Research Article

Non-Tuberculosis Mycobacteria In Phthisio-Pulmonological Practice In The Republic Of Uzbekistan

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ABSTRACT

Background: With the widespread increase in the incidence of mycobacteriosis, a thorough study of this disease is required in order to develop algorithms for diagnosis, treatment and monitoring. It has been shown that patients with local immunodeficiency - chronic obstructive pulmonary disease, bronchiectasis, cystic fibrosis, pneumoconiosis and other respiratory diseases

Methods: The study involved 14544 patients who had applied to the Republican Specialized Scientific and Practical Medical Center of Phthisiatry and Pulmonology under the Ministry of the Health of Uzbekistan. The ATS / IDSA diagnostic algorithm was used as the criteria for non-tuberculosis mycobacteria detection. Sputum, bronchoalveolar lavage fluid, feces, pleural fluid, surgical material, punctate, urine were taken for biological analysis.

Results: Out of 14544 patients, non-tuberculosis mycobacteria were detected in 38 (0.26%) patients; while Mycobacterium avium complex - in 17 (44.7%), among men - in 26 (68.4%), among women - in 12 (31.6%). Non-tuberculosis mycobacteria were predominantly excreted in sputum - in 27 (71.2%) patients, less often in urine - in 6 (15.7%) and from bronchoalveolar fluid - in 2 (5.3%), in isolated cases in pleural fluid, gastroduodenal lavage and from the operating material.

Conclusion: According to the results of a retrospective study of the treatment results of 14544 patients who underwent inpatient therapy for the period from 2009 to 2019. with suspected respiratory TB, it was revealed that NTMB are detected annually in biological samples of 4.0 patients.

Keywords: non-tuberculous mycobacteria, DNA strips, GenoType Mycobacterium AS / CM, Mycobacterium avium Complex.

INTRODUCTION

The incidence of tuberculosis (TB) in the Republic of Uzbekistan, according to the expert commission of the World Health Organization (2018), tends to decrease. So, in 2011, it was 52.4 per 100 thousand population, and already in 2018 - 42.5 per 100 thousand population [23]. Thanks to the introduction of modern high technologies, cases of detection of non-tuberculous mycobacteria (NTMB) are registered in the country; nevertheless, the problem of NTMs has not been sufficiently studied, and the records of diseases caused by NTMs are not kept. According to the American Thoracic Association (ATS), the incidence of NTMs in the US and the European Region from 2006-2007. was 0.3 - 0.8 per 100 thousand population [8.9], and in 2014 - from 2.3 to 3.9 per 100 thousand population [4,7,20]. The growth in the incidence of mycobacteriosis is evidenced by the data of studies conducted in England, Spain, Japan, Great Britain, Brazil, the Netherlands [5,9,24]. According to the studies of a number of foreign authors, there are territorial differences in the species diversity of

NTMs - both in developed and developing countries [3,21]. According to a number of authors, in the Russian Federation, the USA, parts of the regions of Southeast Asia and most of the European region, Mycobacterium avium Complex (MAC) is more often found [16,22,15].

There is still no official statistics on the registration and recording of diseases caused by NTMB, both in the Russian Federation and in other republics of the post-Soviet space, including the Republic of Kazakhstan, in the Republic of Uzbekistan; accordingly, it is not possible to reliably estimate the incidence rate.

To date, more than 120 types of NTM are known, 15 of them are considered conditionally pathogenic for humans (MAC, M.kansasii, M.abscessus, M.chelonae, M.fortuitum, M.genavense, M.haemophilum, M.immunogenum, M. malmoense, M.marinum, Mmucogenicum, M.scrofulaceum, M.smegmatis, M.szulgai, M.ulcerans, M.xenopi) [14]. Of greatest interest is Mycobacterium avium complex (MAC), which is a common NTMB, and causes disease, the clinical

manifestations of which are similar to TB [13,17,18]. According to a case-control population study conducted in Denmark, it was found that in patients with any type of chronic respiratory diseases, the risk of NTM damage to the respiratory organs increased 16.5 times [1]. Moreover, NTM and the causative agent of tuberculosis can cause both combined and monoinfection, which is difficult to differentiate using standard diagnostic methods used in the practice of phthisiatric institutions. Thus, mycobacteriosis is currently a serious problem for both clinicians and laboratory specialists. A significant variety of pathogens, the complexity of the preanalytical stage, the ambiguity of microscopic examination and cultivation methods, the introduction of fundamentally new identification methods into practice require the development of regulatory documents governing the algorithms for laboratory diagnostics and identification of etiologically significant NTMs.

METHODS
Study population
The retrospective study included 47019 diagnostic samples isolated from 14544 patients who underwent inpatient treatment in departments for the period from 2009 to 2019. with suspected respiratory tuberculosis. The age of the patients varied from 20 to 60 years (mean age 46.1 ± 6.5 years). By gender distribution: men were - 9599 (65.9%), women - 4945 (34%). The ATS / IDSA diagnostic algorithm, which included:
- two or more positive sputum cultures for NTM from different samples (if the study of the original sputum sample did not give a result, it was repeated) or
- at least one positive culture for NTMB bronchoalveolar lavage fluid (BALF)
- histopathological changes in transbronchial or other lung biopsy with a positive culture for NTMB or at least
- one positive sputum culture or culture of BALF on NTM [9].
Table 1 presents diagnostic samples isolated from patients and sent for microbiological research to the National Reference Laboratory (NRL).

Material design
Diagnostic specimens were processed using N-acetyl-L-cysteine sodium hydroxide (NALC) in a class 2 biosafety cabinet [19]. Microscopic examination of sputum smears stained by the Ziehl-Nelsen or Auramine-O method (the method is nonspecific, therefore a positive sample test result (ABC +) may indicate the presence of both TB and NTMB in the sample) [2]. Cultivation was carried out on liquid culture medium.

Table 1: Biological material used for study

| Sputum	BALF	Feces	Pleural fluid	Surgical material	Punctuate	Urine
31112 (66.1%)	847 (1.8%)	743 (1.5%)	688 (1.4%)	2121 (4.5%)	485 (1%)	11023 (23.4%)

Middlebrook 7H9 (base broth 0.47% w / v 7 ml) with inoculation of 0.5 ml of decontaminated diagnostic sample (BACTEC ™ MGIT ™ 960 System, Becton Dickinson, USA) [11,12]. The identification of the culture of NTMB isolated during growth on days 4–42 was carried out using SDMPT64 (SD Bioline TB Ag MPT64 test, Korea) [17]. The species of NTMB cultures was carried out using the GenoType Mycobacterium AS / CM version 1.0 DNA * strip hybridization technology. The test procedure consisted of three stages: DNA isolation from cultures grown on a solid or liquid medium, multiplex amplification (PCR) with biotinylated primers, reverse hybridization of the DNA of the strain under study with DNA probes specific for the mycobacteria species immobilized on a nitrocellulose strip; further, the results were taken into account by comparing those that appeared on the strip (Hain Lifescience GmbH, Nehren, Germany) [10]. Statistical analysis and data collection were carried out in the MS Excel © interface using the Epi DataAnalysis © version 3.1 [6]. The research was conducted according to the principles of the World Medical Association Declaration of Helsinki.

RESULTS
As a result of identification of NTM cultures using GenoType Mycobacterium AS / CM, 9 types of NTM were isolated. The total number of NTMs for the period from 2009 to 2019 was 38 (0.26%) cases. As can be seen from table 1, two thirds of mycobacteriosis are slow-growing NTMB, which amounted to 26 (68.4%). Among the slow-growing, the most common were the NTM species belonging to the Mycobacterium avium complex (MAC) - 17 (44.7%), represented by similar microorganisms: non-photochromogenic species *M.avium* - 8 (21%) and *M.intracellulare* - 9 (23.6%), cathochromogenic *M. gordonae* were less common - in 8 (21%) cases. *M. kansasii* was detected in only one case, which amounted to 2.63%. Fast-growing non-tuberculous mycobacteria were isolated in 12 (31.6%) samples. Among them, *M.fortuitum* - 5 (13.1%) and *M.chelonae* - 4 (10.5%) prevailed. *M.marinum,*

M.septicum and *M.immunogenum* were encountered in isolated cases.
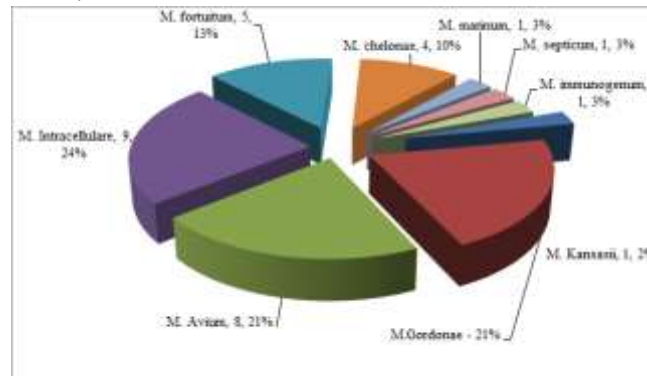
Fig.1: Frequency of isolation of various types of non-tuberculous mycobacteria in patients examined for the period 2009 - 2019.

It should be noted that non-photochromogenic and cathochromogenic NTMs deserve the greatest attention, which most often cause mycobacteriosis against the background of impaired natural immunity, especially in patients with any type of chronic respiratory diseases, such as COPD, BEB (bronchiectasis), cystic fibrosis, pneumoconiosis, including tuberculosis of various localization. The results obtained are comparable with the data of the world and domestic literature [9,21,16].

In terms of gender composition, NTMs were more common among men (26, 68.4%) than among 12 women (31.6%). It should be noted that the largest number of patients with NTMB was in the middle age, in women - 45.5 years, in men with a median of 52 ± 5.4 years.
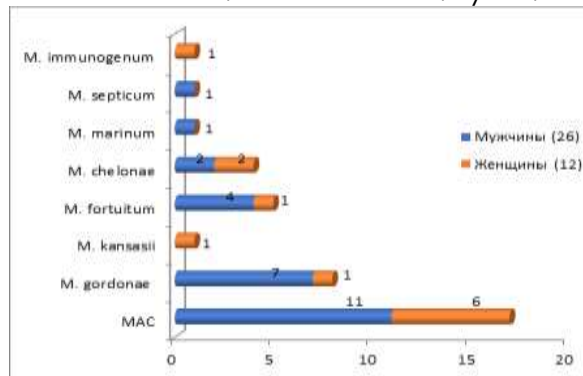
Fig.2: Distribution of cases of NTMB detection by gender composition, taking into account species

There was no significant difference in the detection of Mycobacterium avium complex in men and women, as in diagnostic samples of men: MAC occurred in 11 (42.3%) cases, and in women - in 6 (50%).

Table 2: shows the frequency of isolation of various types of non-tuberculous mycobacteria in diagnostic samples.

	Sputum	BALF	Gastro-lavage	Fecs	Plural fluid	Surgical material	Punctu ate	Urine
M. marinum	1 (2,6%)							
M.intracellulare	8 (21,1%)							1 (2,6%)
M.gordonae	5 (13,3%)					1(2,6%)		2 (5,3%)
M.fortuitum	4 (10,5%)				1(2,6%)			
M.chelone	3 (7,9%)							1 (2,6%)
M.avium	4 (10,5%)	1 (2,6%)	1 (2,6%)					2 (5,30)
M.septicum		1 (2,6%)						
M.immunogenum	1 (2,6%)							
M.kansassii	1 (2,6%)							
Bcero	27 (71,2%)	2 (5,3%)	1 (2,6%)	0	1 (2,6%)	1 (2,6%)	0	6 (15,7%)

M.gordonae and M.fortuitum were more common in men than in women, which amounted to 7 (26.9%) and 4 (15.3%), versus 1 (8.3%) and 1 (8.3%)) in women, however, *M.chelonae* was more common in women - in 2 (16.6%) cases, and in men - in 2 (7.7%) cases. The rest of the NTMBs were encountered in isolated cases.

It can be seen from the table that NTMB were excreted mainly in sputum - in 27 (71.2%) patients, less often in urine - in 6 (15.7%) and from bronchoalveolar fluid - in 2 (5.3%). In isolated cases, NTMBs were detected in pleural puncture, gastroduodenal lavage and in one patient from the surgical material who was operated on for fibrocavernous tuberculosis.

CONCLUSION

According to the results of a retrospective study of the treatment results of 14544 patients who underwent inpatient therapy for the period from 2009 to 2019. with suspected respiratory TB, it was revealed that NTMB are detected annually in biological samples of 4.0 patients. The total number of patients with NTMB for the period from 2009 to 2019 amounted to 38 (0.26%); at the same time. Mycobacterium avium Complex is the most common NTMB - 17 (44.7%), which is comparable with the data of the world literature. GenoType Mycobacterium AS / CM is a reliable tool for determining the species of NTMs and is recommended by WHO to continue monitoring the spectrum of NTMs circulating in the Republic of Uzbekistan, which will undoubtedly allow a specialist to timely verify the pathogen. We believe it is necessary to study NTMB deeper and in detail with the subsequent modification of the protocol, taking into account the species and further formation of the diagnostic algorithm and treatment regimens in the territory of the Republic of Uzbekistan.

AUTHOR CONTRIBUTIONS

Nargiza Parpieva undertook analyses; Sultanov Sanjar led the laboratory component; Anvarova Ekaterina led the precipitins component; Babamatova Khilola led the component for selected bacterias; all authors contributed to the analysis, and to drafts and edits of the manuscript.

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