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Research Paper

Redefining MDR-TB: Comparison of *Mycobacterium tuberculosis* clinical isolates from Russia and Taiwan

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ABSTRACT

Multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis are global challenges due to the limited number of effective drugs for treatment. Treatment with less than 4–5 effective drugs might lead to the further emergence of drug resistance and poor clinical outcomes. For better prediction of treatment outcomes, we compared drug-resistance profiles of consecutive clinical MDR *Mycobacterium tuberculosis* isolates from high- and low-burden settings.

This was a retrospective cohort study. We analysed 225 and 229 MDR isolates from Moscow (Russia) and Taiwan, respectively, obtained between 2014 and 2015. Drug susceptibility testing was performed by the Bactec MGIT 960 automated system and the agar proportion method. Detection of resistance-associated mutations in the *M. tuberculosis* genome was carried out by an array and/or sequencing of selected loci.

The principal differences between resistance profiles of MDR isolates in the two countries were the percentages of pre-XDR (40.9% vs. 14.8%) and XDR (34.7% vs. 1.7%) isolates, both of which were significantly higher in Moscow isolates. Forty-eight (33%) of 147 MDR and pre-XDR Russian isolates fall into a group with less than four effective drugs, which accounts for 40% ($N = 120$) of these isolates. The other 60% in this group were XDR strains ($N = 72$). Consequently, the average number of effective anti-tuberculosis drugs for MDR-TB treatment was lower for Russian isolates (3 vs. 7). Furthermore, a notable percentage (9%) of isolates resistant to kanamycin harboured mutations in the *whiB7* locus, which was not detected by molecular tests targeting common mutations in the *rrs* and *eis* loci. We found that 98.2% and 45.9% of MDR isolates from Moscow and Taiwan, respectively, were resistant to streptomycin.

Molecular tests for detecting resistance to drugs other than rifampicin, isoniazid, fluoroquinolones, and second-line injectable drugs are needed for individualized therapy. The conventional MDR treatment schemes most probably fail in these cases due to the limited number of effective drugs.

1. Introduction

The World Health Organization (WHO) world tuberculosis (TB) report of 2017 indicated that two-thirds (61%) of the 10.4 million new TB cases and nearly a quarter of the 490,000 new cases of multidrug-resistant TB (MDR-TB), with 6.2% also being classified as extensively drug-resistant TB (XDR-TB), were detected and reported in 2016 (WHO, 2017). MDR- and XDR-TB are worldwide challenges due to a limited number of effective drugs for treatment. The Russian Federation is considered to be an MDR-TB high-burden country with 18–40% prevalence of MDR-TB among new cases, and possessing a TB incidence rate of 65 per 100,000, as estimated in 2013 (Yablonskii et al., 2015). In Taiwan, the TB incidence rate was 43 per 100,000 in 2016. A drug resistance surveillance report from the Taiwan Centers for Disease Control (CDC) (Chuang et al., 2016) identified the following first-line TB drug resistance percentages for new and retreated cases: isoniazid (INH): 9%, 18%; rifampin (RMP):2%, 10%; ethambutol (EMB): 2%, 7%; streptomycin (STR): 8%, 12%; and MDR-TB: 1%, 6%. Approximately

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120 MDR-TB cases are registered annually in Taiwan.

The loss of susceptibility to the most important bactericidal second-line drugs, fluoroquinolones (FQs) or injectable drugs (SLIDs), is associated with an increased probability of an unfavourable outcome (Cegielski et al., 2014). Furthermore, the use of less than 4–5 effective drugs during treatment leads to the further emergence of drug resistance (Falzon et al., 2017).

Rapid and accurate drug susceptibility testing (DST) is a cornerstone of the successful treatment of MDR-TB and the prevention of subsequent development of acquired resistance to drugs used in the regimen (Cegielski et al., 2014). Drug-resistant *M. tuberculosis* is associated with mutations in several genes, including *rpoB* for RMP; *katG*, *oxyR-ahpC*, and the *inhA* regulatory region for INH; *embB* for EMB; *rpsL* and *rrs* for STR; *gyrA* and *gyrB* for FQs; and *rrs* and the promoter of *eis* for KAN, AMK, and CAP (Campbell et al., 2011). Genotypic drug-susceptibility tests have advantages over conventional phenotypic DSTs in turnaround time and in their ability to detect disputed mutations that nevertheless confer clinically significant low-level resistance (Miotto et al., 2018). However, the development of molecular assays, their interpretation, and the clinical use of whole genome sequencing data are complicated by an insufficient understanding of the genetic mechanisms of antibiotic resistance, which demands massive genotype-phenotype correlation studies and mutation analysis experiments (Heyckendorf et al., 2018). Surveillance and epidemiological studies allow for the identification of current drug resistance and can estimate the diagnostic methods needed in clinical laboratories to detect comprehensive drug resistance profile.

To determine the challenges for routine DST of MDR-TB in different settings, we compared drug resistance profiles of MDR *M. tuberculosis* isolates from high- and low-burden regions, – Moscow, Russian Federation and Taiwan, and identified drug resistance-conferring mutations in common genes.

2. Material and methods

According to the Ethics Committee of the Moscow Research and Clinical Centre for Tuberculosis Control, this research did not require ethics approval. All samples used in this study were coded and lacked personal information about the patients, especially names and addresses, to maintain their anonymity. According to the Taiwan Communicable Disease Control Act, TB is one of the notifiable diseases, and specimen collection for laboratory testing is mandatory. This study did not require ethics approval, and participant consent was not required.

2.1. Clinical isolates

From 2014 to 2015, 229 and 225 consecutive MDR *M. tuberculosis* isolates were tested in the Reference Mycobacteriology Laboratories of the Taiwan Centers for Disease Control and the Moscow Research and Clinical Center for Tuberculosis, respectively. The initial isolate was selected for each patient.

2.2. Mycobacterial culture identification and drug susceptibility testing

Decontaminated specimens were inoculated in solid and liquid culture. Cultures were deemed negative after incubation for 42 days without the isolation of any mycobacteria. The MPB64 antigen and DST of *M. tuberculosis* were detected as previously described (WHO 2003; Taiwan CDC, 2017). In Taiwan, *M. tuberculosis* isolates were subjected to DST using the proportion method with 7H10 and 7H11 medium (Becton, Dickinson and Company, Spark, MD, USA). Drug resistance was defined as the growth of 1% of colonies in a drug-containing medium. The critical concentrations of the tested drugs in 7H10 were INH, 0.2 μg/ml; RMP, 1 μg/ml; STR, 2 μg/ml; EMB, 5 μg/ml; CAP, 10 μg/ml; ofloxacin (OFX), 2 μg/ml and moxifloxacin (MFX), 0.5 μg/ml. The critical concentrations of the tested drugs in 7H11 were amikacin (AMK), 6 μg/ml; kanamycin (KAN), 6 μg/ml; para-aminosalicylic acid (PAS), 8.0 μg/ml and ethionamide (ETH), 10 μg/ml. Pyrazinamide (PZA, 100 μg/ml) was tested using the Bactec MGIT 960 as described previously (WHO, 2008). In Russia, MTB DST for RMP, INH, EMB, STR, PZA, OFX, MFX, KAN, AMK, PAS, and ETH was performed using the Bactec MGIT 960 as described previously (Sharma et al., 2011; WHO, 2008). MTB DST for CS was performed on Löwenstein–Jensen solid medium using a critical concentration of 30 μg/ml.

2.3. Microarray analysis and gene sequencing

DNA was isolated using an automated DNA isolation station from Freedom EVO® Clinical (Tecan Group Ltd., Männedorf, Germany). Fragments of the *katG*, *inhA*, *tlyA*, and *whiB7* genomic loci were amplified with their corresponding pairs of primers (Table A.1). The 30 μl reaction volume contained 3 U of Qiagen HotStarTaq DNA polymerase, 1 × Qiagen PCR buffer, 3 mM $MgCl_2$ (all three from Qiagen GmbH, Hilde, Germany), 200 μM each dNTP (Evrogen LLC, Moscow, Russia), 1 μM each primer, and 1 μl of the DNA sample. The PCR cycling conditions were as follows: initial denaturation at 95 °C for 4 min; 40–50 cycles of denaturation at 95 °C for 40 s, annealing at primer-specific temperature (Table A.1) for 30 s, and extension at 72 °C for 30 s; and final extension at 72 °C for 5 min. Fragments were purified from agarose gels using a MinElute gel extraction kit (Qiagen GmbH, Hilde, Germany) and subjected to dideoxy sequencing using ABI Prism BigDye Terminator version 3.1 (Applied Biosystems, Foster City, CA, USA). In Taiwan, PCR products were verified using a high-performance DNA analyser (eGene HDA-GT12, Qiagen) and sent for sequencing (Gemonics, Taiwan). The analysis of the *rpoB*, *katG, inhA, ahpC, gyrA, gyrB, rrs, eis*, and *embB* genes associated with resistance in Russian isolates was performed by DNA microarray as previously described (Zimenkov et al., 2016).

3. Results

3.1. Characteristics of study isolates

Comparable numbers of consecutive MDR *M. tuberculosis* isolates were collected in Taiwan and Moscow from 2014 to 2015. Of the 229 Taiwan isolates, 83.4% (191/229) were simple MDR (susceptible to FQs and SLIDs), 14.8% (34/229) were pre-XDR (26 MDR plus FQ- and 8 MDR plus SLID-resistant), and 1.7% (4/229) were XDR. Of the 225 Russian isolates, 24% (55/225) were simple MDR, 35% (78/225) were pre-XDR (21 MDR plus FQ- and 71 MDR plus SLID-resistant), and 41% (92/225) were XDR (Table 1). Russian isolates had a high resistance rate to EMB (64%), PZA (76%), and STR (98%) (Table 1 and Table A.2). PAS resistance was found in 78 (34.7%) of the Russian isolates and 13 (5.7%) of the Taiwanese isolates. Cycloserine resistance was rare, and only 3 and 2 cycloserine-resistant isolates were found in the Russian and Taiwanese isolates, respectively. Russian isolates were resistant to a larger number of tested drugs. The expected value, or distribution mode, of efficient drugs for Russia and Taiwan MDR isolates was 3 (N = 54, 24%) and 7 (N = 69, 30%) drugs, respectively (Fig. 1).

A slight increase in resistance was observed when comparing primary and retreated cases both in Russia and Taiwan (Fig. A.1). The most significant difference was found for XDR prevalence in Russian isolates: 25% (N = 26) and 43% (N = 52) for primary and retreated cases, respectively. The Beijing genotype was the most frequent genotype detected in the two collections, accounting for 90% (N = 202) and 55% (N = 125) of the Russian and Taiwanese isolates, respectively. Correlation of resistance with genotype revealed only a slight shift towards more resistant forms for the Beijing isolates, particularly Beijing B0/W-148 (Mokrousov et al., 2012) sublineage isolates (N = 97) compared to other Beijing isolates (N = 105) in the Russian collection (Fig. A.2).

Table 1
Drug-resistance profiles of multidrug-resistant Mycobacterium tuberculosis isolates.

	Russian MDR isolates (N = 225)	Taiwanese MDR isolates (N = 229)
Simple MDR[a]	55 (24.4%)	191 (83.4%)
Pre-XDR	92 (40.9%)	34 (14.8%)
MDR resistant to SLID	71	8
MDR resistant to FQs	21	26
XDR[b]	78 (34.7%)	4 (1.7%)
MDR with additional resistance to		
PZA	172 (76.4%)	64 (27.9%)
EMB	145 (64.4%)	124 (54.1%)
STR	221 (98.2%)	105 (45.9%)
Resistant to PZA + EMB + STR	121 (53.8%)	50 (21.8%)

MDR, multidrug resistant, XDR, extensively drug resistant, PZA, pyrazinamide, EMB, ethambutol, STR, streptomycin, SLID, second-line injectable drugs, FQs, fluoroquinolones.

[a] Isolates resistant to at least rifampicin and isoniazid.

[b] MDR isolates with additional resistance to one SLID and any FQ.

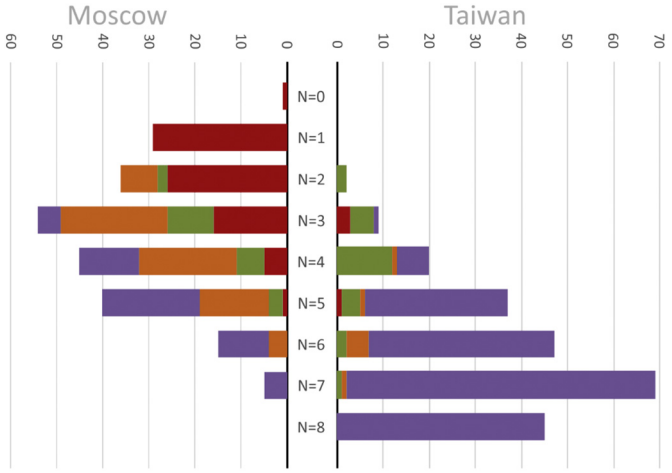

Fig. 1. The distribution of clinical isolates according to the number of effective drugs (N). Violet – pure MDR, Orange – MDR and additional second-line injectable drug resistant, Green – MDR and fluoroquinolone resistant, Red – XDR cases.

3.2. Detection of drug-resistant mutations

3.2.1. First-line drugs resistance mutations

The difference between the two collections was also observed when resistance mutation frequencies were analysed (Table A.3). The canonical *rpoB* S531 L, associated with high-level RMP resistance, was identified in 87% and 66% of Russian and Taiwanese isolates, respectively. The *rpoB* disputed mutations (L511P, D516Y, H526C, H526L, H526N, and L533P), associated with borderline and low-level RMP resistance, were found in 4% and 11% of Russian and Taiwanese isolates, respectively. Previously described mutations, found in RMP-resistant but rifabutin-susceptible isolates, were identified in 6% (14/225) and 22% (51/229) of Russian and Taiwanese isolates, respectively (Table A.3) (Dheda et al., 2017; Jamieson et al., 2014; Rukasha et al., 2016; Van Ingen et al., 2011).

A high diversity of mutations conferring INH resistance was observed in Taiwanese isolates. A canonical single KatG S315 T substitution was found in 73% and 53% of Russian and Taiwanese isolates, respectively. Including single, double, and concurrent with the *inhA* promoter mutations, the occurrence of S315 T mutations increased to 98% and 59% in the Russian and Taiwanese isolates, respectively. The S315 T substitution was previously reported to have a prevalence of 83%–94% (Campbell et al. 2011; Vilchèze and Jacobs, 2014). The single *inhA* regulatory region mutation c(−15)t, leading to low-level INH resistance (Kambli et al., 2015), was found in 25% of Taiwanese isolates and in only 2% of Russian isolates. The combination of *inhA* regulatory region c(−15)t and *katG* S315 T mutations, which contribute to high-level INH resistance (Kambli et al., 2015), was found in 6% and 19% of Taiwanese and Russian isolates, respectively. Of the 229 Taiwanese isolates, 27 (12%) had no mutations using commercial molecular tests for detecting INH resistance. However, 17 isolates harbouring various mutations in the *katG* and *inhA* genes and in the *inhA* regulatory region were identified using Sanger sequencing (Table A.3).

EMB resistance in *M. tuberculosis* is predominantly associated with *embB* gene mutations (Starks et al., 2009). Various substitutions at codon 306 of the *embB* gene were found in 45% (102/225) and 23% (53/229) of Russian and Taiwanese isolates, respectively. Isolates with an *embB* M306 mutation but still phenotypically susceptible to EMB were found in 26% (27/102) and 11% (6/53) of Russian and Taiwanese isolates, respectively. The *embB* M306 V was the most frequent mutation identified (Table A.3).

3.2.2. Second-line drug resistance mutations

A substantial difference in the prevalence of resistance to second-line drugs between the two collections did not allow us to make reliable conclusions on resistance mutation spectra differences. FQ-resistant isolates accounted for 99 and 30 of the Russian and Taiwanese isolates, respectively. For SLIDs, the difference was even more significant: 149 and 12 isolates were resistant to at least one SLID for Russia and Taiwan, respectively.

Regarding FQ resistance, substitution D94G in GyrA was the most prevalent among resistant strains in both Russia (38%) and Taiwan (50%). No mutations were found in the *gyrB* gene in Taiwanese isolates; however, 8 Russian isolates harboured mutations in the *gyrB* gene, including 4 isolates that had concurrent mutations in the *gyrA* gene (Table A.3). In accordance with previous studies, three GyrB substitutions (D500H, D500N, and N538D) were identified in FQ-resistant isolates (Aubry et al., 2006; Malik et al., 2012), and GyrB A543T was found in the susceptible stain. No novel *gyrA* and *gyrB* mutations were identified in this study. FQ-resistant isolates with no mutations in both *gyrA* and *gyrB* accounted for 2 and 1 of the Taiwanese and Russian isolates, respectively.

KAN resistance caused by the *rrs* a1401g mutation and various *eis* promoter mutations were identified in 85% (122 of 149) of Russian isolates. Unexpectedly, *eis* mutations (56%) were more abundant than the *rrs* mutation (29%) (Table A.4). Mutations in the *eis* locus were generally associated with resistance to KAN and susceptibility to AMK and CAP. However, some discrepancies between the two sets of isolates were observed. Of the isolates with an *eis* c(−12)t mutation, 20 of 24 Russian isolates were phenotypically resistant to KAN; however, all Taiwanese isolates with the same mutation remained susceptible (Table A.4). Furthermore, a weaker correlation of the *rrs* a1401g mutation with CAP resistance was observed in Taiwanese isolates – 4 out of 9 were susceptible, compared to 3 out of 42 for Russian isolates.

Additional analysis of SLID-resistant isolates with no mutations in the *rrs* and *eis* loci (27 from Russia and 1 from Taiwan) revealed mutations in the *tlyA* gene in 3 out of 6 CAP-resistant isolates (2 for Russian and 1 for Taiwan), which were still susceptible to KAN and AMK.

Of the remaining 22 isolates, 19 were resistant to KAN and susceptible to the other two SLID drugs, 2 were resistant to KAN and CAP, and 1 was resistant to all three drugs. Mutations in the 5′ untranslated region of the *whiB7* gene were identified in 13 (9% of total KAN-resistant, N = 144) isolates. Of the KAN monoresistant isolates, 9 and 3 bore a237g and g298 t mutations (numbering from transcription start site), respectively. A single isolate with a (−7)c deletion in the *whiB7* promoter region was resistant to KAN and CAP (Table A.4). Furthermore, 12 Russian isolates with various profiles of phenotypic SLID resistance had no mutation in the *rrs*, *eis*, *tlyA*, or *whiB7* genes.

4. Discussion

In this study, we identified the principal differences between resistance profiles of MDR *M. tuberculosis* isolates from two high- and low-incidence settings. The diversity of the identified resistance profiles calls into question the usefulness of the MDR and XDR definitions themselves: we identified isolates resistant to nearly all drugs but susceptible to either FQs or SLIDs and, thus, annotated these isolates as MDR, following the guidelines. Conventional MDR treatment schemes most probably fail in these cases due to the limited number of effective drugs, as five effective anti-TB drugs should be administered during the intensive phase for a patient with MDR-TB (Falzon et al., 2017). The high correlation of MDR with resistance to other first-line drugs in Russian isolates suggests that using PZA (Zhang et al., 2012) and EMB for treatment (Caminero, 2008) may be futile. Similarly, 98.2% of Russian isolates were resistant to STR, whereas 54% of Taiwanese isolates were STR susceptible. This difference is likely due to the different histories of TB treatment in the two countries.

The diversity of mutations associated with RMP and INH resistance was higher in Taiwanese isolates, and the prevalence of the common *rpoB* S531L and *katG* S315T double mutation was higher in Russian isolates. This finding could be explained in part by the spread of specific successful genotypes (Mokrousov et al., 2012, 2017) associated with MDR and XDR. Indeed, 43% of the studied Russian isolates belonged to the Beijing B0/W-148 clone (Mokrousov et al., 2012). On the contrary, a substantial amount of the mutations found in Taiwanese isolates were most probably associated with phenotypic low-level, or borderline, resistance to RMP (10%) and INH (30%).

Novel substitutions in the *KatG* gene (A312V, L458C, W412*, W341R) were identified, adding to the growing number recently reported mutations in this gene (Greif et al., 2012; Kandler et al., 2018; Mitarai et al., 2012; Torres et al., 2015). Another identified *KatG* substitution, Q461P, was described earlier (Mitarai et al., 2012) and was associated with low-level resistance to INH (Brossier et al., 2016). The *inhA* regulatory region mutations, which are also associated with low-level INH resistance, were found in 30% of Taiwanese isolates. The previously reported prevalence of these mutations ranged from 12% (Domínguez et al., 2016) to as high as 91% (Perdigao et al., 2008). Since the frequency of such mutations was only approximately 2% in Russian isolates, a high dose of INH may not be an option for treatment (Dheda et al., 2018).

The proportion of pre-XDR and XDR isolates was substantially higher in MDR isolates from Russia. In our study, XDR prevalence was 35%, which is substantially higher than the reported average of 6% (WHO, 2017). These results could be due to unintentional sample selection bias and need to be investigated further using countrywide surveillance analysis. Nevertheless, this finding raises a question about the usefulness of identifying resistance to RMP alone (Garfein et al., 2015) or to RMP and INH, both of which are common in several molecular tests (Heyckendorf et al., 2018).

Analysis of drug resistance-conferring mutations supported the value in detecting mutations in the *gyrB* and *eis* loci for second-line drug susceptibility testing. Whereas the *rrs* (a1401g) mutation leads to cross-resistance to all three SLIDs, mutations in the *eis* gene promoter, enhancing its transcription, lead to low-level monoresistance to KAN (Zaunbrecher et al., 2009). In addition to the high proportion of *eis* promoter mutations (~40%), we identified a significant proportion (~9%) of isolates with mutations in the *whiB7* region in the Russian collection (Reeves et al., 2013). Two of the most prevalent mutations (a237g and g298t), located upstream of the *whiB7* coding sequence, were identified in different KAN-resistant, but AMK- and CAP-susceptible, isolates obtained from different patients, thus confirming its role in KAN resistance. *WhiB7* regulation is not completely understood, but the current proposed mechanism is based on transcription attenuation with the direct involvement of protein factors (Burian and Thompson, 2018). The identified consensus mutations, at positions 298 and 237 from the transcription start site, could lead to changes in RNA secondary structure or stability as well as to alterations in the binding of regulatory proteins.

Although novel mutations were identified in this study, drug-resistant isolates without mutations in known loci were also found. The misdiagnosis of drug resistance due to unknown mechanisms and disputed mutations may lead to inappropriate drug therapy for TB patients. As a result, this practice could promote further drug resistance and lead to poor clinical outcomes (Wells et al., 2013). Therefore, conventional DST cannot yet be completely substituted with molecular methods, and a comprehensive analysis of novel drug-associated mutations and the confirmation of disputed mutations' impact on drug susceptibility are needed.

The differences between the observed mutation profiles also lead to the conclusion that the sensitivities of molecular tests are region-specific and could depend on the epidemiologic situation, history of TB treatment, healthcare system, or level of TB burden. For example, the microarray test developed in Russia (Zimenkov et al., 2016) had a lower sensitivity of resistance detection in Taiwanese isolates — the sensitivity for INH resistance detection was ~88% and 100% for Taiwanese and Russian isolates, respectively. It should be noted, however, that this is not an accurate estimation, as only MDR isolates were analysed.

The nucleic acid amplification tests GenoType MTBDR*plus/sl* (Hain Lifescience, Nehren, Germany) and Xpert MTB/RIF (Xpert) (Cepheid, Sunnyvale, CA) have been recommended by the WHO for RMP/INH resistance detection (Gu et al., 2015). Xpert is a simple and rapid method; however, only RMP resistance occurring from mutations in *rpoB* codons 507–533 is analysed. GenoType MTBDR*plus* focuses on the mutations in *rpoB* and *katG* and the *inhA* regulatory region. Although the probes in GenoType MTBDR*plus* detect the most frequent resistance-conferring mutations, some important mutations identified in this study could not be clearly detected. Furthermore, GenoType MTBDR*sl* focuses on FQ and SLID resistance-mediating mutations, but some of these mutations could not be identified as well. Among 149 KAN-resistant isolates, 9% of Russian isolates had WhiB7 mutations, which are not targeted by any modern molecular tests. Based on these results, DNA sequencing is still necessary for drug-resistance detection.

Observed differences in resistance patterns and mutation profiles also confirmed that a shorter treatment scheme is not applicable for MDR-TB in high burden countries, as suggested previously (Dheda et al., 2018). It should be noted also that new and repurposed drugs not analysed in this study, including bedaquiline, delamanid, linezolid, clofazimine, and beta-lactams (Sotgiu and Migliori, 2015; WHO, 2018), were available, however with limitations, for the treatment of MDR and XDR TB cases in Russia and Taiwan.

Although the isolates collected in Taiwan were population-based, a limitation of this study was the sample selection criteria. We did not separate primary cases from chronic and relapse cases for several reasons. First, the criteria for distinguishing primary and chronic infections are not very reliable due to the high incidence of migration in the Moscow region. Second, the national surveillance database in the Russian Federation is in its initial stages of development, and therefore, a new resident of the Moscow region with chronic TB could be identified and treated as a primary TB case. Finally, the diagnostic laboratory does not necessarily possess demographic information about the patient, as its main task is the rapid identification of *M. tuberculosis*, determining its resistance profile, and finding effective drugs for treatment. Thus, our study provides information about the diversity of clinical *M. tuberculosis* isolates in Russia and Taiwan and the spectra of tests that are needed for individualized treatment (Bastos et al., 2014) when MDR tuberculosis is identified (Cox et al., 2018).

5. Conclusion

The main aim of diagnostics in low-burden tuberculosis settings in Taiwan is the detection of resistance to FQs and SLIDs for MDR-TB so as

to prevent the evolution of pre-XDR isolates to a more resistant form. However, for MDR-TB cases in Moscow, comprehensive susceptibility testing to first- and second-line agents is needed for effective individualized therapy, preventing the emergence of resistance, and reducing the transmission of drug-resistant TB.

Conflict of interest

There are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2018.12.031.

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