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COMPARATIVE ASESSMENT OF BACTERIAL EXCRECTION DEGREE DEPENDING FROM SENSIVITY PROFILE OF MYCOBACTERIUM TUBERCULOSIS TO ANTITUBERCULOSIS DRUGS

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Introduction

The term "successful pathogen" [1] has been introduced into medical science recently. It means the causative agent of the disease that has gained global significance, and in the evolution process formed mechanisms for protection against innate and acquired immunity, as well as treatment. "Successful pathogens" include, first of all, mycobacterium tuberculosis (MTB), human immunodeficiency virus, hepatitis, herpes. According to the WHO, these agents infected several billion people across the planet.

Tuberculosis (TB) remains a serious health problem. According to statistical reporting, in recent years, the incidence of TB in Ukraine has improved. The incidence of active forms of TB in 2016 amounted to 54.7 cases per 100 000 populations, compared with 2014 - 70.5 and with 2011 - 75.9. At the same time, the incidence rate of contact persons at the hotbeds of TB infection increased and amounted to 9.3 cases per 1000 contacts in 2016, compared with 2014 - 6.7 and from 2011 - 5.5 [2,3] . This situation actualizes work in the hotbeds of TB infection, taking into account modern principles and methods.

The presence of MTB in the patient's sputum sample is one of the main symptom of respiratory TB that help to detect and identify a pathogen, to study its properties, and set the diagnose. The indicator of bacterial excrection has a trend to increase and in 2016 was 31.9 per 100 000 populations, compared with 2014 - 31.0 and from 2011 - 29.1 [2,3]. The assessment of bacterial excretion has an important prognostic value, determines the severity of the disease and the effectiveness of treatment [4,5].

One of the important part of the epidemiological component of TB pathomorphosis in modern conditions is the change in important biological properties of the pathogen, not only in the form of increased frequency and extension of MTB resistance to anti-tuberculosis drugs (ATD), but also changes in manifestations of vital activity - virulence, massivity and growth rate of culture, etc.[6,7].

The effect of resistance on the properties of MTB and their relationship is not definitively determined. For example, experiments using the biological method confirmed differences in the virulence of different strains of MTB and various resistant profiles [8, 9], as well as MTB resistant to isoniazid (INH), have low virulence for experimental animals [10]. More recent studies demonstrate the complexity of relations between genetic mutations that are responsible for the resistance and properties of bacteria [11,12].

In Ukraine classic bacteriological methods are used: Ziehl–Neelsen bacterioscopy, culture studies on Löwenstein–Jensen media (L-J), and also since 2012 the more modern methods, culture studies on liquid media with use of BACTEC MGIT-960 automated system and Xpert MTB/RIF molecular-genetic testing. Any culture technique determines MTB phenotypic features, Xpert MTB/RIF determines genotypic ones.

Purpose: to determine the growth intensity of MTB depending on profile of resistance to ATD, to assess the bacterial excretions, to compare the data of the phenotypic and genotypic methods, to carry out the correlation analysis of the results.

Materials and methods

The results of the phenotypic and genotypic tests of 148 samples have been analyzed. The material of the study was sputum collected for diagnosis of new case of TB. The results of one first portion were assessed. All cases were registered in Kharkiv region in 2016 with firstly diagnosed lung TB, proved by a positive culture test. The samples were divided into groups according to the results of a drug susceptibility test (DST). Thus, group 1 included 52 samples with conserved susceptibility to ATD, group 2 included 38 samples with monoresistance to INH and polyresistance to INH and streptomycin (such an aggregation of profiles became possible due to wide distribution of resistance to streptomycin, and the exclusion of this medication from standard TB treatment regimens), group 3 included 58 samples with multidrug resistant (MDR) profile, i.e. resistance to INH and rifampicin (RIF).

The standard assessment of positive inoculation: - single colonies– 1-19 colonies;- 1+ - 20-100 colonies; - 2+ - 100-200 colonies; - 3+ - 200-500 colonies; - 4+ - over 500 colonies (too numerous to count).

According to order of the Ministry of Health No.250 dated 21.05.2007 the bacterial excretion is assessed as: scanty for single colonies, moderate for 20 to 100 colonies (1+), massive for 100 and over (2+,3+,4+) [13]. This assessment is important for epidemiological control over TB infection hotbeds; thus, the housing of patients with massive and moderate bacterial excretion refers to dangerous hotbeds (category I), with scanty excretion to less dangerous hotbeds (II category).

The standard assessment of positive molecular genetic testing by quantities of DNA copies: very small, small, moderate, great quantity.

Correlation analysis (by Spearman and Kendall) has been made in SPPS static program.

Results

In the group with conserved sensitivity of MTB to ATD by inoculation results the following bacterial excretions have been noted: 30,76% - scanty, 28,84% - moderate, 40,38% - high. In the group with MTB resistance to INH scanty bacterial excretion was identified in 36,84%, moderate in 39,17%, massive in 23,68%. In the group with MDR TB: scanty in 37,93%, moderate in 22,41% and massive bacterial excretion in 39,65%. (Table 1.)

Massive bacterial excretion was more often identified in patients with conserved sensitivity and multiresistance to ATD, in patients with resistance to INH

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scanty and moderate bacterial excretion is noted more often.

Table 1. Results of L-J culture test

Group	Quantity of samples	Scanty bacterial excretion		Moderate bacterial excretion		Massive bacterial excretion	
	n	n	%	n	%	n	%
1	52	16	30,76	15	28,84	21	40,38
2	38	14	36,84	15	39,47	9	23,68
3	58	22	37,93	13	22,41	23	39,65

Table 2. Results of Xpert MBT/RIF

Group	Quantity of samples	Negative result		Very small quantity of material		Small quantity of material		Medium quantity of material		Great quantity of material	
		n	%	n	%	n	%	n	%	n	%
1	52	4	7,7	3	6,25	13	27,08	16	42,1	15	31,25
2	38	6	15,8	5	15,62	9	28,12	11	34,37	7	21,57
3	58	5	8,6	6	11,32	15	28,3	14	26,41	17	32,07

In the molecular genetic testing 15 negative results have been noted, which comprised 7,7% in 1 group, 15,8%, in 2 group, 8,6% in 3 group. Thus, the culture study has etyologically confirmed TB diagnosis in more than 10% of patients with the negative result of Xpert MTB/RIF. All samples have the inoculation result of up to 20 colonies.

92,5% of cases had the positive results of Xpert MTB/RIF in 1 group: very small quantity in 6,25%, small in 27,08%, medium quantity in 42,1%, great quantity in 31,25%. In 2 group 84,2% of positive results have been noted: 15,62% - very small quantity, 28,12% - small, 34,37% - medium quantity, 21,57% - great quantity. In 3 group 91,4% of positive results have been noted: very small quantity in 11,32%, small in 28,3%, medium quantity in 26,41%, great quantity in 32,07%. (Table 2.) Thus, the result very small quantity is 4,3 % and 9,4% more frequent in group 2 than in groups 1 and 3 respectively, the results small quantity are distributed evenly in all groups 27-28%, medium quantity is 7,73% and 15,7% more frequently noted in group 1 than in groups 2 and 3, respectively, great quantity is 10% more often in groups 1 and 3, than in group 2.

Correlation analysis showed a strong and significant correlation between the indicated results, the Spearmen correlation is 0.75, Kendall - 0.66, with the reliability level $p<0.01$.

An incomplete match of the results of the genotypic and phenotypic studies is observable. This may be because the Xpert MTB/RIF methodology is automated, and the credibility of the results of inoculation on solid L-J media depends on the quality of the reagents, conditions of preparation of the medium and the skills of the laboratory specialists.

Discussions

In the group with conserved susceptibility of MTB, the genotypic and phenotypic methods revealed a significantly higher rate of bacterial excretion than in the group with isoniazid resistance. The same high rates of bacterial excretion were observed in the group with MTB resistance to isoniazid and rifampicin, which indicates a higher epidemic risk for patients with sensitive and multidrug-resistant tuberculosis.

Scanty and moderate bacterial excretion was significantly more frequent in the group with isoniazid resistance. In addition, the phenotypic method made it possible to add more than 15% of cases of bacterial excretion in this group, which suggests the need to take into account all the diagnostic methods for objectifying the indications for hospitalization and the amount of work in the hotbets of infection.

The massive bacterial excretion was more often identified in patients with conserved sensitivity and multiresistance to ATD, in patients with resistance to INH scanty and moderate bacterial excretion is noted more often.

Incorporation of Xpert MTB/RIF results with information on the amount of MTB genetic material in the patient's sputum sample, as well as the presence of MTB resistance, in assessing the of hotbeds of tuberculosis infection will make it possible to improve epidemiological surveillance, by significantly shorter time of analysis.

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Keywords: Mycobacterium tuberculosis, tuberculosis, MDR tuberculosis, Xpert MTB/RIF, bacterial excretion, infection control.

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