman, J. Grosset, P. Hauduroy, Mycobacteria: Laboratory methods for testing drug sensitivity and resistance, Bull. World Health Organ. 29 (1963) 565–568.
[11] B.S. Reisner, A.M. Gatson, G.L. Woods, Evaluation of mycobacteria growth indicator tubes for susceptibility testing of *Mycobacterium tuberculosis* to isoniazid and rifampin, Diagn. Microbiol. Infect. Dis. 22 (1995) 325–329.
[12] P. Jureen, J. Werngren, S.E. Hoffner, Evaluation of the line probe assay (LiPA) for rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*, Tuberculosis (Edinb.) 84 (2004) 311–316.

[13] D. van Soolingen, P.W. Hermans, P.E. de Haas, D.R. Soll, J.D. van Embden, Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: Evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis, J. Clin. Microbiol. 29 (1991) 2578–2586.
[14] Z. Sun, J. Zhang, X. Zhang, S. Wang, Y. Zhang, C. Li, Comparison of gyrA gene mutation between laboratory-selected ofloxacin-resistant *Mycobacterium tuberculosis* strains and clinical isolates, Int. J. Antimicrob. Agents 31 (2008) 115–121.
[15] C. Xu, N. Kreiswirth, S. Sreevatsan, J.M. Musser, K. Drlica, Fluoroquinolone resistance associated with specific gyrase mutation in clinical isolates of multidrug-resistant *Mycobacterium tuberculosis*, J. Infect. Dis. 174 (1996) 1127–1130.
[16] J.D. Thompson, D.G. Higgins, T.J. Gibson, CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, Nucleic Acids Res. 22 (1994) 4673–4680.
[17] A. Sandgren, M. Strong, P. Muthukrishnan, B.K. Weiner, G.M. Church, M.B. Murray, Tuberculosis drug resistance mutation database, PLoS Med. 6 (2009) e2.
[18] S. Sreevatsan, X. Pan, K.E. Stockbauer, N.D. Connell, B.N. Kreiswirth, T.S. Whittam, et al, Restricted structural gene polymorphism in the *Mycobacterium tuberculosis* complex indicates evolutionarily recent global dissemination, Proc. Natl. Acad. Sci. USA 94 (1997) 9869–9874.
[19] M.H. Hazbon, M. Brimacombe, M. Bobadilla del Valle, M. Cavatore, M.I. Guerrero, M. Varma-Basil, et al, Population genetics study of isoniazid resistance mutations and evolution of multidrug-resistant *Mycobacterium tuberculosis*, Antimicrob. Agents Chemother. 50 (2006) 2640–2649.
[20] R.C. Chan, M. Hui, E.W. Chan, T.K. Au, M.L. Chin, C.K. Yip, et al, Genetic and phenotypic characterization of drug-resistant *Mycobacterium tuberculosis* isolates in Hong Kong, J. Antimicrob. Chemother. 59 (2007) 866–873.
[21] V. Kapur, L.L. Li, S. Iordanescu, M.R. Hamrick, A. Wanger, B.N. Kreiswirth, et al, Characterization by automated DNA sequencing of mutations in the gene (rpoB) encoding the RNA polymerase beta subunit in rifampin-resistant *Mycobacterium tuberculosis* strains from New York City and Texas, J. Clin. Microbiol. 32 (1994) 1095–1098.
[22] E.R. Dalla Costa, M.O. Ribeiro, M.S. Silva, L.S. Arnold, D.C. Rostirolla, P.I. Cafrune, et al, Correlations of mutations in *katG*, *oxyR-ahpC* and *inhA* genes and in vitro susceptibility in *Mycobacterium tuberculosis* clinical strains segregated by spoligotype families from tuberculosis prevalent countries in South America, BMC Microbiol. 9 (2009) 39.
[23] A. Von Groll, A. Martin, P. Jureen, S. Hoffner, P. Vandamme, F. Portaels, et al, Fluoroquinolone resistance in *Mycobacterium tuberculosis* and mutations in *gyrA* and *gyrB*, Antimicrob. Agents Chemother. 53 (2009) 4498–4500.
[24] M. Setareh, L.P. Titov, L.K. Surkova, High level association of mutation in KatG315 with MDR and XDR clinical isolates of *Mycobacterium tuberculosis* in Belarus, Acta Microbiol. Immunol. Hung. 56 (2009) 313–325.
[25] J. Bazira, B.B. Asiimwe, M.L. Joloba, F. Bwanga, M.I. Matee, Use of the GenoType® MTBDRplus assay to assess drug resistance of *Mycobacterium tuberculosis* isolates from patients in rural Uganda, BMC Clin. Pathol. 10 (2010) 5.
[26] A.N. Umubyeyi, L. Rigouts, I.C. Shamputa, K. Fissette, Y. Elkrim, P.W. de Rijk, et al, Limited fluoroquinolone resistance among *Mycobacterium tuberculosis* isolates from Rwanda: Results of a national survey, J. Antimicrob. Chemother. 59 (2007) 1031–1033.
[27] A. Aubry, N. Veziris, E. Cambau, C. Truffot-Pernot, V. Jarlier, L.M. Fisher, Novel gyrase mutations in quinolone-resistant and -hypersusceptible clinical isolates of *Mycobacterium tuberculosis*: Functional analysis of mutant enzymes, Antimicrob. Agents Chemother. 50 (2006) 104–112.
[28] F. Maruri, T.R. Sterling, A.N. Kaiga, A. Blackman, Y.F. van der Hejden, C. Mayer, et al, A systematic review of gyrase mutations associated with fluoroquinolone-resistant *Mycobacterium tuberculosis* and a proposed numbering system, J. Antimicrob. Chemother. 67 (2012) 819–831.
[29] Y. Hu, X. Ma, E.A. Graviss, W. Wang, W. Jiang, B. Xu, A major subgroup of Beijing family *Mycobacterium tuberculosis* is associated with multidrug resistance and increased transmissibility, Epidemiol. Infect. (2010) 1–9.
[30] F. Doustdar, A.D. Khosravi, P. Farnia, M.R. Masjedi, A.A. Velayati, Molecular analysis of isoniazid resistance in different genotypes of *Mycobacterium tuberculosis* isolates from Iran, Microb. Drug Resist. 14 (2008) 273–279.
[31] D. Hillemann, S. Rusch-Gerdes, E. Richter, Evaluation of the GenoType MTBDRplus assay for rifampin and isoniazid susceptibility testing of *Mycobacterium tuberculosis* strains and clinical specimens, J. Clin. Microbiol. 45 (2007) 2635–2640.
[32] D. Hillemann, S. Rusch-Gerdes, E. Richter, Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin–capreomycin, and ethambutol resistance testing of *Mycobacterium tuberculosis* Strains and Clinical Specimens, J. Clin. Microbiol. 47 (2009) 1767–1772.
[33] O. Ignatyeva, I. Konstsevaya, A. Kovalyov, Y. Balbanova, V. Nikolayevskyy, K. Toit, et al, Detection of resistance to second-line antituberculosis drugs by use of GenoType MTBDRsl assay: A multicenter evaluation and feasibility study, J. Clin. Microbiol. 50 (2012) 1593–1797.