THE EFFECTIVENESS OF HAIN MTBDR IN DETERMINING THE RESISTANCE OF M. TUBERCULOSIS TO ANTI-TUBERCULOSIS DRUGS

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Summary

According to the WHO, about 10 million people fall ill with tuberculosis every year, 1.2-1.4 million of them die. Drug-resistant forms of tuberculosis make a significant contribution to this statistic - 483,000-639,000 new cases of multidrug-resistant (MDR-TB) and extensively drug resistant (XDR-TB) tuberculosis are recorded annually.

This study was performed to evaluate the sensitivity and specificity of molecular-genetic diagnostic method Hain MTBDR in comparison to cultural methods of determining *M. tuberculosis* drug resistance.

Materials and methods. 3527 sputum specimens from TB patients were investigated by Hain MTBDR and cultural methods (BACTEC and solid Lowenstein-Jensen medium) during 2018-2019.

Results. 25 inconsistencies were found between the results of Hain MTBDR and cultural methods, which accounted for 0.7% of all tests performed. The sensitivity of Hain MTBDR in this study was 99.7%, and specificity - 99.6%. Most of the inconsistencies in the results of molecular genetic and cultural research methods (12 cases) accounted for the determination of resistance to fluoroquinolones. Of these, mutations in the gyrA gene were found in 10 cases, corresponding to resistance to fluoroquinolones, which were not confirmed subsequently.

Conclusions. Hain MTBDR is a fast, highly sensitive, highly specific and relatively economical method for diagnosing drug resistance of *M. tuberculosis*.

Key-words: Molecular genetic method, cultural method, chemoresistant forms of tuberculosis.

Резюме. Эффективность Hain MTBDR в определении устойчивости M. Tuberculosis к противотуберкулезным препаратам

Ежегодно по данным ВОЗ туберкулёзом заболевает около 10 млн. человек, 1,2-1,4 млн. из них умирают. Немалый вклад в данную статистику вносят химиорезистентные формы туберкулёза – ежегодно регистрируется 483000-639000 новых случаев мультирезистентного (МРТБ) и расширенно резистентного (РРТБ) туберкулёза.

Целью данного исследования было оценить чувствительность и специфичность молекулярно-генетического метода диагностики Hain MTBDR по сравнению с культуральными методами определения устойчивости *M. tuberculosis* к противотуберкулезным препаратам.

Материалы и методы. Было обследовано 3527 образцов мокроты пациентов больных туберкулёзом, полученных в течение 2018-2019 гг., с помощью Hain MTBDR и культуральных методов исследования (в системе BACTEC и на среде Левенштейна-Йенсена).

Результаты. Среди обследованных образцов мокроты было найдено 25 несовпадений между результатами Hain MTBDR и культуральных исследований, что составило 0,7% от всех проведенных исследований. Чувствительность Hain MTBDR в данном исследовании составила 99,7%, а специфичность – 99,6%. Большинство несовпадений результатов молекулярно-генетических и культуральных методов исследований (12 случаев) приходилось на определение устойчивости к фторхинолонам. Из них в 10 случаях были обнаружены мутации в гене gyrA, соответствующие устойчивости к фторхинолонам, которые впоследствии не подтвердились.

Выводы. Hain MTBDR является быстрым, высокочувствительным, высокоспецифичным и относительно экономным методом диагностики лекарственной устойчивости *M. tuberculosis*.

Ключевые слова: молекулярно-генетический метод, культуральный метод, химиорезистентные формы туберкулёза.

Background. According to the WHO, about 10 million people fall ill with tuberculosis every year, 1.2-1.4 million of them die. Chemoresistant forms of tuberculosis make a significant contribution to this statistic - 483,000-639,000 new cases of multidrug-resistant (MDR-TB) and extensively drug resistant (XDR-TB) tuberculosis are recorded annually. That is, approximately every 17th patient in the world develops multidrug-resistant or extensively drug resistant tuberculosis [1]. In this regard, an important issue is the fastest determination of *M. tuberculosis* (MTB) drug resistance with the aim of timely initiating treatment with individual regimens using second-line drugs and preventing the expansion of drug resistance.

Cultural research methods (the gold standard for determining the resistance of MTB) are essentially phenotypic, so they cannot determine the resistance to anti-tuberculosis drugs as soon as possible. XDR-TB diagnostics is a two-step process. In the first

stage, resistance to first-line drugs is determined, and in the second stage, resistance to second-line drugs is determined [2].

Over the past decade, the diagnosis of drug-resistant tuberculosis has been significantly improved with the introduction of molecular genetic research methods [3]. These methods can be divided into 2 main categories: linear probe methods and sequencing methods. The first are GeneXpert MTB / RIF (Cepheid, USA) and Hain MTBDR (Hain Life Sciences, Germany) [4], which have received worldwide distribution due to their high sensitivity, specificity and economic efficiency. Sequencing methods, despite their higher sensitivity and specificity, have not been so widely used because of their high cost, which prevents their widespread use in countries with a high tuberculosis burden as part of national tuberculosis control strategies.

The Hain test uses hybridization technology to detect mutations associated with MTB resistance to both the first and second line drugs. Detection of certain mutations in the rpoB gene corresponds to suspected resistance to rifampicin (R), katG and inhA to isoniazid (H), gyrA and gyrB to fluoroquinolones (Q, in particular to levofloxacin (Lfx) and moxifloxacin (Mfx)), rrs and eis - to aminoglycosides.

Earlier studies, systematized in two meta-analyzes [5, 6], noted a wide variation in the sensitivity of this method from 96% (for R) to 44% (for kanamycin (Km)) and specificity from 99% (for H, R, Km and amikacin (Am)) up to 79% (for ethambutol). Due to this variation in the diagnostic value of molecular genetic diagnostic methods, culture tests remain the gold standard for determining the drug resistance of tuberculosis.

This study was performed to evaluate sensitivity and specificity of molecular-genetic diagnostic method Hain MTBDR in comparison to cultural methods of determining M. tuberculosis drug resistance.

Materials and methods. 3527 sputum specimens from TB patients were investigated by Hain MTBDR and cultural methods (BACTEC and solid Lowenstein-Jensen medium) during 2018-2019. Hain MTBDR was used to determine resistance to R (deletions and mutations in the rpoB gene), H (deletions and mutations in the katG and inhA genes), Q (deletions and mutations in the gyrA and gyrB genes) and aminoglycosides (deletions and mutations in the rrs, eis genes). The results obtained when performing culture tests and Hain MTBDR were compared with each other. Statistical data processing was performed using Microsoft Office Excel 2010 and Statistica 8.0.

Results. Among sputum samples, 25 inconsistencies were found between the results of Hain MTBDR and culture tests, which accounted for 0.7% of all the studies performed (Table 1).

From the data obtained it is clear that most of the differences in the results of molecular genetic and culture tests (12 cases) accounted for the determination of resistance to fluoroquinolones. Of these, in 10 cases, mutations were found in the gyrA gene, corresponding to resistance to fluoroquinolones, which subsequently was not confirmed phenotypically by using culture drug susceptibility tests.

The false negative Hain MTBDR results, that is, the absence of detected mutations responsible for drug resistance combined with phenotypical detection of mutations, were observed mainly in the diagnosis of H resistance (4 cases) and aminoglycosides (Km - 3 cases, Cm - 3 cases).

The sensitivity of Hain MTBDR in this study was 99.7%, and specificity was 99.6%.

Discussion. The Hain MTBDR method is becoming more and more popular in bacteriological laboratories of tuberculosis institutions, since it has a sufficiently high sensitivity and specificity and can quickly detect the resistance of MTB to anti-tuberculosis drugs.

The sensitivity and specificity of the Hain MTBDR method in this study was higher compared with the results obtained in the studies of Feng et al. (2013) and Bai et al. (2016) [5-6], however, this may be associated with a significantly smaller number of tests carried out than in two meta-analyzes.

The omission of resistance to H may be due to the fact that Hain MTBDR does not determine the S315N mutation, which, meanwhile, occurs in almost a quarter (23.8%) of H-resistant MTB [7].

The omission of aminoglycoside resistance can be explained by the same reason. Detection of mutations in the eis and rrs genes provides a high level of specificity of the study and a sufficient level of its sensitivity [5, 6], however, mutations encoding resistance to aminoglycosides may be contained in other genes not identified by Hain MTBDR, in particular, gidB [11] .

Preservation of sensitivity to fluoroquinolones even in the presence of mutations in the GyrA gene was also demonstrated in a number of studies [8–10], where it was suggested that substitution of C-8-methoxy and C-8 halogen in the chemical structure of the new generation of fluoroquinolones (levofloxacin and moxifloxacin) provides their higher bactericidal and bacteriostatic efficacy with a lower minimum

Table 1.

Comparison of Hain MTBDR and culture tests results

Drug	Hain MTBDR	Mutations	Number of cases	Culture tests
R	resistant	deletion wt8 in rpoB gene	1	sensitive
H	sensitive	-	4	resistant
Q	sensitive	-	1	resistant to Lfx
		-	1	resistant to Lfx, Mfx
	resistant	deletion wt2 and mutation MUT2 in gyrA gene	1	resistant to Lfx
		deletion wt2 and mutation MUT2 in gyrA gene	1	sensitive to Lfx, Mfx
		deletion wt2 and mutation MUT1 in gyrA gene	3	sensitive to Lfx, Mfx
		deletion wt3 in gyrA gene	1	sensitive to Lfx
		deletion wt3 in gyrA gene	1	sensitive to Lfx, Mfx
		deletion wt3 and mutation MUT3C in gyrA gene	1	sensitive to Lfx
		deletion wt3 and mutation MUT3C in gyrA gene	1	sensitive to Lfx, Mfx
		deletion wt3 and mutation MUT3A in gyrA gene	1	sensitive to Lfx, Mfx
Km	sensitive	-	3	resistant
	resistant	deletion wt2 in eis gene	1	sensitive
Cm	sensitive	-	3	resistant
	resistant	mutation MUT1 in rrs gene	1	sensitive

inhibitory concentration, therefore mutations in the GyrA gene can provide resistance to these fluoroquinolones only in low concentrations, whereas in the standard dosage their antimicrobial activity will be maintained.

Conclusions. Hain MTBDR is a fast, highly sensitive, highly specific and relatively economical method for diagnosing drug resistance of M. tuberculosis. A small percentage of differences between the results of molecular genetic and phenotypic (cultural) research methods can be explained by the fact that Hain MTBDR does not detect rare mutations that determine resistance to anti-tuberculosis drugs, as well as the fact that mutations in the gyrA gene with the introduction of a new generation of fluoroquinolones detect resistance to low concentrations of drugs only.

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