ONLINE SERVICE FOR INTERPRETATION OF THE RESISTANCE PREDICTION RESULTS TO BEDAQUILINE BY THE MOLECULAR DATA

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ABSTRACT

Background. *Bedaquiline is a new and promising anti-tuberculosis drug, however resistance can develop with long-term use. This is mainly due to mutations in the atpE and mmpR genes in M. tuberculosis (MTB).*
The aim of the work. *The aim of the research was to test a system for automated interpretation of results for predicting resistance to bedaquiline by the molecular data.*
Materials and methods. *DNA was isolated from strains of M. tuberculosis in the Irkutsk region and Yakutia. The total quantity of DNA samples was 27 strains from Yakutia and 21 strains from the Irkutsk region. The study of MBT genomes was carried out on the DNA previously obtained by the authors in the territories of the Irkutsk region (n = 5), Yakutia (n = 4), Buryatia (n = 3), Zabaykalskiy kray (n = 4) and the Far East (n = 8). We used the BSATool program to detect bedaquiline resistance based on Sanger sequencing and genomic data. Sanger sequencing analyzed the atpE and mmpR genes, and whole genome sequencing examined mutations in the same sequences, as well as additionally in mmpL5, mmpS5, Rv0678, Rv1979c, and pepQ.*
Results. *Complete agreement between the phenotypic and genotypic analysis of resistance to bedaquiline was found for three strains from Yakutia. One genome with significant mutations associated with resistance to bedaquiline was identified. A conclusion was made about the relatively low prevalence of mutations that may induce resistance to this antibiotic, which coincides with the data of other studies in Russia. A conclusion was made about the importance of molecular analysis of target genes with subsequent detection of resistance to bedaquiline in silico.*

Key words: *bedaquiline, sequencing, resistance, genes, atpE, mmpR, mmpL5, mmpS5, Rv0678, Rv1979c, pepQ*

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ОНЛАЙН-СЕРВИС ДЛЯ ИНТЕРПРЕТАЦИИ РЕЗУЛЬТАТОВ ПРИ ПРОГНОЗИРОВАНИИ УСТОЙЧИВОСТИ К БЕДАКВИЛИНУ ПО МОЛЕКУЛЯРНО-БИОЛОГИЧЕСКИМ ДАННЫМ

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РЕЗЮМЕ

Обоснование. *Бедаквилин – новый и многообещающий противотуберкулёзный препарат, однако при длительном лечении к нему развивается устойчивость. Это связано преимущественно с мутациями в генах atpE и mmpR у M. tuberculosis (МБТ).*

Цель работы. *Апробация системы автоматизированной интерпретации результатов при прогнозировании устойчивости к бедаквилину на основе молекулярно-биологических данных.*

Материалы и методы. *ДНК выделяли из штаммов M. tuberculosis, циркулировавших в Иркутской области и Республике Саха (Якутии). Общее количество исследованных ДНК составило 27 штаммов из Якутии и 21 штамм из Иркутской области. Исследование геномов МБТ было проведено на ДНК штаммов, полученных авторами ранее на территориях Иркутской области (n = 5), Республики Саха (Якутия) (n = 4), Республики Бурятия (n = 3), Забайкальского края (n = 4) и Дальнего Востока (n = 8). Для выявления устойчивости к бедаквилину на основе нуклеотидной последовательностей генов и геномных данных мы использовали программу BSATool. При использовании секвенирования по Сэнгеру анализировались гены atpE и mmpR, при полногеномном секвенировании исследовались мутации в этих же последовательностях, а также дополнительно в mmpL5, mmpS5, Rv0678, Rv1979c и pepQ.*

Результаты. *Обнаружено полное соответствие фенотипических и генотипических результатов оценки устойчивости к бедаквилину для трёх штаммов из Якутии. Кроме того, при анализе геномных данных обнаружен один геном со значимыми мутациями, способными вызвать устойчивость к бедаквилину. Делается вывод об относительно низком распространении мутаций, способных вызвать устойчивость к этому антибиотику, что совпадает с данными других исследователей в России. Сделано заключение о важности молекулярно-биологического анализа генов-мишеней с последующим выявлением устойчивости к бедаквилину in silico.*

Ключевые слова: *бедаквилин, секвенирование, резистентность, гены, atpE, mmpR, mmpL5, mmpS5, Rv0678, Rv1979c, pepQ*

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Tuberculosis is an infectious disease caused by pathogenic mycobacteria belonging to the class Actinobacteria, order Actinomycetales, family Mycobacteriaceae, forming the *Mycobacterium tuberculosis* complex group [1]. In 2019, the World Health Organization (WHO) reported 10 million new cases and 1.2 million deaths [2]. However, the emergence and spread of multi-drug resistant (MDR) and extensively drug resistant (XDR) strains of *Mycobacterium tuberculosis* up to half a million annually [3] requires the introduction of new anti-TB drugs into treatment regimens. Bedaquiline is a new and promising antituberculosis drug, but as with other drugs, it is possible that *M. tuberculosis* (MTB) may develop resistance to it with long-term treatment. Specifically, mutations in the *atpE* gene prevent drug interaction with its target, ATP synthase, and mutations in the efflux pump repressor *mmpR* (*Rv0678*) lead to accelerated drug evacuation from the microbial cell [4–6]. Despite the involvement of a significant number of genes in resistance to bedaquiline and clofazimine (including cross-resistance), mutations in the *atpE* and *mmpR* genes (*Rv0678*) are determinant in the majority of clinical isolates [7]. Considering these results, a Sanger sequencing of these genes was used to analyse the developed software package.

An online service for automated interpretation of sequencing data and prediction of pyrazinamide resistance has been previously developed by us [8]. The service is available at https://bsatool.ru. In the current study, we present an extension of the capabilities of the BSATool software package for automated interpretation of results in predicting bedaquiline resistance based on molecular biological data, including Sanger sequencing and whole genome sequencing.

MATERIALS AND METHODS

The strains were obtained from the bacteriological laboratories of the Irkutsk Regional Clinical Tuberculosis Hospital (IRCTB) and E.N. Andreev Scientific and Practical Centre "Phthisiatry" (SPC "Phthisiatry"). Phenotypic sensitivity of MTB isolates to antituberculosis drugs (ATDs) was determined by absolute concentrations on Levenshtein – Jensen medium (IRCTB and SPC "Phthisiatry") and on Middlebrook 7H9 medium using an automated system Bactec MGIT 960 (Becton, Dickinson and Company, USA), including sensitivity to bedaquiline (SPC "Phthisiatry"). Strains were inactivated *in situ*. DNA isolation was performed according to the previously described method [9].

The total number of DNA samples examined included 27 strains from Yakutia and 21 strains from the Irkutsk region. The study of MBT genomes was carried out on the MBT strains previously obtained by the authors in the territories of the Irkutsk region ($n = 5$), Yakutia ($n = 4$), Buryatia ($n = 3$), Zabaykalskiy kray ($n = 4$) and the Far East ($n = 8$). Therefore, our study included a set of DNA samples representing different regions and territories. The structure of polymerase chain reaction (PCR) primers for the amplification of *atpE* and *mmpR* genes was developed independently by the authors. The following primers were used for the *atpE* gene: 1305F 5'-*TCGAAGAGGAACACCACTAG* and 1305R 5'-*GGACAATCGCGCTCACTTC*. The primers Bdq678F 5'-*CACGCTTGAGAGTTCCAATCA* and Bdq678R 5'-*ACCGCATCAACAAGGAGTGA* were used for the *mmpR* (Rv0678) gene. These oligonucleotides were designed to amplify PCR fragments of 368 and 679 base pairs, respectively.

PCR parameters were set, according to previously published protocols [8]. Sanger sequencing was performed using a domestic genetic analyzer Nanofor-05 (Syntol, Russia). Genomic libraries were prepared using DNA Flex kit (Illumina, USA). Full genome sequencing of samples was performed on NextSeq 550 sequencer (Illumina, USA) also using reagents v. 2.5 and flow cell (high output) at 300 cycles.

Primary data processing included removal of short low-quality sequences and technical fragment cut-offs and was performed according to previously published methods [10]. Sanger sequencing and full-genome sequencing were performed at the Center for Development of Progressive Personalized Health Technologies (shared research facility of the Scientific Centre for Family Health and Human Reproduction Problems (Irkutsk).

To reveal resistance to bedaquiline by Sanger sequencing, we used the BSATool software [8], which analysed mutations in *atpE* and *mmpR*. To reveal resistance to bedaquiline, mutations in the same genes (*atpE* and *mmpR*) and additionally in *mmpL5*, *mmpS5*, *Rv0678*, *Rv1979c* and *pepQ* were analysed using BSATool genomic data. Based on the above analyses, potential resistance to bedaquiline was identified in an *in silico* model. The clinical significance of the detected mutations in causing drug resistance was assessed according to the mutation catalogue recommended by the World Health Organization as a reference database [11].

RESULTS

Analysis of Sanger sequencing results in manual mode and by the BSATool software system

Of the 27 strains obtained from the Republic of Sakha (Yakutia), 9 were classified as extensively drug-resistant strains or their precursors (pre-XDR), 10 were sensitive to all ATDs, and the rest showed multidrug resistance [11]. Resistance to bedaquiline was found in only three pre-XDR strains.

When analysed by Sanger sequencing and using the BSATool service, significant mutations with the potential to cause resistance to bedaquiline were revealed in the same three strains. Based on the results of microbiological studies, these strains were registered as XDR.

Among the 21 strains from Irkutsk, 10 were classified as pre-XDR and the remaining 11 strains showed multidrug resistance. Among all these strains, only two had significant mutations that can induce resistance to bedaquiline, both in manual Sanger sequencing analysis

and using the BSATool service. Notwithstanding, due to the lack of a methodology for determining resistance to bedaquiline in the bacteriological laboratory of the IRCTB, these strains were not assigned XDR status.

Figure 1 shows an example of DNA analysis of four strains using the BSATool software package, one of which contains a mutation that can induce resistance to bedaquiline.

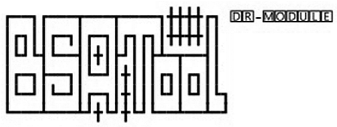
Analysis of the results of whole genome sequencing by BSATool software package

Manual analysis of the whole-genome data was not feasible due to the large amount of information. The study analysed the genome of 24 MTB strains, including those from the Irkutsk Region, the Republic of Sakha (Yakutia), the Republic of Buryatia, the Trans-Baikal Territory and the Far East. A significant mutation 2223444C/T in the *Rv1979* gene was only revealed in one strain, which belongs to the most virulent subtype B0/W148 of the Beijing genotype [12]. It is important to emphasize that the remaining strains analyzed by whole-genome sequencing did not show resistance to bedaquiline as part of *in silico* prediction.

DISCUSSION

Assessment of resistance to anti-TB drugs using molecular biological methods requires an individual approach depending on the prevalence of clinically relevant mutations. The hotspots for rifampicin are centered on a small 81 bp (base pair) long stretch of DNA and the most feasible method is to reveal them by real-time PCR. This method has already been successfully used in the practice of medical diagnostics for almost a decade.

For other ATDs, the situation with clinically relevant mutations is not as favourable, which requires the use of sequencing of multiple gene nucleotide sequences. The use of software systems that can reveal significant mutations in the results of whole-genome sequencing or Sanger sequencing significantly expands the possibilities of using these molecular biological methods in clinical practice. The relatively low frequency of revealed mutations causing resistance to bedaquiline is consistent with the results of studies conducted by colleagues in the European part of Russia [13]. All the mutations identified were observed in patients receiving long-term bedaquiline treatment as part of MDR/XDR tuberculosis chemotherapy regimens.



BSATool - Bacterial SNP Annotation Tool
(c) V.Sinkov, Irkutsk, Russia, 2017-2023
Version: 0.3.170723

Query	Query name	Query start	Query end	Query lenght	Query direction	Mutations	Intersected regions
Q1	825F.fa	1461033	1461317	284	-->	0	IGR_1460997_1461044,Rv1305,IGR_1461291_1461320
Q2	825R.fa	1461007	1461277	270	<--	0	IGR_1460997_1461044,Rv1305
Q3	850F.fa	1461039	1461311	272	-->	0	IGR_1460997_1461044,Rv1305,IGR_1461291_1461320
Q4	850R.fa	1461011	1461303	292	<--	0	IGR_1460997_1461044,Rv1305,IGR_1461291_1461320
Q5	860F.fa	1461032	1461317	285	-->	1	IGR_1460997_1461044,Rv1305,IGR_1461291_1461320
Q6	860R.fa	1461017	1461266	249	<--	1	IGR_1460997_1461044,Rv1305
Q7	870F.fa	1461043	1461320	277	-->	0	IGR_1460997_1461044,Rv1305,IGR_1461291_1461320
Q8	870R.fa	1461006	1461260	254	<--	0	IGR_1460997_1461044,Rv1305

Query	Query name	Locus	Gene	Genome position(s)	Type	Mutation	Codon	Amino Acid	Change	Description	Drug	DR	DR Info
Q1	825F.fa	-	-	-	-	-	-	-	-	-	-	-	-
Q2	825R.fa	-	-	-	-	-	-	-	-	-	-	-	-
Q3	850F.fa	-	-	-	-	-	-	-	-	-	-	-	-
Q4	850R.fa	-	-	-	-	-	-	-	-	-	-	-	-
Q5	860F.fa	Rv1305	atpE	1461242	SNP	c.198C>G	Caa/Gaa	Q66E	missense	Probable ATP synthase C chain AtpE	BDQ	R	I66M
Q6	860R.fa	Rv1305	atpE	1461242	SNP	c.198C>G	Caa/Gaa	Q66E	missense	Probable ATP synthase C chain AtpE	BDQ	R	I66M
Q7	870F.fa	-	-	-	-	-	-	-	-	-	-	-	-
Q8	870R.fa	-	-	-	-	-	-	-	-	-	-	-	-

FIG. 1.
Analysis of the results of Sanger sequencing of the atpE gene for two strands in four different strains: a significant mutation associated with resistance to bedaquiline was revealed in strain 860; no resistance-associated mutations were revealed in the other strains

CONCLUSION

Molecular biological analysis of nucleotide sequences of target genes followed by *in silico* resistance assessment against bedaquiline using the BSATool automated analysis system (publicly available) may be recommended primarily for laboratories performing Sanger sequencing. The low frequency of mutations associated with resistance to bedaquiline may be explained by the recent use of the drug in the treatment of patients and, consequently, by the small number of MTB strains resistant to bedaquiline. The validation of the proposed approaches for predicting bedaquiline resistance in clinical samples from patients with repeated courses of MDR/XDR tuberculosis treatment confirms the reliability of the obtained data.

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Conflict of interest

The authors of this article declare no conflicts of interest.

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